

# IDENTIFICATION OF CHRONIC OILING SOURCES IN CALIFORNIA

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## **EXECUTIVE SUMMARY**

Rapid identification of petroleum samples is critical for effective response to mystery oil spills. In California, mystery spills (spills with an unknown source) may be anthropogenic or may be related to productive natural petroleum seeps. In this report, we provide two types of information to help with identification of mystery spill samples in California. In Chapter 1, we present a new method of quickly comparing quantified aspects of an oil “fingerprint” from a mystery sample to a large database of potential sources and other samples. Using simple relative percent difference among a series of biomarker ratios (compared using programming language R), we were able to quickly narrow down potential matching samples that could be confirmed using more standard fingerprinting techniques. In Chapter 2, we present information on specific groups of matching samples presumed to be related to natural seeps in California, identified in part using methods described in Chapter 1. These 10 groups include samples from tar balls and oiled wildlife, and in four cases are linked to specific source seeps. This is one of few reports linking mystery samples to specific seeps, and we also present information on some seeps off central California that previously were not well documented. These groups of matching samples provide a starting point for comparison of future mystery samples, and provide a greater understanding of the dynamics of chronic oiling from seeps in California.

## **ACKNOWLEDGMENTS**

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## Chapter 1

### Use of a biomarker ratio library to aid in rapid identification of mystery oil spill samples

#### INTRODUCTION

Oil spills of unknown origin pose logistical and legal challenges for responders. Mystery spills may be anthropogenic (e.g., from offshore bilge cleanings, or undetected leaks from offshore production facilities or shipwrecks), or natural (from natural petroleum seeps). Determining the probable source of mystery spills is critical for effective response.

In California, where productive natural petroleum seeps occur offshore (Kvenolden and Cooper 2003), tarballs and oiled wildlife are often reported without a known source (Hostettler et al. 2004, Henkel et al. 2014), and it is often assumed that these events are natural. However, anthropogenic mystery spills have also been documented, most notably the chronic leaking of the shipwreck *S.S. Jacob Luckenbach*, resulting in the presumed oiling of >50,000 seabirds in central California (Hampton et al. 2003). Thus, a method to quickly identify the potential source of mystery oil samples is highly desirable, to distinguish natural from anthropogenic spills.

Various techniques can be used to assess and compare the chemistry of oil samples. The most widely used technique is use of gas chromatography and mass spectrometry (GC/MS), and comparison of total ion and/or extracted ion chromatograms, or oil “fingerprints” (Bayona et al. 2015, Wang et al. 2016). In the United States, the standard method (ASTM 5739-00) for comparison of oil sample extracted ion chromatograms (EICs) is to visually overlay the chromatograms to look for differences in “biomarker” peaks (ion peaks whose height and shape are determined by the composition of the ancient biological community from which the oil was derived). This standard is used by the California Department of Fish and Wildlife Office of Spill Prevention and Response Petroleum Chemistry Lab (OSPR-PCL) and the U.S. Coast Guard Marine Safety Laboratory (USCG-MSL), among others.

Increasingly, newer chemometric methods are also being employed to quantify the similarity of samples. Among these methods, calculation and comparison of the ratios of pairs or groups of biomarkers has become a standard practice (Shen 1984, Urdal et al. 1986, Wang et al. 1998, Christensen and Tomasi 2007, Mulabagal et al. 2013). The accepted European standard protocol (CEN TR1552-2) uses (among other diagnostic steps) comparison of a set of “normative” biomarker ratios, using the absolute difference of ratio peak heights divided by the mean of the peak heights (percent difference). Based on acceptable instrument/lab repeatability variation of 5%, the CEN protocol recommends an overall threshold of 14% for the difference between any one ratio for “matching” samples (Kienhuis et al. 2016). The field of petroleum chemometrics is rapidly evolving, and there are many other newer analytical methods (in addition to biomarker ratios) that have been proposed (Christensen and Tomasi 2007, Rodgers and McKenna 2011, Bayona et al. 2015), however, we will not attempt a full review of additional methods here.

While both qualitative and quantitative methods have advantages, if one is wishing to identify a mystery sample there are potential challenges with quickly comparing the mystery sample to a library or database of many samples of known origin. In Europe, the COSIWeb online database has recently been developed to quickly compare various aspects of chemical profiles of different petroleum samples (Dahlmann and Kienhuis 2016); however, no such system is currently available in the United States. Large datasets of biomarker ratios have been numerically compared, often using Principal Components Analysis (PCA) or other multivariate analyses (Christensen and Tomasi 2007, Peters et al. 2008, Lobão et

al. 2010, Liu et al. 2013). Although the use of PCA is scientifically sound and provides excellent discriminatory power to identify matching oil samples, this tool also has several drawbacks: 1) PCA requires a substantial database of potential source samples, 2) it is mathematically complex (requiring a statistical software package), and perhaps most importantly, 3) the results vary depending on the number and variability of samples in the database.

U.S. Geological Survey (USGS) geochemists, working with tar balls and other oil recovered in California, have identified a set of 19 biomarker ratios that are fairly stable and resistant to weathering and biodegradation, and has built a substantial database of biomarker ratios (Hostettler et al. 2004, Peters et al. 2008, Lorenson et al. 2009, 2011). The OSPR-PCL, using the same techniques, has also built a database of >170 oil/tar samples. Importantly, these databases include ratios for samples from known potential sources, including offshore production platforms, shipwrecks, and known seeps. Here we report on a simple method (using mean relative percentage difference) to quickly compare biomarker ratios of a mystery sample to a library of other biomarker ratios. This method avoids the drawbacks of PCA, in that it is mathematically simple and provides consistent results regardless of the number of samples in the database. This method is not intended to quantifiably identify samples as matching or non-matching, but simply to quickly narrow down the possibly suspects. This method has allowed us to quickly identify many matching oil samples, resulting in a greater understanding of several sources of chronic oiling (as described in Chapter 2).

## **METHODS**

### *Sample Preparation*

We used a variety of samples from tarballs, oiled birds, natural seeps, and offshore oil production platforms, collected in California between 1997 and 2012. Oil/tar samples were extracted using dichloromethane, base-washed, and the solvent exchanged to n-hexane. For GC/MS, samples were then separated into aliphatic and aromatic fractions using a liquid chromatography column with neutral alumina. Sample concentration for each fraction was adjusted to a standard value (20mg/ml), and each fraction was spiked with an internal standard to validate the retention time in the chromatograph.

### *GC/MS Analysis and Visual Comparison*

For each sample analyzed by GC/MS, a Total Ion Chromatogram (TIC) and a series of Extracted Ion Chromatograms (EIC) were generated for each fraction (aliphatic and aromatic). This resulted in 33 (24 aromatic and 9 aliphatic) chromatograms that could be compared using the visual overlay method (ASTM 5739-00).

Samples were considered to be a match (i.e., share a common source) if their chromatograms showed that they had all of the same compounds and that they were present in the same amount. Weathering and biodegradation (consumption of certain compounds by microbes) can cause some compounds to be lost from oil samples before other compounds (e.g., steranes then hopanes disappear in heavily degraded samples; Peters and Moldowan 1993). Thus, if chromatograms matched except for a few compounds that may have differed due to differential weathering or biodegradation, samples may still have been considered to match.

### *Biomarker Ratio Calculation*

To be able to calculate biomarker ratios, fractionated samples were re-run through GC/MS with a slower retention time to provide much greater detail for certain compounds, and Selected Ion Monitoring (SIM) mass chromatograms were generated. From these detailed chromatograms, up to 19 biomarker ratios were calculated (Table 1-1).

**Table 1-1.** Biomarker ratios used for comparison of oil samples (per Lorenson et al. 2009).

Ratio Number	Ratio	Ratio Number	Ratio
1	del 13C	11	C <sub>24</sub> /C <sub>23</sub> TT
2	Ts/T <sub>m</sub>	12	C <sub>26</sub> /C <sub>25</sub> TT
3	C <sub>26</sub> /Tet (triplet)	13	C <sub>31</sub> S/H
4	C <sub>28</sub> /C <sub>29</sub>	14	C <sub>29</sub> H/H
5	PAH-RI	15	C <sub>35</sub> /C <sub>34</sub> S
6	C2D/C2P	16	BNH/H
7	C3D/C3P	17	OI/H
8	C <sub>28</sub> /C <sub>29</sub> TT	18	G/H
9	C <sub>20</sub> /C <sub>23</sub> TT	19	C <sub>29</sub> Ts/C <sub>29</sub> H
10	C <sub>22</sub> /C <sub>21</sub> TT		

These biomarkers were chosen to correspond to those compiled for other California samples by USGS (Lorenson et al. 2009). OSPR-PCL chemists were trained by USGS chemists to calculate these same biomarker ratios so that the results between the two labs would be comparable. Peak heights or areas were determined using the instrument software and the ratios in table 1-1 were calculated. It should be noted that these ratios are not the same as the “normative diagnostic ratios” recommended by the European CEN protocol (Kienhuis et al. 2016), which use a different set of biomarkers. Ratio #1 is the ratio of Carbon<sub>13</sub> to Carbon<sub>12</sub>, and was calculated independently by an outside lab. In many cases, this ratio was not calculated for our samples. All known underwater (marine) petroleum seeps in California originate in Monterey Formation Shale, and the oil is thus referred to as Monterey Formation crude. A primary interest and first step in fingerprinting mystery samples in California is to determine if the oil is Monterey Formation crude; if it is not, then it is presumed to be anthropogenic (not from a natural seep). If the oil is consistent with Monterey Formation crude, further analysis is required to determine the likely source, which in addition to seeps can include produced oil and bunker fuels derived from Monterey Formation crude. However, the Carbon<sub>13</sub> ratio is remarkably consistent among Monterey Formation sources and thus provides little discriminatory capability among Monterey Formation oils. For highly degraded samples (either biodegraded or weathered), some peaks (e.g., hopanes) may have been missing and these ratios could not be calculated.

### *Biomarker Ratio Comparison*

To quantitatively compare ratios of different samples, we developed a simple script in programming language R (R Core Team 2014) to calculate Mean Relative Percent Difference (MRPD), where Relative Percent Difference (RPD) is:

$$RPD = \text{abs}(x_1 - x_2) / (x_1 + x_2) / 2$$

(with  $x$  being a biomarker ratio) and the MRPD is the mean of up to 19 RPD values calculated for each paired comparison. If ratio values are missing in one or both of the two samples being compared, that ratio is ignored, and the mean of the remaining RPD values is used. The R script inputs a csv file, computes this calculation comparing any one sample to every other sample in a database, and outputs another csv file with MRPD comparing the row of choice against all other rows. These results can then be sorted by MRPD (low to high) for a list of most likely potential matches to the mystery sample. We used this method to compare samples in a database of >170 OSPR-PCL samples, and also compared some samples to a larger library of USGS ratios from sources in California (675 samples; Lorenson et al. 2009). After samples with low MRPD relative to the mystery sample (or test sample) were identified, chromatograms were then examined using the standard visual overlay method described above to determine if they indeed matched.

We used an iterative process to initially assess the usefulness of this tool, then once many mutually-matching samples were identified, we used these groups of samples with a presumed common source to analyze the characteristics of matching samples in greater detail. Initially, paired samples with MRPD of <0.15 (15%) were visually assessed, but if matching samples were found we continued comparisons down the list (higher MRPD values) until samples no longer matched. We also assessed analytical variability in two ways: 1) comparing 5 replicate samples (from the Cosco Busan oil spill source) with separate GC/MS runs and ratio calculation, and 2) calculation and comparison of ratios from 31 splits of samples previously analyzed by USGS. Finally, we also developed a similar R script to create a matrix of MRPD values for pairwise comparison of all samples in our OSPR-PCL biomarker ratio library, and create a dendrogram (using R function “hclust” for hierarchical cluster analysis, using a matrix of pairwise MRPD values as the dissimilarity matrix) to visually assess the relationships among samples based on MRPD.

## RESULTS

### *Analytical Variability*

Variability can be introduced into calculation of biomarker ratios at several levels, including sample collection and preparation, instrument differences, and differences among chemists measuring biomarker peaks to calculate ratios. Our first assessment of variability was among 5 splits of the same bunker fuel oil sample (from the Cosco Busan oil spill), each analyzed separately for 10 pairwise MRPD values. We found that variability of MRPD among these samples was quite low (mean MRPD =  $0.04 \pm 0.02$  SD). At the next level, we re-analyzed (GC/MS) and calculated biomarker ratios for splits of 31 samples previously analyzed by USGS using similar methods. For these samples, we found greater variability, with a mean MRPD of 0.12 (0.09 SD). This sample set included one outlier MRPD value of 0.55 (all other values were  $\leq 0.22$ ). We have not yet assessed these differences in further detail to determine the cause of the higher differences between OSPR-PCL and USGS-derived values.

### *Matching Groups*

Using the iterative method described above of visually comparing chromatograms for pairs of samples with low MRPD values, we found several groups of mutually matching samples (Appendix A). We used the three largest groups (Groups A, B, and C) to further investigate the usefulness of MRPD values for identifying potentially matching samples. Samples from these groups were all consistent with weathered Monterey Formation crude with degradation of rank 5 (Peters and Moldowan 1993), with

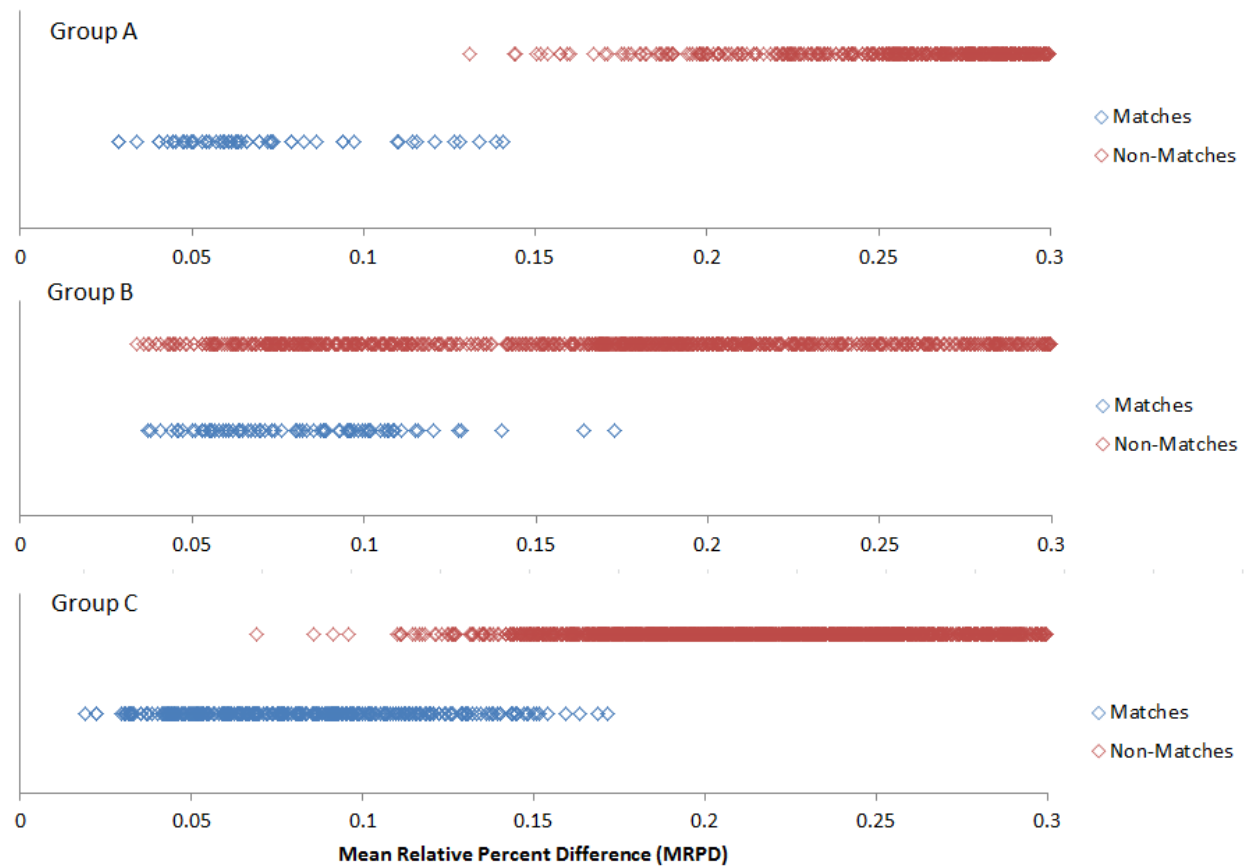
the exception of one sample that was rank 3 (in Group B). Additional details on these groups is provided in Chapter 2.

Ratios for samples in Groups A, B, and C were each compared to every other sample in the OSPR-PCL ratio library ( $n = 173$  samples; 172 comparisons for each sample), to assess whether thresholds could be established for matching vs. non-matching samples. Within Group A ( $n = 11$  matching samples), mean MRPD among matching samples was  $0.072 (\pm 0.029)$  with a range of 0.029 to 0.141. For non-matching samples, mean MRPD was  $0.536 (\pm 0.246)$  with a range of 0.131 to 1.121. This resulted in only minor overlap of matching and non-matching samples (Fig. 1). Within Group B, matching samples ( $n = 13$ ) had a mean MRPD of  $0.082 (\pm 0.028)$  with a range of 0.037 to 0.173. Non-matching samples had a mean MRPD of  $0.389 (\pm 0.216)$ , and a range of 0.034 to .0966. The lower range for non-matching samples was considerably lower than for Group A, and many non-matching samples had MRPD values that overlapped with matching samples (Fig. 1). Overall, there were 21 non-matching samples that overlapped with the 13 mutually matching samples. Of the non-matching samples, about half ( $n = 11$ ) were missing ratio 4 ( $C_{28}/C_{29}$ ), but the others had generally similar ratios to the matching samples in Group B. However, based on visual comparisons of chromatograms, these samples were not consistent with the Group B matching samples. Within Group C ( $n = 26$  matching samples), mean MRPD among matching samples was  $0.085 (\pm 0.033)$ , with a range of 0.019 to 0.172. Non-matching samples had a mean MRPD of  $0.427 (\pm 0.208)$ , with a range of 0.069 to 1.009. There was a moderate amount of overlap of MRPD values between 0.10 and 0.15 for non-matching and matching samples (Fig. 1-1).

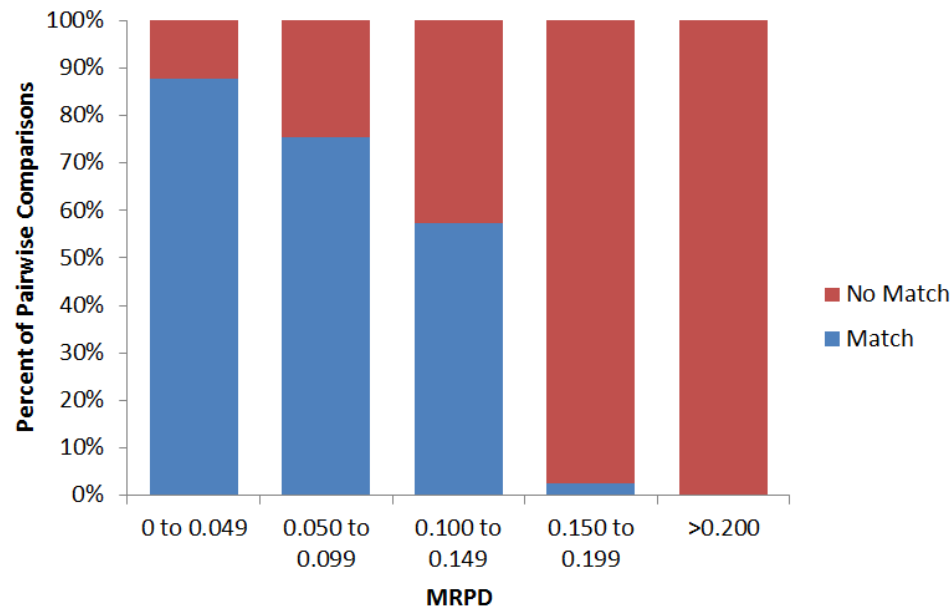
Among 866 pairwise comparisons of matching samples, the maximum MRPD value was 0.173. Most matching samples had MRPD values of  $<0.15$  (Fig. 1-2), and most non-matching samples had MRPD values of  $>0.15$ .

To visually compare relationships among samples, we plotted a dendrogram based on MRPD (Fig. 1-3). This dendrogram appears to provide good discrimination of different groups of mutually matching samples. The highest level of discrimination (first split at the top of the graph) seemed to accurately divide mostly produced oil samples (platform samples and oil spill samples) on left side of the graph (left of the left most Group B sample), and mostly presumed natural seep samples on the right side.

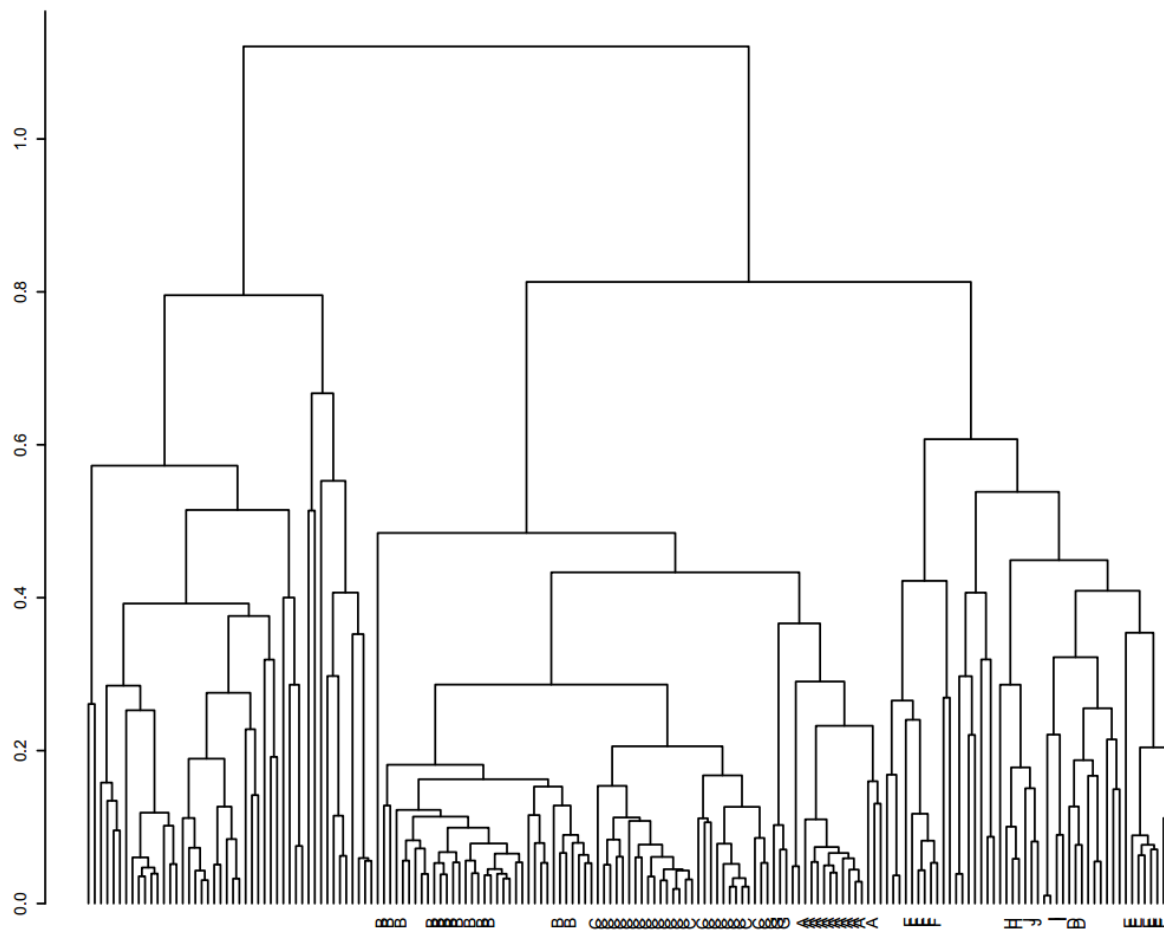
As a preliminary assessment of the relative usefulness of different biomarker ratios to distinguish among samples, we plotted the mean ratio values for ratio numbers 2 through 18 for each group (Group A, B, and C) and the standard deviation of the mean; to allow for easy comparison on a shared y-axis, the mean values for each group were divided by the grand mean for that ratio among samples from all three groups (Fig. 1-4). Overlap of standard deviation error bars indicates that some ratios are not distinct among groups, while ratios number 6 ( $C_{2D}/C_{2P}$ ), 10 ( $C_{22}/C_{21}$  TT), 11 ( $C_{24}/C_{23}$  TT), and 16 (BNH/H) were completely distinct among the three groups.



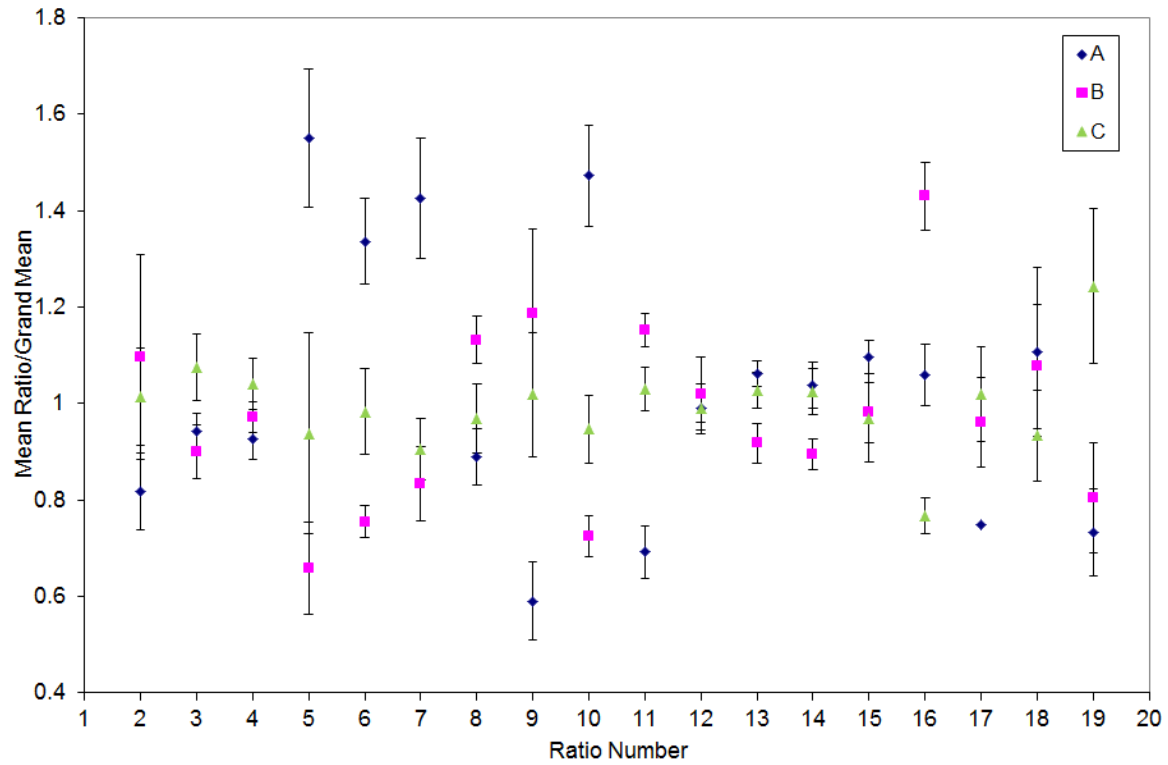
**Figure 1-1.** Mean Relative Percent Difference (MRPD) between matching (blue) and non-matching (red) samples in three groups (Groups A, B, C) analyzed, based on comparison of biomarker ratios. Y-axes are dimensionless; matching and non-matching samples are simply offset for ease of viewing. X-axes are truncated at 0.3; values for non-matching samples continued to much higher values.



**Figure 1-2.** MRPD for pairwise comparison of matching and non-matching samples, expressed categorically.



**Figure 1-3.** Dendrogram showing relationships among all samples tested in the OSPR-PCL ratio library. Letters along the x-axis show the Group (from left to right, B, C, G, A, F, H, J, I, D, E) to which those samples belong.



**Figure 1-4.** Representation of variability among ratio values (ratios 2 through 19) within three groups (Groups A, B, and C) of matching samples. Mean ratio values for each group were divided by the grand mean of that ratio for all groups, to allow for easy comparison on the same y-axis. Error bars are one standard deviation.

## DISCUSSION

Our method of quantitatively comparing biomarker ratios using Mean Relative Percentage Difference (MRPD) performed well at quickly identifying potential matching oil samples that could then be confirmed using a visual overlay comparison. This method provides results (MRPD) that are standardized and consistent (regardless of number of samples being compared), and thus is preferable to more complex multivariate approaches that may require a “training set” and provide results that vary depending on the other samples analyzed.

We found that among 866 pairwise comparisons, the maximum MRPD value for matching samples was 0.173, and most matching samples had MRPD values  $<0.15$ . However, if the goal is to identify any matching samples, we conservatively recommend visually comparing samples with MRPD values of  $<0.20$ . It should be noted that in practice, it should not typically be necessary to visually compare a mystery sample to a large number of other samples, if an existing library of known samples exists that includes which samples are already known to match. If a potential match is found using MRPD values and the match is confirmed, additional visual comparisons are not required. Similarly, if a mystery sample is visually confirmed to not match a sample in the ratio library that is in a group of mutually matching samples, the mystery sample would be known to not match any samples in that group and visual comparisons to those samples could be skipped. Thus, even comparing all samples with a  $\text{MRPD} < 0.20$  may be a fairly quick process. Using these methods, we identified several groups of matching samples and matched several tar ball or wildlife samples to specific sources for the first time (discussed in Chapter 2).

This method (use of MRPD) is one of several that could potentially be used to quickly screen mystery samples. Potential adjustments to our methods could be investigated further, including the way that missing ratios are treated (currently not included in calculations), and which particular ratios are used. We used ratios that were previously determined to be robust and resistant to degradation (Lorenson et al. 2009), but we found that some ratios performed better than others at discriminating among the three groups we analyzed. Further analysis may warrant re-examination of which ratios are most appropriate to identify oil samples. Importantly, if different ratios are proposed for standardized testing, they should be agreed upon by any agencies or other groups planning on comparing samples, so that use of existing libraries or databases can be used to help identify mystery samples (e.g., Dalhmann and Kienhuis et al. 2016). Analyses of ratios using other methods could also be considered, for example regression analysis incorporating confidence limits for each ratio (Daling et al. 2002) and cross-plots of one ratio to another (Wang et al. 2016).

Although our use of biomarkers ratios quantifies aspects of oil fingerprints, we do not recommend quantification of biomarker ratios alone as a means of declaring samples as matching or not matching. As our comparisons within Group B showed, there may be significant overlap in similarity of biomarker ratios for samples that differ in other ways. We strongly recommend the continued use and refinement of techniques such as this to quickly compare samples quantitatively to database of many samples, but detailed visual comparison of chromatograms and chemists’ interpretation of weathering and biodegradation patterns should be used to confirm matches.

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## Chapter 2

### Sources of Chronic Oiling in California

#### INTRODUCTION

While marine oil spills are recognized as a significant threat to wildlife and wildlife habitats in California, chronic oiling from natural seeps also affects a substantial number of birds and other wildlife (Henkel et al. 2014, OSPR unpubl. data). Understanding spatial and temporal trends in chronic oiling of wildlife or tar ball events helps OSPR and other oil spill responders assess the likelihood that a mystery oiling event is the result of natural seepage rather than an anthropogenic source. For example, knowing that tar balls from natural seeps are rarely found north of central California during summer months may lead to an immediate investigation of a tar ball event in northern California during summer, whereas a tar ball event in central California during winter may be considered normal, and not warrant an investigation.

Beached bird monitoring programs, including BeachCOMBERS (Santa Cruz County south to Los Angeles County) and Beach Watch (Sonoma County south to San Mateo County) that monitor both deposition of dead oiled wildlife and deposition of tar balls provide useful baseline information on trends in chronic oiling (Roletto et al. 2003, Nevins et al. 2009; Henkel et al. 2014). While information on general spatial and temporal trends in chronic oiling is useful, more detailed information on trends and magnitude of oiling from particular seeps can lead to more effective response decisions and more accurate information that response agencies can provide to the public. If a mystery oiling event is investigated and pinpointed to a particular seep as a likely source, this provides much greater confidence that the event was indeed from a natural seep, rather than assuming that any weathered Monterey Formation crude oil is likely from a natural seep.

Here, we present information on spatial and temporal trends of oiling from known and suspected natural seeps. Using methods described in Chapter 1, we identified several groups of samples believed to each be from common sources. The OSPR-PCL will continue to expand these groups as more samples are compared. Through identification of these groups (and potential future groups), mystery spill source identification can be greatly expedited through comparison of mystery samples to representative samples from these known groups (and if a match is found, obviating the need for biomarker ratio analysis described in Chapter 1). However, if samples are compared to known groups and no matches are found, we recommend use of biomarker ratios to further investigate potential sources from a greater diversity of potential sources.

#### METHODS

Using oil samples submitted to the OSPR-PCL from oiled wildlife (primarily birds and sea otters [*Enhydra lutris*]) and tar balls, we used standard oil fingerprinting methods (GC/MS and visual overlay of chromatograms) and biomarker ratio analysis as described in Chapter 1 to identify matching samples. All samples were consistent with weathered Monterey Formation crude with degradation of rank 5 (Peters and Moldowan 1993), with a few exceptions as noted in the results. Many mystery samples have not been matched to any other sample (and are not included in this summary), but many others have been matched to one or more other samples. Here, we present information on groups of matching samples in which at least one sample had biomarker ratios measured and included in our OSPR-PCL ratio library (to allow for widespread comparison to other samples), and samples were present from at least two different events (or one event in addition to a potential source). Thus, additional groups have been identified with mutually-matching samples, but are not included here if biomarker ratios have not yet

been calculated for a sample from the group. In addition, we did not include samples from known anthropogenic spill events.

Using comparison (MRPD) of biomarker ratios as described in Chapter 1, we also compared representative samples from each group to a library of USGS ratios from sources in California (675 samples; Lorenson et al. 2009). This provided information on possible similar samples warranting further investigation, but in most cases splits of USGS samples were not re-analyzed to confirm matches using visual comparison of chromatograms. Information on USGS samples with similar biomarker ratios is provided as preliminary background information.

## RESULTS

We have currently identified 10 groups of samples presumed to be from different natural seeps in California. Groups were arbitrarily assigned the identifying letters A through J. Each group is discussed in detail on subsequent pages, and additional information on biomarker ratios and approximate relatedness of groups (based on a dendrogram) is provided in Chapter 1. Four of the groups have identified sources of particular seeps between Monterey County and Santa Barbara County. More information on previously undescribed seeps is provided in Appendix B.

Many samples analyzed were collected during “events” considered to be above baseline conditions and warranting investigation. In many cases, samples from one event were found to belong to multiple groups, and/or identified groups as well as other non-matching samples. One event with only one group represented was a “Central Coast Tar Ball Event” in February 2002, in which all samples identified to group were found to be in Group B (with a likely source near Point Conception; see Group B details below). On five occasions, samples from multiple groups were found on the same day at the same location (Table 2-1). These data appear to indicate that sources for Groups A and D (currently unknown) are likely to be in a similar geographic area, and possibly near the source of Group C (known to be off northern San Luis Obispo County; see Group C below).

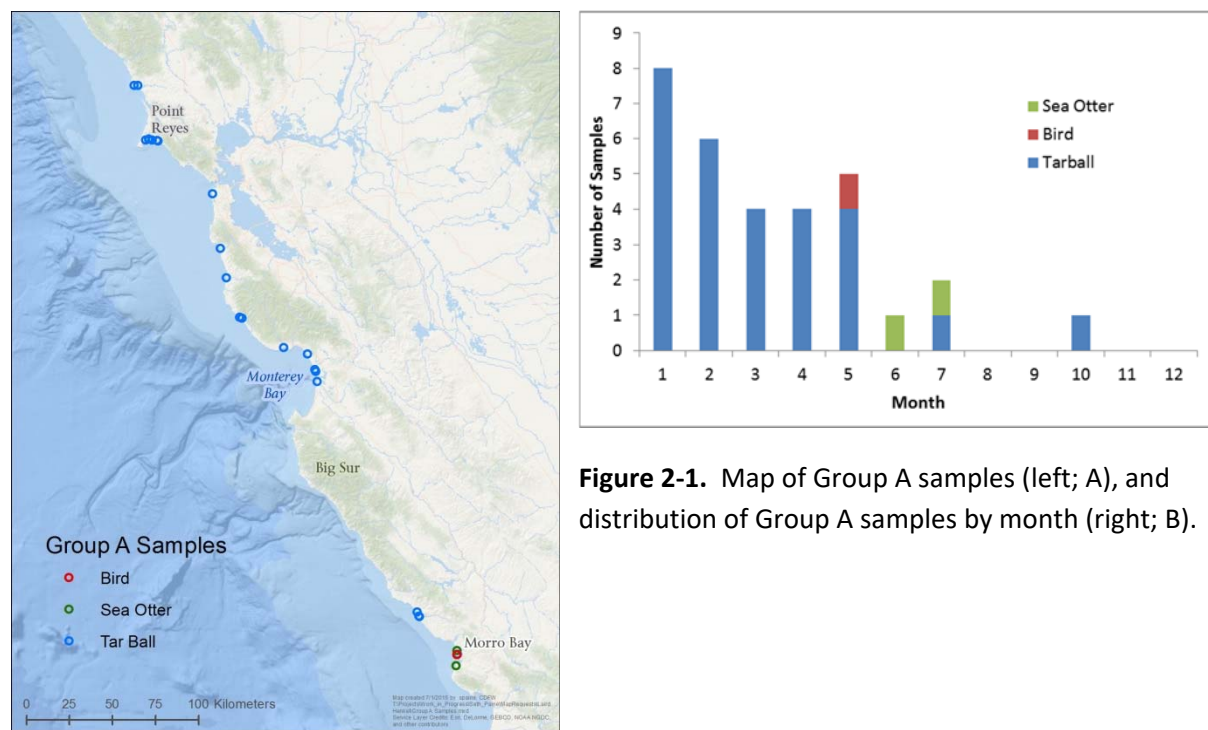
**Table 2-1.** Oiling events in which samples were collected on the same day at the same location, and subsequently identified to more than one group.

Location	County	Date	Groups	Sample Type
Limantour Beach	Marin	2/25/2007	A, C	Tar ball
Drakes Beach	Marin	4/14/2007	A, D	Tar ball
Cove Beach	San Mateo	1/28/2008	A, D	Tar ball
Morro Strand	San Luis Obispo	3/7/2011	A, C	Tar ball
Morro Strand	San Luis Obispo	4/26/2011	A, C	Tar ball
Morro Spit	San Luis Obispo	7/2/2012	A, C	Sea Otter

## Group A

Source: Unknown (possibly Big Sur area)

Group A included 31 mutually matching samples collected between 2007 and 2013. Most samples were tar balls, although samples were also collected from one bird and two sea otters (all 3 animals were from the Morro Bay area). Most samples were collected between January and May, although the two sea otters were collected in June (2009) and July (2012). Sample collection location ranged from Sonoma County in the north (the northernmost sample from any group) to San Luis Obispo County in the south. Comparison of biomarker ratios to USGS samples resulted in several samples with MRPD values  $<0.10$ , all of which were tar ball samples primarily from central California (except for three samples from San Miguel Island). These USGS samples were in USGS Family 33. The source for this group is unknown although a tar whip collected at a seep off Big Sur matched this group. However, other samples collected at this seep (which we refer to as the Lopez Seep) did not match Group A, and are included in Group F (see information on Group F for more information on the Lopez Seep). It is possible that the tar whip collected at the Lopez Seep came from another (un-sampled) seep nearby. The geographic and temporal distribution of samples in this group seems to be consistent with a source in the central coast area. Additional at-sea sampling of oil from seeps off Big Sur may help identify the source for Group A.

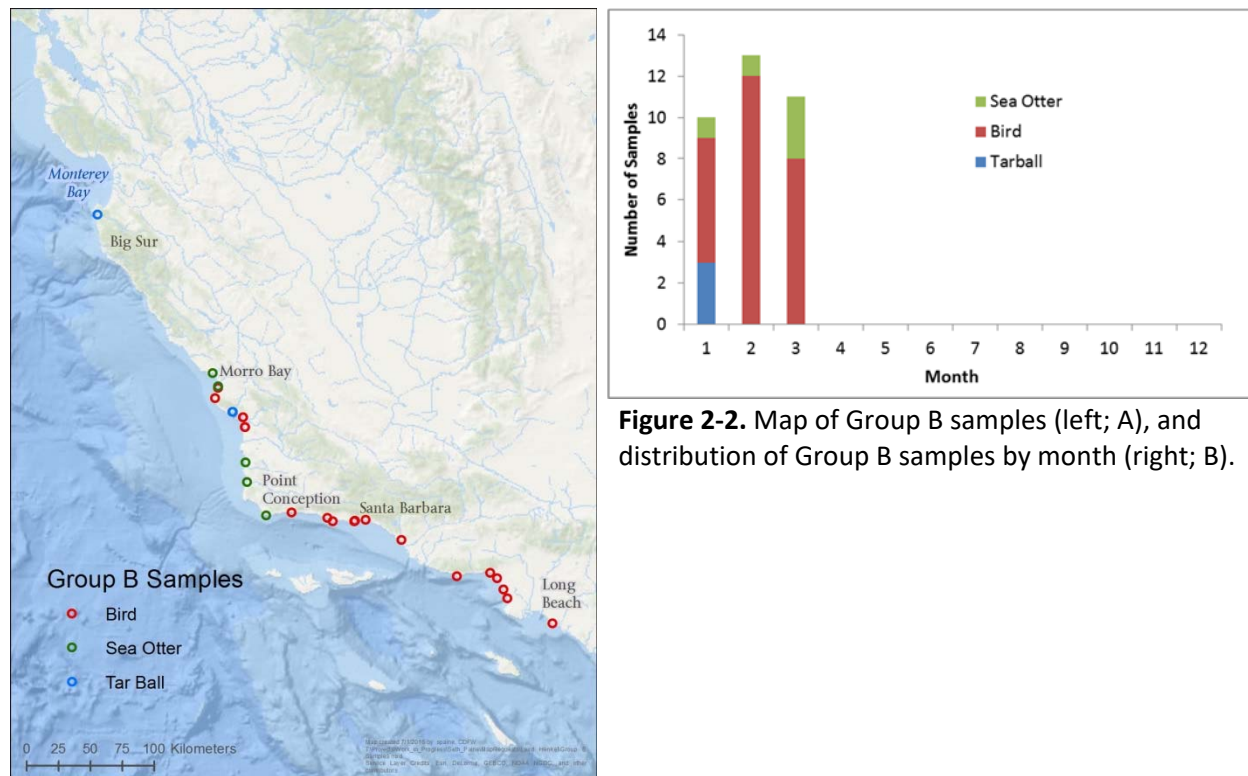


**Figure 2-1.** Map of Group A samples (left; A), and distribution of Group A samples by month (right; B).

## Group B

Source: Not confirmed, but likely seeps off Point Conception

Group B included 34 mutually matching samples collected between 2002 and 2013. The majority of samples ( $n = 26$ ) were from birds, and all samples were collected during January, February, or March. Samples were collected from Monterey County in the north to Orange County in the south, with most samples coming from San Luis Obispo and Santa Barbara counties. Comparison of biomarker ratios to USGS samples resulted in many ( $>70$ ) samples with MRPD values  $<0.10$ , although as discussed in Chapter 1, this group has significant overlap in ratio values with other non-matching samples (possibly from related seeps). The USGS samples with similar ratios included tar balls (primarily from Santa Barbara County) and several seep samples off Point Conception, and were in USGS Family 22. We compared Group B samples to several known seep samples, and while some seep samples were similar to Group B samples, none were exact matches. However, based on the similarity between Group B samples and Point Conception seep samples, and the geographic distribution of Group B samples, it seems reasonable to hypothesize that the source for Group B is a seep in the Point Conception area that we have not yet directly compared to Group B. Point Conception is an area of significant seep activity, with many different individual seeps in the area. Further investigation of Group B and related samples is warranted.

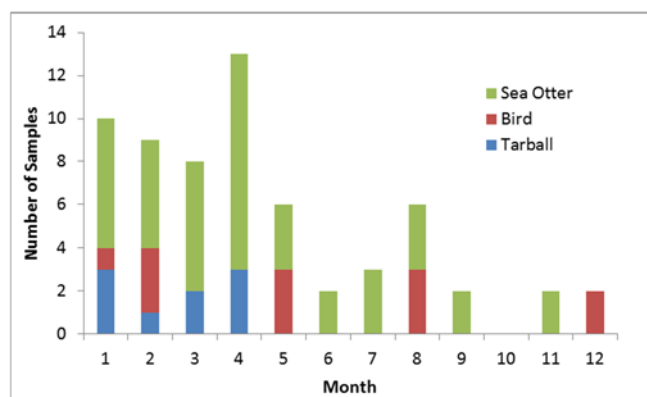
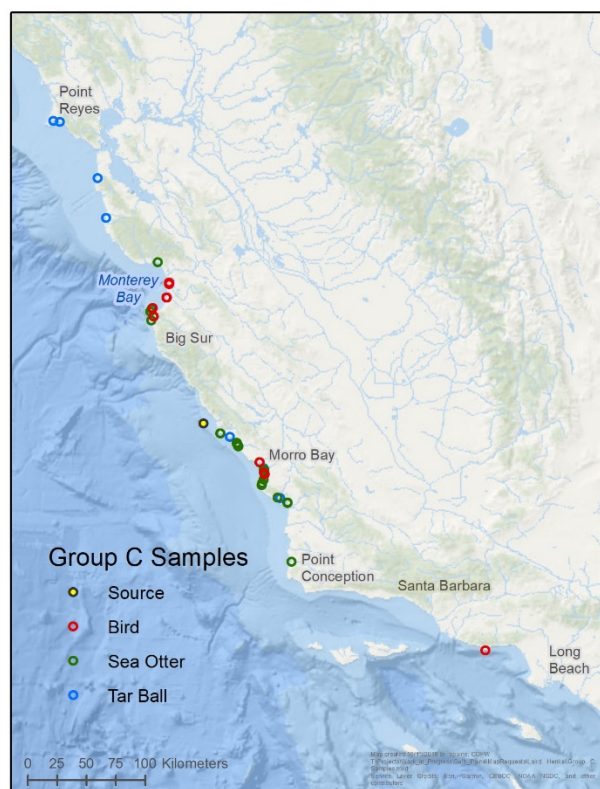


**Figure 2-2.** Map of Group B samples (left; A), and distribution of Group B samples by month (right; B).

### Group C

Source: Piedras Blancas Seep (35.74 N, 121.44W)

Group C (the largest group) included 63 mutually matching samples collected between 1997 and 2012. The majority of samples ( $n = 42$ ) were from sea otters. Samples were collected during all months except October, but with a peak from January through April (during which all tar ball samples were collected). Samples were collected from Marin County in the north to Los Angeles County in the south, with the majority of samples coming from San Luis Obispo County. Comparison of biomarker ratios to USGS samples resulted in only one comparison with a MRPD value  $<0.10$ , a tar ball sample from San Mateo County in USGS Family 22. Another USGS sample had an MRPD value of approximately 0.15 and was investigated as possible source sample. A split of this sample was analyzed and found to match Group C, but the source (a historic shoreline seep at Pirate's Cove in Avila Beach, San Luis Obispo County) was no longer an active seep. Subsequently, USGS (T. Lorenson, pers. comm.) reported a marine seep farther north off Point Piedras Blancas in northern San Luis Obispo County (which we refer to as the Piedras Blancas Seep), and OSPR personnel followed up and sampled oil at the surface in July, 2012; oil samples from this seep were found to match Group C. The predominance of oiled sea otter samples from San Luis Obispo County in the group is consistent with a local source.

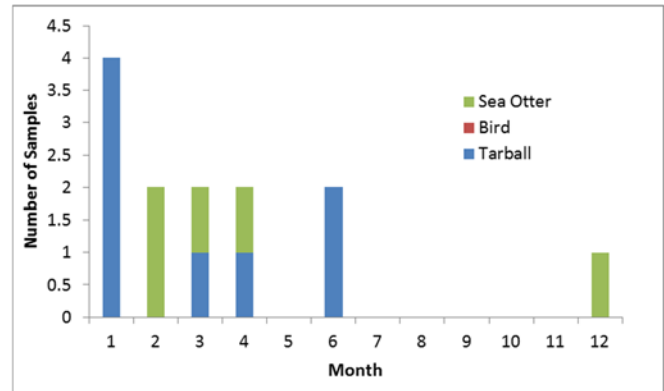
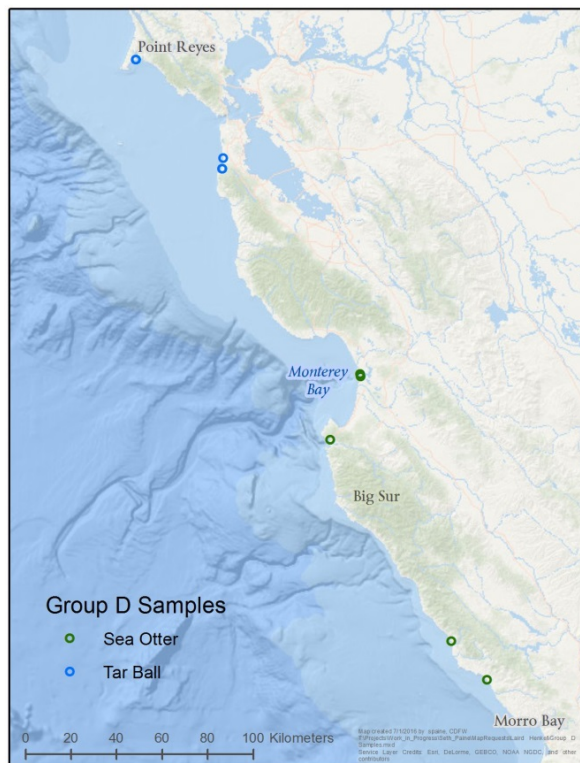


**Figure 2-3.** Map of Group C samples (left; A), and distribution of Group C samples by month (right; B).

## Group D

Source: Unknown

Group D included 13 mutually matching samples collected between 2003 and 2011, all of which were tar balls or from sea otters. Most samples were collected between January and April. Samples were collected from Marin County in the north to San Luis Obispo County in the south. Comparison of biomarker ratios to USGS samples resulted in only one comparison with a MRPD value  $<0.10$ , a tar ball sample from Vandenberg Air Force Base (northern Santa Barbara County) in USGS Family 212. The source for Group D is unknown, but based on the geographic distribution of samples, may be in the central coast area.

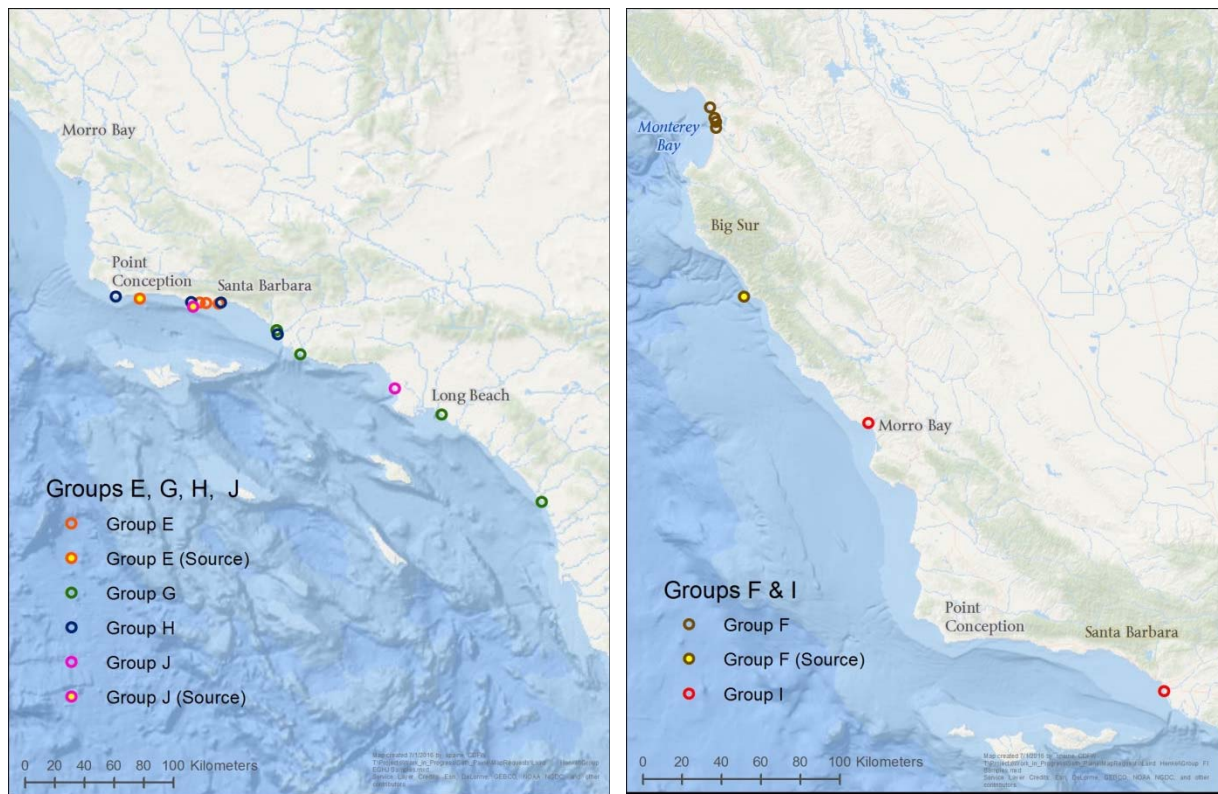


**Figure 2-4.** Map of Group D samples (left; A), and distribution of Group D samples by month (right; B).

## Group E

Source: Gaviota Seep 02-67 (34.44 N, 120.27 W)

Group E included 6 mutually matching samples collected between 2003 and 2011, two of which were seep samples and the other four were from oiled birds. The birds were all collected in Santa Barbara County during March (2009 and 2010; Fig. 2-5a). Bird samples matched samples collected from one or more seeps off Gaviota, in Santa Barbara County. These samples were collected by USGS, but splits were provided to the OSPR-PCL for analysis. When comparing MRPD of Group E samples to the USGS database, we found only two samples with MRPD <0.10, which were these two seep samples (seep 02-67 and seep 04-33, also referred to as Sacate Seeps), in USGS Family 212.



**Figure 2-5.** Maps of samples in groups E, G, H, and J (left, A), and groups F and I (right, B).

**Group F**

Source: Lopez Seep (36.02 N, 121.62 W)

Group F included 7 mutually matching samples collected between 2006 and 2013, three of which were seep samples, two were from oiled birds, and two from otters. All four animals were collected within Monterey Bay (Fig. 2-5b); the two otters both during February, and the birds during August and December. One of the otters was collected alive, and became well known as “Olive” after cleaning and rehabilitation. All samples (wildlife samples and seep samples) were highly degraded (rank  $\geq 6$ ). Seep samples were collected by OSPR from the surface of the water in November 2010. These samples were weathered sheen and mousse, whereas a fresher tar whip collected at the same time and location was found to be in Group A. However, based on the extensive sheen present, it is assumed that Group F samples were associated with a seep at or near that location. Comparison of ratios to the USGS samples resulted in one sample with MRPD  $<0.10$ , which was identified as a seep sample from “Point Sur”, with an inland location approximately between the Lopez Seep and Pfeiffer Seep farther north off Big Sur. The USGS location is presumably approximate. This USGS sample does not have an identified USGS Family. Further sampling and investigation of seeps off Big Sur may shed additional light on this group.

**Group G**

Source: Unknown

Group G included 6 mutually matching samples collected between 2008 and 2012, two of which were tar balls, and four of which from oiled birds. Four samples were collected in Ventura County, one in Orange County, and one in San Diego County (the southernmost sample identified to a group; Fig. 2-5a). All samples were collected during February and March, except one tar ball sample (from San Diego County) collected in July. No USGS samples had MRPD values  $<0.10$  and the source for this group is unknown (based on geographic range of samples, the source is likely south of Point Conception).

**Group H**

Source: Unknown, but possibly seep west of Coal Oil Point.

Group H included 4 mutually matching samples collected in 2011 and 2014, two of which were from oiled birds, one from an oiled otter, and one was a tar ball. The three wildlife samples were collected in Santa Barbara County, and the tar ball was collected in Ventura County (Fig. 2-5a). Two samples were collected in March, and two (the bird samples) in July. Comparison of Group H samples to USGS ratios resulted in 15 USGS samples with MRPD values  $<0.10$ , from USGS Family 14. These samples were primarily tar balls from the general vicinity of Coal Oil Point in Santa Barbara County, and also included one USGS seep sample from west of Coal Oil Point (sample 03-194, 34.45 N, 120.14 W). This USGS seep sample has not yet been directly compared to OSPR-PCL group H samples, but this comparison could confirm the source of this group.

**Group I**

Source: Unknown

Group I included only two matching samples, both from oiled sea otters: one from August 2003 in San Simeon, and one from October 2006 in Cayucos (both in San Luis Obispo County; Fig. 2-5b). Comparison of sample ratios to USGS samples did not result in any MRPD values  $<0.10$ , and the source for this group is unknown. Sample chromatograms were reported to appear “similar” to Group C, but ratios are quite different between Groups C and I.

**Group J**

Source: Trilogy Seep, near Coal Oil Point (34.39 N, 119.88 W)

Group J included only two matching samples, on oiled bird sample (from Manhattan Beach in Los Angeles County, in March 2011), and a seep sample (Fig. 2-5a). The seep sample (provided by USGS) is from the Trilogy Seep, off Coal Oil Point, near Platform Holly. Comparison of Group J samples to USGS sample ratios resulted in 18 samples with MRPD  $<0.10$ , all except for one in USGS Family 211. These samples were primarily tar ball samples from the vicinity of Coal Oil Point, but also include the USGS sample for the Trilogy Seep and a sample collected at Platform Holly.

## DISCUSSION

It is typical for mystery tar ball events or wildlife oiling events to be reported in California during winter months (Henkel et al. 2014, OSPR unpubl. data). It has been assumed that winter weather patterns resulting in northward winds and current result in tar balls from significant seeps off southern and northern California being deposited as far north as the San Francisco Bay area (Hostettler et al. 2006, Peters et al. 2008). In addition, extreme winter weather may affect the discharge from natural seeps (Henkel et al. 2014). Most of the samples in our identified seep groups were also collected during winter months, although samples collected at various other times of the year likely indicate more localized effects of seeps.

Ours is the first study we are aware of to link tar ball or oiled wildlife samples to specific seeps off central California. We identified a large numbers of samples linked to the Piedras Blancas seep off northern San Luis Obispo County, and a lesser number of samples linked to the Lopez Seep off Big Sur, and the Gaviota and Trilogy seeps in the Santa Barbara Channel. In addition, seeps off Point Conception are likely the source for another large group of samples. Given the productivity of seeps in the Santa Barbara Channel, it is somewhat surprising that we identified many more samples associated with the Piedras Blancas seep farther north. However, we want to stress that our sampling of tar ball and oiled wildlife samples was not random or representative, but was biased by various projects that requested sample analysis. In particular, a study of chronic oiling of wildlife in the Monterey Bay Area (Nevins et al. 2009) and other sampling requested by the BeachCOMBERS and Beach Watch programs resulted in a bias of more samples from Point Reyes south through Monterey County, and many fewer samples from southern California. However, it should also be noted that north of Sonoma County, reports of tar balls or miscellaneous oiled wildlife are rare, so there were very few samples available to be tested from northern California. Additional sampling of tar balls and oiled wildlife from southern California may result in more matches to seeps in the Santa Barbara Channel or farther south. However, given the large number of samples associated with the Piedras Blancas seep, monitoring of this seep (e.g., with regular aerial surveys) might be warranted to better understand the productivity of this seep.

Within each group, tar ball samples tended to occur farther north along the coast, sea otter samples tended to be more localized (presumably closer to the source), and bird samples somewhat variable in geographic distribution (as noted above, these locations should not necessarily be considered to be representative of the geographic extent for that group). There are differences in how each of these matrices are related to the origin of the oil. Tar balls move only with surface currents (driven largely by wind), and thus could potentially be used for hindcasting the source of the oil based on recent weather patterns. However, tar balls can also persist on shore for long periods of time, and can become covered (and subsequently uncovered) by sand on beaches, introducing uncertainty in the timing of transport of the oil from the origin. Depending on how heavily oiled they are, birds and otters are not likely to persist for a long period of time while oiled (thus the timing of oiling can be more narrowly estimated), but they can potentially move long distances after oiling, introducing uncertainty in geographic proximity of the oil source (and wildlife can also be oiled by tar balls on the water or on shore that have already moved some distance from the source). Thus, estimating a likely source location based on location of samples may be prone to substantial uncertainty, although having a large dataset of samples provides a better indication of where a likely source might be located.

Groups without an identified source are presumed to be from natural seeps, given that they are consistent with weathered Monterey Formation crude and there are no known other chronic sources of

Monterey Formation crude. However, there is a slight possibility that there could be an unknown anthropogenic source for one or more of these groups, such as an unknown shipwreck. Using methods described in Chapter 1, we have compared samples from these groups to several known major seeps, including the Pfeiffer Seep off Big Sur (36.20 N, 121.87 W), onshore seeps at Carpenteria Bluffs, and the El Segundo seeps in Santa Monica Bay. But other seeps, either in the marine environment or inland (with the capability of flushing to the ocean during winter rains) may yet be unsampled.

Additional investigation of seeps, including documentation of little-known or unknown seeps, and sampling of oil from known seeps, may help identify additional sources for mystery samples. In addition, continued research on chemistry of mystery oil samples could potentially link mystery samples to known seeps with different chemical fingerprints, if extensive weathering may account for differences between mystery samples and source samples.

By identifying these groups of mutually-matching samples, we now have the potential to more accurately identify new mystery samples. We recommend initial comparison of mystery samples in California against representative samples in these (or future) known groups. If matches are identified, more detailed searches (e.g., as described in Chapter 1) would not be needed. However, if no matches are found to known groups, calculation of biomarker ratios and comparisons using methods described in Chapter 1 is recommended. We also recommend continuing analysis of mystery samples to expand known groups of matching samples, identify new groups, and identify additional seep sources for mystery samples.

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**Appendix A.** Mean biomarker ratios for samples in 10 groups of matching samples (SD in parentheses), and number of samples per group.

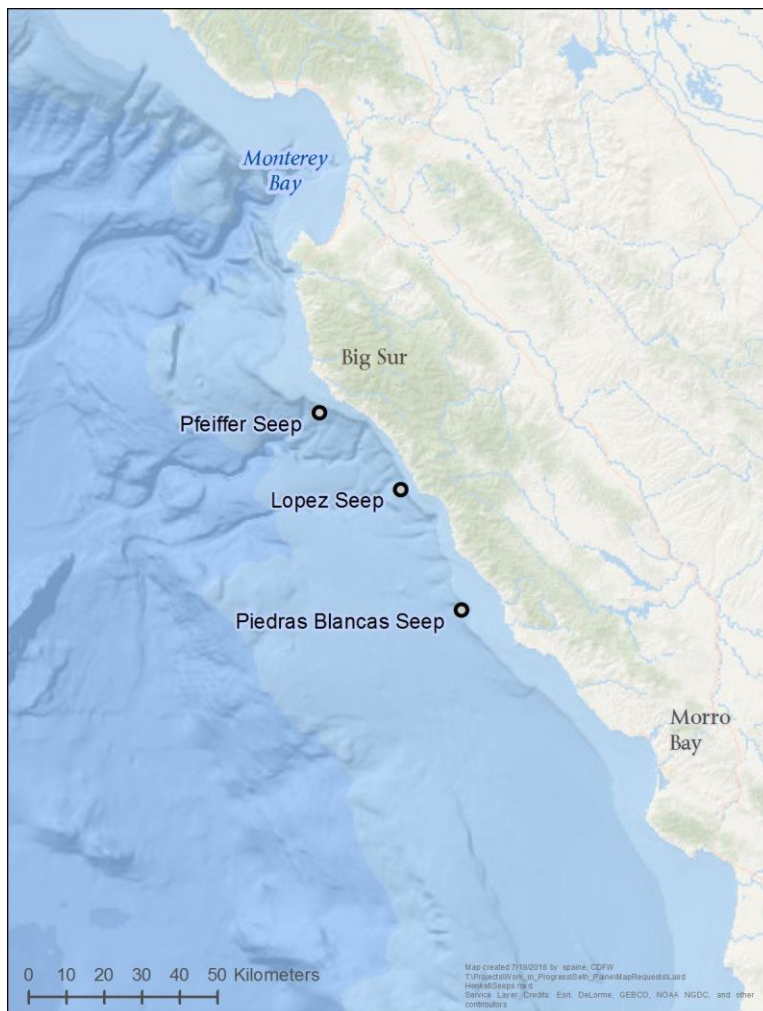
Note that ratio 1 was measured only in a subsample from each group, if at all.

	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H	Group I	Group J
<b>n samples with ratios</b>	11	13	26	2	5	5	3	2	2	2
<b>1) del 13C</b>	-22.95 (0.08)	-23.30 (0.14)	-22.93 (0.34)	n/a	-22.5 (0)	n/a	n/a	n/a	n/a	-22.8 (0)
<b>2) Ts/T<sub>m</sub></b>	0.19 (0.02)	0.26 (0.05)	0.24 (0.02)	0.35 (0.01)	0.42 (0.02)	0.35 (0.02)	0.28 (0.02)	0.44 (0.01)	0.27 (0)	0.37 (0.04)
<b>3) C<sub>26</sub>/Tet (triplet)</b>	3.43 (0.14)	3.28 (0.20)	3.91 (0.25)	5.09 (0.12)	4.27 (0.24)	4.74 (0.13)	5.21 (0.11)	5.65 (0.21)	4.40 (0.28)	5.15 (0.07)
<b>4) C<sub>28</sub>/C<sub>29</sub></b>	1.03 (0.05)	1.08 (0.03)	1.16 (0.06)	1.18 (0.01)	n/a	n/a	1.31 (0.08)	1.17 (0)	1.14 (0.07)	1.2 (0)
<b>5) PAH-RI</b>	110.03 (10.15)	46.72 (6.74)	66.59 (14.79)	36.05 (4.31)	25.00 (1.58)	22.78 (1.83)	120.00 (13.00)	6.19 (0.27)	40.50 (2.12)	9.35 (0.92)
<b>6) C2D/C2P</b>	3.37 (0.22)	1.90 (0.09)	2.48 (0.22)	1.64 (0.03)	0.52 (0.08)	0.37 (0.08)	3.70 (0.15)	0.84 (0.03)	1.40 (0.13)	1.09 (0.09)
<b>7) C3D/C3P</b>	3.95 (0.34)	2.31 (0.22)	2.51 (0.18)	1.57 (0.08)	1.07 (0.05)	1.06 (0.06)	3.55 (0.35)	1.02 (0.04)	1.57 (0.01)	1.43 (0.16)
<b>8) C<sub>28</sub>/C<sub>29</sub> TT</b>	0.64 (0.04)	0.82 (0.04)	0.70 (0.05)	0.75 (0.02)	0.92 (0.02)	0.75 (0.02)	0.72 (<0.01)	0.81 (0.04)	0.71 (0.02)	0.78 (0.01)
<b>9) C<sub>20</sub>/C<sub>23</sub> TT</b>	0.09 (0.01)	0.19 (0.03)	0.16 (0.02)	0.22 (0)	0.25 (0.01)	0.29 (0.02)	0.15 (0.02)	0.28 (0.01)	0.21 (0.01)	0.24 (0.03)
<b>10) C<sub>22</sub>/C<sub>21</sub> TT</b>	0.96 (0.07)	0.47 (0.03)	0.62 (0.05)	0.40 (0)	0.28 (0.01)	0.28 (0.01)	0.66 (0.02)	0.32 (0.01)	0.45 (0.01)	0.33 (0.01)
<b>11) C<sub>24</sub>/C<sub>23</sub> TT</b>	0.26 (0.02)	0.43 (0.01)	0.38 (0.02)	0.52 (0.01)	0.58 (0.01)	0.67 (0.01)	0.38 (0.01)	0.65 (0.06)	0.47 (0.01)	0.58 (0.01)
<b>12) C<sub>26</sub>/C<sub>25</sub> TT</b>	0.73 (0.02)	0.76 (0.05)	0.73 (0.04)	0.75 (0.01)	0.76 (0.06)	0.76 (0.01)	0.68 (0.03)	0.86 (0.01)	0.75 (0.02)	0.83 (0.01)
<b>13) C<sub>31</sub>S/H</b>	0.61 (0.02)	0.52 (0.02)	0.59 (0.02)	0.52 (0.01)	0.44 (0.09)	0.99 (0.06)	0.59 (0.02)	0.48 (0.03)	0.65 (0.05)	0.51 (0.01)
<b>14) C<sub>29</sub>H/H</b>	0.82 (0.04)	0.71 (0.02)	0.81 (0.04)	0.67 (0.01)	0.67 (0.10)	1.87 (0.07)	0.69 (0.04)	0.66 (0.02)	0.91 (0.04)	0.67 (0)
<b>15) C<sub>35</sub>/C<sub>34</sub> S</b>	2.06 (0.06)	1.85 (0.12)	1.82 (0.17)	1.36 (0.07)	1.21 (0.05)	1.63 (0.06)	1.38 (0.01)	1.38 (0.08)	1.53 (0.48)	1.62 (0.08)
<b>16) BNH/H</b>	0.93 (0.06)	1.26 (0.06)	0.68 (0.03)	0.65 (0.01)	0.37 (0.07)	1.02 (0.18)	0.39 (0.02)	0.58 (0)	0.66 (0)	0.77 (0.02)
<b>17) OI/H</b>	0.03 (<0.01)	0.04 (<0.01)	0.04 (<0.01)	0.05 (0.01)	0.05 (0.01)	0.21 (0.01)	0.05 (0.01)	0.09 (0.01)	0.07 (0.01)	0.08 (0.01)
<b>18) G/H</b>	0.12 (0.02)	0.12 (0.01)	0.10 (0.01)	0.07 (0.02)	0.07 (0.02)	0.32 (0.03)	0.04 (0.02)	0.08 (0)	0.13 (0)	0.09 (0.01)
<b>19) C<sub>29</sub>Ts/C<sub>29</sub> H</b>	0.11 (0.01)	0.12 (0.02)	0.19 (0.02)	0.24 (0.02)	0.16 (0.02)	0.12 (0.01)	0.15 (0.01)	0.20 (0.02)	0.15 (0.02)	0.16 (0.02)

## Appendix B

### Information on Seeps off Central California

While many petroleum seeps in California have been previously described (Wilkinson 1971, Lorenson et al. 2009), most available information is focused on seeps in southern California (south of Point Conception). Here, we provide basic information on three substantial seeps off central California (between Monterey Bay and Point Conception) that have not been previously described in detail. We provide names for these seeps based on the closest significant landmark on shore. All locations (Fig. B-1) are approximate, and based on observations of slicks on the surface of the water; actual seep locations are presumed to be very close to the consistently observed slick locations. Additional aerial surveys off the central coast would be helpful to determine if there are additional undocumented seeps in this area.



**Figure 1.** Map of petroleum seeps off the Big Sur coastline, central California.

### Pfeiffer Seep

Location: 36.204 N, 121.872 W

The Pfeiffer Seep is approximately 6.5 km southwest of Pfeiffer Point/Pfeiffer Beach (or 6.1 km southwest of Cooper Point) in Big Sur, Monterey County. This location is offshore of the continental shelf at a depth of 440 m, near the top of the submarine Sur Canyon. Slicks at the surface at this location have been noted for many years, including by biologists (CDFW and NOAA) conducting aerial surveys for marine birds and mammals. Oil from the surface was sampled by CDFW-OSPR (L. Henkel and J. Ames) in November 2010 (OSPR PCL submittal 053-10). Oil observed at that time was primarily silvery sheen and rainbow sheen, with some small low-density tar balls/mousse (Fig. B-2). Biomarker ratios were calculated for some of these samples (see Chapter 1), but no matches were found to mystery spill samples (and no MRPD values were <0.10 in comparison to USGS samples). Samples were consistent with weathered Monterey Formation crude oil.

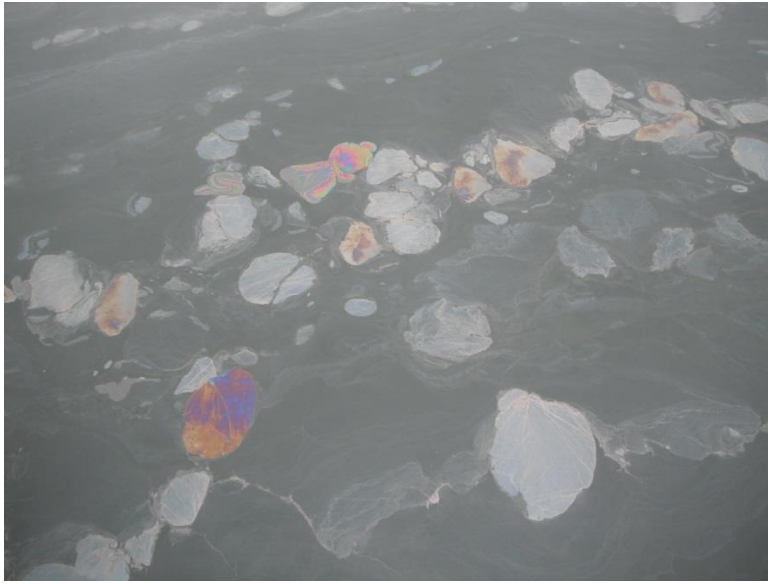


**Figure B-2.** Sheen observed at the Pfeiffer Seep in November 2010.

### Lopez Seep

Location: 36.025 N, 121.627 W

The Lopez Seep is approximately 5.4 km west of Lopez Point in southern Big Sur (near Lucia), Monterey County. This location is near the head of the submarine Lucia Canyon, at a depth of approximately 390 m. Like the Pfeiffer Seep, slicks have been observed at the surface at this location for many years. Oil was sampled at the surface at this location by CDFW-OSPR (L. Henkel and J. Ames) in November 2010 (OSPR PCL submittal 053-10). Oil observed at that time was primarily silvery sheen and rainbow sheen, weathered into discrete pads (Fig. B-2). Some tar balls were also seen and collected, and as noted in Chapter 2, a tar whip was also collected. The tar whip was approximately 30 cm long and was not sheening in the water. Most samples from this location were identified as Group F, but the tar whip was identified as Group A (see Chapter 2). Group F samples were all highly degraded.



**Figure B-3.** Sheen observed at the Lopez Seep in November 2010.

Piedras Blancas Seep

Location: 35.740 N, 121.443 W

The Piedras Blancas Seep is approximately 16.5 km northwest of Point Piedras Blancas, San Luis Obispo County. This location is near the edge of the continental shelf at a depth of approximately 250 m. This seep was first reported by Tom Lorenson of USGS, who observed oil at this location during a research cruise in 2011. A wildlife aerial survey team from U.C. Santa Cruz (P. Capiolo and J. Davis) under contract to CDFW-OSPR flew over the location in January 2012 and confirmed a sheen extending approximately 5 km (from 35.751 N, 121.499 W to 35.739 N, 121.434 W). Oil at the seep was sampled by CDFW (M. Crossland and B. Meyer) on July 5, 2012 (PCL submittal 023-12). Oil observed at the surface included weathered mousse and silvery sheen, with some tar balls (Fig. B-4). Oil samples from this location were identified as consistent with Group C, and thus matched a large number of historic wildlife and tar ball samples (see Chapter 2).

Additional samples (PCL submittal 024-12) were collected on July 6, 2012 approximately 13 km northwest of the presumed seep location, at 35.837 N, 121.557 W by CDFW (M. Crossland and K. Hare). These samples were similar to the Piedras Blancas Seep samples, but not identical. It is not known whether these samples are related to another unidentified seep. Additional field work in this area would be useful.



**Figure B-4.** Oil at the Piedras Blancas Seep as seen from an aerial survey in July 2012 (left) and at the surface in July 2012 (right).

#### Literature Cited

- Lorenson, T.D., F.D. Hostettler, R.J. Rosenbauer, K.E. Peters, J.A. Dougherty, K.A. Kvenvolden, C.E., Gutmacher, F.L. Wong, and W.R. Normark. 2009. Natural offshore oil seepage and related tarball accumulation on the California coastline – Santa Barbara Channel and the southern Santa Maria Basin; source identification and inventory. USGS Open-File Report 2009-1225, 116 p.
- Wilkinson, E.R. 1971. California Offshore Oil and Gas Seeps. California Division of Oil and Gas, Sacramento, CA. 11 p.