



## Abstracts for Poster Presentations

Organized by Track

## Gulf Ecology

### Determining Bioindicators for Coastal Tidal Marsh Health Using the Food Web of Larvae of the Greenhead Horse fly (*Tabanus nigrovittatus*)

**D. Bhalerao\***, D. Swale, L. Foil, C. Husseneder

Louisiana State University Agricultural Center, Baton Rouge, LA

Coastal marshlands of the Gulf of Mexico are of economic and ecological importance but highly threatened by natural and anthropogenic impacts. Current methods to determine the health of marshlands are laborious, time consuming and expensive. Our study focusses on identifying invertebrate bioindicators of marsh health. The greenhead horse fly *Tabanus nigrovittatus* Macquart is native to coastal marshlands from Texas to Nova Scotia. The larvae are apex invertebrate predators and their development is dependent on the food web in the soil. Population crashes in adults and larvae of this horse fly species were observed after the Deepwater Horizon Oil Spill in eastern Louisiana, which was affected by the oil spillage. We hypothesize that the food web of greenhead horse fly larvae was impacted by the oil spill and that components of the food web can be used as bioindicators of marsh health. Firstly, to identify components of the food web that are important to sustain larval development we performed DNA metabarcoding of the larval guts and the surrounding sediment. Families of insects and fungi were highly abundant in the guts and sediment. We found no significant difference in larval diets from varied locations suggesting that tabanid larvae collected at different sites but during the same season have similar diets in Louisiana marshes. Seasonal variation in the larval diets will be evaluated in a separate study. Comparing food webs at sites where larvae were present and sites where they were absent will identify taxa that are crucial to the food web sustaining larval development. Secondly, sediment toxicity, oil content and biochemistry will be correlated to abundance of components in the larval food web to determine factors that may have contributed to the decline of larvae in eastern Louisiana following the oil spill. The results of this study will be used to develop an inexpensive PCR tool to detect marsh health of the entire eastern United States.

### The Lingering Effect of Crude Oil from the Macondo Oil Spill on the Community Composition of Epiphytes

**S. Blonder\***, M. Parsons

Florida Gulf Coast University, Fort Myers, FL

Oil spills can result in deleterious impacts in many coastal ecosystem components, including microalgae. Many microalgae thrive as epiphytes living on macro-algae or seagrass hosts. These epiphytic algae make up part of the microbiome that also includes associated micro- and meso-grazers and benthic microalgae living on the sediment surface (edaphic). Epiphytic algae communities have a great influence on the surrounding micro-benthic community as well as the overall ecosystem. There is little research, however, on how oil impacts epiphytic communities. The objective of this study was to determine how the community composition of epiphytic microalgae and their grazers changes due to the influence of natural crude oil exposure. The potential influence of oil exposure was tested by examining 28 epiphyte samples collected from the Chandeleur Islands (Louisiana) in September 2015, at sites with exposure to low, moderate, to high levels of oil during the Macondo oil spill. The community composition of diatoms, dinoflagellates, cyanobacteria, protozoans and larger zooplankton were identified to the lowest taxonomic units possible, and quantified. Environmental parameters,

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host distinctions, and PAH levels were compared against the biomass, abundance, and diversity of the epiphyte community through ANOSIM and SIMPER analyses in PRIMER. Due to the lack of existing data on the impacts of oil exposure on these communities, this study provides baseline research for future studies. It is important to understand how these communities are impacted because they can be used as indicators of ecosystem health and resilience, as well as potential biomarkers of oil pollution.

## Integrity of the Mouse Blood-Brain Barrier is Reduced by Polyaromatic Hydrocarbons

**W. Burggren**<sup>1</sup>, D. Ho<sup>2</sup>

<sup>1</sup>Biological Sciences, University of North Texas, Denton, TX, <sup>2</sup>Clinical Investigation, Tripler Army Medical Center, Honolulu, HI

A common outcome of oil spills is exposure to volatile organic compounds. Such toxicants may have narcotic and carcinogenic effects for humans and terrestrial vertebrates. While the gross morphological, physiological and toxicological effects of oil-derived compounds are being intensively studied, the more subtle effects on behavior are poorly understood. While there are many mechanisms by which toxicants can affect behavior, the blood-brain barrier represents an important defense in keeping toxicants and other neurologically active substances from penetrating the central nervous system. Consequently, any substance that disrupts the integrity of the blood-brain barrier has the potential for altering behavior. We have previously detected several such substances off-gassing from Deep Water Horizon oil, including the model polyaromatic hydrocarbon (PAH), benzo-a-pyrene (BaP). In these experiments, we cultured monolayers of mouse brain microvascular endothelial cells (BMECs) - one of the primary constituents of the mammalian blood-brain barrier - in 96-well plates that were fitted with printed circuits. We continuously measured the transendothelial electrical resistance (TEER) of the monolayer (an indication of the barrier function integrity of endothelial cells) using the Applied BioPhysics ECIS system. After the cells formed a monolayer (~24-30 hours after cell inoculation), BaP was introduced into the cell medium, creating concentrations of 1, 10, 100 and 1000 nM. All BaP concentrations produced a significant ( $P < 0.05$ ) reduction in mouse BMEC barrier integrity in a concentration-dependent manner, with percent reductions in TEER ranging from 15-25% measured 20 hours after first exposure. These experiments confirm that BaP, and perhaps other polycyclic aromatic hydrocarbons, have the potential for producing subtle behavior as well as morphological and physiological effects in mammals through interference with the blood-brain barrier's integrity.

## A Spatiotemporal Analysis of Hepatic and Biliary PAHs in Groupers from around the Gulf of Mexico

**B. Carr**<sup>\*1</sup>, E. Pulster<sup>1</sup>, A. Garcia<sup>1</sup>, M. Armenteros<sup>2</sup>, S. Murawski<sup>1</sup>

<sup>1</sup>University of South Florida, St. Petersburg, FL, <sup>2</sup>University of Havana, Havana, Cuba

Groupers are among the most economically important species groups in the Gulf of Mexico. Understanding their contaminant load and population health is important in sustaining the fishery. This study measured polycyclic aromatic hydrocarbon (PAH) concentrations in bile and liver samples of multiple grouper species, with the goal of better understanding the spatiotemporal distribution and variability of PAH contamination throughout the Gulf of Mexico. Bile and liver samples were collected from a number of grouper species during a gulf-wide survey conducted over a seven-year period from 2011 to 2017. Liver tissues were extracted using the QuEChERS method and quantified using GC-MS/MS. Biliary PAHs were quantified using high pressure liquid chromatography equipped with a

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fluorescence detector (HPLC-FLD). This data set establishes a gulf-wide baseline of PAH levels in groupers, which is critical in assessing impacts on these economically important species as a result of any future oil spills. Spatial patterns, species differences and size/sex comparisons are provided and interpreted in this paper.

## The Effect of Temperature and UV on Marine Protozoa Following Exposure to Chemically Dispersed Crude Oil

**S. Cosgrove**, M. Moison, E. J. Buskey  
UTMSI, Port Aransas, TX

Environmental parameters such as temperature and UV light can affect the growth and survival of planktonic organisms. Marine protozoa are often understudied, yet form an essential part of the marine food web, serving as consumers of phytoplankton and as prey of larger zooplankton. This study aims to investigate the impact of crude oil on the growth and community structural changes of various protozoan species when exposed to different temperatures and light conditions. Two methods of experimentation were employed to test the effect of Louisiana light sweet crude oil at concentrations of 1ppm, 5ppm and 10ppm and the dispersant Corexit 9500A with a dispersant to oil ratio of 1:20. We ran 7 day mesocosm experiments within 120 gal. tanks using natural protozoan communities obtained from the Aransas Shipping Channel in South Texas to determine alterations in community diversity and species richness. This larger scale experimentation method was implemented over two seasonal periods (June and December) allowing for an average 15 °C difference in the ambient seawater temperature. On a smaller scale, heterotrophic dinoflagellate species were exposed to chemically dispersed crude oil in 2L quartz glass bottles placed in a temperature controlled UV transparent Plexiglas incubator, and exposed to varying light conditions (full spectrum, no light, no UV-B). The Plexiglas incubator experiment was run at both ambient temperature of 25 °C and 10°C below. This allows for the testing of the combined effect of varying light conditions and temperature. Following exposure to chemically dispersed crude oil under these varying environmental conditions, daily survival, mortality and potential recovery rates of protozoan species were determined. Under all environmental scenarios, heterotrophic dinoflagellates in particular exhibited a higher level of tolerance and recovery, potentially influencing the planktonic food chain.

## Tales Told by Two Hundred Tilefish: A Baseline Health Assessment of the Gulf of Mexico 2015-2017

**K. Deak**\*<sup>1</sup>, L. Dishaw<sup>2</sup>, S. Snyder<sup>1</sup>, S. Murawski<sup>1</sup>

<sup>1</sup>University of South Florida, St Petersburg, FL, <sup>2</sup>USF Children's Research Institute, St Petersburg, FL

To quantify baseline health of demersal teleosts in the oil-rich Gulf of Mexico, an extensive demersal long-line survey was conducted in United States, Cuban, and Mexican waters from 2015-2017. Using Golden tilefish (*Lopholatilus chamaeleonticeps*), an array of biomarkers was examined in tissue and blood samples to screen for toxicologically relevant endpoints of petrochemical exposure. These targets included: immune system markers (lysozyme, hematocrit, leukocrit, and differential white blood cell counts), oxidative stress effects (sorbitol dehydrogenase, thiobarbituric reactive substances, and superoxide dismutase) and cumulative genotoxicity (erythrocyte abnormality classifications). Using multivariate analysis, fish collected from regions potentially impacted by the Deepwater Horizon oil spill in the vicinity of the De Soto Canyon had statistically significant differences in biomarker response

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from fish from similarly platform-rich regions in Texas and Mexico. This includes a suppression of plasma lysozyme response, elevation of monocyte counts, nearly absent antioxidant production, low levels of liver damage, and a lower frequency of blebbed erythrocyte nuclei in De Soto Canyon fish, despite elevated levels of liver and biliary polycyclic aromatic hydrocarbons. This suggests the development of resiliency mechanisms in this area. Conversely, these markers responded nearly identically in samples collected from the other oil fields. Sex differences were also observed Gulf-wide, with statistically significant elevation of plasma lysozyme and sorbitol dehydrogenase in females, possibly linked to protection during spawning seasons. This dataset is the most extensive compilation of Golden tilefish health currently known in the Gulf of Mexico and can be utilized to rapidly assess the impact of future spill events across this dynamic ecosystem.

## The Marsh Periwinkle (*Littoraria irrorata*) as an Indicator of Deepwater Horizon Oil Spill Effects

**D. R. Deis**<sup>1</sup>, J. W. Fleeger<sup>2</sup>, S. M. Bourgoin<sup>3</sup>, D. S. Johnson<sup>4</sup>, I. A. Mendelssohn<sup>5</sup>, Q. Lin<sup>2</sup>, A. Hou<sup>6</sup>

<sup>1</sup>Atkins, Jacksonville, FL, <sup>2</sup>Louisiana State University, Baton Rouge, LA, <sup>3</sup>Atkins, Tallahassee, FL, <sup>4</sup>Virginia Institute of Marine Science, Gloucester Point, VA, <sup>5</sup>Louisiana State University, Baton Rouge, LA, <sup>6</sup>Louisiana State University, Jacksonville, LA

The Deepwater Horizon oil spill resulted in varying degrees of oiling in the salt marshes of northern Barataria Bay, Louisiana, USA. This study examines the effects of moderate and heavy marsh oiling compared to reference (without visible oiling) stations, and recovery, on *Littoraria irrorata*, the salt marsh periwinkle, from 2.5 to 7.5 years after the spill. After an initial population decline associated with oiling, the population density of *L. irrorata* increased at all oiling categories with the highest density of *L. irrorata* at moderately-oiled sites compared to both reference and heavily-oiled stations. *Spartina alterniflora* stem density recovered within two years after the spill at the moderately-oiled stations facilitating recovery of *L. irrorata* density approximately one year later. *L. irrorata* average shell length and length-frequency distributions are potentially sensitive indicators of the health and recovery of the marsh. Average shell length increased linearly from 16.5 mm at the beginning of the study to 19.0 mm at 7.5 years at reference sites. However, snails at both the moderately-oiled and heavily oiled sites increased in average shell length through year 4 of the study, but then decreased. The difference between the oiling categories is associated with a decline in the relative abundance of the larger adults (shell size 21-26 cm) at oiled sites. This discrepancy could mean that either *L. irrorata* individuals are not surviving to these larger sizes, are growing more slowly than expected, or that adults leave the oiled area prior to growth to these larger sizes. Larger *L. irrorata* may be emigrating from the oiled areas.

## Oiling Effects on Oxygen and Hydrogen Sulfide Depth Profiles in Coastal Marine Sediments

**M. E. Feeney**<sup>\*1,2</sup>, M. W. Lindsey<sup>1,2</sup>, M. L. Parsons<sup>1,2</sup>, A. Robertson<sup>3,2</sup>, H. Urakawa<sup>1,2</sup>

<sup>1</sup>Florida Gulf Coast University, Fort Myers, FL, <sup>2</sup>Alabama Center for Ecological Resilience, Dauphin Island, AL, <sup>3</sup>University of South Alabama and Dauphin Island Sea Lab, Dauphin Island, AL

Coastal sediment microbial communities play important roles in nutrient cycling and primary production in estuaries. Past studies have documented a range of responses to oiling in these sediment microbial communities, but there is need for a better understanding of the biotic and abiotic factors

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involved in the differing responses. We hypothesized that coastal oiling diminishes the oxygen supply to mudflat and coastal subtidal sediment and creates reduced conditions. In the present study, the physicochemical response of subtidal sediment cores to the addition of water-accommodated fractions (WAF) of crude oil was investigated through microelectrode profiles. Sediment samples were collected from subtidal zones in both the Chandeleur Islands, Louisiana and Estero Bay, Florida. The sediment microbial communities from the Chandeleur Islands represent an opportunity to study previously oiled communities, as they were exposed to the Deepwater Horizon oil spill in 2010. The sediments of Estero Bay serve as controls in which no oil contamination has been previously recorded. As expected, hydrogen sulfide increased with depth in the anoxic parts of the sediment. Overall responses of oxygen and hydrogen sulfide to WAF exposure varied depending on the sediment properties and other limiting factors. The maximum hydrogen sulfide concentration observed in each core ranged between 104  $\mu\text{mol/L}$  and 8319  $\mu\text{mol/L}$ , and the maximum oxygen penetration depth observed ranged between 0 mm and 5.75mm. Even under the undiluted WAF exposure, respiration and photosynthesis processes of benthic microbial communities were not completely halted. Generally, WAF exposure promoted hydrogen sulfide production. Higher concentrations of hydrogen sulfide were observed in sediment cores from Estero Bay than those from the Chandeleur Islands, possibly due to differing sediment types, microbial communities and benthic fauna.

## Quantification of Functional Marker Genes for Denitrifying Microbial Populations in the Chandeleur Islands Impacted by the 2010 Gulf of Mexico Oil Spill

**N. Y. Flourney**<sup>1</sup>, P. Crawford<sup>1</sup>, C. Tatariw<sup>2</sup>, B. Mortazavi<sup>2</sup>, P. A. Sobecky<sup>1</sup>

<sup>1</sup>University of Alabama, Tuscaloosa, AL, <sup>2</sup>Dauphin Island Sea Lab, Dauphin Island, AL

Barrier island ecosystems provide protection by reducing storm surges, dissipating wave energy, and economically through services such as fisheries, water catchment, and water quality. As these ecosystems are deteriorating and threatened in this century, services provided to humans are being valued monetarily to communicate their importance. Events such as the 2010 Gulf of Mexico oil spill, act as catalysts to accelerate deterioration and further loss of these vital ecosystem services. The oil spill impacted the Chandeleur Islands, barrier islands in Louisiana waters located forty miles south of Gulfport, MS. Island chain vegetation; i.e., *Avicennia germinans* and native *Spartina alterniflora* was heavily damaged as a result of the oil spill. As oil was deposited differentially, it was important to investigate the microbiology of oil-impacted areas as marsh vegetation is directly linked to microbe-driven ecosystem services such as denitrification, a nitrogen (N) cycle pathway. The objectives of this study were: i) characterize the biodiversity of microorganisms; ii) quantify denitrifying microbial populations using functional marker genes; and iii) measure rates of denitrification during a one-year period. Eco-functional marker genes *narG*, *nirS*, *norB*, *nosZ*, and *nrfA* were selected to represent denitrification. Three different marsh sites were selected for study based upon estimated amounts of prior oiling. Highest rates of denitrification were in September while the lowest rates were observed in February. The highest *nirS* abundance was detected for two of the three sites (Site 1 and 2) in September while Site 3 exhibited the highest abundance in November. Similarly, the highest abundances observed for *norB* and *nosZ* varied by site and by month. Weathered oil was also detected in some of the marsh sediment cores and chemically typed to Macondo oil. Studies such as this one are designed to characterize the barrier island microbial biodiversity and N cycle processes to better understand long-term effects disturbances such as the 2010 oil spill pose to an ecosystem service that contributes to maintaining marine water quality. This is especially important in light of the fact that weathered oil continues to be observed seven years post spill.

## The Role of the Plant-Microbe Relationship in Oiled Salt Marsh Management

S. K. Formel<sup>\*1</sup>, V. Elango<sup>2</sup>, J. H. Pardue<sup>2</sup>, S. A. Van Bael<sup>1</sup>

<sup>1</sup>Tulane University, New Orleans, LA, <sup>2</sup>Louisiana State University, Baton Rouge, LA

The 2010 Deepwater Horizon oil spill is being heavily investigated to understand the effects of the spill on communities of macro- and microorganisms. In particular, the foundational salt marsh grass, *Spartina alterniflora*, is studied with regards to the effects of oil pollution because of the central role it plays in salt marsh function. However, the response of the plant has never been studied in association with the microbial associates of the plant. We hypothesize that the microbiome of *S. alterniflora* is integral to the persistence of the grass in oiled environments and propose that the microbiome should be considered in management of oiled salt marshes. Here we report our preliminary results from a greenhouse experiment in which *S. alterniflora* was grown in mesocosms contaminated with naturally weathered Macondo-252 oil. We examine changes in the microbial communities of *S. alterniflora* and plant physiology through time and between the rhizosphere, root, and leaf tissues. Additionally, we describe how oil constituents are changing through time and as they infiltrate the plant tissues, depending on the presence or absence of the plant and select microbial taxa. Effects of the oil are slow to manifest, supporting the idea that long-term research is necessary after an oil spill. The response of these communities, and the ecosystem they support, is key to understanding the oil spill and developing efficient management strategies in affected salt marshes.

## Patch Structure and Diel Vertical Migration of Zooplankton in Hypoxic Waters Measured with *in-situ* Imaging and Multibeam Acoustics

A. T. Greer<sup>1</sup>, I. Church<sup>2</sup>, L. M. Chiaverano<sup>3</sup>, C. Briseno-Avena<sup>4</sup>, K. Axler<sup>4</sup>, F. J. Hernandez<sup>3</sup>, R. K. Cowen<sup>4</sup>, W. M. Graham<sup>1</sup>

<sup>1</sup>University of Southern Mississippi, Stennis Space Center, MS, <sup>2</sup>University of New Brunswick, Fredericton, NB, Canada, <sup>3</sup>University of Southern Mississippi, Ocean Springs, MS, <sup>4</sup>Oregon State University, Newport, OR

The northern Gulf of Mexico (nGOM) represents a biologically productive coastal ecosystem influenced by large amounts of freshwater input that, under weak wind conditions, can generate strong stratification in the summer. Stratification, in conjunction with oxygen consumption in deeper waters, leads to extensive hypoxia across the Louisiana shelf and Mississippi Bight. To understand the ecological effects of hypoxia, high resolution spatial distributions of different zooplankton taxa are needed. Multibeam acoustics, which are used extensively in hydrographic surveys, have the potential to resolve zooplankton patches on fine-scales and in 3D, but their use in ecological studies requires high quality data on taxonomic composition and oceanographic properties to determine the dominant contributors to backscatter. To examine the ecological effects of hypoxia and evaluate the utility of the multibeam to resolve distributions, we towed the *In Situ* Ichthyoplankton Imaging System (ISIS, equipped with a CTD, dissolved oxygen, and chl-a fluorescence sensors) while simultaneously collecting day/night multibeam water column backscatter (Reson Seabat 7125, 400 kHz) along a 9 km transect following the 20-m isobath. Multibeam data were processed to make them directly comparable to the imaging system (i.e., compressed into 2D). The imaging system detected a variety of diel vertical migration behaviors: shrimp aggregated within the hypoxic zone during the day and migrated to mid-water column at night, while larval fishes tended to reside in the upper portion of the water column

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during both day and night. The multibeam data showed diel variability in acoustic backscatter that was likely driven by the shrimp migrations. We find that zooplankton have taxon-specific responses to hypoxic waters, which likely plays a role in the overall community response to this widespread summer phenomenon in the nGOM. Future work aims to use the 3D data produced by the multibeam to resolve patches across larger areas of the nGOM.

## Impacts of Crude Oil and Dispersant on Marine Microbial Biofilms: Implications for the Preservation of Historic Gulf of Mexico Shipwrecks

J. L. Salerno<sup>1</sup>, **R. Mugge**\*<sup>2</sup>, B. Little<sup>3</sup>, J. Lee<sup>3</sup>, L. J. Hamdan<sup>2</sup>

<sup>1</sup>George Mason University, Manassas, VA, <sup>2</sup>University of Southern Mississippi, Ocean Springs, MS, <sup>3</sup>U.S. Naval Research Laboratory, Stennis Space Center, MS

Episodic deposition of hydrocarbons and chemical dispersant may disrupt deep-sea benthic ecosystems, including artificial reefs formed by historic shipwrecks and their associated organisms. Laboratory experiments were performed to determine the impacts of crude oil, dispersed crude oil, and dispersant on the community structure and function of microorganisms in seawater (SW) and biofilms on carbon steel, a common material for metal ship hulls. Steel corrosion was monitored to investigate the potential impact of spill contaminants on steel shipwreck preservation. Experimental aquaria were filled with SW, incubated at 4°C, and carbon steel disks (CSDs) were placed in each tank. Tanks were amended with crude oil, dispersed oil, dispersant or no treatment, and monitored weekly for up to 16 weeks for temperature, salinity, and dissolved oxygen. SW and CSD biofilms were sampled biweekly to characterize shifts in bacterial communities via Illumina MiSeq sequencing of 16S ribosomal RNA gene amplicons. Predicted and sequenced metagenomes were analyzed to examine impacts of oil and dispersant on metabolic function of biofilms. Gammaproteobacteria, Alphaproteobacteria, and Flavobacteria dominated SW and biofilm communities. Bacterial community structure in SW and biofilms differed significantly between treatments. Biofilms amended with oil and dispersed oil exhibited increased OTUs affiliated with hydrocarbon-degrading microorganisms and CSDs from these treatments had increased metal loss compared to controls and dispersant treatments. Dispersant-amended biofilms showed a decrease in biodiversity and genes associated with hydrocarbon degradation. These findings indicate that exposure to spill contaminants can disrupt biofilms colonizing metal hulls and enhance steel corrosion, potentially impacting shipwreck preservation.

## Larval Gulf Menhaden (*Brevoortia patronus*) Diet, Growth and Condition during an Anomalous High Freshwater Discharge Event

**A. M. Hoover**\*<sup>1</sup>, C. Briseño-Avena<sup>2</sup>, L. Chiaverano<sup>1</sup>, A. L. Deary<sup>3</sup>, F. Hernandez<sup>1</sup>

<sup>1</sup>University of Southern Mississippi, Ocean Springs, MS, <sup>2</sup>Oregon State, Corvallis, OR, <sup>3</sup>NOAA, Seattle, WA

The Gulf Menhaden commercial fishery is the largest by weight in the northern Gulf of Mexico (GOM). Efforts to manage stocks of fisheries importance have recently evolved toward ecosystem management approaches, which consider multiple biotic and abiotic environmental influences. Physical parameters such as salinity and temperature, as well as biological parameters such as phytoplankton and zooplankton community structures can impact larval fish survival and recruitment. In January 2016, historic levels of rainfall within the upper Mississippi River Basin prompted the earliest

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opening of the Bonnet Carré Spillway, a water control structure upriver of New Orleans, LA, that diverts water from the Mississippi River through Lake Pontchartrain, and into the northern GOM. This unprecedented winter opening of the spillway coincided with the spawning season and larval ingress period for Gulf Menhaden. The Consortium for Coastal River Dominated Ecosystems (CONCORDE), along with several other research consortia, collected physical and biological data aboard the *RV Point Sur* from February 10-12, 2016 in order to assess the biophysical impacts of this atypical discharge event in the Mississippi Bight. Distinct water masses were identified during the cruise using a suite of physical and chemical observations, and subsurface plankton samples were collected in each water mass using a 60-cm bongo net sampler fitted with 202- $\mu\text{m}$  and 333- $\mu\text{m}$  mesh nets. Here we examine the potential impacts of the spillway opening on the planktonic foodweb as it pertains to larval Gulf Menhaden. Our analysis includes comparisons among water masses of zooplankton diet and assemblage structure, and larval Gulf Menhaden diet, growth and condition. Overall, our goal is to better understand how biophysical factors influence larval Menhaden survivorship and recruitment dynamics in the GOM.

## The Occurrence and Distribution of Large Siliceous Particles on the Mississippi-Alabama Shelf During Spring

**S. Kelso**\*<sup>1</sup>, A. Chaplin<sup>1</sup>, J. W. Krause<sup>1</sup>, C. Briseño-Avena<sup>2</sup>, A. L. Deary<sup>3</sup>, F. J. Hernandez<sup>4</sup>

<sup>1</sup>Dauphin Island Sea Lab, Dauphin Island, AL, <sup>2</sup>Hatfield Marine Science Center, Oregon State University, Newport, OR, <sup>3</sup>National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Seattle, WA, <sup>4</sup>Department of Coastal Sciences, University of Southern Mississippi, Ocean Springs, MS

Diatoms are an important coastal phytoplankton group as they can dominate algal biomass and contribute to primary production in some of the most productive regions for fisheries. Individual diatoms are large among phytoplankton (e.g.  $\sim 0.02 - 0.10$  mm), but too small for direct consumption by many fishes or larvae unless they are in large chains or aggregates. We present data on the abundance of large siliceous particles (LSP, e.g. diatom chains, aggregates) measured on size-fractionated (0.2 - 4.8 mm) mesozooplankton samples from the Mississippi-Alabama shelf spring 2016 bloom period. Copepods (dominant group in abundance and biomass) have siliceous mandibles and can retain consumed diatoms in their guts; hence we used total copepod counts and measurement of silica per copepod to correct for this artifact. Among the five size-fractions, the LSP biomass per volume swept showed no statistically significant differences, suggesting that LSP size may be on the order of millimeters, consistent with the lengths of large-diatom chains observed with concurrent *in-situ* imagery. LSP represented  $\ll 1\%$  of the total silica quantified on 1.2  $\mu\text{m}$  filters with samples drawn from 10-L Niskin bottles (which typically under-sample rare particles like LSP); this too is consistent with *in-situ* imagery data showing large-diatom chain abundances of  $\sim 2 - 5 \text{ L}^{-1}$ . The implications for these findings are: 1) despite their abundance, LSP likely have a disproportionately important role on silica and organic matter export due to increased size and settling efficiency; 2) LSP may escape predation by smaller grazers which are similar in size and the rarity of LSP lessens exposure to small fishes and larvae; 3) if aggregated by physical mechanisms such as those occurring in plankton thin layers, these LSP may be abundant enough to be efficiently consumed by higher trophic organisms (e.g. fish or larvae), increasing the transfer efficiency from primary producers to higher trophic levels.

\* Student presenter

## Competition in a marine teleost is altered by exposure to crude oil

**A. J. Khursigara\***, J. L. Johansen, A. J. Esbaugh

The University of Texas at Austin, Port Aransas, TX

Fish form social hierarchies in which some fish become dominant over subordinate fish. These hierarchies become more prominent when resources are limited in the environment, whereby dominant fish experience reduced social stress while monopolizing resources. This leads to higher fitness and survival in dominant fish, while subordinate fish experience chronic stress that leads to a cascade of physiological impairments that can result in ecological death. A number of studies have shown that exposure to low concentration of polycyclic aromatic hydrocarbons (PAHs) can reduce physiological parameters such as aerobic scope and swim performance. Our previous work has shown that in a dyad setting, oil exposure can predispose an individual to social subordination. Based on this, we hypothesized that oil exposed individuals would be at a competitive disadvantage in a larger group setting, which would result in reduced growth and elevated chronic stress indicators. There was no difference observed in an 8 week growth test utilizing satiating feeding regimes, however when resources were limited, individuals exposed to environmentally realistic oil concentrations had reduced specific growth rate as well as a significantly reduced standard metabolic rate. These data demonstrate that the physiological impairments imposed by sub-lethal oil exposure can impact downstream ecological performance.

## Isotopic and Mercury Analyses of Coastal Seabirds Collected from Louisiana in 2010 during the Deepwater Horizon Natural Resource Damage Assessment

**K. Lamb\***<sup>1</sup>, M. Polito<sup>1</sup>, R. Brasso<sup>2</sup>

<sup>1</sup>Louisiana State University, Baton Rouge, LA, <sup>2</sup>Southeast Missouri State University, Cape Girardeau, MO

Understanding the foraging ecology and trophodynamics of biocontaminants among marine vertebrate species is critical in addressing their conservation and management concerns. Stable isotope analysis is commonly used to estimate diets and resource use as marine predators retain isotopic abundance of animal tissues that reflect the same biomarkers found in their diet and habitat. Accumulation of mercury is common in tissues of top marine predators like seabirds. Trophic magnification factors (TMF) correlate mercury concentrations with trophic structure and helps estimate the biomagnification potential of mercury. Using samples collected from coastal Louisiana from June to December 2010 by Deepwater Horizon Natural Resource Damage Assessment (NRDA) we examined the relative foraging ecology and trophodynamics of total mercury (Hg) of four coastal seabird species: Brown Pelican (*Pelecanus occidentalis*), Laughing Gull (*Leucophaeus atricilla*), Royal Tern (*Thalasseus maximus*) and Black skimmers (*Rynchops niger*). To quantify the relative trophic positions of the seabirds, oyster and blue crab samples were also analyzed. Relative trophic position of each species was established using stable isotope analysis where nitrogen isotopic values ( $\delta^{15}\text{N}$ ) infer trophic level and diets, while carbon isotopic values ( $\delta^{13}\text{C}$ ) trace trends in inshore vs. offshore habitat usage. While data analysis is ongoing, preliminary results indicate high correlation between trophic level and total mercury concentration. Further analysis will quantify this observation and provide insight into the extent of biomagnification and bioaccumulation in coastal Louisiana.

\* Student presenter

## Evaluating the Accuracy of Metabarcoding Based Biodiversity Analysis

**F. Leasi**<sup>1</sup>, J. L. Sevigny<sup>1</sup>, H. Bik<sup>2</sup>, K. S. Diaz<sup>1</sup>, P. Montagna<sup>3</sup>, J. L. Norenburg<sup>4</sup>, M. Reuscher<sup>3</sup>, M. Rohal<sup>3</sup>, T. Schuelke<sup>2</sup>, W. K. Thomas<sup>1</sup>

<sup>1</sup>University of New Hampshire, Durham, NH, <sup>2</sup>University of California, Riverside, Riverside, CA, <sup>3</sup>Texas A&M University Corpus Christi, Corpus Christi, TX, <sup>4</sup>Smithsonian National Museum of Natural History, Washington, DC

Accurate estimates of biodiversity are required for ecosystem monitoring programs and understanding ecosystem function. This is especially true for meiofauna: a hyperdiverse community of microscopic organisms that play fundamental ecological roles, underpin ecosystem functioning, and are ubiquitous in every aquatic habitat on earth, but for which identification is labor intensive, time consuming, and costly. Recent studies suggest that metabarcoding - DNA sequence analysis of a common “orthologous” gene - is a cost- and time-effective method to disentangle meiofauna diversity. However, the efficiency of metabarcoding meiofauna has not been well tested, with most analyses coming from samples of only select community members. Here, we aim to test metabarcoding on a higher diverse community using different laboratory and computational methods. We compare the results obtained from different metabarcoding approaches on approximately a thousand individuals belonging to seven phyla; each specimen was previously morphologically identified at the species level. Results suggest that biodiversity estimates are strongly biased by (i) the primers and/or 18S rDNA region used, (ii) the specific taxon, and (iii) the bioinformatics pipeline applied. Such biases affect our understanding of species composition, and the ecological correlations within and among communities. With the goal of achieving a standard operational procedure that assesses biodiversity of meiofauna accurately and consistently, we explore different methods (preservation, extraction, library-preparation, and bioinformatics pipelines) to obtain, sequence, and analyze environmental DNA with a multi-locus approach. Preliminary results suggest that these procedures will help achieve a more accurate and inclusive biodiversity estimation, understand the links between marker genes and ecosystem functions, and responses to sediment contamination.

## How were Microphytobenthic Communities Influenced by the Deepwater Horizon Oil Spill?

**M. W. Lindsey**\*<sup>1</sup>, M. L. Parsons<sup>1</sup>, A. Robertson<sup>2</sup>

<sup>1</sup>Florida Gulf Coast University, Fort Myers, FL, <sup>2</sup>University of South Alabama and Dauphin Island Sea Lab, Dauphin Island, AL

Crude oil contains many different compounds that can alter multiple components of marine ecosystems, including the microphytobenthos (MPB). Changes in the MPB community structure can impact the benthic food web, including higher trophic level organisms that serve as economically important fisheries in the Northern Gulf of Mexico (GoM). As the impacts of crude oil exposure on MPB community dynamics are poorly known, the purpose of this study was to compare subtidal MPB community responses (i.e. changes in biodiversity, resilience, and recovery) of a previously-exposed population (Chandeleur Islands, Louisiana), to a naïve population (Estero Bay, Florida) in controlled laboratory microcosm experiments (6.5 cm diameter sediment cores). This experiment occurred over the course of five weeks during the summer of 2017 (July and August). Eighteen sediment cores (9 from each study site) were exposed to three water-accommodated fraction (WAF) crude oil treatments (100%, 50%, and 0% WAF). Over the course of the experiment, photosynthetic measurements were taken for Optimum Yield and Total Maximum Fluorescence using a Diving PAM. Subsamples were also

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collected for microscopic examination of the MPB community. We hypothesize that some communities of MPB would be hindered by the crude oil WAF, while others would be stimulated; there may also be a shift in species abundances between the MPB communities. The results will also provide data to assess MPB community recovery. Lastly, the results can provide insight into potential impacts, if any, of MPB changes to mesograzers and higher trophic level organisms.

## Predicting Disturbance-Driven Impacts on Ecosystem Services in Coastal Wetlands

S. Rajan<sup>1</sup>, P. Crawford<sup>1</sup>, A. Kleinhuizen<sup>2</sup>, C. Tatariw<sup>2</sup>, B. Mortazavi<sup>2</sup>, P. Sobecky<sup>1</sup>

<sup>1</sup>University of Alabama, Tuscaloosa, AL, <sup>2</sup>University of Alabama, Dauphin Island Sea Lab, Tuscaloosa, Dauphin Island, AL

Natural and human-induced disturbances pose significant threats to the health and long-term productivity of Alabama coastal wetlands. As wetlands are a vital state resource, decisions on management, restoration, and remediation require actionable data if socio-economic demands are to be balanced with efforts to sustain these habitats. In 2010, the BP oil spill was a large and severe disturbance that threatened coastal Gulf ecosystem services. The largest marine oil spill to date served to highlight fundamental gaps in our knowledge of oil-induced disturbances and the resiliency and restoration of coastal Alabama wetland functions. To address these gaps, a year-long mesocosm study was conducted to investigate oil-induced effects on (i) plant-microbial interactions, (ii) microbial and plant biodiversity, and, (iii) the contributions of microbial genetic biodiversity to ecosystem services. In this study, *Avicennia germinans* (black mangrove), a C3 plant that grows from the tropics to warm temperate latitudes, were grown with or without mono- and polyculture mixtures of *Spartina alterniflora*, a C4 plant. At an interval of 3-months, 1.9 L m<sup>-2</sup> of Louisiana sweet crude oil was introduced as a pulse disturbance. Molecular-based analyses of microbial community biodiversity, genetic diversity, and functional metabolic genes were compared to controls (i.e., no oil disturbance). To assess the oil-induced effects on the nitrogen (N) cycle, measurements of denitrification and N fixation processes were conducted. Our results showed that community diversity and phylogenetic diversity significantly changed and that the oil disturbance contributed to the creation of niches for distinct microbial types. The abundance of N-fixing microbial types increased as the abundance of denitrifying microbial types decreased as a result of the oil disturbance. As denitrification is an ecosystem service that directly contributes to removing nitrate (NO<sub>3</sub><sup>-</sup>) loading to coastal zones, impairment of this process is detrimental to the long-term health and productivity of the Gulf of Mexico. Our results are designed to investigate controlling factors and yield insights to aid decision-makers in their ongoing management efforts to restore wetlands along the Alabama coast and elsewhere.

## The Effects of *Geukensia granosissima* on *Spartina alterniflora* and Nitrogen Cycling in a Louisiana Salt Marsh

A. McDonald\*<sup>1</sup>, K. Ebinger<sup>2</sup>, A. Chelsky<sup>3</sup>, A. Rietl<sup>3</sup>, B. J. Roberts<sup>3</sup>

<sup>1</sup>Eckerd College, St. Petersburg, FL, <sup>2</sup>University of Colorado, Boulder, CO, <sup>3</sup>Louisiana Universities Marine Consortium (LUMCON), Chauvin, LA

Coastal wetlands provide many services including storm protection, nutrient cycling, fisheries production, and long-term carbon storage but are being lost as a result of multiple natural and anthropogenic stressors. Ecosystem engineers modify, maintain, and create habitats by modulating the

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availability of resources to other species, thereby exerting a large influence over local abiotic conditions. In Louisiana saltmarshes, the marsh cordgrass *Spartina alterniflora* and the gulf ribbed mussel *Geukensia granosissima* are coexisting ecosystem engineers that may form a mutualistic relationship that could enhance marsh stability and enhance living shoreline restoration efforts. We examined the impact of *G. granosissima* density and biomass on *S. alterniflora* above- and below-ground biomass and stoichiometry, soil nutrient and carbon pools, and potential nitrification and denitrification (denitrification enzyme assay) rates for ten 0.25m x 0.25m x 10cm deep plots along a ~150m stretch of marsh edge at Sister Lake, LA. We selected plots to provide a gradient of mussel biomass based on visible densities on the surface. Plots were separated into soil, aboveground plant parts, belowground roots, and mussels for processing in the lab. *G. granosissima* surface densities were not a good indicator of total density in the plots (often >10x higher), but we were successful in sampling a broad range in both density and biomass (12-fold and 43-fold, respectively). *S. alterniflora* root biomass, % live roots, and root % N content all increased with mussel density and biomass. Both aboveground, belowground N pools and therefore total plant N pool also increased with *G. granosissima* density and biomass. Neither nitrification nor denitrification potentials were correlated with mussel density/biomass but nitrification increased with soil redox and DEA with soil N content. These results are consistent with *G. granosissima* potentially increasing the overall available nitrogen pool in marsh soils and ultimately leading to increases in total, and particularly, belowground, *S. alterniflora* biomass and N content and further suggest the possible utility of incorporating them into future living shoreline restoration efforts.

## Short-term Changes in Mesozooplankton Abundance and Community Structure in Response to the Presence of Dispersed Crude Oil: A Mesocosm Study

**M. Moison**, S. Cosgrove, E. J. Buskey  
UTMSI, Port Aransas, TX

Mesocosm experiments were performed to investigate the impact of crude oil on Gulf of Mexico mesozooplankton communities. The aim of this study was to determine the effects of crude oil and dispersant treated crude oil on natural zooplankton assemblages over a 7-day exposure period. Twelve mesocosms were filled with 500 liters of natural channel water and spiked with 0.5-5mm plankton from net tows. All treatments (10 ppm Louisiana sweet crude oil; 5ppm and 10ppm of dispersed Louisiana sweet crude oil with a ratio of 1:20 Corexit 9500 to oil at low (20°C) and high (27°C) temperatures) were established in triplicate. Mesozooplankton from glutaraldehyde-preserved samples were enumerated using a stereomicroscope for analyzing shifts in community structure and diversity, and under UV illumination for quantifying the ingestion of crude oil droplets. Zooplankton populations at the start of the experiment were largely dominated by the copepod *Acartia tonsa*. The communities did not show a significant difference between the control treatment and the 5 ppm treatment, whereas all zooplankton communities were affected by the presence of oil in the 10 ppm mesocosms during the first days of the experiment. The zooplankton diversity decreased, with an increased dominance of Polychaeta larvae and harpacticoid copepods (*Euterpina* sp.) and a decrease of calanoid copepods. Moreover, low temperatures increased the toxicity of dispersed crude oil in the 10 ppm mesocosms. All species of copepods were found to be able to ingest crude oil, especially the largest adult calanoids (*Acartia tonsa*, *Centropages* sp. *Parvocalanus* sp. and *Paracalanus* sp.) with a high ingestion rate of crude oil droplets. Overall the shift in mesozooplankton assemblages due to the presence of dispersed crude oil can be expected to affect the young stages of fish larvae that would ingest them.

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## Feeding Behaviors of *Americamysis bahia*: Observations, Experiments, and the Impact on Crude Oil

**A. Nihongi**<sup>1</sup>, S. Wahlig<sup>2</sup>, J. Rubio<sup>2</sup>, J. R. Strickler<sup>2</sup>

<sup>1</sup>School of Freshwater Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI, <sup>2</sup>University of Wisconsin-Milwaukee, Milwaukee, WI

Mysids are found in water bodies all over the world. They are important food sources for fish including within the Gulf of Mexico. The majority of mysids are omnivores and opportunists. We observed close up feeding behaviors of *Americamysis bahia* using a high-speed video camera, holography, and a Schlieren system. We investigated how mysids detect and handle their prey. We then investigated whether the presence of crude oil in the water column will affect the process of *A. bahia* while searching and locating their prey. Additionally, we observed the fecal pellets of *A. bahia* when they were fed with hexadecane or octane.

## Benthic Foraminifera as Indicators for Environmental Baselines and Anthropogenic Influence on the Northern Cuban Continental Slope

**B. O'Malley\***, P. T. Schwing, M. Armenteros, D. J. Hollander

University of South Florida, St. Petersburg, FL

Benthic foraminifera (BF) are single-celled calcareous protists sensitive to environmental change and commonly used as indicators of many types of pollution (e.g. petroleum, heavy metals). The majority of the northern coast of Cuba is characterized by a narrow continental shelf with deep water (>1500 m) within 10-15 km of the coastline. In this study, BF are used to assess pollution gradients off the northern coast of Cuba that serve as baselines for potential petroleum blowouts and a current assessment of anthropogenic influence primarily from population and industrial centers associated with embayment/estuaries (e.g. Havana and Mariel Bays). Cores were collected along a 190 km east-to-west transect along the northern coast of Cuba and, where possible, along inshore to offshore transects. BF were stained, extracted, and identified at species level to determine density, species richness, heterogeneity, and the Foraminiferal Index of Environmental Impact (FIEI). The foraminiferal assemblages and indices from Cuban samples were compared to those from deep-water sites affected by oil spills from the northern (Deepwater Horizon, 2010) and the southern (Ixtoc, 1979-80) Gulf of Mexico. Considering Cuba's growing economy, burgeoning tourist industry, and increased exploration of deepwater petroleum reserves, this study offers not only a perspective on potential existing anthropogenic influences on near shore and deep-water systems, but also provides environmental and ecologic baseline measurements in the case of a future petroleum accident.

## Growth Promotion of Dinoflagellates by Oil-degrading Bacteria Isolated from Oil Polluted Sites after the Texas City "Y" Oil Spill

**B. Park**, H. Bacosa, Z. Liu, T. Severin, D. L. Erdner, E. J. Buskey

Marine Science Institute, Port Aransas, TX

The association between phytoplankton blooms and oil spills is still controversial despite extensive studies. Crude oil leads to variation in bacterial communities and metabolism, and this variation can

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affect phytoplankton growth and species composition. However, studies of the effect of bacterial communities exposed to crude oil on phytoplankton growth have rarely been conducted. As the first step of this study, we isolated oil-degrading bacteria from sediment samples collected from two oil-contaminated sites near Galveston Island after the Texas City “Y” oil spill in 2014, and investigated variation in dinoflagellate growth after addition of these bacteria. A total of seven oil-degrading bacterial cultures were established, and, in preliminary tests, two (C1-T3 and E1-Gal-T2) of these bacterial cultures exhibited clear growth promoting effects on three dinoflagellate cultures; axenic *Amphidinium carterae* and *Peridinium sociale*, and xenic *Karenia brevis*. The growth promoting effect on dinoflagellates had a positive correlation with the inoculated bacterial density; addition of C1-T3 and E1-Gal-T2 at the highest bacterial densities ( $10^6$  or  $10^7$  cells mL<sup>-1</sup>) showed higher growth enhancement of three dinoflagellate cultures, compared to additions of lower densities. To investigate the factors affecting the potential growth-promoting effect of these bacteria, nutrient limited f/2 medium was prepared by removing one of the nutrient components (nitrogen, phosphorous, trace metals or vitamins, and E1-Gal-T2 was inoculated into these nutrient limited media, containing *P. sociale*, at a concentration of  $10^7$  cell mL<sup>-1</sup>. E1-Gal-T2 did not show any growth promoting effect on *P. sociale* under nitrogen, phosphate, or vitamins-limited conditions, but clear growth promoting effect was observed in trace metal-limited media. These findings suggest that these bacteria are capable of enhancing the growth of dinoflagellates through releasing substances associated with trace metals.

## LADC-GEMM: Towed-hydrophone Surveys of the Northern Gulf of Mexico

**C. Pierpoint**<sup>1</sup>, N. Sidorovskaia<sup>2</sup>, L. Scala<sup>3</sup>, D. Comeaux<sup>4</sup>

<sup>1</sup>Seiche Limited, Dinas Cross, Pembrokeshire, United Kingdom, <sup>2</sup>University of Louisiana, Lafayette, LA,

<sup>3</sup>Seiche Limited, Bradworthy, United Kingdom, <sup>4</sup>ASV Global, Broussard, LA

The Littoral Acoustic Demonstration Center, Gulf Ecological Monitoring and Modeling (LADC-GEMM) consortium carried out towed-hydrophone, passive acoustic surveys for marine mammals in 2015 and 2017. The study area in the Northern Gulf of Mexico included the site of the Deepwater Horizon catastrophe. Hydrophone arrays were towed by relatively quiet unmanned surface vehicles (USVs) controlled from a support vessel. Marine mammals were detected both in real-time via a telemetry link, and during offline analysis of continuous sound recordings. We present the distribution of sperm whales and delphinid species encountered during these surveys, and an estimate of sperm whale density. [Research supported by BP/GOMRI Consortium grant.]

## Polycyclic Aromatic Hydrocarbons in Sediment and Gafftopsail Catfish (*Bagre marinus*) Collected in the Bay of Campeche

**E. L. Pulster**<sup>1</sup>, A. Gracia<sup>2</sup>, S. Murawski<sup>1</sup>

<sup>1</sup>University of South Florida, St. Petersburg, FL, <sup>2</sup>Universidad Nacional Autónoma de Mexico, Mexico City, Mexico

The Bay of Campeche contains the majority of the Petroleos Mexicanos (PEMEX) oil rigs and was the location of the world’s third worst oil spill occurring in 1979, the Ixtoc I. Following the spill, PEMEX established a 4,000 km<sup>2</sup> exclusion zone around the spill site, only allowing vessel traffic directly related to oil platform operations. Gafftopsail catfish, *Bagre marinus*, are common in the Gulf of Mexico and were found to be abundant within the exclusion zone. In 2015, sediment and gafftopsail catfish ( $n=20$ ) were collected during a gulf-wide survey from within the exclusion zone in the Bay of Campeche. Bile,

muscle and liver tissues were analyzed for 46 polycyclic aromatic hydrocarbons (PAHs) using HPLC-FLD and GC tandem mass spectrometry. Sediment ( $\Sigma$ PAHs 190-230  $\mu\text{g}/\text{kg}$ ) and biliary benzo[a]pyrene (2.7 - 470  $\mu\text{g}/\text{kg}$ ) were similar in magnitude. Whereas muscle ( $\Sigma$ PAHs  $760 \pm 200 \mu\text{g}/\text{kg}$ ), liver ( $\Sigma$ PAHs  $1,900 \pm 800 \mu\text{g}/\text{kg}$ ) and biliary naphthalene (430 - 35,000  $\mu\text{g}/\text{kg}$ ) were several orders of magnitude higher than sediment concentrations. To our knowledge, this is the first report of PAH levels in fish collected from within the exclusion zone in the Bay of Campeche.

## Impacts of Deep Water Horizon Oil Exposure on the Southern Ribbed Mussel (*Geukensia granosissima*): Implications for Salt Marsh-Stabilizing Facilitation

**A. H. Quade**<sup>\*1</sup>, D. S. Johnson<sup>2</sup>, J. W. Fleeger<sup>3</sup>, D. R. Deis<sup>4</sup>, Q. Lin<sup>3</sup>, A. Hou<sup>3</sup>, S. A. Graham<sup>5</sup>

<sup>1</sup>Nicholls State University, Thibodaux, LA, <sup>2</sup>Virginia Institute of Marine Science, Gloucester Point, VA,

<sup>3</sup>Louisiana State University, Baton Rouge, LA, <sup>4</sup>Atkins, Jacksonville, FL, <sup>5</sup>Biological Sciences, Nicholls State University, Thibodaux, LA

The Mississippi River Delta, the largest and most valuable coastal wetland complex in the contiguous United States, provides numerous ecosystem services to the state of Louisiana, the region, and the nation. However, the provision of one service, oil and gas extraction, critically altered the structure and function of this ecologically and economically important ecosystem when the Deepwater Horizon (DWH) offshore drilling rig exploded and led to the release of approximately 5 million barrels (~800 million L) of crude oil into the northern Gulf of Mexico in 2010. Barataria Bay, located in the central portion of the Delta, was among the areas most severely impacted, with approximately 120 km of oiled coastline and documented negative effects on wetland ecosystems and estuarine dependent species. The primary objective of this research was to investigate the chronic impacts of DWH oiling on the population structure of the southern ribbed mussel (*Geukensia granosissima*), a keystone salt marsh species that facilitates ecosystem function and stability through plant-invertebrate mutualism. Five mussel surveys were conducted in May through October of 2017 at salt marsh sites in northern Barataria Bay that received either moderate oiling, heavy oiling, or no visible oiling. Preliminary results show reduced juvenile and total *G. granosissima* abundance in oiled compared to reference sites, indicating incomplete salt marsh recovery seven years after initial DWH oiling. Long-term negative impacts on *G. granosissima* communities at oiled sites further suggest chronic alterations to ecosystem structure, which may affect ecosystem function, and ultimately influence salt marsh stability.

## Microphytobenthos as Indicators of Oiling and Food Web Dynamics

**N. N. Rabalais**<sup>1</sup>, W. Morrison<sup>2</sup>, G. Woods<sup>2</sup>

<sup>1</sup>Louisiana State University, Baton Rouge, LA, <sup>2</sup>Louisiana Universities Marine Consortium, Chauvin, LA

We hypothesized that phytoplankton accessory pigments would be altered in the sediment surface, along with the microphytobenthos (MPB) community, in response to hydrocarbon concentrations and especially in the summer when hydrocarbons may be volatilizing due to higher air temperatures. We took microphytoplankton benthos samples in spring, summer and fall in 2015 through 2017 to test this hypothesis. HPLC analysis was paired with microscopic counts to determine the community composition. Initial results show a preponderance of diatoms, but also green algae and cyanobacteria (some of which may be toxin producers). Chlorophyll a (Chl a) concentrations were greater than 25 mg g dry sed<sup>-1</sup> in many samples. Units of mg g dry sed<sup>-1</sup> normalize for all sediments in the sample, usually to 2-mm depth, which is not easily compared to mg cm<sup>-2</sup> in (Fleeger et al. 2015 MEPS). Initial results do

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not indicate seasonal differences, but not all data are completed. Variability in Chl a concentrations toward the end of four years post-spill (Fleeger et al. 2015) was high as were values from our study, which covered 2015 to 2017. Our study did not sample from any “highly” oiled sites. Other variables of importance are water level on the day of sampling and for several days before, incident radiation, sediment grain size and hydrocarbon concentration. We also sampled long sediment cores, which were dated for sediment accumulation rate, in each of Terrebonne Bay, Barataria Bay (west) and Barataria Bay (east in the area of Port Sulphur). Three longer cores were taken within the Port Sulphur area. From these, we predicted that sediment microphytobenthos as identified by phytoplankton accessory pigments would correspond to phytoplankton counts, hydrocarbon components, and other sediment characteristics. The phytoplankton pigments in these cores degraded rapidly with depth and may not be useful for historic analyses.

## The Impacts of an Expanded Set of Meiofaunal Genome References on the Analyses of Metagenomic Samples Collected from the Gulf of Mexico

**J. L. Sevigny**\*<sup>1</sup>, F. Leasi<sup>1</sup>, H. Bik<sup>2</sup>, K. Diaz<sup>1</sup>, P. Montagna<sup>3</sup>, J. Norenburg<sup>4</sup>, M. Reuscher<sup>3</sup>, M. Rohal<sup>3</sup>, T. Schuelke<sup>2</sup>, W. K. Thomas<sup>1</sup>

<sup>1</sup>University of New Hampshire, Durham, NH, <sup>2</sup>University of California, Riverside, Riverside, CA, <sup>3</sup>Texas A&M University Corpus Christi, Corpus Christi, TX, <sup>4</sup>Smithsonian Institution, Washington, DC

Meiofauna (benthic metazoans < 2mm) encompass an extremely broad and diverse set of creatures. They are important contributors to ecosystem functioning through their roles in nutrient cycling, sediment stability, and food web interactions. Shifts in meiofaunal biodiversity may reflect environmental changes, which can be revealed almost instantly thanks to the organisms’ short generation time and low dispersal capability. Surveying these communities is fundamental to the assessment of natural and anthropogenic stresses, restoration, pollution monitoring, and ecosystem health. Current molecular studies typically employ metagenetic sequence analyses, but are very limited due to a historically low representation of meiofaunal genomic data in public repositories and the lack of adequate genetic markers to capture population level changes. Here we report on an expanded set of reference genomes and discuss novel bioinformatics workflows to investigate meiofaunal communities. These new genomes encompass 165 individuals representing 76 unique taxa and include the first genomes available for Gastrotricha, Nemertea, and Sipuncula. We have established standard operating procedures for meiofauna preservation, molecular investigations (including DNA extraction and sequencing), and bioinformatic workflows for individual genome assemblies, which will facilitate future sequencing projects. We demonstrate the influence of these new resources on metagenomic studies through comparative analyses of samples collected from the Gulf of Mexico. Furthermore, we report on the role of genetic markers and loci, such as complete mitochondrial genomes and polymorphic microsatellites, to investigate population level changes in meiofaunal communities. These results allow us to evaluate the progress towards a more detailed analysis of meiofauna community structure with a more inclusive array of taxa, fostering our understanding of how anthropogenic stresses, such as oil spills, impact the ecosystem.

\* Student presenter

## Role of Hydrocarbon Seeps in Structuring the Microbial Community

**A. Subramaniam**<sup>1</sup>, A. Juhl<sup>1</sup>, J. Montoya<sup>2</sup>, M. Aleman<sup>3</sup>

<sup>1</sup>Lamont Doherty Earth Observatory, Palisades, NY, <sup>2</sup>Georgia Institute of Technology, Atlanta, GA,

<sup>3</sup>University of Southern California, Los Angeles, CA

The Northern Gulf of Mexico is a dynamic and diverse ecosystem that is driven by a complex circulation pattern and varied physical and chemical forcings. Previously we have shown that the abundant natural hydrocarbon seeps along the continental slope enhance surface phytoplankton chlorophyll concentrations. We used spectrofluorometric techniques, flow cytometry, and High Performance Liquid Chromatography measurements of phytoplankton pigments from various depths to measure microbial concentrations and composition in the northern Gulf of Mexico. The Custom Laser Active Spectroscopic System (CLASS) was used to uniquely quantify cyanobacteria using phycobilifluorescence while the flow cytometer was used to quantify five microbial groups: picoeukaryotes, heterotrophic bacteria, heterotrophic nanoflagellates, *Synechococcus* spp., and *Prochlorococcus*. Statistical analysis show that the microbial populations at natural seep locations clustered separately from those at nonseep locations. We will present the results of this study in the context of the interaction between natural hydrocarbon seeps, hydrographic forcings, and nutrient availability and discuss the biogeography of phytoplankton in the Northern Gulf of Mexico.

## Epiphytic and Sediment Diatom Production in Mississippi Sound

**M. Tabor**\*<sup>1</sup>, L. A. West<sup>2,3</sup>, E. Cox<sup>2</sup>, J. Cebrian<sup>2,3</sup>, J. W. Krause<sup>2,3</sup>

<sup>1</sup>Athens State University, Athens, AL, <sup>2</sup>Dauphin Island Sea Lab, Dauphin Island, AL, <sup>3</sup>University of South Alabama, Mobile, AL

Shallow subtidal regions lining Mississippi Sound (coastal Alabama and Mississippi) are highly productive across trophic levels. A variety of species including seagrasses and benthic microalgae (BMA; e.g. diatoms, dinoflagellates, cyanobacteria) contribute to benthic primary production. Regional studies have shown that eutrophication may negatively affect seagrass density but not the net benthic primary production; this suggests that BMA productivity is quantitatively significant despite considerably lower areal biomass than seagrass. Distributions of primary production among phytoplankton have important implications for material and energy flow in pelagic systems, but despite the known importance of BMA productivity, the contributions of specific groups (e.g. diatoms) are largely unquantified. Here we report the benthic primary production in Grand Bay (Mississippi Sound) and infer the contributions of benthic and epiphytic diatoms. Bare sediment cores and sea grass with sediment cores were collected from a 1-m subtidal zone and incubated in a mesocosm. After the samples had acclimated for two days, half of the cores were dosed with Germanium to arrest diatom division. Three days after Ge addition, we measured gross and net primary production in the water column and sediments to compare rates in both treatments. Within the benthos, there was significantly more microalgal biomass (i.e. Chlorophyll) than in the water column but microalgal biomass among treatments and zones (i.e. water column vs. benthos) remained consistent. While a majority of the system productivity occurred in the water column, we determined that most of the benthic productivity was driven by epiphytic diatoms residing on seagrass. In one of the repeated trials, sediment diatoms appear to have a significant contribution. On the time scale of our experiments, our results suggest that diatoms alone may be responsible for up to 75% of benthic productivity at this site.

\* Student presenter

## The Effects of Crude Oil on Northern Gulf of Mexico Salt Marsh Nitrogen Cycling

**D. Tollette\***, C. Tatariw, B. Mortazavi

University of Alabama, Dauphin Island Sea Lab, Tuscaloosa, Dauphin Island, AL

Coastal salt marshes provide many ecological services including the removal and transformation of nutrients from coastal waters. Denitrification, a microbially mediated process removes nitrate ( $\text{NO}_3^-$ ) that would otherwise contribute to eutrophication in coastal waters. Dissimilatory nitrate reduction to ammonium (DNRA) is also utilizes  $\text{NO}_3^-$  but transforms it into ammonium which is then retained in the system. By affecting these two processes, oil spills can impact the ecosystem services marshes provide. The objectives of our study were two-fold: to determine (i) the extent to which denitrification and DNRA are affected by oil spills in marshes, and (ii) if changes in these processes to new inputs of hydrocarbons are similar at previously contaminated sites and at sites with no history of oil contamination. We collected sediments from three sites subjected to a range of hydrocarbon contamination following the 2010 Deepwater Horizon oil spill. Of the three sites, one was moderately oiled (Chandeleur Islands, LA) while marshes sampled at the other two (Dauphin Island, AL and Dog River, AL) were not contaminated during the spill. Sediments from each site were incubated with and without crude oil and rates of denitrification and DNRA were measured with the isotope pairing technique on sediment slurries. Denitrification and DNRA rates were similar across sites (Two-way ANOVA,  $p = 0.086$  and  $p = 0.865$ , respectively) and between controls and oil treated sediments (Two-way ANOVA,  $p = 0.656$  and  $p = 0.695$ , respectively). These results indicate that denitrification and DNRA rates were similar across sites regardless of contamination history. Moreover, the addition of oil at these sites did not affect rates of denitrification or DNRA across the sites. Taken together these results suggest that the addition of new oil to sediments from these sites does not impact denitrification or DNRA rates regardless of oiling history.

## Juvenile Assemblages of Families Lutjanidae and Serranidae in the Gulf of Mexico, with Respect to the Loop Current and other Hydrographic Features

**S. Velez\***<sup>1</sup>, J. A. Moore<sup>2</sup>

<sup>1</sup>Florida Atlantic University, Boca Raton, FL, <sup>2</sup>Florida Atlantic University, Jupiter, FL

The Gulf of Mexico (GoM) is a dynamic environment with a variety oceanographic processes taking place. These features include; the Loop Current, cyclonic and anticyclonic eddies, and the Mississippi River Plume. They were directly responsible for the extent to which the Deepwater Horizon Oil Spill (DWH) spread throughout the Gulf and are important drivers of the biological processes within the GoM. The relationship these features have on the long-term community assemblages of Families Lutjanidae and Serranidae has been of great interest from both biological and economic standpoints. These families represent some of the most economically important fisheries in the GoM. Identifying the role these features play in the transportation of larval and juvenile nearshore species to offshore environments is vital to resource managers. Using historical data collected shortly after the DWH Oil Spill via the NOAA Natural Resource Damage Assessment (NRDA) in 2011, the faunal composition and abundance of these families were analyzed. In conjunction with these data, cruises conducted by the Deep Pelagic Nekton Dynamics of the Gulf of Mexico (DEEPEND) Consortium from 2015-2017, were analyzed to identify any changes in faunal composition in an attempt to characterize what oceanographic processes affect these assemblages. In comparing these two studies, we have identified seasonal differences in the faunal composition and abundance of these two families within the pelagic environment. Ontogenetic changes were also observed in the genus *Pristipomoides* of family

\* Student presenter

Lutjanidae, where high numbers of larger individuals who were found far from suitable habitats. This suggests an increased ability to remain in the pelagic ecosystem and stall the settling process until a suitable habitat can be found.

## DEEPEND: Molecular Evidence for Environmental Change in the Deep-Sea in the Gulf of Mexico

**M. Weber\***, R. I. Eytan  
Texas A&M Galveston, Houston, TX

It was long believed, that while sea surface temperatures fluctuate greatly, little variation occurs in the deep-sea. This longstanding belief has been challenged as long-term monitoring efforts have uncovered evidence of rapid alterations in deep-sea temperatures. Furthermore, recent studies have suggested that benthic deep-sea communities are currently being altered as a result climatic changes. Historic fluctuations in population size often reveal the effects of major ecological events on the genetic diversity of a population or a species. These fluctuations can be inferred through the use of molecular data. Global climate conditions have varied greatly since the last glacial maxima, approximately 20,000 years ago, leading to alterations in global currents, oceanic temperatures, and sea level. Several studies have uncovered strong evidence of recent genetic bottlenecks in coastal marine fishes attributed to these changes in the marine environment. In this study we sought to answer whether fluctuations in the population sizes of deep-sea fishes mirror those found in coastal/shallower water. Evidence of recent population expansions in deep-sea fishes would suggest that the deep-sea environment is more volatile than previously imagined, while widespread evidence of stable populations in these fishes would suggest otherwise. To answer this question, we employed several different methods of analysis utilizing DNA sequence data; frequency based and tests that make use of the topology and branch lengths of genes trees to infer changes in population size over time. These methods can test for population expansion, population bottlenecks, and exponential growth. These analyses were performed on twenty species of deep-sea fish to identify general trends in the deep-sea environment.

## Benthic Diatom Population Responses to the Oiling of Louisiana Coastal Marshes

**J. A. Zingre\***, M. Parsons  
Florida Gulf Coast University, Fort Myers, FL

\* Student presenter

## Monitoring, Data Management & Analysis

Heat Flux of Upper Ocean in the Northern Gulf of Mexico in the LASER January 2016 and SPLASH April 2017

**M. Barzegar paiin lamouki**\*<sup>1</sup>, D. Bogucki<sup>1</sup>, B. Haus<sup>2</sup>, S. Mehta<sup>2</sup>, S. Minming<sup>2</sup>, M. Segler<sup>1</sup>

<sup>1</sup>Texas A&M University-Corpus Christi, Corpus Christi, TX, <sup>2</sup>University of Miami, Miami, FL

The upper ocean absorbs most of the sun's energy. The ocean and atmosphere are in direct contact with each other and the resulting heat flux has an important role in the weather and climate changes. Wind-induced shear, microscale wave breaking and surface film have affected the heat flux created in the upper ocean. In this study, we have collected the instantaneous vertical temperature profiles by the uprising Vertical Microstructure Profiler (VMP) near the frontal area. Analyzed profiles were compared to theoretical profiles calculated by considering the surface cooling, solar heating and combined effect parameters [1] of the top 4 centimeters of the ocean surface. In addition, we have studied how the salinity and density profiles vary near the front area in the upper ocean. The data used for our studying was collected during the LASER and SPLASH experiments in the northern Gulf of Mexico on January 2016 and April 2017, respectively.

[1] Soloviev, A.V. and R. Lukas, 2014. The Near-Surface Layer of the Ocean: Structure, Dynamics, and Applications (Second edition), Springer, NY, 552 pp.

Sedimentary Signatures of the 2010 Deepwater Horizon Event: A Time Series Analysis

**S. R. Carter**\*<sup>1</sup>, R. Larson<sup>1,2</sup>, P. Schwing<sup>2</sup>, C. Holmes<sup>3</sup>, K. Yang<sup>4</sup>, G. Brooks<sup>1</sup>

<sup>1</sup>Eckerd College, St. Petersburg, FL, <sup>2</sup>University of South Florida, St. Petersburg, FL, <sup>3</sup>Environchron, Tallahassee, FL, <sup>4</sup>Hong Kong Baptist University, Kowloon Tong, Hong Kong

Following the 2010 Deepwater Horizon (DwH) event, a depositional pulse was documented in bottom sediments in the Desoto Canyon region of the Gulf of Mexico. This massive sedimentation event resulted in a ~1 cm thick surface layer deposited within ~5 months, recorded by a significant initial increase in excess <sup>234</sup>Th inventories and mass accumulation rates. The sedimentological signature of the depositional event was recorded differently in two sedimentologic regimes. On the western, siliciclastic-dominated side of the Desoto Canyon, the event was manifested as an initial increase in calcium carbonate content and strong deviations in silt-sized sediments. On the eastern, carbonate-dominated side of the Desoto Canyon, the event was recorded as an initial decrease in calcium carbonate content coupled with variations in silt-sized sediments. All sites demonstrate noticeable changes in sediment texture and composition when compared to pre-event sediments down-core. These results are consistent with a rapid change of sedimentation pattern immediately after the DwH event, followed by a gradual return to pre-event conditions over the subsequent ~6 years. Sediments also exhibit a systematic coarsening since ~1990. This study aims to provide insight into the long-term preservation potential of the sedimentological signature of this event and similar depositional events, whether natural or anthropogenic, that may have been preserved in the sedimentary record.

\* Student presenter

## Oil Slick Thickness Measurement with a Novel Mechanical Sampler

**O. Garcia**<sup>1</sup>, G. Graettinger<sup>2</sup>, L. DiPinto<sup>3</sup>, D. Garcia<sup>1</sup>, H. Fort<sup>4</sup>, D. Han<sup>5</sup>, C. Hu<sup>6</sup>, J. Morris<sup>7</sup>, V. Kourafalou<sup>8</sup>, S. Sun<sup>6</sup>

<sup>1</sup>Water Mapping, LLC, Gulf Breeze, FL, <sup>2</sup>NOAA, Seattle, FL, <sup>3</sup>NOAA, Silver Spring, MD, <sup>4</sup>Abt Associates, Boulder, CO, <sup>5</sup>NOAA, St. Petersburg, FL, <sup>6</sup>University of South Florida, St. Petersburg, FL, <sup>7</sup>Abt Associates, Boulder, FL, <sup>8</sup>University of Miami, Miami, FL

Although it is important to measure oil slick thickness because it is a crucial parameter to calibrate and validate remote sensing estimates and to help assess the amount of spilled oil, in practice such a measurement has been notoriously difficult for a number of reasons. Here, after several attempts of using different techniques developed by the oil industry to sample and measure oil slick thickness, we have developed a new electro-mechanical device for collection of undisturbed floating surface oil. This device consists of a floating apparatus that allows the surface water (and the floating oil) to flow freely through a surface profiler tube. Samples collected by this device are then used to quantify the thickness of the oil using two different methods. The first method consists of a photographic and imaging analysis of the sample, and the second method is a chemical separation of the oil contained in the sample which is then measured volumetrically. During laboratory experiments and in synoptic tests, we also used two other common methods for measuring oil thickness (sorber pads and dip plate) to compare results from all three measurement techniques. Results obtained by the oil sampler device show the capacity of sampling oil films from 5 $\mu$ m to 2mm, suggesting the advantage of its operations on a wide range of thicknesses. Additionally, samples collected from this new device can be used for later chemical analysis of the oil itself. This device has been calibrated in the lab and tested in the Northern Gulf of Mexico where oil slicks were frequently observed. During three different field campaigns, sample collection and measurement over this site showed thicknesses ranging from 5 $\mu$ m to 300 $\mu$ m.

## Abundance and Deformity of Foraminifera as an Effective Monitoring Scale for Contamination in the Persian Gulf

**S. Haghshenas**<sup>1</sup>, M. Risk<sup>2</sup>

<sup>1</sup>University of Tehran, Tehran, Islamic Republic of Iran, <sup>2</sup>McMaster University, Durham, ON, Canada

Hydrocarbon contamination is an important issue of concern in the Persian Gulf, where the whole body of water is heavily influenced by oil and gas developments on both northern and southern coastlines. As the number and volume of developments in this important body of water continue to rise, coastal ecosystems are increasingly threatened by this type of heavy contamination impacts. Moreover, fin-fish cage culture is getting more and more popular all over the gulf with considerable potential amount of impact on marine habitats. Oil spills are not only annoying and unsightly, but many of the compounds can be extremely toxic. Monitoring and evaluation of oil spills should be a regular part of managing the coastlines. One of the best and cheapest monitoring schemes available is using bio-indicators. Benthic foraminifera communities can be used as bio-indicators of coastal oil contamination as contact with hydrocarbons can induce growth deformities in their shapes. On the other hand, fish farm contamination is of a big importance for future developments and foraminifera can act as a fantastic monitor in this regard. These indicators are of short lifespans with quick response to environmental condition fluctuations and can easily be found in carbonate sediments in most of the parts along the study coastline. This study provides a few examples of foram deformation in response

to oil leakage. The responses to the organic waste from fish farms are also described. Foram abundance and morphology can be a useful tool in assessing environmental impacts.

## Determining the Variability and Accuracy of High Frequency Radar Surface Currents in the Mississippi Bight

**L. E. Hode\***, S. D. Howden, A. R. Diercks

The University of Southern Mississippi, Stennis Space Center, MS

High frequency radar-derived surface currents provide hourly observational data for the Mississippi Bight. This near real time data aids in oil spill tracking, water quality assessments, circulation studies, and model validation. Identifying seasonal patterns, variability timescales, and measurement error enables more efficient use of the data. As such, we performed a principal component time series analysis of the HFR data to determine the primary modes of surface current variability in the Mississippi Bight for 2010-2016. The first three modes explain 71.9% of the variance and reflect only weak seasonality with the strongest variance occurring in summer. In addition, we calculated RMS error between hourly HFR currents and surface drifters from the Lagrangian Submesoscale Experiment (LASER) for January through April, 2016. Time spent within the study area varied significantly depending on each drifter's release point, and trajectory and velocity differences with the HFR data coincided with a drifter's initial position in the study area. Finally, we compared resultant velocities from two algorithms designed to combine radial current velocities into surface current maps in order to isolate regions within the Mississippi Bight where one algorithm outperformed the other. This analysis serves to update prior climatological and principal component time series work and to reassess error calculations using concurrent drifter data. Though seasonal patterns agree largely with prior climatological values and directions, the inclusion of four more years of data, an alternative combination algorithm, and a larger drifter dataset emphasize regional variability within the study area. In the event of a future oil spill, estimation of seasonality and characterization of sub-regions could assist in oil dispersal tracking.

## Do Methane Seeps on the Northern US Atlantic Margin Stimulate Chlorophyll-a Concentration Levels?

**Y. Li\*<sup>1</sup>**, H. Gao<sup>1</sup>, A. Skarke<sup>2</sup>

<sup>1</sup>Texas A & M University, College Station, TX, <sup>2</sup>Mississippi State University, Starkville, MS

Numerous methane seeps have been identified across the world, which contribute significantly to the total amount of hydrocarbons discharged into the marine environment. On the one hand, leaked gas provides an additional source of nutrients for phytoplankton and zooplankton growth. On the other hand, the microbial oxidation of hydrocarbons consumes oxygen and contributes to acidification, negatively affecting these plankton. The concentration level of Chlorophyll-a (Chl-a)—as an assessor of phytoplankton biomass—can be used to monitor the status of phytoplankton in a region. However, the response of Chl-a to gas seeps has not been thoroughly investigated. Recently, widespread methane seeps were discovered on the northern US Atlantic margin through a multibeam sonar survey. The Chl-a in the northern US Atlantic margin can also be affected by ocean upwelling, and by the Gulf Stream—a warm and swift Atlantic Ocean current originating in the Gulf of Mexico. To evaluate whether methane seeps plays a role in regulating Chl-a in this region, this study collected the monthly Chl-a and Sea Surface Temperature (SST) products derived from the Moderate Resolution Imaging

\* Student presenter

Spectroradiometer (MODIS) from 2001 to 2016 (with a 4-km spatial resolution) for the northern US Atlantic margin. Then, the Empirical Orthogonal Function (EOF) method was applied to these datasets to generate the dominant spatial and temporal (seasonal and annual) patterns of Chl-a and SST variability. Finally, analysis of the spatio-temporal patterns of Chl-a and SST around gas seeps was conducted to understand the response of Chl-a to methane leakage.

## Improved Measurements of Waves Spectra and Stokes Drift using a Novel Miniature Lagrangian Wave Buoy: Observations from SPLASH 2017

**S. Mehta**\*<sup>1</sup>, B. K. Haus<sup>1</sup>, A. Smith<sup>1</sup>, T. M. Ozkomen<sup>1</sup>, N. J. M. Laxague<sup>2</sup>, J. V. Pol<sup>3</sup>, A. V. Pol<sup>3</sup>

<sup>1</sup>University of Miami, Miami, FL, <sup>2</sup>Columbia University, Palisades, NY, <sup>3</sup>INGU Solutions, Calgary, AB, Canada

Wave measurements are crucial for obtaining accurate forecasts of ocean and coastal wave conditions for scientific and engineering applications including the design, construction, safety, and operational planning of ports and harbors. They are also necessary for oil-spill response planning to estimate the transport contribution due to Stokes drift and for determining operational protocols during a response. Here we are exploring the feasibility of using very low cost, easily and rapidly deployable wave buoys in open ocean using different configurations. Wave observations presented here were made during the Sub mesoscale Processes and Lagrangian Analysis on the SHelf (SPLASH) experiment, in the Louisiana Bight from the R/V Walton Smith (UM), during late April 2017 using miniature Lagrangian wave buoys, configured in a collaboration between SUSTAIN lab (UM) and INGU Solutions Canada (INGU). The exceptionally small size (1.5" diameter) and low mass enables INGU's Pipers™ to move with the flow, and capture water state and wave data (e.g. three-dimensional linear accelerations, rotation rates, and magnetic properties along with skin temperature and pressure). Ten Pipers™ were deployed in pairs (one directly attached to an undrogued CARTE drifter and one tethered to its spoke), multiple times for about 2.5 to 3 hours, under different conditions in light to moderate winds, wind-sea and swell, throughout the campaign. Preliminary data analysis shows that tethered configuration showed more realistic sea surface evolution with the inertial subrange of the observed energy spectra extending up to comparatively higher wavenumbers than other traditional platforms). The Pipers™ hence provide a more versatile, robust, and accurate retrieval of the intrinsic wave frequencies for a range of spatial scales thereby serving as a valuable addition to the current wave measuring instruments for a variety of open water bodies (lakes, rivers, estuary, tidal channel and open ocean) for short term in-situ deployments.

## Parallel Factor Analysis for the Determination of Polycyclic Aromatic Hydrocarbons in the Gulf of Mexico

**N. Mujumdar**, K. Trieu, A. Santana, S. Wise, A. D. Campiglia  
University of Central Florida, Orlando, FL

The abundant release of crude oil into the Gulf of Mexico by the Deepwater Horizon (DWH) event has raised great concerns over potential ecosystem impacts. Polycyclic aromatic hydrocarbons (PAHs) are some of the most common and toxic pollutants encountered in oil spills that pose severe health risks. Presently, monitoring is restricted to 16 PAHs (EPA-PAHs), but it is well understood that this list omits many toxic PAHs. Among the "forgotten" PAHs, isomers with molecular weight 302 are of particular concern due to their high toxicological properties. The chromatographic analysis of high-molecular

\* Student presenter

weight-PAHs (HMW-PAHs) is challenged by similar retention times and virtually identical mass fragmentation patterns. The selectivity of fluorescence HPLC detectors is modest for resolving co-eluting isomers. The goal of this research is to solve this problem by processing room-temperature fluorescence excitation-emission matrices (RTF-EEMs) with parallel factor analysis (PARAFAC). This presentation will demonstrate that coupling three-dimensional data formats with multi-way calibration algorithms allows for the quantification of HMW-PAHs in samples of unknown composition. RTF-EEMs are directly recorded from sample extracts with the aid of commercial instrumentation. By assessing PARAFAC's three-way deconvolution routine, HMW-PAHs are directly determined from RTF-EEMs with no need of further experimental steps. The accuracy, precision, sensitivity, selectivity and limits of detection of the developed methodology are presented for HMW-PAHs determined in synthetic mixtures of known composition, standard reference materials and Gulf of Mexico extracts.

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## The Environmental Monitoring of Operational Discharges of Oil and Gas Activity in the North Sea: Linking Field Monitoring to Risk Assessment

**D. Pampanin**<sup>1,2</sup>, S. J. Brooks<sup>3</sup>, S. Sanni<sup>1,2</sup>, E. Lyng<sup>1</sup>, B. Einar Grøsvik<sup>4</sup>, R. C. Sundt<sup>5</sup>

<sup>1</sup>International Research Institute of Stavanger, Randaberg, Norway, <sup>2</sup>University of Stavanger, Stavanger, Norway, <sup>3</sup>Norwegian Institute for Water Research, Oslo, Norway, <sup>4</sup>Institute of Marine Research, Bergen, Norway, <sup>5</sup>Statoil ASA, Stavanger, Norway

Oil and gas companies operating on the Norwegian Continental Shelf are required to carry out environmental monitoring to obtain information on the actual environmental impacts of their activities and to give authorities a better basis for regulation. Scientists, operators and regulators have worked cooperatively for two decades in this program, for implementing knowledge, developing methods and tools to manage the offshore produced water (PW) discharges. A multidisciplinary approach (e.g. chemistry, biology, modelling and risk assessment) is in use at present to monitor discharges and reduce risks. In 2015, the Norwegian Environmental Agency published new guidelines as result of the research activity performed since 1995. The new requirements have been applied for the first time in 2017, the holistic approach shows a significant improvement in the scientific outcomes of the monitoring, in a cost-efficient way. Aiming to integrate biomarkers into probabilistic risk assessment for oil based discharges, data from a previous survey have been analysed using biomarkers in fish species as risk indicators. Recent publications from our research group showed how to bridge the gap between biomarker and whole organism responses related to oil based discharges. The "biomarker bridge" concept allows translating biomarker results to higher order effects. It is therefore possible to use biomarkers to provide information regarding the environmental impact and the risk related to oil based discharges. In the 2014 case study, the monitoring was carried out in a field with no actual discharge of PW. The impact of drill cuttings and other sediment sources were expected as the main types of contamination. A different biomarker response pattern than commonly associated with PW appeared. This case study confirmed the use of monitoring biomarker responses offshore as risk indicators in the procedures for Environmental Risk Assessment of PW discharges.

## The NOAA Satellite and Information Service, Marine Pollution Surveillance Program

**E. Ramirez**

NOAA, College Park, MD

The NOAA/NESDIS Satellite Analysis Branch (SAB) monitors for accidental and intentional crude oil discharges in U.S. waters on a 24/7 basis. A combination of multi-spectral and Synthetic Aperture Radar (SAR) imagery is scanned daily in near-real time to look for possible oil anomalies. A variety of analysis techniques are applied and ancillary datasets are consulted to rule out false positive oil signatures. The goal of the Marine Pollution Surveillance Report (MPSR) is to supply responders with the location and extent of the oil, and relative oil thickness if possible, to assist with damage assessment and mitigation. The full suite of products includes maps, shapefiles, and a KML. Primary customers include the National Ocean Service (NOS), the U.S. Coast Guard (USCG), and the Bureau of Safety and Environmental Enforcement (BSEE). Oil reports are currently disseminated through email to a closed distribution list, but new recipients can be added upon request. SAB is also able to place special satellite imagery requests for oil spill monitoring purposes when asked.

## Evaluation of Potential Impacts of Oil Platforms on the Marine Environment in the Northwestern Gulf of Mexico

**S. Sun**<sup>\*1</sup>, Y. Liu<sup>2</sup>, C. Hu<sup>1</sup>

<sup>1</sup>University of South Florida, St Petersburg, FL, <sup>2</sup>Nanjing University, Nanjing, China

The Gulf of Mexico (GoM) is a major hydrocarbon province, which contributes to more than 98% of the outer continental shelf oil production in the United States. The potential impacts of offshore oil drilling and production platforms on the marine environment, however, are generally unknown, yet these platforms may introduce contaminants into the sea via accidental spillage. Moreover, there are numerous platforms that have been deactivated over the past decades, which may also impact the marine environments for the same reason. In this study, we attempt to assess the potential impacts of both active and deactivated oil platforms on the marine environment through the use of satellite remote sensing data and systematic analyses. Specifically, a new dataset has been formed from a previous study to combine the database from the Bureau of Safety and Environmental Enforcement and results from remote sensing, where the timeline of installation, production, and decommission/abandonment of each oil platform is established. Using this dataset, we evaluate satellite-derived chlorophyll-a concentration and light diffuse attenuation (an index of water clarity) around the oil platforms and, when possible, evaluate their potential changes before platform installation, during production, and after decommission. Same assessment is repeated for nearby locations away from oil platforms as the control sites. We believe that this is the first time that such a systematic evaluation is conducted on the potential impacts of oil platforms on the marine environment of the northwestern Gulf of Mexico.

## Using Satellite Images to Characterize the Galveston Bay Tidal Plume

**K. M. Thyng**

Texas A&M University, College Station, TX

Galveston Bay is important as a major international port, is lined by petrochemical industries, supports aquaculture, and is home to many natural fish species. There are occasional oil spills in the Bay, such as

\* Student presenter

the March 2014 Texas City “Y” spill. It also experienced major flooding during and after Hurricane Harvey in August 2017, bring toxins and pollutants into the bay and subsequently onto the shelf. Water mostly exits the Bay through the main shipping channel, where an ebb-tide jet forms a tidally-driven plume over the Texas shelf. The details of the resultant bay plume are important for mixing and transport, and therefore determine, in part, where material in the water will travel after leaving the bay. Satellite imagery is used to characterize the bay plume under different forcing conditions, given that the bay plume is typically visible due to entrained sediment. I distill the forcing mechanisms of winds, river input, and tides down to typical scenarios, then inter-compare the scenarios with the resultant plume characteristics. I correlate the plume size, shape, and position with the forcing mechanisms. Better understanding the effect of the forcing mechanisms on plume behavior will enable better prediction of the plume location based on meteorological and other conditions, which in turn will enable better response to spill and flooding events.

## A New Drifter to Track Ocean Pollutions and the Very Surface Circulation

**N. Wienders**

Florida State University, Tallahassee, FL

Currents at the very surface of the ocean transport debris and contaminants such as oil, red tides or toxic river plumes. Though few observations have been made of currents at the very surface (within the upper few centimeters) of the ocean, it is understood that substantial shear can exist between the surface and depths of one to several meters deep, where most of the observations approximating the surface current are taken (e.g., from drifters with drogues, upward-looking ADCPs, or coastal radar). Not only is the velocity structure of the upper few meters poorly observed and its dynamics not fully described, but it is also unresolved in ocean models. As a consequence, for many applications involving numerical calculations of surface transport of biological materials and oil, crude parameterizations are often used to adjust ocean model currents to account for the unresolved vertical shear due to wind and waves. We have designed a new instrument which can for the first time, measure and monitor the ocean surface circulation within an inch of the surface where specific processes occur (the Stokes Drift). The drifters are disks about 6 inches in diameter and small enough to measure the effect of the smallest gravity waves. Powered by batteries and/or solar panels, an accelerometer activates the antennas in clear sight of the satellites at any time. The drifters are about 20 percent buoyant so they are only partially immersed to allow for GPS reception and satellite data transmission while minimizing the wind drag. The drifters will transmit time, position and optional data stream (temperature, salinity, etc.) via satellite at user-programmable intervals. To ensure the drifters will not be affected by flipping from waves, they have GPS and satellite antennas on both sides.

## Innovative Technologies used for Ocean Observing

**T. R. Wims**

Independent Consultant, Lanham, MD

The Gulf of Mexico is important to the United States and all nations in the region for food security, energy, recreation, and fisheries and weather observation. Understanding the pulse of ocean life in the Gulf is vital to the region. The surface and undersea observation of the Gulf would improve our understanding of the region. Existing technologies can be brought to bear on this problem. Lessons learned, and technological advances used in the Ocean Observatories Initiative (OOI) could be applied

\* Student presenter

to implement and increase under sea observations in the Gulf. The OOI manages and integrates data from the over 800 instruments deployed in the Atlantic and Pacific Oceans, in seven arrays. Instruments are located on a myriad of platforms including gliders, AUVs, surface buoys, profilers, inductive mooring cables, and seafloor junction boxes. Overall there are nearly 75 models of specialized instrumentation used throughout the OOI that collect over 200 unique data products. The types of instruments used in the arrays are Fluorometers, HD Digital Cameras, Acoustic Doppler Current Profilers, Bio-acoustic sonar and many more. The data collected from these instruments can be analyzed and used to support reef surveys and fisheries movement. The instruments and technologies can also be used to predict and analyze the impact of storms, seismic events and manmade events like oil spills, on our reef systems. Each year, instrument manufacturers improve their instruments, which in turn allows the OOI to increase its ocean observing capabilities, therefore, increasing data collection capability and our understanding of the Gulf of Mexico. The way we use ocean observing technologies, is just as important as the technologies themselves. Instruments deployed on moorings can be moved to collect information, in anticipation of seasonal events and migratory patterns of sea life. Underwater autonomous vehicles can travel long distances while collecting data.

## Versatile Instrument for Rapid Collection of Luminescence Data from Polycyclic Aromatic Compounds in Oil-Contaminated Environmental Samples

**S. Wise**, A. Santana, K. Trieu, L. Violante, A. Campiglia  
University of Central Florida, Orlando, FL

Crude oil spills such as the Deepwater Horizon event contribute to contamination of seawater and ocean sediments with polycyclic aromatic hydrocarbons (PAHs). While low molecular weight PAHs are known to have acute toxic effects, high molecular weight PAHs (HMW-PAHs) of 302 g/mol or greater are more mutagenic and carcinogenic, and bioaccumulation of these lipophilic compounds is likely. Their toxicity and the ability of microbes to biodegrade them will depend on the specific structure of the molecules. Monitoring and remediation strategies, therefore, depend on the ability to identify, quantify, and differentiate among the HMW-PAH isomers. Fluorescence spectroscopy provides a highly specific technique that can quantify and differentiate isomers in complex samples that would not be well-resolved by chromatographic techniques due to their overlapping retention times and similar mass spectroscopy fragmentation patterns. We describe a versatile instrument that can produce fluorescence excitation-emission matrices for samples at room temperature, 77 K, and 4.2 K. At these lower temperatures spectral features sharpen considerably, and it becomes possible to quickly distinguish isomers by measuring over relatively short spectral ranges. The instrument also measures luminescence lifetimes on millisecond timescales, such as the phosphorescence exhibited by sulfonated PAHs. This enables the identification and quantification of other toxic molecules potentially present in environmental samples based on their phosphorescent lifetimes. The authors acknowledge financial support from The Gulf of Mexico Research Initiative (Grant 231617-00). The views expressed are those of the authors and do not necessarily reflect the view of this organization.

## Physical & Chemical Connectivity

### Evaluation of Molecular Ratios as Geochemical Proxies for Source Characterization and Tracking Weathering Processes of Hydrocarbons in Coastal Sediments Impacted by Deepwater Horizon Oil spill

P. L. Adhikari, E. Overton, M. S. Miles, B. Meyer  
Louisiana State University, Baton Rouge, LA

Molecular ratios of select group of hydrocarbons have been widely used to assess hydrocarbon emission sources, however, their application for complex environmental samples and weathered oil residues has been criticized. The unprecedented release of crude oil during the Deepwater Horizon (DWH) oil spill, followed by numerous sampling events, provide comprehensive time-series data on hydrocarbon distribution and persistence. These unique data can be used to investigate the reliability of the quantitative molecular ratios as geochemical proxies for source diagnostics and tracking weathering processes in the coastal sediments. In this study, we utilized ~1750 time-series surface sediment samples collected from various locations in the Coastal Louisiana during 2010-2016 by the Coastal Waters Consortium (CWC) investigators. The sediment samples were analyzed for alkanes, PAHs and petroleum biomarker compounds. Biomarker patterns in the sediment samples were used to confirm MC252 contamination and to determine the degree of weathering. The MC252 contaminated sediment samples were then selected to calculate 27 quantitative molecular ratios (alkanes and PAHs) for binary plots and statistical analyses. The results showed that the commonly used parent PAHs-based diagnostic ratios do not efficiently separate sources of hydrocarbon in sediments. In contrast, alkyl-PAHs' ratios and their molecular profiles were more useful than the parent PAHs for source diagnostic. The results also showed that, five out of 27 molecular ratios varied significantly in an interaction with sampling years and biomarker-based oil weathering patterns (A, AB, or B). Similarly, 23 molecular ratios varied significantly with sampling years and 18 of them varied significantly with biomarker weathering patterns. These results showed that the majorities of the molecular ratios do change with time and with oil weathering, however, the five robust molecular ratios, including pyrogenic index and  $BaPy/(BaPy+BePy)$ , which did not change throughout the sampling period seem to be more reliable to assess the sources of hydrocarbon. Similarly, the variable molecular ratios can be statistically modeled and used as geochemical proxies to determine the degree and type/nature of natural attenuation in future spills.

### Characterization of Chemical Fractions from MC252 Oil

C. A. Miller, III<sup>1</sup>, A. Alqassim<sup>\*2</sup>, M. Wilson<sup>2</sup>, J. Wickliffe<sup>2</sup>, J. Oswald<sup>2</sup>, D. Pangeni<sup>3</sup>, E. Overton<sup>3</sup>

<sup>1</sup>Global Environmental Health Sciences, Tulane University, New Orleans, LA, <sup>2</sup>Tulane University, New Orleans, LA, <sup>3</sup>Louisiana State University, Baton Rouge, LA

Crude oil is a complex mixture of chemicals with variable toxic properties. Some chemicals in oil are well studied for their toxicological properties, but most have not been characterized. We developed a screening system with the potential to identify novel toxic molecules within crude oil taken directly the MC252 well. We used an aryl hydrocarbon receptor signaling assay coupled with chemical fractionation and analysis to identify bioactive oil chemicals. Several four ringed aromatic hydrocarbons were identified as potent and more abundant signaling molecules in this screen, and several of these compounds were alkylated to varying degrees. The most active candidates that we have identified

\* Student presenter

belong in the chrysene family. Individual methylated chrysenes were compared with unsubstituted chrysene in human aryl hydrocarbon signaling assays in a recombinant yeast model system and in cytotoxicity assays using HepG2 (human hepatocarcinoma) cells. 4-methyl chrysene was an exceptionally strong activator of aryl hydrocarbon receptor signaling. 5-methyl chrysene displayed average potency in aryl hydrocarbon signaling assays but was the most cytotoxic. Current studies are designed to examine the persistence of these compounds as oil ages and weathers in the environment. We propose that chrysenes are toxicologically relevant molecules within crude oil for organisms that utilize aryl hydrocarbon receptor signaling pathways.

## Time-Resolved Phosphorescence Spectroscopy for the Isomeric Determination of Polycyclic Aromatic Sulfur Heterocycles with MW 234 in the Gulf of Mexico

**S. Arif\***<sup>1</sup>, M. Al-Tameemi<sup>1</sup>, A. Campiglia<sup>1</sup>, W. Wilson<sup>2</sup>, S. Wise<sup>2</sup>

<sup>1</sup>University of Central Florida, Orlando, FL, <sup>2</sup>National Institute of Science and Technology, Gaithersburg, MD

Due to the complex matrix composition of oil contaminated sites, the undoubtful identification and quantitative determination of environmental pollutants often requires the sequence of high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). A classic example is the analysis of the sixteen polycyclic aromatic hydrocarbons included in the priority pollutants list of the U.S. Environmental Protection Agency (EPA-PAHs). Since considering the EPA-PAHs alone can lead to drastic underestimations of potential toxic effects of oil spills, a strong case can be made for including hetero-aromatic compounds in risk assessments of contaminated sites. PASHs exist in an even greater variety of chemical structures than PAHs and, because of the asymmetry imposed by the heteroatom, the number of PASHs isomers with the same molecular weight (MW) is usually large. The large number of isomers which increases the difficulty of separation and identification by chromatographic approaches. Herein, we demonstrate the capability to differentiate individual PASHs isomers of MW 234 g mol<sup>-1</sup> via vibrational spectroscopy at liquid nitrogen (77 K) and liquid helium (4.2K) temperatures. We present phosphorescence spectra with fingerprint information for specific isomer identification at the parts-per-billion (ng.mL<sup>-1</sup>) concentration levels. Characteristic phosphorescence lifetimes provide an additional qualitative parameter for the unambiguous and accurate determination of MW isomers in oil spill samples. We demonstrate that the relatively long phosphorescence decays of PASHs facilitate the time discrimination of strong fluorescence interference from PAHs and methylated-PAHs often present in environmental samples exposed to oil spills. Acknowledgements: M. Al-Tameemi, S. Arif and A. D. Campiglia acknowledge financial support from The Gulf of Mexico Research Initiative (Grant 231617-00). The views expressed are those of the authors and do not necessarily reflect the view of this organization.

## Thirty-six Year Depositional History of Ixtoc-1 Oil Spill Hydrocarbons: Persistence, Sources and Potential Impacts

**T. Bartlett\***, I. Romero, N. Zenzola, B. Carr, G. Ellis, E. Goddard, D. Hollander  
University of South Florida, College of Marine Science, Saint Petersburg, FL

Approximately 3 million barrels of oil were released in 1979 when the exploratory oil well Ixtoc-1 experienced a blowout. The goal of this study is to identify chemical signatures of Ixtoc-1 oil in marine sediments to better understand the long-term preservation of environmentally important chemical

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compounds associated with oil spills. Sediment cores were collected at four sites in Campeche Bay in the southern Gulf of Mexico, subsampled in 2 and 5mm increments, and analyzed for biomarkers (hopanes, steranes, and diasteranes), n-alkanes (C10-37), and polycyclic aromatic hydrocarbons (PAHs). We determined biomarker, alkane, and PAH ratios to differentiate inputs from recent oil exploration, buried Ixtoc oil, and marine and riverine sources. The four sediment cores were collected in diverse areas of Campeche Bay with different proximity to the coastline and presence of petroleum industry: 23-92 is the deepest site (3737m water depth) with inputs primarily from marine sources; Ixtoc-1 (60m water depth) was recovered from an area of active and intense petroleum exploration known as “the exclusion zone”; IXW-250 (583m water depth) is located 80 km west from the Ixtoc-1 wellhead and has indications of buried Ixtoc oil; LT-1 (16m water depth) is located 45 km from the mouth of the Laguna de Términos and has primarily riverine inputs. The upper 5mm of the Ixtoc-1 core shows alkane patterns typical of crude oil associated with recent oil exploration. We found potential Ixtoc oil buried at 38mm core depth in IXW-250; at this depth the Ts/Tm (trisinorhopane 18a/trisinorhopane 17a) ratio is similar to a reference Ixtoc oil sample. LT-1 has carbon preference indices (CPIs) nearly five times greater than Ixtoc oil at 34mm indicative of buried riverine input. 23-92 has distinct Ts/Tm ratios and CPI representing primarily marine inputs. Our results demonstrate the long-term preservation of organic chemicals buried in the seafloor from various sources (oil releases, marine, riverine, etc.) and can improve the long term assessment of other spills such as the Deepwater Horizon spill.

### Exposure and Uptake of Oil Hydrocarbons and Oxygenated Oil Weathering Products in Fish Egg Incubation Experiments Using Weathered Oil

**E. Beirne**<sup>1</sup>, S. Katz<sup>1</sup>, B. Clark<sup>2</sup>, D. Nacci<sup>2</sup>, R. Ricker<sup>3</sup>, G. Baker<sup>3</sup>, C. Aeppli<sup>1</sup>

<sup>1</sup>Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, <sup>2</sup>USEPA Environmental Effects Laboratory, Narragansett, RI, <sup>3</sup>NOAA Office of Response and Restoration, Santa Rosa, CA

Oil weathering that occurred after the Deepwater Horizon spill has resulted in persistent oxygenated oil weathering products. These products potentially impacted fish embryo development. In order to characterize typical exposure regimes and resulting bioaccumulation of oxygenated oil weathering products for fish embryos, we conducted incubating experiments with fish eggs (Atlantic killifish; *Fundulus heteroclitus*) and weathered Deepwater Horizon oil. We used two different exposure systems: oil-coated gravel columns and high-energy water accommodated fractions (HEWAFs). Three separate oil treatments were used: an evaporated crude oil, a slick oil collected during the Deepwater Horizon oil spill response, and an isolated oxygenated fraction of this oil. We measured PAHs, alkanes, and carboxylic acids in the aqueous phase, in fish eggs, and in passive sampler chips that were co-exposed with the fish eggs. Carboxylic acids have been shown to be indicator oil weathering compounds. We will present preliminary data showing exposure levels of oil hydrocarbons as well as oxygenated weathering products. These results will help to design and interpret experiments to determine the toxicity of such weathering products.

## Temporal Varying Spatial Patterns in Mobile Bay Outflow

**J. W. Book**<sup>1</sup>, S. M. Parra<sup>2</sup>, B. Dzwonkowski<sup>3,4</sup>, S. Dykstra<sup>3,4</sup>, S. J. Warner<sup>5</sup>, J. N. Moum<sup>5</sup>, S. D. Howden<sup>6</sup>

<sup>1</sup>U.S. Naval Research Laboratory, Stennis Space Center, MS, <sup>2</sup>American Society for Engineering

Education Postdoctoral Fellow at the U.S. Naval Research Laboratory, Stennis Space Center, MS,

<sup>3</sup>University of South Alabama, Dauphin Island, AL, <sup>4</sup>Dauphin Island Sea Lab, Dauphin Island, AL, <sup>5</sup>Oregon

State University, Newport, OR, <sup>6</sup>University of Southern Mississippi, Stennis Space Center, MS

Seven moorings were used in April 2016 to observe the spatial structure of the riverine driven outflow of Mobile Bay, which is a major source of fresh water to the Mississippi Bight coastal shelf. Three moorings with 6 km spacing were deployed 8 km from the outlet to form a fan across the Mobile Bay outflow path. The center mooring of the fan array was a specially designed Bio-Optical Physical Pop-up Environmental Reconnaissance System (BOPPERS) that measured thermohaline and optical properties of the entire water column at varying intervals. The western-most mooring of the fan array also formed the nearshore point of a four mooring array extending offshore towards the south at 10 km spacing. Together these six short-term moorings formed a bent “L” shape and complemented the long-term observations made by one Dauphin Island Sea Lab mooring located another 10 km to the west of the offshore line. All moorings measured ocean currents throughout the water column with acoustic Doppler current meters. Some moorings were equipped with temperature and salinity sensors and with temperature microstructure sensors at discrete depths and with bottom pressure sensors. The profiling BOPPERS mooring showed the complexity of the outflow structure as strong changes in vertical thermohaline structure sometimes occurred as quickly as within the 3-hour time gap between profiles. Pulses of low salinity plume water in phase with the tides were observed by the line of moorings extending offshore, but the spatial pattern of the pulses varied over the length of the deployment. During neap tides, much lower salinity waters were observed 25 km from the coast than at 15 km from the coast. During spring tides this pattern reversed to the more standard one of lower salinity pulses at 15 km than at 25 km. We plan to analyze these patterns to better understand the varying influences of tides, wind, and bottom friction on the spatial and temporal patterns of this important outflow.

## Applying Measures of Local Turbulence from Simulations to Correlate Droplet Sizes Across Experimental Configurations and Lengthscales

**C. P. Booth\***, J. Leggoe, Z. M. Aman

The University of Western Australia, Crawley, Australia

Full scale experiments of deep-water blowouts are intractable from both a financial and environmental perspective. As such data from laboratory and mesoscale experiments has been scaled to the full field using functions based on the Weber, Reynolds and Ohnesorge Numbers that have been calculated based on the bulk parameters of the system. One limitation with this approach in the past is that there has been a question surrounding whether or not data from apparatus that are distinctively different to a jet, for example a stirred cell or autoclave, correspond to the turbulence generated in a jet. This work utilizes simulation data to obtain measures of the local turbulence and compares the available data to obtain functions that enables direct correlation of droplet size using general and volumetric parameters across the various apparatus.

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## Recent Sedimentation Patterns Off Northwest Cuba: Comparison with Deepwater Horizon and Ixtoc-1 Impacted Sediments

**G. R. Brooks**\*<sup>1</sup>, R. A. Larson<sup>2</sup>, M. Armenteros-Almanza<sup>3</sup>, M. Diaz-Asencio<sup>4</sup>, A. Martínez-Suárez<sup>3</sup>, G. Hidalgo-Perez<sup>5</sup>, P. Schwing<sup>6</sup>, K. Yang<sup>7</sup>, D. J. Hollander<sup>6</sup>

<sup>1</sup>Eckerd College, Saint Petersburg, FL, <sup>2</sup>Eckerd College/USF, Saint Petersburg, FL, <sup>3</sup>Centro de Investigaciones Marinas, Universidad de La Habana, Habana, Cuba, <sup>4</sup>Centro de Estudios Ambientales de Cienfuegos, Ministerio de Ciencia, Tecnología y Medioambiente, Cienfuegos, Cuba, <sup>5</sup>GEOCUBA Estudios Marinos, Habana, Cuba, <sup>6</sup>USF, Saint Petersburg, FL, <sup>7</sup>Hong Kong Baptist University, Hong Kong

Sediment cores were collected along the coastline, continental shelf and slope (to ~1500 m water depths) of northwest Cuba between November 2016 and May 2017 to investigate sediment sources, distribution patterns and transport mechanisms/pathways across the Cuban continental margin, and for comparison to Deepwater Horizon (DwH) and Ixtoc-I impacted sediments in the NE and SW Gulf of Mexico (GoM) respectively. Coastal cores consist primarily of carbonate sands and muds near the base, transitioning upward to organic-rich muds. Some contain surficial overwash deposits. Cores collected at the immediate shoreline contain variable siliciclastics (island-derived sediments). Offshore cores (between ~300 and 1500 m water depths) contain a basal carbonate-rich unit similar to coastal cores. Adjacent to major bays (e.g., Habana, Mariel) carbonate units are capped by siliciclastic-rich sediments. Cores off westernmost Cuba exhibit little to no siliciclastic-rich sediments. <sup>234</sup>Th<sub>xs</sub> was detected in the surface of all offshore cores, extending to ~1 cm in easternmost cores compared to 2-4 mm in westernmost cores, reflecting active deposition within the last ~5 months (<sup>234</sup>Th<sub>xs</sub>) and an increase in island-derived sediment input off major bays to the east. Work is ongoing to fully characterize the last ~100 years of sediment accumulation using <sup>210</sup>Pb<sub>xs</sub>. Curiously, little if any siliciclastic-rich sediments were found on the inner shelf between the coastline and ~300 m water depths. The lack of sediments on the inner shelf suggests a general disconnect in sediment exchange between the nearshore and offshore. Initial observations suggest NW Cuba sedimentation patterns are distinctly different than those in the NE and SW GoM.

## A New *in vitro* Exposure Device to Assess the Health Impacts of Oily Marine Aerosols on the Human Respiratory System

**L. Chandrala**<sup>1</sup>, N. Afshar-Mohajer<sup>2</sup>, K. Nishida<sup>3</sup>, Y. Ronzhes<sup>1</sup>, V. K. Sidhaye<sup>3</sup>, K. Koehler<sup>2</sup>, J. Katz<sup>1</sup>

<sup>1</sup>Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD, <sup>2</sup>Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, <sup>3</sup>Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD

Aerosolization of oil as waves break up oil slicks is one of the transport pathways of oil-spill matter into the atmosphere. Our recent studies have shown that the size distribution of these oily droplets ranges from submicron to micron scales. Due to their long residence time in the air, the nano-aerosols may pose health risks to oil-spill workers and downstream communities. Although numerous epidemiological studies have established that exposure to fine particles is associated with increased cardiopulmonary morbidity, the health implications of inhaling crude-oil aerosols remain largely unknown. To assess the health impact of the nano-oil droplets, whose toxicity might involve both size and chemical composition, we have designed and implemented a novel *In vitro* Oily Aerosol Exposure and Microscopy System (IOAEMS). It exposes human lung cell cultures to laboratory-generated oily aerosols in a controlled environment. Its unique feature is the integration of a microscopy system, which enables direct observation on the culture throughout the exposure. In the present study, fully-

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differentiated patient-derived primary bronchial epithelial cells are exposed to aerosolized crude oil and crude oil-dispersant mixtures at various concentrations. Aerosols at realistic concentrations mimicking those produced by the breaking wave are generated by a single-jet Collison nebulizer. The size distributions of these nano and micron-scale aerosols are characterized by a scanning mobility particle sizer and an aerodynamic particle sizer. The entire system is maintained at 37°, and the air is humidified and mixed with 5% CO<sub>2</sub> to maintain physiological pH levels. The time evolution of the cells is visualized using an EMCCD camera equipped with 20X long-distance objective. Ongoing tests determine paracellular permeability, protein abundance, and cell viability to assess the toxicity of aerosolized oil on bronchial epithelial cells. The live imaging elucidates the evolution of the monolayer integrity.

## Seasonal Variation of DOC Concentration Predicted from CDOM Absorption Coefficients in the Northern Gulf of Mexico

**B. Kelly**, A. Chelsky, E. Bulygina, B. Roberts

Louisiana Universities Marine Consortium, Cocodrie, LA

Dissolved organic carbon (DOC) is an important component of the carbon cycle in coastal waters and is heavily influenced by riverine and estuarine inputs. Historically it has been necessary to measure [DOC] directly, however newer techniques have taken advantage of the high level of correlation between [DOC] and optically derived colored dissolved organic matter (CDOM) absorption coefficients. System dependent relationships can be established by performing multiple linear regressions on measurements of CDOM absorption coefficients at specific wavelengths and measured [DOC], from which surface [DOC] can be accurately predicted throughout the system where optical measurements can be obtained. In April, July, September, and October 2017 we sampled surface water from 80+ sites over an area of ~100,000 km<sup>2</sup> along the Louisiana-Texas shelf in the northern Gulf of Mexico. [DOC] was measured on filtered water samples using a Shimadzu TOC-VCSH analyzer using standard techniques. Spectral absorbance of the filtered water samples was measured using a UV-Vis spectrophotometer and was used to calculate CDOM absorption coefficients. We evaluated the CDOM absorption coefficient-DOC relationships and the precision with which concentrations can be estimated from CDOM for each cruise and across all sampling periods. This study provides an evaluation of viability of scaling up CDOM absorption coefficient and remote-sensing derived estimates of DOC concentrations to the scale of the LA-TX shelf ecosystem and how these relationships vary with season and hurricanes.

## Copepods Toxicity of Photo-irradiated Oil: The Role of PAHs and Oxygenated Photo-products

**H. Chen**\*<sup>1,2</sup>, C. Aeppli<sup>1</sup>

<sup>1</sup>Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, <sup>2</sup>Wheaton College, Norton, ME

Previous research showed that photo-oxidation of oil slicks on the sea surface led to the formation of oxygenated photoproducts after the 2010 Deepwater Horizon oil spill. However, it is not yet fully understood how this photooxidation influenced the toxicity of oil residues. The goal of this research was to investigate the toxicity of photo-oxidized oil residues towards marine copepods. Source oil samples were irradiated using natural sunlight in a greenhouse for up to 40 days. PAHs and carboxylic acids were analyzed by GC/MS, and the fraction of polar oil residues was quantified using TLC-FID. Low-

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energy water-accommodated fractions (WAFs) were prepared for toxicity test, including copepods mortality test and Microtox test. Chemical analysis showed that irradiated samples contained more polar compounds and formed and less aromatic compounds than dark controls exist. Toxicity test using copepods (*Acartia tonsa*) showed that the acute toxicity of irradiated oil was consistently higher than or equal to that of dark controls, although these samples contained more PAHs than the irradiated ones. Furthermore, the toxicity of irradiated samples initially increased from the 10-days to the 20-days irradiation samples, but then decreased in the 40-days irradiation samples. Overall, our results suggest that polar oil photoproducts exhibit a significant acute toxic effect for copepods.

## Molecular Characterization of Interfacial Material Isolated from Environmental Samples Impacted by the Deepwater Horizon Oil Spill

**H. Chen**<sup>1</sup>, A. C. McKenna<sup>1</sup>, C. A. Davis<sup>1</sup>, R. P. Rodgers<sup>1,2</sup>

<sup>1</sup>National High Magnetic Field Laboratory, Tallahassee, FL, <sup>2</sup>Future Fuels Institute, Tallahassee, FL

Deepwater Horizon (DWH) oil spill on April 20, 2010, released ~3,190,000 barrels of light, sweet crude oil into the Gulf of Mexico. Once released into the environment, the oil underwent physical and chemical processes that transformed oil compounds into a recalcitrant, poly-functional, oxygen-rich mixture. A small fraction of these oxidized transformation products behaves like surfactants and collectively designated as interfacial material. These species generate a layer at the oil/water interface that can stabilize the emulsion, thereby reducing the effectiveness of remediation and recovery efforts. However, the majority of these species are not detectable by routine gas chromatography (GC) techniques due to their high polarity (low volatility) and when combined with their complexity, requires advanced analytical techniques for their molecular characterization. Here, the interfacial material was isolated from field samples (e.g., tar balls, burn residues) collected up to seven years after the DWH spill via an in-house isolation method. (1) Subsequent characterization by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) revealed tens-of-thousands of interfacially active species in weathered field samples. The species are enriched in oxygen, whereas the inactive and whole oil species are composed of abundant hydrocarbon, nitrogen, and sulfur-containing species. The isolation, identification, and characterization of interfacially-active compounds derived from DWH residues highlight the compounds that form the oil/water interface and dictate transition from oil-soluble compounds to water-soluble compounds. The molecular-level data of these interfacially active species in the field samples offers new insights into the formation of field emulsions and mousses and weathering-induced compositional changes for petroleum contaminants in the environment.

1. J. M. Jarvis, R. P. Rodgers, W. K. Robbins. (2014). Acknowledgement: Work supported by NSF DMR 11-57490, and BP/Gulf of Mexico Research Initiative.

## Phytoplankton Derived Transparent Exopolymer Particle Response to Oiling

**L. K. Cole**<sup>\*1,2</sup>, J. W. Krause<sup>1,2</sup>, J. Latham<sup>3</sup>, K. Thamtrakoln<sup>3</sup>

<sup>1</sup>Dauphin Island Sea Lab, Dauphin Island, AL, <sup>2</sup>University of South Alabama, Mobile, AL, <sup>3</sup>Rutgers University, New Brunswick, NJ

Transparent exopolymer particle (TEP) concentrations in marine systems depend in part on the quantity of dissolved organic carbon (DOC) precursors released by phytoplankton as well as abiotic factors which allow DOC to aggregate. Shifts in the size distribution of TEP may alter the timing of TEP

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facilitated aggregation of marine debris into particles both large and dense enough to be removed from the water column. Size and quantity of these DOC precursors may be affected by both the abundance (biomass loading) and diversity of the phytoplankton population. We investigated the impact of oil-exposure on TEP size frequency distribution using a newly developed auto-imaging FlowCAM based approach. Experiments were conducted on three recently isolated, representative phytoplankton cultures from the Gulf of Mexico; a diatom (*Skeletonema* spp.), a dinoflagellate (*Prorocentrum* spp.) and a chlorophyte (*Tetraselmis* spp.). Each species was exposed to three concentrations of water accommodated fraction of crude oil (WAF) as well as WAF chemically enhanced with dispersant (CEWAF) in experiments designed to test the hypotheses that 1) higher biomass loads, and/or 2) species diversity will increase the resilience of the community to perturbation. Preliminary results show significant differences in the response across species, with *Tetraselmis* showing the most resistance to WAF and CEWAF at concentrations of  $\geq 10$ ppm. In addition, *Tetraselmis* showed a higher frequency of small TEP ( $< 50\mu\text{m}$  diameter) and a lower frequency of large TEP ( $> 50\mu\text{m}$  diameter) in CEWAF treatments relative to WAF and controls, while *Skeletonema* showed the opposite. As TEP abundance and reactivity are intricately linked with the biological pump, a shift to smaller and more abundant TEP may be an important mechanism affecting the timing of oil removal from the water column via particle aggregation and subsequent sedimentation.

### LES Simulations of Multiphase Plumes with Crossflow

**W. K. Dewar**<sup>1</sup>, N. Wienders<sup>1</sup>, A. Poje<sup>2</sup>, A. Fabregat<sup>2</sup>, T. Ozgokmen<sup>3</sup>

<sup>1</sup>FSU, Tallahassee, FL, <sup>2</sup>CUNY-SI, Staten Island, NY, <sup>3</sup>RSMAS-UM, Miami, FL

Large eddy simulations of rotating, stratified plumes in cross flows are discussed. Parameters describing the plume are modeled after those of the DwH event, and large-scale flows of several cm/s are included. Solutions with and without bottom layers are compared and contrasted for both rotating and non-rotating settings. Dependences of the solution on rotation are described.

### Scales of Re-suspension in the Northern Gulf of Mexico

**A. R. Diercks**<sup>1</sup>, V. L. Asper<sup>2</sup>, S. F. DiMarco<sup>3</sup>, J. P. Chanton<sup>4</sup>, U. Passow<sup>5</sup>

<sup>1</sup>University of Southern Mississippi, Abbeville, MS, <sup>2</sup>University of Southern Mississippi, Stennis Space Center, MS, <sup>3</sup>Texas A&M University, College Station, TX, <sup>4</sup>Florida State University, Tallahassee, FL, <sup>5</sup>University of California at Santa Barbara, Santa Barbara, CA

Time series data of size-specific in-situ settling speeds of marine snow (moored flux cameras), particle size distributions (profiling camera), currents (various current meters) and stacked time series flux data (sediment traps) were combined to recognize resuspension events of different scales and magnitudes. Deep ( $> 1,000$  m) benthic nepheloid layers (BNLs), created by resuspension events ranging from small-scale-local, to small-scale-far-field to hurricane-scale, were investigated in the northern GoM during fall 2012 to summer 2013. Local small-scale resuspension is suggested for events in which low POC content combined with high lithogenic silica (LSi) flux, combined indicators for resuspension, at 30 mab (meter above bottom) compared to the flux at 120 mab, suggests local resuspension. Flux data also indicated two other small-scale resuspension events, which occurred at some distance at shallower depths, rather than locally. Presumably, inertia driven resuspension introduced material from the shallow areas surrounding the stacked traps. This material was laterally transported as midwater BNLs leaving a resuspension signal in POC and LSi flux at 120 mab, but not at 30 mab. The passage of hurricane Isaac

over the sampling area caused a larger scale resuspension event of high magnitude that lasted a few days and was recorded in both traps. Based on our data we suggest that small scale resuspension events, driven by inertial currents, can be imagined as high frequency “noise” on the seasonal curve of flux over time which is dominated by productivity, food webs, mesoscale circulation, riverine input and larger resuspension events. We suggest that these events may appreciably impact transport and redistribution of lithogenic and organic sedimented material. Especially in regions with varying topography, such events when occurring on a sea mount or salt dome may carry matter farther afield. The frequency and duration of the occurrence of these small-scale events is currently unknown, however, based on current meter and profiling camera measurements, could happen as frequently as daily.

## Stratigraphic Fingerprinting and Source Identification of Oil Present in Recent Marine Sediments from the Southern Gulf of Mexico

**K. N. Duong\***, J. Radovic, A. Jaggi, S. Larter, T. Oldenburg  
University of Calgary, Calgary, AB, Canada

The southern Gulf of Mexico (sGoM) is one of the largest regions in the world for petroleum exploration. This region contains many sedimentary basins producing hydrocarbons that have been classified by organic geochemical based biomarkers into four main oil families by Guzman-Vega and Mello (1999). In 1979, a large oil blowout occurred in this region at the Ixtoc I exploratory well, which released a massive amount of crude oil (530 million liter) into shallow water (50 m water depth). However, the ultimate fate of the released oil is not fully understood due to insufficient data and lack of proper monitoring procedures at that time. The goal of this project is to improve the understanding of the spatiotemporal distribution of hydrocarbons in the sGoM environment, by stratigraphically characterizing the organic molecular markers in the sediment samples, to identify their sources, and assess the contribution of natural and anthropogenic hydrocarbon inputs. To that end, aromatic, aliphatic and biomarker compound concentrations were determined by GC-MS, and their distributions and ratios at different strata intervals in the sediment from five different cores compared to those in potential oil sources in the sGoM. We observed ubiquitous, but varying levels of hydrocarbon levels in the samples, many of which were characterized by unresolved complex mixture “humps”, an indicator of degraded oil. This suggests constant oil inputs, possibly from natural seeps. However, specific ratios of triterpene biomarkers, and dibenzothiophenes, in certain downcore samples, correspond to the unique fingerprint of Ixtoc-I reference oil, a member of the Tithonian oil family of the Campeche basin. We suggest that these compounds are potential proxies for the identification of Ixtoc-I oil in the sGoM sediments. The results of this study also serve as a good analog for the long-term monitoring of the oil released after the Deepwater Horizon blowout.

## The Effect of Tidal and Super Tidal Motions on Sediment Resuspension

**J. Earls\***, M. Buijsman, D. Wallace  
University of Southern Mississippi, Stennis Space Center, MS

This study discusses the effect of tidal and super tidal motions on the resuspension of sediments on the continental shelf in the Mississippi Bight shallower than 60 meters. Hydrocarbon molecules from natural seeps and man-made oil spills will adsorb to both suspended sediment in the water column and already deposited sediment on the continental shelf. High frequency motions then resuspend these

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oiled sediments back into the water column where they could be dispersed by currents. Our objective is to discover what processes cause the sediment resuspension. We will analyze existing data sets from SEED and GOMRI and collect new ADCP, CTD, and sediment data on the continental shelf. We will estimate shear stresses from wave and current data and correlate these with ADCP derived acoustic backscatter data. One primary goal is to document the characteristics of the (super) tidal barotropic and baroclinic motions as recorded in existing data sets.

## Mississippi River Plume Interaction with Surface Oil in the Northern Gulf of Mexico

**C. R. Edwards**<sup>1</sup>, R. M. Castelao<sup>2</sup>, N. D'souza<sup>3</sup>, K. Ziervogel<sup>4</sup>

<sup>1</sup>Skidaway Institute of Oceanography, University of Georgia, Savannah, GA, <sup>2</sup>University of Georgia, Athens, GA, <sup>3</sup>University of Rhode Island, Narragansett, RI, <sup>4</sup>University of New Hampshire, Durham, NH

A thus far unexplored aspect of marine oil snow (MOS) formation is the role of river plumes carrying dissolved and particulate matter offshore, where the low salinity water and its contents interacts with seep oil and pelagic microorganisms, possibly forming sinking MOS. Oil seeps may be hotspots for MOS formation, as they have been connected to increased primary productivity relative to non-seep sites (D'souza *et al.*, 2016), and increased TEP production at seeps is thought to facilitate MOS formation (Ziervogel *et al.*, 2014). Data from recent glider deployments near a natural seep site (GC600) indicate simultaneous influence of fresh water and hydrocarbon inputs in the upper 200m on the continental slope; analysis of hydrographic and bio-optical data (backscatter, CDOM and chl-a fluorescence) collected by the glider suggests that large particles are formed below the phytoplankton max after the arrival of fresh water at slope sites with seep activity. Patterns in glider-measured chl-a- and CDOM fluorescence will be interpreted using fluorescence emission spectra for phytoplankton pigments and CDOM measured with an aquatic laser fluorescence analyzer and estimates of bacterial abundance from epifluorescence microscopy and flow cytometry. Taking advantage of a combination of satellite, shipboard, and glider-derived data, we will explore the interaction of Mississippi River plume water with hydrocarbons near this natural seep site, as well potential controls on this important but understudied aspect of MOS formation in the northern Gulf of Mexico.

## Using Volcanic Sediment as an Independent Dating Tool of the 1979 Ixtoc-1 Event

**J. Fillingham**\*<sup>1</sup>, E. Fredric<sup>2</sup>, R. Larson<sup>1</sup>, P. Schwing<sup>2</sup>, G. Brooks<sup>1</sup>, D. Hollander<sup>2</sup>

<sup>1</sup>Eckerd College, St. Petersburg, FL, <sup>2</sup>University of South Florida, St. Petersburg, FL

The 1982 El Chichón volcanic eruption created a distinctive sedimentary layer in the southern Gulf of Mexico. (SGoM) This sudden sedimentation event resulted in elevated concentrations of volcanic glass in sediment cores collected throughout the (SMoG). In the western-most region of the southern gulf, volcanic glass makes up < 40% of the sediment. While concentrations of volcanic glass are high in the southwest region, volcanic glass abundance diminishes to the east. The ash cloud from the 1982 El Chichón eruption initially blew east in the first days of the eruption. The prevailing trade winds forced the cloud west in the coming days, allowing only minor incursions over the westernmost gulf and minor distribution in the eastern direction. The ash cloud was transported primarily west rather than east in the days and months following the eruption. This data is consistent with the El Chichón eruption being the primary contributor to the volcanic glass in the southern gulf during this time period. Based on the radiometric decay of <sup>210</sup>Pb<sub>xs</sub>, age models have been constructed to estimate of the age of sediment. These models only provide general ages, and yield considerable variations in age depending on the

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model. The available data for the southern gulf region showed considerable variation among age models, with a positive relationship between depth down core and margin of error. While other volcanic events did occur during the late 1970s and early 1980s, only the 1982 El Chichón eruption ejected the large amount of volcanic sediments in the correct region that would produce the evident volcanic glass layer. The volcanic layer, with a known deposition period of early 1982, was used as an independent tool to define the best age model and isolate the precise age of deposition of the Ixtoc-1 event.

## Determining the Acute and Sublethal Toxicity of Oxygenated Hydrocarbons in Weathered Oil

**N. Forziati**\*<sup>1</sup>, D. Nabi<sup>2</sup>, C. Aeppli<sup>2</sup>

<sup>1</sup>Simmons College, Boston, MA, <sup>2</sup>Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

It has been found that persistent oxygenated hydrocarbons (OxHCs) formed after the Deepwater Horizon oil spill as a result of oil weathering. However, OxHCs are generally not factored into risk assessment of oil spills, and little is known about how OxHCs are contributing to the toxicity of weathered oil. Therefore, we investigated toxicity of OxHC using three toxicity assays: Microtox toxicity assay, a reactive toxicity assay, and copepod toxicity assays. We found that OxHC model compounds had significant baseline toxicity, and that their toxicity is additive with that of naphthalene, a representative polycyclic aromatic hydrocarbon (PAH). In addition to baseline toxicity, we found that OxHCs exhibited reactive toxicity. Reactive toxicity is characterized by reaction of chemicals with biomolecules such as proteins. Lastly, we found that an OxHC fraction isolated from weathered oil led to a reduction in respiration rate for *Acartia tonsa*. Our results suggest that OxHCs may have substantial effects on organisms that are currently not taken into consideration in oil spill damage and risk assessment.

## The Effect of Removing Carbon Limitation on Diatom Aggregation and Physiological Responses When Exposed to Oil

**J. Genzer**\*, L. Bretherton, J. Hillhouse, S. Setta, M. Kamalanathan, A. Quigg  
Texas A&M University at Galveston, Galveston, TX

Anthropogenic emissions of carbon dioxide are fueling changes in ocean chemistry, commonly known as ocean acidification; this may reduce the stress of carbon limitation on silicifying diatoms. These phytoplankton are key contributors to marine snow formation through the production of exopolymeric substances (EPS). Diatoms are also thought to produce EPS as a means of protection from harmful substances, such as spilled oil. While little is known about the effects of oil spills on phytoplankton, even less has been studied about the potential future implications of these events under ocean acidification conditions. Using two centric diatoms, *Thalassiosira pseudonana* and *Chaetoceros diversum*, we conducted a series of roller tank experiments to mimic deep water processes. Four treatments were used, including a control, enhanced  $p\text{CO}_2$  levels, a water accommodated fraction (WAF) of oil, and enhanced  $p\text{CO}_2$  with WAF. We found treatment specific marine snow as well as marine oil snow formation with larger aggregates forming under elevated  $p\text{CO}_2$  conditions compared to controls and irregular morphologies when WAF was present. Photosynthetic efficiency ( $F_v/F_m$ ) was lowered in the WAF treatments compared to control and  $p\text{CO}_2$ . Similarly, alterations to the photosynthetic apparatus were also observed. The differences in aggregate morphology across

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treatments shows that further study is necessary to examine their composition and the factors which are important in driving their formation. The greater loss of oil and cells to aggregates in ambient conditions suggests that EPS produced by *T. pseudonana* may be affected in future ocean conditions, especially in polluted waters. Additional measurements suggest that the treatments used affected diatom production of EPS, proteins and lipids, and their enzymatic activities. The results of these studies reveal glimpses into the future story of phytoplankton marine snow production and how they will react to harmful conditions, such as oil spills.

## Small Scale Biological Responses of Phytoplankton Species Exposed to Oil and Surfactant

**J. Hillhouse**<sup>1</sup>, J. Genzer<sup>1</sup>, L. Bretherton<sup>1</sup>, A. Williams<sup>1</sup>, M. Kamalanathan<sup>1</sup>, Z. Finkel<sup>2</sup>, A. Quigg<sup>1</sup>

<sup>1</sup>Texas A&M University at Galveston, Galveston, TX, <sup>2</sup>Mount Allison University, New Brunswick, NB, Canada

Multiple small-scale experiments were carried out using ten phytoplankton species in order to examine their cellular responses to treatments designed to simulate the Gulf of Mexico's conditions following the Deepwater Horizon oil spill. Experiments included species of chlorophytes, centric and pennate diatoms, and cyanobacteria to examine a broad range of microalgae. Each species was subjected four treatments: a control, a water accommodated fraction of oil (WAF), a WAF chemical enhanced with surfactant (CEWAF) and a dilute CEWAF (DCEWAF). The cultures were then incubated for two weeks and sampled to monitor changes in the concentration of oil (EOE, estimated oil equivalence), chlorophyll *a* relative fluorescence intensity and chlorophyll *a* fluorescence induction and relaxation (FIRE) curve parameters ( $F_v/F_m$ ,  $\sigma_{PSII}$ ,  $\rho$ , and  $\tau_i$ ). We found that the growth rates were more affected by the pollutants than photosynthetic efficiency. All centric diatoms were sensitive to each treatment, while pennate diatoms were tolerant to WAF and DCEWAF, and the cyanobacteria and chlorophyte species were not affected by any treatment. These results reveal a wide range of responses across the phytoplankton species studied, and confirms previous results from large scale mesocosms that communities are not affected as a whole but rather at the individual level.

## An Unstructured-Grid Coupled Hydrodynamic-Wave-Sediment Model for the Barataria Bay, Louisiana

**H. Huang**, S. Sorourian, L. Cui, D. Justic

Louisiana State University, Baton Rouge, LA

A newly developed, triangular-grid, hydrodynamic-wave-sediment coupled modeling system is applied to the Barataria Bay, Louisiana in order to simulate gravitational-, tidal-, wind-driven, and wave-driven circulations in estuarine environment and adjacent inner continental shelf. The simulated flow field is subsequently used to predict suspended sediment concentration and passive Lagrangian particle transport pathway from Mississippi and Atchafalaya Rivers and Davis Pond River Diversion. The modeling system is comprised of the Finite-Volume Coastal Ocean Model (FVCOM), FVCOM-SWAVE (an unstructured-grid variant of the third-generation surface wave model SWAN), and FVCOM-SED (an unstructured-grid sediment erosion, transport, and deposition model based on the Community Model for Coastal Sediment Transport developed by the USGS and its implementation in ROMS). All three models employ the same finite-volume numerical algorithm and the same unstructured triangular mesh grid, making them easier to couple without introducing interpolation error. The ocean model has

been modified to incorporate the wave radiation stresses due to horizontal gradients of the momentum flux of surface waves and enhancement of bottom drag in shallow water due to wave-current interaction. The wave model ingests surface currents and water levels from the ocean model. Numerical modeling results indicate that in Barataria Bay most of the waves are locally generated. In addition, current induced bottom shear stress dominates the various passes between the bay and the continental shelf and the deep shipping channel, while wave induced bottom shear stress dominates all shallow lakes and bayous and is the main force for sediment resuspension in these areas. Some initial sediment transport numerical experiment results are reported and they will be used to study oil-sediment particle interactions and oil deposition/resuspension in response to frontal and tropical disturbances.

### A Practical Oxygen Enrichment System for Mesocosms without Vigorous Mixing

**A. H. Knap**<sup>1</sup>, R. Windham<sup>2</sup>, S. T. Sweet<sup>1</sup>, M. Morales-McDevitt<sup>1</sup>, G. Bera<sup>1</sup>, P. H. Santschi<sup>2</sup>, U. Passow<sup>3</sup>, A. Quigg<sup>2</sup>, T. L. Wade<sup>1</sup>

<sup>1</sup>Texas A&M University, College Station, TX, <sup>2</sup>Texas A&M University, Galveston, TX, <sup>3</sup>University of California Santa Barbara, Santa Barbara, CA

Mesocosm studies allow provide the opportunity to determine processes and mechanisms in a controlled environment, however they also have some limitations. One possible limitation is oxygen consumption by microbes that can create hypoxia or anoxia conditions in the absence of mixing. The ADDOMEx consortium used 18 (110) liter mesocosms for a 360-hour experiment to better elucidate mechanisms for marine oil snow (MOS) formation and sedimentation. A major concern was prevention of anoxia in this long term mesocosms experiment without altering the marine snow flocs through vigorous stirring, mixing, or aeration. Accurately determining carbohydrates and proteins which make up the MOS is a major objective of our experiments requiring collection of large amounts of unaltered MOS. During our 360 hr. mesocosm experiment we measured dissolved oxygen (DO) concentrations 1 m below the surface and 1 m above the bottom. At the start of our experiment (0 hr) the average DO concentrations were similar in the controls, WAF and DCEWAF tanks and averaged 8.15, 8.01 and 8.00 mg/L, respectively. However, after 96 hours the DO in the control, WAF and DCEWAF averaged 9.00, 7.26 and 5.50 mg/l, respectively with one WAF treatment at 4.18 mg/L. It was apparent that processes (e.g. MOS formation, oil biodegradation, etc.) reduced the DO in the WAF and DCEWAF mesocosms while microbial growth was increasing the DO in the controls. We had anticipated this issue and had developed and tested an O<sub>2</sub> enrichment system. The system uses medical grade oxygen to maintain adequate DO in the mesocosms by diffusion of the oxygen from saturated water inside an inverted funnel. All treatments including the control were oxygenated for 1 hr. After oxygenation, the WAF mesocosms DO average was 8.63 mg/ L and the DO was 6 mg/l or higher throughout the rest of the experiment. The O<sub>2</sub> enrichment system is inexpensive and easily modified for use in various mesocosm designs.

### Evaluating Sub-mesoscale Frontal Eddies over a Broad, Low-energy Continental Shelf

**D. Kobashi**, R. Hetland

Texas A&M University, College Station, TX

Sub-mesoscale frontal eddies over the Texas-Louisiana (TXLA) Shelf are simulated based on a high-resolution non-assimilative regional ocean model. We use an assessment of simulated salinity fields as

a way to investigate if the model is able to accurately simulate the structure of frontal eddies. The model successfully reproduces instantaneous and mean salinity structure both horizontally and vertically against multi-year densely sampled CTD data. However, the model demonstrates a poor performance in predicting salinity perturbation defined as the difference between the instantaneous salinity and climatological salinity for all salinity profiles between 2008 and 2011; the difference in salinity gradients between the model and observation are equivalent to an additional, significant Gaussian error added to the observed salinity. The model is able to reproduce the magnitude and character of salinity gradients associated with frontal eddies, an energetic eddy field with spatial scales too small to accurately characterize using conventional observational techniques. However, due to their chaotic nature, these eddies are not always predicted at the right time and place, resulting in significant errors in predicting salinity fields. The intensity and distribution of frontal eddies changes seasonally; they are more active in summer than in fall and they are most intense off the central and western Louisiana. The model performance varies spatially and temporarily. The simulated salinity has higher errors in summer than in fall and the highest model error is near the Mississippi River and the lowest errors are off Texas. The most active area of the frontal eddies coincides with the area of the summer hypoxic water suggesting their potential implications for ecosystems of the shelf.

## The Use of Single-Phase Scale-Resolving Simulations to Estimate Droplet Sizes Produced by an Oil-in-Water Jet

**J. Leggoe**, C. P. Booth, Z. M. Aman

The University of Western Australia, Crawley, Australia

Deep-water blowouts produce a plume of oil droplets that interact with the surrounding ocean as they ascend, where the fate of these droplets is strongly linked to their size. The droplet size distribution (DSD) is determined by the primary breakup of the jet and the secondary breakup of these original droplets in the highly turbulent near-field surrounding the oil jet. As full-scale experiments are intractable, laboratory and mesoscale experiments have been conducted to obtain data as to how oil jets breakup when entering quiescent water. The data from these experiments are then used to construct scaling laws which can then be used to predict the DSD that will form from a full-scale event, taking into account the application of dispersants. A potential weakness of these scaling laws is that they use dimensionless quantities, like the Weber and Ohnesorge Numbers, which are defined using the bulk parameters of the system. As the phenomena of droplet formation from the jet and the breaking of droplets is governed by the local turbulent flow fields it is argued that a more accurate measure of the DSD can be obtained using information describing the local flow fields. These measures can be difficult to experimentally obtain so we look at using Scale Resolving Computational Fluid Dynamics to accurately predict the resolved scales of the turbulent flow in the very near field of a jet and compare measures of the instantaneous turbulence against data published in the literature.

## Horizontal Material Dispersion by Boundary Layer Currents

**J. Liang**

Louisiana State University, Baton Rouge, LA

Most previous studies on buoyant material dispersion in the ocean focus on the effect of submesoscale, mesoscale or basin-scale currents. To study the effect of oceanic surface boundary layer flows on material dispersion, a coupled modeling framework that includes a large eddy simulation

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model for three-dimensional turbulent water flows and a Lagrangian particle model for materials is developed. I will show that boundary layer current, wave-induced Stokes drift, turbulence, and particle buoyancy all contribute to dispersion. In shear and Langmuir turbulence, dispersion is between diffusive and ballistic for neutrally buoyant particles and is diffusive for buoyant particles.

## Molecular Responses to Alkylated Chrysenes Found in Crude Oil in Murine Lung and Liver Cells

**R. C. Lichtler**<sup>\*1</sup>, M. J. Wilson<sup>1</sup>, B. M. Meyer<sup>2</sup>, D. Pangeni<sup>2</sup>, E. B. Overton<sup>2</sup>, J. K. Wickliffe<sup>1</sup>

<sup>1</sup>Tulane University, New Orleans, LA, <sup>2</sup>Louisiana State University, Baton Rouge, LA

Polycyclic aromatic hydrocarbons (PAHs) are chemicals that are abundant in the environment, can be generated through the process of incomplete combustion, and are relatively common hydrocarbons in crude oil. Some PAHs are known to be hazardous to human health due to their environmental persistence and carcinogenic properties. Although there are many types of PAHs, individual PAHs may produce different biochemical and toxicological responses in biological systems depending on their chemical structures. Regulatory assessments are based on a few, unsubstituted PAHs that have been widely used for toxicity testing, but there has been comparatively little research on substituted PAHs such as those with one or more alkyl side groups. In order to determine if alkylated PAHs elicit molecular responses that are toxicologically relevant to those induced by the well-studied parent compounds, we exposed metabolically competent murine lung and liver cells to specific PAHs (i.e. chrysenes) found in Deepwater Horizon MC252 oil. We found that methylchrysenes induced marked molecular responses as did the unsubstituted parent compound chrysene. Responses were benchmarked against the reference PAH, benzo[a]pyrene. These results indicate that future studies and regulatory testing should include substituted PAHs and mixtures of PAHs in toxicity assessments.

## Analysis of 18S rRNA Defined Eukaryotic Community Composition in Coastal and Offshore Gulf of Mexico upon Exposure to Oil and Dispersants

**G. Lin**<sup>\*1,2</sup>, S. Setta<sup>3</sup>, A. M. Achberger<sup>2</sup>, S. M. Doyle<sup>2</sup>, A. Quigg<sup>3</sup>, J. B. Sylvan<sup>2</sup>

<sup>1</sup>Ocean University of China, Qingdao, CHINA, <sup>2</sup>Texas A&M University, College Station, TX, <sup>3</sup>Texas A&M University Galveston campus, Galveston, TX

On April 20, 2010 the Deepwater Horizon drilling rig exploded in the northern Gulf of Mexico, causing an unprecedented oil spill in US waters. As part of the clean-up response, chemical dispersants (e.g. Corexit<sup>®</sup> 9500), which disperse oil into the water column by breaking down surface oil slicks, were used. However, the influence of oil and dispersants on marine organisms, and in particular, microbial eukaryotes, remains unclear. Further, while the Deepwater Horizon spill largely impacted deep waters, most oil spills occur at the surface, and the majority of work to date has focused on bacterial community response at depth. To generate data on the response of the eukaryotic community to oil and dispersant exposure in coastal and offshore seawater of the Gulf of Mexico, we analyzed 18S rRNA gene diversity in three mesocosm experiments performed with the water accommodated fraction of oil with and without Corexit. For each mesocosm, four treatments were set up: (1) a seawater only control, (2) seawater with the water accommodated fraction of oil (WAF), (3) seawater with WAF and Corexit in a 1:20 ratio (chemically enhanced WAF, CEWAF), and (4) a diluted CEWAF treatment (DCEWAF). Samples were collected every 12 hours for 4 days and filtered sequentially onto 10  $\mu\text{m}$  and then 0.2  $\mu\text{m}$  filters. DNA was then extracted and prepared 18S rRNA gene libraries for Illumina MiSeq

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sequencing. For the 0.2  $\mu\text{m}$  fraction, there was more overlap in community composition at the beginning among the four treatments followed by divergence into separate groups. In contrast, the larger eukaryotes (10  $\mu\text{m}$  fraction) were more mixed, especially at the later time points. Eukaryotic diversity was greater in the control and WAF treatments relative to the treatments with dispersant. Coastal communities were less diverse and had a higher abundance of Bacillariophyceae, compared to open ocean communities, which had higher abundance of heterotrophic flagellates (*e.g.* Bicoecia and Chrysophyceae).

## Production of Exopolymeric Substances and the Roles in Oil Transport in Water-Accommodated Fraction of Oil and Corexit/Oil Contaminated Mesocosms

**P. Lin**<sup>1</sup>, C. Xu<sup>1</sup>, L. Sun<sup>1</sup>, K. A. Schwehr<sup>1</sup>, A. Yard<sup>1</sup>, T. L. Wade<sup>2</sup>, A. H. Knap<sup>2</sup>, P. G. Hatcher<sup>3</sup>, A. Quigg<sup>1</sup>, P. H. Santschi<sup>1</sup>

<sup>1</sup>Texas A&M University Galveston Campus, Galveston, TX, <sup>2</sup>Texas A&M University, College Station, TX, <sup>3</sup>Old Dominion University, Norfolk, VA

Exopolymeric substances (EPS) are released by algae and bacteria under normal conditions, but often at higher concentrations under stressful conditions, *e.g.*, petroleum contaminated environment, as a life strategy to protect from acute toxicity. This ameliorating mechanism can cause an either positive or negative feedback on the re-distribution of petroleum in the water column. We conducted a 16-day long-term mesocosm experiment, using light cycle (12 h: 12h light: dark), coastal seawater and natural microbial consortia, in three treatments: 1) a water accommodated fraction of oil (WAF), 2) a diluted chemically-enhanced WAF with addition of oil dispersant Corexit (DCEWAF) in a ratio of 1:20, and a 3) control (seawater only). Colloidal (3 kDa- 0.4  $\mu\text{m}$ ), suspended particulate matter (SPM, > 0.4  $\mu\text{m}$ ) and sinking marine oil snow (MOS, > 1 mm) were collected and extracted to examine the short-term (4-day) and long-term (16-day) responses of microbes with respect to EPS production and composition, in the three fractions. Furthermore, it was of great interest how the EPS composition is related to the petroleum distribution in the water column versus in the sinking MOS. Information obtained from the current study will provide important information to resolve how production of amphiphilic EPS is affected by petroleum contamination, and how oil in WAF/DCEWAF treatments is associated with colloids, SPM or sinking MOS.

## Comparison between Ambient and Enhanced Nutrient Mesocosms in Short and Long Term Experiments

**M. E. Morales-McDevitt**\*<sup>1</sup>, T. Wade<sup>2</sup>, G. Bera<sup>2</sup>, D. Shi<sup>2</sup>, A. Quigg<sup>2</sup>, A. H. Knap<sup>2</sup>

<sup>1</sup>Oceanography, Texas A&M University, College Station, TX, <sup>2</sup>Texas A&M University, College Station, TX

Among the questions that emerged from the Deepwater Horizon (DWH) oil spill was the role that external sources of nutrients (*e.g.* river influx) in the biodegradation of oil. The ADDOMEx consortium conducted five mesocosm experiments with coastal (M1, M2, M4, M5) and open ocean water (M3), with ambient and added (enhanced) nutrient concentrations. Mesocosm experiments were short term (3 to 4 days -M2, M3, M4) and long term (16 days -M5). In all instances, seawater was mixed with Macondo Surrogate oil plus Corexit (20 to 1) in baffled recirculation tanks (BRT) to generate water accommodated fractions (WAF) and chemically enhanced water accommodated fraction of oil (CEWAF) treatments respectively. A 10-fold dilution of CEWAF (DCEWAF) was also made while transferring the mixture from the BRT to the mesocosm tanks. M3 and 4 were augmented with f/20 nutrient medium

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added at time zero. M2 and M5 had ambient nutrients (M1 is not shown). Dissolved inorganic nitrogen, phosphorous and silicate concentrations were measured every 24 hours throughout the experiment. Neither nutrient was ever completely depleted in any of the experiments. The removal rate of nitrogen for M2 from time zero (0.3 mg/L) ranged from  $-0.04$  to  $-0.018 \text{ hr}^{-1}$ , the control having the highest values. In contrast, M5 had an initial nitrogen concentration at time zero (0.4 mg/L) was  $-0.004 \text{ hr}^{-1}$  in all treatments. The differences in experiment length and planktonic communities could explain the disparities in the removal rate between M2 and M5. In M3 and M4, nitrogen consumption was not as obvious as in the other two experiments, yet it is important to consider their high DIN concentration. Still, removal rates in the DCEWAF and CEWAF were higher than in any other experiment ( $-0.02 \text{ hr}^{-1}$  to  $-0.004 \text{ hr}^{-1}$ ). In M2 and M5 the N:P ratio decreased with time suggesting that nitrogen was limiting. In the WAF and DCEWAF treatments of M3 and M4, the N:P retained a stable N:P which indicates that neither nutrient became limiting. The decrease of silicate over time suggests its uptake by a growing diatom community.

## Investigating Multiple Inputs of Oil Residues to Gulf of Mexico Beaches

**A. E. Morrison\***, H. K. White

Haverford College, Haverford, PA

Seven years after the Deepwater Horizon (DWH) incident, oil residues from the spill persist on Gulf of Mexico beaches. Additional anthropogenic inputs of oil to the beach environment include offshore industrial activity and asphalt from parking lots and roads. Oil residues from natural sources such as oil seepage and asphalt volcanism have also been collected from beaches in 2017. In this study we document the distribution and chemical composition of oil residues from these multiple sources using gas chromatography with flame ionization detection (GC-FID) and mass spectrometry (GC-MS), as well as thin layer chromatography with flame ionization detection (TLC-FID). This study provides insight into the variable chemical composition of the different types of oil residues present on Gulf of Mexico beaches as well as information regarding their long-term fate.

## The Influence of Hydrostatic Pressure on Microbial Motility and Hydrocarbon Chemotaxis

**K. K. Mullane\***, D. H. Bartlett

Scripps Institution of Oceanography, UCSD, La Jolla, CA

Pressures of the magnitude present at the depth of the Deepwater Horizon (DPWH) oil spill inhibit the motility of mesophilic bacteria<sup>1,2</sup>. We are assessing the impact of pressure on motility and hydrocarbon chemotaxis in hydrocarbon-degrading bacteria, including those obtained from the DPWH oil spill. The long-term goal of this research is to understand these impacts in terms of hydrocarbon biodegradation in the deep sea. We hypothesize that at the pressures present at depths greater than 1000 meters, motility will be inhibited, except where high pressure adaptation has evolved. Two sets of strains containing both piezo (pressure)-sensitive and piezotolerant bacteria belonging to the orders Oceanospirillales (*Alcanivorax*, and *Halomonas*) and Alteromonadales (*Marinobacter*, and *Shewanella*) are being examined at pressures ranging from 0.1 - 50 megapascals (MPa). The growth of these strains under the anaerobic conditions that develop within standard pressure vessels was greatly enhanced under nitrate respiratory conditions. Most of the strains were observed to be motile via a single polar flagellum. Qualitative analyses of alkane and aromatic hydrocarbon chemotaxis have made use of glass

\* Student presenter

serum vials with pressurizable septa containing hydrocarbons as the sole carbon source in a defined medium with 0.3% agar. Under these incubation conditions many of the strains exhibited vigorous motility and chemotaxis. Future studies will include capillary assays of chemotaxis as a function of pressure, and through a collaboration with Dr. Masayoshi Nishiyama from the University of Kyoto, Japan, the use of high pressure microscopy to measure pressure effects on strain swimming speed, cell displacement, and fraction of motile cells. Cumulatively, these analyses will enable a better understanding of the impacts of pressure on microbial motility and hydrocarbon chemotaxis in the deep sea.

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### Transcriptome-wide Responses of Aggregates of the Diatom *Odontella aurita* to Oil

Y. Liang<sup>1</sup>, D. Nanjappa<sup>1</sup>, C. Brown<sup>1</sup>, U. Passow<sup>2</sup>, A. Quigg<sup>3</sup>, A. Irwin<sup>1</sup>, Z. Finkel<sup>1</sup>

<sup>1</sup>Mount Allison University, Sackville, NB, Canada, <sup>2</sup>University of California Santa Barbara, Santa Barbara, CA, <sup>3</sup>Texas A & M, Galveston, TX

Phytoplankton aggregates played an important role in the formation of marine oil snow after the Deepwater Horizon oil spill event. The molecular response of phytoplankton aggregates in response to oil is poorly characterized. Here we use RNAseq to analyze the transcriptome-wide responses of aggregates of the marine diatom *Odontella aurita* exposed to two different types of oil in a roller table experiment. *O. aurita* was incubated in quadruplet roller tanks at 22°C in Macondo and Refugio oil (1% vol: vol). We identify a common set of 353 genes that are differentially expressed under both Macondo and Refugio oil exposure relative to the control. The complexity of marine oil snow assays resulted in a sampling design spread over two days and over different times of the day; differential expression detected across pseudo-replicates was almost entirely independent of the effect of the oil treatments. Differential expression is often more extreme in the Refugio versus the Macondo treatment; indicating that differences in the physical and chemical characteristics of the source oil influences the molecular response. KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) enrichment analysis indicate that oil exposure leads to a down-regulation of carbon fixation, photosynthesis and photosynthesis related proteins. Analyses of the up-regulated genes is less straightforward (no KEGG enrichment was detected) but GO enrichment analysis identified a number of genes associated with stress responses, replication and transcription, cytoskeletal and ruffle membrane related processes. These results indicate that oil exposure leads to a reduction of photosynthesis and carbon fixation gene expression and stimulates a stress response that may include changes to the cytoskeleton and cell membrane. More work is required to determine the general applicability of these identified biomarkers of oil exposure to other diatoms and phytoplankton taxa.

## Microbial Community Composition of Gulf of Mexico Sediments in Response to Crude Oil

**U. T. Nguyen**\*<sup>1</sup>, S. A. Lincoln<sup>1</sup>, A. Valladares Juárez<sup>2</sup>, M. Schedler<sup>2</sup>, J. L. Macalady<sup>1</sup>, R. Muller<sup>2</sup>, K. H. Freeman<sup>1</sup>

<sup>1</sup>Penn State University, University Park, PA, <sup>2</sup>Institute of Technical Biocatalysis, Hamburg University of Technology, Hamburg, Germany

About 3-31% of oil released from the 2010 Deepwater Horizon (DWH) disaster was deposited onto deep Gulf of Mexico (GOM) sediments, where biodegradation is potentially the main process regulating the fate of the spilled oil. However, information on the dynamics of deep sediment microbial communities in response to oil spill is limited, especially under high pressures. Here, we investigated crude oil biodegradation in laboratory incubation under simulated seafloor conditions, with 13 different core top GOM sediments collected at water depths from 60-1500 m. After 18 days, changes in oil components were analyzed with gas chromatography-mass spectrometry, and 16S-rRNA genes of microbial communities were sequenced on Miseq PE Illumina using universal primers 515F/806R targeting V4 hypervariable regions. In all samples, original diversity decreased as oil degraders increased their abundances. Proteobacteria was the most dominant phylum, followed by Bacteroides and Firmicutes. Gene prediction based on phylogenetic investigation of communities by reconstruction of unobserved states method (PICRUST) showed positive correlations between copy numbers of hydrocarbon-degrading genes and the depletion in total n-alkanes and total polycyclic aromatic hydrocarbons ( $r^2 = 0.2$  to  $0.8$ ). While shallow sediments selected for *Alcanivorax* and *Marinobacter*, deep sediments had high abundance of *Colwellia*, *Oleispira*, and *Pseudoalteromonas*, known genera of oil-degraders. Constrained analysis of principal coordinates (CAP) further showed separate clusters of post-incubation shallow and deep communities. A subset of deep sea samples incubated at 4<sup>0</sup>C at both *in-situ* pressure (9.4, 11, 15 MPa) and surface pressure (0.1 Mpa) revealed variation in oil degrader compositions, along with stochastic behavior in hydrocarbon degradation according to pressure change, suggesting that pressure was an important factor shaping microbial communities. Overall, our study advances the understanding of how natural deep water microbial ecology responds to crude oil disturbance under simulated deep sea conditions, which could help guide bioremediation decision makers in case of future deep sea spills in the GOM.

## Targeted Analysis of Ketone-Containing Transformation Products in Photo-Ox Specific Microcosms and Field Samples

**S. F. Niles**\*<sup>1</sup>, H. Chen<sup>2</sup>, A. M. McKenna<sup>2</sup>, L. C. Krajewski<sup>1</sup>, Y. E. Corilo<sup>2,3</sup>, R. P. Rodgers<sup>1,2</sup>, A. G. Marshall<sup>1,2</sup>

<sup>1</sup>Florida State University, Tallahassee, FL, <sup>2</sup>National High Magnetic Field Laboratory, Tallahassee, FL,

<sup>3</sup>Future Fuels Institute, Tallahassee, FL

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry has revealed tens-of-thousands of oxygen-containing transformation products in field samples collected after the Deepwater Horizon spill. The technique is uniquely suited for the analysis of oxidized species in weathered oil samples due to its inherent high resolution (to resolve hundreds of peaks per nominal mass) and mass accuracy (100-300 ppb), which allows elemental composition assignment for thousands of species per analysis, based on mass measurement alone. However, although selection of ionization method provides coarse chemical specificity, it does not enable the unambiguous assignment of chemical functionality. Here, we apply a previously developed method for the specific isolation, derivatization, and molecular characterization of ketone-containing species in petroleum<sup>1</sup> to the characterization of polyfunctional

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oxidized species isolated from photo-ox specific microcosms and those isolated from field samples. The technique allows for the selective isolation of ketone-enriched fractions by strong anion exchange (SAX) chromatography, and subsequent derivatization via Ampliflex keto-reagent and mass spectral characterization unambiguously identifies ketone-containing transformation products. The work presented is the first step in understanding the contribution of specific chemical functionalities to the tens of thousands of oxidized transformation products observed in field samples. 1. Krawjewski, *et al. Energy Fuels* 2017, 31 (10), 10674-10679. Acknowledgement: Work supported by NSF DMR 11-57490, and BP/Gulf of Mexico Research Initiative.

## Nutrient Amended Biodegradation of Hydrocarbon Contamination along Canada's Labrador Coast

**S. M. C. Murphy\***, C. R. J. Hubert  
University of Calgary, Calgary, AB, Canada

Newfoundland and Labrador's offshore region, an area larger than the Gulf of Mexico's outer continental shelf, is the focus of ongoing northern oil exploration via high-resolution seismic surveys along the Labrador coast. Future oil production and heavy shipping traffic into Hudson Bay increase this region's risk of an oil spill in the marine environment. Whether indigenous microbial communities in this marine corridor can effectively biodegrade spilled hydrocarbons, naturally or via nutrient amendment, remains poorly understood. To demonstrate the microbial response within this corridor (Labrador Shelf through to Hudson Bay), O<sub>2</sub> consumption and CO<sub>2</sub> production were monitored over time in the headspace of sediment microcosms amended with diesel or crude oil with different concentrations of limiting nutrients. Sequencing of 16S rRNA gene amplicon libraries using the Illumina MiSeq platform was used to determine baseline community composition within environmental samples, and to monitor community changes associated with biodegradation in the microcosm incubations. Labrador Shelf sediments amended with diesel (0.1% v/v) and incubated at 4°C experienced greater O<sub>2</sub> consumption and CO<sub>2</sub> production relative to unamended controls. Treatments with higher concentrations of nitrogen and phosphorous (4.7mM N, 1.5mM P) show elevated O<sub>2</sub> depletion and CO<sub>2</sub> production compared to those with ambient nutrient levels (i.e. seawater: 15µL N, 2µL P). Microcosms amended with a larger volume of diesel (1% v/v) and a high nutrient level experienced the fastest response, with complete removal of headspace O<sub>2</sub>; demonstrating the ability of the sediment microbial community to respond to heavy contamination. These results indicate the potential for natural attenuation catalyzed by indigenous microbial communities in the event of a spill associated with shipping or oil production along the Labrador coast. Nutrient amendment as a strategy for enhanced bioremediation is likely to be effective in expediting near-shore clean up in the area.

## Direct Fractionation Methods for MC252 Crude Oil Coupled with an Aryl Hydrocarbon Receptor Signaling Assay Identifies Novel Compounds of Toxicological Interest

**D. Pangeni\***<sup>1</sup>, E. Overton<sup>1</sup>, C. Miller<sup>2</sup>

<sup>1</sup>Louisiana State University, Baton Rouge, LA, <sup>2</sup>Tulane University School of Public Health and Tropical Medicine, New Orleans, LA

Crude oils are chemically complex mixtures consisting of thousands of compounds that differ greatly in their chemical properties and activities in biological systems. Most of the chemical constituents in oils are not fully characterized and have not been extensively examined for toxicity. We developed a

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screening system with the potential to identify novel toxic molecules within MC252 oil by coupling chemical fractionation with an aryl hydrocarbon receptor signaling assay. The fractionation method provided chemical class separation of the complex mixture into saturates (S), Aromatics (A), Resins (R), and Asphaltenes (A). Fractions that produced the greatest responses in the aryl hydrocarbon receptor signaling assay were then sub-fractionated using normal and reverse phase HPLC. The greatest bioactivities identified were present in the aromatic (A) fractions. Polycyclic aromatic hydrocarbons (PAHs) reside in the A fractions and are of interest due to their carcinogenic and other toxic activities. However, several of the PAHs identified in this screen were alkylated and less is known about this subset of compounds. The alkyl PAHs will be assessed for effects on gene expression, mutagenesis, and cytotoxicity to provide for better understanding of their potential effects. We conclude that this reiterative “fractionate and assay” approach may be generally useful for identifying bioactive compounds in fresh and aged oil samples from other sources.

### DEEPEND: The Impact of *in situ* Data Assimilation in a Numerical Model on the Characterization of the Biophysical Habitat

**B. Penta**<sup>1</sup>, S. deRada<sup>1</sup>, T. Sutton<sup>2</sup>, M. Johnston<sup>2</sup>, R. Milligan<sup>2</sup>, C. Easson<sup>2</sup>, A. Cook<sup>2</sup>, K. Boswell<sup>3</sup>, C. Lembke<sup>4</sup>, D. English<sup>4</sup>

<sup>1</sup>U.S. Naval Research Laboratory, Stennis Space Center, MS, <sup>2</sup>Nova Southeastern University, Dania Beach, FL, <sup>3</sup>Florida International University, North Miami, FL, <sup>4</sup>University of South Florida, St. Petersburg, FL

The Deep-Pelagic Nekton Dynamics of the Gulf of Mexico consortium (DEEPEND) uses observational and multi-model approaches to characterize biophysical variability and investigates the dynamics of deep-pelagic animal assemblages at multiple temporal and spatial scales. Run in near-real time, at 1/25° horizontal-resolution, the HYCOM Gulf of Mexico (HYCOM-GOM) ocean model was used to support the five DEEPEND research cruises (2015-2017) and aid adaptive sampling strategies. Post-cruise, three additional simulations were performed with the HYCOM-GOM configuration to better understand biophysical relationships in the Northeastern Gulf of Mexico. First, a hindcast “nature run” was conducted with no data assimilation. Next two data assimilative runs were completed. One assimilated data from publicly available sources (e.g., the NOAA Global Telecommunication System [GTS]), and the other assimilated *in-situ* CTD and glider data collected during the cruises. We analyzed the impact of the various assimilated data on the model results and on the characterization of the biophysical habitat and associated environmental drivers.

### Comparison of Mixing Energy from High and Low Energy Oil in Water Preparations to that Found in the Marine Environment

**A. Redman**<sup>1</sup>, T. Parkerton<sup>2</sup>, K. Daly<sup>1</sup>, L. Zhao<sup>3</sup>, M. Boufadel<sup>3</sup>

<sup>1</sup>ExxonMobil Biomedical Science, Inc, Annandale, NJ, <sup>2</sup>ExxonMobil Biomedical Science, Inc, Spring, TX, <sup>3</sup>New Jersey Institute of Technology, Newark, NJ

The potential hazards of oil exposures to aquatic life are evaluated in standardized laboratory toxicity tests under controlled conditions to compare different oils and calibrate effect models that support extrapolation of lab results to assess realistic field exposures. Typical systems involve the mixing of oil in water under different degrees of mixing energy to promote the dissolution and entrainment of oil constituents in the exposure media. Recent use of the high energy water accommodate fraction

(HEWAF) dosing system has provided hazard data intended to characterize potential impacts of oil spills. This system involves the blending of oil in water at high speeds (>10,000 RPM) for short times (30-60 seconds) to produce aqueous test media. The concentrations of dissolved and particulate (droplet) oil constituents in this complex exposure system varies during the preparation procedure and over the course the exposure period as droplets rise to the surface and as volatile constituents are lost and are replenished through droplet dissolution. Therefore, concerns have been raised regarding the reproducibility and interpretation of data developed with this dosing technique. The maximum energy dissipation rate,  $\epsilon$ , is commonly used to relate formation of droplets to mixing methods. The  $\epsilon$  of the HEWAF test system was compared to the  $\epsilon$  for other common preparation systems (e.g., low energy stirring), and typical environmental processes related to oil spill fate (e.g., deepwater blowout, breaking waves, etc). Further, theoretical droplet size distributions are compared to reported droplet size distributions using mechanistic modeling. The results show agreement between the theoretical droplet size distributions associated with high energy mixing and empirical measurements. However, the mixing energy found in the HEWAF system are more than four orders of magnitude higher than energetic conditions found in the environment resulting in transient droplet distributions that have limited environmental relevance. Therefore, data developed with the HEWAF system must be interpreted with caution.

## Behavior of Sea Spray Particles in Very High Wind Conditions

**G. Rivera**\*<sup>1</sup>, S. Shawver<sup>1</sup>, B. Haus<sup>1</sup>, L. Shen<sup>2</sup>, W. Drennan<sup>1</sup>

<sup>1</sup>University of Miami, Miami, FL, <sup>2</sup>University of Minnesota, St. Paul-Minneapolis, MN

Sea spray forms when bubbles burst as a result of breaking waves. These spray particles are then ejected into the atmosphere from the ocean surface in the form of aerosols. Understanding how air-sea fluxes behave in high wind conditions is still a relatively new area of research that requires further investigation. Ortiz-Suslow used the Air-Sea Interaction Saltwater Tank (ASIST) at the Rosenstiel School of Atmospheric Science (RSMAS) to collect data on the influence of large spray droplet production on air-sea fluxes in high wind conditions. Here we extend this work to measure the variability of sea spray concentration and velocity as a function of particle size, height and wave phase (crest to trough to crest). Measurements from both salt and fresh water are reported in a variety of wind speeds to 25 m/s, both with and without added long waves. The results provide input to LES numerical models for spray above waves.

## Comparison of Exopolymeric Substances (EPS) with Transparent Exopolymeric Particles (TEP) and Microgels in Mesocosms

**P. H. Santschi**<sup>1</sup>, U. Passow<sup>2</sup>, W. Chin<sup>3</sup>, M. Chiu<sup>3</sup>, C. Xu<sup>1</sup>, P. Lin<sup>1</sup>, L. Sun<sup>1</sup>, K. A. Schwehr<sup>1</sup>, A. Quigg<sup>1</sup>

<sup>1</sup>Texas A&M University at Galveston, Galveston, TX, <sup>2</sup>University of California Santa Barbara, Santa Barbara, CA, <sup>3</sup>University of California at Merced, Merced, CA

Exopolymeric substances (EPS), produced by microorganisms and precursors of transparent exopolymeric particles (TEP), are ubiquitous and abundant in the ocean and have an important role in marine snow and/or marine oil snow (MOS) formation, colloid and particle dynamics and energy transfer in the ecosystem. TEP are operationally defined as particles (>0.4  $\mu\text{m}$ ) that can be stained with Alcian blue, while EPS, are measured chemically as the sum of the main components: polysaccharides and proteins, while minor components (lipids and nucleic acids) are ignored. Marine microgels in the

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filter-passing fraction are reversibly formed from EPS and determined by dynamic light scattering and flow cytometry. Mesocosm experiments provided the opportunity to compare the 3 different forms in a water accommodated fraction of oil (WAF) and a control with only coastal seawater. Our results revealed a significant correlation ( $p < 0.001$ ,  $r = 0.70$ ,  $n = 29$ ) between concentrations of TEP and EPS extracted from suspended particulate organic matter. EPS electrostatically held on particle surfaces extractable by EDTA, however, accounted for  $< 1\%$  of TEP. This has important implications as most EPS is not electrostatically bound to particles, and thus, not extractable by EDTA and Alcian blue might stain acidic polysaccharides in particles indiscriminately. Microgel measurements complement EPS and TEP results. Results from ongoing laboratory intercalibration experiments using incubations of natural microbial consortia in seawater amended with glucose and inorganic nutrients (N and P) at Redfield ratios will shed further light into such comparisons. Results from these studies will provide important information to resolve the relationship between TEP, EPS and microgels, and their role in regulating petroleum transport in the ocean.

## Characterization of Particulate Formation in and Rheological Properties of Suspensions of Microbial Consortia Exposed to Crude with and without Dispersant

**H. Schawe**\*<sup>1</sup>, A. R. White<sup>2</sup>, J. Sheng<sup>2</sup>

<sup>1</sup>Veterans Memorial High School, Corpus Christi, TX, <sup>2</sup>Texas A&M University-Corpus Christi, Corpus Christi, TX

An unprecedented volume of dispersant was injected directly into the well head during the Deepwater Horizon oil spill. The dispersant caused significant reduction of oil droplet size in the subsurface plume and subsequently affected their rising velocities, as well as adversely impacted natural oil-degradation processes by bacteria and microbial assemblage. It has also been shown that dispersant can inhibit formation of microgels consisting of extracellular polymeric substances (EPS) secreted by bacteria. Here, we consider how dispersant (Corexit 9500A) influences the ability of bacterial consortia to alter their surroundings through EPS and particulate formation. Consortia capable of consuming oil sampled from the Deepwater Horizon site, Port Aransas, TX, and off shore from Louisiana are cultured in conditions where their carbon source is either crude oil, oil with Corexit, or Corexit only. In addition to monitoring consortia growth rates under different carbon sources, particulate sizes are measured in situ using dynamic light scattering (DLS). Additionally, the bulk rheological, including viscoelastic, properties of the culture will be characterized with a dynamic shearing rheometer (DSR). Although all three treatments promote consortia growth, treatments containing crude oil and Corexit with DOR 1:20 cause the production of substantial nanometer particles O(10-100) nm. Control experiments containing crude and Corexit without bacteria yield particle sizes at the micron scale, revealing these nanoscale particles are strongly related to microbial activities rather than the physical breakup of oil. The changes in particulate sizes and rheological properties would have significant impacts on the hydrodynamics and droplet dynamics following the spill. These results are important for understanding how bacteria following the Deepwater Horizon oil spill could have affected the bulk compositional and rheological properties and could help inform future modeling efforts.

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## The Variability of Winds Observed near Submesoscale Fronts

**M. Shao**\*<sup>1</sup>, B. K. Haus<sup>1</sup>, D. G. Ortiz-Suslow<sup>1</sup>, N. J. M. Laxague<sup>2</sup>, B. Lund<sup>1</sup>

<sup>1</sup>RSMAS, Miami, FL, <sup>2</sup>Columbia University, New York City, NY

Sub-mesoscale fronts (SF) may mediate the air-sea interaction but seldom observation has been done. An investigation of air-sea interaction was conducted in the northern part of Gulf of Mexico, where SF happened frequently during winter. The field data consist of twin flux tower, accompanied with marine X-band radar measurement, in the expedition in Jan-Feb 2016. Surface wind acceleration (deceleration) in several across fronts transect was observed, which was considered as mesoscale front features. Momentum budget analysis showed that turbulence stress divergence, closely related with the local SST, was the dominated term, which is consistent with the vertical mixing mechanism. Coherent eddies near SF was detected by wavelet method, which may act as the mixing role. Momentum and heat flux was calculated from eddy covariance and bulk method. The comparison between them indicates that momentum flux was closely related with local SST and structure near the front. Directly observed sensible heat fluxes were generally 1-2 times larger than the expected flux, derived from a commonly used bulk algorithm, which indicated SF may significantly accelerate the energy exchange between the ocean and atmosphere. In particular cases, positive and negative heat flux appeared in different side of SF. These new features observed bear significant implication to air-sea parameterization and sub-mesoscale energy cascade.

## Light Rare Earth Element Depletion during Deepwater Horizon Methanotrophy

**A. M. Shiller**<sup>1</sup>, E. Chan<sup>2</sup>, D. Joung<sup>1</sup>, M. Redmond<sup>3</sup>, J. Kessler<sup>2</sup>

<sup>1</sup>University of Southern Mississippi, Stennis Space Center, MS, <sup>2</sup>University of Rochester, Rochester, NY,

<sup>3</sup>University of North Carolina-Charlotte, Charlotte, NC

Light rare earth elements (LREEs: La, Ce, Pr, and Nd) have generally been thought not to have a biological role. However, recent work has demonstrated that the LREEs are essential for at least some methanotrophs, being co-factors in the XoxF type of methanol dehydrogenase (MDH). We show here that dissolved LREEs were significantly removed in a submerged plume of methane-rich water during the Deepwater Horizon (DWH) well blowout. Furthermore, incubation experiments conducted with naturally methane-enriched waters from hydrocarbon seeps in the vicinity of the DWH wellhead also showed LREE removal concurrent with methane consumption. Metagenomic sequencing of incubation samples revealed that LREE-containing MDHs were present. Our field and laboratory observations provide further insight into methanotrophy and the abundance of certain types of bacteria during the DWH blowout. Additionally, our results are the first observations of direct biological alteration of REE distributions in oceanic systems. In view of the ubiquity of LREE-containing MDHs in oceanic systems, our results suggest that biological uptake of LREEs is an overlooked aspect of the oceanic geochemistry of this group of elements previously thought to be biologically inactive. We explore the implications of these observations and whether they might be relevant to upper ocean REE cycling and distributions.

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## Mixing and Wind-response in Submesoscale Fronts

**A. Solodoch\***, J. Molemaker, K. Srinivasan, L. Bertero  
UCLA, Los Angeles, CA

We conducted high-resolution observations of (submesoscale) transient fronts in the Gulf of Mexico from a small vessel equipped with novel instruments. In repeated transects of a front sourced from a Mississippi river plume, a towed instrument array allowed resolution of about 1 meter, complementing measurements from an on-board ADCP. Concurrently a drifting ADCP, and additional tracked drifters were deployed. From our measurements, integrated with wind measured by weather buoys, we find that the propagation of the River plume front is consistent with an inertial response model. In addition we quantify the rate and routes of vertical mixing on both sides of the front.

## Advection of *Karenia brevis* Blooms from the Florida Panhandle towards Mississippi Coastal Waters

I. M. Soto<sup>1</sup>, **M. K. Cambazoglu**<sup>1</sup>, A. D. Boyette<sup>1</sup>, K. Broussard<sup>2</sup>, D. Sheehan<sup>3</sup>, S. D. Howden<sup>1</sup>, A. M. Shiller<sup>1</sup>, P. J. Fitzpatrick<sup>4</sup>, R. A. Arnone<sup>1</sup>, P. F. Mickle<sup>2</sup>

<sup>1</sup>University of Southern Mississippi, Stennis Space Center, MS, <sup>2</sup>Mississippi Department of Marine Resources, Biloxi, MS, <sup>3</sup>Alabama Department of Public Health, Mobile, AL, <sup>4</sup>Mississippi State University, Stennis Space Center, MS

*K. brevis* blooms from the Florida Panhandle region are often advected westward towards the Mississippi-Alabama coast; however, there is interannual variability in their presence and intensity in Mississippi coastal waters. The 2015 *K. brevis* bloom was compared to the 2007 Florida Panhandle *K. brevis* bloom, which showed a westward advection pattern, but did not intensify along the Mississippi coast. A combination of *in-situ* cell counts, flow cytometry, and ocean color satellite imagery from the Moderate Resolution Imaging Spectroradiometer onboard the Aqua satellite was used to detect and delineate the blooms in 2007 and 2015. Two different regional applications of NCOM-Navy Coastal Ocean Model were used to understand the circulation and transport pathways. A Lagrangian particle tracking software was used to track the passive movement of particles released at different locations for both bloom events. Ancillary data (e.g., nutrients, wind, salinity, river discharge) from local buoys, monitoring stations and coincident oceanographic cruises were also included in the analysis. The *K. brevis* blooms reached the Mississippi coast both years; however, the bloom in 2007 lasted only a few days and there is no evidence that it entered the Mississippi Sound. Two major differences were observed between both years. First, circulation patterns in 2015 resulting from an intense westward-northwestward that persisted until December allowed for continuous advection, whereas this pattern was not evident in 2007. Second, local river discharge was elevated throughout late fall 2015 while 2007 was below the average. Thus, elevated discharge may have provided sufficient nutrients for bloom intensification. These results illustrate the complex, but important interactions and connectivity in coastal zones. The circulation analysis allowed to explore transport pathways for other pollutants such as oil or dispersants.

## Langmuir Turbulence Interaction with Crosswind Currents and Bottom Boundary Layer Turbulence

**A. Tejada-Martinez**<sup>1</sup>, J. Zhang<sup>2</sup>

<sup>1</sup>Civil and Environmental Engineering, University of South Florida, Tampa, FL, <sup>2</sup>University of South Florida, Tampa, FL

Results are reported from large eddy simulations (LES) of a shear flow in an unstratified finite-depth water column driven by a surface wind stress and a constant crosswind pressure gradient. The Craik-Leibovich vortex force in the LES equations serves to generate Langmuir turbulence characterized by Langmuir circulation (LC) consisting of parallel counter rotating vortices aligned downwind. The vortex force parameterizes the interaction between surface gravity waves and the wind-driven shear current resulting in LC at the surface of the ocean. In the present formulation the wind and waves are taken to be aligned. The crosswind pressure gradient induces a crosswind current and thus a net current (wind plus pressure gradient-driven) which is misaligned with the wind/wave direction. In LES without crosswind pressure gradient, LC is generated at the surface and over time penetrates deeper into the water column while also growing in width, resulting in LC reaching the bottom of the water column. In LES with constant pressure gradient, the growth of LC can be interrupted by the crosswind current's bottom boundary layer. Turbulent ejections from this boundary layer interact with the LC leading to a coherent structure characterized by a blend between Langmuir turbulence (associated with the LC) and bottom-generated shear turbulence. Diagnostics of the turbulence structure and budgets of the Reynolds shear stress will be presented. Analysis will emphasize the near-bottom portion of the water column where Langmuir turbulence can play an important role in sediment suspension and in the mixing of sediments with oil droplets entrained from the surface.

## Velocity Measurements from a Drifting Sediment Trap in the Northeastern Gulf

**A. M. Thurnherr**<sup>1</sup>, A. R. Diercks<sup>2</sup>, J. Montoya<sup>3</sup>

<sup>1</sup>Lamont-Doherty Earth Observatory, Palisades, NY, <sup>2</sup>University of Southern Mississippi, Hattiesburg, MS, <sup>3</sup>Georgia Tech, Atlanta, GA

Measurements from floating sediment traps drifting 10s to 100s of meters below the sea surface are often interpreted as Lagrangian measurements with uniform horizontal flow across the entire water column between the traps and the sea surface. Both vertical shear in the horizontal velocity field and wind drag of the surface buoys will break the Lagrangian assumption. In order to assess these effects for drifting-trap measurements collected near 100m in the northeastern Gulf, a floating sediment trap was instrumented with an Acoustic Doppler Current Profiler as well as an ALEC electromagnetic current meter to measure the ocean velocities relative to the trap. The trap was deployed during the recent ECOGIG EN600 cruise where the instruments collected data during three deployments lasting 25hr, 79hr and 76hr, respectively. Here we present an analysis of the resulting data.

## Modeling Sediment Resuspension and Methane Distribution in the Deep Gulf of Mexico under the Effect of Near-Seabottom Flow

**Y. Wang\***<sup>1</sup>, P. Roland<sup>2</sup>, A. L. Dissanayake<sup>1</sup>, K. Ziervogel<sup>3</sup>, C. Meile<sup>1</sup>

<sup>1</sup>Department of Marine Sciences, University of Georgia, Athens, GA, <sup>2</sup>National School for Water and Environmental Engineering, Strasbourg, FRANCE, <sup>3</sup>Ocean Process and Analysis Laboratory, University of New Hampshire, Durham, NH

The benthic environment in the Gulf of Mexico is affected by cold seeps. The distribution of oil and gas (mostly methane) released from the seafloor is affected by near-bottom currents, which in turn shapes the magnitude and location of biogeochemical processes in the water column. Here, we focus on the effect of flow in the benthic boundary layer at GC 600 in the northern Gulf of Mexico at a water depth of ~1250 m. We quantify fluid flow by numerical modeling based on ADCP time series data measuring flow velocities 15-30 m above the bottom and high-resolution bathymetry data in a 500 x 500 x 100 m<sup>3</sup> simulation domain. Flow velocities are determined from the Navier-Stokes equations; turbulence is modeled using a k-ε model. Flow velocities are used to assess sediment resuspension and the distribution of dissolved methane around seeps. To estimate sediment resuspension, we compare simulated flow velocities above the seafloor with experimentally determined critical shear velocities. We calculate the percentage of time the simulated flow velocities exceed this threshold to map the spatial distribution of resuspension probability. To quantify dissolved methane concentration, we simulate the conditions representative of the seep Mega Plume 1 with the Texas A&M Oilspill Calculator (TAMOC). TAMOC predicts the methane dissolution rates from bubbles and their trajectories as they rise in the water column and they are used to estimate the methane mass added into the water at different locations in the domain. The distribution of dissolved methane is then computed based on the ambient fluid velocities. Our results show that seafloor topography has a significant effect on the near-bottom flow velocity, resulting in variations in flow velocities across the domain and with predominant flow direction. Simulated resuspension is episodic and uneven across the domain. Dissolved methane distribution is significantly affected by the flow field, spreading predominantly towards the SE.

## Forming Mechanism of Ordovician Microbial Carbonate Reservoir in Northern Slope of Tazhong Uplift, Tarim Basin, China

**Y. Wang\***, H. Chen

China University of Geosciences, Wuhan, China

Microbial carbonate reservoirs is one of the hot research areas. Though the observation of microbialite's core and casting thin sections of the northern slope of middle Tarim Basin, we can conclude that the microbialites of study area is given priority to fracture-pore type reservoir. Though the porosity and permeability analysis of full diameter core, we find that the microbialites are good gas reservoirs. On the analysis of the formation mechanism and characteristics of microbialites, combined with stress sensitive experiment and imaging logging, we summarized the advantage factors of the forming reservoir space of microbialites, firstly, the generation of native dolomite can promote the dissolution of calcite. The microbialites of the study area is given priority to eogenetic karstification, and parts superposition late hypogenic karstification, improving the reservoir physical properties. Secondly, rich organic matter content is not only caused dissolution by organic acid which is generated by microbialites in the burial diagenetic stage, but also reduce the ultimate strength of microbialites which could easy to form cracks under the tectonic movement. Therefore, the reservoir space of microbialites in the northern slope of middle Tarim Basin is mainly secondary role. Though the

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determination of rock compressibility, it is concluded that microbialites' compaction resistance is commonly, but algae crumbs skeleton is advantageous to the protection of the algal hole. The strongest ability to resist compaction is silicified microbialites, followed by dolomitization.

## Hydrocarbon Charging History within Shun 1 Strike-slip Fault Belt of the Super-deep Reservoir, Tarim Basin, China

**Y. Wang\***, H. Chen

China University of Geosciences, Wuhan, China

Shunbei area is a major breakthrough of Tarim basin in the oil and gas exploration. By taking advantage of the oil and gas geochemical characteristics of Yijianfang Formation in Shunbei area, combined with microscopic fluorescence spectrum parameters and fluorescence color, we put forward that the study area experienced multiple hydrocarbon filling and the crude oil of early filling experienced a certain degree of biodegradation. The oil is high maturity and natural gas is crude oil associated gas. We concluded that the source of oil and gas in shunbei area is the same with shunnan area which is from Cambrian yuertusi formation source rocks by using isoprenoids parameters and biomarkers in oil-oil correlation. Through the fault which broking from Cambrian formation to Ordovician formation, the hydrocarbon had upward migrated to the Ordovician reservoirs. Along shunbei area to collect samples for fluid inclusion testing, the cold cathodelight and fluorescent observation, we put forward that there are three episodes of oil charging and one episode of gas charging: Late Caledonian, Late Hercynian, Late Himalaya, the gas charging occurred in Late Himalaya. Analysed the API degree of oil inclusions and crude oil in shunbei area, we realized that the largest contribution of shunbei area's hydrocarbon accumulation is the period of hydrocarbon charging in Late Himalaya.

## Simultaneous PLIF and PIV Measurements on Refractive Index Matched Immiscible Buoyant Oil Jet Fragmentation in Water

**X. Xue\***, J. Katz

Johns Hopkins University, Baltimore, MD

Subsurface oil well blowout generate immiscible turbulent buoyant oil jets, which breaks up into a cloud of oil droplets. Understanding of the fragmentation is essential for evaluating the spreading of the oil jet and its interaction with the surrounding water in the near field, and for determining the droplet size distribution needed for modeling the subsequent transport of the oil. There is limited experimental data on the near field behavior of the opaque oil jet because of the inability to perform phase distribution measurements there. Injecting silicone oil into sugar water, which have the same refractive index, as surrogates for crude oil and water, respectively, enables us to observe the breakup process. The dynamic similarity is maintained by keeping nearly the same interfacial tension as well as density and viscosity ratios. The mixing process is visualized by simultaneous applications of planar laser induced fluorescence(PLIF) by premixing the oil with dye, and particle image velocimetry (PIV), by seeding both phases with particles. The PLIF images are used for measuring the droplet sizes as well. The Reynolds number is varied by changing the velocity, and Weber number effects are evaluated by varying the interfacial tension as well. Results show that with increasing Reynolds number, the jet spreading angle evaluated from the PLIF images increases, and its centerline velocity decreases at a faster rate. Beyond about 7 nozzle diameters, the turbulence peaks at the center of the jet, and its magnitudes scales with centerline velocity. As expected, the oil ligament fragmentation occurs

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primarily in regions of high strain-rate fluctuations in the near field shear layer and at the end of the potential core. This latter moves closer to the nozzle, and the resulting characteristic droplet sizes decrease with increasing Reynolds number. In both cases, the fragmentation process generates compound droplets, each containing multiple layers of oil and water.

## Decision-making in Bacteria: An Integrated Chemotactic Response to Multiple Chemical Cues

**X. Zhao\***, R. M. Ford

The University of Virginia, Charlottesville, VA

Microorganisms played a significant role in the degradation of hydrocarbons released into the Gulf of Mexico following the Deepwater Horizon blowout. Many of these marine species are motile and exhibit chemotaxis toward hydrocarbons. Oil droplets, which contain a mixture of hydrocarbons, were formed when a dispersant was injected into the oil plume. The limited aqueous solubility of the hydrocarbons generates chemical gradients near the oil-water interface that attract (repel) chemotactic bacteria to accumulate (disperse) around the oil droplets, and thereby enhance (diminish) the biodegradation process. Transport properties such as the chemotactic sensitivity coefficient and chemotaxis receptor constant provide a quantitative measure of the extent to which chemotaxis contributes to the overall degradation process. However, these properties have not been evaluated under conditions which involve multiple chemical sources of attractants and repellents. In this work, mathematical models for the chemotactic velocity of *Escherichia coli* in response to multiple stimuli were derived from fundamental principles. The intrinsic signal transduction mechanism at the molecular level was integrated into this multi-scale model. A convection-free microfluidic device was employed to create a constant gradient between the source and sink, a critical factor for accurate assessment of transport properties incorporated into the model. Simulated bacteria distributions were generated from the multiple stimulus model in the presence of chemoattractant alpha-methylaspartate, chemorepellent nickel ion, and both together. Under the condition of a single chemoeffector, a larger concentration gradient resulted in a greater chemotactic response. In the case of multiple chemoeffectors, attractant and repellent counteracted each other when mixed together, and complemented each other when imposed on opposite sides. Marine bacteria were also studied and transport parameters were obtained by fitting experimental results with the theoretical model.

## Multistep Baroclinic Instability during a Loop Current Eddy Shedding Event

**L. Hiron**, L. K. Shay, B. Jaimes de la Cruz

RSMAS/University of Miami, Miami, FL

The forcing behind the detachment of a Loop Current eddy is analyzed using a mooring array grid that provides zonal and meridional velocities, temperature, and pressure of the full water column for the March – June 2010 period. Previous studies have been focused on the instabilities and eddy-mean flow interactions along the Loop Current's boundary, but no attention has been given to the development of instabilities in the Loop Current bulge. Observations have shown that the input of kinetic energy (KE) through the Yucatan Channel result in the deepening of the Loop Current and, by geostrophic adjustment, increases its available potential energy (APE). However, we note that the constant input of KE and APE initially inhibits the conversion of APE to KE, which leads to an accumulation of APE over the Loop Current bulge. Once the Loop Current reaches a maximum depth  $\sim 1100$  m, the core of the LC

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becomes baroclinically unstable and detaches an anticyclonic eddy that propagates southward. Once the eddy detaches, the depth of the Loop Current decreases by 50 m, indicating a release of APE from the mean flow. With the eddy's core detachment, APE and relative vorticity are advected southward, far from the LC bulge, and a meander is formed on the LC boundary. The meander will then intensify an adjacent cyclone located on the east side of the LC, and lead to the LCE detachment. Therefore the initial inability of APE to convert to KE seems to be the first step in the baroclinic instability that results in an LCE shedding.

## Science for Response

### The Effect of Dispersion Retention on Surface Oil During the Deepwater Horizon Oil Spill

J. Skancke, C. Beegle Krause  
SINTEF, Trondheim, Norway

The subsea dispersant injection (SSDI) response technique was applied at the well during the Deepwater Horizon (DWH) oil spill. The purpose of SSDI is to reduce the size of oil droplets that enter the water column. Smaller droplets take longer to rise through the water column, causing them to surface over a larger area, leading to a thinner oil film more susceptible to natural dispersion. What is unclear is to what extent dispersants applied to the wellhead will remain with the oil or to what extent it will leach out as droplets rise through the water column. If droplets surface together with dispersant, a secondary effect of SSDI would be enhanced natural surface dispersion by waves due to the oil's lower interfacial tension. Here, we investigate the effect of varying degrees of dispersant retention on the fate of surface oil in a simulation of the DWH oil spill. The DWH-scenario is simulated with the OSCAR oil spill model, which incorporates 3D ocean currents for oil transport and handles the initial release conditions through a coupled plume and droplet size model. Multiple scenarios are run where the degree of surfactant-retention is varied from high to low. When the oil reaches the sea surface, natural dispersion by breaking waves is calculated using an updated version of a previously published model for the droplet size of oil under breaking waves which uses the oil-water interfacial tension as a parameter. In this way, surfacing oil with a lower interfacial tension is more susceptible to subsequent entrainment and is more rapidly removed from the surface. The impact of retained dispersant is compared to observational data of surface oil in areas close to the release point.

### Comparing aquatic toxicity of Oil: PETROTOX model vs Measured

G. Bera<sup>1</sup>, T. Parkerton<sup>2</sup>, A. Redman<sup>3</sup>, N. Truner<sup>4</sup>, D. Renegar<sup>4</sup>, J. Sericano<sup>1</sup>, A. Knap<sup>1</sup>

<sup>1</sup>Texas A&M University, College Station, TX, <sup>2</sup>Exxon Mobil, Houston, TX, <sup>3</sup>Exxon Mobil, New Jersey, NJ,

<sup>4</sup>NOVA Southeastern University, Fort Lauderdale, FL

In this study, we compared the CROSERF Water Accommodated Fraction (WAF) procedure with two alternative techniques in which crude oil is passively dosed via partitioning from silicone tubing or O-rings. Using fresh Macondo surrogate oil (MC552), WAFs were prepared at a 30 mg/L loading using each dosing approach to first investigate oil dissolution kinetics which was monitored using fluorometry and expressed as estimated total oil equivalents (EOE). Subsequent experiments with each dosing method were then conducted at oil loadings of 10, 30, 100 and 360 mg/L. Following equilibration, the dosed aqueous test media was analytically characterized by measurement of total polycyclic aromatic and aliphatic hydrocarbons using GC-MS and dissolved hydrocarbons using GC-FID biomimetic solid phase microextraction (BE). Results indicated that equilibrium was achieved within 72 h for all dosing methods. Total measured concentrations of speciated hydrocarbons were fit to an oil solubility model to estimate dissolved and droplet oil concentrations for each treatment. The composition and concentration of dissolved hydrocarbon concentrations was found to be consistent between dosing methods and varied with oil loading depending on component aqueous solubility. Detailed GCxGC compositional input obtained on this oil was used as input to the PETROTOX model to calculate dissolved toxic units (TUs) at each oil loading as an improved exposure metric for toxicity

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prediction. Predicted TUs at each loading were shown to be well correlated to both EOE and BE measurements. Implications of this work for conducting and interpreting oil toxicity tests are discussed.

## Seasonal Variability of Triplets Deformation and Current Field Kinematic Properties from LASER and GLAD Experiments

**M. Berta**<sup>1</sup>, A. Griffa<sup>1</sup>, A. Poje<sup>2</sup>, T. Ozgokmen<sup>3</sup>

<sup>1</sup>CNR-ISMAR, La Spezia, Italy, <sup>2</sup>CUNY, New York, NY, <sup>3</sup>RSMAS (UM), Miami, FL

A crucial aspect of hydrocarbons dispersion by ocean currents is represented by the evolution of tracer patches. In order to characterize the shape of a material surface and the kinematic properties responsible for its deformation, we investigate the evolution of surface drifter triplets at submesoscales. In particular we study the effects of the submesoscale flow seasonal cycle on triplets evolution and flow field kinematic properties. For the winter time we focus on the cloverleaf pattern deployment during January 2016 in the DeSoto Canyon in the framework of LASER (LAgrangian Submesoscale ExpeRiment). The evolution of the LASER triplets in winter will be compared to the submesoscale triplets behavior observed in summer in the same area during the GLAD (Grand LAgrangian Deployment) experiment. In both experiments hundreds of drifters were launched O(100m) apart from each other over an area of about 10km x 10km in a few hours. The rapidness of the high-density deployments over a very localized area makes these datasets a unique opportunity to observe and analyze triad evolution at submesoscales. We expect that the space and time scales of triplets deformation will vary seasonally, due to the intensification of submesoscales in winter. Moreover, triplet deformation will be characterized computing a “compressibility” parameter that quantifies the different roles of strain rate and convergence. The parameter is very useful for intercomparison between different experimental and model results.

## Air Entrainment and Bubble Plume in Wave Breaking

**Q. Gao**<sup>\*1,2</sup>, W. Drennan<sup>3</sup>, B. Haus<sup>3</sup>, L. Shen<sup>1,2</sup>

<sup>1</sup>Department of Mechanical Engineering, University of Minnesota, Minneapolis, MN, <sup>2</sup>St. Anthony Falls Laboratory, University of Minnesota, Minneapolis, MN, <sup>3</sup>Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL

Air entrainment and bubble plume induced by breaking waves play a key role in the transport of spilled oils, especially under high wind conditions. In this study, we perform high-fidelity numerical simulations to investigate the entrainment process and the dynamics of bubble plume. In the simulations, breaking waves and the bubbles larger than the grid size are directly captured using the coupled level-set and volume-of-fluid method. Subgrid-scale bubbles that cannot be resolved explicitly are modeled using a four-way coupled polydispersed two-fluid model. In the computation, subgrid-scale bubble entrainment, breakup, and coalescence are accounted for so that the bubble size spectrum is precisely captured. Based on the simulation data, we analyze the air entrainment process, bubble size spectrum, void fraction distribution, and bubble plume evolution. The simulate results are shown to agree with experiment measurement.

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## Predicting the Behaviors of Oil Compounds within a few Kilometers of the Macondo Wellhead over the Course of the Deepwater Horizon Timeline

**J. Gros**<sup>1</sup>, A. L. Dissanayake<sup>2</sup>, I. Jun<sup>1</sup>, L. Zhao<sup>3</sup>, M. C. Boufadel<sup>3</sup>, C. M. Reddy<sup>4</sup>, J. S. Arey<sup>5</sup>, S. A. Socolofsky<sup>1</sup>  
<sup>1</sup>Texas A&M University, College Station, TX, <sup>2</sup>University of Georgia, Athens, GA, <sup>3</sup>New Jersey Institute of Technology, Newark, NJ, <sup>4</sup>Woods Hole Oceanographic Institution, Woods Hole, MA, <sup>5</sup>Eawag, Duebendorf, Switzerland

Understanding the behavior of petroleum fluids in the vicinity of the broken well during the Deepwater Horizon disaster is still a challenge. Sampling of such large water masses can only be fragmentary, and different hypotheses have been presented regarding the route followed by different fractions of the petroleum mass emitted (gas and liquid). Model simulations contribute to a better understanding of what happened in 2010 and could ultimately help guide response to future subsurface oil releases, where conditions will likely differ from Deepwater Horizon. In previous work, we developed a multiphase buoyant plume model for a release of petroleum fluids in the deep sea (Gros *et al.*, PNAS, 2017). This model considers the coupled effects of the changing pressure, temperature, and compositions of droplets and bubbles on their properties. The model predicts in detail the behaviors of >100 compounds, enabling validation against field observations with unprecedented detail. However, the model has previously been applied only to a single day representative of the period after the riser pipe was cut at the wellhead (June 4 - July 15, 2010). Here, we report on new simulations with and without subsea dispersant injection over the course of the Deepwater Horizon disaster that shed new light on the predicted effect of dispersant. Whereas methane would essentially dissolve in all scenarios considered, the toxic BTEX compounds appear to lie in a narrow range of aqueous solubilities that are particularly affected by the droplet size reduction brought by dispersant injection during this event. Dispersant injection is therefore predicted to have a potent effect on air quality at the sea surface and the distribution of these volatile toxic compounds within the water column. We also investigated the effects of the riser cut on the behaviors of other petroleum compounds during different periods of the disaster.

## Airdropped Drifter Design for the Tracking of Oil Spills and Surface Currents

**C. Guigand**, G. Novelli, T. Ozgokmen  
University of Miami, RSMAS, Miami, FL

Building up on the recent CARTHE drifter development, we are focusing on designing a low-cost biodegradable airdropped version. Air transport and delivery of small drifters may be more cost effective in some situations where the theater of operation is far enough from shore or needs to be reached very promptly (i.e. oil spill). This design could facilitate synoptic drifter array deployments even in challenging sea conditions. The proposed parachute system is partly made with natural burlap fabric and cotton straps. The overall package is enveloped in to a water-soluble protection shell that would liberate the drifter from the parachute within minutes of ocean impact.

## Modular Synthesis of Amphiphilic Grafted Nanoparticles (AGNs) for Oil Spill Remediation

**C. B. Keller\***, M. Ejaz, S. M. Grayson  
Tulane University, New Orleans, LA

Amphiphilic block copolymers can be organized (self-assembled) into various complex architectures for tailored applications like oil encapsulation. Primarily, the field of amphiphilic block copolymers focuses on linear copolymers. Strictly linear copolymers are subject to a critical concentration for self-assembly to occur and can sometimes require high concentrations of polymer to form. The research herein aims to successfully study the encapsulation efficiency of well-defined amphiphilic block copolymers of poly<caprolactone>-*b*-poly<oligo ethylene glycol methacrylate> grafted from the surface of SiO<sub>2</sub> nanoparticles. This body of work will discuss the synthesis of these copolymers in a “controlled” fashion utilizing the techniques of surface-initiated ring opening and atom transfer radical polymerizations. Through tuning the conditions of polymerization for each block, the length of either block can be tailored to vary internal void volume of the inner hydroscopic corona. Structure of the cleaved polymer, as well as the degree of control over the block lengths, can be analyzed via nuclear magnetic resonance (NMR), matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), infrared spectrometry (IR), thermogravimetric analysis (TGA), and gel permeation chromatography (GPC). These grafted copolymers will provide insight into a system with a “forced” void volume resultant from a bulky outer hydrophilic shell enhancing oil encapsulation.

## High Resolution SAR Remote Sensing of Oil Seepage during SPLASH

**S. Lehner**<sup>1</sup>, A. Soloviev<sup>2</sup>, C. Dean<sup>2</sup>, W. Perrie<sup>3</sup>, J. Kluge<sup>2</sup>, E. Schwarz<sup>4</sup>, P. Schuler<sup>5</sup>

<sup>1</sup>German Aerospace Center (DLR), Wessling, GERMANY, <sup>2</sup>Nova Southeastern University, Dania Beach, FL, <sup>3</sup>Bedford Institute of Oceanography, Halifax, NB, CANADA, <sup>4</sup>German Aerospace Center, Neustrelitz, Germany, <sup>5</sup>Company Oil Spill Response, Houston, TX

Oil seepages and spills change the roughness of the sea surface and can thus be easily detected by synthetic aperture radar (SAR), causing dark areas on the satellite images due to reduced backscatter. Oil spills cause sheen and a change of colour on optical imagery such as observed on Sentinel-2 data. During a field experiment in the Gulf of Mexico at the estuary of the Mississippi river in April 2017, we collected a variety of different SAR and optical datasets both showing persistent oil seeps south of the Mississippi delta. We investigated oil spill imaging under different environmental conditions as well as for different radar wave lengths and polarizations together with optical and in situ data. We acquired radar data from the two C-band satellites, RADARSAT-2 and Sentinel-1, as well as TerraSAR-X band imagery and compared the detected oil to in situ data as part of the Consortium for Advanced Research on Transport of Hydrocarbon in the Environment (CARTHE/GoMRI) Submesoscale Processes and Lagrangian Analysis on the Shelf (SPLASH) research cruise. During the cruise oil was detected visually directly at the surface and described by the NOAA scale. It could be shown that the size of visually detectable oil is much smaller than the dark areas caused by the smoothing of the sea surface by an extended monomolecular layer. We compare the extent of the detected dark area for different environmental situations, i.e., in different wind speed, sea state and tidal front situations, which can be derived directly from the imagery. Using TerraSAR imagery with multiple polarization we look at properties of the oil spill. Sentinel-1 data with large areal coverage show that the influence of the oil seepage can be traced for distances larger than 100km. The interaction between the Mississippi front

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and the seepage is investigated. Using recent satellite data as well acquired after the hurricane season we map the latest developments of the situation.

## Measurement of Body Burden by Polycyclic Aromatic Hydrocarbon Concentrations in Adult Southern Flounder Exposed to South Louisiana Crude Oil

**C. A. Miller**, R. L. Medvecky, T. A. Sherwood, K. Main, D. L. Wetzel  
Mote Marine Laboratory, Sarasota, FL

The Deepwater Horizon blowout caused significant immediate damage to exposed organisms; however, the sub-lethal impacts of the spill on offshore and near shore biota are still not fully characterized. In a continued attempt to understand the responses in benthic Gulf fish species, such as Southern Flounder (*Paralichthys lethostigma*), a laboratory experiment was designed using Deepwater Horizon surrogate oil-contaminated sediment. Adult flounder were chronically exposed to South Louisiana crude oil (SLC) treated sediment (3g oil/kg sediment) over a 30 day period. In a secondary experiment designed to demonstrate how the stress of chronically exposed fish can affect their ability to process an acute exposure, a replicate set of flounder were exposed to a chemically enhanced water accommodated fraction (CEWAF) at 1 ppm immediately following the 30-day sediment exposure. Following both exposures, the fish were sacrificed and uptake of polycyclic aromatic hydrocarbon (PAH) concentrations were measured in the liver and whole body. Results will be used to discuss the impacts on individuals and potential population level implications.

## Cardiovascular Responses of the Red Drum (*Sciaenops ocellatus*) to the Combined Environmental Stressors of Hypoxia and Crude Oil

**D. Nelson**\*<sup>1</sup>, A. J. Esbaugh<sup>2</sup>, D. A. Crossley II<sup>1</sup>

<sup>1</sup>University of North Texas, Denton, TX, <sup>2</sup>University of Texas at Austin - Marine Science Institute, Port Aransas, TX

In the Gulf of Mexico, fish experience both environmental and anthropogenic stressors which potentially act synergistically to affect an organism. Prior studies on the effect of crude oil have shown deleterious effects on multiple organisms at the level of the cardiovascular system. However, questions remain regarding the combined actions of oil exposure and environmental stressors such as hypoxia. We investigated how convective oxygen transport is impacted by both crude oil and hypoxia in the red drum (*Sciaenops ocellatus*). We simultaneously measured heart rate, ventral aortic blood flow and dorsal aortic pressures in juvenile red drum. Our working hypothesis was crude oil and hypoxia will result in greater negative effects of cardiovascular function that would be predicted due to a sum of these stressors alone. Baseline heart rates was  $51 \pm 6$  bpm in 100% air saturation water with a cardiac output of  $48.18 \pm 6.22$  mL/min/kg and mean dorsal aortic pressures of  $2.99 \pm 0.18$  kPa. Hypoxic exposure caused a bradycardia in fish reducing heart rate to  $29 \pm 3$  bpm, cardiac output to  $25.84 \pm 2.4$  mL/min/kg and an increase in mean dorsal aortic pressure to  $3.36 \pm 0.31$  kPa. This study is ongoing. This study is supported by the GoMRI RECOVER Consortium to A.R. and D.C.

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## Near-Infrared Spectroscopic Reactor for On-Line Monitoring of Gas Biodegradation at High Pressure

**N. Noirungsee**\*<sup>1</sup>, C. Norton<sup>2</sup>, S. Hackbusch<sup>1</sup>, J. Viamonte<sup>1</sup>, P. Bubenheim<sup>1</sup>, R. Müller<sup>1</sup>, A. Liese<sup>1</sup>

<sup>1</sup>Hamburg University of Technology, Hamburg, Germany, <sup>2</sup>Ruhr University Bochum, Bochum, Germany

Methane was the main gaseous hydrocarbon released from the wellhead at 1500-meter depth during The Deepwater Horizon incident in 2010. Aerobic methane-oxidizing bacteria (methanotrophs) were responsible for biodegradation of methane in the water column [1, 2]. However, the effect of high hydrostatic pressure of the deep sea and on aerobic methane biodegradation is largely unknown. High pressure reactors for biological experiments were constructed at Hamburg University of Technology. Nitrogen gas is employed as a pressurizing medium to achieve high hydrostatic pressure characteristic of the deep sea. However, the challenge of measuring aerobic methane biodegradation in high-pressure reactor, lies in subsampling of gas at high-pressure. In this study, a new optical system has been developed based on an optical flow cell for near infrared (NIR) spectroscopic analysis of gas mixtures at high pressure [3]. NIR light is transmitted via optical fiber through the reactor, and the absorbance is measured with spectrometer. The optical system was directly mounted onto a high-pressure reactor. This system allows measurement of methane partial pressure in a high-pressure gas mixtures bypassing the subsampling step. Aerobic methane-oxidizing bacteria were cultivated in the reactor equipped with the optical system, and methane biodegradation under high pressure was monitored in real-time from the removal of methane from the headspace.

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## Surface Dispersion Measurements Combining Drone-Tracked Drift Cards and GPS-Drifters

**G. Novelli**, C. Guigand, N. J. M. Laxague, S. Mehta, B. K. Haus, T. M. Özgökmen  
University of Miami, Miami, FL

Understanding the dispersion of oil spills is critical to improve operational models and optimize mitigation efforts. GPS-tracked drifters and fluorescent dye have been used in the past to investigate ocean transport characteristics in, or below, the mixed-layer. However, these observations may not always be relevant for material like oil slicks, floating in the upper centimeters at the surface of the ocean. Here we report on an experiment involving the simultaneous deployment, and subsequent tracking for 4 hours and more than 4 km, of a 50m-diameter patch of 600 drift cards, surface and near-surface drifters, representing the motion of material at depths of 0.01m, 0.05m, and 0.60m respectively. The experiment was carried out in the Louisiana Bight from the RV Walton Smith, during the Submesoscale Processes and Lagrangian Analysis on the SHeLF (SPLASH) campaign in April 2017. A consumer camera-equipped drone, launched and retrieved from the ship at intervals, recorded the drift cards trajectories, while the drifter positions were acquired by GPS every 5 minutes. The different

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transport patterns observed by the instruments drifting at different depths will be discussed in relation to the presence of Langmuir circulation and shallow density fronts. The use of drones in oceanographic research is very promising for collecting high-resolution geo-referenced data at the ocean surface.

## Modeling the Time Dependent Vertical Mixing and Dispersion of Oil

**J. M. R. Restrepo**<sup>1</sup>, J. Ramirez<sup>2</sup>, S. Moghimi<sup>3</sup>

<sup>1</sup>Oregon State University, Corvallis, OR, <sup>2</sup>Universidad de Colombia, Sede Medellin, Medellin, Colombia,

<sup>3</sup>Portland State University, Portland, OR

We introduce a time dependent model for the mass exchange between sea surface and subsurface oil. The primary modeling challenge addressed here relates to upscaling microscopic dynamics to the larger operational scales of the oil transport model. Upscaling is accomplished by filtering and stochastic parametrization, rather than by assuming homogeneity and stationarity of microscale processes. The two mass transport mechanisms we consider are the droplet distribution dynamics and the competition of buoyancy and wave and wall-bounded turbulent mixing. The model is then incorporated into a Shallow-water Oil Model that couples ocean dynamics and the chemical dynamics of the oil itself. The model is being designed for forecasting at spatio-temporal scales relevant to environmental issues.

## *Lysmata wurdemanni* as a Proxy for Deep-Water Column Micronekton in Hydrocarbon Toxicity Studies

**A. Renegar**<sup>1</sup>, N. Turner<sup>1</sup>, G. Bera<sup>2</sup>, B. Riegl<sup>1</sup>, A. Knap<sup>2</sup>, J. Sericano<sup>2</sup>, S. Sweet<sup>2</sup>

<sup>1</sup>Nova Southeastern University, Dania, FL, <sup>2</sup>Texas A&M University, College Station, TX

This research is part of a series of studies for the Deep-sea Risk Assessment and Species Sensitivity to WAF, CEWAF and Dispersant project (D-TOX), designed to advance the understanding of hydrocarbon toxicity in several ecologically important deep-sea zooplankton/micronekton. However, there are multiple challenges to conducting experiments with these organisms, and the use of a proxy organism allows comparison and validation of experimental results. This study examines the suitability of the peppermint shrimp, *Lysmata wurdemanni*, as an experimental proxy for several ecologically important deep-sea zooplankton/micronekton. This crustacean species occurs in shallow coastal marine environments throughout the western Atlantic, Caribbean and Gulf of Mexico, is similar in size to the mesopelagic organisms previously tested, and is readily available via commercial aquaculture. The effects of crude oil on *L. wurdemanni* will be assessed in a 48-h constant-exposure multi-concentration toxicity test utilizing a passive dosing system, and acute effects will be evaluated both during exposure and at the end of the 48-h exposure period. Experimentally derived LC50 data will be compared to that previously observed for Euphausiidae, *Janicella spinacauda*, *Systellaspis debilis*, *Sergestes* sp., *Sergia* sp. and *Americamysis bahia*. In addition to evaluation of *L. wurdemanni* as a surrogate for mesopelagic crustaceans, this study will provide new hydrocarbon toxicity data on a commercially important coastal crustacean species.

## Hydrocarbon Toxicity to *Porites divaricata*: Comparison of Observed and Predicted Thresholds

**A. Renegar**<sup>1</sup>, N. Turner<sup>1</sup>, G. Bera<sup>2</sup>, B. Riegl<sup>1</sup>, R. Dodge<sup>1</sup>, A. Knap<sup>2</sup>, P. Schuler<sup>3</sup>

<sup>1</sup>Nova Southeastern University, Dania, FL, <sup>2</sup>Texas A&M University, College Station, TX, <sup>3</sup>Oil Spill Response USA Inc., Fort Lauderdale, FL

Shallow water coral reef ecosystems have an elevated chance of exposure to hydrocarbons due to their close proximity to the coastline. Previous research to evaluate hydrocarbon toxicity to corals and coral reefs has generally focused on community level effects, and a significant data gap exists on the toxicity thresholds of hydrocarbons to corals at higher levels of resolution. Targeted toxicity studies are therefore vital to accurate assessment of coral resilience to hydrocarbon exposures. Previous experiments have evaluated the toxicity of 1-methylnaphthalene and phenanthrene to the scleractinian coral *Porites divaricata*, and the critical target lipid body burden was determined using the experimentally derived LC50 and the target lipid model. This is then used to estimate threshold concentrations of crude oil exposure. Here, the effect of an additional single hydrocarbon, toluene, and of crude oil is assessed in 48-h constant exposure multi-concentration toxicity tests with *P. divaricata* in order to assess the accuracy of modeled threshold concentrations. Macondo surrogate oil (MC252) will be passively-dosed via silicone tubing, similar to the approach used for single hydrocarbons. Acute and sub-acute effects (color, polyp extension/retraction, mucus production, tissue loss/mortality, and photosynthetic efficiency via PAM fluorometry) are evaluated both during exposure and during a post-exposure recovery period. EC50 and LC50 estimates are based on sub-acute and acute effects at the end of the 48-h exposure period. The comparison of predicted to observed toxicity is a key focus. The toxicity thresholds determined in this study will provide needed data for modeling impacts of potential hydrocarbon concentrations and exposures. This information can then be used in Net Environmental Benefit Analysis (NEBA) or Spill Impact Mitigation Assessment (SIMA) of predicted impacts and response options in coral reef environments.

## Soft Boom Oil Containment and Recovery System

**C. T. C. Smith**

ALGAENTIS HazMat Sponge, Pagosa Springs, CO

The size and scope of the 2010 oil release in the Gulf of Mexico revealed serious shortcomings in the standard approach to oil spill control. The Algaentis HazMat Soft Boom Oil Recovery and Containment System is based on the use of floating booms constructed from open-cell urethane foam, floating in front of and attached to Hard Boom, which channels the oil sheen into the soft hydrophobic/oleophilic Boom. The Soft Boom employs a special polymer that exhibits a surface that rejects water [hydrophobic] and attracts oil [oleophilic], with open-cell [spongiform] structure in cylinders 6" to 24" in diameter and 6' to 10' in length. In water, these booms naturally float high and retain their buoyancy as they adsorb the hydrocarbons, which are generally less dense than water. U.S. Coast Guard and BP Strike Team observed that the 12" Soft foam booms would adsorb up to nine liters of light to medium gravity crude per cubic meter of foam, and maintain containment, while tethered to Hard Boom, in moderate to rough off-shore wave conditions. The Gulf Oil Spill was characterized by surface floating oil sheen and submerged emulsified oil and "tar balls", dispersed below the surface water, rather than appearing on the surface as an oil film or "slick". The emulsified oil and tar ball collection "Curtain Net" we deployed contained hydrophobic/oleophilic "open cell" foam blanket material, in the form of a flat foam sheet, approximately 1" - 2" in thickness, sandwiched between a closed sleeve of polypropylene

\* Student presenter

mesh netting, in various void sizes down to a ½" void. This open Curtain Net sleeve, containing the hydrophobic/oleophilic blanket, was attached to the bottom of the hard boom, floating behind the soft boom, to control and suspend the Curtain Net, suspended below the Hard Boom, on a horizontal steel cable. We designed a floating system that will pick up the oil-loaded boom, & physically squeeze the oil out of the boom. Recovered oil then passes through on-board separation equipment, to remove traces of water, and captured oil will be stored in the vessel & transported to port for refining. The cleaned boom can then be continuously returned directly to water duty, to continue its work in spill remediation, or clean the boom, and return the Haz Mat Soft hydrophobic/oleophilic Boom to storage, for use in future incidents control.

## Impact of Vertical Resolution in Lagrangian Analysis on the Shelf

**P. Spence**<sup>1</sup>, G. Jacobs<sup>2</sup>, B. Bartels<sup>1</sup>, T. Campbell<sup>2</sup>, T. Smith<sup>2</sup>

<sup>1</sup>Vencore Corp., Stennis Space Center, MS, <sup>2</sup>Naval Research Laboratory, Stennis Space Center, MS

Fresh water outflows from the Mississippi River southwest passage strongly affect coastal processes in the Mississippi Bight region. Strong vertical stratification results that controls the mixing of momentum flux from the atmosphere. This situation presents a challenging problem for numerical models to represent the vertical structure and associated physics. We use the Consortium for Advanced Research on Transport of Hydrocarbon in the Environment (CARTHE) Submesoscale Processes and Lagrangian Analysis on the Shelf (SPLASH; <http://carthe.org/splash/>) field experiment in April, May 2017 during which several dense surface drifter deployments were conducted in the area. A 1 km horizontal resolution Navy Coastal Ocean Model (NCOM) of the Gulf of Mexico (GoM) was run during the experiment. The vertical grid is comprised of 49 total layers; 34 terrain-following (sigma) layers above 550 meters and 15 lower z-levels. The sigma coordinate structure has higher resolution near the surface with the surface layer having 0.5 m thickness. Initiated in 2012, this model of the GoM has been running in real-time in support of multiple CARTHE at sea experiments (GLAD 2012, SCOPE 2013, LASER 2016). This simulation uses measurements made by the US Geological Survey along the northern GoM to estimate riverine discharge into the model basin. Prior work had indicated the model vertical structure was not sufficient. A local nested NCOM at the same 1 km resolution is set up over the SPLASH experiment area to understand the effect of increased vertical resolution. Resolution is increased to 100 and 200 vertical layers. Comparisons of the surface currents of the nested models with SPLASH drifters are made to evaluate the effect of the increased computational vertical resolution. Strong stratification near the surface due to fresh water from Mississippi River runoff is more accurately resolved resulting in improved prediction of near-surface currents when compared to observed drifter speeds.

## Kinetics of Crude Oil Biodegradation under High Pressure Conditions

**J. Viamonte**<sup>\*1</sup>, S. Hackbusch<sup>1</sup>, N. Noirungsee<sup>1</sup>, P. Bubenheim<sup>1</sup>, X. Sun<sup>2</sup>, J. Kostka<sup>2</sup>, R. Müller<sup>1</sup>, A. Liese<sup>1</sup>

<sup>1</sup>Hamburg University of Technology (TUHH), Hamburg, Germany, <sup>2</sup>Georgia Institute of Technology, Atlanta, GA

After the accident of the Deepwater Horizon (DWH) platform in the Gulf of Mexico (GoM), large amounts of crude oil and gaseous hydrocarbons were released into the deep sea. Consequently, the spilled oil was directly affected by *in-situ* physicochemical conditions (pressure, temperature, outflowing droplet size, addition of chemical dispersant, etc.) and biological factors, such as

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degradation by microorganisms. In order to gain a better understanding of the fate of released hydrocarbons and to complement far field oil spill models, crude oil biodegradation experiments are carried out under comparable DWH blowout pressure conditions. Presence of elevated pressures have been reported to play a significant role over biodegradation efficiency of single crude oil compounds [1]. Evaluations of biodegradation rates for target hydrocarbons are performed in high pressure reactors at 4°C and 150 bar (equivalent to DWH blowout depth of 1500 m) by in-line monitoring of microbial oxygen consumption and performing offline analyses of concentrations of single hydrocarbons. Faster biodegradation rates for target saturates have been observed at higher pressures in experiments using the isolated pressure tolerant model strain *Rhodococcus* PC20 [2]. On the other hand, tests performed with top layer sediments collected near the DWH blowout site containing the bacterial community, suggest that higher pressures hinder the degradation efficiency of target crude oil compounds. Aiming to confirm and complement previous results, current research is focusing on system pressures equivalent to 4000 m (deepest point in the GoM) for different crude oil types. Additionally, effects of Corexit EA9500A and methane on the bacterial degradation abilities are taken into consideration. Altogether, these findings will be combined within the high pressure crude oil biodegradation (HPOB) model, assisting to forecast degradation extent for possible future deep water spills.

[1] Schedler *et al.* 2014. *AMB Express* 4(1):77.

[2] in preparation: Hackbusch, S., Viamonte, J., Noirungsee, N., Dethloff, J., Bubenheim, P., Liese, A. Performance of GoM model organism *Rhodococcus* PC20 at elevated pressure.

## Numerical Modeling of Time-Dependent Subsidence in a Gas Field in Coastal Louisiana

**G. Z. Voyiadjis**, Y. Zhou

Louisiana State University, Baton Rouge, LA

Hydrocarbon production is an important process contributing to the subsidence and wetland losses in coastal Louisiana. Releveling data from National Geodetic Survey show that subsidence along several major oil and gas fields accelerated after production when reservoirs were nearly depleted. The Valentine gas field in coastal Louisiana is chosen to analyze the subsidence during and after production based on 3D finite element modeling. An elastic viscoplastic model is adopted to interpret the accelerated postdepletion subsidence by overcoming the limitation of traditional theories within the framework of poroelastisity and poroplasticity. The elastic viscoplastic model integrates Perzyna's theory of overstress viscoplasticity, an elliptical cap yield surface, Drucker-Prager failure envelope, and a hardening law from critical state theory. The elastic viscoplastic model is applied to both the simplified disc-shaped reservoir and its surroundings. The material properties of the elastic viscoplastic model are calibrated based on a computational technique along with available laboratory and field data from literature. The numerical modeling results can approximately reproduce both the observed subsidence during production and the delayed subsidence after production using the calibrated material properties. The delayed postdepletion subsidence can be attributed to the delayed compaction in both the reservoir and the surroundings. The delayed compaction in the depleted reservoir results from viscoplastic deformation, and the delayed compaction in the surroundings arises from both viscoplastic deformation and poroelastic deformation associated with pore pressure diffusion. After calibrating the finite element model based on the observed subsidence, the time-dependent subsidence in the near future is predicted in the Valentine field.

## Science for Restoration, Management, and Policy

### Growth Responses of Three Dominant Wetland Plant Species to Various Flooding and Nutrient Levels

**S. G. Barrett\***, G. P. Shaffer

Southeastern Louisiana University, Hammond, LA

Coastal Louisiana is experiencing a greater rate of wetland loss than any other wetland system in the United States. This is primarily due to anthropogenic stressors such as flood control levees, backfilling and development of wetlands, and other hydrologic modifications. Methods employed to mitigate wetland loss include the construction of river diversions and assimilation wetlands, which can provide consistent sources of freshwater influx and nutrients to impounded swamps and marshes. It is well known that prolonged flooding causes strain on wetland plant communities and facilitates or exacerbates wetland degradation. However, because river diversions and assimilation wetlands bring high nutrient loads along with freshwater, there is debate over whether prolonged flooding or high influx of nutrients is the primary cause of stress in river diversion and assimilation wetland discharge areas. This mesocosm experiment addresses this question by isolating the effects of flooding and nutrients on the biomass of bald cypress (*Taxodium distichum*), maidencane (*Panicum hemitomon*), and cordgrass (*Spartina patens*) over the course of a growing season. The results of this study provide clarity as to whether flooding stress, high nutrient loads, or both cause a reduction in wetland plant productivity. By evaluating the growth responses of *T. distichum*, *P. hemitomon*, and *S. patens* at varying nutrient regimes, we gain insight on how these more dominant species will react to high nutrient discharges from large river diversions, such as those proposed in Louisiana's 2017 Master Plan.

### Lasting Impacts of Fertilizer Application on Soil Microbial Community in an Oil-Impacted Salt Marsh

**G. Cagle\***<sup>1</sup>, Q. Lin<sup>1</sup>, I. Mendelsohn<sup>1</sup>, J. Fleeger<sup>1</sup>, S. Graham<sup>2</sup>, D. Dies<sup>3</sup>, C. Hess<sup>1</sup>, R. Zhang<sup>1</sup>, A. Hou<sup>1</sup>

<sup>1</sup>Louisiana State University, Baton Rouge, LA, <sup>2</sup>Nicholls State University, Thibodaux, LA, <sup>3</sup>ATKINS North America, Tampa, FL

Planting and fertilizing are common restoration methods following hydrocarbon spills, but it is unclear how these treatments may impact the native soil microbial community. The effects of planting and fertilizing were evaluated in a three-year manipulative field experiment in a marsh in Northern Barataria Bay, Louisiana that was oiled during the Deepwater Horizon oil spill. Changes in soil microbial community structure and diversity during the spring and fall seasons under factorial planting and fertilizing treatments were investigated by 16S Illumina sequencing. Fertilizer application immediately after planting caused a significant decrease in microbial diversity and left a lasting impact in the microbial community structure for at least one year. The organisms that proliferated following fertilizer application are evaluated herein. In subsequent seasons, the effects of planting and fertilizing were not the cause of variability in the microbial community, and time was the strongest driver of microbial community composition variability. Fertilizing bare soil might have caused eutrophic effects in the soil microbial community of Northern Barataria Bay marshes that experienced plant mortality following the Deepwater Horizon oil spill.

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## Fate of Eroding Crude Oil Asphalt and Emulsion in Shallow Marsh Embayments

**W. Coronel\***, V. Elango, J. Pardue

Louisiana State University, Baton Rouge, LA

Marshes impacted by the Macondo spill continue to erode, releasing crude oil in the form of a soft asphalt or emulsion to adjacent shallow embayments. This process moves highly weathered crude oil from the marsh profile to comparatively high energy shallow open water systems where geochemical and microbiological conditions may influence additional weathering. Specifically, we hypothesize that the erosion process can accelerate biodegradation by moving crude oil asphalts and emulsions from a low energy anoxic environment in the marsh to a higher energy oxic environment in these shallow embayments. Sediment cores were taken in summer 2017 across transects which bisect the boundary between a contaminated marsh and Barataria Bay in the vicinity of Bay Jimmy/Bay Batiste where significant oiling occurred on the marsh surface. Cores were analyzed by GC-MS and weathering ratios constructed from 2 and 3-ring PAH compound series (C1-, C2-, C3- and C4-, when possible alkylated naphthalene, phenanthrene, and dibenzothiophenes) in the numerator and 4-ring PAH series, computed from C1-, C2 and C3-chrysene data, in the denominator. Microbial populations are being assessed using Illumina sequencing using MiSeq protocols and non-matrix dimensional scaling will be used to compare microbial community structure. In addition, microbial data will be mined for the abundance of sequences associated with known PAH degraders from genera such as *Halomonas* and *Marinobacter*, previously identified on the marsh surface. Dialysis samplers, placed along the same transect, is measuring nutrients, N and P, and redox indicators like sulfate and iron. Initial results indicate that secondary deposition of crude oil derived PAHs is not occurring uniformly across these embayments. A defined oil layer was absent from offshore cores. Relative PAH weathering, microbial community structure and porewater geochemistry will be discussed to draw conclusions on ongoing weathering of Macondo oil in these systems.

## Supporting Science and Restoration through the Mississippi Based RESTORE Act Center of Excellence (MBRACE)

**K. Darnell**, D. Wiesenburg, L. Bernard

The University of Southern Mississippi, Hattiesburg, MS

The Mississippi Based RESTORE Act Center of Excellence (MBRACE) was designated as Mississippi's Center of Excellence in September 2016. MBRACE is a consortium of Mississippi's four research universities (Jackson State University [JSU], Mississippi State University [MSU], University of Mississippi [UM], and The University of Southern Mississippi [USM]), with USM serving as the lead institution. The mission of MBRACE is to seek sound comprehensive science-and technology-based understanding of the chronic and acute stressors on the dynamic and productive waters and ecosystems of the northern Gulf of Mexico, and to facilitate sustainable use of the Gulf's important resources. MBRACE's Science Plan focuses on the State's directive toward sustainable coastal management through three major thrust areas: (1) monitoring and ocean observations, (2) modeling, and (3) process studies. The first awards funded by MBRACE in Fall 2017 under the Core Research Program focus on understanding oyster reefs and their sustainability. With these awards, researchers from JSU, MSU, UM, and USM are examining how ecological conditions relevant to oysters vary over time and between newly restored oyster reefs and adjacent unrestored oyster reefs in Mississippi Sound. Future funding opportunities will be made through a competitive award process on research topics detailed in the MBRACE Science Plan.

\* Student presenter

## Linking Habitat to Recruitment: Evaluating the Importance of Pelagic *Sargassum* to Fisheries Management in the Gulf of Mexico

**F. Hernandez**<sup>1</sup>, C. Hu<sup>2</sup>, K. Dillon<sup>1</sup>, G. Zapfe<sup>3</sup>, W. Ingram<sup>3</sup>

<sup>1</sup>University of Southern Mississippi, Ocean Springs, MS, <sup>2</sup>University of South Florida, St. Petersburg, FL,

<sup>3</sup>NOAA Fisheries, Southeast Fisheries Science Center, Pascagoula, MS

A major limitation in improving stock assessments for managed species is the general lack of fisheries-independent indices. Currently, few stock assessments include environmental or habitat-related parameters, even though a goal of NOAA Fisheries is to move towards Ecosystem-Based Fisheries Management (EBFM) approaches. Holopelagic *Sargassum* is a presumed "nursery habitat" for numerous commercially and recreationally targeted fish species, including Gray Triggerfish (*Balistes capriscus*), Greater Amberjack (*Seriola dumerili*), Mahi mahi (*Coryphaena hippurus*), and Tripletail (*Lobotes surinamensis*), among others. In spite of its importance, quantitative assessments of nursery function are lacking for *Sargassum*, and little is known about the environmental and climatic factors that drive variability in *Sargassum* biomass and distribution. Here we present an overview of a new three-year project funded by the NOAA RESTORE Science Act Program that will evaluate the nursery function and importance of *Sargassum* to fisheries in a useful context for resource managers. Using a combination of remote sensing observations, vessel surveys, and lab analyses, our objectives are to: 1) quantify *Sargassum* variability in distribution and biomass at gulf-wide scales in the northern Gulf of Mexico, and understand the environmental controls of such variability; 2) quantify the nursery-role function of *Sargassum* relative to temporal/spatial variability, habitat morphology, and alternative open water habitats; and 3) develop and test the efficacy of remote sensing and field-derived habitat indices for inclusion in stock assessments of managed species associated with *Sargassum*. Here we also present preliminary results on the spatial variability in *Sargassum* faunal assemblages from our first cruise in July 2017. Overall our deliverables will provide a habitat-specific context for stock assessments and EBFM initiatives by filling a data void with regards to the fisheries contribution of *Sargassum*.

## Visualizing the Distribution of Petrogenic PAHs in *Spartina* and *Avicennia* Tissues from Barataria Basin, Louisiana

**K. Sebastian\***, V. Elango, J. Pardue

Louisiana State University, Baton Rouge, LA

Vegetation in marsh environments can sequester polycyclic aromatic hydrocarbons (PAHs) by partitioning from the air phase through the waxy cuticle on the leaf surface. Recent studies from Macondo oil-impacted marshes has demonstrated that cycling of low molecular weight petrogenic PAHs occurs through the cuticle and other leaf tissue. Equilibration with leaf tissue appears to be rapid and there is evidence from coupled measurements in passive samplers and vegetation that transport of these compounds can occur to adjacent marshes via an air route. At present, our understanding of these cycling phenomenon in marshes is very coarse and limited by grab samples across the entire leaf. In addition to bulk chemical measurements of alkylated PAHs in leaf tissue, multi-photon confocal microscopy may be used to visualize distributions of PAHs that fluoresce between 350 and 390 nm across the 2-D depth of *Spartina* and *Avicennia* leaf tissue. At this wavelength range, alkylated phenanthrenes are the primary PAH that fluoresces. In this paper, imaging will be presented that documents the distribution of PAHs across the leaf tissue including specialized tissues such as salt

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glands and stomata on the adaxial and abaxial leaf surface. Imaging and the observed fluorescence will be quantified and compared with GC-MS measurement of specific PAHs. Initial images show relatively uniform distributions within the middle lamella tissue between epidermal *Avicennia* cells below the cuticle. Little or no fluorescence was observed associated with salt gland structures or the interior of the epidermal cells, themselves. These images show that passage of PAHs across the cuticle into deeper cell tissues occurs quickly, after a few hours of exposure to oil vapors. These images provide further evidence that the cuticle serves as the entry point of PAHs into leaf tissue, not the sole reservoir for these compounds.

## Hepatic Accumulation of Polycyclic Aromatic Hydrocarbons and Prevalence of Hepatic and External Skin Lesions in Golden Tilefish from the Gulf of Mexico

**S. M. Snyder**\*<sup>1</sup>, E. L. Pulster<sup>1</sup>, S. B. Fogelson<sup>2</sup>, S. A. Murawski<sup>1</sup>

<sup>1</sup>University of South Florida, St Petersburg, FL, <sup>2</sup>Fishhead Labs LLC, Stuart, FL

From 2011 to 2017, the Center for Integrated Modeling and Analysis of Gulf Ecosystems (C-IMAGE) has conducted a multidisciplinary study evaluating the prevalence of fish disease and polycyclic aromatic hydrocarbon (PAH) contamination in the Gulf of Mexico as a result of the 2010 Deepwater Horizon blowout. Annual demersal longline cruises, including a Gulf-wide survey, sampled fishes to evaluate tissue accumulation of PAHs and microscopic evidence of lesions associated with varying levels of accumulation. Golden tilefish (*Lopholatilus chamaeleonticeps*), an important recreational and commercial fish species, were selected as a target species due to preliminary results showing relatively high levels (1-195 ug/g w/w) of total PAH accumulation in liver tissue compared to other demersal Gulf of Mexico fishes. Elevated PAH levels in golden tilefish may be related to their burrow-forming and benthophagous lifestyle. The goal of this study was to investigate the correlation between levels of hepatic accumulation of PAHs in golden tilefish and prevalence of abnormal microscopic changes (e.g. neoplasia) in the liver and skin. Liver samples were analyzed using QuEChERS extraction followed by gas chromatography tandem mass spectrometry (GC-MS/MS) to quantify PAHs and alkylated homologs present in the hepatic parenchyma. A subset of hepatic samples collected from fish with high and low concentrations of liver PAHs was processed for histological analysis. In addition, the frequency of skin lesion occurrence was also observed, recorded and sampled for histopathology. The data from this research provides valuable insight into the negative health effects of chronic PAH exposure to Gulf of Mexico golden tilefish tissues due to environmental contamination.

## Using IUCN Data to Prioritize Conservation Needs in the Gulf of Mexico

**K. Strongin**\*<sup>1</sup>, B. Polidoro<sup>1,2</sup>, C. Linardich<sup>2</sup>, G. M. Ralph<sup>2</sup>, K. E. Carpenter<sup>2</sup>

<sup>1</sup>Arizona State University, Tempe, AZ, <sup>2</sup>IUCN Marine Biodiversity Unit, Norfolk, VA

A comprehensive understanding of the status of complete clades of marine organisms in the Gulf of Mexico is critical to the sustainability of the region and its marine resources. The current threats and extinction risk for 1307 species present across the Gulf of Mexico and based on IUCN Red List Categories and Criteria, were analyzed. These species included all known marine bony shore fishes, marine birds, sharks, marine reptiles, habitat building species (corals, mangroves, and seagrasses), and select invertebrates (sea cucumbers, cephalopods, and sea snails). Analyses showed that 6% of all assessed marine species in the Gulf of Mexico are currently listed in a threatened category, 8% are listed as Data Deficient, and 83% are listed as Least Concern. Given the low percentage of endemics,

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many species present in the Gulf are impacted by threats that occur both within and outside of the Gulf. For example, 34% of marine species present in the Gulf are directly impacted by both large-scale and small-scale fisheries practices; however, the intensity of fishing pressure can vary widely across species' ranges in the Gulf, the Caribbean or the Atlantic. Other anthropogenic impacts, including development, removal of prey, tourism, dredging, shipping, drilling, and habitat loss, collectively affect another 28% of marine species, but also with varying intensity across their global ranges. These findings highlight the need for further research on the impacts of known threats on marine populations both within and outside of the Gulf, as well as the connectivity of marine populations across the Caribbean and Atlantic. Moving forward, these results will help to better focus conservation measures for Gulf species and for the reduction of both regional and global threats to marine biodiversity.

## Social & Ecological Resilience

### Supplementing Public High School Students' Access to Science Education and Careers: The Emerging Scholars Environmental Health Sciences Academy

**H. Covert**<sup>1</sup>, A. Adkison<sup>2</sup>, J. Langhinrichsen-Rohling<sup>3</sup>, L. Perrault<sup>1</sup>, I. Ramkissoon<sup>3</sup>, E. Sisskin<sup>4</sup>, G. Stewart<sup>4</sup>, J. Wickliffe<sup>1</sup>, M. Lichtveld<sup>1</sup>, D. Ilunga Tshiswaka<sup>4</sup>

<sup>1</sup>Tulane University, New Orleans, LA, <sup>2</sup>Escambia County School District, Pensacola, FL, <sup>3</sup>University of South Alabama, Mobile, AL, <sup>4</sup>University of West Florida, Pensacola, FL

U.S. college graduation rates in science, technology, engineering and math (STEM) are insufficient for maintaining the country's technological innovation and economic growth. Moreover, women and certain racial/ethnic groups are underrepresented in STEM. Inquiry-based supplemental science programs are one means of expanding access for girls and minorities to STEM education and careers. Started in 2013 with funding from the Gulf Region Health Outreach Program, the Emerging Scholars Environmental Health Sciences Academy is a supplemental summer science program for high school students in Mobile, New Orleans, and Pensacola. Based at universities in these cities, the academy seeks to expand access to environmental health science education and careers for public high school students, most of whom attend under-resourced schools. This presentation will discuss the academy's educational model of learning, applying and experiencing science; describe each program site's unique characteristics; present some successful outcomes; and provide lessons learned for implementing a supplemental science program for high school students. Finally, we will discuss how providing opportunities for students to learn about and practice scientific problem-solving may enhance the development of the adaptive capacity necessary for resilience.

### *in vitro* Oily Marine Aerosol Exposure Alters Human Bronchial Epithelial Function

**K. Nishida**<sup>1,2</sup>, L. D. Chandrala<sup>1</sup>, N. Afshar-Mohajer<sup>2</sup>, K. Koehler<sup>2</sup>, J. Katz<sup>1</sup>, V. Sidhaye<sup>1,2</sup>

<sup>1</sup>Johns Hopkins University, Baltimore, MD, <sup>2</sup>Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

The 2010 Deepwater Horizon was the largest accidental marine oil spill to date leading to approximately 4.9 million oil barrels and 1.9 million gallons of dispersant in the Gulf of Mexico (Gray *et al.* 2014). Excess oil and oil with dispersant that is not removed forms oil slicks on the ocean surface. Crashing waves aerosolize oil slicks, creating nano to micron sized oil droplets capable of entering human airways. An evaluation of oil spill research from 1968 to 2015 revealed less than 1% of the selected publications investigated how oil spills effect humans (Murphy *et al.* 2016). Our research adds to this deficiency by understanding how aerosolized oil effects the human bronchial epithelium at the molecular level. Utilizing the novel In Vitro Oily Aerosol Exposure and Microscopy System (IOAEMS) we expose differentiated patient derived primary Human Bronchial Epithelial cells (NHBE) to clinically relevant oil concentrations and/or oil with dispersant to assess airway epithelial function. Specifically, we are interested in determining if either oil alone or oil with dispersant alters epithelial barrier function, cell motility or mucociliary clearance- functions which are critical in basic monolayer epithelial physiology. Determining if these exposures can influence airway epithelial function will allow us to both elucidate potential therapeutics strategies to address this, and guide policy in response to oil spills.

## Framework for Assessing Oil Spill Chemical Risks to Children During Beach Play

**H. M. Solo-Gabriele**<sup>1</sup>, A. Ferguson<sup>2</sup>, K. D. Mena<sup>3</sup>, M. Gidley<sup>4</sup>, R. Guerrero<sup>5</sup>, N. Kumar<sup>6</sup>, P. Tarwater<sup>3</sup>

<sup>1</sup>University of Miami, Coral Gables, FL, <sup>2</sup>University of Arkansas for Medical Sciences, Little Rock, AR,

<sup>3</sup>University of Texas Health Science Center at Houston, School of Public Health, El Paso, TX, <sup>4</sup>University

of Miami (CIMAS), Key Biscayne, FL, <sup>5</sup>University of Texas Health Science Center at Houston, School of

Public Health, Houston, TX, <sup>6</sup>University of Miami, Miami, FL

The Beach Exposure And Child HEalth Study (BEACHES) focuses on quantifying exposures and associated health risks of oil spill chemicals (OSCs) during child beach play. We hypothesize that physical features of a beach influence OSCs distribution and hence children's exposure on the beach with respect to their time-space interaction. This program will test this hypothesis through three projects. Project 1 will assess children's time-space interaction and their activities on the beach that will influence OSCs exposure. As part of this project, beach-related activities will be quantified at four beach sites to evaluate influences of beach characteristics on play activities. Digital video-recording/video-translation will be used to evaluate child beach play coupled with a sand adhesion study to improve estimates of dermal exposures. Project 2 will assess distributions of OSCs. Specifically, algorithms will be developed to compute time-location specific concentrations of OSCs in air, water, and beach sands using readily-available OSC data. These algorithms will provide location and time-specific OSC concentrations at oil impacted beaches. These concentrations will be validated with respect to outputs from fate and transport models. The algorithms will be contextualized in terms of beach characteristics and child beach play so that OSCs concentration distributions can be assigned to play locations and associated activities as documented in Project 1. Project 3 will integrate the results from Projects 1 and 2 to develop a risk assessment modeling platform that considers cumulative (multiple contaminants with same health end point) and aggregate (ingestion, dermal, and inhalation) risks. Statistical methods based upon Monte Carlo approaches will be used to provide exposure and associated risk uncertainty. Sensitivity analysis will be conducted to provide information on data gaps and where further research would provide the greatest benefit.

## Communicating the Expected Frequency of Extreme Floods to the Public: Insights from Recent Events in the Gulf of Mexico Region

**C. Tucker\***, J. Trepanier

Louisiana State University, Baton Rouge, LA

Recent meteorological events have shown how devastating rainfall can be to life and property. Recurrence intervals, or return periods, are estimates of how often these events happen and can be used to communicate the likelihood of occurrence to individuals. A commonly used recurrence interval is the 100-year storm. Communicating the meaning of the 100-year storm is often lost on the public. It means the event has a one percent (1%) chance of occurring in any given year, not the often-confused meaning of "will happen once every 100 years". In fact, over the course of six months (March-August 2016), Louisiana received two 100-year storm events, and over the course of three years, East Texas received three 500-year events (2015-2017), and of these five events, only one was a named storm (Hurricane Harvey, 2017). Each event caused more than \$100 million in residential and commercial damage, the largest oil refinery in the nation (Baytown Refinery, ExxonMobil) was shut down, and more than 100 people have perished as a result. This study identifies two problems currently existing in communicating probabilities of occurrence: (1) the varied methods in estimating occurrence

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probabilities leads to widely different results, and (2) the lack of transparency in the data and methods used by the scientists blinds the public from the truth. They are often given one number and told to trust it without any mention of uncertainty or limitations. Recent events from Texas and Louisiana are used to test different methods for estimating recurrence intervals. A discussion on the use of probabilities of occurrence instead of return periods is included as a way to better communicate with the public.