FLORIDA INTERNATIONAL UNIVERSITY, UNIVERSITY OF NORTH FLORIDA, AND NOVA SOUTHEASTERN UNIVERSITY:
Assessing impacts of oil exposure to deep sea ecosystems of the Gulf of Mexico using sharks and scavengers as integrative models

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SCIENCE ACTIVITIES

General Summary

The deep sea ecosystems of the Gulf of Mexico are poorly known, especially the populations and communities of top predators, like sharks. These species and ecosystems, however, could have been impacted by the Deepwater Horizon Oil Spill. Our primary goals were to 1) document, as much as possible, baseline conditions of the ecosystem using indicator species (sharks and benthic scavengers), and 2) assess the impacts of the spill on physiology of indicator species, abundance of deep sea sharks and scavengers, population and community structure, and trophic interactions. To meet these goals, we proposed five complementary objectives. Our progress on each of these are listed below:

1. Assess and monitor the accumulation of hydrocarbon products in pelagic and deep water shark tissues to obtain an integrative, temporal assessment of the exposure of their diverse prey to sub-surface oil and *2. Assess and monitor the accumulation of hydrocarbon products in deepwater teleosts and benthic scavengers to determine their exposure to sub-surface oil.*

At least 1 of the 4 PAH biomarker assays have been run on a total of 476 deepwater fish from the northeast Gulf of Mexico; a total that includes 63 jawless fish (i.e., hagfish; scavengers), 217 elasmobranchs, and 195 bony fish. Overall, the results of this study support the hypothesis that deepwater fishes from the northeast Gulf of Mexico are exhibiting physiological signs of heightened exposure to PAHs, likely originating from the Deepwater Horizon Oil Spill. It remains unclear whether the greater levels of PAH exposure in deepwater fish from oiled sites are capable of causing higher-level effects such as cell- or organ-level abnormalities because the range of biomarkers observed in these individuals are consistent with those observed in coastal fish from only moderated contaminated locations.

3. Asses and monitor abundance, community composition, and population structure of deep-water shark communities.

Because this goal overlapped considerably with the research goals of a block grant to Dr. Grubbs at Florida State University, we decided to combine ship time and overall project objectives. Details on our findings relative to Goal 3 are detailed in the Final Report by D. Grubbs (FSU).

4. Assess trophic interactions of deepwater communities and monitor changes as exposure to oil increases.

We have analyzed stable nitrogen and carbon isotope values for 1043 individuals from impacted and less impacted areas of the Gulf of Mexico. We found relatively little variation in N or C stable isotope values in the community, which somewhat compromised its value as an indicator of trophic change. Stable isotopes would be a better tool if more extensive baseline data were available (rather than contrasts of impacted vs unimpacted sites). Nonetheless, there are initial indications of possible trophic shifts in the northern Gulf for two shark species that are abundant. Ongoing work by Florida State should help elucidate whether this might be induced by the spill.

5. Train the next generation of shark biologists and marine ecologists while enhancing collaborations among FIO member institutions.

This project resulted in collaborations among FIU, UNF, Nova, FSU, and Mote. We also brought in collaborators from University of West Florida and VIMS. The project resulted in field and lab experiences for dozens of undergraduate and graduate students from these, and other, institutions and will result in at least three graduate degrees.

Results and scientific highlights

Trophic interactions

We investigated the trophic interactions of deep-sea sharks, teleosts, and scavengers (agnthans and invertebrates) of the Gulf of Mexico using stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis. Isotopic studies of sharks were supplemented with stomach contents analysis. Samples were collected from more than 1000 individuals captured at depths between 200 - 1200 m along the northern slope of the Gulf of Mexico (NGS; near the DwH) and the west Florida slope (WFS; far from the DwH). Although we detected some spatial, temporal, and inter-species variation in mean trophic positions of sharks based on stable isotopes, there was a high degree of trophic overlap among species, between locations, and through time. Stomach contents analysis further suggested relatively similar diets among the taxa with sufficient sample sizes at the level of broad taxonomic categories for prey. We found that stable isotope values of *Squalus* cf. *mitsukurii*, the most commonly captured elasmobranch, varied between sample regions, through time and also with sex and size.

In general, the isotopic niches of scavengers (crabs, isopods, hagfish) were considerably larger than those of either sharks or teleosts. This was particularly pronounced in the NGS, were the isotopic niche of scavengers was much bigger than that of either fish group or scavengers found in the WFS. While scavengers occupied similar isotopic niche space in both regions, sharks and teleosts in the WFS had, in general, lower $\delta^{15}N$ values.

Interestingly, $\delta^{15}N$ values increased between April 2011 and 2012 in the NGS, but not the WFS, for shark species with adequate sample sizes. An identical pattern was found for the one species of teleost, *Urophycis floridana,* that was captured in adequate numbers across both regions during both April cruises. For giant isopods, $\delta^{15}N$ values were similar from April 2011 to April 2012 in the NGS but declined in the WFS. Without further baseline data from pre-spill communities of the NGS it is not possible to determine if these trophic shifts might be related to the DwH event. This study, however, provides the first characterization of the trophic interactions of deep-sea sharks, teleosts, and scavengers in the Gulf of Mexico and establishes system base lines for future investigations and possible lingering impacts from the DwH.

Shark population genetics

Population genetic structure was determined for tiger sharks, a large apex predator species that is likely to have a strong influence on community dynamics. Tiger sharks are relatively common in the Gulf of Mexico and therefore likely to have been exposed to the oil spill, directly and/or as upper level trophic consumers. Tiger sharks also show diverse habitat use ranging from coastal to pelagic environments, deep diving ability (documented to 820m depth) and a highly migratory life history, providing a good model for energy transfer between diverse horizontal and possibly vertical habitats.

Population structure was determined using 10 nuclear microsatellite loci and mitochondrial control region sequences to gain a multi-marker perspective on population connectivity between the Gulf of Mexico and Florida Atlantic coast. Both sets of markers show no significant differentiation among these populations, suggesting both high male and female-mediated connectivity over this region, presumably facilitated by long-distance movements of individuals between these areas. Given the strong connectivity between the Gulf of Mexico and Florida Atlantic, potential exposure to oil/dispersant is not likely limited to tiger sharks just in the vicinity of the oil spill. Sub-lethal impacts from direct exposure, therefore, has the potential

to affect the population status of tiger sharks in regions far removed from direct oil/dispersant contact if impacts of exposure are intense.

Shark DNA Barcoding

Many deep sea sharks are poorly known, and the taxonomy of several species remains unclear. As part of this project, we used genetic barcoding analyses (mitochondrial COI locus) to check the morphology-based, field determined identity of 245 samples (individual sharks) captured on deep-sea fishing gear over 3 cruises, and resolve any taxonomic issues with difficult-to-identify species. DNA barcoding revealed the samples to derive from 15 shark species constituting 10 genera and 8 families. With few exceptions, the barcode identifications were consistent with morphology-based identifications, and there was no evidence for the presence of previously undescribed cryptic species within the Gulf. Taxonomy of deep-sea species, however, remains unclear at a global scale and samples from our cruises are being used to help resolve some of these taxonomic questions.

Exposure

The biomarker component of this project examined exposure of deepwater fish in the northeastern Gulf of Mexico to polycyclic aromatic hydrocarbons (PAHs), the most toxic constituents of petroleum, in order to assess the impact of the Deepwater Horizon oil spill on the biology of these species. UNF conducted laboratory analysis of three biomarkers of PAH exposure: 1) activity of cytochrome P4501A1 (Cyp1A1), a key enzyme that is involved in the metabolism of PAHs and is generally induced when fish are exposed to elevated concentrations of these compounds; 2) presence of biliary fluorescent aromatic compounds (FACs), metabolites of PAHs that are commonly used as indicators of increased metabolism of oil constituents; and 3) occurrence of micronuclei, erratic "extra" nuclei that occur in greater numbers in erythrocytes of fish that have been exposed to genotoxic chemicals such as PAHs. The occurrence of hepatic PAH-DNA adducts, covalent bonds that can form between PAHs and DNA and cause DNA damage in organisms exposed to these compounds, was also examined in a small subset of shark samples but continuation of this work was put on hold due to time considerations. However, samples for conducting these assays have been archived for future analysis.

In general, most bony fish exhibited low, but measurable levels of Cyp1a1 activity. However, there were significant differences between the range of levels observed in conspecifics from reference and oil-impacted locations. These differences suggested that bony fish residing in oil-impacted sites are exhibiting signs of greater exposure to Cyp1a1-inducing compounds compared to reference site individuals, but that overall exposure levels may not be high enough to cause higher-levels impacts on cell and organ function. Alternatively, it may indicate that the deepwater bony fish examined in this study normally exhibit much lower levels of Cyp1a1 activity than their shallow water counterparts and that the differences observed between sites is of greater concern. Cyp1a1 activity levels in elasmobranchs examined in this project were generally lower than those observed in bony fish. Like bony fish, there were significant differences between Cyp1a1 activity in sharks from reference sites compared with conspecifics from oil-impacted locations, suggesting greater exposure to Cyp1a1-inducing compounds in the latter (particularly for *Squalus* cf *mitsukurii*). Temporal trends in data support the hypothesis that sharks from oil-impacted locations are exhibiting physiological signs of elevated PAH exposure but whether these levels are capable of causing higher levels effects remains unclear. No micronuclei were detected in any shark samples. Therefore, there is no evidence for chromosomal damage in northern Gulf sharks.

Cruises & field expeditions

Ship or Platform Name	**Class** (if applicable)	**Chief Scientist**	**Objectives**	**Dates**
Weatherbird II	R/V	Dean Grubbs	BP spill benthic longline sampling	April 5-20, 2011
Weatherbird II	R/V	Dean Grubbs	BP spill benthic longline sampling	August 24 – September 1, 2011
Weatherbird II	R/V	Dean Grubbs	BP spill benthic longline sampling	March 30 – April 16, 2012

1) Peer-reviewed publications

In review

Churchill, D., M. R. Heithaus, Jeremy J. Vaudo, D. R. Grubbs, and J. I. Castro. In review. Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexico revealed through stable isotope and stomach contents analysis. Deep Sea Research II

In preparation

Churchill, D., M. R. Heithaus, Kirk Gastrich, and D. R. Grubbs. Spatial and temporal variation in trophic interactions in deep water communities in the Gulf of Mexico. Target Journal: Marine Ecology Progress Series

Churchill, D., M. R. Heithaus, Kirk Gastrich, and D. R. Grubbs. Variation in trophic position of common large-bodied scavengers in deep sea habitats of the Gulf of Mexico.

Grubbs RD, J Gelsleichter, M Heithaus, K Gastrich, C Cotton. In. prep. Demersal elasmobranch assemblages associated with DeSoto Canyon and the continental slope of the eastern Gulf of Mexico relative to the Deepwater Horizon oil spill. Target Journal: Deep Sea Research II, to be submitted January 2013

Grubbs RD, J Gelsleichter, M Heithaus, K Gastrich, C Cotton. In. prep. Community structure of deep demersal fishes in the northeastern Gulf of Mexico. To be submitted summer 2013

Cotton, C F, R D Grubbs, J A Musick. Submitted. Reproduction and embryonic development in two species of North Atlantic squaliform sharks, Centrophorus cf. niaukang and Etmopterus princeps: evidence of matrotrophy? Submitted to Deepsea Research II

White, W T, D A Ebert, G J P Naylor, H Ho, P Clerkin, A Verissimo, C Cotton. In review. Revision of the genus Centrophorus (Squaliformes: Centrophoridae): Part 1 - Redescription of Centrophorus granulosus (Bloch & Schneider), a senior synonym of C. acus Garman and C. niaukang Teng

Moura, T, E Jones, C F Cotton, S B Irvine, R K. Daley, M W Clarke, P Lorance, K Jakobsdottir, L J López-Abellán, P Crozier, G Diez, I Fossen, J E Dyb, R B Severino, P Pascual-Alayón, H Dobby, I Figueiredo. In

prep. Large- scale distribution of three deep-water Squaliforms integrating data on sex, maturity and environment.

Veríssimo A, Cotton C, Burgess G, Buch R, Guallart J, Gailbraith J. In prep. A revision of the gulper sharks (genus Centrophorus) in North Atlantic waters

Bernard, A.B., K. Feldheim, B. Wetherbee, M. Heithaus and M. Shivji. Population structure of tiger sharks inferred from microsatellite and mitochondrial markers. Target Journal: Molecular Ecology (planned submission Summer, 2013).

Gelsleichter J, AE Leary, RD Grubbs, MR Heithaus. Impacts of the Deepwater Horizon Oil Spill on deepwater shark populations in the northeast Gulf of Mexico. To be submitted to DSRIII.

Gelsleichter J, AE Leary, RD Grubbs, MR Heithaus. Multibiomarker assessment of PAH exposure and effects in deepwater fish populations impacted by the Deepwater Horizon Oil Spill.

2) Presentations and posters

Title	**Presenter**	**Authors**	**Meeting or Audience**	**Abstract published (Y/N)**	**Date**
Assemblages of large demersal fishes associated with DeSoto Canyon and the continental slope waters in the Eastern Gulf of Mexico with reference to the Deepwater Horizon Oil Spill.	Grubbs	R. Dean Grubbs[1], Jim Gelsleichter[2], Michael R. Heithaus[3], Kirk Gastrich[3], Charles Cotton[4]	Ocean Sciences	Y	February 2012
Demersal elasmobranch assemblages associated with Desoto Canyon and the continental slope of the eastern Gulf of Mexico relative to the Deepwater Horizon oil spill	Grubbs	R. Dean Grubbs[1], Jim Gelsleichter[2], Michael R. Heithaus[3], Kirk Gastrich[3], Charles Cotton[4]	American Elasmobranch Society/ American Society of Ichthyologists and Herpetologists	Y	August 2012
Effects of the Deepwater Horizon Oil Spill on Deepwater Fish Populations from the Northeast Gulf of Mexico.	Gelsleichter	Gelsleichter J, Leary AE , Drakos H, Davis M, Gonzalez de Acevedo M, McCallister M, Ford R, Heithaus ME, Grubbs RD	2012 meeting of the Southern Division of the American Fisheries Society, Biloxi, MS.	N	January 2012
Effects of the Deepwater Horizon Oil	Gelsleichter	Gelsleichter J, Leary AE , Drakos H, Davis	2012 meeting of the American Elasmobranch Society	N	August 2012

Spill on Deepwater Shark Populations from the Northeast Gulf of Mexico.		M, Gonzalez de Acevedo M, McCallister M, Ford R, Heithaus ME, Grubbs RD			
Effects of the Deepwater Horizon Oil Spill on Deepwater Fish Populations from the Northeast Gulf of Mexico.	Gelsleichter	Gelsleichter J, Leary AE, Heithaus M, Grubbs RD	Gulf of Mexico Oil Spill and Ecosystem Science Conference	N	January 2013

3) Data

See attached files BP_CRUISES_DATABASE_060712 and Deep Sea-15. We are still in the process of unifying genetic, ecotoxicology, and trophic interactions databases. We foresee no issues with this process for permanent archiving of data.

PARTICIPANTS AND COLLABORATORS

4) Project participants

First Name	Last Name	Role in Project	Institution	Email
Jose	Castro	Co-Principal Investigator	Mote	jcastro@mote.org
Michael	Heithaus	Principal Investigator	FIU	heithaus@fiu.edu
Jim	Gelsleichter	Principal Investigator	UNF	Jim.gelsleichter@unf.edu
Mahmood	Shivji	Principal Investigator	NOVA	mahmood@nova.edu
Kirk	Gastrich	Co-Principal Investigator	FIU	Kgastric@fiu.edu
Dean	Grubbs	Principal Investigator	FSU	dgrubbs@bio.fsu.edu
Chip	Cotton	Co-Principal Investigator	VIMS	cottonc@savannahstate.edu
Annebelle	Brooks	Field Crew	Cape Eluthra	annabellebrooks@beibahmas.org
Rachel	Decker	Filed Crew	FIU	Decker.rachel@gmail.com
William	Bemis	Field Crew	Cornell	Web24@cornell.edu

MENTORING AND TRAINING

5) Student and post-doctoral participants *

First Name	Last Name	Post-doc / PhD / MS / BS	Thesis or research topic	Institution	Supervisor	Expected Completion year
Diana	Churchill	PhD	Trophic Structure of Deepwater Communities	FIU	Heithaus	2014
Jeremy	Vaudo	Postdoc	Trophic structure	FIU	Heithaus	2012
Andrea	Reategui	BS	Field collections	FIU	Heithaus	2012
Robin	Sarabia	MS	Field collection	FIU	Heithaus	2012
Shomen	Mukherjee	Postdoc	Field collections	FIU	Heithaus	2011

Jeremy	Vaudo	Postdoc	Field Collections/Lab analysis	FIU	Heithaus	2012
Derek	Burkholder	PhD	Field Collections	FIU	Heithaus	2012
Nicole	Chavarry	BS	Field Collections	FIU	Heithaus	2012
Christine	Testerman	PhD	Shark Genetic Population Structure	NSU	Shivji	2013
Andrea	Bernard	PhD	Shark Genetic Population Structure	NSU	Shivji	2013
Kimberley	Atwater	MS	Shark DNA Barcoding Analyses	NSU	Shivji	2014
Mike	McCallister	MS	Field collections	UNF	Gelsleichter	August 2012
Ryan	Ford	MS	Field collections	UNF	Gelsleichter	August 2012
Brenda	Anderson	MS	Field collections	UNF	Gelsleichter	May 2013
Arianne	Leary	BS	Laboratory analysis	UNF	Gelsleichter	May 2012
Monica	Collazos	BS	Laboratory analysis	UNF	Gelsleichter	May 2013
Melissa	Gonzalez De Acevedo	BS	Laboratory analysis	UNF	Gelsleichter	May 2012
Hilary	Drakos	BS	Laboratory analysis	UNF	Gelsleichter	May 2012

*FSU Participants and students on cruises included in Grubbs' report

6) Student and post-doctoral publications, if planned

In review

Churchill, D., M. R. Heithaus, Jeremy J. Vaudo, D. R. Grubbs, and J. I. Castro. In review. Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexico revealed through stable isotope and stomach contents analysis. Deep Sea Research II

In preparation

Churchill, D., M. R. Heithaus, Kirk Gastrich, and D. R. Grubbs. Spatial and temporal variation in trophic interactions in deep water communities in the Gulf of Mexico. Target Journal: Marine Ecology Progress Series

Churchill, D., M. R. Heithaus, Kirk Gastrich, and D. R. Grubbs. Variation in trophic position of common large-bodied scavengers in deep sea habitats of the Gulf of Mexico.

Bernard, A.B., K. Feldheim, B. Wetherbee, M. Heithaus and M. Shivji. Population structure of tiger sharks inferred from microsatellite and mitochondrial markers. Target Journal: Molecular Ecology (planned submission Summer, 2013).

7) Student and post-doctoral presentations and posters, if planned

Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date
Effects of the Deepwater Horizon Spill on the trophic interactions of deep-sea and associated species of the Gulf of Mexico	Diana Churchill	Chuirchill, D., M. R. Heithaus,	American Elasmobranch Society/ American Society of Ichthyologists and Herpetologists	Y	August 2012
Spatial and temporal variation in trophic interactions of deep-sea fishes and invertebrates of the Gulf of Mexico	Diana Churchill	Chuirchill, D., M. R. Heithaus,	American Elasmobranch Society/ American Society of Ichthyologists and Herpetologists	Planned	Summer 2013

8) Images

**Note: Pictures of species sampled during cruises included with D. Grubbs report. Additional images and video clips available upon request.*

DSCF6971 Alejandra Mickle 31 March 12 Sorting shark specimens

DSCF7024 Alejandra Mickle 31 March 12 A large sixgill shark being brought on board for non-lethal sampling. Sharks were fitted with satellite transmitters and released.

P4140127 Andrea Reategui 14 April 2011 Giant isopod collected from deep sea waters of the northern Gulf of Mexico. Analyses are currently underway to determine whether such scavengers may be sentinels of ecosystem change.

P4140199 Andrea Reategui 14 April 2011 Fish trap filled with hagfish slime.

P4150275 Andrea Reategui 14 April 2011 Retrieval of a deepsea longline

13) Continuing Research

If you are continuing this research under another grant, please include granting authority and title of award and a very brief synopsis (2-3 sentences).

FIU is currently seeking research funding to continue work; collaborative efforts will continue with D. Grubbs, who is funded under the Deep-C consortium. UNF is currently subcontracted to conduct biomarker assays on fishes collected throughout the northeast Gulf as part of a 3-year follow-up study on the impacts of the Deepwater Horizon Oil Spill. This work is supported by grant from Gulf of Mexico Research Initiative to Florida State University (DEEP-C: Deepsea to Coast Connectivity in the Eastern Gulf of Mexico). Data from the current study will be compared to results from this future work.