UNIVERSITY OF SOUTH FLORIDA, COLLEGE OF MARINE SCIENCE

ASSESSING THE CONCENTRATION AND THE MOLECULAR AND ISOTOPIC COMPOSITION OF DEEP-SEA SUBMERGED OILS IN THE NORTHERN GULF OF MEXICO

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PRINCIPAL INVESTIGATOR

SCIENCE ACTIVITIES

1) General Summary

Narrative (1 pages maximum): Please provide a brief overview of the project and goals supported during the conduct of this project. Be sure to highlight any 'lessons learned' that could be applied to other/future oil spill related projects (e.g., management, data support, logistics, etc.). Listing accomplishments against project activities, objectives and milestones in bulleted form is acceptable.

The goal of this project were to determine the concentration and chemical composition hydrocarbons throughout the water column focusing on the different layers comprising the deep-sea plumes/intrusions. Utilizing techniques in molecular organic and isotope geochemistry this research aims to develop a "chemical fingerprint" for the subsurface oil that future researchers can trace the flow and cycling of oil within the marine waters and sediments and within marine ecosystems and habitats. During two research cruises, August and December 2010 we were be able to evaluate how the concentration and chemical composition subsurface hydrocarbons are changing over time and space. This study of subsurface oils is also vitally needed for determining environmental baselines, assessing the persistence of the subsurface hydrocarbons in the plume layers with time and transport away from the blowout site, understanding ecosystem responses and evaluating the causes of biological and chemical changes associated with pre- and post-impact assessments and restoration. . The outcomes are related to the goals below:

1. Determine the concentration and chemical composition of hydrocarbons (e.g., molecular abundance and distribution of organic compounds) in the different layers that comprised the deep-sea plumes

2. Develop a "chemical fingerprint" for the subsurface oil, using a combination of molecular distribution and CSIA so that future researchers can trace the cycling of oil within the marine waters and sediments and within marine ecosystems and habitats.

3. Evaluate how concentration and chemical composition subsurface hydrocarbons are changing temporally and spactially in the Northern Gulf of Mexico (GoM).

4. Evaluate how concentration and chemical composition surface and subsurface oils are potentially toxic to photoautotrophic organisms (algae and bacteria) the Northern Gulf of Mexico (GoM)

Note: Because the subsurface hydrocarbons plumes has disappeared by the time of the December 2010 cruise (see results) and because we were detecting abundant and widespread hydrocarbons distributions in the surface sediments, I requested to Dr. Bill Hogarth (Project Director for the BP-FIO block grant) that a potion of the remaining funds be repurposed to intensify the study of hydrocarbons in the complimentary sediment study entitled "Assessing the impact of the Deepwater Horizon oil spill on sediments and benthic communitities on the west Florida Shelf". Rather than waste time consuming and expensive analyses on samples that were consistently showing low, background hydrocarbons concentrations, Dr. Hogarth agreed to the repurposing of the research funds to focus on sediments. For scientific consistency, results from the sediment hydrocarbon study are presented in the "complimentary" sediment studies final report, authored by Hollander and Schwing.

Lessons Learned:

The use of an *in-situ* large-volume filtering device at the depths where the sub-surface plumes were located was critical for the success of the project. Compound-specific isotopic analyses of the n-alkanes provided the "chemical finger-printing" technique to indisputably link the origin of

the sub-surface plumes of hydrocarbon to a source from the DwH blowout. Coupling total petroleum hydrocarbon concentrations to "real-time" bioluminescence toxicity assays and mutigencity tests has proven to be a powerful set of tools in documenting the effect of the Deepwater Horizon on primary producers. Because the concentration of sub-surface hydrocarbon had dropped to background signals by December 2010, 5 months after the DwH blowout was stopped, greater emphasis on settling particles, their composition and concentrations, would have provided a more direct link to the processes responsible for and the occurrence of sedimentary oil deposition.

2) Results and scientific highlights

Narrative (2 pages maximum): This should be a summary of significant results (positive and negative) and conclusions during the conduct of this project. Listing science results and highlights in bulleted form is acceptable. In each case, please explain the impact of the result.

Results presented by each goal are:

1. To determine the concentration and chemical composition of hydrocarbons (e.g., molecular abundance and distribution of organic compounds) in the different layers that comprised the deep-sea plumes.
    - Total petroleum hydrocarbon (TPH) concentrations and distribution were measured along two transects (DSH and PCB) during two cruises: August 2010 (4 sites) and December 2010 (5 sites). For comparison, results were compared to similar samples collected in May 2010. Approximately 10 water depths were sampled per site using a large-volume filtering apparatus to capture enough material for chemical finger printing using compound specific isotopic analyses. Between 100 – 300 liters of sea water were filtered per sampling depth.
    - Along the DSH transect, between 26-45 nM ENE of the DWH blowout site, May and August 2010, three distinct layers enriched in total petroleum hydrocarbons were observed: at eh the surface, in two subsurface plumes located at 400 m and 100-1200 meters water depth.
    - In May and August 2010, TPH concentrations varied between 75 and 800 ug/l. TPH concentrations at the surface were up to 400 ug/l, at ~400 m water depth TPH was 350-425 ug/l and between 1000-12000 m water depth TPH ranged from 625 – 785 ug/l.
    - In May and August 2010, samples from other water depth (above and below the 400 m and 1000-1200 m plumes) had a TPH that did not fall below 75 ug/l.
    - In May and August 2010, both sub-surface plumes at 400 and 1000-1200 m) showed nearly identical molecular distributions. Both plumes were concentrated in the High Molecular Weight (HMW) n-alkanes with carbon numbers greater than C-26. Only in surface samples were both Light and heavy molecular weight hydrocarbons present.
    - In December 2010, all sites from the two transects lines showed low TPH concentrations in the range between 10 – 50 ug/l and showed no distinct surface or sub-surface enrichment.

2. Develop a "chemical fingerprint" for the subsurface oil, using a combination of molecular distribution and Compound-specific Isotopic Analyses (CSIA) so that future researchers can trace the cycling of oil within the marine waters and sediments and within marine ecosystems and habitats.

- Using CSIA, Macondo 252 source oil had a molecular isotopic composition of n-alkanes that exhibited four "finger-print" characteristics: 1) the range of $\delta^{13}C$-values for all hydrocarbons varied by up to 4‰, from -29 to -33‰, 2) $\delta^{13}C$ values of C16- C25 n-alkanes were constant at ~-30‰, 3) $\delta^{13}C$ values of C27-C34 n-alkanes showed a continuously decreasing trend from -30 to -33‰, and 4) $\delta^{13}C$ values of n-alkanes >C34 showed a trend to increasing values back up to -30‰
- Surface samples collected in May and August 2010 showed these exact CSIA $\delta^{13}C$ characteristics and were determined to be sourced from the Macondo 252 oil
- Subsurface hydrocarbons at 400 and 1000-1200 m water depth were concentrated in HMW n-alkanes >C-26 and showed the exact same CSIA trends characteristics of decreasing $\delta^{13}C$ values between C27 – C34 and were determined to be sourced from the Macondo 252 oil
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3. Evaluate how concentration and chemical composition subsurface hydrocarbons are changing temporally and spatially in the Northern Gulf of Mexico (GoM).
    - In May and August 2010, the water column profiles of TPH looked identical and varied between 75 and 800 ug/l.
    - For both May and August 2010 samples, TPH concentrations in the shallower plume at ~400 m water depth were 350-425 ug/l whereas in the deeper plume at 1000-12000 m water depth TPH ranged from 625 – 785 ug/l.
    - In May and August 2010, independent of distance from the blowout (from 25 nM DSH 10 to 70 nM PCB 06), the TPHs within the plumes were dominated by High Molecular Weight (HMW) n-alkanes with carbon numbers greater than C-26. Only in surface samples were both light and heavy molecular weight hydrocarbons, C18 – C36, present.
    - In December 2010, all sites from the two transects lines showed low TPH concentrations in the range between 10 – 50 ug/l and showed no distinct surface or sub-surface enrichment.
    - Hydrodynamic modeling of sub-surface flow was consistent with disappearance of TPH concentrations indicating that took a minimum of 4 months for the sub-surface plumes to flow out of the northern Gulf of Mexico or be degraded once the blowout was sealed.

4. Using bioluminescent assays, evaluate how concentration and chemical composition surface and subsurface oils are potentially toxic to photoautotrophic organisms (algae and bacteria) the Northern Gulf of Mexico (GoM)
    - Bioluminescent bacteria and algae were exposed and incubated with surface and sub-surface plume water rich in TPHs.
    - Of the 14 samples measured, 21.4% were toxic to bacteria via Microtox (surface waters only) and 23% were toxic to phytoplankton via QwikLite (subsurface waters only)
    - Of the 14 samples measured , 43% were mutagenic by the Inducttest (surface and subsurface)
    - Microtox assay shown to be more sensitive to oil, QwikLite more sensitive to dispersant

3) Cruises & field expeditions

Ship or Platform Name	**Class** (if applicable)	**Chief Scientist**	**Objectives**	**Dates**
Weatherbird II		David Hollander	Collect water column samples and sediment core samples along the PCB and DSH transects	August 2010

Weatherbird II		David Hollander	Collect water column samples and sediment core samples along the PCB and DSH transects	December 2010

4) Peer-reviewed publications, if planned (Note: a special section will focus on student and post-doctoral publications)
    a. Published, peer-reviewed bibliography (Copies of the papers are requested

Joye, S.B., I. Leifer, I.R. MacDonald, J.P. Chanton, C.D. Meile, A.P. Teske, J.E. Kostka, L. Chistoserdova, R. Coffin, D.J. Hollander, M. Kastner, J.P. Montoya, G. Reheder, E. Solomon, T. Treude and T.A. Villareal, 2011, Technical Comment on "A Persistent Oxygen Anomaly Reveals the Fate of Spilled Methane in the Deep Gulf of Mexico" by Kessler et al., *Science*. 332/6033 DOI: 10.1126/science.1203307 .

    b. Manuscripts submitted or in preparation (Please note target journal, and anticipated date of publication or submission)

Hollander, D.J., R. Larson, I. Romero, P. Schwing, K. Watson, N. Zenzola; D.W., Brooks, G., Hastings, J. Chanton, J. Kostka. W. Overholt, Mechanisms of Sedimentary Oil Deposition in Deep-Sea in the aftermath of the Deepwater Horizon Blowout Event, to be submitted to Science, May 2013.

Hollander, D.J. K. Freeman, G. Ellis, A. Diefendorf, E. Peebles, and E. Goddard, On the origin and fate of sub-surface petroleum hydrocarbon plumes associated with the Deepwater Horizon blowout in the Northern Gulf of Mexico, rejected from *Nature and Science, to be resubmitted to Organic Geochemisty, expected early summer 2013.*

Paul, J., D.J. Hollander, P. Coble, K. Daly, S. Murasko, D. English, L. McDaniels, and C. Kovach, Toxicity and Mutagenicity of Gulf of Mexico Waters Contaminated by Petroleum Hydrocarbons during the Deepwater Horizon Oil Spill, rejected from Nature and Science, to be submitted to *ES&T (Environmental Science and Technology),* June *2013.*

5) Presentations and posters, if planned (Please provide copies of each) (Note: a special section will focus on student presentations)

Title	**Presenter**	**Authors**	**Meeting or Audience**	**Abstract published (Y/N)**	**Date**
The Science Since the Spill	Hollander, D.J	Hollander, D., Ellis, G., Peebles, E., Paul, J., Goddard, E.,	Smithsonian's National Museum of Natural History (NMNH) and the Centers for Ocean Sciences Education Excellence (COSEE), Invited Presentation in the *Changing Tides* lecture	Video Simulcast	March 2011

			series		
Origins and Fate of Oil in the Northern Gulf of Mexico: Ongoing Chemical, Biological and Geological Perspectives	Hollander, D.J.	Hollander, D., Ellis, G., Peebles, E., Paul, J., Goddard, E.,	National Science Teachers Association (NSTA) and the Centers for Ocean Sciences Education Excellence (COSEE), Invited Presentation		April 2011
Why subsurface oils associated with the BP Deepwater Horizon blowout are so long-lived and remain toxic?: A molecular organic geochemical perspective,	Hollander, D.J	Hollander, D., Freeman, K. Ellis, G, Diefendorf, A., Goddard, E., Peebles, E., and Paul, J.P.,	Keynote Lecture, Gordon Research Conference on Chemical Oceanography,	N	August 2011
Characterizing the Nature, Molecular Distribution and Isotopic Composition of Sub-Surface Oil Over Space and Time	Hollander, D.J.	Hollander, D., Ellis, G., Peebles, E. and Paul, J., Goddard, E.,	FIO- BP's 10M PI's Meeting	N	Sept. 2011
Deposition, Distribution and Fate of Macondo Oil in the Sediments of the Northern Gulf of Mexico,.	Hollander, D.J.	Hollander, D.J., B. Flower, D. Naar, R. Weisberg, K. Daly, I. Romero, M. Robert,	NSTC- SOST Meeting,	y	October 2011
Long-Lived, Sub-Surface Layers of Toxic Oil in the Deep-Sea: A Molecular Organic and Isotopic Geochemical Approach to Understanding their Nature, Molecular Distribution, Origin and Impacts to the Northern Gulf of Mexico,	Hollander, D.J	Hollander, D., Freeman, K., Ellis, G., Diefendorf, A., Peebles, E. and Paul, J.,	American Geophysical Union- National Meeting	Y	Dec. 2011
Organic geochemical evidence for oil spill impacts on fish in the Gulf of Mexico: Comparative and quantitative analyses	Hollander, D.J.	Romero, I. C., Hollander, D. J., Patterson, W. Quintana-Rizzo, E., Kane, A. Murawski, S.,	ASLO National Meeting, Salt Lake City	Y	February 2012

of polycyclic Aromatic hydrocarbons,		Peebles, E. B., Ellis, G., Zenzola, N., Torres, J. J.			
Deposition, distribution and fate of Macondo oil in the sediments of the Northeastern Gulf of Mexico	Hollander, D.J.	Hollander, D. J., Flower, B., Larson, R. Brooks, G. Romero, I., Zinzola, N. Means, Z.	ASLO National Meeting, Salt Lake City	Y	February 2012
Rapid increase in accumulation rate and shift in sedimentary regime in the NE Gulf of Mexico following the 2010 BP blowout event	Brooks, G. .	Brooks, G. R., Larson, R. A., Hollander, D. Flower, B. P., Hastings, D., Valente, A., Hill, K. ., Moore, C. Romero, I.,	ASLO National Meeting, Salt Lake City	Y	February 2012
Organic Geochemical Signatures of the DWH Oil Spill in the Gulf of Mexico: Potential Ecologic Consequences in Fish Communities	Hollander, D.J.	Romero, I., D. J. Hollander, W. Patterson, E. Quintana-Rizzo, A. Kane, S. Murawski, E. B. Peebles, J.J. Torres,	Gordon Research Conference on Organic Geochemistry	N	August 2012
Biogeochemical Radiocarbon analysis of the Gulf Oil Spill: Sediments, Plankton and Coastal Fauna.	Chanton, J.	Chanton, J., J.Cherrier. J. Sarkadee-Adoo, S. B. Joye, D. J. Hollander, W. M. Graham, C.A. Brunner, S. Bosman1, A. Mickel1, G. Brooks, R. Larson, D. Hastings,	American Geophysical Union- National Meeting	Y	Dec. 2012
Testing the Mechanisms of Sedimentary Oil Deposition in Deep-Sea.	Hollander, D.J.	Hollander, D.J., G. R. Brooks, D. J. Hollander, R. Larson, I. Romero, P. Schwing, K. Watson, N. Zenzola; D.W., Hastings, J. Chanton, J. Kostka. W.	Gulf of Mexico Oil Spill & Ecosystem Science Conference,	Y	Jan. 2013

		Overholt,			
Shifts in Reef Fish Community and Trophic Structure Following the Deepwater Horizon Oil Spill	Patterson, W.	Patterson, W., J. Tarnecki, C. Jagoe, I. Romero, D. Hollander, A. Kane, M. James,	Gulf of Mexico Oil Spill & Ecosystem Science Conference,	Y	Jan. 2013
Radiocabon analysis of the Gulf Oil Spill	Chanton, J.	Chanton, J., J. Cherrier, J. Sarkodeeadoo, W.M. Graham, S. Joye, D. Hollander, C. Brunner,	Gulf of Mexico Oil Spill & Ecosystem Science Conference,	Y	Jan. 2013
Deposition and persistance of polycyclic aromatic hydrocarbons from the first DEEPWATER oil spill: from deep-sea sediments to fish communities (Gulf of Mexico, USA)	Romero, I.	Romero, I., D.J. Hollander, G.R. Brooks, W. Patterson, S.W. Ross, J.J. Torres, A.S. Kane, S. Murawski, P. Schwing, E. Quintana-Rizzo, R.A. Larson, E.A. Goddard, N. Zenzola,	IMOG (International Meeting of Organic Geochemists)	Y	Sept. 2013
Elucidating the mechanism of sedimentary oil deposition following the Deepwater Horizon blowout event in the Northern Gulf of Mexico	Hollander, D.J.	Hollander, D.J., Romero, I.C., Schwing, P.T., Larson, R.A., Watson, K., Zenzola, N., Murawski, Brooks, G.R., Hastings, D.W., Chanton, J., Kostka, J., Overholt, W.,	IMOG (International Meeting of Organic Geochemists)	Y	Sept. 2013

(Note: All presentations from the PI and Scientific participants will be available immediately upon request)

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

- Non-Pier Reviewed:

ScienceMagazine: News and Analyses, 2013, BP Research Dollars Yield Signs of Cautious Hope, www.sciencemag.org, 8 FEBRUARY 2013 VOL 339 SCIENCE

Nature News and Comments Article: Dirty blizzard buried Deepwater Horizon oil, 26 January 2013, *Nature* doi:10.1038/nature.2013.12304

- (Sampling site map will be submitted to FIO by the end of the week, 4/13/2013)

7) 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.

- (Water column and sediment core sample locations and allotment will be submitted to FIO by the end of the week, 4/13/2013)

PARTICIPANTS AND COLLABORATORS

8) 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
David	Hollander	Principal Investigator	USF-CMS	davidh@usf.edu
John	Paul	Scientific participant- Water Column Toxicity to Photoautotrophs	USF-CMS	John Paul <jpaul@marine.usf.edu
Katherine.	Freeman	Scientific participant- Molecular Isotopic Analyses of Hydrocarbon	Penn State University	Kate Freeman <khf4@psu.edu>
Ernst	Peebles	Scientific	USF-CMS	Ernst Peebles

		participant-Water Column Sampling		<epeebles@usf.edu>
Ethan	Goddard	Technical Support	USF- CMS	Ethan Goddard <egoddard@mail.usf.edu>
Greg	Ellis	Technical Suppoort	USF-CMS	gellis@mail.usf.edu
Will	Patterson	Scientific Participant-Fisheries Ecology	University of South Alabama-Daufin Island Marine Laboratory	William Patterson <wpatterson@disl.org>

MENTORING AND TRAINING

9) 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
Isabel	Romero	Post-Doc	Sedimentary Hydrocarobn in the Aftermath of the Deepwater Horizon Blowout	USF-CMS	Hollander	2014
Patrick	Schwing	Post-Doc	Benthic Habitiat and Community Changes as a Result of the Deepwater Horizon Blowout Event	USF-CMS	Hollander	2014
Rebekka	Larson	Ph.D.	Short-lived Radioisotope Dating of Sediments Deposited During the Deepwater Horizon Blowout Event	USF-CMS	Hollander	2014
Katherine	Watson	MS	Large-scale tracking of oil-derived hydrocarbons after the Deepwater	USF-CMS	Hollander	2013

			Horizon blowout: Reconstructing spatial and temporal petroleum distribution			
Nicola	Zenzola	BS-Senior Thesis	Determinations of the Composition of Petyroleum Hydrocarbons in the Sediment of the Northern Gulf of Mexico	Eckerd College	Hollander-Major Supervisor	2012

10) 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)

Romero, I.C., Schwing, P.T., Larson, R.A., Brooks, G.R., Hastings, D.W., Hollander, D.J., Hydrocarbon deposition in deep-sea sediments following the 2010 Deepwater Horizon Blowout. Submitted to *Deep Sea Research*, Deadline May 1, 2013.

Romero, I., D.J. Hollander, G.R. Brooks, W. Patterson, S.W. Ross, J.J. Torres, A.S. Kane, S. Murawski, P. Schwing, E. Quintana-Rizzo, R.A. Larson, E.A. Goddard, N. Zenzola, Concentration and composition of Polycyclic Aromatic Hydrocarbons in mesopelagic fish in the Gulf of Mexico: Evidence of 2010 Deepwater Horizon oil spill exposure, to be submitted to Plos1, expected submission early summer 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**
Assessing the Fate and Impact of the BP Oil on Deep-Sea Sedimentary Environments and Benthic Communities: NE Gulf of Mexico	Larson, R.	Larson, R. A., G. R. Brooks, B. P. Flower, D. Hollander, D. W. Hastings, I. Romero, A. Valente, K. Hill, C. Moore,	ASLO National Meeting, Salt Lake City	Y	February 2012
Large-scale tracking of oil-derived hydrocarbons after the	Watson, K.	Watson, K., Romero, I. Murawski, S.,	Gulf of Mexico Oil Spill & Ecosystem Science Conference,	Y	Jan. 2013

Deepwater Horizon blowout: Reconstructing spatial and temporal petroleum distribution,		Hollander, D.J.,			
Spatio-Temporal Concentrations And Composition Of Polycyclic Aromatic Hydrocarbons In Fish: Evidence For DwH Oil Spill Impact On Mesopelgic And Outer Shelf-Reef Fish In The Gulf Of Mexico	Romero, I.	Romero, I., D.J. Hollander, W. Patterson, S. W. Ross, A. S. Kane, S. Murawski, E. Quintana-Rizzo, E.B. Peebles, E.A. Goddard, J. J. Torres,	Gulf of Mexico Oil Spill & Ecosystem Science Conference,	Y	Jan. 2013
Deposition and persistence of polycyclic aromatic hydrocarbons from the first DEEPWATER oil spill: from deep-sea sediments to fish communities (Gulf of Mexico, USA)	Romero, I.	Romero, I., D.J. Hollander, G.R. Brooks, W. Patterson, S.W. Ross, J.J. Torres, A.S. Kane, S. Murawski, P. Schwing, E. Quintana-Rizzo, R.A. Larson, E.A. Goddard, N. Zenzola,	IMOG (International Meeting of Organic Geochemists)	Y	Sept. 2013

(Note: Student and Post-Doctoral Presentations will be made available immediately upon request)

12) 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.

(See abbreviated visuals, All presentations are available to FIO and GoMRI promotions upon request)

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).

Funding Authority: GoMRI- C-IMAGE and Deep-C

- Complete all water column analyses from cruises conducted during 2011 and 2012
- Expand spatial domain to include both the DeSoto Canyon and the region to the WSW of the DwH blowout site
- High resolution (mm scale) sampling & analyses of sediments
- Develop CSIA-δ^{13}C and δD fingerprint for PAHs

- Link biological & ecosystem impact studies to Hydrocarbon Distribution and CSIA