Florida A&M University and University of West Florida

Assessing the impact of the Deepwater Horizon oil spill on coastal waters of the Florida panhandle: water, sediment and fish.

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SCIENCE ACTIVITIES

1) General Summary

At the time the present work was proposed, the northern coast of the Gulf of Mexico was threatened by oil from the Deepwater Horizon well. Oil had impacted the Louisiana, Mississippi and Alabama coasts, and begun to appear on beaches and barrier islands in northwest Florida. The spill was ongoing, and the magnitude of the threat to fisheries and the coastal environment was unknown. Therefore, we planned a broad sampling campaign to assess hydrocarbon concentrations in sediments and water, and to assess potential impacts on fishes in the area. Some sampling by UWF and FAMU had already been done during the early period of the oil release, and the present funding allowed additional collections and analyses to begin later in the summer of 2010.

Water, sediments and biota were sampled at two National Estuarine Research Reserves (Apalachicola and Grand Bay NERRs) and several Florida State Parks along the northern Gulf of Mexico, beginning in June 2010 and continuing thru 2011 or 2012, depending on location. Samples were also collected in a heavily oiled area off Barataria Bay, Louisiana in the spring of 2011. Water and sediments, including beach sands, were analyzed for oil components (particularly PAHs). Initially, some water samples were also monitored for routine parameters including nutrients, DOC, salinity and chlorphyll, as it was uncertain what the extent, magnitude and effects of the spill might be. Fish tissues were sampled for measurement of biomarkers of oil exposure and effect, including cytochrome p450-1A induction, and enzymes associated with detoxification and oxidative stress. Coquina clam (*Donax* spp.) tissues were sampled as indicators of sandy beach surf zone contamination with PAHs.

We coordinated our sampling efforts with other FIO-funded projects, specifically **"**Tracing the Intrusion of the GOM-2010 Oil Spill on Coastal and Marine Food Webs using Radiocarbon and Stable Isotopes", J. Chanton, FSU et al. and "Uncoupling of Autotrophy and Heterotrophy: Effects of the Deepwater Horizon Oil Spill on Microbial Food Webs", W. Jeffrey, UWF, et al. This integration included sampling at common locations and times, and sharing data and

findings. Co-PI Jagoe also participated in two additional FIO-funded projects, "Acute Effects of Oil on Northern Gulf of Mexico Reef Communities", (with W. Patterson, UWF) and Impacts from MC252 oil on ecologically and commercially important plankton of the Gulf of Mexico", (with D. Rumbold, FGCU). These two projects and the present study applied similar biomarker analyses on reef fishes, plankton, and inshore fishes, respectively. Since these studies use similar lab methods, several FAMU students have been working jointly on all three projects and sharing efforts and resources in both the field and laboratory. We anticipate that these efforts at integration among multiple studies will facilitate interpretation of results among the projects, and provide a more complete and holistic picture of the impacts due to the oil spill.

2 Results and scientific highlights

Water and sediments were collected biweekly at oil-impacted areas of Perdido Key and Santa Rosa Island, FL from June 2010 thru June 2011, and at longer intervals through spring 2012. Water and sediments were also collected quarterly at St Andrews and Topsail Beach State parks. Water samples were collected at weekly to monthly intervals at the Apalachicola and Grand Bay, NERRs beginning in June 2010 through January 2011. In May 2011, we also collected samples at Bay Jimmy LA, an area that was heavily oiled during the spill event and remains highly contaminated. A total of about 200 fish were collected in 2010-2011, representing resident inshore species such as hardhead catfish, mullet, croaker, seatrout and spot. Fish sampling focused on three locations: Apalachicola NERR, Florida (no oiling), Grand Bay NERR, Mississippi (light oiling during and immediately after the spill) and Bay Jimmy, Louisiana (heavy oiling, oil still present in sediments and marshes). Livers were excised in the field, immediately frozen on dry ice, and stored at -80C until analyzed for enzyme biomarkers. The remainder of the carcass was frozen and stored at -18 C. Although not included in our original project description, coquina clams were also sampled at monthly intervals at several of our sampling locations in northwest Florida as a bioindicator species that would concentrate PAHs.

As discussed above, Coquina, *Donax variabilis*, were chosen as an indicator species for the sandy beaches of the panhandle. More intensive sampling was conducted at the UWF beach property on Santa Rosa Island and Perdido Key State Park. PAH concentrations in water quickly declined to non-detect levels after the well was capped and oil releases ended. Additional analyses of some water samples for parameters including DOC, nutrients, chlorophyll and bacterioplankton revealed no trends that could be associated with oil exposure. PAH concentrations in sand in the surf zone of panhandle beaches also declined to non-detect in spring 2011. PAH concentrations in *Donax variabilis* clam tissues also declined in 2011. These clams were good indicators of PAH pollution, showing concentration factors relative to sand PAH levels ranging from 60 for Chrysenes to 300 for Naphthalenes. See section 12) Images (below) for additional figures summarizing these results.

Three enzymes were selected as biomarkers for petroleum hydrocarbon exposure and effects: ethoxyresorufin-o-dethylase (EROD), glutathione-S-transferase (GST), and superoxide dismutase (SOD). EROD provides a direct measure of cytochrome p450-1A activity, so EROD and GST can be used to assess induction of the phase 1 and phase 2 metabolic pathways responsible for the processing and excretion of toxic organic compounds (including PAHs and

other hydrocarbons) in fish and other animals. SOD detoxifies reactive oxygen species that are generated by exposure to PAHs and other oil components, and provides an additional measure of response to petroleum exposure. A number of previous studies have shown that these enzymes are upregulated in response to exposure to petroleum hydrocarbons and PAHs. For enzyme analyses, 50-100 mg of sample (wet weight) was homogenized in cold Tris-DTT-glycerol buffer, centrifuged at 10000g, and the supernatant (S9 fraction) used for enzyme analyses. EROD was assayed by following the formation of resorufin from ethoxyresorufin by fluorimetry, using minor modifications of published methods (Eggens and Galgani 1992; Vehniainen et al 2012). GST was measured spectrophotometrically using the conjugation of 1-chloro-2,4,dinitrobenzene (CDNB) to glutathione (Habig et al. 1974). SOD was determined by measuring the inhibition of the reaction of a tetarazolium dye (WST-1) with superoxide anions produced by the xanthine/xanthene oxidase reaction using kits purchased from Sigma-Aldrich. All assays were performed in triplicate in 96-well plates in multimode plate readers. Enzyme activities were normalized to protein concentrations in the s9 fractions; protein was measured according to Bradford (1976).

Across all fish species sampled, GST and SOD activities were 2-3 times higher at the Grand Bay and Bay Jimmy sites than in Apalachicola Bay. EROD activities were lowest in fish from ANEER and Bay Jimmy, but nearly 5-10 times higher in fish from Grand Bay. Among species, the highest EROD activities were found in pinfish and hardhead catfish, and the lowest in croaker and seatrout. Hardhead catfish and spot had the highest SOD activities, and the highest GST activities were found in hardhead catfish and mullet. However, detailed interspecies comparisons are difficult as not all species were collected at all locations, raising the possibility that the observed differences may reflect environmental conditions at the various sampling sites, rather than actual differences among species. For species collected at multiple sites, SOD and GST activities were significantly higher in mullet from Grand Bay than those from Apalachicola, SOD and GST activities in hardhead catfish from Bay Jimmy and Grand bay were not different. Taken together, the results suggest that some, but not all of the biomarkers responded as expected; work in this area is continuing.

LaTrisha Allen, FAMU, will use results from the biomarker studies as part of her doctoral dissertation. She has completed two years in the Ph.D program at FAMU, with graduation expected by 2015. A proposal to complete her work, which will compare of biomarker results from fish, plankton and mollusks, and continue sampling in the same locations, has been funded by the NOAA Environmental Cooperative Science Center. Another FAMU student, Judith Sarkodee-Adoo, who was supported by the Chanton et al. project that used carbon isotopes to trace oil intrusion into food webs (as mentioned in section 1 above), used tissues from fish collected for the present project for her MS thesis work; she competed her degree in summer 2012. In addition to the FIO funded work, an additional FAMU student, Jessica Wise, used otoliths extracted from our sampled fish for a MS thesis project, funded by NOAA's Environmental Cooperative Science Center. This project has been completed and her thesis was successfully defended in spring 2012.

3) Cruises and Field Expeditions

Samples were collected at field sites at beaches and nearshore waters from July 2010 through Spring 2012. As this was a coastal/nearshore project, we collected our samples from beaches or marshes, or using small outboard boats. No ship time was used.

4) Peer-reviewed publications, if planned (Note: a special section will focus on student and post-doctoral publications)

Snyder, R.A., G. Exline, C. Welch, A. Vestal, R. Pelot, M. Ederington-Hagy, F. Hileman. Coquina *Donax variabilis* as indicators of coastal PAH pollution along sandy beach shorelines. (submitted for review).

5) Presentations and posters

Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date
Coquina *Donax variabilis* as indicators of coastal PAH pollution along sandy beach shorelines.	Snyder	Snyder, R.A., G. Exline, C. Welch, A. Vestal, R. Pelot, M. Ederington-Hagy, F. Hileman.	Coastal and Estuarine Research Federation	yes	Nov 2011

6) Other products or deliverables

Sand and water monitoring data are posted on the CEDB website: http://uwf.edu/cedb/

7) Data:

Two attachments are included with this report. One is a spreadsheet containing the metadata for the fish collected to date for biomarker analysis. Biomarker data generated from these samples to date are stored on multiple computers at FAMU. The other is a text document describing the samples collected and processing protocols for beach sand, water and Coquina clams collected along the Florida panhandle. After publication, data files for water, sand, fish and coquina tissue samples will be forwarded to the Harte Institute for archival purposes.

PARTICIPANTS AND COLLABORATORS

8) Project participants

First Name	Last Name	Role in Project	Institution	Email	Gender	Race	Citizenship
Richard	Snyder	Co-PI	UWF	rsnyder@uwf.edu	Male	White	USA
Melissa	Hagy	Technician	UWF	mederingtonhagy@uwf.edu	Female	White	USA
Fredrick	Hileman	Faculty	UWF	fhileman@uwf.edu	Male	White	USA

Charles	Jagoe	Co-PI	FAMU	charles.jagoe@famu.edu	Male	White	USA
Jennifer	Cherrier	Co-PI	FAMU	Jennifer.cherrier@famu.edu	Female	White	USA

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	Gender	Race	Citizenship
Lauren	Campanella	BS	Field sampling and PAH analysis	UWF	Snyder	2014	Female	White	USA
Marie	Gaona	BS	Field sampling and PAH analysis	UWF	Snyder	2012	Female	White	USA
Chelsea	McCurry	BS	Field sampling and PAH analysis	UWF	Snyder	2016	Female	White	USA
Jessie	Rosenbalm	MS	Field sampling and PAH analysis	UWF	Snyder	2012	Female	White	USA
Kaitlyn	Toebe	BS	Field sampling and PAH analysis	UWF	Snyder	2014	Female	White	USA
Alexandra	Vestal	BS	Field sampling and PAH analysis	UWF	Snyder	2014	Female	White	USA
Christina	Welch	BS	Field sampling and PAH analysis	UWF	Snyder	2014	Female	White	USA
Gracie	Barnes	BS	Field sampling and PAH analysis	UWF	Snyder	2014	Female	White	USA
Robert	Pelot	BS	PAH analysis	UWF	Snyder	2014	Male	White	USA
Christopher	Maxwell	BS	Field sampling and PAH analysis	UWF	Snyder	2014	Male	White	USA
Preston	Shisgal	BS	Field sampling and PAH analysis	UWF	Snyder	2014	Male	White	USA
LaTrisha	Allen	PhD	Field Sampling and Biomarker Response	FAMU	Jagoe	2015	Female	Afr Am	USA
Kali	Farris	MS	Field Sampling and Biomarker Responses	FAMU	Jagoe	2012	Female	Afr Am	USA
Diana	Johnson	MS	Field Sampling and Biomarker Responses	FAMU	Jagoe	2012	Female	Afr Am	USA
Jessica	Wise	MS	Field Sampling and Biomarker Responses	FAMU	Jagoe	2012	Female	Afr Am	USA

10) Student and post-doctoral publications, if planned

Wise, J.S. 2012. Trace metal otolith analysis of Gulf of Mexico fishes. MS thesis, Florida A&M University, May 2012 (degree completed)

Allen, L. Comparison of biomarkers of effect and exposure in fish and invertebrates after the Deepwater Horizon Oil Spill. PhD dissertation, anticipated completion 2015

Allen, L., Jagoe, C. et al. Biomarkers of oil exposure and effect in estuarine fish from the northern Gulf of Mexico after the Deepwater Horizon oil spill. Anticipated submission to Environmental Pollution by December 2013.

11) Student and post-doctoral presentations and posters, if planned (Please provide copies of each)

Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date
Trace metal otolith analysis of Gulf of Mexico fishes	Wise	J Wise	6th NOAA Educational Partnership Program Forum	Y	Mar 2012
Biomarker responses in estuarine fish after exposure to Deepwater Horizon oil	Allen	L. Allen, C. Jagoe et al.	Annual Meeting of the Society of Environmental Toxicology and Chemistry (planned submission May 2013)	Not yet	Nov 2013

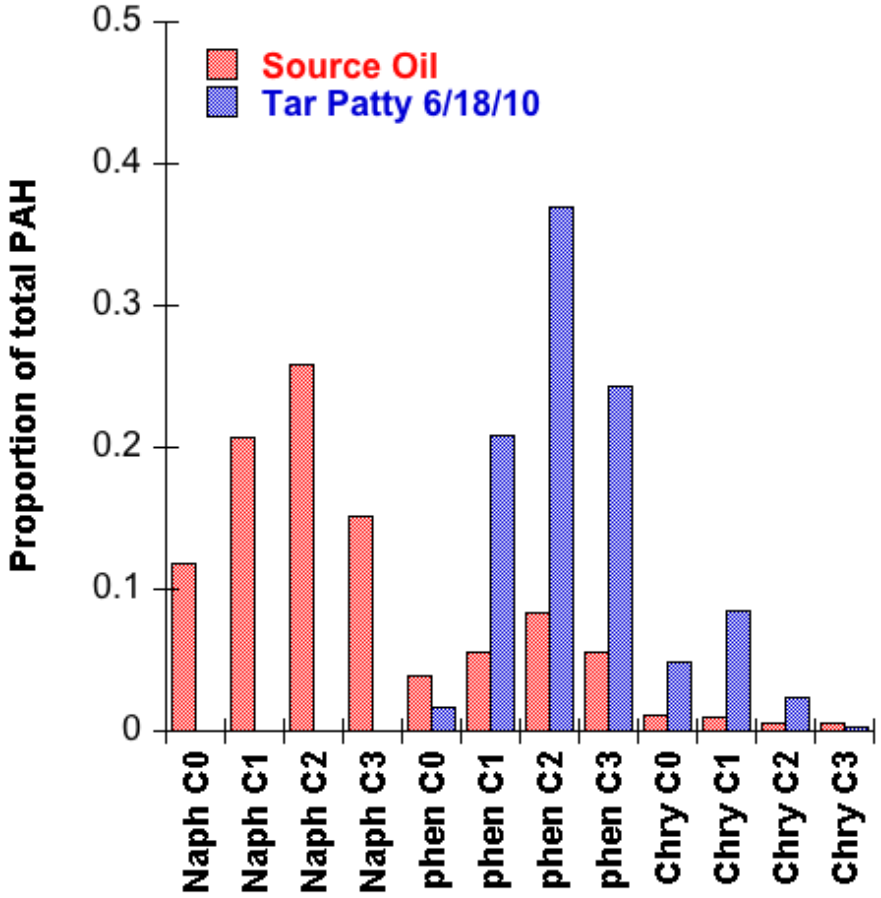
12Figures

Coquina clams from Florida panhandle beaches.

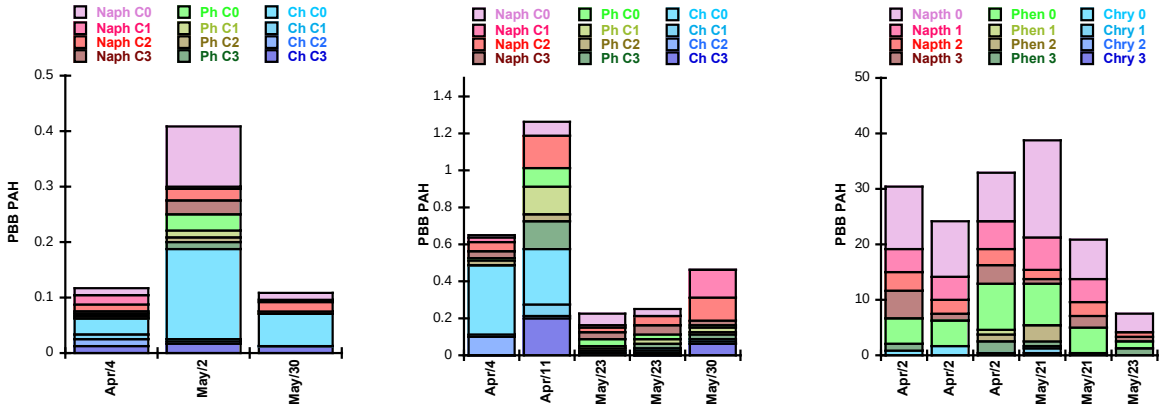
Tar patty and Coquina

Removal of Coquina tissue from shells

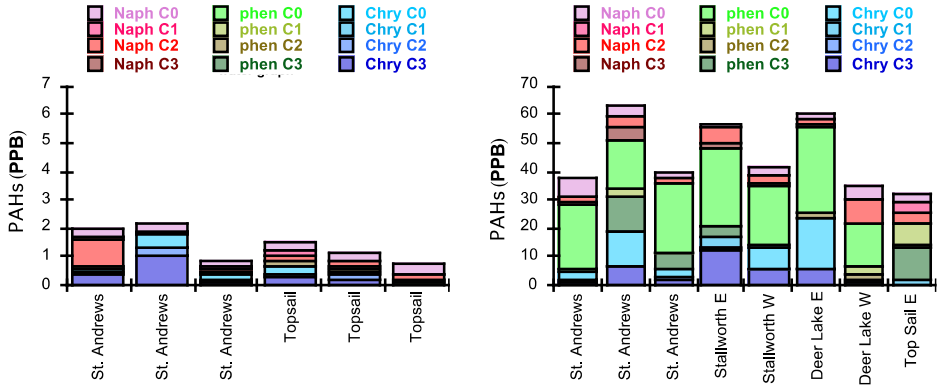
Coquina tissue extraction



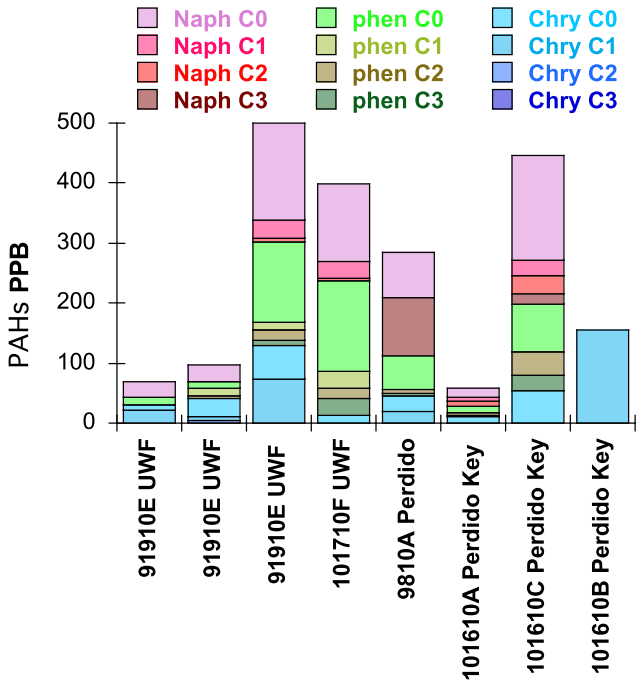
Loss of lighter PAH fractions with weathering. Tar patties are more representative of PAH exposure to Florida Panhandle beaches than the source oil



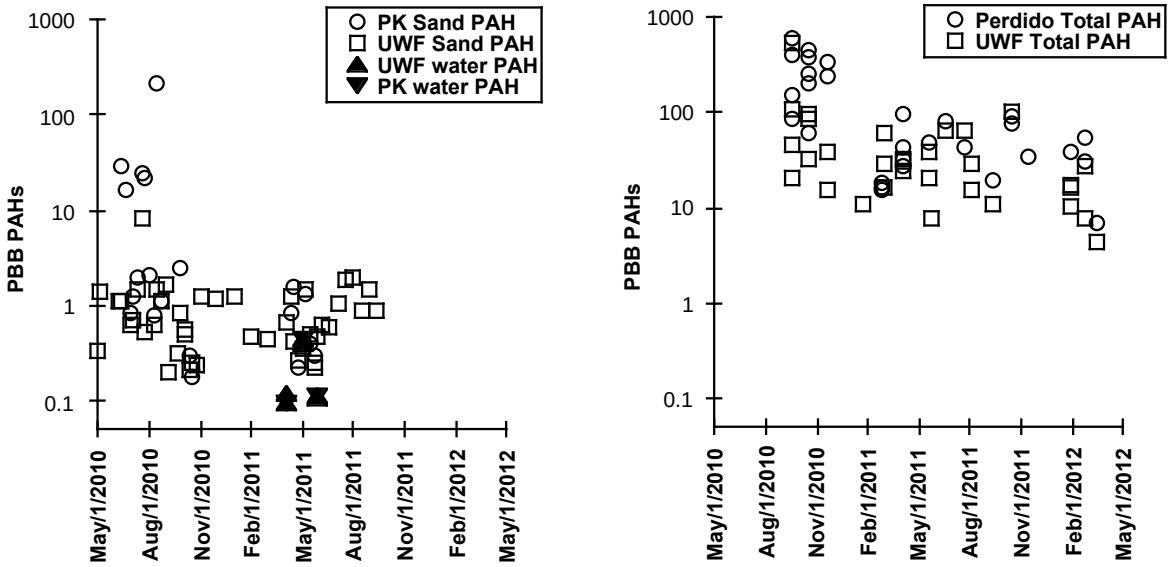
Composition of the selected PAHs in surf zone seawater (left), surf zone sand (middle), and Coquina tissues (right) at the UWF beach property on Santa Rosa Island during April and May of 2011.



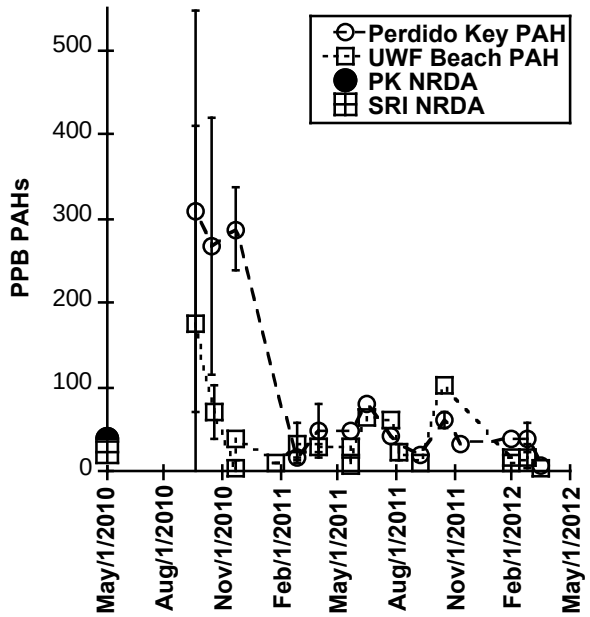
Sand (left) and Coquina (right) PAH concentrations for March 2011 sampling at St. Andrews State Park, and Topsail Beach sites (Stallworth E & W, Deer Lake E & W, Top Sail E).



Variation among replicates taken at the UWF property Santa Rosa Island and Perdido Key in September and October of 2010.



PAH concentrations in surf zone sand (left) and Coquina tissues (right)from May 2010 to May 2012. Coquina collections did not start until September 2010, water analyses were non-detect after June 2010, and sand analyses were non-detect after September 2011. High sand concentrations were coincident with oil mats washing up on the beaches.



Time series of Coquina tissue concentrations as means and standard deviations of replicate samples. Tissue concentrations were high in the fall of 2010 following deposition of oil mats on these beaches, but dissipated by the following spring. At the end of the series levels were approaching detection limits for the constituent molecules, and were similar to Coquina samples taken by Escambia County, FL officials prior to oil impacts for the NRDA process on Perdido Key (PK) and Pensacola Beach, Santa Rosa Island, FL (SRI).

Time series data for PAH concentrations in sand (top) and coquina tissues (bottom).

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).

LaTrisha Allen's doctoral work at Florida A&M University will continue/build on the present study. Her continuing research is funded through "Environmental Cooperative Science Center, A Regional Ecosystem Approach for the Conservation and Sustainable Management of Coastal and Marine Resources", Cooperative agreement NA11SEC48100001 with NOAA's Educational Partnership Program and Florida A&M University (Sept 2011-August 2016)