FLORIDA GULF COAST UNIVERSITY: Impacts from MC252 Oil on Ecologically and Commercially Important Plankton of the Gulf of Mexico

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SCIENCE ACTIVITIES

1) **General Summary:** The Macondo well blowout occurred on 20 April 2010. It was capped 86 days later on 15 July 2010 after releasing up to 4.9 million barrels of oil. Immediate concern focused on mechanical toxicity of oil adhering to large animals causing physical injury or death. However, a more insidious and potentially more ecologically significant impact was chemical toxicity to planktonic organisms, particularly specialized plankton, termed neuston, that live at the sea surface. Surface-active compounds, both natural and anthropogenic, often forms a film at the sea surface microlayer (SSML) that can be enriched in a numerous toxicants including petroleum hydrocarbons, particularly PAHs. Thus, there was a concern that the SSML could be contaminated from: oil rising to the surface during the event, COREXIT both injected at depth and aerial-sprayed, and PAH residues from burning oil. Following the discovery of sub-surface plumes, there was also the lingering concern that the SSML could be contaminated from small droplets of oil (10-50 μm) slowly rising to the surface over the coming month(s) possibly at great distances from the well-head, having traveled with currents at depth. In August 2010, these concerns were raised at meeting sponsored by Unified Area Command to obtain feedback on their monitoring plan, i.e., the so-called "Sub-Surface Oil Sampling Plan". Although their response was positive, i.e., that they recognized the risk of a contaminated SSML, to our knowledge there was no coordinated effort by a governmental agency to sample the SSML after the blowout.

Accordingly, when grant funds became available (4 October 2010), we contracted with AEOS (St. Petersburg, FL) to fabricate a remotely controlled, battery-powered unmanned surface vehicle with a rotating Teflon drum that could collect SSML and sub-surface water uncontaminated with oil from the sampling vessel (adapted from existing designs: O. Wurl, pers. comm.; A. Stortina, pers. comm.). A total of 68 water samples (including 12 field blanks and 2 field duplicates) were collected during 6 trips (3 in northern Gulf and 3 off SW Florida as reference site; see cruise and field operations). In addition, 18 samples each of neuston and plankton were collected (along with 2 field duplicates). These samples were then subjected to the following analyses:

1. Ex situ screening bioassays of SSML and subsurface water (0.5 m and mid-depth) using :
    - Newly developed chronic (24-hr exposure) Microtox type assay (*Vibrio fischeri* bacteria),
    - 48-hr exposure of copepods (*Acartia tonsa*)
    - 48- hr exposure of early life stages (ELS) of urchins (*Lytechinus variegatus*)
2. Chemical assays of water for PAHs (in collaboration with France's Centre of Documentation, Research and Experimentation on Accidental Water Pollution) and a component of COREXIT (i.e., DOSS; contract laboratory: ALS | Environmental, Kelso, WA)
3. Analyses of wild-caught neuston and plankton for biomarkers of exposure and effects
    - Chemical assays of PAHs residues in bulk neuston/plankton
    - Morphological examination of targeted taxa in neuston/plankton (e.g., copepods, finfish)
    - Biochemical assays on bulk neuston/plankton for enzyme induction (e.g., EROD, etc.; in collaboration with Florida A&M University)

Additionally, to validate the bioassays, we conducted toxicity tests on copepods, urchin and red drum (*Sciaenops ocellatus*) embryos exposed to chemically-enhanced water accommodated fraction (CE-WAF) of Source Oil B (A0031B) that was finally obtained from BP's consultant, AECOM (Fort Collins, CO) in August 2011.

These various lines of evidence were synthesized and reconciled through a weight-of-evidence (WOE) procedure to assess the possibility of lingering toxicity from MC252 oil on plankton.

Results and scientific highlights Sub-samples of Source Oil B were artificially aged by heating and then combined with COREXIT9500 to produce a chemically enhanced-water accommodated fraction (CE-WAF) based on CROSERF protocols (Aurand & Coelho 2005, Smith 2010); loading rates were 1983 ± 18 mg/L oil and 233 ±2 mg/L COREXIT (n=9 tests). Nominal concentrations in serial dilutions were adjusted based ∑PAH measured in 4 CE-WAFs (mean solubility of oil: 0.29% ±0.09%, n = 4). To assess photo-enhanced toxicity, exposures were done under a 12-hr photoperiod with fluorescent supplemented with UV (315-800 µWcm-2 UVA, 12-28 µWcm-2 UVB).

The copepods were extremely sensitive to the CE-WAF relative to the other two species (Table 1). The least sensitive bioassay for the PAHs was the modified Microtox assay (*Vibrio fischeri*; data not shown).

Table 1. Median 24-hr EC50 / LC50 values (95%CI) for Source Oil B for copepod, urchin and red drum.

Species	Endpoint	No. of Tests	EC_{50} (mg/L)	LC_{50} (mg/L)
Copepod	Mortality	4		0.05 (0.02 – 0.11)
Urchin ELS	Normal Development	2	0.72 (0.24 – 2.21)	
Red Drum ELS	Viable Hatch & Mortality	3	0.78 (0.19 – 3.25)	1.08 (0.22 – 5.28)

Comparisons of these results with published values (Fucik et al. 1995, Neff et al. 2000, McIntosh et al. 2010) or presented at a recent special session on the DWH blowout (SETAC, 11-15 Nov 2012) must be done cautiously where there are inconsistencies in method (e.g., weathering, loading, mixing speeds, etc.), which may have resulted in WAFs with differing compositions, and exposure protocols (e.g., duration, UV, etc.). With that caveat in mind, these toxicity estimates were generally intermediate with results of those studies that observed toxicity.

Ambient water samples, particularly samples of SSML, were found to be toxic when screened for toxicity using the battery of bioassays (Fig. 1). This pattern of toxicity in the SSML, which occurred in samples from both regions, did not correlate with the patterns observed in ∑PAH concentrations in water samples (Fig. 2) or neuston/plankton (Fig. 3). Furthermore, there were no statistically significant vertical or offshore-onshore gradients in ∑PAH concentrations in water or tissue residues. Nor was there a significant regional difference (i.e., northern Gulf and SW Florida) in PAH concentrations in water or tissues. While ∑PAHs concentration in neuston and plankton were sometimes intercorrelated (Fig. 3) they were not correlated with ∑PAHs in water column (Fig. 2). Moreover, based on fluoranthene/pyrene and benz(a)anthracene/chrysene ratios, the majority of PAHs found in the neuston and plankton were of pyrogenic origin.

Dioctyl sulfosuccinate (DOSS), a component of COREXIT used as a tracer, was determined in 28 of the water samples (and 1 field blank and 1 duplicate) and found sporadically (30% were below detection level @ 3µg/L) at average concentration of 7.8 ±8 µg/L and ranging as high as 45 µg/L in the SSML (however, only 21 µg/L was reported in a duplicate analysis of this sample; it is noteworthy that 2.8 µg/L was also reported in the field blank). Two samples contained DOSS ≥20 µg/L, the reporting limit of the EPA monitoring effort, but only the one exceeded the 40 µg DOSS/L Aquatic Life Benchmark (EPA Mathew et al. 2012). DOSS concentrations did not differ statistically vertically or between regions.

Microscopic survey of neuston/plankton for copepods found only one species of copepod, *Centropages typicus* to occur with sufficient frequency to allow for regional comparisons (i.e., >35 individuals in 61% of samples); eggs /larvae of fish occurred at insufficient numbers to allow for regional comparisons. Nonetheless, other than two larval fish with apparent lordosis, gross morphological abnormalities were not observed in either fish larvae or copepods; although prosome length of copepods differed among regions (both male and females from Pensacola were significantly larger) prosome and ratio of prosome to urosome did not appear correlated with bioassay results or PAHs in water or tissues.

Neuston/plankton were assayed for three enzymes that would be expected to be induced by exposure to PAHs: ethoxyresorufin-o-dethylase (EROD), glutathione-S-transferase (GST), and superoxide dismutase (SOD). EROD activity was undetectable in most samples; very low levels of activity were detected only in aliquots from one sample of neuston collected off Ft Myers in May 2011. SOD activity was detected in all samples with median activities of 7.8 and 6.0 units mg-1 protein from Ft Myers and Pensacola, respectively. GST activity was detected in all but one sample with median activity again higher along the Ft Myers transects than Pensacola. Taken together, these findings do not support the idea that biomarker enzyme activities were elevated in plankton from the Pensacola area relative to the Ft Myers area.

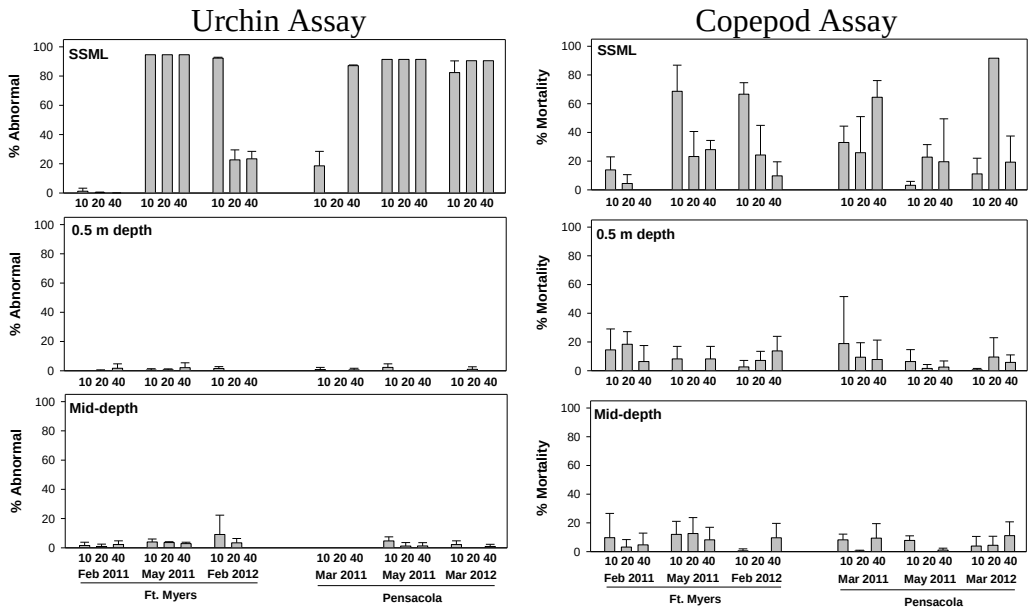

Fig 1. Abnormal development of urchins (left) and mortality of copepods (right) incubated in water collected from different depths (corresponding to different panels) along transects (10 km to 40 km) offshore of Fort Myers and Pensacola, Florida during 2011-2012.

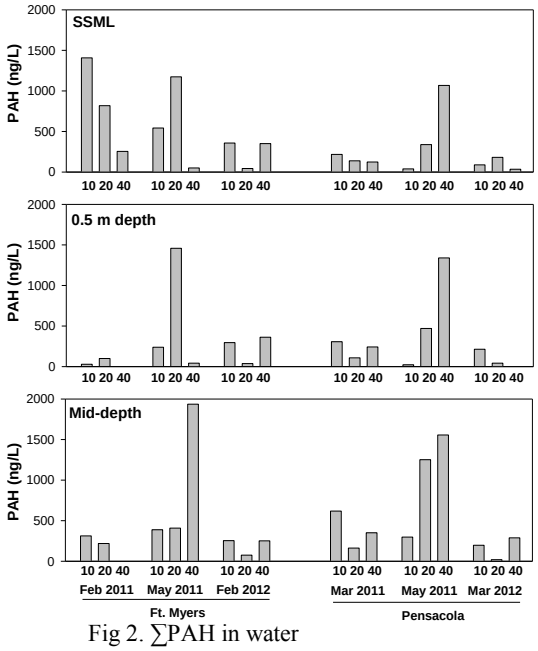

Fig 2. ∑PAH in water

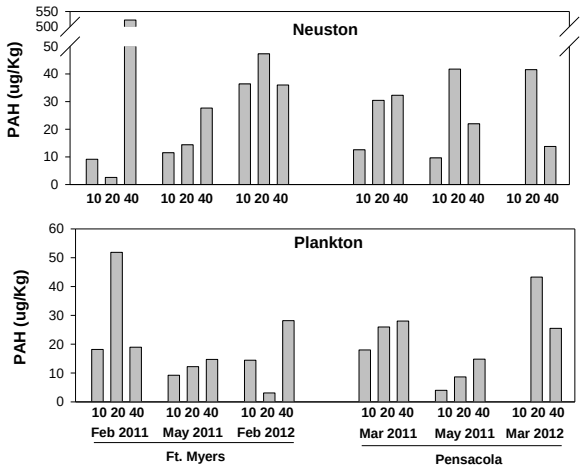

Fig 3. ∑PAH in (μg/kg) in tissue of neuston collected at the surface (top; 150 μm mesh net) and plankton collected in an oblique tow from mid-depth to 0.5 m below the surface (bottom, 150 μm mesh net).

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In closing, the weight of evidence from various measures of exposure and effects (e.g., EC50/LC50s; pattern of toxicity in *Ex situ* screening bioassays; PAH and DOSS concentrations in both water and tissue; morphological examinations and enzyme induction assays of bulk neuston/plankton) when objectively weighted based on attributes of data quality, strength of association and study design (i.e., sample size) do not support lingering impacts on the neuston/plankton. As yet, the toxicant(s) or natural toxin(s) responsible for the observed toxicity in the ex situ bioassays remains unknown. Many compounds, including naturally occurring toxins like brevetoxin, are known to concentrate at the SSML.

2) Cruises & field expeditions

Ship or Platform Name	**Class** (if applicable)	**Chief Scientist**	**Objectives**	**Dates**
C-HAWK; FGCU		Rumbold	Sample along transect from Ft. Myers out to 40 km for water, neuston/plankton	2011-02-15
Boston Whaler; UWF		Rumbold	Sample along transect from Pensacola out to 40 km for water, neuston/plankton	2011-03-17
C-HAWK; FGCU		Rumbold	Sample along transect from Ft. Myers out to 40 km for water, neuston/plankton	2011-05-10
Boston Whaler; UWF		Rumbold	Sample along transect from Pensacola out to 40 km for water, neuston/plankton	2011-05-18
C-HAWK; FGCU		Rumbold	Sample along transect from Ft. Myers out to 40 km for water, neuston/plankton	2012-02-17
Boston Whaler; UWF		Rumbold	Sample along transect from Pensacola out to 40 km for water, neuston/plankton	2012-03-15

- Peer-reviewed publications, if planned (Note: a special section will focus on student and post-doctoral publications)
    - Manuscripts submitted or in preparation (Please note target journal, and anticipated date of publication or submission)

1. Loh, A.N., Campbell, I., Guyomarch, J., Le Floch, S., Rumbold, D.R. *In Prep*. PAH concentrations in surface waters from the BP Deep-Horizon oil spill. Journal: ES&T
2. Loh, A.N., Campbell, I., Workinger, K., Rumbold, D.R. *In Prep*. Polycyclic aromatic hydrocarbon concentrations in neuston and plankton samples from the coastal waters off Pensacola and Fort Myers, Florida. Journal: ES&T
3. Jogoe et al. *In Prep.* Biomarkers of polycyclic aromatic hydrocarbon in neuston and plankton collected from Gulf of Mexico following the DWH blowout. Environmental Pollution.
4. Rumbold, D.G., Jagoe, C., Barreto, J., Loh, AN, Tolley, SG., Volety, A. *In Prep*. Weighing the evidence of lingering toxicity of MC252 oil to Gulf of Mexico plankton in the two years following the DWH blowout. Environmental Toxicity and Chemistry.

3) Presentations and posters, if planned (select copies are attached) (Note: a special section will focus on student presentations)

Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date
What level of human toxicity is engendered by a Marine oil spill?	Barreto, J.,	Griffith A., Torres, J., Sweeney, J., and Barreto, P.	Oceans: Protecting our lives and livelihood. Naples Florida.	no	Sept. 2010
Impacts from MC252 Oil on ecologically and commercially important plankton of the Gulf of Mexico	Rumbold, D.G.	Jagoe, C., Barreto, J., Loh, AN, Tolley, SG., Volety, A.	Invited presentation to the Centre of Documentation, Research and Experimentation on Accidental Water Pollution, (CEDRE) Brest, France	no	June 30, 2011
Polycyclic aromatic hydrocarbon concentrations and bioassays of sea-surface microlayer and subsurface water collected from the Gulf of Mexico off Pensacola and Fort Myers, FL.	Loh, A.N.	Campbell, I., Guyomarch, J., Le Floch, S., Workinger, K., Gant, D., Barreto, P., Barreto, J., Rumbold, D.	33th Annual Meeting of Society of Environmental Toxicology and Chemistry. Long Beach, CA.	yes	Nov 11-15, 2012
Polycyclic aromatic hydrocarbon concentrations in neuston and plankton samples from the coastal waters off Pensacola and Fort Myers, Florida.	Campbell, I.	Workinger, K., Rumbold, D., Loh, A.N.	33th Annual Meeting of Society of Environmental Toxicology and Chemistry. Long Beach, CA.	yes	Nov 11-15, 2012
Characterization and detection of polycyclic aromatic hydrocarbon in water	Loh, A.N.	Campbell, I., Guyomarch, J., Le Floch, S., Workinger, K., Coughlin, M., Rumbold, D.	Submitted for SETAC Europe 23rd Annual Meeting, Glasgow, Scotland		12-16 May 2013

4) Other products or deliverables
Please list (for example: maps, models, tools) and indicate where they can be located/obtained.

5) Data

*Please provide a spreadsheet indicating the **metadata and ancillary information on the location and status of the archived samples**. Also, indicate if there are any issues with respect to data archiving schedule and plan. If you have a lot of metadata, representative samples will suffice. This will all be incorporated into the GoMRI database at some point in the future.*

Example shown below:

Trip No.	Date	Collection Location	Sample No.	Purpose	Contents	Container	Collection Method	Time	Site	Lat/Long	Depth	Archive sample Location
1	2011-02-15	Ft. Myers, FL	1	QC	DI H2O	2 L	Pour over rotating drum	07:35		N/A	N/A	dry lab freezer Vester
1	2011-02-15	Ft. Myers, FL	2	QC	DI SW	2 L	Pour over rotating drum	07:45		N/A		dry lab freezer Vester
1	2011-02-15	Ft. Myers, FL	3	Bioassay / Chemical Analysis	Sea Water	2 L	Sub-Surface	10:02	25 mi	N 26°10 W 82°13	60 ft	dry lab freezer Vester
1	2011-02-15	Ft. Myers, FL	4	Bioassay / Chemical Analysis	Sea Water	2 L	SSML	10:22-11:28	25 mi	N 26°10 W 82°13	60 ft	dry lab freezer Vester
1	2011-02-15	Ft. Myers, FL	5	Bioassay / Chemical Analysis	Sea Water	2 L	0.5 m	10:22-11:28	25 mi	N 26°10 W 82°13	60 ft	dry lab freezer Vester
1	2011-02-15	Ft. Myers, FL	6	Plankton Analysis	Neuston	white lid	150 µm net w/ floats	10:51-10:58	25 mi	N 26°10.461 W 82°13.421	60 ft	KW @ Vester

PARTICIPANTS AND COLLABORATORS

6) Project participants

Please list the participants of your project, their role(s) and contact information. No personal information will be released. **Note: Student/educational information will be collected elsewhere in this report**.*

** We understand one person may fulfill more than one role; please list all applicable roles using the following standardized titles: Principal Investigator, Co-Principal Investigator, Scientific Participant, Technician, Lab Assistant, Administrative Support.*

First Name	Last Name	Role in Project	Institution	Email
Charles	Jagoe	Co-Pi coordinating students in biochemical biomarker assessment of plankton	FAMU	charles.jagoe@famu.edu
Jose	Barreto	co-PI coordinating students in bacteria bioassays	FGCU	Jbarreto@fgcu.edu
Ai Ning	Loh	co-PI coordinating technician and students in PAH/dispersant analyses	FGCU	anloh@fgcu.edu
Aswani	Volety	co-PI coordinating students in bioassays, toxicity testing and to a lesser extent morphological/cytogenetic assessment of plankton	FGCU	avolety@fgcu.edu
Greg	Tolley	co-PI coordinating students in bioassays, toxicity testing and morphological/cytogenetic assessment of plankton	FGCU	Gtolley@fgcu.edu
Julien	Guyomarch	Collaborator on analytical determinations	Centre of Documentation, Research and Experimentation on Accidental Water Pollution (CEDRE)	Julien.Guyomarch@cedre.fr

MENTORING AND TRAINING

7) Student and post-doctoral participants

Please list the student participants of your project, their educational role, and other information. No personal information will be released.

First Name	Last Name	Post-doc / PhD / MS / BS	Thesis or research topic	Institution	Supervisor	Expected Completion year
Kelsey	Workinger	MS	Tox tests; Cytogenetic, Morphological aberrations in plankton	FGCU	Rumbold	2013
Morris	Brad	BS	bioassays	FGCU	Rumbold	2011
Nathalie	Goupil	MS	Copepod assays	Univ. Brittany	Rumbold	No longer working on project
Drew	Liddick	MS	Non-thesis-RA: Field collections, Tox tests, brood stock maint.	FGCU	Rumbold	2014
Jeff	Devine	MS	Non-thesis-RA: Field collections, brood stock maint.	FGCU	Rumbold	2013
Christopher	Lienhardt	BS	Analytical determination of PAHs	FGCU	Loh	2012
Brian	Lumley	BS	Analytical determination of PAHs	FGCU	Loh	2011
Dawn	Gant	BS	Bacterial assay	FGCU	Barreto	2012
Kali	Farris	MS	Non-thesis-RA: Enzyme Induction	FAMU	Jagoe	2012
LaTrisha	Allen	PhD	Comparison of oil effects on biomarkers in fish and invertebrates	FAMU	Jagoe	2015

8) Student and post-doctoral publications, if planned

a. Published, peer-reviewed bibliography (Copies of the papers are requested)
b. Manuscripts submitted or in preparation (Please note target journal, and anticipated date of submission or publication)

1. Workinger, K., Loh, A.N. and Rumbold, D.G. *In Prep*. Toxicity of MC252 oil to copepods and early-life stages of a sea urchin and finfish. Mar. Pollution Bull.

9) Student and post-doctoral presentations and posters, if planned (copy attached)

Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date
Toxicity of MC252 oil to copepods and early-life stages of a sea urchin and finfish.	Workinger, K.,	Loh, A.N. and Rumbold, D.G.	33th Annual Meeting of Society of Environmental Toxicology and Chemistry. Long Beach, CA.	yes	November 11-15, 2012

10) Images

Please attach high-resolution images and provide details including a description of the image, location, credit, date, etc. Of note: Image may be used in FIO or GoMRI promotions, so please make sure you have rights to use the image.

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 will continue/build on the present work. She is funded by "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 2012)