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Using Comprehensive Two-Dimensional Gas Chromatography to the Explore the Geochemistry of the Santa Barbara Oil Seeps

FINAL REPORT
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1. Project Summary

The development of comprehensive two-dimensional gas chromatography (GC × GC) has expanded the analytical window for studying complex mixtures like oil. Compared to traditional gas chromatography, this technology separates and resolves at least an order of magnitude more compounds, has a much larger signal to noise ratio, and sorts compounds based on their chemical class; hence, providing highly refined inventories of petroleum hydrocarbons in geochemical samples that was previously unattainable. In addition to the increased resolution afforded by GC × GC, the resulting chromatograms have been used to estimate the liquid vapor pressures, aqueous solubilities, octanol-water partition coefficients, and vaporization enthalpies of petroleum hydrocarbons. With these relationships, powerful and incisive analyses of phase-transfer processes affecting petroleum hydrocarbon mixtures in the environment are available. For example, GC × GC retention data has been used to quantitatively deconvolve the effects of phase transfer processes such as water washing and evaporation. In short, the positive attributes of GC × GC-analysis have led to a methodology that has revolutionized the analysis of petroleum hydrocarbons.

Overall, this research has opened numerous fields of study on the biogeochemical "genetics" (referred to as petroleomics) of petroleum samples in both subsurface and surface environments. Furthermore, these new findings have already been applied to the behavior of oil at other seeps as well, for petroleum exploration and oil spill studies.

2. Final Report

2.1 Introduction. The goals of this work were to investigate the geochemistry of the Santa Barbara oil/methane seeps (Figure 1) with GC × GC. With this technology, we sought to provide unprecedented insights into the processes that act on petroleum in subsurface and surface environments. In the these environments, the composition of petroleum can be altered by chemical, physical, and biological processes such as oxidation, photolysis, evaporation, dissolution, and biodegradation, that are collectively termed "weathering". Distinguishing the role of each process is important for understanding the biogeochemical cycling of petroleum in nature, whether it is from a geologic, economic, or environmental perspective.

In general, marine oil seeps are naturally occurring, steady state chemostats and therefore offer advantageous laboratories for petrochemical studies. The Santa Barbara seeps are considered some of the most active in the world and are ideal for several reasons: the large volumes of oil seeping; the proximity to critical economic, residential, and recreational areas; and the existing collaborative ties that we have with Professor David Valentine (UCSB) and his group.

Before the results are presented, an overview of GC × GC is necessary. Compared to traditional gas chromatography (GC), which tends to focus on a few select compounds or a compound class that is readily quantifiable, GC × GC separates and resolves at least an order of magnitude more compounds; has a much larger signal to noise ratio; and sorts compounds based on their chemical

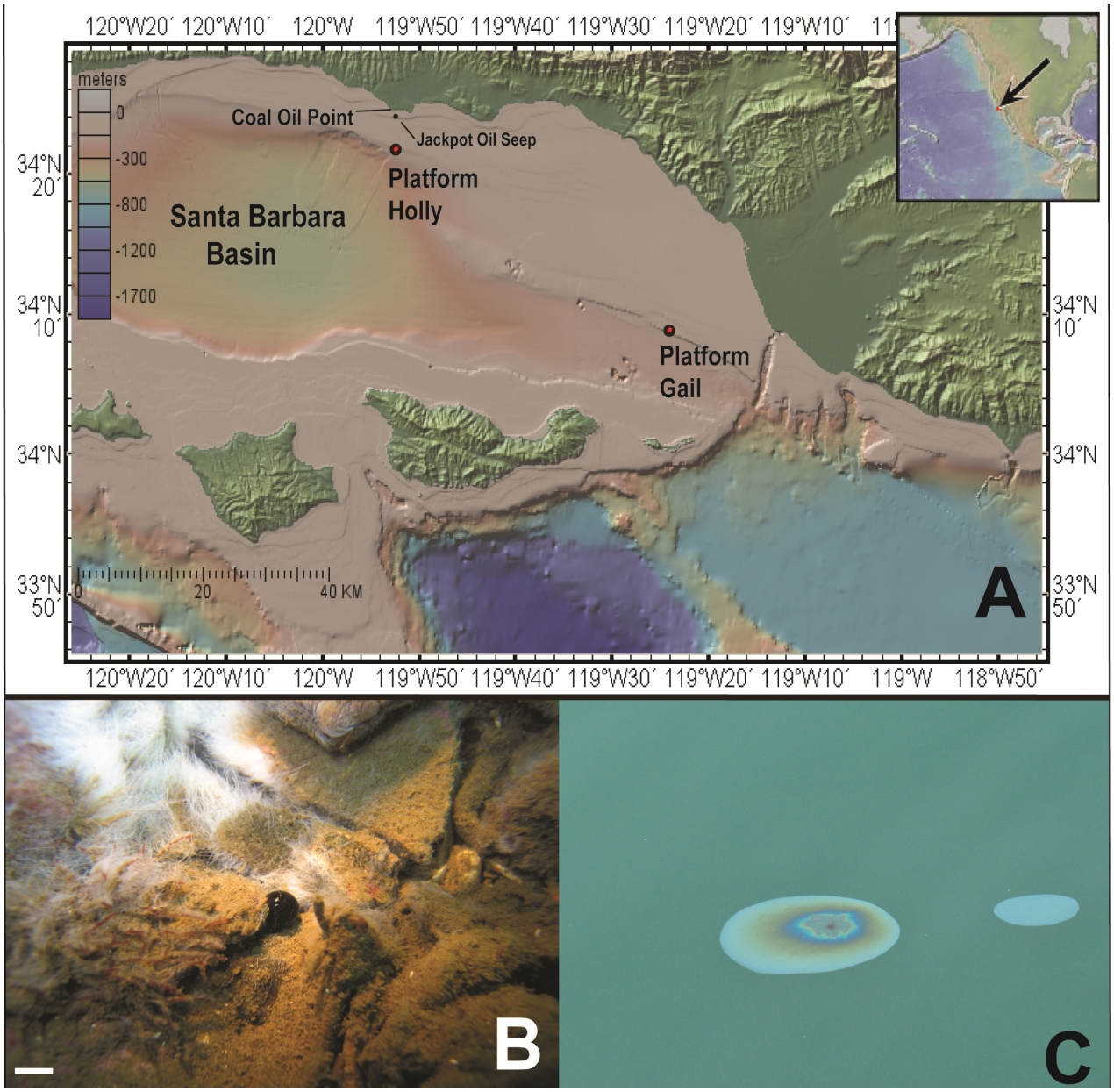

Figure 1. *(A) Map of the Santa Barbara Channel. (B) Image of oil seeping from the sea floor at Jackpot Seep (Scale bar = 1-cm). White threads are filamentous bacteria. (C) Image of seep oil once reaching the sea surface over Jackpot Seep.*

class. It therefore provides highly refined inventories of petroleum hydrocarbons in geochemical samples that were previously unattainable (Frysinger et al. 2003; Nelson, et al. 2006; Reddy et al., 2002). Coupling GC × GC with a flame ionization detector (FID) and with a time of flight mass spectrometer (TOF-MS) allows for the quantification and spectral identification of many of these resolved compounds (Ventura et al., 2007). Using petroleum from the Monterey formation, which is the source oil of the Santa Barbara seeps, we regularly quantify and obtain mass spectra for thousands of compounds in a single oil sample (Figure 2).

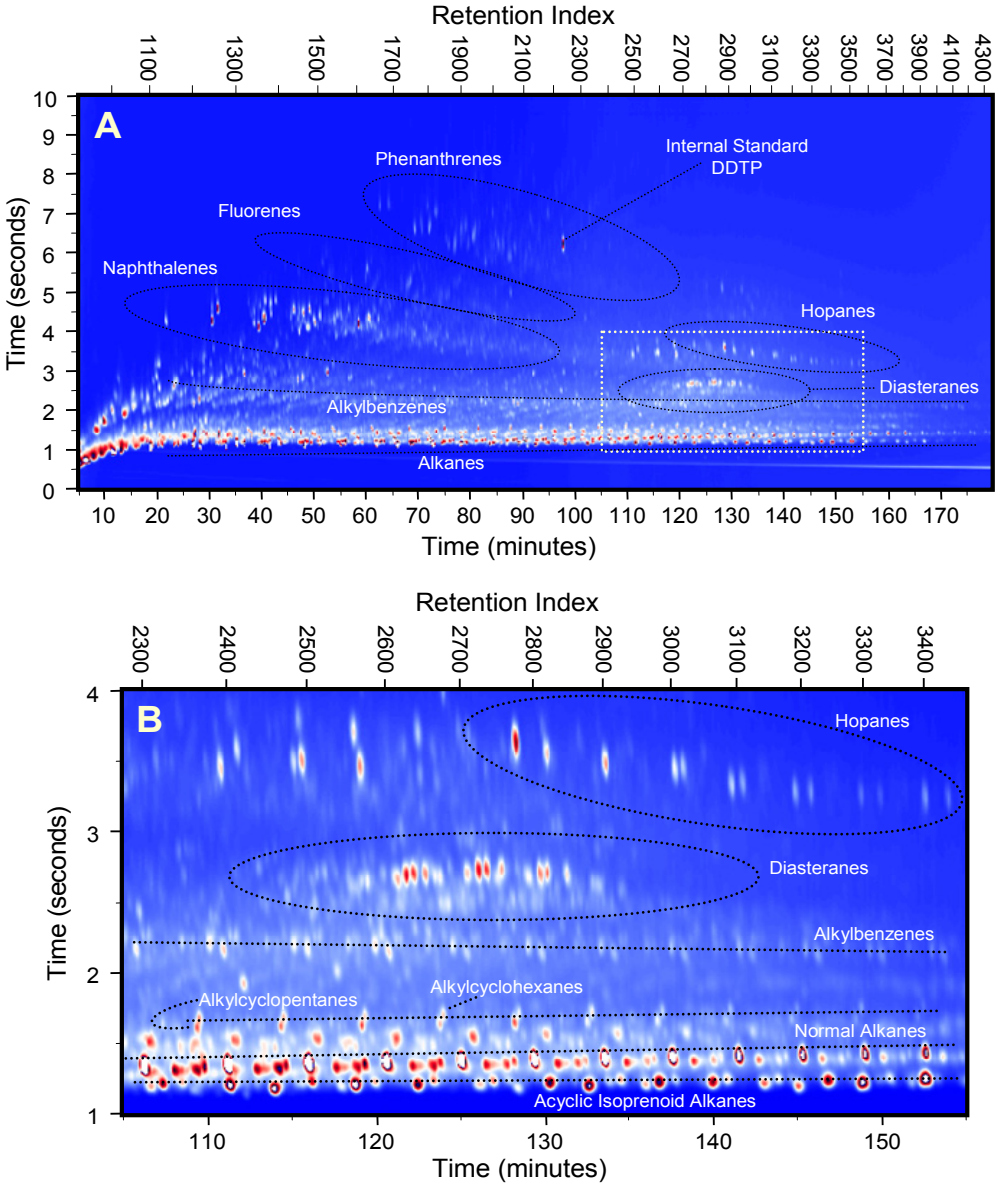

Figure 2. *GC×GC-FID chromatograms of oil (Monterey Formation) collected from Platform Holly, located offshore the University of California at Santa Barbara (UCSB; Figure 1A). (A) is a full chromatogram and (B) is a blow-up of the white box in (A). The data are displayed as color-contour plots, with blue representing low signal, white representing medium signal, and red representing a high signal. A volatility-based separation with a nonpolar polydimethylsiloxane column produces essentially a boiling point separation along the x-axis. A polarity-based separation with a 50% phenyl-substituted polydimethylsiloxane column yields chemical class-type separation along the y-axis. In the second dimension, the least polar petroleum classes observed in this sample, the branched and normal alkanes, have the least retention on the second column and are located at the bottom of the chromatogram. The most polar classes, multi-ring PAHs, have the greatest retention on the second column and are located near the top of the chromatogram. The cycloalkanes appear in bands just above the branched alkanes. Other petroleum components of interest highlighted here are the diasterane and hopane biomarkers. Retention indices in all three panels are consistent with carbon number, i.e. RI = 1000 is n-C_{10}, RI = 1500 is n-C_{15}, etc.*

Overall, this technique is significantly improving our present understanding of petroleum geochemistry by enabling quantitative analysis, identification, and tracking of thousands of compounds between different samples.

Due to the generosity of the Seaver Foundation and the WHOI Director of Research, we were provided funds to purchase a GC × GC coupled to a time of flight mass spectrometer (GC × GC/TOF-MS). The award from the Seaver Foundation was contingent upon this DOE grant being funded. This instrument has delivered powerful data not only for this project but also for other research initiatives in our laboratory including several related projects in collaboration with Schlumberger Doll Research. We have received $500,000 in gifts from this company based on initial data that we generated with the GC × GC/TOF-MS.

2.2 Results from prior support.

This project has been very successful and provided new insights into petroleum geochemistry. An overall review of the major milestones is presented below. Please note that in the summer of 2007, we participated on a two-week-long cruise on the *R/V Atlantis* focused solely on the biogeochemistry of oil seeps along the California coastline. This cruise included 12 dives of the *DSV Alvin;* we went on three of them. Many different types of samples were collected; two studies from these samples will be discussed in this report.

(a) Method development of GC × GC. As demonstrated in Figure 2, GC × GC can provide nearly complete composition data for petroleum hydrocarbons. However, the potential wealth of physical property information contained in the corresponding two-dimensional chromatograms has remained largely untapped. Several years ago, we developed a simple but robust method to estimate GC × GC retention indices for petroleum hydrocarbons. By exploiting *n*-alkanes as reference solutes in both dimensions, we were able to calculate retention indices that were insensitive to the uncertainty in the enthalpy of gas-stationary phase transfer for a suite of representative components (Arey et al., 2005). The resulting two-dimensional retention indices can then be used to estimate the liquid vapor pressures, aqueous solubilities, octanol-water partition coefficients, and vaporization enthalpies of a wide range of petroleum hydrocarbons which, in turn, can be used to investigate phase transfer processes affecting petroleum hydrocarbon mixtures in the environment. One key result of these powerful relationships developed from these retention indices is that the exact compound structure of each hydrocarbon in each complex mixture does not have to be fully identified in order to model the effects of phase transfer processes on the complete mixture. Only the retention times for both dimensions need to be known. This dramatically expands the number of compounds that can be used to model processes like water-washing or gas washing, which are typically limited to less than 20 compounds that are within one or two compound classes in traditional GC.

To first test this approach, we chose to use samples collected after the *Bouchard 120* oil spill (Arey et al., 2007a, b), which occurred in April 2003. The composition of this oil and what seeps out at Santa Barbara are quite similar. In the *Bouchard* study, we collected oil-covered rocks from one beach over a six-month period, which allowed us to test both weathering patterns and the kinetics. We first mapped hydrocarbon vapor pressures and aqueous solubilities onto the compositional space explored by GC × GC chromatograms of weathered samples (Figure 3). Then we developed

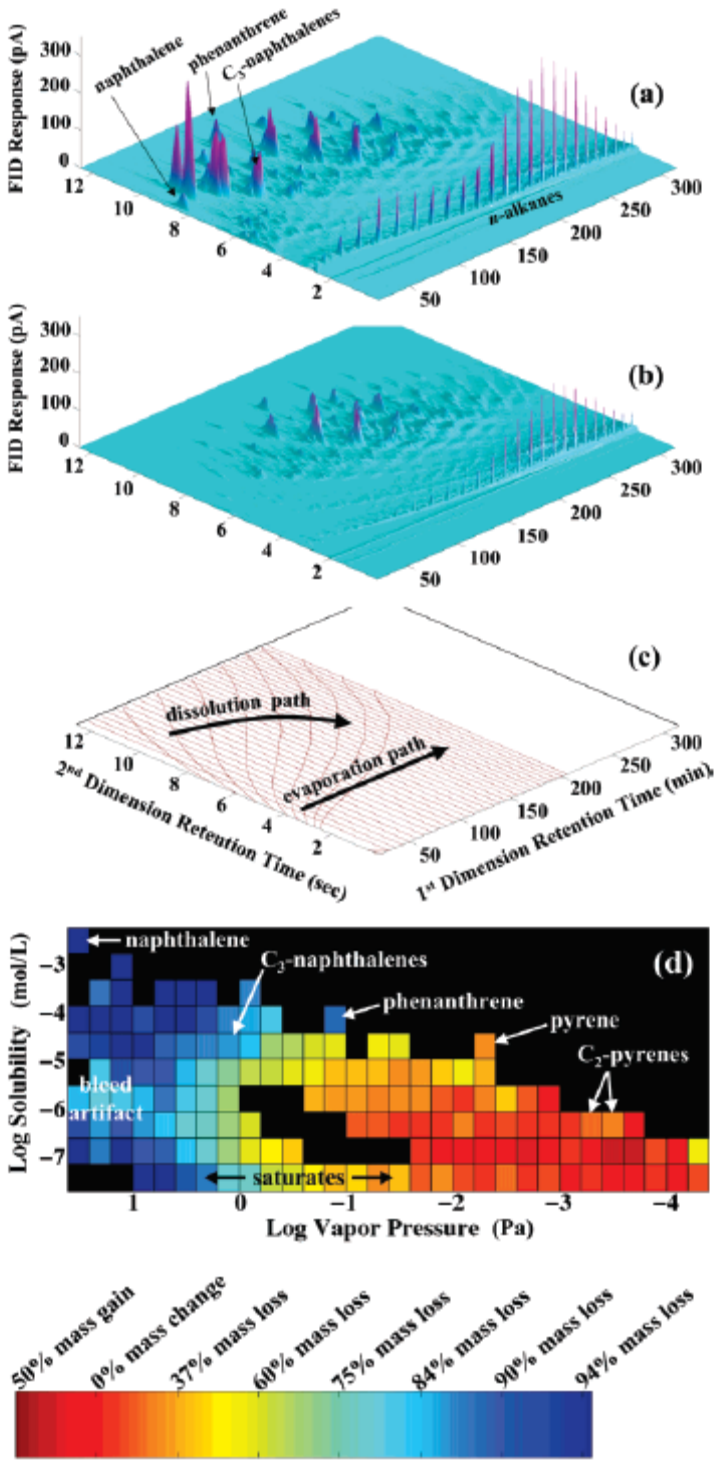

Figure 3. *Conversion of a GC×GC chromatogram into a Mass Loss Table (MLT). (a) Mountain plot of the complete GC×GC chromatogram of original Bouchard 120 oil. Because GC×GC-FID has similar response factors for different hydrocarbons, mountain plots accurately convey the relative mass abundances of different compounds. (b) Mountain plot of a sample weathered for 60 days. (c) Contours of hydrocarbon vapor pressure (straight lines) overlaid with contours of hydrocarbon aqueous solubility (curved lines). Evaporation and dissolution were expected to remove hydrocarbon mass along a path that is perpendicular to the corresponding partitioning property contour lines, as shown by the bold arrows. Finite element cells were delimited by these contours and their intersections. (d) The day 60 MLT, created by calculating the mass ratios of finite element cells in this sample with respect to corresponding cells in the original oil.*

methods to decouple quantitatively mass loss patterns associated with evaporation and dissolution using mass loss tables (MLTs). The compositional complexity of oil, traditionally considered an obstacle, was now an advantage. We exploited the large inventory of chemical information encoded in oil to differentiate robustly signatures of mass transfer to air and water. Therefore, these MLTs reflect mass removal rates of thousands of different compounds. By comparison, conventional ratios reflect information about only a handful of compounds. With this new approach, we evaluated mass transfer models (Arey et al., 2007b) that more properly account for evaporation, dissolution, and degradation of oil in the subsurface and surface environment. We used this approach extensively as described in the next section.

(b) Using GC×GC to investigate petroleum weathering in natural marine seeps. This aspect of the project required the most effort and embodies the overall theme of the project. Hence, we will expand on these results more than others discussed in this final report.

To compare the molecular composition of petroleum samples that have undergone biological and physical weathering during their step-wise ascent from a subsurface reservoir (Figure 1A) to the sea floor (Figure 1B) and then to the sea surface (Figure 1C), each sample was analyzed by GC × GC (Figure 4).

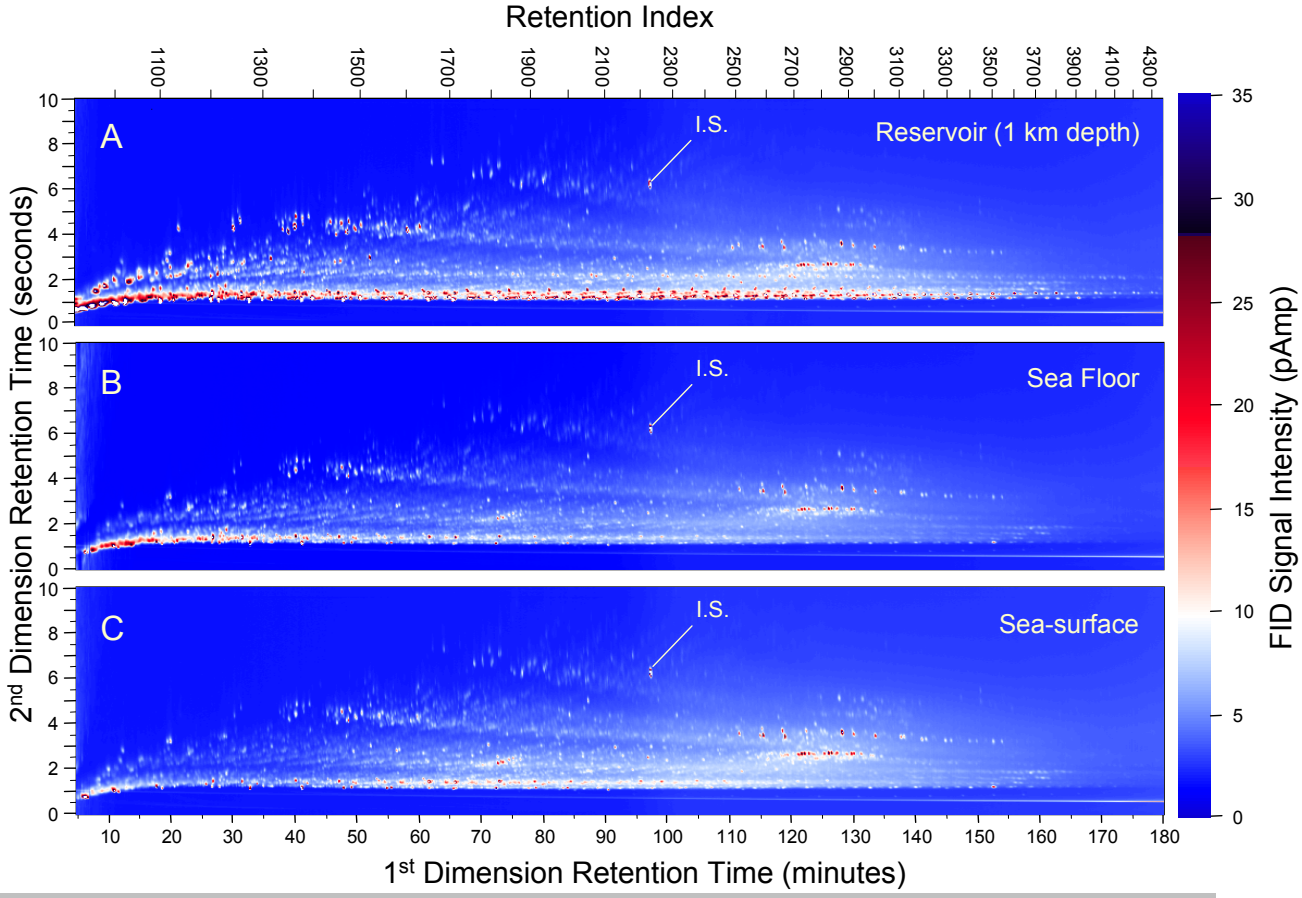

Figure 4. *GC × GC-FID chromatograms of (A) crude oil from Platform Holly, (B) oil collected as it was emerging from the sea floor at Jackpot Seep, and (C) a droplet of oil collected as it was spreading on the sea surface above Jackpot Seep. I.S. denotes the elution position of the internal standard, DDTP.*

Oil analysis of reservoir sample. Chromatographic analysis of the reservoir sample (Figure 4A) resolved thousands of compounds. Resolved compounds range in size containing 10–43 carbons. Normal-alkanes, alkylbenzenes, alkylnaphthalenes, acyclic isoprenoids, and other branched alkanes collectively represent 40% of the reservoir petroleum hydrocarbon mass.

Subsurface transformations by anaerobic biodegradation. To identify the compositional changes between the reservoir source oil and the oil emitted from the sea floor, we prepared a difference chromatogram of the sea floor sample minus the reservoir sample (Figure 5A). This figure reveals that several compound groups were degraded during their ascent from the reservoir to the sea floor. Losses of *n*-alkanes and other saturates, select aromatics including low-molecular-weight alkyl-substituted, naphthalenes, and alkyl-substituted benzenes were observed. Compound classes displaying the greatest losses between the subsurface and sea floor include many of the components present at high concentration in the reservoir oil, such as *n*-alkanes, branched alkanes, acyclic isoprenoids, monocyclic alkanes, alkylbenzenes, and alkylnaphthalenes. The observed losses of these compound classes are typical of biodegradation, and the differences between the seeping and reservoir petroleum hydrocarbon compositions were attributed to biodegradation in the subsurface. This interpretation was tested independently by examining the chromatograms for evidence of hydrocarbon evaporation or dissolution during this transition (Figure 6). This analysis was possible because GC × GC uniquely enables the quantitative elucidation of hydrocarbon phase transfers to air (evaporation or gas washing) and water (dissolution) (as described earlier). A comparison of sea floor oil and reservoir oil samples showed no systematic evidence of significant mass losses due to evaporation or water-washing (Figure 6B). This supports our interpretation that biodegradation, rather than gas-washing or water-washing in the reservoir, caused the observed compound losses.

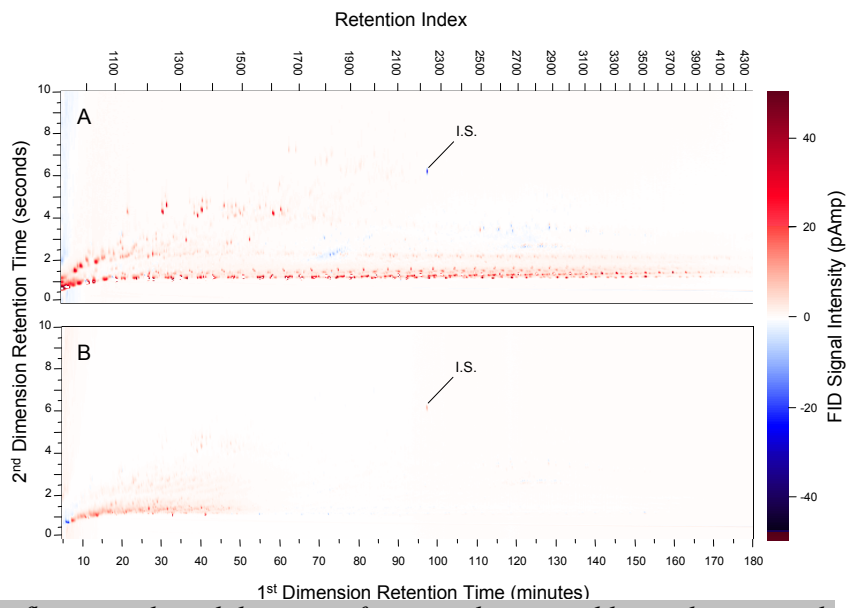

Figure 5. *Difference chromatograms produced by (A) subtracting the chromatogram of a sea floor sample (Figure 4B) from the chromatogram of a reservoir sample (Figure 4A) and (B) by subtracting the chromatogram of a sea surface sample (Figure 4C) from the chromatogram of a sea floor sample (Figure 4B). The base-plane appears white, while compounds at a higher concentration in the reservoir sample (A; Figure 4A) and the sea floor sample (B; Figure 4B) appear red. Compounds at higher concentrations in the sea floor sample and the sea surface sample appear blue and compounds with little difference in concentration in both samples vanish. These difference chromatograms highlight both the (i) biodegradation of abundant hydrocarbons that occurs in the subsurface as well as (ii) the physical processes acting to alter the hydrocarbon composition of the crude oil from the time it reaches the sea floor until its eventual transit to the sea surface.*

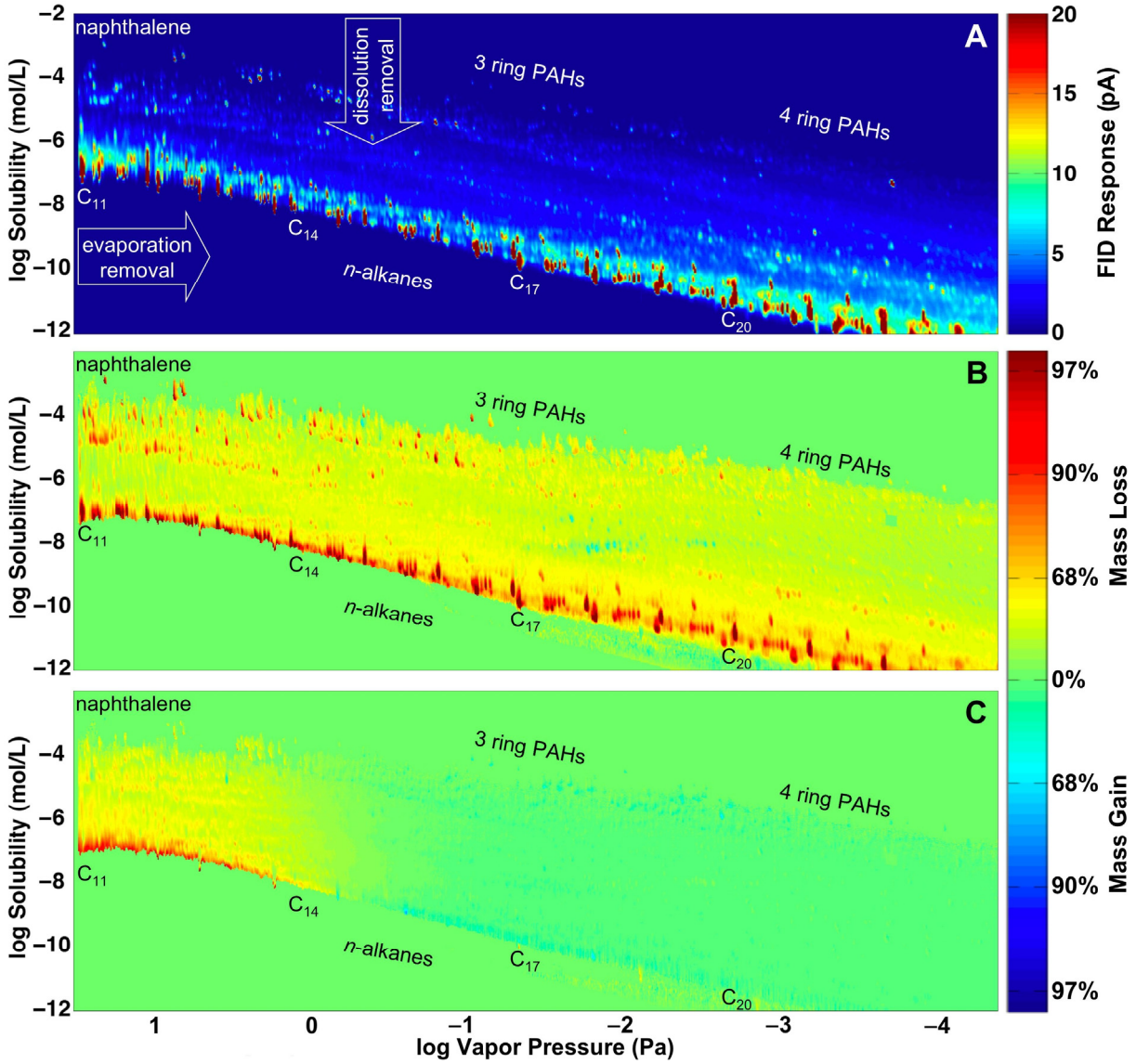

Figure 6. *GC×GC chromatograms elucidate signatures indicative of hydrocarbon phase transfer to air and water (Arey et al., 2005). In each panel, the 1st and 2nd dimension retention times have been directly transformed into hydrocarbon volatility and aqueous solubility, respectively. (A) The reservoir sample chromatogram is transformed into a map of composition (detector response) plotted with respect to volatility (x-axis) and aqueous solubility (y-axis) of the analytes. Labeled arrows indicate the expected paths of mass transfer signatures; evaporation and dissolution should progressively remove mass along the axes of volatility and solubility, respectively (Arey et al. 2005). In the subsequent panels, hydrocarbon compositional changes with respect to volatility and aqueous solubility are depicted for (B) the reservoir sample to sea floor sample transition and (C) the sea floor sample to sea surface sample transition. Observed trends of mass loss and mass gain suggest that: (B) the sea floor sample is heavily biodegraded with respect to the reservoir sample, rather than evaporated or dissolved. There is no clear evidence for phase transfer in the subsurface; supporting the interpretation, that biodegradation is the responsible mass loss process. By contrast, (C) the sea surface sample is partly evaporated relative to the sea floor sample, showing an estimated 10% TPHs loss.*

To identify biodegradation trends among compounds less abundant than those visualized in difference chromatograms (Figures 5A and B), an algorithm was developed to track compounds between similarly sourced samples, based on GC × GC retention times. This algorithm tracked the concentration changes of a large number of individual compounds from one sample to another, in an automated fashion. Using the retention time information of detected peaks, the algorithm searched for peaks having closely matched retention times between two chromatograms. We assumed that peaks having sufficiently similar retention times would be chemically identical. The algorithm included several criteria designed to eliminate possible false positive matches. Briefly, the algorithm operated as follows. One chromatogram was arbitrarily designated as the "template" sample, and the other as the "target". For each detected peak in the template chromatogram, a search oval was centered on the 1st and 2nd dimension peak retention time. If any other peak lay within the search oval radius, then these peaks were considered confounding and all of them were eliminated from the analysis. If the peak was not eliminated at this step, the search oval was placed onto the target chromatogram at the same coordinates as the candidate peak of the template chromatogram. If a single peak lay within the search oval in the target chromatogram, then this peak was considered a tentative match with the (template) peak that originated the search oval. If zero peaks or multiple peaks were found within the search oval in the target chromatogram, then all of the relevant peaks (in both template and target samples) were eliminated from the analysis. Finally, the assignments of the template and target chromatograms were swapped, and the search procedure described above was repeated. Peaks were considered successfully matched only if they were accepted by the match criteria both before and after the swap. The search oval dimensions were chosen such that they resulted in the largest number of matches between the two chromatograms. For well-aligned chromatograms, the algorithm produced a high rate of successful peak matches using a very small search oval. For example, in a comparison of the reservoir oil to sea floor oil, ~1400 peaks were successfully matched (accounting for >26% of detected peaks in each chromatogram) using an optimal search oval of 0.32 min (1st dimension diameter) by 0.11 sec (2nd dimension diameter). It was estimated that the rate of false positive matches due to the random appearance of new compounds was <0.1%, based on attempted matches between hypothetical samples where the peak distributions were identical to the real samples but with randomized retention time positions. It is important to note that errors or artifacts of the peak delineation tools (Reichenbach et al., 2004) may also lead to false positive peak matches. Measured concentration changes of tracked compounds across samples were considered statistically significant if the observed concentration changes exceeded the estimated uncertainty in the integrated peak volumes.

With this algorithm, we found that there was no obvious microbial preference for saturated or aromatic hydrocarbon loss nor was there a bias based on the initial concentration (Figure 7), molecular weight, volatility, or solubility (Figure 6B). Importantly, this algorithm excludes compounds that are degraded completely, such as most *n*-alkanes and many of other compounds. Based on the sheer number and diversity of degraded compounds, it appears that there is a level of generality in the metabolic preference of anaerobic, hydrocarbon-degrading communities that was not previously known to exist (Spormann et al. 2000), which substantially changed the molecular composition of the reservoir oil prior to reaching the sea floor.

The subsurface environment underlying the seeps is anoxic. As such, anaerobic metabolic processes linked to petroleum consumption are likely to characterize the activity of the subsurface microbial community in this environment. These results provide important insights into the activity of

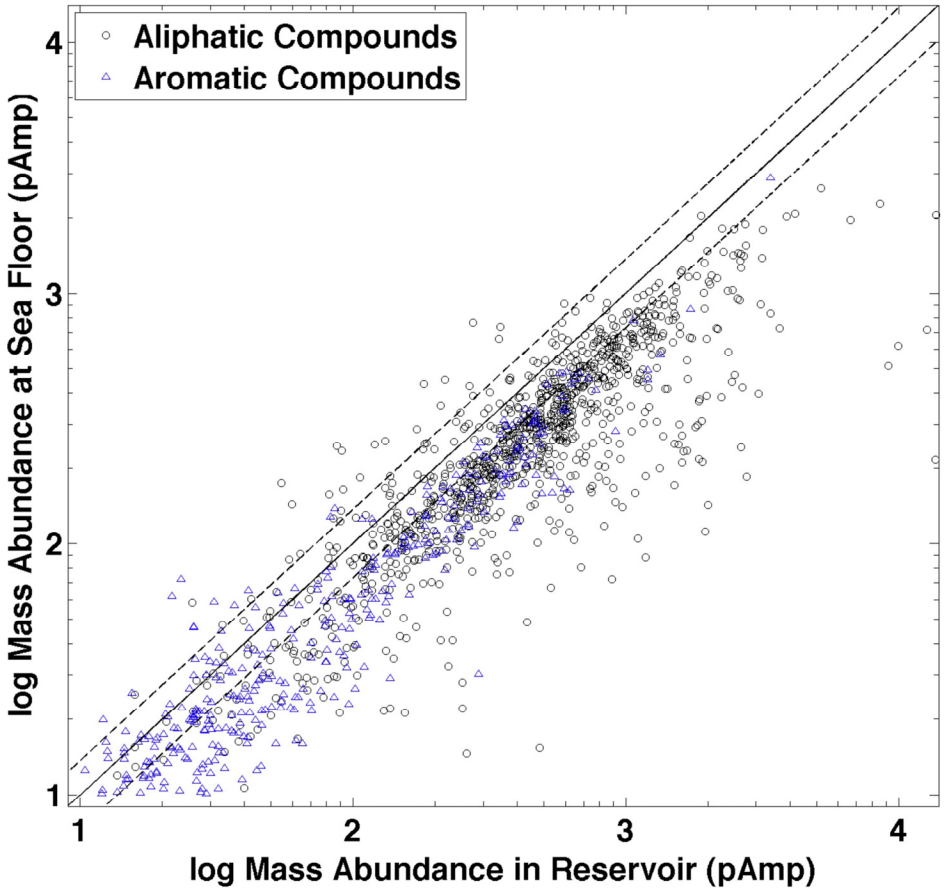

Figure 7. *A comparison of mass abundances of ~1400 individually tracked compounds in the reservoir oil and sea floor seep oil. We estimated an analytical uncertainty of +/- 34% (for the 95% confidence interval) in the mass abundance of each compound, but individual error bars are not included as they diminish visual clarity. Consequently, the mass abundance uncertainty is instead depicted as a 34% error in the 1:1 line (dashed lines). Points falling below the lower dashed line indicate a significant mass loss in the sea floor seep relative to the reservoir oil. Points above the upper dotted line indicate a significant mass.*

petroleum-degrading microbes in the subsurface. Normal alkanes and isoalkanes are removed first during microbial petroleum degradation followed by branched and cycloalkanes and then progressing through alkyl and multi-ring cycloalkanes with multi-ring compounds containing three or more aromatic rings being the most resistant (Peters et al. 2005). Results shown here indicate that biodegradation preference likely acts on a continuum of compounds. That is, hundreds to thousands of compounds appear to be simultaneously metabolized by the microbial community at varying rates, resulting in the distribution of losses shown in Figures 5A and 7. These results support those reported by Larter et al. (2003, 2006), and Prince and Suflita (2007), but our observations dramatically increase the number of hydrocarbons known to be biodegraded. Several unanticipated trends were also observed in the results, including increased recalcitrance of long chain (~C_{38}–C_{42}) isoprenoids with increasing cyclization, equivalent biodegradation of 1- and 2- methylnaphthalenes, and the preferential or simultaneous loss of long chain alkylcyclopentanes and alkylcyclohexanes over short chain analogs. The latter observation seemingly supports those made by Siddique et al.

(2006) and Hostettler et al. (2007). While these samples represent only snap-shots of hydrocarbon biodegradation, the number and diversity of degraded compounds supports a perspective of broad metabolic specificity. These results further demonstrate a capacity for anaerobic microbial communities to consume diverse hydrocarbons beyond those typically investigated, with implications for discovery of novel metabolic actions. The complete or partial biodegradation of over one thousand hydrocarbons, as shown here, stands in stark contrast to only a handful of known pathways for hydrocarbon biodegradation by strictly-anaerobic microbes (Spormann et al. 2000). Some of our lab-based biodegradation studies were published by Wardlaw et al (2011).

Analysis of sea surface chromatograms showed that select hydrocarbon components evaporated very rapidly when the oil bubbled from seeps and reached the sea surface. To interrogate whether evaporation or dissolution acted broadly on the oil emitted from the sea floor seep, we evaluated mass loss trends with respect to hydrocarbon volatility and solubility for the sea surface sample compared to the sea floor sample (Figure 6B and 6C). Duplicate samples at the sea surface show significant mass loss with respect to hydrocarbon volatility, when compared to sea floor samples (mass loss trends are shown for only one of the two sea surface samples; Figure 6C). Based on duplicate samples of droplets escaping from the sea floor, captured during mid-ascent through the water column (not shown), and resting at the sea surface, the ascending oil did not change significantly during traversal of the water column, and experienced rapid evaporation only upon reaching the sea surface. At the sea surface, an estimated 10% oil mass loss occurred within seconds or minutes, giving rise to the strong evaporation signature observed in the sea surface samples.

(c) Oil droplet transport downstream from the oil seeps. To extend past the previous work and examine the fate of oil once it reaches the sea surface, we collected surface sediment aboard the *R/V Atlantis* down current from the seep field (Figure 8). They were analyzed for hydrocarbon content using traditional gas chromatography with flame ionization detection (GC-FID) and GC×GC-TOF-MS, and the resulting spatial variations related to regional current patterns as determined with a high frequency radar array.

To test that the oiled sediments shared the same source as the oil seeps, we compared the hopane biomarkers between sea surface oil at the Trilogy oil seep (one of the distinct seeps at Santa Barbara) and the sediments. Hopane biomarkers have distinct ratios for specific oils and are conserved during weathering (Prince et al, 1994). Excellent matches were found. Visually, this is apparent in Figure 9 where we have depicted the GC × GC chromatograms in the region where hopane biomarkers elute from the source oil and oil extracted from the sediments. While the recalcitrant hopanes reveal very similar patterns of hydrocarbons, significant weathering has occurred in the sediments. Specifically, the early eluting material composed of low molecular hydrocarbons has been removed (Figure 10). And while the hopanes were trace components in the initial oil, they are the dominant compounds in the oil sediment (Figure 10b). (In Figure 10, the hopanes elute at retention index of 3000 in the 1st dimension and 3 seconds in the 2nd dimension).

We suggest that the observed loss pattern (Figure 10b) results from a combination of evaporation, dissolution, and biodegradation. Evaporation and dissolution act on compounds eluting early on the x-axis and late on the y-axis of GC × GC images, respectively, as described earlier (Arey et al 2005, Wardlaw et al 2008). Evaporation would likely be limited to the time oil resides at the sea surface, as opposed to dissolution, which would occur both in the slick and after deposition into the

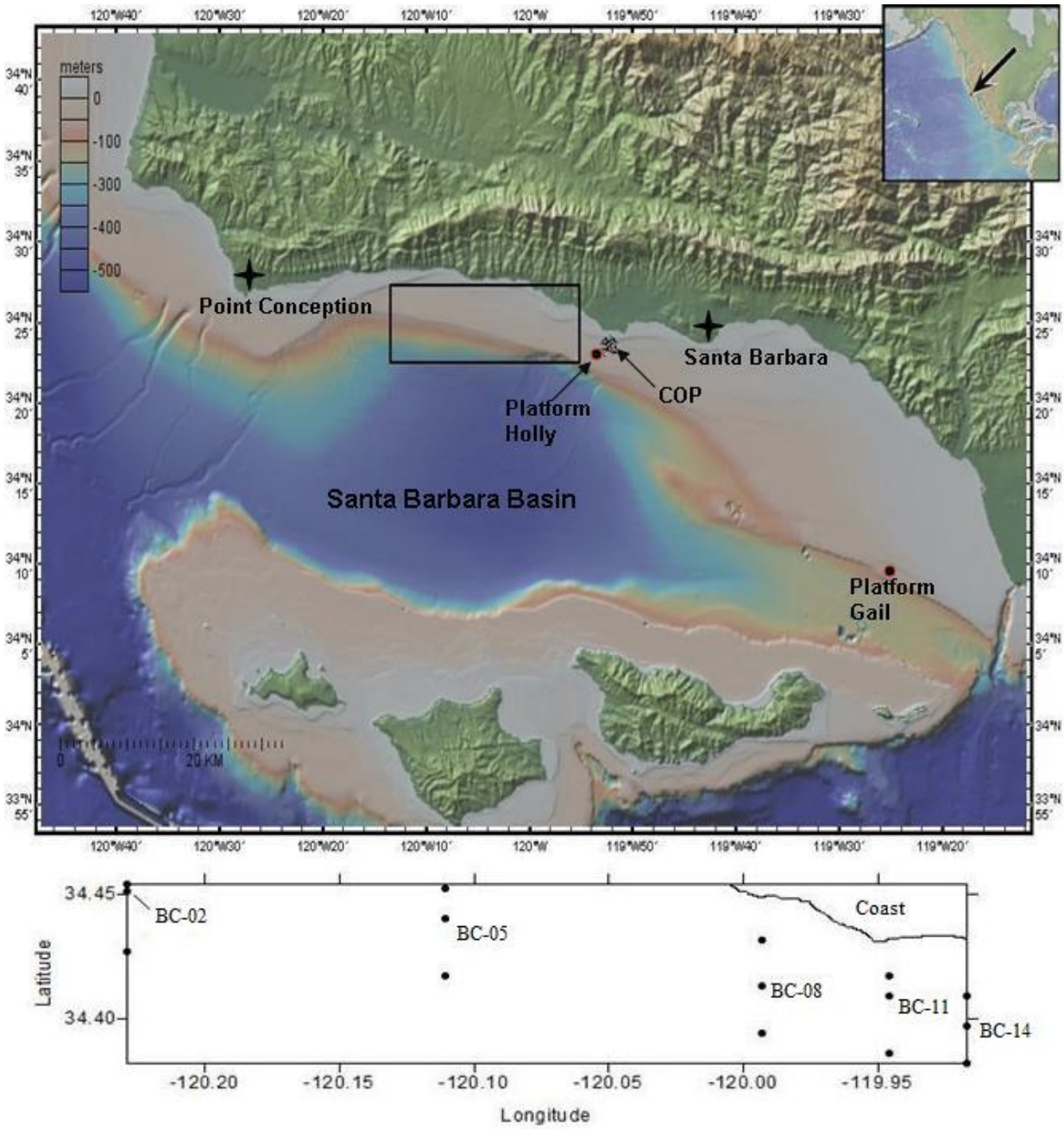

Figure 8. *Map of the study area of oil droplet project and surrounding region near the Santa Barbara oil seeps at Coal Oil Point (COP) seep field at Santa Barbara, as well as reference reservoirs. The area of the rectangular box in the top panel is reproduced beneath the panel with exact locations of sediment sample stations. The stations are arranged numerically with station numbers decreasing with distance from the seep field.*

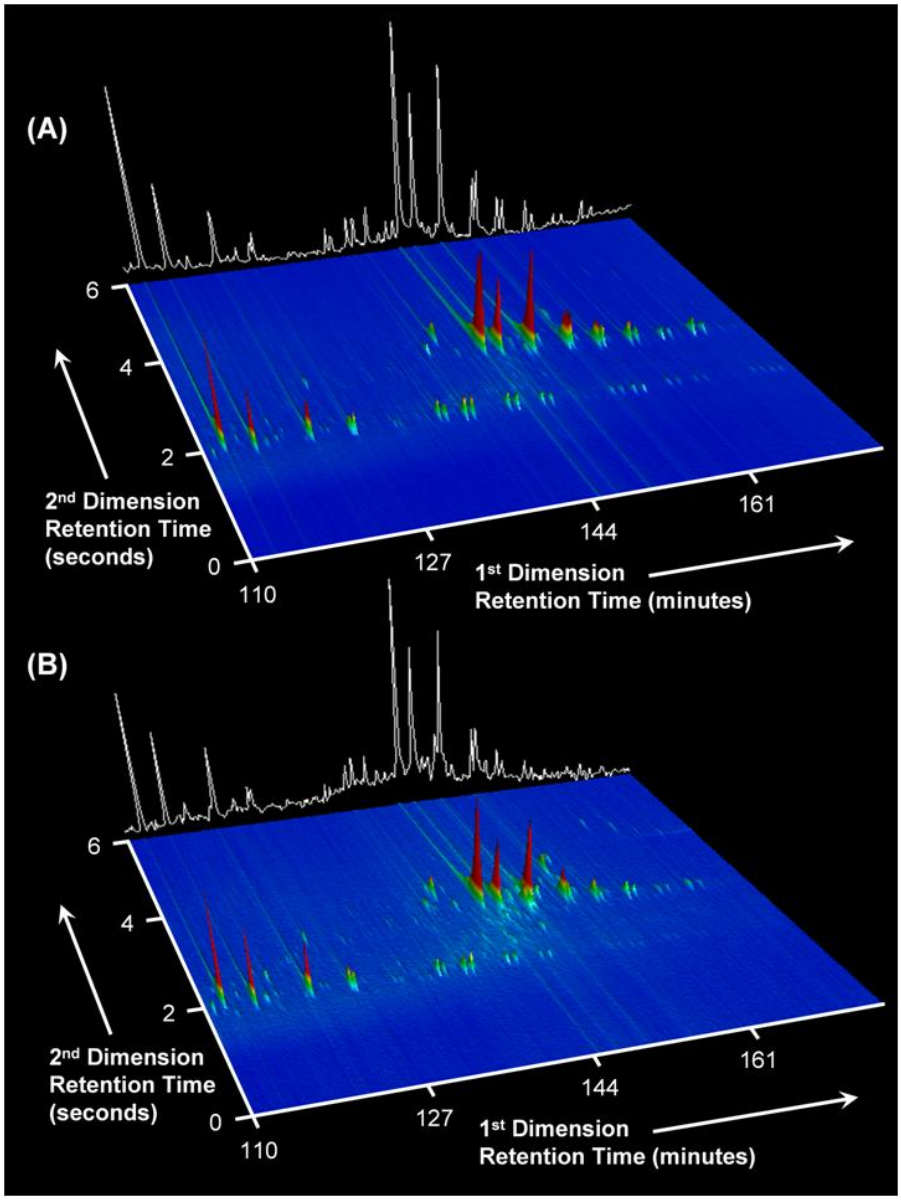

Figure 9. *GC × GC-ToF-MS chromatograms of the hopane biomarker region (ion m/z 191) of (A) surface slick oil collected from the sea surface above the Trilogy seep at Coal Oil Point and (B) sediment sample oil from BC–14. One-dimensional chromatograms are simulated in white at the rear of each two-dimensional chromatogram. The similarity of the images points to the same oil source. Reservoir samples from this region also had similar patterns.*

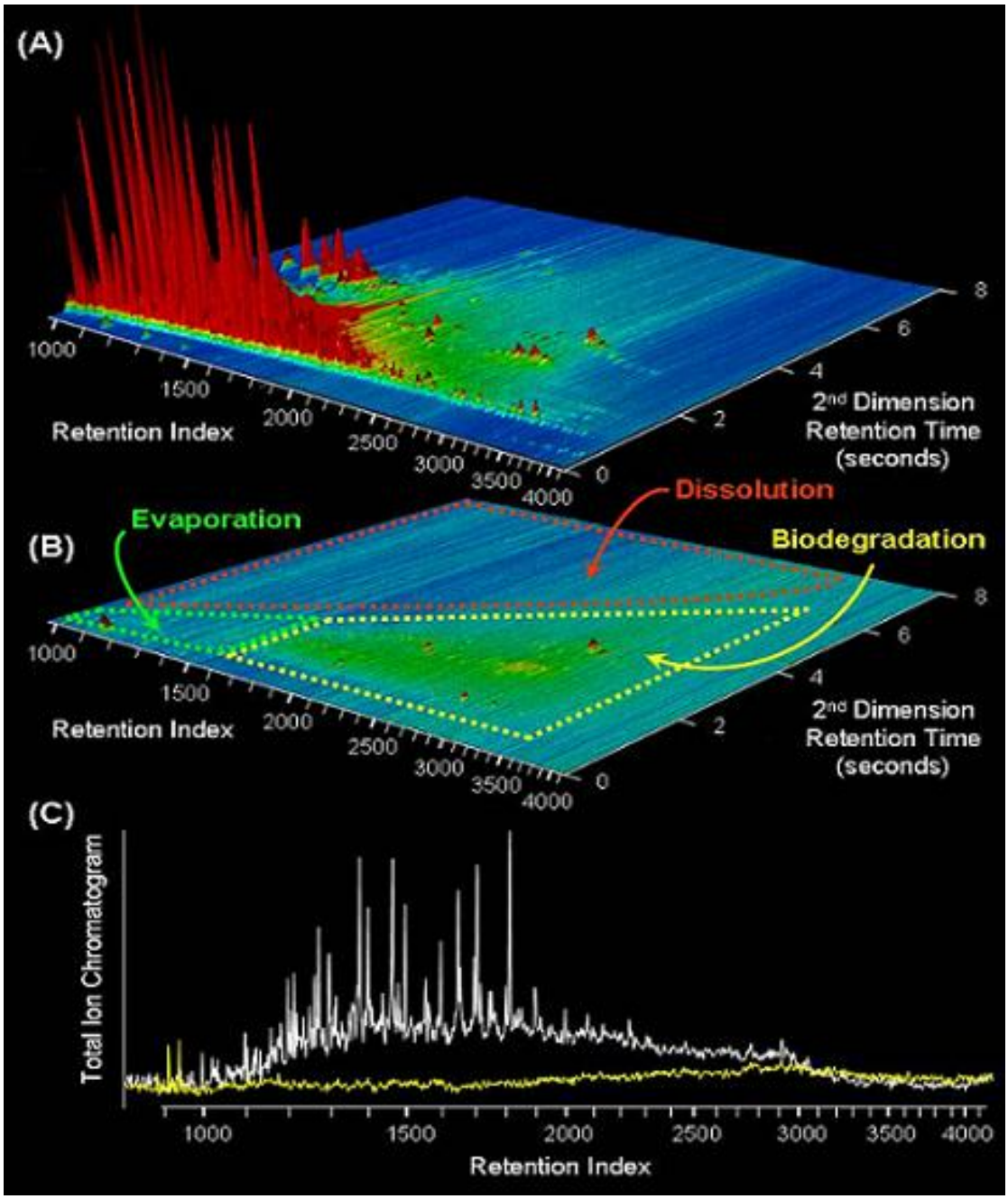

Figure 10. *GC×GC-ToF-MS Chromatograms of (A) surface slick oil collected from the sea surface above Trilogy seep; and (B) sediment sample oil from BC-14, with regions of the chromatogram marked corresponding to the likely associated forms of weathering; and (C) comparison of GC-FID chromatograms for surface slick oil (white) and sample BC-14 sediment oil (yellow), displayed on the same scale.*

sediment. Again, ~10% was found to evaporate within minutes of an oil droplet reaching the sea surface (Wardlaw et al. 2008). However, based on considerations of volatility, evaporation is unlikely to affect significantly compounds less volatile than ~ n-C_{16}, in good agreement with the results presented in Figures 10 and the timescale for transport and deposition. Wardlaw et al. (2008) noted no significant dissolution of hydrocarbons for freshly seeped oils, but the exposure time was much shorter than for these oils, and it seems likely that the more polar polycyclic aromatic hydrocarbons (PAHs) gradually dissolve into seawater during slick migration and after deposition.

Aerobic biodegradation of petroleum in the surface slick and sediments is presumed to remove hydrocarbons remaining after evaporation and dissolution, and may compete with these physical loss processes for some compounds. As discussed previously, we established the perspective of broad metabolic specificity in anaerobic hydrocarbon-degrading communities, reporting the metabolism of over 900 distinct compounds. We suspect that a similar generality in hydrocarbon degradation exists within aerobic microbial communities. Based on the loss pattern in Figure 10, numerous compounds can be linked to biodegradation. These apparently-biodegraded compounds range in size from ~16-42 carbons and include n-alkanes, branched alkanes, isoprenoids, alkyl cyclopentanes, alkyl cyclohexanes, alkyl benzenes, and alkyl napthalenes among others. These compounds constitute a significant portion of the non-weathered material in Figure 10A and implicate biological degradation as an important source of weathering in Figs 10B. Residual hydrocarbons remaining after biodegradation are highly insoluble, non-volatile and generally demonstrate structural complexity such as multiple rings and numerous tertiary carbons – all factors that tend to suppress remineralization.

The spatial distribution of oil content (calculated as the total petroleum hydrocarbon; TPHs) suggests a plume originating from the seeps, with maximum deposition to sediments around 60 m water depth. Using calculated annual current velocities through the seep field and continuing west, this deposition occurs over ~6 hours to 5 days. We suggest the observed plume represents a chronic fallout pattern for heavy oil from the persistent surface slicks, and that average surface currents modulate the distribution of the fallout (Figure 11). Considering the amount of oil and quantity of sediment impacted, we estimate a sediment oil burden of $0.3 – 3\times10^{12}$ g in the study area, equivalent to 8-80 spills of the *Exxon Valdez*.

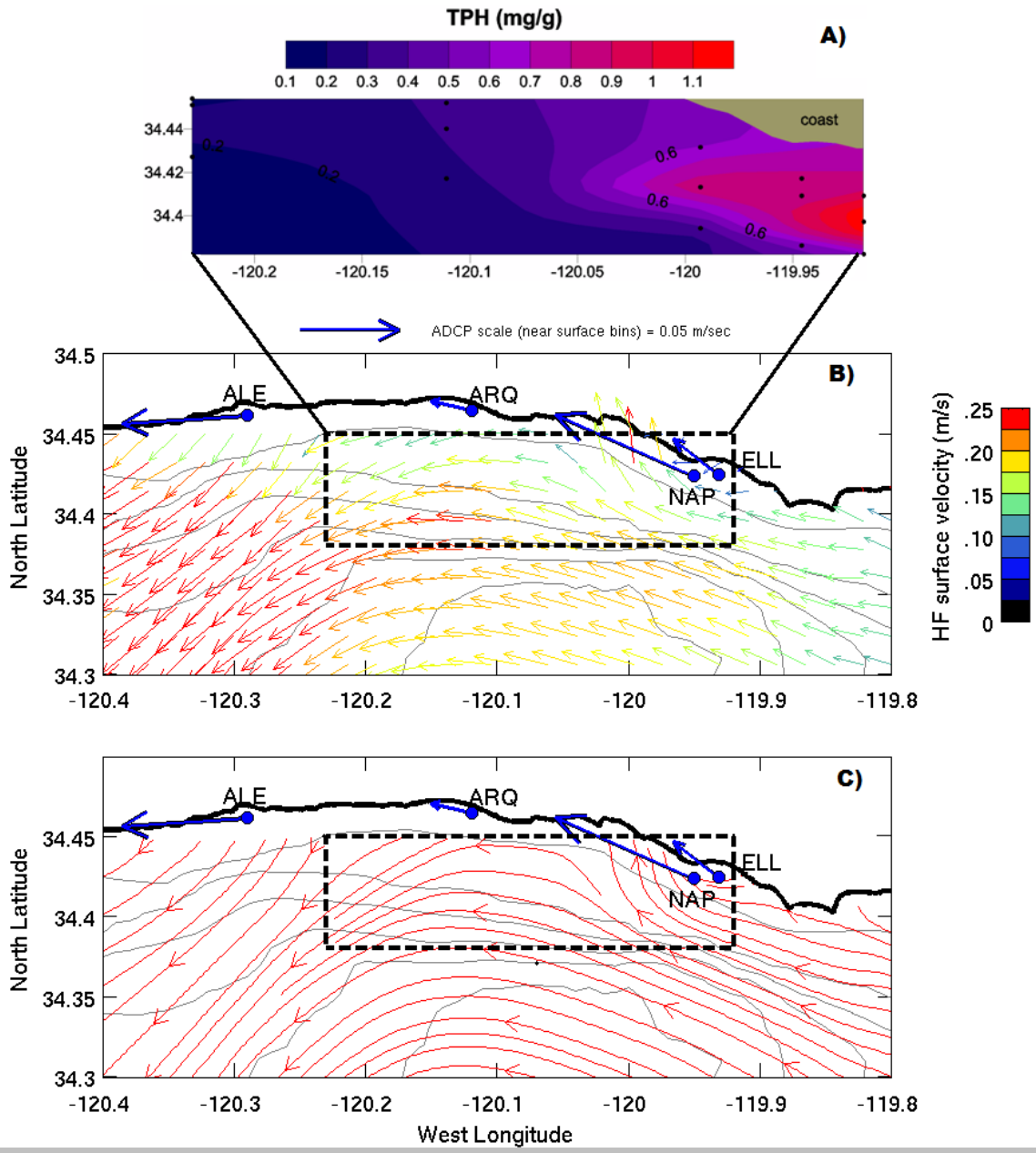

Figure 11. *(A) "Fallout plume" contour plot of TPH concentration for 15 selected sample sites (black dots) shown in conjunction with (B) mean surface current vectors derived from HF radar for 2000-2007 in and around the study area and; (C) particle paths computed from mean current vectors shown in B. Current speeds are color coded according to the color bar to right of the center panel. Blue arrows near shore are current vectors in the upper water column (depth range approximately 2-4 m) from acoustic Doppler current meters deployed along the 15 m isobath. The speed scale for these vectors is indicated above the panel. Fine black lines are bathymetric contours at 50, 100, 200, 300, 400, and 500 m. The lower panel shows particle paths computed from the mean current vectors of the center panel. Arrow heads indicate flow direction. Upstream ends of lines are starting points of particle tracks along HF radar coverage boundary. Particle track beginning near 34.42 °N, 120° W suggests bifurcation of mean flow trajectories: one branch flows shoreward west of COP, the other branch curves offshore.*

(d) Discovery and description of extinct asphalt volcanoes along the southern California margin. During our second dive on the *DSV Alvin*, we collected a rock-like sample from a mound referred to as "Il Duomito", which is about 10 km offshore and at a water depth of 150 to 200 meters (Figure 12). There have been numerous hypotheses on the composition and source of this geologic feature including carbonate, mud, and tar (Keller et al. 2007). We were able to determine, based on simple chemical analysis on the boat that Il Duomito was composed of tar. This led us to investigate a similar feature "Il Duomo" (Figure 12; one of the samples shown in Figure 13), which is several km northwest of Il Duomito. It also was composed of tar. We published this work in *Nature Geosciences* (Valentine et al. 2010).

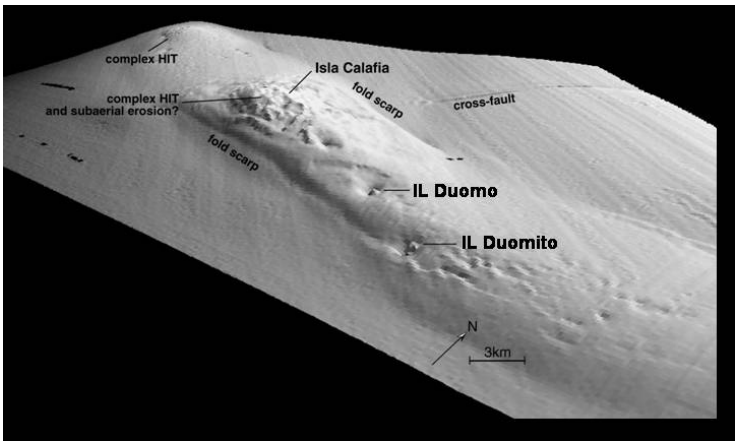

Figure 12. *High resolution shaded relief of the Mid-Channel Trend of the Santa Barbara channel revealing geomorphic features with Il Duomo and Il Duomito (Keller et al., 2007).*

We believe these are extinct asphalt or tar volcanoes. We were excited about this finding, but not entirely surprised as asphalt volcanism is increasingly being recognized as an important process at cold seeps. This process links ancient subsurface carbon reservoirs with more rapid biogeochemical processes at the surface. Both volcanoes occur as mounds closely associated with sediment-laden depressions or pits, suggesting extrusion of liquid petroleum coupled with localized subsidence or gas blowout (Figure 14). The estimated volume of Il Duomo is 5 to 7 $\times 10^4$ m^3 (Figure 15).

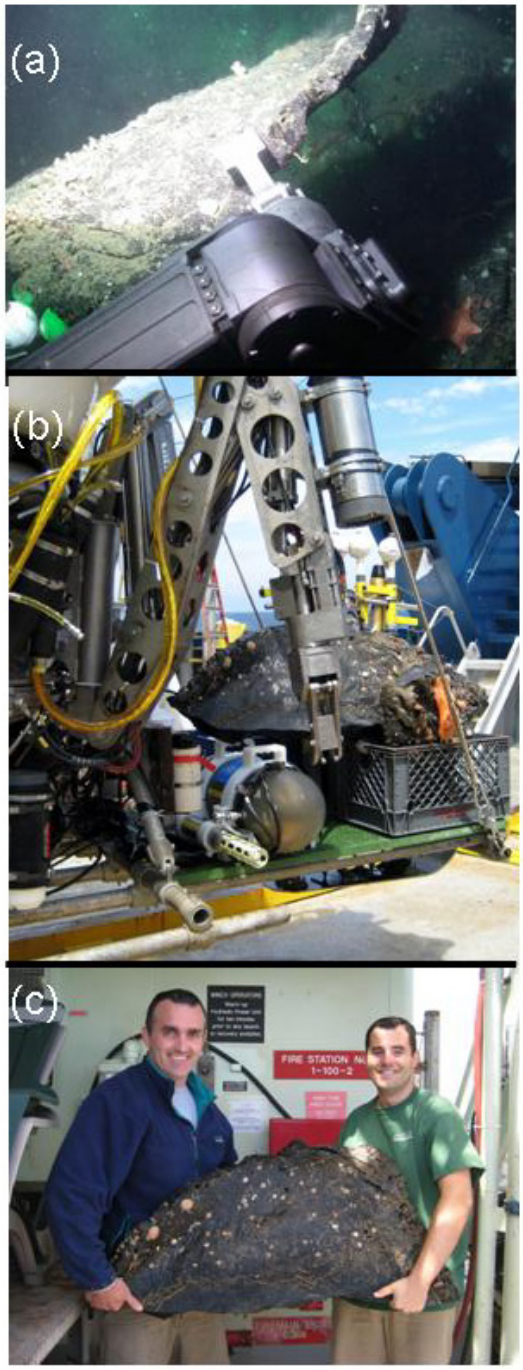

Figure 13*. Photographs from the tar mound research: (a) one tar sample being collected by DSV Alvin's manipulator arm at Il Duomo; (b) the same piece resting in the collection basket of DSV Alvin; (c) Chris Reddy and Dave Valentine holding the same sample from the previous pictures. Note the size but ease of presenting this sample, revealing the low density composition of the tar.*

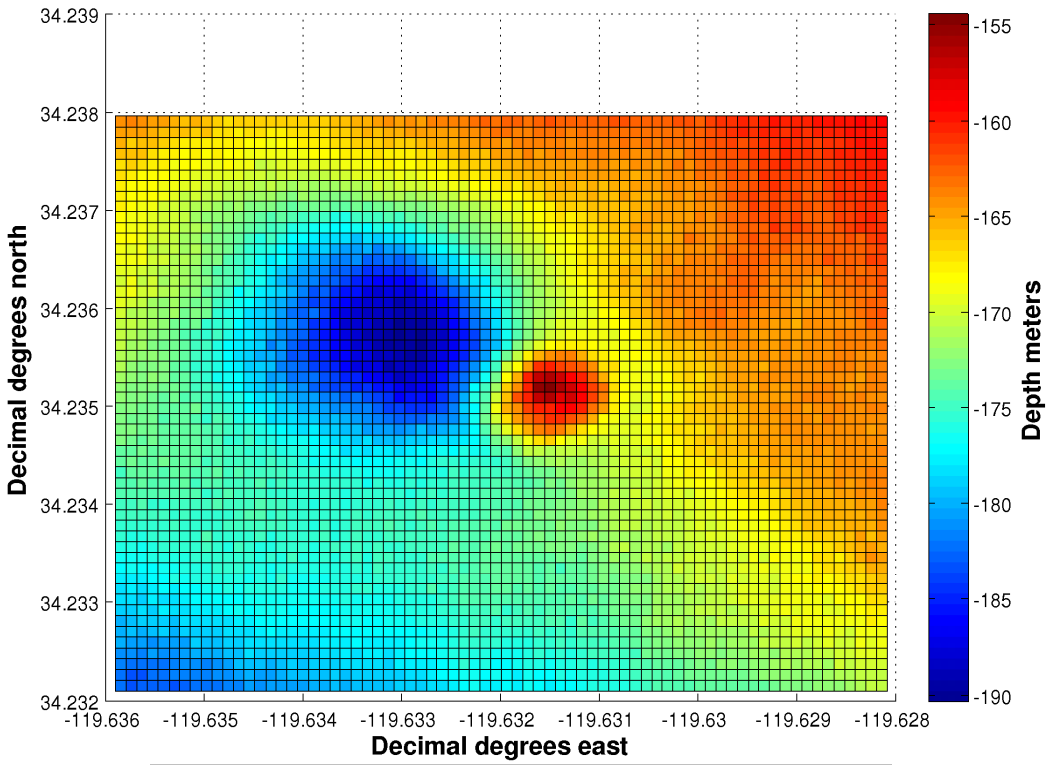

Figure 14. *Bathymetric image of Il Duomo and associated pit.*

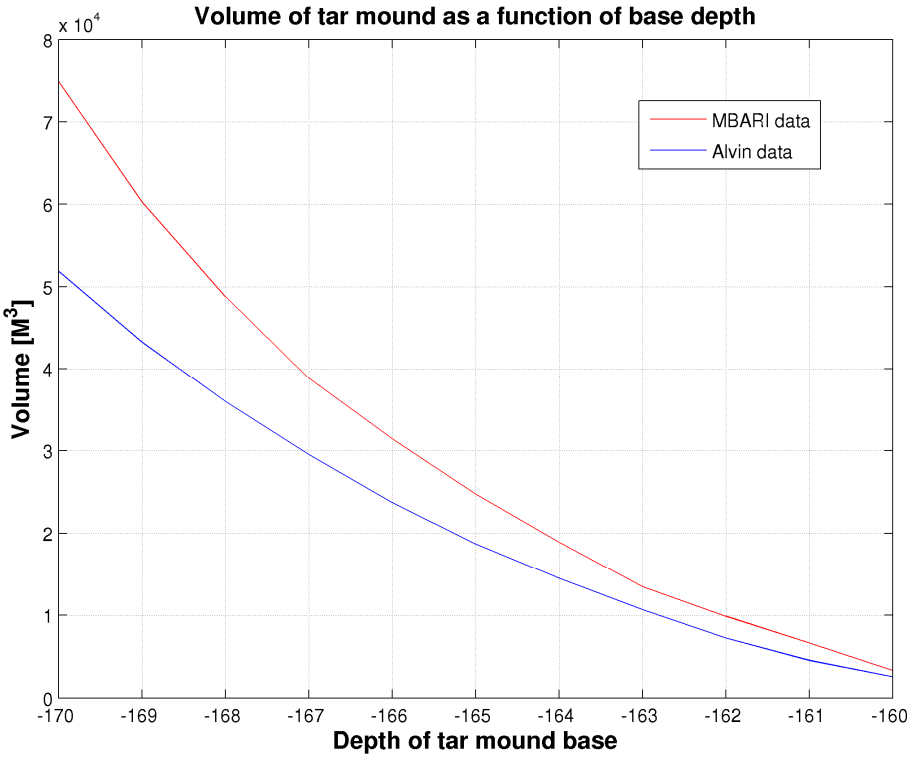

Figure 15. *Volume of Il Duomo as function of base depth. These values were calculated with bathymetric data collected via DSV Alvin during the cruise or with high-resolution data available from Monterey Bay Aquarium Research Institute (MBARI).*

The volcanoes range from 10 to 30 meters in height off the sea floor and may extend below the level of present sediment cover. No active seepage was observed during approximately 10 hours of visual and video surveys from the *DSV Alvin*, but the volcanoes appear to serve as an oasis for benthic life when compared to the surrounding sediment. Four asphalt samples were collected from each site during these surveys and all show remarkable similarity in their structure and chemical composition. Organic carbon comprises 50% of the mass for each sample, with sulfur, hydrogen and nitrogen comprising another 10% in aggregate. Inclusions of fine-grained sediment and microfossils comprise much of the residual mass. Radiocarbon dating of several samples from Il Duomo indicates that the bulk carbonate entreated in the asphalt is ~35 thousand years old. Each sample was analyzed for the stable isotope composition of carbon, nitrogen and sulfur, and results are consistent with a petroleum source from the Miocene-age Monterey Formation. Analysis of extracts by GC × GC also revealed a highly degraded material (Figure 16) and a suite of biomarker hopanes consistent with petroleum from the Monterey Formation. Like the sediment samples from the previous studies, extreme weathering was observed but not as extensive. We believe that this is the first report of extinct asphalt volcanoes along the Pacific margin of North America, and it provides an important window into the activity of cold seeps by capturing important structures that are unlikely to survive into the geologic record.

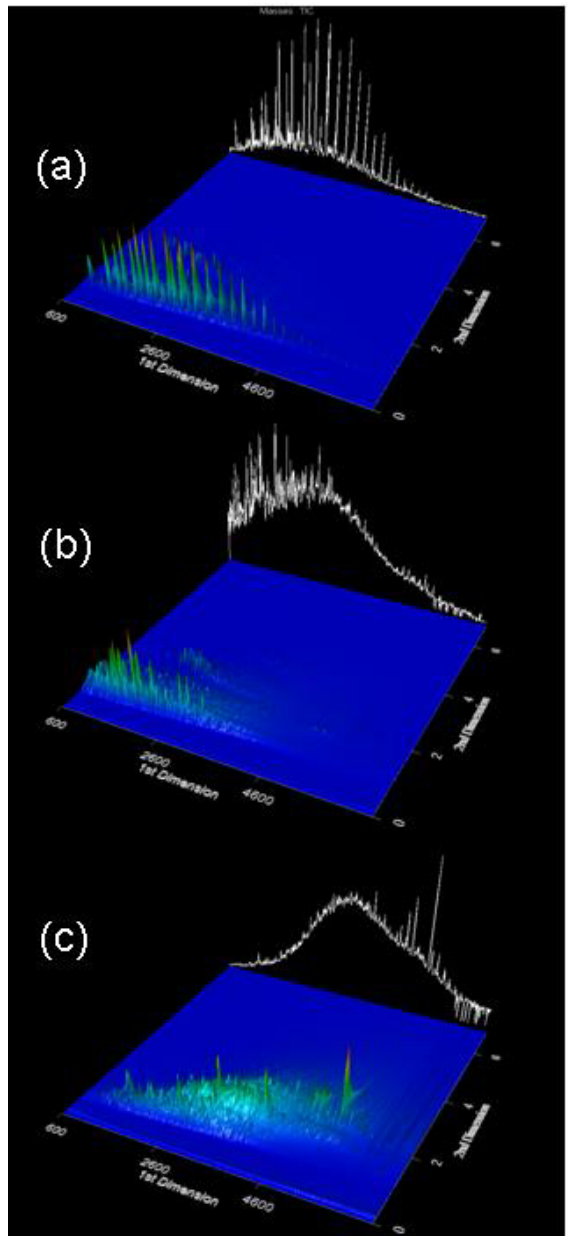

Figure 16. *GC×GC-TOF-MS chromatograms of (a) crude oil from Platform Holly, (b) crude oil collected as it was emerging from the sea floor at Jackpot Seep, and (c) tar mound sample collected from Il Duomo (same sample as Figure 13). Blue corresponds to the base-plane; the abundance of each molecule is proportional to height above the baseline. Notice the extreme changes in the oil composition in these samples. Only the most trace components in the reservoir oil (a) are present in the tar mound sample (c), revealing that most of the molecules (and mass) have been removed.*

(e) Outcomes of this work led to contributions to the response to the <u>Deepwater Horizon</u> disaster.

Based on our experiences in Santa Barbara, my colleagues and I were primed for major contributions to the response to, and damage assessment of, the *Deepwater Horizon* oilspill. This study provided numerous key insights into the science of the *Deepwater Horizon* spill and

the subsequent release of oil and gas from the Macondo well. The initial goal was to study the petroleum hydrocarbons found in subsurface plumes. We identified one plume in late June 2010 at 1100 meters and observed that it was composed of only a select few compounds (Figure 17). That is, of the 1000s of compounds in oil, only six compounds were in the majority. These compounds were benzene, toluene, ethyl benzene, and the three xylenes (ortho-xylene, meta-xylene, and para-xylene, together referred to as BTEX; Figure 18) all of which totaled 75 micrograms per liter. We noted that these compounds have properties that allow them to dissolve in water at levels higher than the other compounds in the oil. In order to achieve these results, we had to collect a pristine sample of oil leaking from the well, which we did by using a specialized sampler (Figure 19) and an underwater remotely operated vehicle (Figure 20). These findings were published in *Science* and the *Proceedings of the National Academy of Sciences* (Camilli et al 2010; Reddy et al 2012). We also estimated the amount of hydrocarbons biodegraded at the sea surface and calculated the amount of total oil released. Overall, this was a successful effort that allowed us to respond quickly to perform science in a time of crisis.

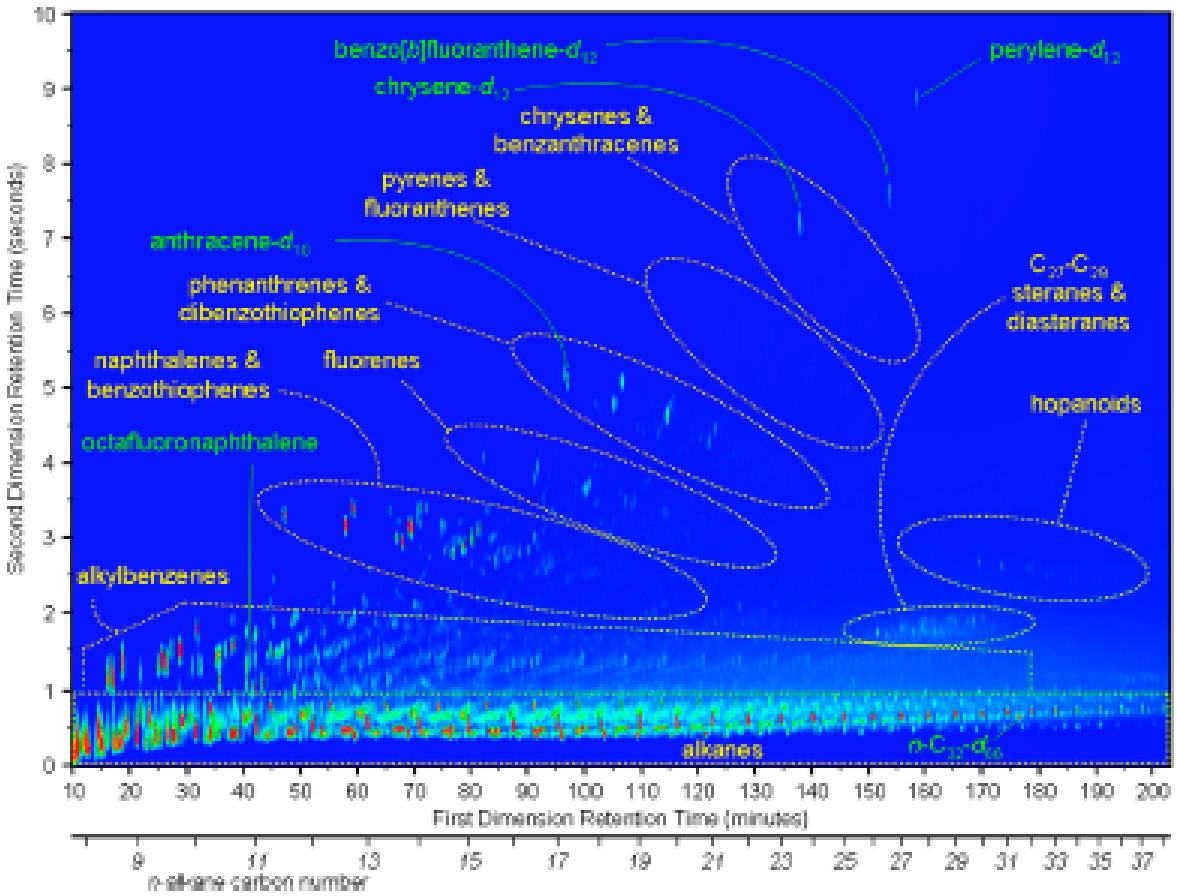

Figure 17. *GC×GC-FID chromatogram of reservoir fluid from Macondo well. This analysis and others by GC×GC have proven invaluable in numerous studies on source identification, photooxidation, and other loss processes*

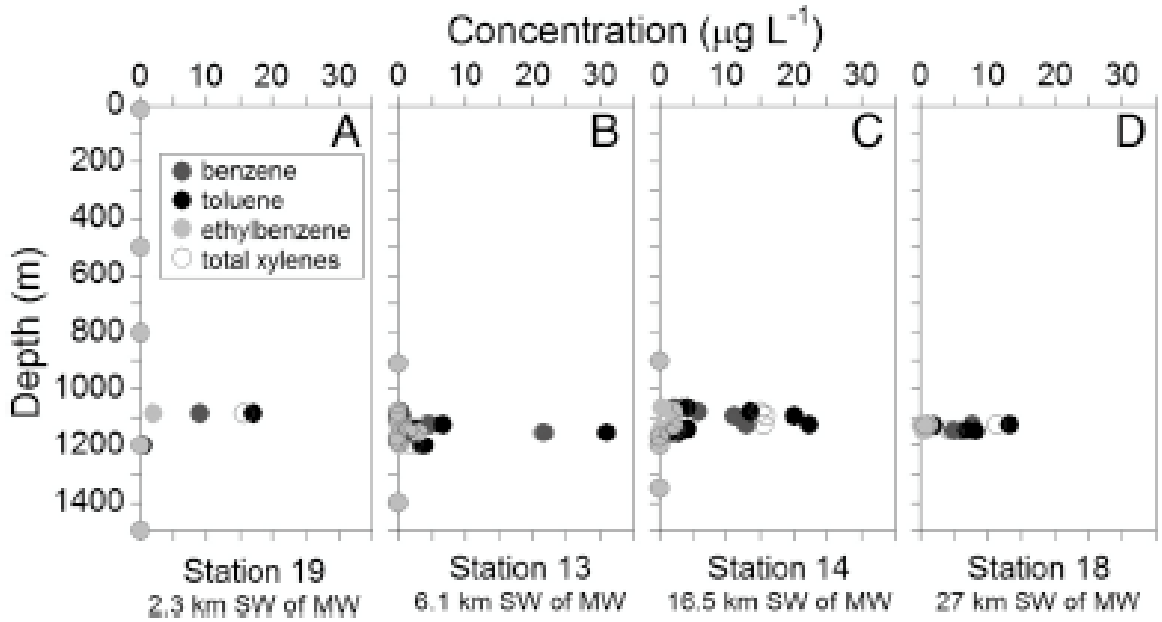

Figure 18. *Concentration profiles of BTEX at several locations in a subsurface plume in late June 2010.*

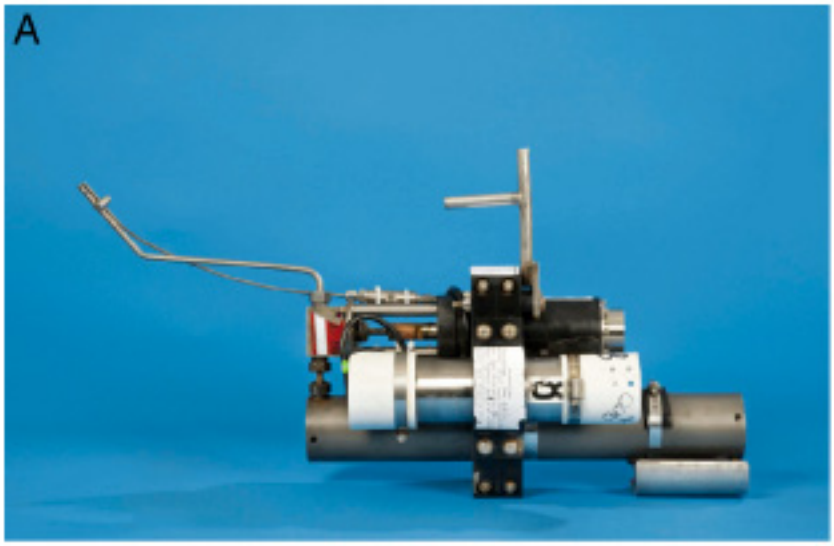

Figure 19. *An isobaric gas-tight sampler that was used to collect reservoir fluids at the Macondo well.*

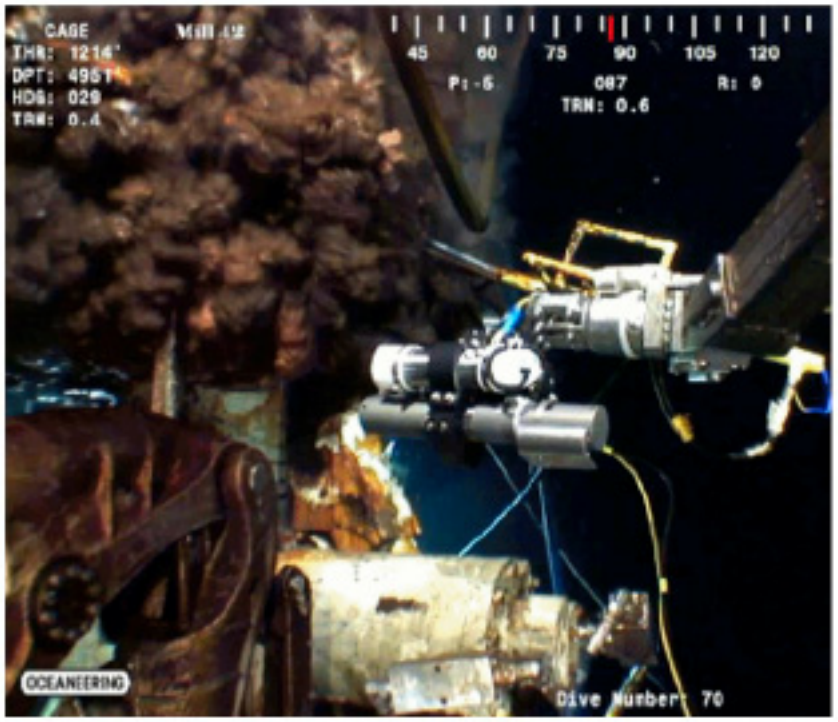

Figure 20. *An image of the isobaric-gas tight sampler collecting oil and gas from the Macondo well.*

3. Summary of Results

This work has provided an extensive description of the compounds found in seeping oil and its fate whether in the sea surface, sediments, or extinct tar mounds. It is the first to differentiate molecular transformations arising from the processes of water washing, evaporation, and biodegradation in the seep environment. Biodegradation is viewed here as a natural attenuator of petroleum hydrocarbon flux and economic value, and acts to decrease seepage in this region through the removal of numerous and diverse hydrocarbons. Anaerobic biodegradation in the subsurface induces mass loss signatures that are dramatically different from the observed evaporation signature at the sea surface. These results provide a new basis for understanding the molecular transformations of petroleum as it migrates from the deep subsurface and becomes influenced by the biosphere, hydrosphere and atmosphere. These processes are especially important given that natural seeps constitute approximately half of all oil inputs to the sea (National Research Council, 2003).

In summary, this project revolutionized the analysis of petroleum hydrocarbons and allowed us to provide a broader understanding the geochemistry of the Santa Barbara oil seeps and petroleum hydrocarbons, in general. These new findings have been applied elsewhere to the behavior of oil at other seeps as well as for petroleum exploration and oil spill studies (as shown during the *Deepwater Horizon* oil disaster).

4. Products

4.1 Presentations resulting from this award.

(a) At WHOI

1. Reddy, C.M. *The discovery of extinct asphalt volcanoes on the southern California margin*, WHOI Exhibit Center Seminar Series, August 25, 2009. WHOI, Woods Hole, MA (presentation).
2. Reddy, C.M. *Natural oil spills.* Visiting Chinese Maritime Students on exchange at Massachusetts Maritime Academy, May 29, 2009. WHOI, Woods Hole, MA (Presentation).
3. Reddy, C.M. *Natural oil spills.* Columbia School of Journalism visitors, May 1, 2009. WHOI, Woods Hole, MA (Presentation).
4. Reddy, C.M. *The Santa Barbara oil seeps: An oil spill every day.* Knight Science Journalism Fellows visit, October 10, 2008. WHOI, Woods Hole, MA (Presentation).
5. Reddy, C.M. *Oil spills in the ocean*. WHOI Trustee's meeting. May 16, 2008. WHOI, Woods Hole, MA. (Presentation)
6. Reddy, C.M. *The Santa Barbara oil seeps.* Visitors from Saint Margaret School, Buzzards Bay, MA. May 7, 2008. WHOI, Woods Hole, MA. (Presentation).
7. Reddy, C.M. *Natural oil spills in the sea.* WHOI Annual Volunteer Luncheon. September 17, 2007. WHOI, Woods Hole, MA. (Presentation).
8. Reddy, C.M. *Oil Spills, natural and unnatural*. WHOI Ocean Science Journalism Fellowship Program. September 14, 2007. WHOI, Woods Hole, MA (Presentation).
9. Reddy, C.M. *Petroleum in the marine environment.* Naval Command College Visit to WHOI. August 31, 2007. WHOI, Woods Hole, MA. (Presentation).
10. Reddy, C.M. *Petroleum in the marine environment.* Naval Staff College Visit to WHOI. May 4, 2007. WHOI, Woods Hole, MA. (Presentation).
11. Reddy, C.M. *Investigating the fate of oil in the marine environment*. Presentation for visitors representing King Abdullah University of Science and Technology. January 25, 2007. WHOI, MA. (Presentation).
12. Reddy, C.M. *Novel views on the fate of petroleum hydrocarbons and alternative fuels in the marine environment*. ONR Site visit, September 25, 2006. WHOI, Woods Hole, MA (Presentation).
13. Reddy, C.M. *Oil spills in the marine environment* Naval Command College Visit to WHOI. August 25, 2006. WHOI, Woods Hole, MA. (Presentation).

(b) Elsewhere

1. Aeppli, C.A., Kellermann M., Valentine, D.L., Lorenson T.D., Nelson, R.K., Reddy, C.M. Oil spill vs. natural seepage: Contrasting weathering characteristics of oil from the Macondo well and Southern California oil seeps. Gulf of Mexico Oil Spill & Ecosystem Science Conference, January 23, 2013. New Orleans, LA. Poster.

2. McKenna, A.M., Lemkau, K.L., Nelson, R.K., Valentine, D.L., Robbins, W.K., Reddy, C.M., Podgorski, D.C., de Corilo, Y., Rodgers, R.P., Marshall, A.G. *Molecular-level characterization of petroleum seeps and asphalt volcanoes from Santa Barbara Basin by FT-ICR mass spectrometry*. American Chemical Society meeting, Philadelphia, PA, August 20, 2012 (presentation).

3. Reddy, C.M. *Oil spills: natural and manmade.* The Gulf of Maine Institute, Newbury, MA, July, 5, 2012 (Presentation). Invited.

4. Reddy, C.M. *An introduction to GC×GC and applications to petroleum processes*. Department of Chemical Engineering, MIT, April 14, 2010. Invited.

5. Reddy, C.M. *Natural oil spills*. Robertson Elementary School, Warwick, RI, April 12, 2010. (Presentation). Invited.

6. Reddy, C.M. *Disentangling oil weathering at a marine seep using GC×GC*. Eastern Analytical Symposium, November 18, 2009, Somerset, NJ. (Presentation with abstract). Invited.

7. Ventura, G.T., Simoniet, B., Nelson, R., Reddy, C. *Resolving the origin of unresolved complex mixtures in hydrothermal petroleum – novel methods for comprehensive two-dimensional gas chromatography.* International Meeting on Organic Geochemistry, Bremen, Germany, September 9, 2009. (Presentation with abstract).

8. Schubotz, F., Ventura, G.T., Nelson, R.K., Reddy, C.M., Hinrichs, K-U. *Deciphering mechanistic details of asphalt degradation and weathering at the ocean floor with GCxGC*. International Meeting on Organic Geochemistry, Bremen, Germany, September 8, 2009. (Presentation with abstract).

9. Schubotz, F., Ventura, G.T., Nelson, R.K., Knittel, K., Wegener, G., Wilhelm, T., Zabel, M., Kasten, S., Boetius, A., Reddy, C.M., Hinrichs, K.-U. *Structure and function of a cold seep nurtured by seepage of heavy oil.* Goldschmidt Conference, Davos, Switzerland, June 25, 2009. (Presentation with abstract). Invited.

10. Reddy, C.M. *Moving past pretty pictures: Analysis of petroleum hydrocarbons by GC×GC*. 6th GC×GC Conference, Portland, OR, May 18, 2009. (Presentation with abstract). Invited

11. Wardlaw, G.D., Reddy, C.M., Nelson, R.K., Valentine, D.L. *Hydrocarbon specificity during aerobic oil biodegradation revealed in marine microcosms with the use of comprehensive two-dimensional gas chromatography*. American Geophysical Union Meeting, December 19, 2008. San Francisco, CA. (Poster with abstract)

12. Reddy, C.M. Nelson, R., Ventura, G., Arey, J.S., Wardlaw, G., Valentine, D. *Disentangling oil weathering at a marine seep using GC×GC: Applications form environmental forensics*. SETAC North America Annual Meeting, November 20, 2008. Tampa, FL. (Presentation with abstract). Invited. Attended.

13. Reddy, C.M., Ventura, G.T., Nelson, R.K., Betancourt, S., Lambertus, G., Mullins, O., Pomerantz, A., Raghuraman, B. *Advances in petroleum analysis via comprehensive two-dimensional gas chromatography*, Federation of Analytical Chemistry and Spectroscopy Society, Reno, NV, September 29, 2008. (Presentation with abstract).

14. Nelson, R.K., Summons, R., Hürlimann, M.D., Ventura, G.T., Reddy, C.M. *Comprehensive two-dimensional gas chromatographic analysis (GC×GC) of bicadinanes in crude oil - A comparison of oils from the Ardjuna Basin, Northwest Java; The Seria Oil Field, Brunei; and two oils from South Texas, USA.* Gordon Research Conference, Environmental Sciences: Water. Holderness School, Plymouth, NH, August 7, 2008. (Invited talk with abstract).

15. Schubotz, F. Nelson, R.K., Ventura, G.T. Reddy, C.M., Henrichs, K.U. *Comparative analysis of asphaltene-rich oils from the Campeche Knolls, deciphered by two-dimensional gas ghromatography*. Gordon Research Conference, Environmental Sciences: Water. Holderness School, Plymouth, NH, August 7, 2008. (Invited talk with abstract).

16. Ventura, G.T., Simoneit, B., Nelson, R.K. Reddy, C.M. *Resolving the origin of unresolved complex mixtures in petroleum-novel methods for comprehensive two-dimensional gas chromatography*. Gordon Research Conference, Environmental Sciences: Water. Holderness School, Plymouth, NH, August 5, 2008. (poster with abstract).

17. Ventura, G.T., Reddy, C.M., Simoneit, B.R.T., Nelson, R.K. *Resolving the unresolved complex mixture of petroleum*. Ocean Sciences Meeting, Orlando, FA March 7, 2008. (Presentation with abstract).

18. Ventura, G.T., Simoneit, B.R., Reddy, C.M., Nelson, R.K., *Resolving the unresolved complex mixtures of petroleum using comprehensive two dimensional gas chromatography-time of flight mass spectrometry.* American Geophysical Union Meeting, December 14, 2007. San Francisco, CA. (Poster with abstract).

19. Valentine, D.L, Reddy, C., Nelson, R.K., Ventura, G.T. *Discovery and description of extinct asphalt volcanoes along the Southern California Margin* American Geophysical Union Meeting, December 13, 2007. San Francisco, CA. (Presentation with abstract).

20. Arey, J.S., Nelson, R.K., Reddy, C. M. *Disentangling oil weathering in the environment using GC × GC.* 234th ACS National Meeting, Boston, MA, August 20, 2007. (Presentation with abstract). Attended.

21. Gaines, R.B., Frysinger, G.S., Reddy, C.M., Nelson, R. K. *Comprehensive 2-D gas chromatography (GC × GC) in environmental forensics*. 234th ACS National Meeting, Boston, MA, August 20, 2007. (Presentation with abstract).

22. Arey, J.S, Nelson, R.K., Nelson, R.K., Wardlaw, G.D., Valentine, D.L. Reddy, C.M. *Disentangling evaporation and water-washing signatures in weathered oil mixtures using comprehensive two-dimensional gas chromatography (GC × GC)* Gordon Research Conference, Environmental Sciences: Water. Holderness School, Plymouth, NH, June 27, 2006. (Poster with abstract).

23. Wardlaw, G.D., Reddy, C.M., Nelson, R.K., Valentine, D. *Petroleum weathering associated with hydrocarbon migration and seepage, a case study from the Santa Barbara Channel*, CA, University of California Toxic Substance Research and Teaching Program, San Diego, CA, April 28, 2006. (Poster with abstract).

4.2 Papers resulting from this award.

2006

Gaines, R. B.; Frysinger, G. S.; Reddy, C. M.; Nelson, R. K. (2006). Oil spill source identification by comprehensive two-dimensional gas chromatography (GC×GC). In *Oil Spill Fingerprinting and Source Identification*, Chapter 5; Wang, Z., Stout, S. Eds.; Elsevier Science: New York.

2007

Reddy,C.M., Nelson, R.K., Sylva, S.S, Xu, L., Peacock, E.E, Raghuraman, B. and Mullins, O.C. (2007). Using GC×GC-FID to identify and quantify olefin-based drilling fluids in crude oils. *J. of Chromatography A*. **1148**, 100-107.

Arey, J.S., Nelson, R.K., and Reddy, C.M. (2007) Disentangling oil weathering using GC×GC. Part 1. Chromatogram analysis. *Env. Sci. and Technol*. **41**, 5738 – 5746.

Arey, J.S., Nelson, R.K., Plata, D.L., and Reddy, C.M. (2007) Disentangling oil weathering using GC×GC. Part 2. Mass transfer calculations. *Env. Sci. and Technol*. **41**, 5747 - 5755.

2008

Mullins, O.C., Ventura, G.T., Nelson, R.K., Betancourt, S.S., Raghuraman, B., Reddy, C.M. (2008) Visible-near-infrared spectroscopy by downhole fluid analysis coupled with two dimensional gas chromatography to address oil reservoir complexity. *Energy and Fuels* **22**, 496-503.

Wardlaw, G., Arey, J.S., Reddy, C.M., Nelson, R.K., Ventura, G.T., Valentine, D.L. (2008) Disentangling oil weathering at a marine seep using GC×GC: Broad metabolic specificity accompanies subsurface petroleum biodegradation. *Env. Sci. and Technol* **42**, 7166-7173.

Farwell, C., Reddy, C.M., Peacock, E.E., Nelson, R.K., Washburn, L., Valentine, D.L. (2008) Weathering and the fallout plume of heavy oil from strong petroleum seeps near Coal Oil Point, CA. *Env. Sci. and Technol.* **43**, 3542–3548.

2010

Valentine, D.L., Reddy, C.M., Farwell, C., Hill, T. Pizarro, O., Yoerger, D., Camilli, R., Nelson, R.K. Peacock, E.E., Clarke, B.A., Roman, C.N., Soloway, M. (2010) Asphalt volcanoes as a potential source of methane to late Pleistocene coastal waters. *Nature Geosciences*, **3**, 345–348.

Camilli, R., Reddy, C.M., Yoerger, D.R., Van Mooy, B.A.S., Jakuba, M.V., Kinsey, J.C. McIntyre, C.P., Sylva, S.P., Maloney, J.V. (2010) Tracking hydrocarbon plume transport and biodegradation at *Deepwater Horizon. Science,* **330**, 201-204.

Ventura, G.T., Raghuraman, B., Mullins, O.C., Nelson, R.K., Reddy, C.M. (2010) Chemical compound class oil fingerprinting techniques using comprehensive two-dimensional gas chromatography (GC×GC). *Organic Geochemistry* **41***,* 1026-1035.

2011

Wardlaw, G.D. , Nelson, R.K., Reddy, C. M., Valentine, D.L. (2011) Biodegradation preference for isomers of alkylated naphthalenes and benzothiophenes in marine sediment contaminated with crude oil. *Organic Geochemistry* **42***,* 630-639.

Ventura, G.T., Hall, G.J., Nelson, R.K. Frysinger, G.S., Raghuraman, B., Pomerantz, A.E., Mullins, O.C., Reddy, C.M. (2011) Analysis of petroleum compositional similarity using multiway principal components analysis (MPCA) with comprehensive two-dimensional gas chromatographic data. *J. Chromatography A.* **1218**, 2584-2592.

2012

Ventura, G.T. , Simoneit, B.R.T., Nelson, R.K., Reddy, C.M. (2012) The composition, origin, and fate of complex mixtures in the maltene fractions of hydrothermal petroleum assessed by comprehensive two-dimensional gas chromatography. *Organic Geochemistry* **45**, 48-65.

Reddy, C.M., Arey, J.S., Seewald, J.S., Sylva, S.P., Lemkau, K.L.*, Nelson, R.K., Carmichael, C.A., McIntyre, C., Fenwick, J., Ventura, G.T., Van Mooy, B.A.S., Camilli, R. (2012) Composition and fate of gas and oil released to the water column during the *Deepwater Horizon* oil spill. *Proceedings of the National Academy of Science,* **109**, 20229-20234.

5.0 References Cited

Arey, J. S.; Nelson, R. K.; Plata, D. L.; Reddy, C. M. Disentangling oil weathering using GC×GC. 2. Mass transfer calculations. *Environ. Sci. Technol.* **2007a**, *41*, 5747–5755.

Arey, J. S.; Nelson, R. K.; Reddy, C. M. Disentangling oil weathering using GC×GC. 1. Chromatogram analysis. *Environ. Sci. Technol.* **2007b**, *41*, 5738–5746.

Arey, J. S.; Nelson, R. K.; Xu, L.; Reddy, C. M. Using comprehensive two-dimensional gas chromatography retention indices to estimate environmental partitioning properties for a complete set of diesel fuel hydrocarbons. *Anal. Chem.* **2005**, *77*, 7172–7182.

Camilli, R., Reddy, C.M., Yoerger, D.R., Van Mooy, B.A.S., Jakuba, M.V., Kinsey, J.C. McIntyre, C.P., Sylva, S.P., Maloney, J.V. (2010) Tracking hydrocarbon plume transport and biodegradation at *Deepwater Horizon. Science,* **2010**, *330*, 201-204.

Frysinger, G. S.; Gaines, R. B.; Xu, L.; Reddy, C. M. Resolving the unresolved complex mixture in petroleum-contaminated sediments. *Environ. Sci. Technol.* **2003**, *37*, 1653–1662.

Hostettler, F. D.; Wang, Y.; Huang, Y.; Cao, W.; Bekins, B. A.; Rostad, C. E.; Kulpa, C. F.; Laursen, A. Forensic fingerprinting of oil-spill hydrocarbons in a methanogenic environment-Manden, ND and Bemidji, MN. *Environ. Forensics* **2007**, *8*, 139–153.

Keller, E.A.; Duffy, M.; Kennett, J.P.; Hill, T. Tectonic geomorphology and hydrocarbon induced topography of the Mid-Channel Anticline, Santa Barbara Basin, California. *Geomorphology* **2007**, *89*, 274-286.

Larter, S. R.; Wilhelms, A.; Head, I. M.; Koopmans, M.; Aplin, A.; Di Primio, R.; Zwach, C.; Erdmann, M.; Telnaes, N. The controls on the composition of biodegraded oils in the deep subsurface: Part I. Biodegradation rates in petroleum reservoirs. *Org. Geochem.* **2003**, *34*, 601–613.

Larter, S. R.; Huang, H.; Adams, J.; Bennett, B.; Jokanola, F.; Oldenburgh, T.; Jones, M.; Head, I. M.; Riediger, C.; Fowler, M. The controls on the composition of biodegraded oils in the deep subsurface: Part II. Geological controls on subsurface biodegradation fluxes and constraints on reservoirfluid property prediction. *AAPG Bull.* **2006**, *90*, 921–938.

National Research Council, *Oil in the Sea III: Inputs, Fates, and Effects*. National Academies Press: Washington, D.C., 2003.

Nelson, R. K.; Kile, B. S.; Plata, D. L.; Sylva, S. P.; Xu, L.; Reddy, C. M.; Gaines, R. B.; Frysinger, G. S.; Reichenbach, S. E. Tracking the weathering of an oil spill with comprehensive two dimensional gas chromatography. *Environ. Forensics* **2006**, *7*, 33–44.

Peters, K. E., C. C. Walters and J. M. Moldowan (2005). The biomarker guide. Cambridge, University Press.

Prince, R. C.; Elmendorf, D. L.; Lute, J. R.; Hsu, C. S.; Halth, C. E.; Senius, J. D.; Dechert, G. J.; Douglas, G. S.; Butler, E. L. 17α(H),21β(H)-hopane as a conserved internal marker for estimating the biodegradation of crude oil. *Environ. Sci. Technol.* **1994**, *28*, 142-145.

Prince, R. C.; Suflita, J. M. Anaerobic biodegradation of natural gas condensate can be stimulated by the addition of gasoline. *Biodegradation* **2007**, *18*, 515–523.

Reddy, C. M.; Eglinton, T. I.; Hounshell, A.; White, H. K.; Xu, L.; Gaines, R. B.; Frysinger, G. S. The West Falmouth oil spill after thirty years: The persistence of petroleum hydrocarbons in marsh sediments. *Environ. Sci. Technol.* **2002**, 36, 4754–4760.

Reddy, C.M., Arey, J.S., Seewald, J.S., Sylva, S.P., Lemkau, K.L.*, Nelson, R.K., Carmichael, C.A., McIntyre, C., Fenwick, J., Ventura, G.T., Van Mooy, B.A.S., Camilli, R. Composition and fate of gas and oil released to the water column during the *Deepwater Horizon* oil spill. *Proceedings of the National Academy of Science*, **2012**, 109, 20229-20234.

Reichenbach, S. E.; Ni, M.; Kottapalli, V.; Visvanathan, A. Information technologies for comprehensive two-dimensional gas chromatography. *Chemom. Intell. Labt. Syst.* **2004**, *71*, 107-120.

Siddique, T.; Fedorak, P. M.; Foght, J. M. Biodegradation of short-chain *n*-alkanes in oil sands tailings under methanogenic conditions. *Environ. Sci. Technol.* **2006**, *40*, 5459–5464.

Spormann, A. M.; Widdel, F. Metabolism of alkylbenzenes, alkanes, and other hydrocarbons in anaerobic bacteria. *Biodegradation* **2000**, *11*, 85–105.

Valentine, D.L., Reddy, C.M., Farwell, C., Hill, T. Pizarro, O., Yoerger, D., Camilli, R., Nelson, R.K. Peacock, E.E., Clarke, B.A., Roman, C.N., Soloway, M. Asphalt volcanoes as a potential source of methane to late Pleistocene coastal waters. *Nature Geosciences* **2010**, *3*, 345–348.

Wardlaw, G., Arey, J.S., Reddy, C.M., Nelson, R.K., Ventura, G.T., Valentine, D.L. Disentangling oil weathering at a marine seep using GC×GC: Broad metabolic specificity accompanies subsurface petroleum biodegradation. *Env. Sci. and Technol*., **2008**, doi: 10.1021/es8013908

Wardlaw, G.D. , Nelson, R.K., Reddy, C. M., Valentine, D.L. Biodegradation preference for isomers of alkylated naphthalenes and benzothiophenes in marine sediment contaminated with crude oil. *Organic Geochemistry* **2011**, *42*, 630-639.