

COMPARISON OF PHYSICAL AND CHEMICAL CHARACTERISTICS OF *IN-SITU* BURN RESIDUE AND OTHER ENVIRONMENTAL OIL SAMPLES COLLECTED DURING THE *DEEPWATER HORIZON* SPILL RESPONSE

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EXECUTIVE SUMMARY

The 2010 *Deepwater Horizon* oil spill was the largest in U.S. marine waters and ultimately involved a number of remedial actions that were unprecedented in scale and scope. Among these was the technique of *in-situ* burning, or the controlled combustion of spilled oil in the environment. While *in-situ* burning had been used for spill response a number of times over the years on both land and water prior to the BP spill in the Gulf of Mexico, the *Deepwater Horizon* burn operations were by far the largest application of the method: an estimated 260,000 barrels (bbl)/10.9 million gallons of oil were burned between April 28 and July 12, 2010. This volume of oil was equivalent to the total volume estimated to have spilled from the *Exxon Valdez*, the nation's largest oil spill prior to the *Deepwater Horizon*.

The scale and duration of the *Deepwater Horizon* release provided an opportunity to both refine and optimize the operational aspects of *in-situ* burning of the oil, and also to observe the behavior of the burn residue that remained after the oil had been combusted. The Macondo wellhead was located at a depth of 1,500 m, and the largest proportion of *in-situ* burns took place within 25 km of the wellhead location. As such, most of the burn residue sank into the deep waters of the Gulf of Mexico.

The fate of the large volume of burn residue was of interest, but not necessarily an operational concern or consideration. However, four months after the wellhead was capped and *in-situ* burn activities had ceased, a chance encounter by a small commercial deepwater shrimp fishery (royal red shrimp, *Hymenopeneus robustus* or *Pleoticus robustus*) with sunken tarballs at 200 m depth resulted in a closure of that fishery by federal officials and renewed interest in the environmental fate of *in-situ* burn residue. Chemical analyses of the deep water tarballs by chemistry labs supporting the spill response suggested that at least some of the tarballs were burn residues associated with response activities.

This study analyzed several oil residue matrices collected during the *Deepwater Horizon* response and from other sources, and compared similarities and differences in physical characteristics and chemical composition with the objective of identifying features that appeared to be unique to *in-situ* burn residue. Samples included Macondo oil collected at the wellhead, tarballs recovered from deep water shrimp trawls, before and after field samples from operational *in-situ* burns during the *Deepwater Horizon* response, before and after laboratory samples from controlled burns performed at Louisiana State University (LSU), and a range of oil residues encountered in various habitats during the response. The results and forensic interpretations will aid in anticipating the environmental fate of burned oil residues and potential impacts to proximal human activities. In addition, with greater understanding of residue behaviors, strategies for potentially recovering neutrally-buoyant or sunken *in-situ* burn residue can be designed and tested.

The sample amounts available for some of the materials were very limited, and restricted the number of analyses (particularly physical) that could be performed. Highest priority physical characterizations performed on the oil residues were density and percent asphaltene content. These parameters were judged to provide information specific to *in-situ* burn residues based on preliminary work with these two parameters performed at LSU. Chemical characterization of all samples was carried out using gas chromatography/mass spectrometry (GC/MS) operated in selected ion monitoring (SIM). The target GC/MS-SIM analytes have been widely used to identify petrogenic, biogenic, and pyrogenic hydrocarbons in a variety of sample matrices. These included selected aromatic hydrocarbons and their alkyl homologs; saturate compounds from C₁₀ to C₃₅; the isoprenoids pristane and phytane; and four groups of oil "biomarkers". The oil biomarkers

included the tri- and pentacyclic hopanes; diasteranes and regular steranes; 14 β (H) steranes; and the triaromatic steroids.

The physical and chemical results were used both directly as well as indirectly, incorporated into indices, to make forensic assessments about the oil residues. For example, petrogenic/pyrogenic indices (Fossil Fuel Pollution Index and Wang Pyrogenic Index) were calculated using aromatic hydrocarbon concentrations. Biomarker ratios were used to determine if tarballs could be sourced back to Macondo oil origins. Asphaltene content appeared to reflect weathered condition of oil residues.

A primary objective of this study was to identify possible indicators that could distinguish *in-situ* burn residues from other oil residues based on physical and chemical analyses of actual *Deepwater Horizon in-situ* burn samples and samples generated from controlled laboratory burns. The results showed that there was no single reliable indicator of *in-situ* burning among those examined. However, partial indicators (e.g., differences in normal alkane profile, enhancement of pyrogenic PAHs, reduction in the modified Fossil Fuel Pollution Index), when considered as an integrated whole, can point to *in-situ* burning as the significant driver of weathering and fate.

The majority of residues from the *Deepwater Horizon* oil spill retained their oil biomarker signatures, making diagnostic ratio analyses possible. The diagnostic ratios were used to:

- 1) Determine if the oil residue was a match to Macondo oil; and
- 2) Determine if there were any differences in the ratios in matching oil residues based on sample matrix and sampling location.

Specifically, a total of 4 out of the 15 diagnostic biomarker ratios did distinguish *in-situ* burn residues from the majority of the other residues. Two of these ratios (C28 aaa-R/C29 aaa-R and C27 BB (S+R)/C28 BB (S+R) + C29 BB (S+R)), may be useful for distinguishing ISB oil from other oil residues and *in-situ* burn tarballs from other tarballs, respectively. Significant differences were also determined in the comparison of sampling locations. These results suggest that weathering of oil residues from the same initial source is highly dependent on the specific environmental compartment in which they reside and the processes driving the weathering within that compartment, but these specificities do not compromise the ability to make match/non-match determinations with the ratios.

It is important to note that the chemical characterization method (GC/MS) and target analyte list used for this report only examines about 5% of the total oil mass. Other newer or more advanced analytical approaches can separate and identify a much larger percentage of oil constituents and hold promise for the future.

The fate of the burn residue from the *Deepwater Horizon in-situ* burn operations might never have been known, were it not for the chance encounters of the single deep water fishery operating in the same general area as most of the burn operations. Impacts to the fishery were minimal and it was quickly re-opened after testing and analysis by federal fisheries managers and seafood safety specialists. However, the experience represents a cautionary footnote for future large-scale *in-situ* burn operations that should be factored into response tradeoff analyses. In addition, the *Deepwater Horizon* episode suggests a need for additional research into burn residue containment or recovery methods that would reduce or eliminate potential fishery impacts associated with sinking residue.

ABSTRACT

An unprecedented volume of crude oil was burned during the *Deepwater Horizon* oil spill response, with an estimated 220,000-310,000 bbl of surface oil consumed by *in-situ* burning over a ten-week period in 2010. Most of the resultant burn residue from these large-scale operations sank in the relatively deep waters of the Gulf of Mexico. However, in late 2010, the deep water royal red shrimp fishery operating north of the Macondo wellhead (also referred to by its lease designation, Mississippi Canyon Block 252, or MC252) and the primary burn zone encountered tarballs at 200 m. At least some of these tarballs were tentatively sourced as *Deepwater Horizon in-situ* burn residue.

This project physically and chemically characterized oil and residue samples from the *Deepwater Horizon* response as well as laboratory samples of source and burned crude oil to provide insights into the changes that occur when oil is burned. *Deepwater Horizon* burn operations were confirmed as the source for tarballs recovered in shrimp trawls. Potential markers of burned crude oil were identified in order to distinguish burn residue from other weathered forms of the same crude oil.

INTRODUCTION

In-situ burning—the controlled combustion of spilled oil in the environment—was one of many remedial techniques employed during the prolonged *Deepwater Horizon* oil spill response in the Gulf of Mexico in 2010. It has been called the oldest cleanup method applied to oil spills (Fingas, 2011), but documentation of its use and scientific evaluation of its efficacy has been spotty and inconsistent over the years. Fingas (2011) listed 45 on-water *in-situ* burns performed during spills or experiments between 1958 and 2008. Although the technique was successfully tested during the landmark *Exxon Valdez* oil spill in Prince William Sound, AK in 1989 (Figure 1), real validation and rigorous scientific evaluation as a response tool occurred in 1993 during a unique experiment off the eastern coast of Canada in 1993. The Newfoundland Offshore Burn Experiment (NOBE, Figure 2) was a collaborative effort among 25 agencies from Canada and the U.S. to conduct two experimental burns of Alberta Sweet crude oil to demonstrate feasibility of burning spilled oil on marine waters, and to collect a wide range of data related to burn parameters and emissions (Fingas et al., 1994a). The NOBE experience was considered to be a logistical and technical success, and yielded a wealth of technical and operational information that has been used as the basis for *in-situ* burn plans and policies ever since.

In-situ burning has been used for spill response a number of times over the years on both land and water. Fingas (2011) provided a comprehensive overview of the state of knowledge just prior to the *Deepwater Horizon* incident and a summary of at-sea applications during oil spill response. In addition to the 45 water-borne examples listed by Fingas, Michel et al. (2005) found it was frequently used on the terrestrial side as well; however, reasonable documentation was available for only 31 of an apparently much larger total. Indisputably, though, the *Deepwater Horizon* burn operations (Figures 3 & 4) were by far the largest application of the method: an estimated 260,000 barrels (bbl)/10.9 million gallons of oil were burned between April 28 and July 12, 2010 (Federal Interagency Solutions Group, 2010). This volume of oil was equivalent to the total volume estimated to have spilled from the *Exxon Valdez*, the nation's largest oil spill prior to the *Deepwater Horizon*.



Figure 1. The *Exxon Valdez in-situ* burn test, March 25 1989. Photo by Alan A. Allen.



Figure 2. NOBE *in-situ* burn experiment, August 1993. Photo by Gary Shigenaka, NOAA.

Oil in the marine environment undergoes a continuous series of compositional changes that are the result of a combination of several processes referred to as weathering (Mills, et al., 1999; Overton et al., 1994; Hollebone, 2015). Weathering processes includes evaporation, dissolution, emulsification, sedimentation, and microbial and photo-oxidation. Weathering, by changing the composition of the original spilled oil, changes the oil's physical and toxic properties, as well as its appearance. As oil weathers, it initially loses volatile components, which are also the most water-soluble components, and the oil becomes more viscous and more likely to glob together as opposed to spreading out in a thin film. Typically, during the weathering process, much of the oil will mix with water and emulsify, forming a viscous mixture that is relatively resistant to rapid weathering changes (Leahy and Colwell, 1990). Consequently, emulsification greatly slows down the weathering processes and is more difficult to remediate by skimming, dispersing or *in-situ* burning.

In-situ burning is preferentially applied to remove fairly fresh oil before the oil emulsifies, although the heat generated during meso-scale or large-scale burns can break oil and water emulsions and feed the fires. Flame temperatures attained during the process of burning crude oil on water vary from 900 to 1200°C. However, the temperature gradient decreases dramatically from the flame, to oil surface (350-500°C), to oil below the surface of the slick (near ambient), to the surface of the water (ambient) (Buist, 1998; Mullin and Champ, 2003). The burning process of fresh oil generally consumes the majority of the oil, upwards of 90%, but does leave unburned, fairly viscous and dense residues with increased asphaltene content. As oil mixes with seawater and forms emulsions, it become more difficult to ignite and is more difficult to burn. Furthermore, continued mixing of unburned residues can contribute to the sinking of these residues.

Despite the scale of the *Deepwater Horizon in-situ* burn operations, there was little organized sample collection and analysis to characterize the physical and chemical changes in the oil occurring as a result of the combustion on the water. This information is important for predicting the fate of burn residues in the marine environment, as well as potential biological effects. The Department of Environmental Sciences of Louisiana State University (LSU) obtained several samples of material related to *in-situ* burns conducted in the Gulf of Mexico in May of 2010. In addition, on-scene personnel of the Emergency Response Division of NOAA arranged for the collection of one before-burn oil and after-burn residue for an operational burn in July of 2010. To our knowledge, these were some of the few sets of such samples collected during the *Deepwater Horizon* response.

A series of other *Deepwater Horizon* oil and residue samples archived by the NOAA chemistry support group at LSU, and samples collected following the report of deep water tarballs being brought up in shrimp trawls, provided the basis for comparative physical and chemical analyses to determine if the *in-situ* burn operations might have been a common thread in several oil residues sampled. Materials generated during controlled laboratory burns could be used as references for these empirical samples to provide insights into environmental fate and behavior of *in-situ* burn residues. These samples and activities represent the basic components of the present research study.



Figure 3. Series of *in-situ* burns during the *Deepwater Horizon* response, June 7, 2010. U.S. Coast Guard photo.



Figure 4. *Deepwater Horizon* *in-situ* burn at dusk, June 12, 2010. U.S. Coast Guard photo.

THE DEEPWATER HORIZON IN-SITU BURN EXPERIENCE

A total of 411 individual burns took place during the *Deepwater Horizon* response between April 28 and July 19, with 376 being judged to have burned significant volumes of oil (see Figures 3 & 4). On June 18 alone, sixteen *in-situ* burns took place (U.S. Coast Guard, 2011). Most of the burns took place within 5-24 km of the wellhead location (Allen et al., 2011). Figure 5 shows the estimated volumes of oil burned on a daily basis, and Figure 6 illustrates the locations of *in-situ* burn operations in the Gulf of Mexico relative to the Louisiana coast and the location of the Macondo wellhead.

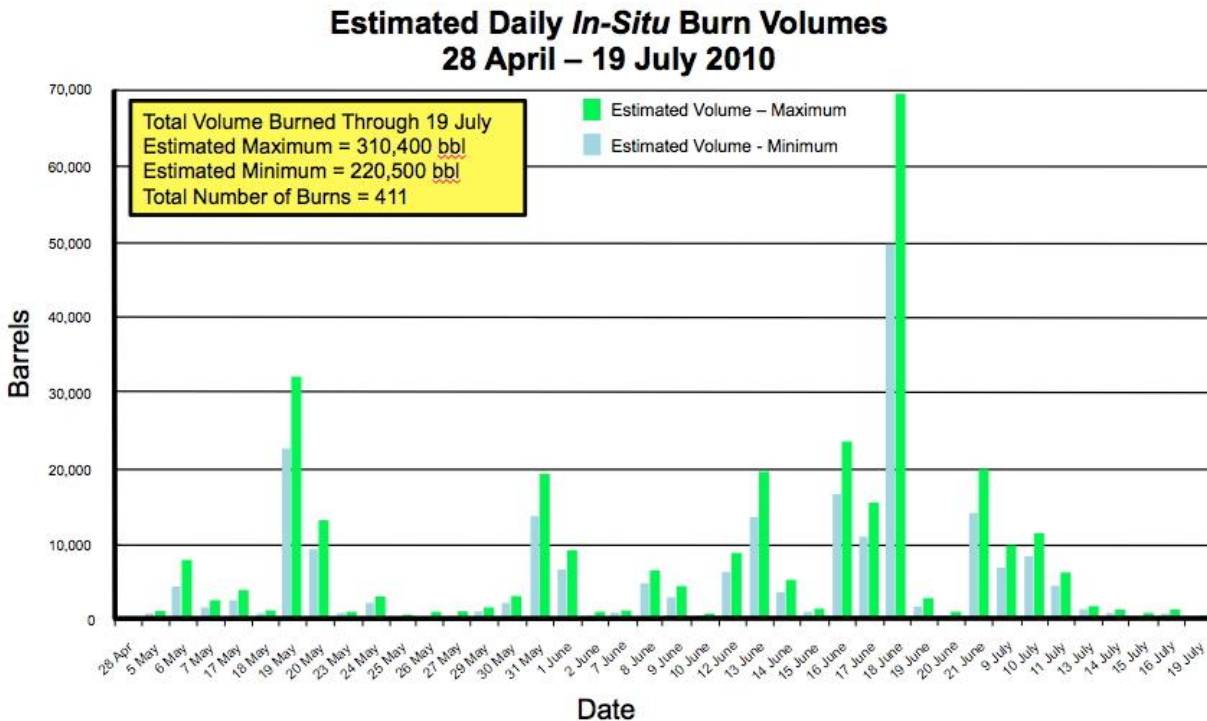


Figure 5. Estimated burn volumes during the Deepwater Horizon, by date. Source: Allen et al., 2011.

The Macondo crude oil released during the *Deepwater Horizon* spill was a light Louisiana crude, with a density measured in the lab of 0.839 g/cm^3 at 15°C . (API gravity 37.2°) (SL Ross Environmental Research, 2010). The practical implication of its density was that the fresh oil floated on the seawater surface of the Gulf of Mexico. In lab studies, the density of the oil increased with increased weathering (evaporation); when the volume of oil was reduced by about 45% through artificial weathering, its density increased to 0.897. As the density of seawater is approximately 1.02-1.03, the weathered Macondo oil would have floated in the Gulf of Mexico as well.

Along the nearshore Gulf coast, some of the weathered oil is believed to have mixed with sand and sediments in the surf zone, and consequently became dense enough to sink and to form submerged oil mats attributed as sources of beached tarballs (Operational Science Advisory Team-2, 2011). However, the oil in the vicinity of the wellhead (roughly 93 km offshore) and where *in-situ* burn operations took place had little opportunity to mix with sediments and floated on the surface of the Gulf. This provided opportunities for large-scale operations like skimming, application of chemical

dispersants, and *in-situ* burning, which are most effective when large contiguous patches of oil are accessible.

Macondo Well Incident, In-Situ Burn Locations

February 11th, 2015



In-situ burn locations provided by BP. Represents all official burns.

Prepared by NOAA's Office of Response and Restoration

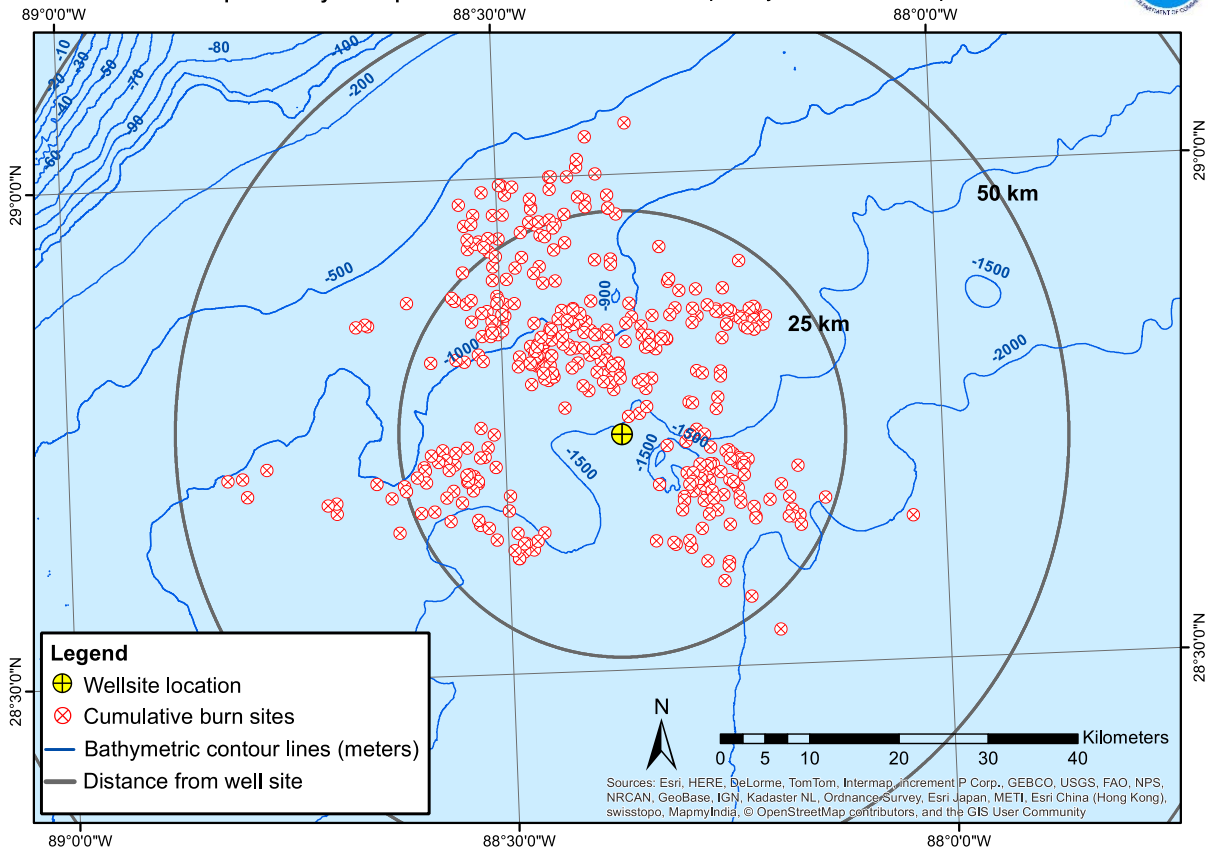


Figure 6. Map showing *in-situ* burn locations relative to Macondo wellhead location and coast of Louisiana. J.B. Huyett, GenWest Systems.

It is well-documented that the physical and chemical characteristics of oil change with combustion, including *in-situ* burning. For example, Environment Canada chemists studying the NOBE burns in 1993 found that the residues from those two experiments were generally lighter than water, but that density was related to burn efficiency. They suggested that following a highly efficient (>99.9%) burn, the resultant residue might be neutrally buoyant. Chemically, Environment Canada determined that the burn residues resembled highly weathered oil, with a substantial loss in mass and a shift to multi-ring polycyclic aromatic hydrocarbons (PAHs) (Fingas et al, 1994b).

The physical properties of burn residues were investigated by Buist et al. (1995), with a specific focus on the changes in density that occur when different oils are burned. The authors noted that this was of particular interest and concern because residues from some accidental *in-situ* burns (e.g., *Haven* and *Honan Jade*) and from large-scale test burns had been observed to sink, while laboratory experiments had shown sinking to be less of a possibility. Buist et al. conducted direct experiments with eight different oil types to determine conditions in which residues of sufficient density to sink in salt water would be created. They found that batch-type *in-situ* burns of thick,

heavier crudes would result in sinking residues. They attributed the increase in density to a process called Equilibrium Flame Vaporization (EFV), in which a fire is fueled by a vapor of constant (mixed) composition over time, fed by an oil source of relatively constant composition—as opposed to a distillation process where oil components are progressively burned off, from lightest to heaviest. The burn process concentrated high molecular weight compounds in the remaining slick, which incrementally increased the density of the residue.

During the *Deepwater Horizon* response, the environmental fate of the *in-situ* burn residue, both short- and long-term, was not routinely monitored. However, the post-response U.S. Coast Guard Incident-Specific Preparedness Review (2011) included in its recommendations for *in-situ* burning (ISB):

Unburned oil or other residue from ISB operations should be recovered and accounted for when evaluating the effectiveness of ISB.

While not formally monitored, U.S. Coast Guard and operational workers did observe and anecdotally communicate changes in the physical characteristics of the Macondo oil once it had been burned: its viscosity and density both appeared to increase (Figures 7 & 8). In fact, *in-situ* burning apparently increased the density of the residual oil to a significant degree—to the point where much, if not most, of the post-burn residue would be heavier than seawater and slowly sink (Figure 9). A U.S. Coast Guard Gulf Strike Team member who witnessed many of the early burn operations recalled:

The residue definitely did sink, it tended to temporarily float in those strings like...in the photo, and within a few minutes post burn would begin to sink. If you tried to touch it with a pole or other object it began to slowly sink. The consistency was sticky like paste. The residue appeared generally the same regardless of burn duration, with some variation if there was non-petroleum debris in it, it had more visible whitish ash (MSTCS Drew Jaeger, USCG Gulf Strike Team).

The apparent tendency of the burn residues to sink rendered them both difficult to remove from the marine environment, and difficult to sample in order to determine physical and chemical changes. Collection protocols for the May 2010 *in-situ* burn samples were not recorded; the July post-burn sample analyzed by NOAA/LSU for this report was obtained as residual material adhered to the fire boom after a burn. As such, it was likely to be intermediate between the pre-burn oil and the fully combusted—and sinking—burn residues pictured in Figure 9. With its known origins, we hoped that this would provide another data point for understanding the range of changes that can occur when oil is burned and serve as a basis of comparison for tarballs and other oil residues of unknown origin.



Figure 7. Burn residue remaining in fire boom at the end of an *in-situ* burn operation, May 2010. U.S. Coast Guard photo, CPO R.J. Schrader.



Figure 8. Burn residue on fire boom following an *in-situ* burn operation, 5 June 2010. U.S. Coast Guard photo.



Figure 9. *In-situ* burn residues just below the surface of the water following a burn operation for the *Deepwater Horizon* oil spill response, May 2010. U.S. Coast Guard photos, CPO R.J. Schrader.

OVERVIEW OF THE PROJECT

The project is divided into six different tasks to provide insights into the changes that may occur when oil is burned and how the physical and chemical characteristics compare to other forms of oil encountered during a spill (e.g., emulsified surface oil, beach- and marsh-stranded oil, etc.). The six tasks are:

- Task 1:** Physical and chemical characterization of surface collected Macondo Well source oil (the riser oil)
- Task 2:** Physical and chemical characterization of *Deepwater Horizon in-situ* burn samples (pre-burn oil and post-burn residue)
- Task 3:** Physical and chemical characterization of deep water (~200 m) tarballs collected by royal red shrimp trawlers
- Task 4:** Burning of surface collected source and emulsified MC252 oil and burning of a surrogate south Louisiana crude oil under controlled conditions
- Task 5:** Physical and chemical characterizations of burn residues resulting from Task 4
- Task 6:** Forensic interpretation of analytical results to characterize physical and chemical changes occurring during the combustion of oil, to include comparison of changes occurring in various weathered oil samples and identification of potential markers for *in-situ* burn residues

The physical and chemical analyses of actual *Deepwater Horizon in-situ* burn samples is the focal point of comparisons for all other oil residue samples of relevant to the spill response. Specific comparisons to be made include:

1. Samples of Macondo well source oil and the same source oil burned under controlled conditions at the NOAA/LSU chemistry support lab in Baton Rouge, LA;
2. Samples of tarballs trawled from depth by deep water shrimp nets in late 2010 and early 2011 north of the blowout location;
3. Other samples of *Deepwater Horizon* oil collected at-sea, in marshes, and from beaches.

As we have noted, there were very small amounts of the actual *Deepwater Horizon in-situ* burn samples (i.e., pre-burn oil and post-burn residue) available, and similarly limited amounts of the Macondo source oil; however, the NOAA chemistry support group at LSU had both of these key materials archived and in possession. Additionally, LSU had already chemically characterized hundreds of samples collected through the duration of the response efforts and beyond (into 2013). A total of 100 different samples, including tarball material from shrimp trawls, had been previously quantitated by LSU to obtain total PAHs. The quantitative data also included oil biomarker profiles that were used to calculate diagnostic ratios for this project. Physical characterization of archived material was carried out if it had not been previously completed and if enough material was available.

These environmental samples established an empirical basis for conceptually modeling how oil changes when it is burned at sea. Analysis of the *in-situ* burn residues and comparison to other oil residues will contribute to our understanding of the longer-term fate of burned oil and identify potential impacts to pelagic and benthic environments, as well as human activities like mid-water and bottom trawling. In addition to the physics and chemistry of the different oil residues, the

project will attempt to define “indicators” that can distinguish an *in-situ* burn residue from other spill related oil residues.

The results of this project will aid in anticipating the environmental fate of burned oil residues and potential impacts to proximal human activities. In addition, with greater understanding of residue behaviors, strategies for potentially recovering neutrally-buoyant or sunken *in-situ* burn residue can be designed and tested.

METHODS

Physical Characterization (Tasks 1, 2, 3 and 5)

Two physical characterizations were performed on the ISB residue or tarballs, density and a percent asphaltene determination. Other parameters, such as resins and viscosity, would also provide important physical characteristics; however, sample quantities were limited, and it was hypothesized that density and percent asphaltene would provide information specific to ISB residues due to some preliminary determinations of these two parameters. Density and percent asphaltene of MC252 source oil were obtained from the literature (Lewan et al., 2014; SL Ross Environmental Research, 2010) and determined by the methods described below for BP surrogate oil (i.e., a light South Louisiana Dorado crude similar to the scarce MC252 oil) used in the laboratory burn experiment, and for samples collected during *in-situ* burn operations. In addition, density and percent asphaltene were determined for 55 and 41 oil residue samples collected throughout the *Deepwater Horizon* response, respectively.

Two methods were used to determine density depending on the physical state of the oil residue being tested. If the ISB residue or tarball material was fluid enough, a pycnometer was used to determine density. In the event that the ISB residue or tarball material was not pliable enough, density was determined by volume displacement (modified ASTM test method D71). Ten milliliters (mL) of deionized (DI) water was poured into a pre-weighed graduated cylinder, the meniscus was marked, and the weight of the cylinder and water was recorded. The cylinder was then emptied and dried in a 100°C oven for 15 minutes. Then, 1 to 2 grams (g) of *in-situ* burn (ISB) residue or tarball (free of extraneous material) was weighed. The ISB residue or tarball material was then placed in the dried cylinder. The cylinder was filled with DI water to 10mL mark and reweighed for a third time. The density of the ISB residue or tarball was the weight of the material divided by the difference in weight of the water. The difference in water weight was converted to volume with the assumption that the density of water is 1 g/mL (e.g. (e.g. 5 g x 1 mL/ 1 g = 5 mL).

Asphaltenes are defined as a broad class of high molecular weight compounds that can be precipitated from oil using pentane, hexane, or heptane and measured as a percentage by weight (Fingas, 2015). Percent asphaltene (modified ASTM D3279-12) was determined by a two stage process. ISB residue or tarballs were first dissolved the ISB residue or tarballs in hexane, and, then in toluene. Approximately 1-to-2 grams (g) of ISB residue or tarball (free of extraneous material) was weighed in a pre-weighed jar. The total mass was recorded and 50 to 60 mL of hexane was then added. The vial was ultra-sonicated for 30 minutes and then allowed to settle for 30 minutes. Pre-labeled filter papers were put in an oven at 100°C for 2 hours to remove any water. The initial weights of the dried filter papers were then recorded. The ISB residues or tarballs dissolved in hexane were carefully decanted to funnels containing the dried and pre-weighed filter papers. The asphaltene collect on the filter paper since they do not dissolve in hexane and any hexane-soluble fraction collected in a vial below the funnel. The filter papers with the collected asphaltene were air dried under a hood and final weights were recorded. To ensure that just asphaltene were being measured, the air dried filter paper was then placed in funnel and rinsed with toluene. A pre-weighed vial below the funnel was used to collect the toluene and the toluene was then allowed to evaporate. The final weight of the vial was recorded and the difference between the before and after vial weight was the mass of asphaltene.

Reproducibility for each test method was determined using replicate analyses and resulting percent relative deviation (%RSD) and standard deviation were calculated. For the density determinations, the average %RSD was 1.49 (average standard deviation of ± 0.013 , n=21) for the pycnometer method, and for the volume displacement method the average %RSD was 12.1 (average standard

deviation of ± 0.13 , $n=8$). Determination of % asphaltenes had an average %RSD of 28 (average standard deviation of 0.56, $n=9$). The higher %RSD and standard deviation are influenced by a decrease in precision and accuracy of samples with low levels of asphaltenes. Statistical comparisons of physical characteristics for Tasks 1, 2, 3, and 5 were carried out using a Student two-tailed t-test with the assumption of equal variance.

Chemical Characterization (Tasks 1, 2, 3, 5, and 6)

Chemical characterization of all samples was carried out using gas chromatography/mass spectrometry (GC/MS) operated in selected ion monitoring (SIM). The GC/MS methodology has been developed specifically for detection and quantification of compounds unique to oil spills. The target GC/MS-SIM analytes are given in Table 1 and are widely used to identify petrogenic, biogenic, and pyrogenic hydrocarbons in a variety of sample matrices. Note that the list includes commonly-found aromatic hydrocarbons and their alkyl homologs, saturate compounds from C_{10} to C_{35} , isoprenoids pristane and phytane, and four groups of oil “biomarkers”. The oil biomarkers include the tri- and pentacyclic hopanes, diasteranes and regular steranes, $14\beta(H)$ -steranes, and the triaromatic steroids. Chemical characterization of samples was performed using an Agilent 7890 GC equipped with an Agilent 5975 inert XL MSD or an Agilent 6890 GC equipped with an Agilent 5973 MSD. Both instrument systems were fitted a 5% diphenyl/95% dimethyl polysiloxane high-resolution capillary column (30 m x 0.25 mm x 0.25 μ m). Instrumental acquisition was identical for both instruments and QA/QC assured that data was comparable between both systems.

Table 1. Targeted Petroleum Hydrocarbon Analytes

Anthracene	Fluoranthene	C-1 Phenanthrenes/Anthracenes
Benz[a]anthracene	Fluorene	C-2 Phenanthrenes/Anthracenes
Benzo[a]pyrene	C-1 Fluorenes	C-3 Phenanthrenes/Anthracenes
Benzo[b]fluorene	C-2 Fluorene s	C-4 Phenanthrenes/Anthracenes
Benzo[e]pyrene	C-3 Fluorenes	Pyrene
Benzo[g,h,i]perylene	Indeno[1,2,3-cd]pyrene	C-1 Fluoranthenes/Pyrenes
Benzo[k]fluorene	Naphthalene	C-2 Fluoranthenes/Pyrenes
Chrysene	C-1 Naphthalenes	C-3 Fluoranthenes/Pyrenes
C-1 Chrysenes	C-2 Naphthalenes	C-4 Fluoranthenes/Pyrenes
C-2 Chrysenes	C-3 Naphthalenes	<u>Saturate Hydrocarbons:</u>
C-3 Chrysenes	C-4 Naphthalenes	nC ₁₀ -nC ₃₅
C-4 Chrysenes	Naphthobenzothiophene (NBT)	<u>Oil Biomarkers:</u>
Dibenz[a,h]anthracene	C-1 NBTs	Hopanes (m/z 191)
Dibenzothiophene (DBT)	C-2 NBTs	Diasteranes & Regular Steranes (m/z 217)
C-1 DBTs	C-3 NBTS	$14\beta(H)$ Steranes (m/z 218)
C-2 DBTs	Perylene	Triaromatic Steroids (m/z 231)
C-3 DBTs	Phenanthrene	

GC/MS data were used to generate information regarding chemical characterization, both qualitative and quantitative, for analyzed samples. Qualitative information included chromatographic profiles of specific compound groups (mainly m/z 57, the normal alkanes), and quantitative data included ratios/indexes to differentiate petrogenic sources from pyrogenic sources and to calculate biomarker diagnostic ratios for the forensic investigation task of the project.

For Tasks 1-3 and 5, the Fossil Fuel Pollution Index (FFPI) and the Wang Pyrogenic Index were both calculated to determine if the PAH signatures in *in-situ* burn residues changed from a petrogenic signature to a more pyrogenic signature. The FFPI was first conceived by Boehm and Farrington (1984) and was later modified by LSU-RCAT to incorporate an expanded list of target aromatic compounds. The modified FFPI was calculated as follows:

$$\text{Modified FFPI} = \frac{((C-1 \text{ Naphs} + C-2 \text{ Naphs} + C-3 \text{ Naphs} + C-4 \text{ Naphs} + C-1 \text{ Fluors} + C-2 \text{ Fluors} + C-3 \text{ Fluors} + \text{DBT} + C-1 \text{ DBTs} + C-2 \text{ DBTs} + C-3 \text{ DBTs} + C-2 \text{ Phens} + C-3 \text{ Phens} + C-4 \text{ Phens} + C-2 \text{ Pyrs} + C-2 \text{ Chrys}) + (0.5 \times (\text{Naph} + \text{Fluro} + \text{Phen} + C-1 \text{ Pyrs} + C-1 \text{ Chrys})))}{(\text{Total Aromatics} - \text{Perylene})}$$

A modified FFPI value closer to one (1.0) represents petrogenic/oil-derived PAHs while a value less than 0.6 represents pyrogenic/combustion-derived PAHs. The Wang Pyrogenic Index was developed after characterizing PAHs in burn residues and soot samples from experimental *in situ* burning of diesel fuel (Wang et al., 1999). The Wang Pyrogenic Index was slightly modified since biphenyl, acenaphthylene, and acenaphthene were not included in the target compound list. Therefore, the modified Wang Pyrogenic Index calculated as follows:

$$\text{Wang Pyrogenic Index} = \frac{(\text{Anthracene} + \text{Fluoranthene} + \text{Pyrene} + \text{Benz[a]anthracene} + \text{Benzo[b]fluoranthene} + \text{Benzo[k]fluoranthene} + \text{Benzo[e]pyrene} + \text{Benzo[a]pyrene} + \text{Perylene} + \text{Indeno[1,2,3-cd]pyrene} + \text{Dibenz[a,h]anthracene} + \text{Benzo[g,h,i]perylene})}{[(\sum \text{Naphthalenes (C0-C4)} + (\sum \text{Fluorenes (C0-C3)} + (\sum \text{DBTs (C0-C3)} + (\sum \text{Phenanthrenes (C0-C4)}))])}]}$$

The Wang Pyrogenic Index for the source diesel was 0.004, between 0.009 and 0.019 for experimental residues, and greater than 0.08 for soot samples (Wang et al., 1999). Therefore, a higher index value represents a pyrogenic source, while a lower index value represents a petrogenic source. Statistical comparisons of chemical characteristics (i.e. GC/MS data) for Tasks 1-3, and 5 were carried out using a Student two-tailed t-test with the assumption of equal variance.

Concentrations of individual analytes were based average response factors calculated from a 5-point calibration curve prepared from a commercially available oil analysis standard (Absolute Standards, Hamden, CT) and using the internal standard method. Alkylated homologs were quantified using the response factor of the parent. Individual internal standards were purchased from AccuStandard (New Haven, CT) and mixed in the LSU lab. Each analytical batch of samples contained a continuing calibration standard (not to exceed $\pm 20\%$ of the original calibration response factors), solvent and instrument blanks (to verify no carry over), and an extract of MC252 source oil. The MS was tuned using PFTBA prior to each analytical batch or every 12 hours. The instrument tune was evaluated and if any parameters were out of acceptable limits, no samples were analyzed until any issues were corrected. All solvents used were of reagent grade or higher.

Controlled Laboratory Burns of MC252 & South Louisiana Crude Oil (Task 4)

At NOAA's request, an initial controlled crude oil burn was performed prior to the initiation of this project on 6 March 2012 using around 5g of MC252 oil. The oil was poured into a crucible and burned with a propane torch for 4 to 5 minutes. The experiment was conducted in triplicate. The initial and final weights of oil and residues were recorded. The residues from this initial burn were chemically characterized by GC/MS. Physical characterization was not possible due to the limited amount of residue remaining after the burn procedure.

A second, larger-scale burn was conducted on 20 February 2014 (Figure 10) using Dorado oil (a light South Louisiana crude oil) as a surrogate source material acquired from BP in place of MC252

oil. The larger-scale burn was an attempt to duplicate the sinking phenomenon believed to be a result of *in situ* burning. Fresh and 15% weathered surrogate oil (500mL) was burned in triplicate in large stainless steel containers (Figure 10a) at the LSU Environmental Health and Safety facility on the Baton Rouge campus. Emulsified MC252 oil collected by skimming operations during the *Deepwater Horizon* response was also burned at this time. All 500mL of each oil was floated on artificial seawater at an average of 34‰ in each stainless steel container. The stainless steel containers (33 cm x 28 cm x 10 cm) were placed in a plastic pool of water to help dissipate heat and reduce spattering of the burning oil. A large propane torch (Figure 10b) was used to ignite the oil, and burn durations were around 5-10 minutes. The stainless steel containers were allowed to cool and were then transported back to the lab where the residues were sampled for physical and chemical characterization described in previous sections.

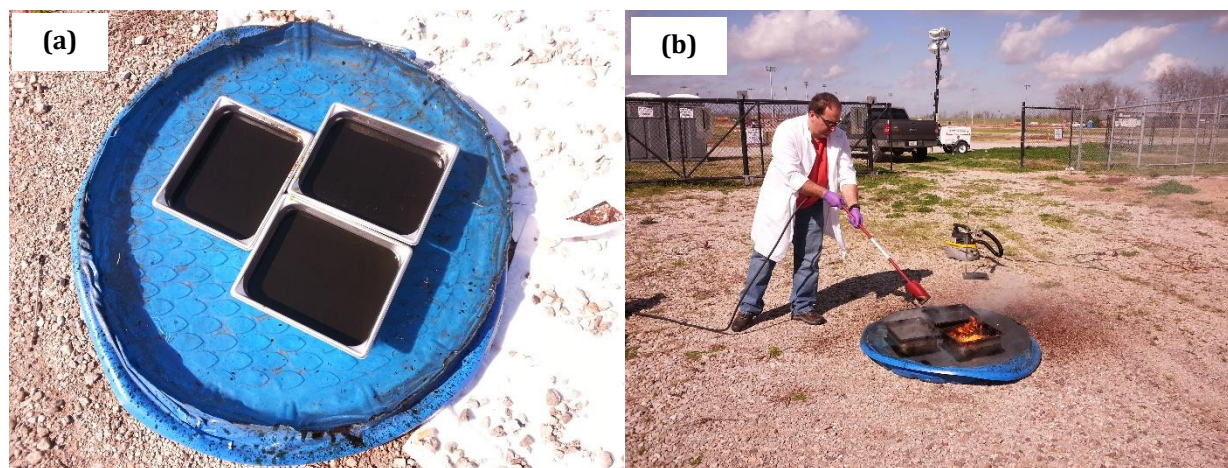


Figure 10. (a) Pans of South Louisiana crude on artificial seawater (left); and, (b) ignition of oil (right).

Forensic Interpretation of Analytical Results (Task 6)

Forensic interpretations included both qualitative and quantitative techniques. Qualitative techniques involved the visual comparison of GC/MS n-alkane profiles, including the nC_{17} /Pristane and nC_{18} /Phytane ratios and the biomarker profiles. The visual comparison was used to detect any characteristic features or obvious differences, which could possibly eliminate candidate sources from MC252 source oil. Match/non-match determinations could be assigned at this point; however, quantitative fingerprinting techniques were used to verify the visual results. The quantitative approach involved the calculation and statistical comparison of certain diagnostic biomarker ratios for each sample and the MC252 source oil. Diagnostic biomarker ratios were used due to the fact that oil biomarkers are more resistant to environmental weathering processes relative to most other oil compounds. These ratios were chosen from the mass spectrometer ion groups of the hopanes; the steranes (diasteranes and regular steranes, and $14(\beta)H$ -steranes); and, the triaromatic steroids (e.g. m/z 191, 217, 218, and 231). A total of 15 diagnostic ratios were calculated for each sample and statistically compared to the same ratios from MC252 source oil. In some instances, only 9 diagnostic ratios were calculated because some of the samples were analyzed prior to addition of two groups of oil biomarkers (the $14(\beta)H$ -steranes and the triaromatic steroids). The foundation and statistical criteria of the diagnostic ratio methodology was adapted from the 2007 edition of *Oil Spill Environmental Forensics: Fingerprinting and Source Identification*, Zhendi Wang and Scott Stout editors, Chapter 7, "Emerging CEN Methodology for Oil Spill Identification" by A.B Hansen, P.S. Daling, L. Faksness, K.R. Sorheim, P. Kienhuis, and R. Duus. The 15 MC252 diagnostic ratios were previously established to be statistically rigorous (Meyer *et al.*, 2014). The diagnostic

ratios were calculated using heights of certain peaks within the hopanes, steranes, and triaromatic steroids profiles. The specific oil biomarker peaks and the 15 diagnostic ratios are provided in Appendix 1. After determining the absolute and critical differences for each ratio in each sample, the sample received a score (the number of “matching” ratios/15 * 100%) that was used to classify the sample as a Match (93-100%), Probable Match (80-92%), Inconclusive (50-79%), and Non-Match (<50%). Only samples in the Match and Probable Match categories were included in the forensic interpretations.

Once the samples had been narrowed down into the Match or Probable Match categories, they were then sorted based on year sampled, sample matrix, and sample location. Sample matrix included ISB oil, ISB tarball, oil, sediment, tarball, and other (i.e. water, mousse, and/or mixed matrix of oil/water/sand). Sample locations included marsh, ocean depth, shore, and water surface. A one-way ANOVA with a post-hoc Tukey-Kramer pairwise comparison was applied to the sample matrix for each of the 15 diagnostic ratios to determine if any of the ratios could distinguish between *in-situ* burn residues and other MC252 oil residues. For the sample location effects, a nested ANOVA with random effects and a variance components covariance structure was carried out for each diagnostic ratio to determine if there were any differences due to location of sample collection. In both cases, the sample number in each matrix or location was highly unbalanced. The mixed procedure (“proc mixed”) in SAS® statistical software, version 9.3, was used to perform the statistical analyses.

RESULTS AND DISCUSSION

Task 1: Physical and chemical characterization of surface-collected Macondo Well source oil (the riser oil)

Values regarding the physical characterization of MC 252 oil were obtained from published literature. The Macondo crude oil released during the *Deepwater Horizon* spill was a light Louisiana crude, with a density measured in the lab of 0.839 g/cm³ at 15° C. (API gravity 37.2°) (SL Ross Environmental Research, 2010). The percent asphaltenes of MC252 oil was calculated by Lewan et al. (2014) to be 0.9-1.0% ± 0.1. MC252 oil that surfaced and subsequently burned in some cases had a significantly different composition than the fluid that flowed from the well blowout at depth. Many of the light hydrocarbons and aromatic compounds (compounds with molecular weights less than naphthalene) were dissolved into the water column during the transit of the oil to the surface. Therefore, the oil burned during *in situ* burn operations would be considered “weathered” since the more volatile and lower molecular weight compounds were already depleted. For this study, the density of weathered MC252 oil increased to 0.88±0.8 (n=7) and percent asphaltenes increased to 1.5±0.5 (n=5).

Table 2. Density of Weathered MC252 Oil

LSU ID#:	Pycnometer Wt (g):	Pycnometer + Sample (g):	Mass of Sample (g):	Volume of Pycnometer (mL):	Density (g/mL):
2010147-122	51.09	60.29	9.20	11.5	0.80
2010147-124	51.22	61.89	10.67	11.5	0.93
2010147-126	51.21	61.99	10.78	11.5	0.94
2010147-128	51.09	59.58	8.48	11.5	0.74
2010147-130	51.21	61.97	10.76	11.5	0.94
2010147-134	51.21	61.70	10.49	11.5	0.91
2010147-136	51.21	61.84	10.63	11.5	0.92
AVERAGE					0.88
STDEV					0.08

Table 3. Percent Asphaltenes of Weathered MC252 Oil

LSU ID#:	Sample Wt (g):	Initial VOA Vial Wt (g):	Final VOA Vial Wt (g):	VOA Vial Wt Difference (g):	% Asphaltenes by Mass
2010147-122 b	1.10	21.37	21.39	0.02	1.4
2010147-126 b	0.97	21.58	21.59	0.01	1.2
2010147-130 b	0.96	21.67	21.69	0.02	2.3
2010147-134 b	1.07	21.50	21.52	0.01	1.4
2010147-136 b	0.97	21.49	21.50	0.01	1.1
AVERAGE					1.5
STDEV					0.5

The chemical characterization of fresh MC252 is shown in Figure 10. MC252 oil is a light, sweet crude oil with a normal alkane hydrocarbon range of nC₁₀ through nC₃₅ (Figure 11, top) and an aromatic profile dominated by the naphthalenes (Figure 11, bottom).

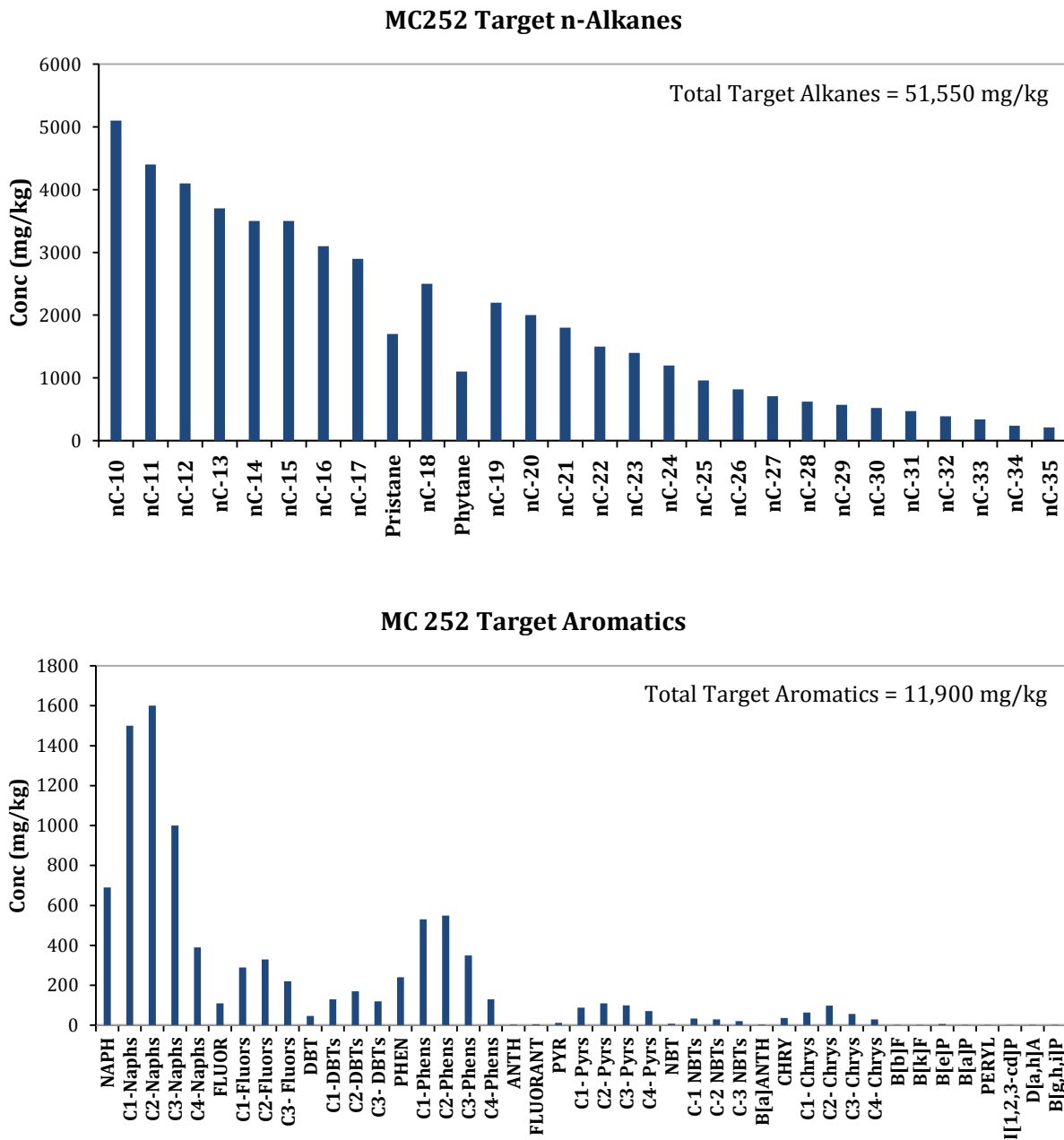


Figure 11. Target analyte profiles for n-alkanes (top) and aromatics (bottom) in fresh MC252 source oil (riser fluid).

Task 2: Physical and chemical characterization of *Deepwater Horizon in-situ* burn samples (pre-burn oil and post-burn residue)

A total of 20 *in-situ* burn samples collected during response operations were analyzed. One batch of 18 samples was received at LSU on 27 May 2010 and two samples, one “before” and one “after” sample, were later received on 20 July 2010. Of these 20, there were 9 sets of before and after samples. The sample set from July 2010 was of limited quantity; therefore, chemical characterization took priority over physical characterization. For the physical characterization of the May 2010 samples (Tables 4 and 5), the average density of samples before *in-situ* burning was 0.88 ± 0.08 (n=7) and the average density of samples after *in-situ* burning was 0.96 ± 0.02 (n=8). The increase in density was significant (p=0.03, Student two-tailed t-test with the assumption of equal variance). The average percent asphaltenes of samples before *in-situ* burning was $1.5\% \pm 0.5$ (n=5) and the average percent asphaltenes of samples after *in-situ* burning was 4.2 (n=5). The increase in percent asphaltenes was not statistically significant (p=0.08, Student two-tailed t-test with the assumption of equal variance). It was assumed that percent asphaltenes would have increased as a result of burning; it did, but did not attain *statistical* significance.

Table 4. Densities for Before and After *In Situ* Burning of MC252 Oil

LSU ID#:	Burn Status	Pycnometer Wt (g):	Pycnometer + Sample (g):	Mass of Sample (g):	Volume of Pycnometer (mL):	Density (g/mL):
2010147-122	Before	51.09	60.29	9.20	11.5	0.80
2010147-124	Before	51.22	61.89	10.67	11.5	0.93
2010147-126	Before	51.21	61.99	10.78	11.5	0.94
2010147-128	Before	51.09	59.58	8.48	11.5	0.74
2010147-130	Before	51.21	61.97	10.76	11.5	0.94
2010147-134	Before	51.21	61.70	10.49	11.5	0.91
2010147-136	Before	51.21	61.84	10.63	11.5	0.92
AVERAGE, BEFORE						0.88
STDEV, BEFORE						0.08
2010147-123	After	51.24	62.21	10.97	11.5	0.95
2010147-125	After	51.09	61.77	10.68	11.5	0.93
2010147-129	After	51.22	62.44	11.22	11.5	0.98
2010147-135	After	51.08	62.46	11.38	11.5	0.99
2010147-137	After	51.05	62.28	11.23	11.5	0.98
2010147-138	After	51.21	62.46	11.25	11.5	0.98
2010147-133	After	51.08	61.78	10.70	11.5	0.93
AVERAGE, AFTER						0.96
STDEV, AFTER						0.02

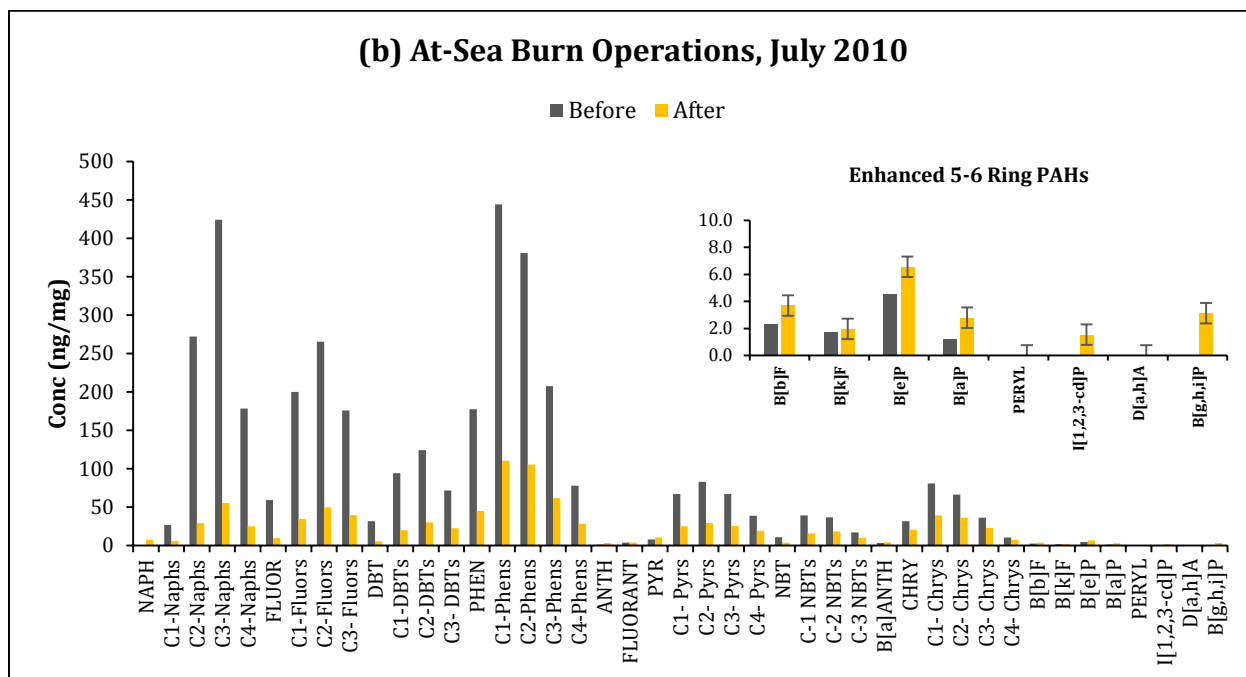
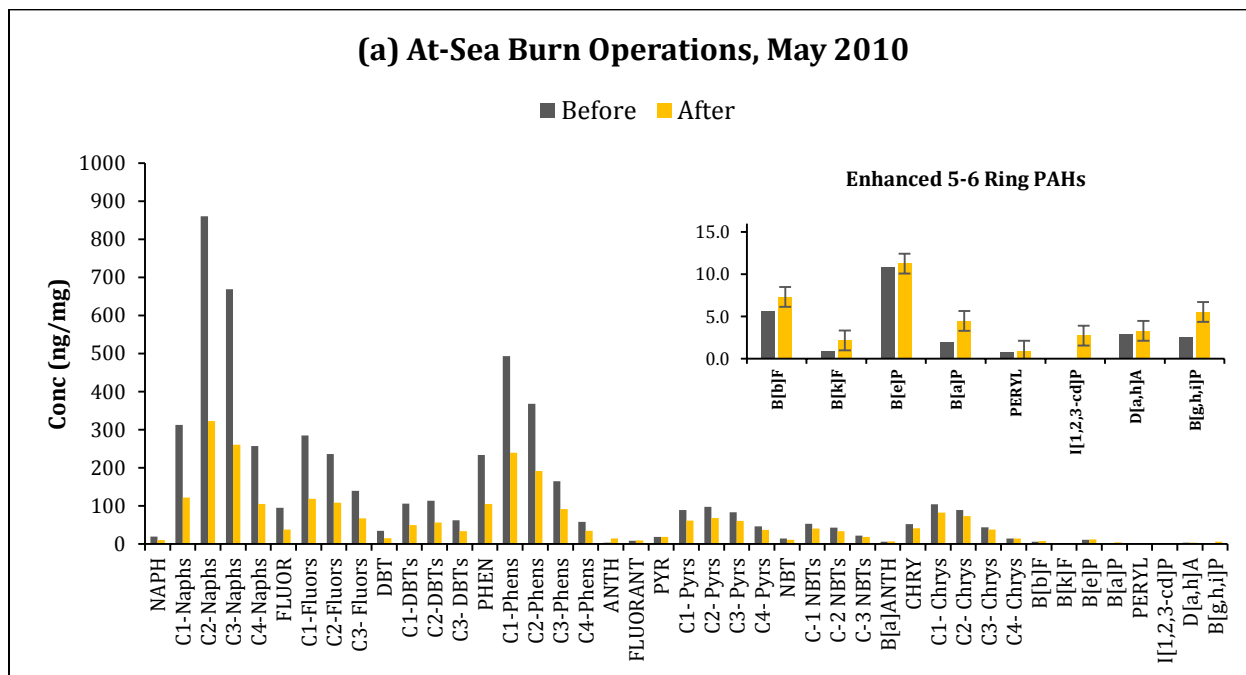
Table 5. Percent Asphaltenes for Before and After *In Situ* Burning of MC252 Oil

LSU ID#:	Burn Status	Sample Wt (g):	Initial VOA Vial Wt (g):	Final VOA Vial Wt (g):	VOA Vial Wt Difference (g):	% Asphaltenes by Mass
2010147-122	Before	1.10	21.37	21.39	0.02	1.4
2010147-126	Before	0.97	21.58	21.59	0.01	1.2
2010147-130	Before	0.96	21.67	21.69	0.02	2.3
2010147-134	Before	1.07	21.50	21.52	0.01	1.4
2010147-136	Before	0.97	21.49	21.50	0.01	1.1
AVERAGE, BEFORE						1.5
STDEV, BEFORE						0.5
2010147-125	After	0.96	24.92	24.95	0.03	3.1
2010147-133	After	1.21	24.97	24.99	0.01	1.2
2010147-135	After	1.18	21.53	21.63	0.10	8.3
2010147-137	After	1.81	25.36	25.47	0.11	6.2
2010147-138	After	0.62	21.86	21.87	0.01	2.2
AVERAGE, AFTER						4.2
STDEV, AFTER						3.0

Table 6 summarizes the chemical characterization of MC252 oil by GC/MS-SIM before and after *in-situ* burning in May and July 2010, and Figure 12 displays the average PAH profile for these samples. The PAH profile for the before sample for July 2010 indicates that it was more of a weathered oil than the May 2010 before samples. The insets in Figure 11 show the enhancement, most of which are significant, of the less-volatile aromatics that are associated with pyrogenic sources (e.g., 5-6 ring PAHs). These compounds were already present in MC252 oil (with the exception of indeno[1,2,3-cd]pyrene); however, their concentrations increased as a result of *in-situ* burning. This increase is also supported by hopane normalized percent decrease or increase for the controlled laboratory burns in Task 4. However, it is important to note that even though these compounds increased as a result of burning, their increase was outweighed by the decrease in the overall total PAH concentration. This finding is consistent with Garrett et al. (2000) and Wang et al. (1999).

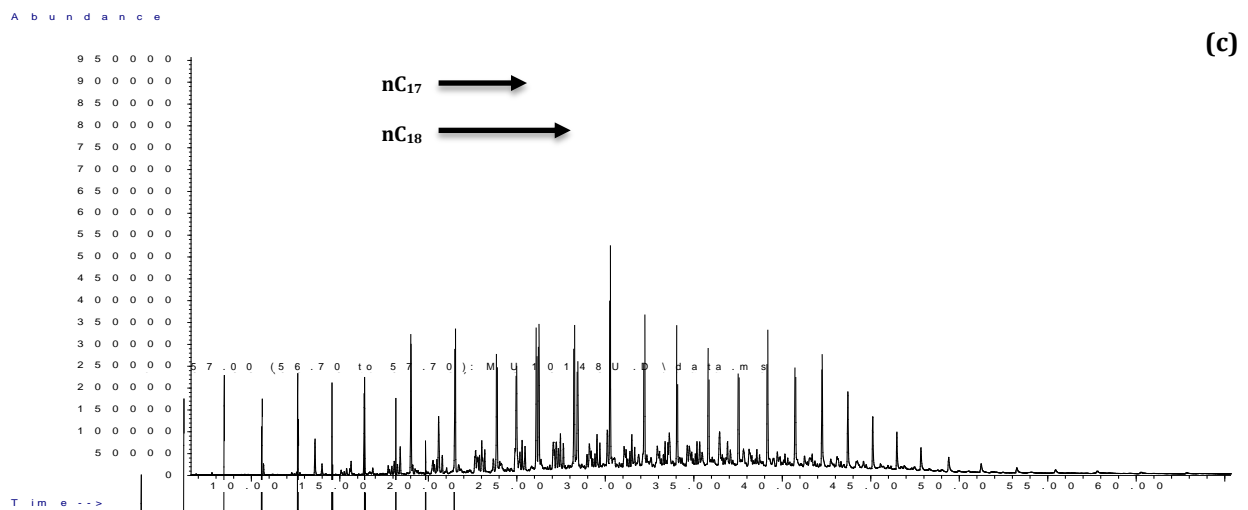
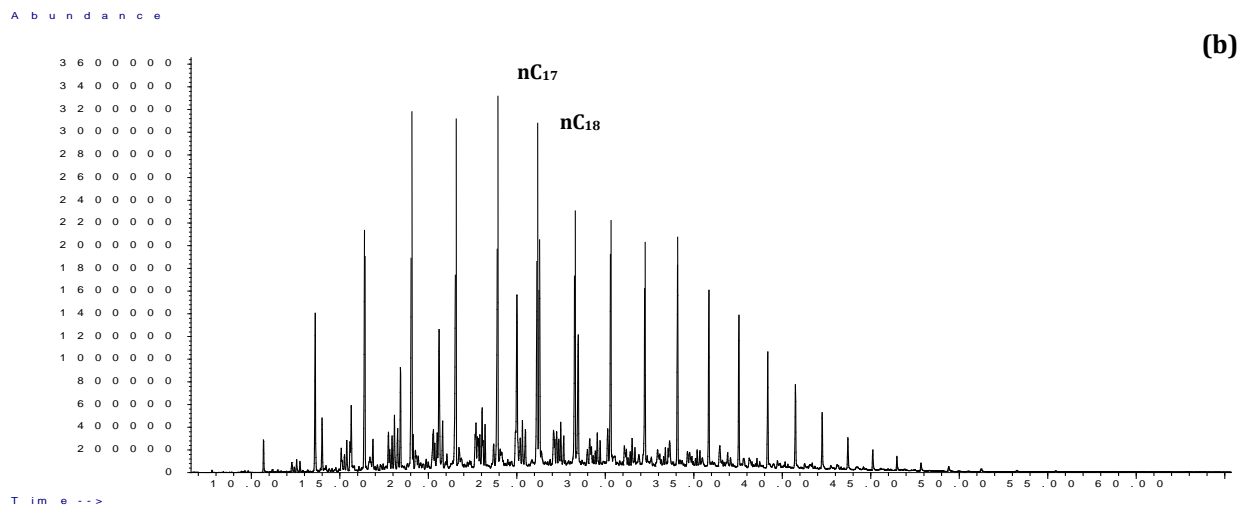
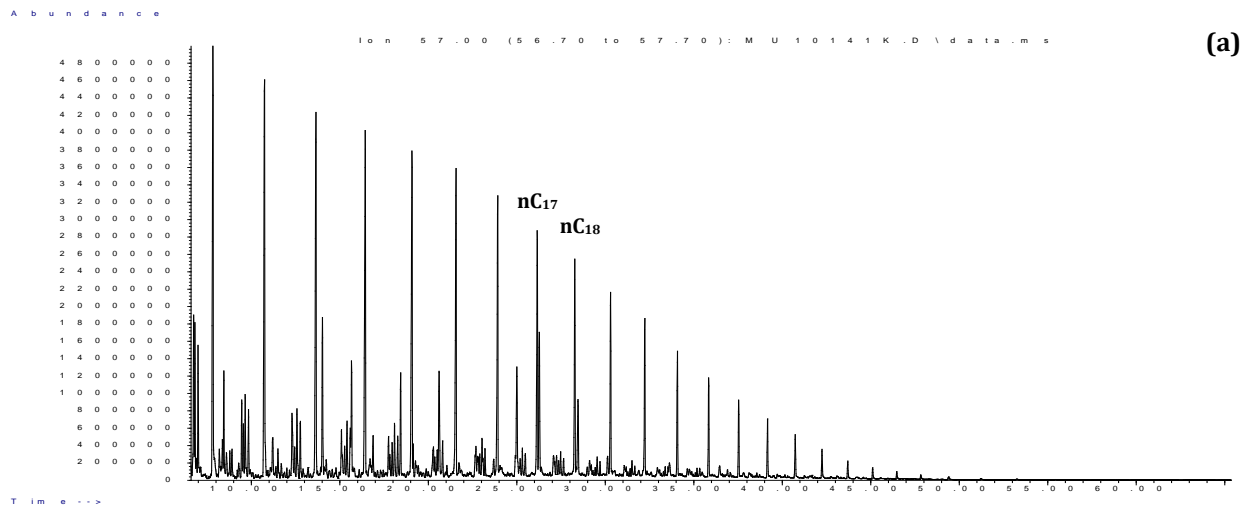
Table 6. Summary of MC252 Oil Before and After *In Situ* Burning

	May 2010		July 2010	
	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>
Avg Total Aromatics (mg/kg)	5,300±900	2,600±1910	3,800	997
Avg Modified FFPI	0.77±0.03	0.65±0.10	0.75	0.64
Avg Wang Pyrogenic Index	0.01±0.002	0.07±0.06	0.01	0.05
Avg Phen/Anth	81±29	29±19	140	14.0
Avg FluorAnt/Pyr	0.46±0.03	0.47±0.09	0.49	0.37
n=	8	10	1	1



Figures 12. PAH profiles and enhancement of 5-6 ring pyrogenic compounds in MC252 oil as a result of *in-situ* burning, May 2010 (a) and July 2010 (b).

Figure 13 shows the changes in the normal alkane profiles of fresh MC252 riser oil, MC252 oil prior to *in-situ* burning, and MC252 oil after *in-situ* burning. *In-situ* burning decreased the average total target aromatics by approximately 49% for May 2010 samples, and approximately 26% for the July 2010 sample set. Once again, the lower percentage for July is reflective of the weathering that had already altered this pre-burn sample.



Figures 13a-c. Alkane profiles of fresh MC252 riser oil (top, a); weathered MC252 oil before *in-situ* burning (middle, b); and, MC252 oil after *in-situ* burning (bottom, c). Note progressively larger prominence of alkanes in the boiling point range of 204°C to 538°C (nC₁₂ to nC₃₅) in (b) from weathering and (c) from *in situ* burning.

The average FFPI of the before *in-situ* burning samples was 0.77 ± 0.03 and the average FFPI of the after *in-situ* burning samples was 0.65 ± 0.10 , indicating a shift from petrogenic to pyrogenic composition. This difference was significant ($p=0.001$; Student two-tailed t-test with the assumption of equal variance). The average Wang Pyrogenic Index of the before *in situ* burning samples was 0.01 ± 0.002 and after *in situ* burning was 0.07 ± 0.06 , also indicating a shift from petrogenic to pyrogenic composition. This difference is also significant ($p=0.01$; Student two-tailed t-test with the assumption of equal variance). Figure 14 is a graphical depiction of the Wang Pyrogenic Index. “Before” samples are clearly separated from most of the “after” samples in the figure. According to Wang et al. (1999), lighter petroleum products and most crude oils fall into the range of 0.00-0.01, which is supported in this instance. The “after” samples are characterized by an increase in the pyrogenic index along with a decrease in the phenanthrene/anthracene ratio; however, the majority fall in the range of what Wang et al. would consider to be heavy oils/fuels and tarballs. There are two “after” samples that align more towards “before” samples, perhaps indicating an inefficient burn.

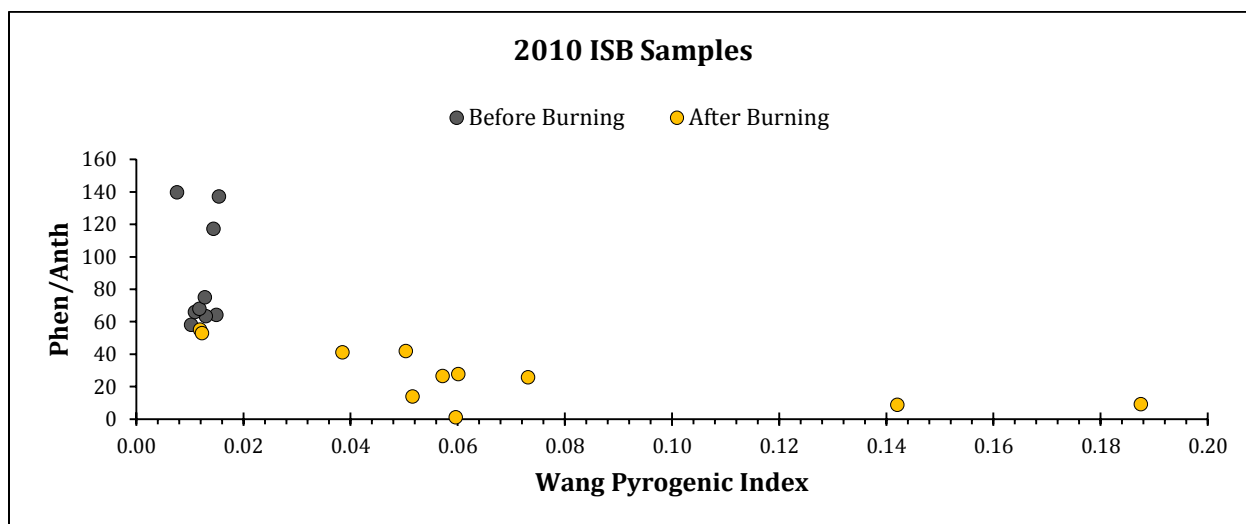


Figure 14. Plot of the Wang pyrogenic index over the ratio of phenanthrene/anthracene for before and after *in situ* burning samples collected in May and July 2010.

Task 3: Physical and chemical characterization of deep water (~200 m) tarballs collected by royal red shrimp trawlers

Royal red shrimp background

The royal red shrimp (*Hymenopeneus robustus* or *Pleoticus robustus*) shown in Figure 15 is a large, deep-water penaeid shrimp that has a wide distribution from the northern U.S. coast to the east coast of South America (Anderson and Lindner, 1971). More specifically, the known range for royal red shrimp in U.S. waters extends from the Atlantic coast of New England, south of Martha's Vineyard, through the Gulf of Mexico and the Caribbean Sea, to French Guiana. They inhabit the upper continental slope region, at depths from 180 to 730 m. (Farfante, 1988).

Unlike other penaeid shrimp species that complete their life cycle within a year, royal red shrimp live for several years (Cascorbi, 2007). Males reach a maximum total length of 180 mm; females, 225 mm (Farfante, 1988).

Few details are known about the life history of the royal red shrimp. Spawning is believed to occur

year-round, but predominantly between January and May, and on the upper regions of the continental slope. Larval forms of the shrimp are unknown (Gulf of Mexico Fishery Management Council, 2004).

The royal red has won favor as a commercial fisheries product for its sweet, juicy flesh and striking red color. The fishery for royal red shrimp serves a niche market, representing a relatively small proportion of the overall shrimp industry in the Southeast U.S. and Gulf of Mexico (Stiles et al., 2007).



Figure 15. Royal red shrimp, *Pleoticus robustus*. Photo by Connecticut Sea Grant.

Royal red shrimp were investigated as an experimental fishery beginning in 1950 in the southeastern U.S. with support from the Bureau of Fisheries, the predecessor federal agency to the NOAA National Marine Fisheries Service (Bullis, 1956). The commercial fishery began officially in the Gulf of Mexico and off Florida's east coast in 1962. According to catch statistics for the Gulf of Mexico cited by Cascorbi (2007), landings of royal red shrimp averaged about 123 MT per year since 1998, or about 0.18% of total Gulf shrimp landings. Of nearly 3,000 vessels permitted for shrimp fishing in the Gulf of Mexico in 2005, only 15 specifically targeted royal red shrimp (Gulf of Mexico Fishery Management Council, 2005). At the time of the *Deepwater Horizon* spill in 2010, NOAA indicated that there were 250 active royal red permits, but that only a few were active.

On November 20, 2010, NOAA received a report from a fisherman who caught tarballs while trawling for royal red shrimp in federal waters off the coast of Alabama. The area had just been re-opened by NOAA to commercial fishing on November 15 after the established sensory testing of 286 finfish and 55 shrimp samples, and chemical analysis of 33 composite samples of 207 finfish and 9 composites of 50 shrimp showed no apparent oil contamination. However, as a result of the

tarballs encountered in the deep water trawl fishery, on November 24, 2010, NOAA closed 4,213 square miles (10,911 sq km) to royal red shrimp fishing only (Figure 16). Fishing for finfish or other penaeid shrimp species was still permitted.

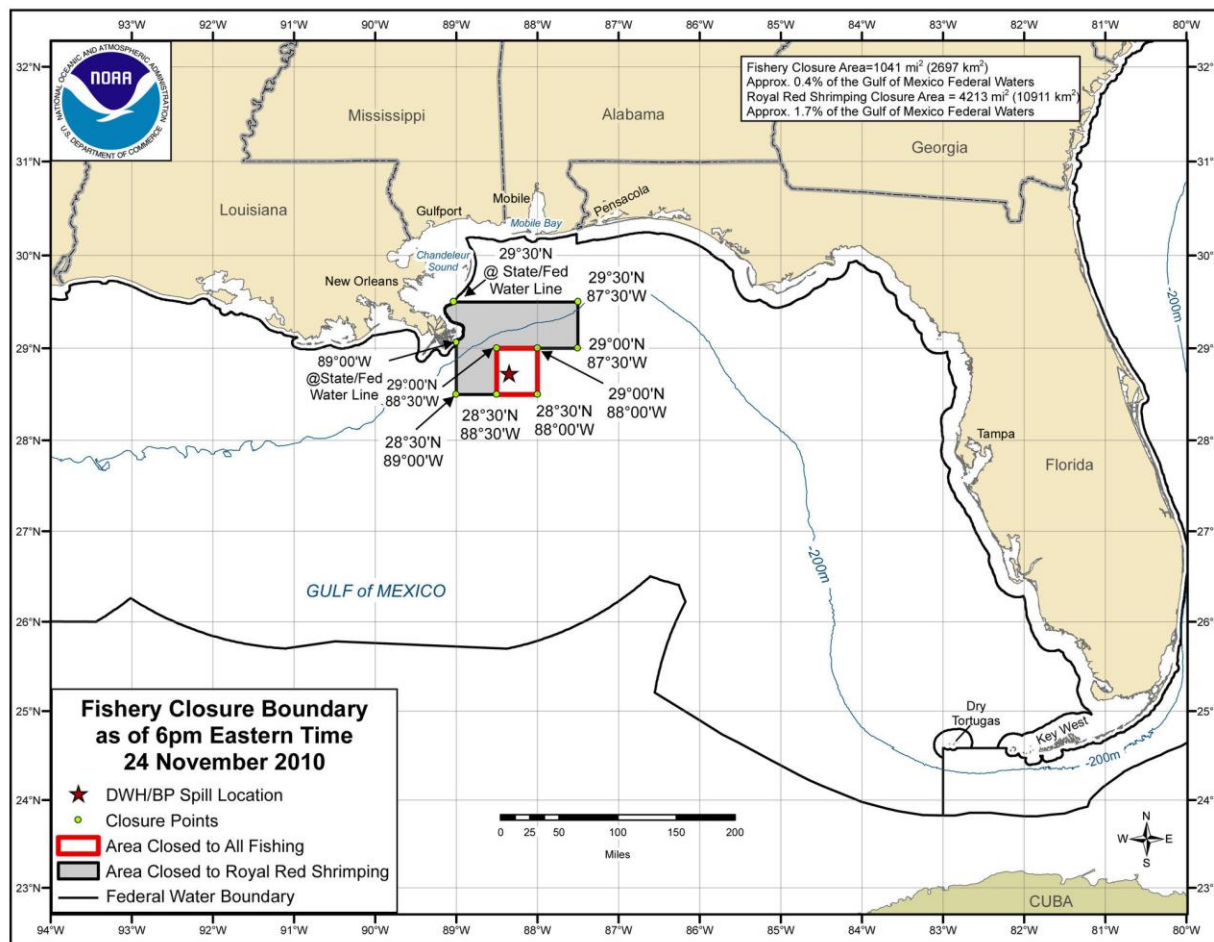


Figure 16. Fishery closure map, 24 November 2010, showing the royal red shrimp closure area due to tarballs. Source: NOAA.

In December 2010 and January 2011, NOAA chartered two fishing vessels to sample royal red shrimp in the closed area over three cruises. The samples were assessed via the sensory and chemical protocols jointly established by the U.S. Food and Drug Administration and NOAA for determining the safety of seafood during the *Deepwater Horizon* response. In addition to targeting PAHs by both gas chromatography and mass spectrometry (GC/MS) and high performance liquid chromatography/fluorescence (HPLC-UVF), samples were also analyzed for dioctyl sodium sulfosuccinate (DOSS), a component of the Corexit dispersants used during the response. Video surveys were also conducted in the closed area to determine the extent of tarball occurrence.

Three of the royal red shrimp samples collected in December failed sensory evaluation by a three-person panel; i.e., they were judged to be tainted. However, chemical analysis failed to show any sign of PAH contamination. The areas where the failed sensory samples originated were resampled in January, and the shrimp were evaluated by a larger (seven-person) sensory panel. All samples passed this round of sensory evaluation.

Chemistry results, summarized in Table 7, from both GC/MS and HPLC-UVF for the edible tissues of the royal red shrimp were below the established levels of concern for all samples collected after the November closure. Based on the sensory and chemistry results, the area was re-opened to all fishing on 3 February 2011.

Table 7. Results for Chemical Analysis of Royal Red Shrimp Sampled in December 2010 and January 2011 (Source: NOAA)

PAHs	Level of Concern (ppb)	Range of values (ppb) GC/MS	Range of values (ppb) HPLC/fluorescence [^]
Naphthalene	123,000	0.63 - 2.0	<2.4
Fluorene	246,000	0.13 - 0.53	<0.41
Anthracene/ Phenanthrene	1,846,000*	0.23 - 1.6	<1.24 - 3.3
Pyrene	185,000	<0.070 - 0.41	<5.7
Fluoranthene	246,000	<0.071 - 0.33	<6.5
Chrysene	132,000	<0.14 - 1.1	<3.7
Benzo(k)fluoranthene	13,200	<0.12 - <0.31	<0.26 - 2.7
Benzo(b)fluoranthene	1320	<0.12 - <0.31	<0.66
Benz(a)anthracene	1320	<0.11 - <0.22	<1.1
Indeno(1,2,3-cd)pyrene	1320	<0.11 - <0.22	<7.7
Dibenz(a,h)anthracene	132	<0.094 - <0.23	<1.8
Benzo(a)pyrene	132	<0.11 - <0.28	<0.96
DOSS	Level of Concern (ppm)	Range of values (ppm)	Range of values (ppm)
Dioctylsulfosuccinate	500	<0.043 - <0.045	---

* Level of Concern for Anthracene and Phenanthrene combined.
[^] HPLC/fluorescence (screening) analyses have higher limits of quantitation than the more sensitive and laborious GC/MS analyses.

Preliminary chemical analysis of some of the tarballs recovered from the trawl catches indicated a possible sourcing to burned Macondo Well oil. Many of the trawl locations where tarballs were encountered were 10 km or less from the primary *in-situ* burn locations.

Three sets of tarballs collected from royal red shrimp trawls were analyzed by LSU. One set was from the vessel *Our Mother* and contained 8 tarballs collected in January 2011. The second set was from the vessel *Aubreigh Marie* and contained 13 tarballs collected in March 2011. The third shrimp trawl set contained five tarballs collected in June 2011. These tarballs were collected ~200 m below the ocean surface and were large, viscous, and dense globs of oily residue encapsulated within a hardened exterior and the tumbling of these tarballs in the trawl nets incorporated shrimp and other trawl debris into the residue (see Figure 17).

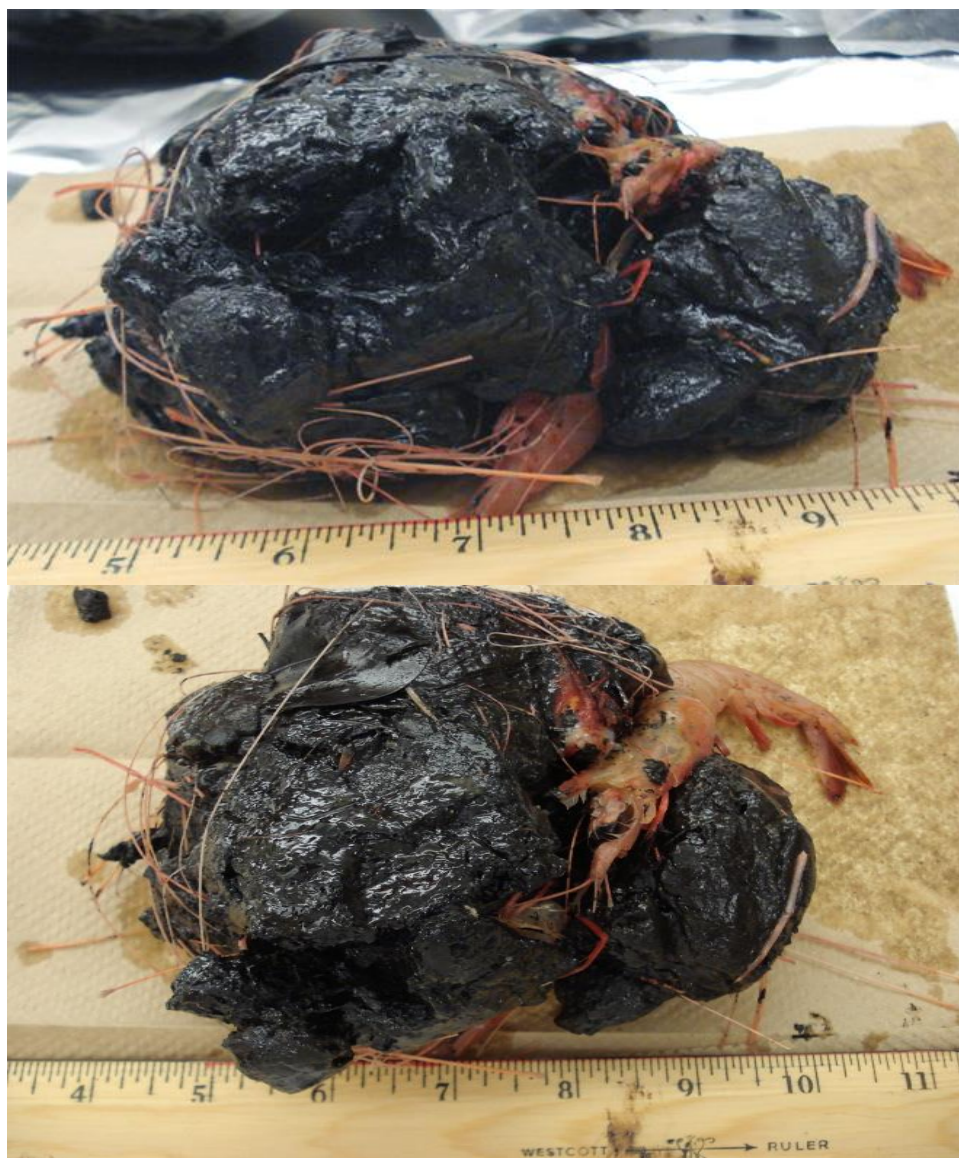


Figure 17. Two views of tarball & shrimp sample recovered in June 2010. LSU photos.

The average density and percent asphaltenes of the shrimp trawl tarballs were significantly different from other MC252 tarballs ($p=0.02$ and <0.001 , respectively; Student two-tailed t-test with assumption of homogenous variance). The average density of the shrimp trawl tarballs was 1.04 ± 0.22 g/mL and the average percent asphaltenes was $22\%\pm 16$ —compared to the density of 0.86 ± 0.33 g/mL and percent asphaltenes of $1.5\%\pm 1.7$ for other MC252 tarballs. Even though there was a significant difference, the variability within each of the tarball groups was high. Regardless of the variability, the trawl tarballs have an average density that is heavier than sea water with a typical density of 1.02 g/mL. Density measurements could be easily affected by any amount of extraneous material incorporated in the tarball, and also by the effect of evaporation of the initial oil. Percent asphaltenes could be affected by compositional differences caused by the efficiency or extent of the burn since *in-situ* burning is not a controlled process which greatly affects the consistency of ISB residues.

The average density of the shrimp trawl tarballs was not significantly higher than the average density of *in-situ* burn samples collected after burning (p=0.27); however, the asphaltenes content was significantly higher (p=0.02) than the percent asphaltenes of the *in-situ* burn samples collected during burn operations. This suggests some other factor influencing these “sinking tarballs” that was not present or determined for samples taken immediately after *in-situ* burning at sea; and, a factor that did not influence other tarballs formed after the *Deepwater Horizon* incident. It is possible that an aspect of the prolonged periods of combustion that caused the increase in density and the eventual tendency of the burn residue to sink and weather at depth enhanced the relative content of asphaltenes in the tarballs that were eventually recovered by shrimp trawls. As mentioned earlier, Buist et al. (1997) suggest that *in situ* burning results in imperfect equilibrium flash vaporization that selectively concentrates and preserves asphaltene fractions of burn residues, regardless of whether the oil was fresh or weathered prior to burning, which results in a progressive increase in the density of the residue. Density and % asphaltenes data are presented in Tables 8 and 9, respectively.

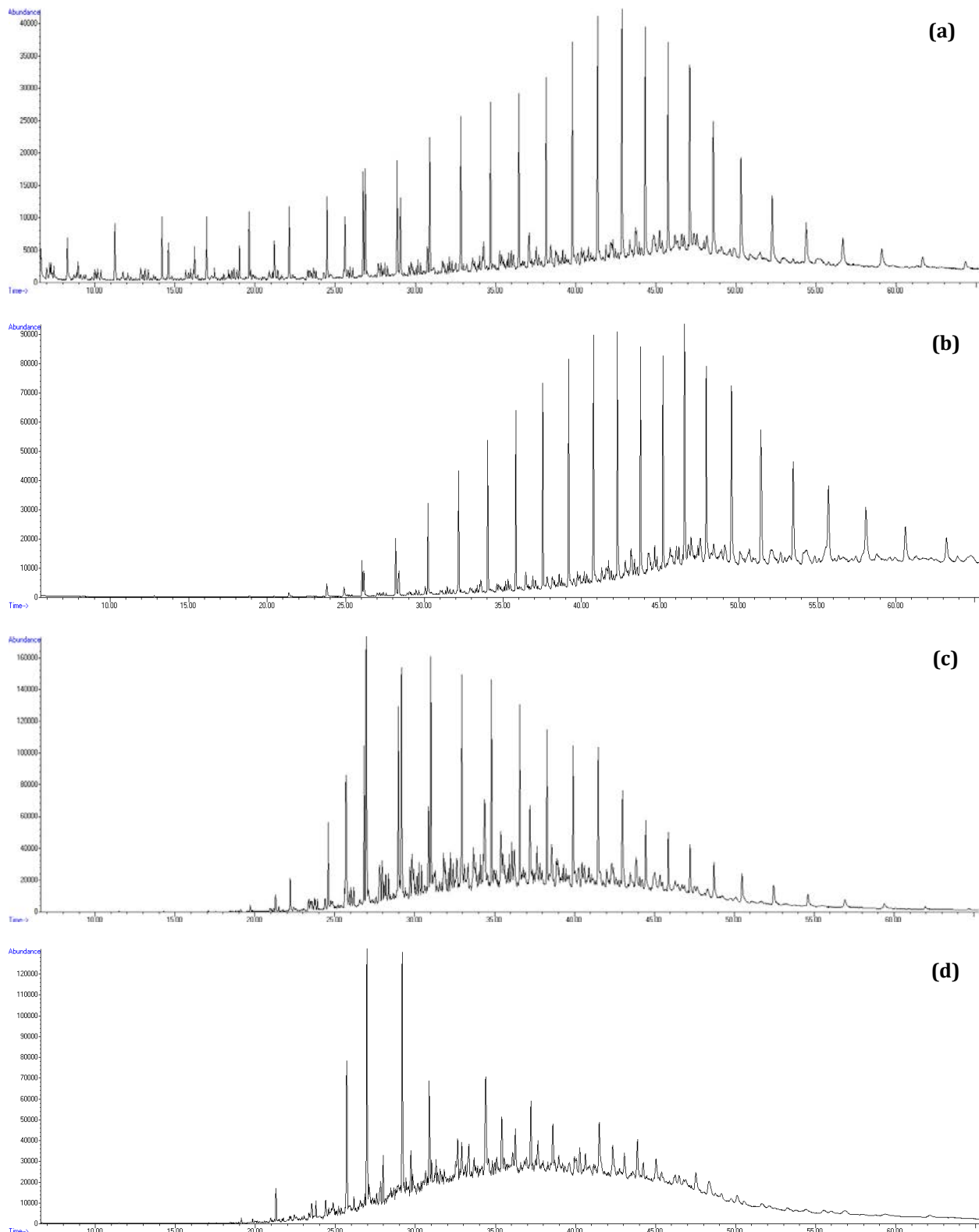
Table 8. Shrimp Trawl Tarball Density Comparisons

Shrimp Trawl Tarballs	Density (g/mL):	ISB After Residue	Density (g/mL):	Other MC252 Tarballs	Density (g/mL):
2011019-01	0.86	2010147-123	0.95	2010134-01	1.85
2011019-02	0.94	2010147-125	0.93	2010134-02	1.04
2011019-03	0.77	2010147-127	0.91	2010134-04	1.12
2011019-04	0.97	2010147-129	0.98	2010201-51	0.95
2011019-05	1.10	2010147-133	0.93	2010228-01	1.17
2011019-06	0.93	2010147-135	0.99	2010228-02	0.69
2011019-07	0.87	2010147-137	0.98	2012249-01	0.57
2011019-08	0.98	2010147-138	0.98	2012250-05	0.56
2011116-01	1.20	AVERAGE	0.95	2012250-10	0.60
2011116-02	0.87	STDEV	0.03	2012258-11	0.56
2011116-03	0.96	n=	8	2012263-01	1.12
2011116-04	1.13			2012265-67	1.28
2011116-05	1.47			2012265-68	0.79
2011116-06	1.33			2012272-02	0.86
2011116-07	1.11			2012278-01	0.67
2011116-08	1.57			2012278-02	0.75
2011116-28	1.25			2012278-03	0.58
2011116-29	1.48			2012289-01	0.58
2011116-30	0.78			2012289-02	0.59
2011116-31	0.95			2012289-03	0.78
2011116-32	0.90			AVERAGE	0.86
2011187-01	0.93			STDEV	0.33
2011187-02	0.93			n=	20
2011187-03	0.92				
2011187-04	0.97				
2011187-05	0.94				
AVERAGE	1.04				
STDEV	0.22				
n=	26				

Table 9. Shrimp Trawl Tarball % Asphaltenes Comparisons

Shrimp Trawl	Asph (wt. %)	ISB After Residues	Asph (wt. %)	Other MC252 Tarballs	Asph (wt. %)
2011019-01	26	2010147-125	3.1	2010131-02	0.81
2011019-02	39	2010147-133	1.2	2010134-01	1.7
2011019-03	35	2010147-135	8.3	2010146-02	2.31
2011019-04	52	2010147-137	6.2	2010228-02	0.59
2011019-05	39	2010147-138	2.2	2011059-01	1.16
2011019-06	36	AVG ISB After	4.2	2012249-01	0.80
2011019-07	30	STDEV	3.0	2012250-09	0.38
2011019-08	41	n=	5	2012250-10	0.42
2011019-28	41			2012265-68	6.6
2011116-02	4.1			2012278-01	0.74
2011116-03	4.3			2012278-02	0.46
2011116-30	4.5			2012278-03	0.62
2011116-31	6.0			2012289-01	0.70
2011116-32	5.8			2012289-02	1.1
2011187-01	13			2012289-03	4.3
2011187-02	4.3			AVG Other	1.52
2011187-03	8.8			STDEV	1.73
2011187-04	18			n=	15
2011187-05	13				
AVG Trawl	22				
STDEV	16.2				
n=	19				

The normal alkane profile of these “sinking” tarballs was significantly different from other MC252 oil tarballs. This profile (Figure 18a) appeared to be unique to tarballs collected at ocean depth and was similar to the normal alkane profile of the simulated distillation (SIMDIS) of MC252 oil at 205°C (Figure 18b). The SIMDIS and shrimp trawl tarballs exhibited evidence of thermal combustion that was absent in the normal alkane profiles of the *in-situ* burn oil residues collected after burning (refer back to Figure 13). The SIMDIS procedure separates oil into various fractions depending on molecular weight and associated boiling points. The shrimp trawl sample appears to have undergone a similar process, thus resulting in an alkane profile similar to the SIMDIS of MC252 oil. Therefore, it is believed that the shrimp trawl tarballs are the result of more efficient *in-situ* burns relative to other field burn residues. The shape of the *in situ* burn tarball profile was very different from tarballs not associated with burning (i.e., weathered residues, Figure 18c) but still considered to be a match to MC252 oil. Figure 18d is the normal alkane profile of microbially-weathered MC252 oil. This profile demonstrates that residues from *in situ* burning were not subjected to the typical weathering processes other oil residues reflect.



Figures 18 a-d. Normal alkane distribution depicting the similarity of a suspected ISB tarball (a) collected in a royal red shrimp trawl and the simulated distillation of MC252 oil at 205°C (b). The alkane distributions in (c) MC252 tarball not associated with *in-situ* burning and (d) microbially-weathered MC252 oil are dissimilar to (a) and (b) even though all four profiles are MC252 oil.

The average FFPI of the shrimp trawl samples was 0.62 ± 0.06 indicating a pyrogenic composition. The average FFPI of the shrimp trawl samples is significantly different from other MC252 tarballs ($p=0.001$; Student two-tailed t-test with the assumption of equal variance). The average Wang Pyrogenic Index of the shrimp trawl samples was 0.06 ± 0.02 , also indicating a more pyrogenic composition. The average Wang Pyrogenic Index is also significantly different from other MC252 tarballs ($p=0.01$; Student two-tailed t-test with the assumption of equal variance). Figure 19 is a graphical depiction of the Wang Pyrogenic Index. According to Wang et al. (1999), lighter petroleum products and most crude oils fall into the range of 0.00-0.01. The majority of the samples plotted in the figure have an index greater than 0.01 and are in the range of what Wang et al. would consider to be heavy oils/fuels and tarballs.

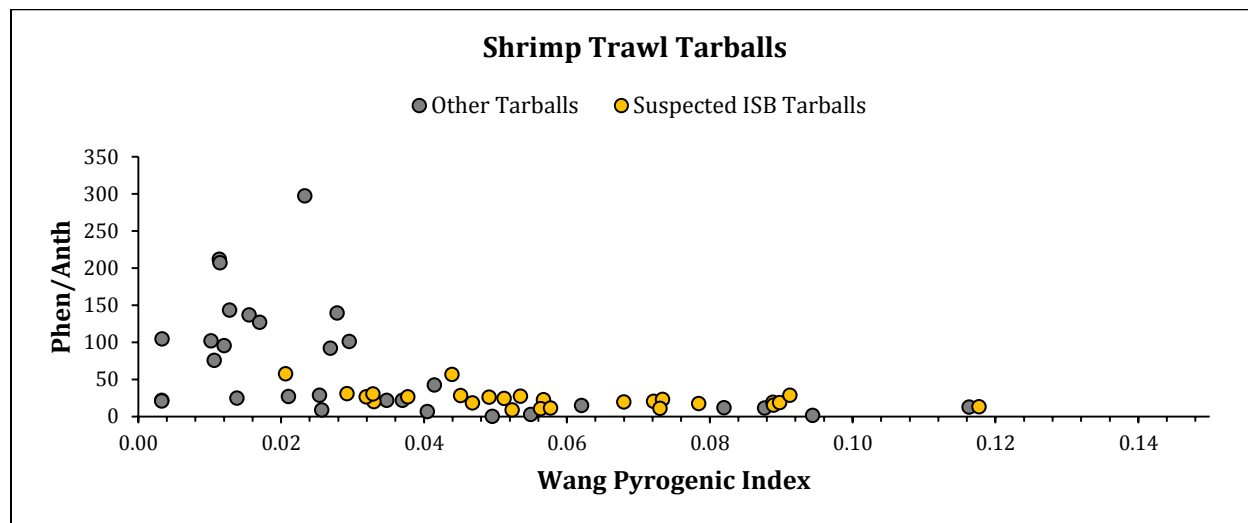


Figure 19. Plot of the Wang pyrogenic index over the ratio of phenanthrene/anthracene for shrimp trawl tarballs suspected to be sinking MC252 residues from *in situ* burning.

All 26 shrimp trawl tarballs were a match (scored 15 out of 15, or 100%) to MC252 oil based on the diagnostic biomarker ratio analyses. Figure 20 displays a comparison of the diagnostic ratio results of the shrimp trawl samples, MC252 source oil, and EPA South Louisiana Crude (SLC) oil standard. The EPA-SLC standard scored 8 out of 15, or 53% and would be considered a non-match. Table 10 provides the values that are plotted in Figure 20. The ability to discriminate between two SLC oils is a demonstration to the utility of the diagnostic ratio analyses employed for this study. Detailed descriptions of the biomarker abbreviations are given in Appendix 1.

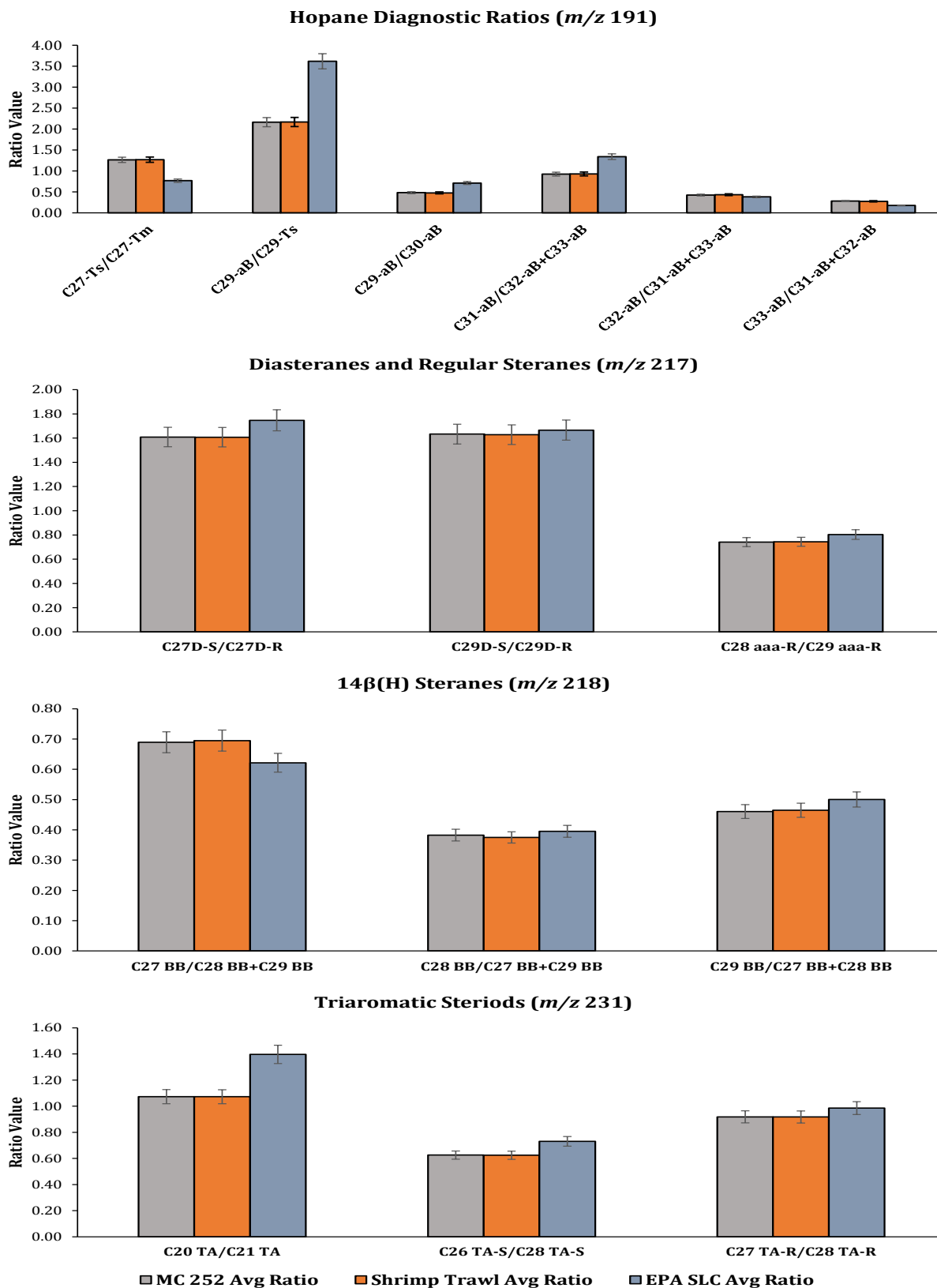


Figure 20. Graphical depiction of diagnostic ratio testing of shrimp trawl samples compared to MC252 source oil and EPA South Louisiana Crude Oil standard. Error bars represent 5% error margin.

Table 10. Diagnostic Biomarker Ratio Analysis Results for Shrimp Trawl Samples

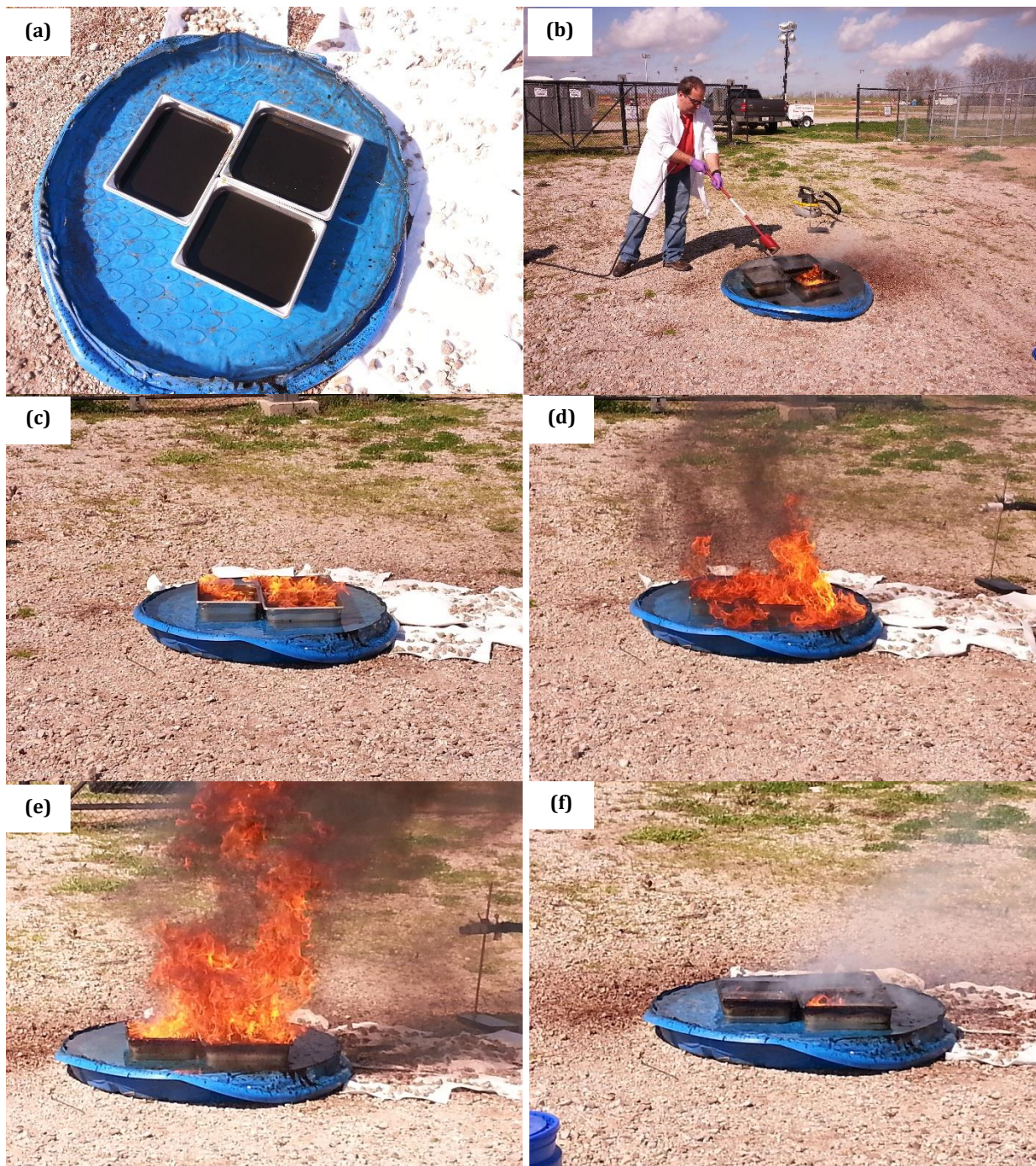
	Shrimp Trawl Ratios (n=5)	MC252 Ratio (n=32)	EPA SLC Ratio (n=4)
Hopanes (m/z 191)	AVG	AVG	AVG
C27-Ts/C27-Tm	1.27	1.26	0.77*
C29-aB/C29-Ts	2.17	2.17	3.62*
C29-aB/C30-aB	0.48	0.48	0.71*
C31-aB/C32-aB+C33-aB	0.93	0.93	1.34*
C32-aB/C31-aB+C33-aB	0.43	0.43	0.38
C33-aB/C31-aB+C32-aB	0.28	0.28	0.18*
Diasteranes and Regular 14a(H)-Steranes (m/z 217)	AVG	AVG	AVG
C27D-S/C27D-R	1.61	1.61	1.75
C29D-S/C29D-R	1.63	1.63	1.67
C28 aaa-R/C29 aaa-R	0.74	0.74	0.80
14B(H)-Steranes (m/z 218)	AVG	AVG	AVG
C27 BB/C28 BB+C29 BB	0.69	0.69	0.62
C28 BB/C27 BB+C29 BB	0.38	0.38	0.40
C29 BB/C27 BB+C28 BB	0.46	0.46	0.50
Triaromatic Steroids (m/z 231)	AVG	AVG	AVG
C20 TA/C21 TA	1.07	1.07	1.40*
C26 TA-S/C28 TA-S	0.62	0.63	0.73*
C27 TA-R/C28 TA-R	0.92	0.92	0.99

* Indicates EPA SLC ratios that are statistically different according to Hansen et al. (2007) and Meyer et al. (2014)

Task 4: Burning of surface collected Macondo Well source oil and emulsified source oil under controlled laboratory conditions

An initial controlled burn of MC252 oil was performed at LSU on 6 March 2012. Around 5g of MC252 oil was placed in a crucible and burned with a propane torch for ~4-5 minutes. The experiment was conducted in triplicate under a laboratory ventilation hood. There was not sufficient residue generated by this method to perform both physical and chemical characterization, so only chemical characterization was completed. Since this burn was performed on a reduced scale, plans were made to conduct a second, larger-scale burn using an increased volume of oil floated on artificial seawater.

The second larger scale burn was conducted on 20 February 2014 at the LSU Environmental Health and Safety facility on the Baton Rouge campus. This burn used 500mL of fresh and 15% weathered surrogate south Louisiana crude oil obtained from BP floated on artificial seawater (avg. salinity of 34‰) in stainless steel containers. Emulsified MC252 oil was also burned in the same manner in this experiment. Each set of burns was performed in triplicate, and burn durations were ~5-10 minutes. Figure 21a-f shows the sequence of the burning process. After the burns were completed, the stainless steel containers were allowed to cool, and were then transported back to the lab where the residues were sampled for both physical and chemical analyses.



Figures 21a-f. Images from the larger-scale burn at LSU in 2014. (a) pans with South Louisiana crude floated on seawater; (b) ignition procedure; (c-e) sequence of mid-burn photos; (f) extinguishment.

Task 5: Physical and chemical characterization of burn residue resulting from Task 4

There was not enough material to complete both the physical and chemical characterization of the initial smaller-scale burn of MC252 oil in 2012; therefore, only chemical characterization was completed for the triplicate burn samples. Normal alkane chromatographic profiles (Figure 22) of the replicates from the smaller-scale controlled burn of MC252 oil exhibited similar chemical characteristics as the *in-situ* burn samples collected during the response to the *Deepwater Horizon*

oil spill, and also resemble the normal alkane chromatographic profile of the shrimp trawl tarball in Figure 18a. For the “after” samples, the modified FFPI was 0.54 ± 0.02 and the modified Wang Pyrogenic Index was 0.13 ± 0.02 . Significant enhancement in the same pyrogenic PAHs as described earlier for Task 2 was also observed and the total target PAHs were reduced from 12,000 mg/kg in fresh MC252 oil to an average of 1,200 mg/kg in the burn residues (a 90% reduction). The PAH profiles for the before and after MC252 from this small-scale burn are shown in Figure 22 along with the PAH profiles from the larger-scale burns.

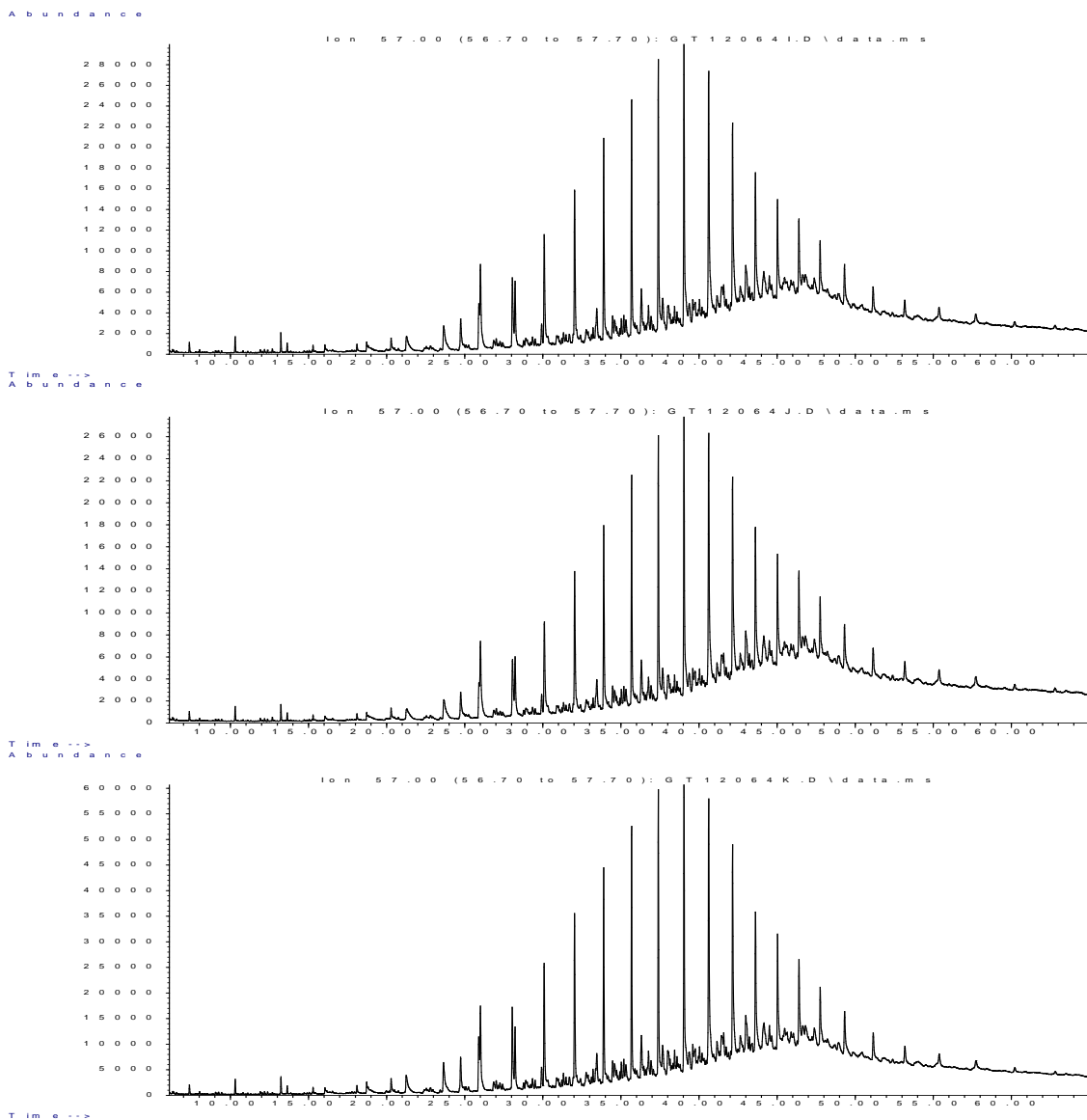


Figure 22. Normal alkane profiles (m/z 57) for three replicate samples from a small-scale lab burn of MC252 oil.

Results of the second, larger-scale burn of surrogate south Louisiana crude oil were quite different from the actual *in-situ* burn samples previously characterized in Task 2 and from the small-scale burn of MC252. There were no significant changes in density or percent asphaltenes. There was no enhancement of the pyrogenic PAHs observed in the actual ISB samples and the smaller scale controlled burn. Only the modified FFPI showed a significant ($p < 0.001$) change. The average FFPI

before burning was 0.79 ± 0.06 and the average FFPI after burning was 0.70 ± 0.02 . Table 11 summarizes the results of the larger-scale controlled burn described above.

Table 11. Summary of Chemical Characteristics from Larger-Scale Controlled Burn

	Fresh Surrogate Oil		15% Weathered Surrogate Oil (by weight)		Emulsified MC252 Oil	
	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>
Avg Total Aromatics (mg/kg)	8,100	3,600±310	9,400	3,400±310	2,700	2,600±270
Avg Modified FFPI	0.83	0.69±0.02	0.83	0.71±0.01	0.73	0.71±0.01
Avg Wang Pyrogenic Index	0.01	0.04±0.01	0.01	0.04±0.004	0.01	0.01±0.0003
Avg Phen/Anth	45.3	13.3±2.0	45.7	11.7±1.2	123	67.4±8.8
Avg FluorAnt/Pyr	0.38	0.60±0.06	0.35	0.80±0.01	0.40	0.32±0.02
n=	1	3	1	3	1	3

	Fresh Surrogate Oil		15% Weathered Surrogate Oil (by weight)		Emulsified MC252 Oil	
	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>	<u>Before</u>	<u>After</u>
Avg Density (n=3)	0.86±0.001	0.84±0.05	0.90±0.002	0.94±0.02	0.98±0.003	0.99±0.01
Avg % Asphaltenes (n)	1.0(1)	1.98±0.67(3)	0.90(1)	1.98±0.68(3)	1.71(1)	2.20±0.34

Figure 23 is a graphical depiction of the Wang Pyrogenic Index. “Before” samples are clearly separated from most of the “after” samples in the figure. Fresh oil before and 15% weathered oil before are on top of each other in the plot (0.006, 45). According to Wang et al. (1999), lighter petroleum products and most crude oils fall into the range of 0.00-0.01, which is supported in this instance. The “after” samples are characterized by an increase in the pyrogenic index along with a decrease in the phenanthrene/anthracene ratio; however, the majority fall in the range of what Wang et al. would consider to be heavy oils/fuels and tarballs. There was very little change in the Wang Pyrogenic Index for the emulsified MC252 oil.

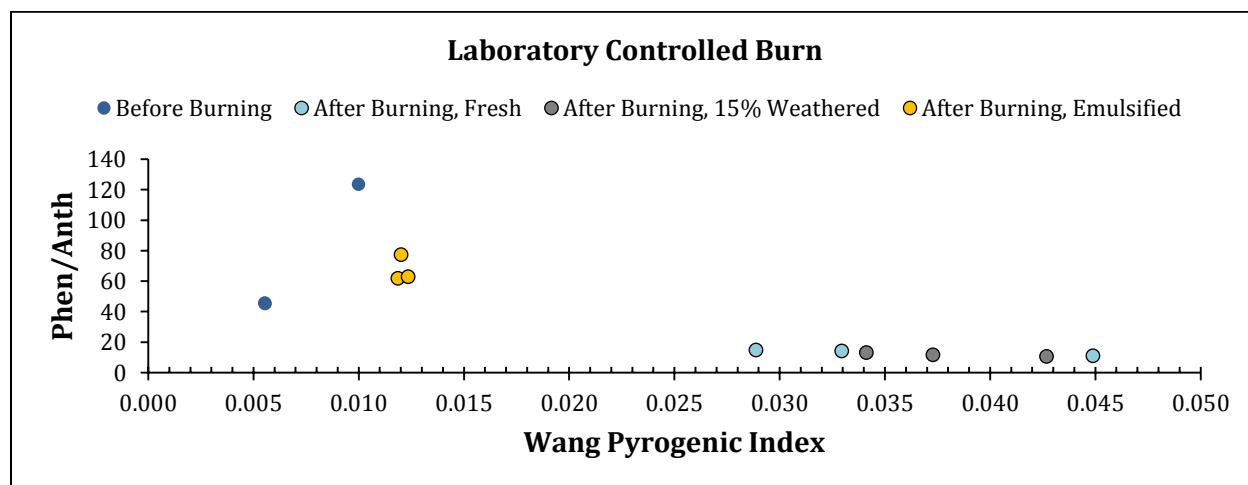
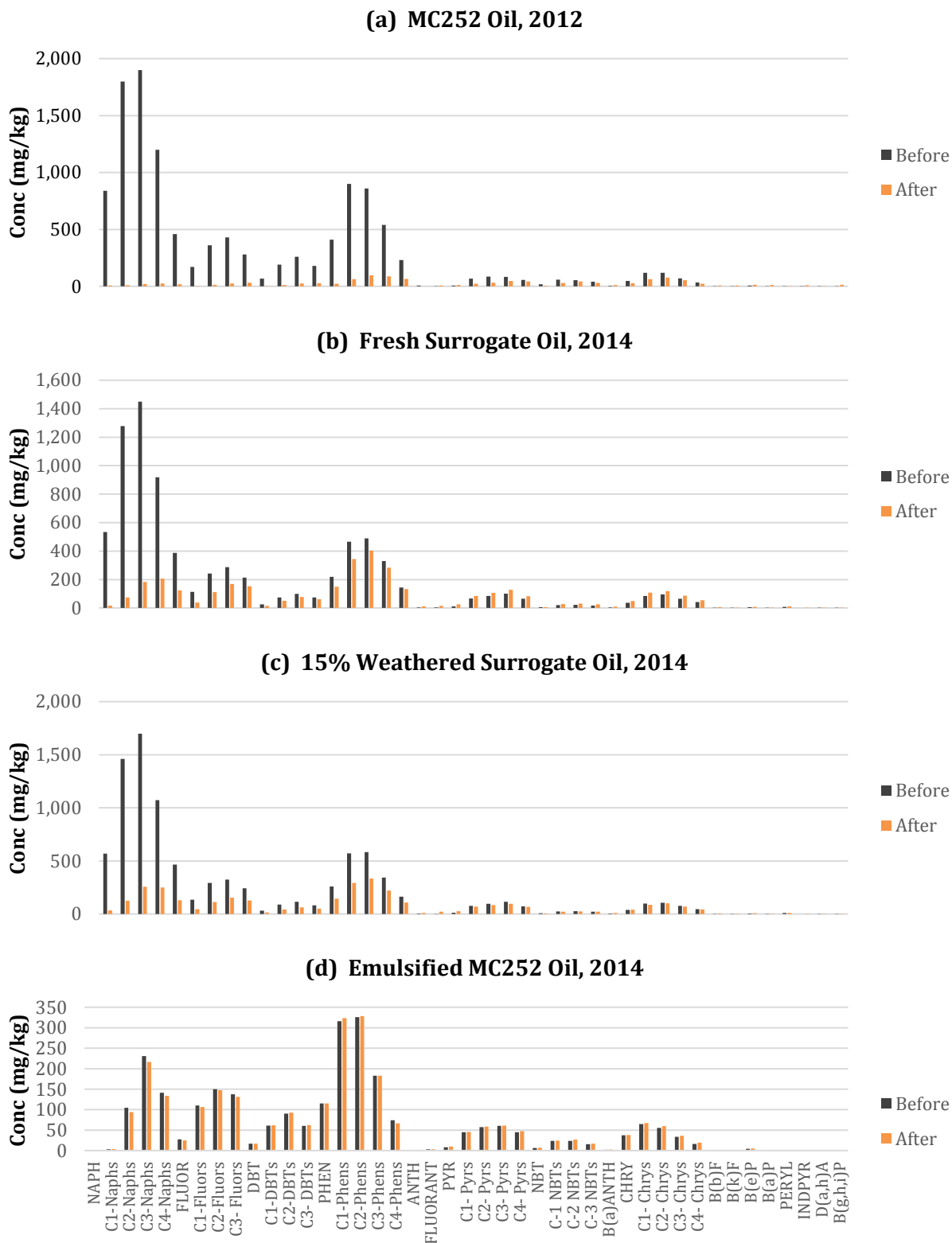


Figure 23. Plot of the Wang pyrogenic index over the ratio of phenanthrene/anthracene for laboratory controlled burning of surrogate crude oil and emulsified MC252 oil.

The difference in results may be due to the use of the surrogate south Louisiana crude oil instead of MC252 oil; unfortunately, this was the only option for burning a larger volume of oil under controlled conditions, as availability of original MC252 riser oil is extremely limited. Controlled *in-situ* burning decreased the average total target aromatics of the fresh surrogate oil by approximately 45%, and the average total target aromatics of the 15% weathered surrogate oil by approximately 36%, both of which are similar for the field *in-situ* burn samples.

Emulsified MC252 oil collected during the *Deepwater Horizon* clean-up operations was also burned during this portion of the laboratory work. The emulsified MC252 oil did not burn well, and as a result, reflected the least amount of change from before to after. There were no significant changes in the emulsified MC252 oil's total aromatics, modified FFPI, and there was no enhancement of the pyrogenic PAHs. Figure 24 shows the PAH profiles for each oil type and post-burn residues tested in the larger-scale controlled burn.

Hopane normalization (Aeppli et al., 2014; Douglas et al., 1996; Garrett et al., 2000; Prince et al., 1994) was applied to the Fresh and 15% Weathered surrogate South Louisiana Crude oil. Using the hopane normalized concentrations, the percent increase or decrease of each target analyte was calculated and are presented in Figure 25. An average 5-point response factor of 0.91 ($R^2=0.9912$) was determined for $17\beta(H),21\beta(H)$ -30-hopane. This response factor was then used to calculate the concentration of $17\alpha(H),21\beta(H)$ -hopane. The hopane normalization factor ($\text{Hopane}_{\text{oil}}/\text{Hopane}_{\text{Weathered}}$) for the Fresh surrogate oil was 0.76, and 0.88 for the 15% Weathered surrogate oil. The results of the hopane normalization of controlled laboratory burns confirms the enrichment of higher molecular weight analytes previously documented in Tasks 2 and 5 (small-scale burn of MC252 oil).



Figures 24a-d. Before and after PAH profiles for controlled burns of four oil types in 2012 and 2014. (a) MC252 riser oil; (b) fresh surrogate South Louisiana crude; (c) weathered surrogate South Louisiana crude; and, (d) emulsified MC252.

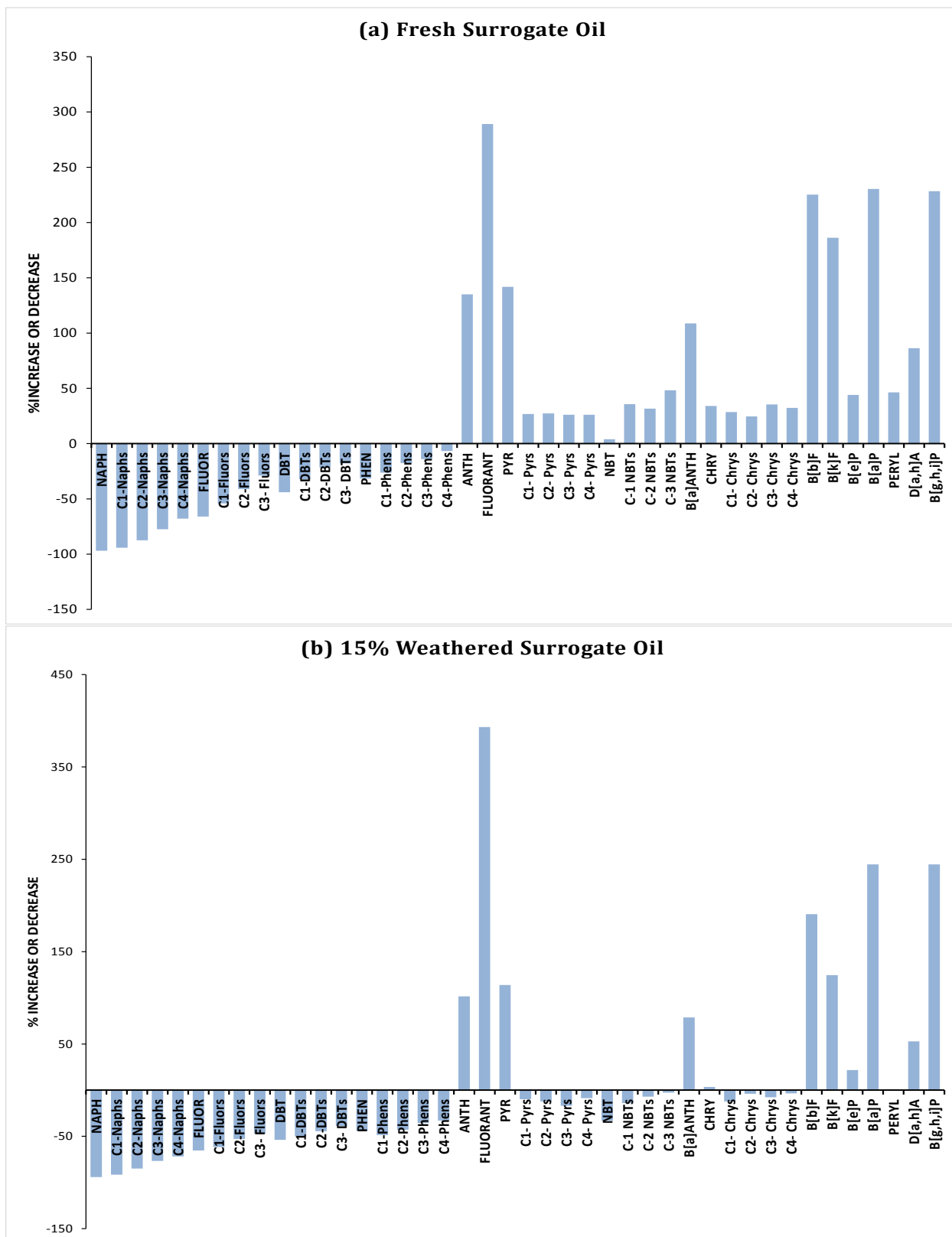


Figure 25. Determination of percent increase or decrease of target analytes based on hopane normalization for (a) Fresh surrogate oil and (b) 15% Weathered surrogate oil.

Task 6: Forensic interpretation of analytical results to characterize physical and chemical changes occurring during the combustion of oil, to include comparison of changes occurring in various weathered oil samples and identification of potential markers for *in-situ* burn residues.

A primary objective of the forensic interpretation of analytical results was to determine if residues from *in-situ* burning during the *Deepwater Horizon* response had measurable characteristics that could distinguish them from other MC252 residues. Results from the other tasks previously discussed indicated that these characteristics included:

- Significant increase in density and percent asphaltenes;
- Enhancement of pyrogenic PAHs;
- Decrease in the FFPI;
- Increase in the Wang Pyrogenic Index; and,
- Evidence of thermal combustion in the normal alkane profile.

However, it appears that all of these discriminating factors must be present to make the distinction between sources with any confidence. Because of this limitation, the forensic interpretation was expanded to include a statistical analysis of sample matrices and sampling locations based on the diagnostic biomarker ratio analyses.

A total of 130 suspected MC252 residues was initially chosen. All samples were grouped according to year, sample matrix (e.g., ISB oil, ISB tarball, oil, other, sediment, or tarball) and sampling location (e.g., marsh, ocean depth, shore, or water surface). These samples were then narrowed to a total of 112 by the diagnostic ratio analyses. Only samples determined to be a statistical match to MC252 oil were used in the matrix and sampling location comparisons. A one-way ANOVA with a post-hoc Tukey-Kramer pairwise comparison, Table 12, was applied to the sample matrix analysis for each of the 15 diagnostic ratios to determine if any of the ratios could distinguish between *in-situ* burn residues and other MC252 oil residues. The Tukey-Kramer comparison separates samples into lettered groups based on the minimum significant difference between means ($p=0.05$). Groups with the same letter have means that are *not* significantly different from each other, and groups with differing letters are significantly different from each other. For the sample location effects, a nested ANOVA with random effects and a variance components covariance structure was carried out for each diagnostic ratio to determine if there were any differences due to location of sample collection. In both cases, the sample number in each matrix or location was highly unbalanced.

A total of 7 out of 15 diagnostic ratios had significant differences between sample matrices. Of these 7 ratios, 4 showed significant differences between *in-situ* burn residues and other MC252 oil residues:

- **The hopane ratio, $C_{31} aB (S+R)/C_{32} aB (S+R) + C_{33} aB (S+R)$, was significantly different for ISB oil and ISB tarball. Both of the *in-situ* burn residues were significantly different from all other matrices except the tarball matrix.**
- **Another hopane ratio, $C_{33} aB (S+R)/C_{31} aB (S+R) + C_{32} aB (S+R)$, for ISB tarball was significantly different from all other matrices; and, ISB oil was significantly different from all other matrices except tarball and oil.**
- **The diasterane and regular sterane ratio, $C_{28} aaa-R/C_{29} aaa-R$, was significantly different when comparing ISB oil and oil residues.**

- The last significant ratio was C27 BB (S+R)/C28 BB (S+R) + C29 BB (S+R) (in the 14(β)H-steranes group). This ratio was significantly different when comparing ISB tarballs to other tarballs.

Table 12. Tukey-Kramer Pairwise Comparisons of Diagnostic Ratios for Different Matrices

C31 aB (S+R)/C32 aB (S+R) + C33 aB (S+R)				C28 aaa-R/C29 aaa-R			
Matrix	Mean	SE	Group	Matrix	Mean	SE	Group
Sediment	1.0533	1.83E-02	A	Oil	0.7825	9.43E-03	A
Other	0.9933	1.83E-02	A,B	Other	0.7767	2.67E-02	A,B
Oil	0.9575	6.48E-03	B,C	Tarball	0.7592	7.40E-03	A,B
Tarball	0.9451	5.08E-03	B,C,D	ISB Tarball	0.7477	9.06E-03	A,B
ISB Oil	0.9309	6.62E-03	C,D	Sediment	0.7400	2.67E-02	A,B
ISB Tarball	0.9300	6.22E-03	D	ISB Oil	0.7361	9.63E-03	B

C33 aB (S+R)/C31 aB (S+R) + C32 aB (S+R)				C27 BB (S+R)/C28 BB (S+R) + C29 BB (S+R)			
Matrix	Mean	SE	Group	Matrix	Mean	SE	Group
ISB Tarball	0.2788	2.90E-03	A	Other	0.7550	3.45E-02	A,B
ISB Oil	0.2743	3.09E-03	A,B	Tarball	0.7500	8.24E-03	A
Tarball	0.2667	2.37E-03	B,C	Oil	0.7340	2.18E-01	A,B
Oil	0.2646	3.02E-03	B,C,D	ISB Tarball	0.7006	1.15E-01	B
Other	0.2467	8.55E-03	C,D	Sediment	0.7000	2.82E-01	A,B
Sediment	0.2400	8.55E-03	D	ISB Oil	0.6900	2.18E-02	A,B

Therefore, C28 aaa-R/C29 aaa-R may be useful for distinguishing ISB oil from other oil residues; and, C27 BB (S+R)/C28 BB (S+R) + C29 BB (S+R) may be useful distinguishing ISB tarballs from other tarballs.

A total of 5 out of 15 diagnostic ratios showed significant differences between sample matrix and sampling location. The statistical analysis did not determine which matrix by sampling location was significant; it was performed to determine if sampling location was a factor in distinguishing some of these oil residues. Sampling location can have an important role because this often governs how oil residues weather. Therefore, this analysis supported the notion that oil residues from the same type of oil may show different characteristics, determined by differences in weathering in various sampling locations, without compromising the ability to make statistical match/non-match determinations with the same ratios.

SUMMARY AND CONCLUSIONS

A primary objective of this study was to identify possible indicators that could distinguish *in-situ* burn residues from other oil residues based on physical and chemical analyses of actual *Deepwater Horizon in-situ* burn samples. Unfortunately, while there is no single reliable indicator of *in-situ* burning, partial indicators (e.g., different normal alkane profile, enhancement of pyrogenic PAHs, and reduction in the modified Fossil Fuel Pollution Index), when considered as an integrated whole, can point to *in-situ* burning as a significant driver of weathering and fate. Table 3 shows some of the characteristics assessed in this study. Notable in these results is the enhancement in asphaltenes content for known burn residues and the tarballs recovered from deep water shrimp trawls.

Table 13. Comparison of Physical and Chemical Characteristics for Different Oil Matrices

	MC252 Riser	SLC Pre- Burn	SLC Post- Burn	ISB Oil Pre-Burn	ISB Oil Post-Burn	Shrimp Tarballs	Other MC252 Tarballs
Density g/mL	0.84	0.86	0.84	0.88	0.96	1.04	0.86
Asphaltene %	0.9-1.0	1.0	1.98	1.5	4.2	22	1.5
FFPI	0.83	0.79	0.70	0.77	0.65	0.62	0.59
Wang Pyrogenic Index	0.004	0.01	0.04	0.01	0.07	0.06	0.04

Even the use of a series of partial indicators must be employed cautiously: comparison of the normal alkane profile, for example, has the caveat that the same profile was not observed in oil residues collected immediately after *in-situ* burning. The distinctive profile appeared only in tarballs that had been recovered from trawls two hundred meters below the ocean surface. The asphaltenes content of the shrimp trawl tarballs was significantly higher than all the other samples, which indicates that asphaltenes content positively correlated with the tendency of those oil residues to sink. Density was a factor but does not appear to be as critical as the percent asphaltenes with respect to sinking tarballs.

The majority of residues from the *Deepwater Horizon* oil spill retained their oil biomarker signatures, making diagnostic ratio analyses possible. The diagnostic ratios were used to:

- 1) Determine if the oil residue was a match to MC252 oil; and
- 2) Determine if there were any differences in the ratios in matching oil residues based on sample matrix and sampling location.

A total of 4 out of the 15 diagnostic biomarker ratios did distinguish *in-situ* burn residues from the majority of the other residues. Two of these ratios, C28 aaa-R/C29 aaa-R and, C27 BB (S+R)/C28 BB (S+R) + C29 BB (S+R), may be useful for distinguishing ISB oil from other oil residues and ISB tarballs from other tarballs, respectively. Significant differences were also determined in the comparison of sampling locations. These differences suggest that weathering of oil residues from the same initial source is highly dependent on the specific environmental compartment in which they reside without compromising the ability to make match/non-match determinations with the same ratios.

The *in-situ* burning research described in this report combined retrospective analysis of samples collected during the *Deepwater Horizon* response with new laboratory work and interpretation to increase the relevance and applicability of the physical and chemical results. The methodological refinements improved our abilities to distinguish burn products from other weathered oil matrices and helped to confirm the generalized source attributions to burn operations that were made shortly after tarballs were encountered in the Gulf of Mexico royal red shrimp fishery. It is important to note that the chemical characterization method (GC-MS) and target analyte list used for this report only examines about 5% of the oil mass. Other analytical methods, such as GCxGC-FID (Aeppli et al., 2014) or APPI FT-ICR MS, have become standard instrument systems for analyzing crude oil. Both have advantages over GC-MS, in that they can separate and identify a much larger percentage of oil compounds; however, it cannot be denied that GC-MS is still relevant and is one of the most recognized instrument systems for characterizing crude oil in the environment.

The *Deepwater Horizon* spill experience validated the operational feasibility and practical application of *in-situ* burning as an open water response method. Like nearly everything else about this incident, the scale of *in-situ* burn operations was unprecedented: between 28 April and 19 July 2010, over 400 individual burns took place, consuming an estimated 220,000-310,000 bbl of oil. This is roughly equivalent to the total volume for the *Exxon Valdez* oil spill in 1989.

Implicit in these numbers is the large volume of *in-situ* burn residue that was generated during the *Deepwater Horizon* operations. Using the standard *in-situ* burn efficiency estimates of 90-95 percent, the amount of residue resulting from the operations potentially ranged from 11,000-33,000 bbl. This is a substantial amount of petroleum hydrocarbons introduced into a relatively small portion of the marine environment—although it should be noted that natural seeps contribute an estimated 560,000 to 1.4 million bbl of oil into the waters of the Gulf of Mexico (National Research Council, 2003) each year (note seep locations in Figure 26).

The fate of the burn residue from the *Deepwater Horizon in-situ* burn operations might never have been known, were it not for the chance encounters of the single deep water fishery operating in the same general area as most of the burn operations (Figure 26). Impacts to the fishery were minimal and it was quickly re-opened after testing and analysis by federal fisheries managers and seafood safety specialists. However, the residue and fishery interactions that occurred in 2010 and 2011 represent a cautionary footnote to large-scale *in-situ* burn operations that should be factored into tradeoff analysis for future spill responses. Further, the *Deepwater Horizon* experience suggests a need for additional research into burn residue containment or recovery methods that would reduce or eliminate potential fishery impacts associated with sinking residue.

On a final note, we recommend that the Environmental Unit for a spill response where burn operations are regularly taking place should collaborate with the Operations Section to collect, analyze, and archive paired samples of pre- and post-burn oil to facilitate the process of source identification of oil residues encountered during a response. In doing so, a solid empirical database of physical and chemical characteristics would be built over time to permit a better understanding of the changes occurring to oil during *in-situ* burning, the circumstances in which residues will float/sink, and the specific markers and physical features that might be used to uniquely identify burn residues.



February 11th, 2015

Macondo Well Incident, In-Situ Burn Locations

In-situ burn locations provided by BP. Represents all official burns. Prepared by NOAA's Office of Response and Restoration

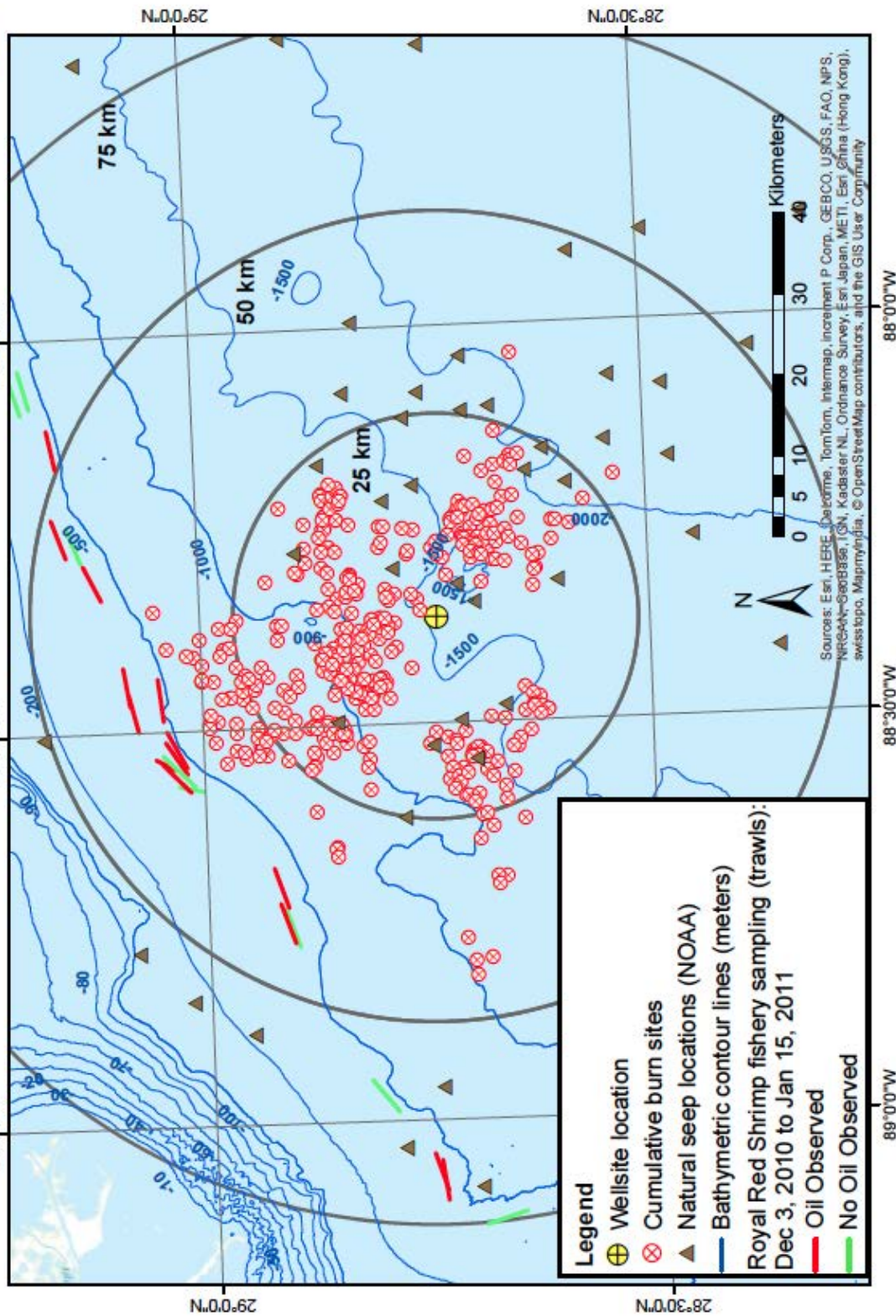


Figure 26. Composite map showing locations of the Macondo wellhead, *in-situ* burn operations, royal red shrimp trawls with preliminary source identifications, and known hydrocarbon seeps. Numbers in boxes denote approximate tarball counts in each quadrant. Prepared by J.B. Huyett, Genwest Systems.

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APPENDIX 1

DIAGNOSTIC BIOMARKER RATIOS

Petroleum Biomarkers Used For Calculating MC252 Diagnostic Ratios

Abbreviation	Compound Name	<i>m/z</i> Value
C27 Ts	C27 18 α (H)-22,29,30-trisnorneohopane	191
C27 Tm	C27 17 α (H)-22,29,30-trisnorhopane	191
C29 aB	C29 17 α (H),21 β (H)-30-norhopane	191
C29 Ts	C29 18 α (H)-30-norneohopane	191
C30 aB	C30 17 α (H),21 β (H)-hopane	191
C31 aB (S+R)	C31 17 α (H),21 β (H)-22(S+R)-homohopane	191
C32 aB (S+R)	C32 17 α (H),21 β (H)-22(S+R)-bishomohopane	191
C33 aB (S+R)	C32 17 α (H),21 β (H)-22(S+R)-trishomohopane	191
C27D Ba-S	C27 13 β (H),17 α (H),20S-diasterane	217
C27D Ba-R	C27 13 β (H),17 α (H),20R-diasterane	217
C29D Ba-S	C29 24-ethyl-13 β (H),17 α (H),20S-diacholestane	217
C29D Ba-R	C29 24-ethyl-13 β (H),17 α (H),20R-diacholestane	217
C28 aaa-R	C28 24-methyl-5 α (H),14 α (H),17 α (H), 20R-cholestane	217
C29 aaa-R	C29 24-ethyl-5 α (H),14 α (H),17 α (H), 20R-cholestane	217
C27 BB (R+S)	C27 5 α (H),14 β (H),17 β (H)-cholestane (20R+20S)	218
C28 BB (R+S)	C28 24-methyl-5 α (H),14 β (H),17 β (H)-cholestane (20R+20S)	218
C29 BB (R+S)	C29 24-ethyl-5 α (H),14 β (H),17 β (H)-cholestane (20R+20S)	218
C20 TA	C20-triaromatic steroid (pregnane derivative)	231
C21 TA	C21-triaromatic steroid (homopregnane derivative)	231
C26 TA-S	C26-triaromatic steroid,20S (cholestane derivative)	231
C28 TA-S	C28-triaromatic steroid,20S (ethylcholestane derivative)	231
C27 TA-R	C27-triaromatic steroid,20R (methylcholestane derivative)	231
C28 TA-R	C28-triaromatic steroid,20R (ethylcholestane derivative)	231

Diagnostic Ratios Chosen for MC252 Source Oil

Ratio	<i>m/z</i> Value
C27 Ts/ C27 Tm	191
C29 aB/C29 Ts	191
C29 aB/C30 aB	191
C31 aB(S+R)/C32 aB(S+R) + C33 aB(S+R)	191
C32 aB(S+R)/C31 aB(S+R) + C33 aB(S+R)	191
C33 aB(S+R)/C31 aB(S+R) + C32 aB(S+R)	191
C27D Ba-S/C27D Ba-R	217
C29D Ba-S/C29D Ba-R	217
C28 aaa-R/C29 aaa-R	217
C27 BB(R+S)/C28 BB(R+S) + C29 BB(R+S)	218
C28 BB(R+S)/C27 BB(R+S) + C29 BB(R+S)	218
C29 BB(R+S)/C27 BB(R+S) + C28 BB(R+S)	218
C20 TA/C21 TA	231
C26 TA-S/C28 TA-S	231
C27 TA-R/C28 TA-R	231