MOTE MARINE LABORATORY: EFFECTS OF THE DEEPWATER HORIZON OIL SPILL ON EPIPELAGIC AND LARGE COASTAL SHARKS AND TELEOSTS OF THE GULF OF MEXICO

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SCIENCE ACTIVITIES

1) General Summary

This project led by Mote Marine Laboratory and the University of North Florida was designed to assess the impacts of the Deepwater Horizon oil spill on certain species of epipelagic and coastal sharks and other fishes inhabiting the northeast Gulf of Mexico. We sought to determine the sublethal effects of petroleum-derived contaminants, particularly polycyclic aromatic hydrocarbons (PAHs), using key biomarkers in blood and other tissues from these species. We also sought to understand patterns of movement in epipelagic fishes of the Gulf, to detect any novel behavior as a result of the spill.

A total of 27 species of large Gulf fishes were sampled during the two years of the study. Collections from these specimens comprised 2,791 individual samples of various tissue types including blood, muscle, liver, bile, gill, kidney, gonads, genetics, vertebrae, and stomach contents. Eight separate research cruises involving 24 project participants and 23 students were used to make these collections. Limited tagging was conducted because overall catch rate was low (< 5 fish per 100 hooks) and specimens were needed for lab analysis. One longfin mako was the first of this species to be satellite-tagged anywhere in the world, and this shark showed significant movement out of the Gulf into the northwest Atlantic in a period of three months. Attempts to obtain blood samples from whale sharks were unsuccessful in US Gulf waters but the technique was applied successfully in Mexican Gulf waters; however, permitting regulations did not allow us to transport these samples to the US for analysis. Further attempts to obtain blood samples from free-swimming whale sharks are planned.

Overall, laboratory assays conducted by Drs. J Gelsleichter (UNF), D Wetzel (Mote) and C Walsh (Mote) do not support the hypothesis that epipelagic teleosts such as swordfish and yellowfin tuna from the northeast Gulf of Mexico exhibit physiological signs of excessive exposure to PAHs originating from the Deepwater Horizon oil spill. Comparisons between sharks from the northeast Gulf of Mexico and reference site data, on the other hand, suggest that some species of northeast Gulf sharks are exposed to comparatively higher levels of petrogenic PAHs, but it remains unclear if these compounds originated from the Deepwater Horizon oil spill or from other, more common sources of petroleum contamination. One species in particular, the silky shark, showed relatively higher indications of PAH exposure; since this species is commonly found around oil/gas rigs in the Gulf, it is unclear whether these higher levels are due to acute exposure such as the DWH spill or chronic exposure from the habitat. Follow-up work is currently underway to conduct comparisons of PAH biomarkers in individuals of the same species from reference and oil-impacted locations to address these questions. This work will include the completion of additional biomarker tests on the samples obtained in this study.

Lessons learned in this study include: 1) funding levels for a project of this magnitude need to be higher to be able to process the thousands of samples available, for without more complete analyses of, for example, PAH body burdens, it is difficult to directly correlate exposure level with biomarker results; 2) highly migratory species are, by definition, very mobile animals that may elude efforts to collect them and, quite possibly, the source of the problem – oil spills – itself; and 3) research ship infrastructure (ship availability, operating speed, etc.) in the Gulf may limit the ability to conduct comprehensive field collections as envisioned in this project.

2) Results and scientific highlights

- Using a variety of research platforms, collections were made of epipelagic and coastal sharks and rays and epipelagic teleost fishes in offshore and inshore waters of the northeast Gulf of Mexico November 2010 - June 2012. A total of 3,984 hooks were set using pelagic longlines, bottom longlines and drumlines, resulting in a total catch of 195 fish (overall CPUE = 4.89 fish/100 hooks). These comprised: 17 species of sharks; swordfish (*Xiphias gladius*), white marlin (*Tetrapturus albidus*) and yellowfin tuna (*Thunnus albacares*); and seven other species of epipelagic fishes. A total of 2,791 different tissue samples were collected from these specimens for laboratory analysis. In general, gross observations of collected specimens indicated they were relatively disease-free with no obvious lesions, growths, discolorations or other grossly visible problems.
- Due to relatively low CPUE, nearly 100% of the catch was brought aboard for tissue sampling, with the exception of swordfish caught in pelagic longline closed areas. These could not be tagged due to NOAA/NMFS regulations. Therefore, few fish were tagged in this study. Among satellite-tagged sharks was a longfin mako (*Isurus paucus*), the first of this species to be sat-tagged, which showed movement from the Gulf into the northwest Atlantic over a three-month period. Whale sharks (*Rhincodon typus*) did not appear in the study area as hoped but attempts to collect blood from free-swimming animals were successful in Mexico, where we did not have permits to transport samples to the US for analysis. Therefore, this component of the study awaits future developments.
- Polycyclic aromatic hydrocarbon (PAH) body burdens for offshore and coastal elasmobranchs and select teleosts were analyzed. Generally, detectable levels were not found except in one silky shark, *Carcharhinus falciformis* (1.63 ug/g lipid) and one pelagic stingray, *Pteroplatytrygon violacea (*0.05 ug/g lipid), indicating low exposure and/or elimination of PAHs prior to sampling of the specimens.
- Activity of cytochrome P4501A1, a key enzyme involved in PAH metabolism, was low but measurable in most teleost fishes, i.e., mean levels of EROD activity ranged from ~2-6 pmol resorufin/min/mg protein in virtually all teleosts that were examined. The single exception to this was the oilfish (*Ruvettus pretiosus*), which exhibited mean EROD activity of 11.4 ±4.18 pmol resorufin/min/mg protein (n = 11), with moderately high levels of enzymatic activity (22-43 pmol/min/mg protein) in three individuals. With this exception, EROD activity levels were generally comparable with measurements of EROD activity in teleosts from non-impacted sites that have been measured in previous studies. Therefore, with possible exception of a few aforementioned individuals, data on Cyp1a1 activity provides little support for the hypothesis that pelagic teleosts of this study were exposed to appreciable levels of PAHs as a result of the Deepwater Horizon Oil Spill.
- Cyp1a1 activity levels in epipelagic sharks were generally lower than those observed in teleosts, i.e., mean EROD activity levels ranged from below the limit of detection to 2.5 ±0.5 pmol/min/mg protein, the latter of which was observed in silky sharks. In contrast to data from teleosts, these results may actually reflect increased PAH exposure in at least *C. falciformis* because measurements of EROD activity have been shown to typically fall below ~1 pmol/min/mg protein in sharks from reference locations, but in northeast Gulf silky sharks sampled in this study, most ranged 2-6 pmol/min/mg protein. It is unclear whether the higher level of Cyp1a1 activity in these individuals represents exposure to oil from the Deepwater Horizon Oil Spill or to other, more traditional sources of petroleum contamination as these levels were comparable with those reported in other shark species from typical “industrialized” sites (~2-3 pmol/min/mg protein).
- Concentrations of biliary FACs were also examined in most teleosts and sharks collected for this study. Mean biliary FAC concentrations in teleosts ranged ~100,000-400,000, ~50-3,000, and ~400-2,250 ng/mL for naphthalene-like, pyrene-like, and benzo(a)pyrene-like metabolites, respectively. Since these values are consistent with those measured in teleosts from locations contaminated with PAHs via traditional, non-catastrophic sources (i.e., shipping activity, natural oil seeps, nearby

locations of petroleum terminals), it is likely that they reflect exposure to PAHs originating from these sources rather than from the Deepwater Horizon oil spill.

- Mean biliary FAC concentrations in northeast Gulf elasmobranchs ranged from ~50,000-245,000, ~190-5,400, and ~490-2,400 ng/mL for naphthalene-, pyrene-, and benzo(a)pyrene-like metabolites, respectively. Although no published values are available for comparison with these measurements, comparison with data from sandbar sharks collected from southwest Florida (mean biliary FAC concentrations of 111,056 ±40,899, 105 ±56.53, and 7,89 ±5,189 for naphthalene-, pyrene-, and benzo(a)pyrene-like metabolites, respectively, n = 5) suggest that northeast Gulf sharks may have been exposed to greater amounts of lower molecular weight PAHs and lower amounts of high molecular weight PAHs, a hallmark of greater exposure to petrogenic PAHs. However, as for data on EROD activity, it remains unclear if evidence for greater exposure to petrogenic PAHs in northeast Gulf sharks reflects the impacts of the Deepwater Horizon Oil Spill or exposure to non-catastrophic sources of these compounds.
- To determine if chromosomal abnormalities indicative of exposure to genotoxic chemicals such as PAHs, we examined the presence of micronuclei in blood smears of all sharks sampled. No micronuclei were detected, providing no evidence for chromosomal damage of the sampled sharks.
- DNA damage was assessed by measuring Comet tail moments and intensities in whole blood from eight shark species and one teleost (i.e., swordfish, *Xiphias gladius*). Median tail moments for all samples were within the range of the healthy control cells and median tail intensities were slightly elevated above the healthy control cells for most samples. Cell dispersion for both tail moments and intensities ranged from healthy to damaged. There were no statistical differences between species, collection dates or location.
- Several different measures of immune function were determined from plasma collected from elasmobranch and teleost species for evaluation as potential biomarkers of exposure to oil spill contaminants. These included activity of several enzymes (lysozyme, superoxide dismutase, glutathione-S-transferase), reactive oxygen and nitrogen species (ROS/RNS) and total uric acid in the plasma. Results indicated these plasma biomarkers are markedly variable among species, and thus are species-specific responses. Silky sharks, however, had consistently higher values for SOD, ROS/RNS, and uric acid than other elasmobranch species. These observations may possibly suggest a higher level of oxidative stress occurring in the immune system of this shark species.
- Reproductive hormones were evaluated in sampled specimens. Highest values of inhibins A and B were seen in shortfin mako (*Isurus oxyrinchus*), silky, dusky (*C. obscurus*) and sandbar (*C. plumbeus*) sharks and in mahi mahi (*Coryphaena hippurus*) and escolar (*Lepidocybium flavorunneum*). No trends were observed for any specific collection area. Anti-Mullerian hormone values were consistently low across all species with little variability.
- Cytokine concentrations of IFN-γ, IL-2, IL-10 and TNF-α varied significantly among specimen, including among individuals of the same shark species. A baseline could not be established, however, some trends were notable. Measurable levels of IFN-γ were detected in all of the assayed shark serum and it was consistently the most highly expressed cytokine among the four tested. IL-10 exhibited the lowest concentration in all samples, except BU-005. IL-2 was highly expressed in SB-003, possibly indicating immune system stress as it is a major inducer of T lymphocyte proliferation in other organisms. The presence of the cytokine genes in carcharhinid sharks (previously undocumented) was partially confirmed by performing reverse-transcription PCR with degenerative primers for each gene, designed from known teleost, mammal, and human sequences and comparing the resulting gel band with the expected molecular weight of the fragment. The sequence homology of these isolated gene fragments with the corresponding human, mouse, and teleost cytokine genes will be determined, to validate their presence in carcharhinids.

3) Cruises & field expeditions

Ship or Platform Name	**Class** (if applicable)	**Chief Scientist**	**Objectives**	**Dates**
Weatherbird II		R Hueter	Collect tissue samples, tag specimens	7-11 Nov 2010
Bellows		J Morris	Collect tissue samples, tag specimens	7-10 Dec 2010
Weatherbird II		R Hueter	Collect tissue samples, tag specimens	23-28 Apr 2011
Ocean		R Hueter	Collect tissue samples, tag specimens	19-27 May 2011
Weatherbird II		R Hueter	Collect tissue samples, tag specimens	20-26 Oct 2011
Eugenie Clark		J Morris	Collect tissue samples, tag specimens	8-10 Nov 2011
Weatherbird II		R Hueter	Collect tissue samples, tag specimens	24-30 Apr 2012
Yellowfin		R Hueter	Collect tissue samples, tag specimens	28-29 Jun 2012

4) Peer-reviewed publications

a. Published, peer-reviewed bibliography (copy provided as separate attachment)

Hueter B (2012) Whale shark aggregation areas. *In* Beyond the Horizon: A Forum to Discuss a Potential Network of Special Ocean Places to Strengthen the Ecology and Culture of the Gulf of Mexico (KB Ritchie and WE Kiene, eds), p 40-41. Proceedings of the Forum: May 11-13, 2011, Mote Marine Laboratory, Sarasota, Florida. Available at http://www.mote.org/clientuploads/4nadine/beyondhorizon/BTH%20Forum%20Proceedings%20Final.pdf and at http://issuu.com/lawsonmitchell/docs/bth_forum_proceedings_final/3 (last accessed 11 December 2012).

b. Manuscripts submitted or in preparation

In prep: Gelsleichter J, Leary A, Long MC, Piercy AN, Hueter RE, and Kerstetter D. Multibiomarker assessment of PAH exposure and effects in epipelagic fishes from the northern Gulf of Mexico. To be submitted to a toxicology-themed journal such as *Aquatic Toxicology*, *Marine Environmental Research*, *Chemosphere*, or *Science of the Total Environment* before May 2013.

In prep: Yordy J et al. Baseline values for plasma biomarkers and plasma electrophoresis profiles in several species of elasmobranch fish. Target journal: *Journal of Wildlife Disease of Journal of Zoo and Wildlife Medicine.* Planned date of submission: March 2013.

In prep: Hueter R, J Tyminski and J Morris. First satellite-tagging of a longfin mako. Target journal: *Fishery Bulletin*. Planned date of submission: March 2013.

5) Presentations and posters (copies provided as separate attachments)

Title	**Presenter**	**Authors**	**Meeting or Audience**	**Abstract published (Y/N)**	**Date**
Effects of the Deepwater Horizon Oil Spill on Epipelagic and Large Coastal Sharks of the Gulf of Mexico	R Hueter	R Hueter and J Gelsleichter	FIO Workshop of FIO/BP grant recipients, Orlando, FL	N	16 Sep 2010

Whale shark aggregation areas	R Hueter	R Hueter	"Beyond the Horizon" conference, Sarasota, FL	Y	12 May 2011
Mote Marine Laboratory Gulf Research Supported by the Guy Harvey Ocean Foundation	R Hueter	R Hueter	Gulf of Mexico Fisheries Symposium, St. Petersburg Beach, FL	N	15 Sep 20122

6) Other products or deliverables
None presently

7) Data
Data are provided in a series of spreadsheets accompanying this report. All samples are accounted for in Custody Sheets housed at Mote Marine Laboratory. Samples that were collected but not analyzed are archived at various laboratories as shown on the corresponding Custody Sheet.

PARTICIPANTS AND COLLABORATORS

8) Project participants

First Name	Last Name	Role in Project	Institution	Email
Robert	Hueter	Principal Investigator	Mote Marine Laboratory	rhueter@mote.org
James	Gelsleichter	Co-Principal Investigator	University of North Florida	jim.gelsleichter@unf.edu
Dana	Wetzel	Scientific Participant	Mote Marine Laboratory	dana@mote.org
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MENTORING AND TRAINING

9) Student and post-doctoral participants

First Name	**Last Name**	**Post-doc / PhD / MS / BS**	**Thesis or research topic**	**Institution**	**Supervisor**	**Expected Completion year**
Andrew	Piercy	Post-doc	PAH effects in Gulf fishes	University of North Florida	J Gelsleichter	2012
Michael	McCallister	MS	Marine environmental biology	University of North Florida	J Gelsleichter	2012
Arianne	Leary	BS	Marine environmental biology	University of North Florida	J Gelsleichter	2012
Matthew	Long	BS	Marine environmental biology	University of North Florida	J Gelsleichter	2013
Erin	Pulster	PhD	Risk assessment of exposure to oil	University of South Florida	F Jaward	2014
Kristina	Deak	BS	Biochemistry	Northeastern University	D Wetzel	2012
Kate	English	BS	Lysozyme analysis in plasma	University of California, Santa Cruz	C Walsh	
Monica	Schmidt	BS	RNA isolation from	University of	C Walsh	

			elasmobranch blood	Wisconsin-Parkside		
Jayne	Gardiner	PhD	Shark senses	University of South Florida	P Motta R Hueter	2012
Courtney	Conklin	BS	Biology	University of Alabama, Birmingham		
Kelly	Lieske	BS	Biology	University of California, San Diego		
Travis	Moore	MS	Pelagic fish ecology	Nova Southeastern University	D Kerstetter	2014
Leslie	Wade	MS	Marine mammal ecology	University of South Florida	D Wetzel	2011
Jeremy	Vaudo	Post-doc	Marine behavioral ecology	Florida International University	M Heithaus	2012
Diana	Churchill	MS	Marine behavioral ecology	Florida International University	M Heithaus	
Ryan	Ford	BS	Biology	University of North Florida	J Gelsleichter	
Connor	Capizzano	BS	Biology	University of Rhode Island		
Ivan	Maulana	BS	Biology	Wesleyan University		
Daniel	Geary	BS	Biology	Auburn University		
Connor	White	BS	Biology	College of William & Mary		
Sohail	Khambisi	MS	Pelagic fish biology	Nova Southeastern University	D Kerstetter	
Amber	Ferguson	MS	Fish anatomy	University of South Florida	P Motta	2015
Nadia	Sandoval	MS	Shark population genetics	Universidad Nacional Autonoma de Mexico		

10) Student and post-doctoral publications
None presently

11) Student and post-doctoral presentations and posters (copies provided as separate attachments)

Title	**Presenter**	**Authors**	**Meeting or Audience**	**Abstract published (Y/N)**	**Date**
Assessment of Exposure to Cytochrome P4501a1-inducing Pollutants in Pelagic Fishes from the Western North Atlantic and Gulf of Mexico through Analysis of Liver EROD Activity (oral).	A Piercy	A Piercy, J Gelsleichter, M McCallister, D Kerstetter, R Hueter	2012 meeting of the Southern Division of the American Fisheries Society, Biloxi, MS.	N	January 2012

Effects of Deepwater Horizon Oil Spill Evaluated by Fixed Wavelength Fluorescence (FF) of Polycyclic Aromatic Hydrocarbons (PAH) Metabolites in Fish Bile of Coastal and Epipelagic Species (poster).	A Leary	A Leary, J Gelsleichter, R Ford, B Anderson, J Drymon, R Hueter	2012 meeting of the Southern Division of the American Fisheries Society, Biloxi, MS.	N	January 2012
Evaluating the effects of the Deepwater Horizon Oil Spill on coastal and pelagic species in the Gulf of Mexico via polycyclic aromatic hydrocarbon metabolite detection in bile with fixed wavelength fluorescence (oral).	A Leary	A Leary, J Gelsleichter, J Drymon, R Hueter	2012 annual meeting of the American Elasmobranch Society	N	August 2012
Effects of the Deepwater Horizon Oil Spill on Epipelagic fish populations in the northeast Gulf of Mexico (poster).	M Long	M Long, A Leary, J Gelsleichter, J Drymon, R Hueter	Gulf of Mexico Oil Spill and Ecosystem Science Conference	N	January 2013

12) Images

Images are provided as separate attachments.

13) Continuing Research

The University of North Florida (J Gelsleichter) is currently subcontracted to conduct biomarker assays on fishes collected throughout the northeast Gulf as part of a three-year follow-up study on the impacts of the Deepwater Horizon Oil Spill. This work, which 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), will compare results with those of the current study. In addition, many aspects of this research will be ongoing at Mote Marine Laboratory (D Wetzel) with the University of South Florida GRI consortium CIMAGE, to investigate offshore and nearshore fishes for immune function, DNA damage, fertility effects and body burdens of polycyclic aromatic hydrocarbons.