

SUBTIDAL STUDY NUMBER 8

Study Title: Mussel Tissue and Sediment Hydrocarbon Data
Synthesis

Lead Agency: NOAA

INTRODUCTION

The goals of project Subtidal Study #8 are (1) to evaluate the internal consistency of sediment and mussel tissue hydrocarbon data, and (2) to objectively identify the presence of Exxon Valdez petroleum hydrocarbons in these samples. The first goal is necessary to minimize the effects of errors in sample collection, documentation, and analysis that are inevitable with a large number of samples collected for several different projects, and that are chemically analyzed using a complex procedure. The more of these errors that can be objectively identified, the greater will be the power of subsequent statistical tests. The second goal is necessary to provide an objective evaluation of the persistence and geographic extent of petroleum hydrocarbon contamination of these samples. This evaluation will provide a common reference for the participating projects, will minimize duplication of expensive analytical effort, and will provide the most comprehensive view of contamination possible with these data.

Inconsistent hydrocarbon data are identified using computer-based statistical methods to identify groups of samples that are clearly biased systematically, or that have been clearly exposed to extraneous contamination unrelated to the oil spill. Computer-based methods are necessary because thousands of sediment and mussel tissue samples have been analyzed for 63 independent analytes each. However, these methods are also very powerful just because of the large number of samples involved. Once identified, these samples may be excluded from subsequent statistical tests, which may greatly enhance the power of these tests.

The presence of Exxon Valdez petroleum hydrocarbons in analyzed samples is objectively determined using a computer-based pattern recognition method called principal component analysis (PCA). This method provides an objective and consistent way of determining the presence and relative amount of oil in samples, and works particularly well with NRDA oil spill samples because the oil spill is by far the major source of hydrocarbons found in Prince William Sound after March 1989.

Once Exxon Valdez petroleum hydrocarbons have been objectively and reliably identified in samples, the results can be mapped to yield a picture of the overall extent of contamination. By including

results from all the projects that collected sediment or mussel tissue samples, the most complete and detailed maps of oil contamination will be prepared, providing a common reference for the participating projects. This, in turn, will provide scientific investigators and the general public with the most accurate indication of the persistence and geographic extent of oil-contaminated sediments and mussels.

OBJECTIVES

- A. Develop appropriate criteria for the final acceptance of hydrocarbon data prior to further analysis.
- B. Calculate a hydrocarbon summary index that expresses quantitative amount and qualitative character of all hydrocarbons detected in sediment and mussel tissue samples.
- C. Provide PIs with evaluated sediment and mussel tissue hydrocarbon summaries in the form of tables, charts, graphs and maps.
- D. Prepare a comprehensive interpretation of sediment and mussel tissue hydrocarbon data identifying patterns of contamination across all the NRDA projects that generated these samples.

METHODS

- A.Sampling methods: No samples will be collected by this project.
- B.Standard operating procedure requirements: See Data Analysis, below.
- C.Quality assurance and control plans: See Data Analysis, below.
- D.Information required from other investigators: Hydrocarbon analysis data and associated sampling data from the Technical Services #1 database.
- E.Safety requirements: N/A
- F.Animal health and welfare: N/A

DATA ANALYSIS

Evaluation of Hydrocarbon Data Consistency

Hydrocarbon analytical data will be examined for conformance with two expectations. First, hydrocarbon concentrations in replicate samples are expected to be more or less similar. Second, samples collected from a priori control sites are not expected to contain hydrocarbons characteristic of crude oil.

A. Replicate Sample Similarity

The procedure described below includes two basic parts; identification of "deviant" samples, followed by an examination of the way these deviant samples are distributed among batches of samples analyzed. Samples may be deviant for many very legitimate reasons. However the samples identified as deviant should not be contained within a very few batches of samples analyzed. If they are, then the batches comprising these deviant samples merit close examination.

Great dissimilarity in hydrocarbon concentrations among samples that are replicates may result from patchiness in the way hydrocarbons are distributed in the matrix sampled, or from systematic bias introduced during sampling, sample storage, or sample analysis. Dissimilarity arising from the way hydrocarbons are distributed in the matrix should be preserved, because one object of sampling is to describe this distribution, so attempts to eliminate such dissimilarity will introduce bias into the data. However, dissimilarity arising from systematic bias should be minimized, to realize the power of subsequent statistical tests.

The samples that contain deviant hydrocarbon concentrations, when compared with their respective replicate samples, should be nearly randomly distributed among all collected samples, if the deviance arises from the way hydrocarbons are distributed in the matrix sampled. Consequently, a very non-random distribution of such dissimilar samples may be taken as an indication of systematic bias. For example, if all the deviant samples identified were analyzed on the same day at the same laboratory, then introduction of systematic bias would be suspected. We will therefore determine the probability that such deviant samples have the distribution observed among sample batches, or catalogues, using the procedure following, and assuming a random distribution among catalogues. Highly unlikely associations of such samples will be removed from consideration until the distribution of the remaining deviant samples is plausible. In this way, systematic bias will be identified and reduced, without compromising the integrity of the remaining data.

1. Identification of Deviant Samples

To determine the probability of an observed distribution of deviant samples among catalogues on the assumption of an underlying random distribution, the deviant samples must first be identified. After these deviant samples are identified, the distribution of them among sample analysis catalogues can be compared with random distributions of the same number of deviant samples.

Each sample is analyzed for 63 different hydrocarbons. A sample will be considered deviant if the concentrations of more than 9 of these are simultaneously very different, when compared with respective concentrations in the remaining replicate samples.

The justification for this criterion, together with a quantitative definition of what is meant by "very different", is contained in the following procedure that will be used to identify deviant samples.

For each hydrocarbon, the logarithm of the squared range of the hydrocarbon for each set of replicate samples is plotted against the logarithm of the median for that set. (Sets of replicates that have zero range for the hydrocarbon considered are not included.) The log-log plot accounts for the expected increase in the variance of each hydrocarbon at higher concentration. A linear regression line is calculated for this plot, and the replicate sets associated with the highest 5% of positive deviations from this regression line are identified. (Only positively deviant replicates on the plot are identified because these have the largest ranges; the negatively deviant replicates are those that agree most closely for the hydrocarbon under consideration.) Within each set of replicates in the highest 5%, the sample with the deviant hydrocarbon concentration is identified, and that sample is given a score of 1. A tally is then made of the number of hydrocarbons having a score of 1 in each sample of each set of replicates. Thus, each replicated sample will contain some number, n , of hydrocarbon concentrations that are identified as deviant using the above procedure.

If the distribution of these deviant hydrocarbon concentrations were random within and among samples, then each hydrocarbon has a 5% probability of being deviant in each sample. The probability, P , that a sample will contain n deviant hydrocarbons simultaneously under these assumptions is:

$$P = \binom{k}{n} (0.05)^n (0.95)^{k-n}$$

where $k = 63$ is the number of hydrocarbons analyzed in the sample. According to equation 1, the probability that more than 9

hydrocarbons are simultaneously deviant within a sample is less than 0.2% ($k=63$, $n=10$). This means that the above procedure will misidentify less than 0.2% of the samples as deviant, if instances of deviation really are randomly distributed within and among samples. If deviant samples are identified, they are flagged, and the above process is reiterated using a log-log plot that does not include replicates of the flagged samples. The process is reiterated because exclusion of the flagged samples and their replicates changes the regression line of the log-log plot for each hydrocarbon, which may then reveal new deviant samples. Reiterations of the process continue until no additional deviant samples are revealed. Thus, the above provides an objective way of identifying deviant samples.

2. Identification of Suspect Catalogues

Samples may be deviant due to the way hydrocarbons are distributed in the matrix sampled, or due to systematic bias. To evaluate these alternatives, we examine the way deviant samples are distributed among sample catalogues, based on an approach that is analogous with eq. 1. Given j samples identified as deviant among a total of J samples initially considered, the probability P that a catalogue containing L samples of which m are deviant is:

$$\frac{\binom{L}{m} \binom{J-L}{j-m}}{\binom{J}{j}}$$

assuming the underlying distribution of deviant samples among catalogues is random. These probabilities are calculated for each catalogue, and the plausibility of the observed probabilities is evaluated using a chi-square test. An estimate of chi-square is calculated as:

$$\sum \frac{(P_{obs} - P_{exp})^2}{P_{exp}}$$

where h is the number of catalogues considered. If this estimate is higher than the critical value of chi-square at $\alpha = 0.05$ and $h-2$ degrees of freedom, then all the deviant samples associated in the least probable catalogue are flagged as systematically deviant. A new estimate of chi-square is calculated for the remaining catalogues, where both j and J are reduced by the m and L , respectively, of the excluded catalogue. The new estimate of chi-square is compared with the critical value, and the process is reiterated until the chi-square estimate is less than the critical value. The catalogues that contain samples flagged as systematically deviant are listed as type I suspect catalogues.

B. Evaluation of Hydrocarbons in Control Site Samples

A second list is obtained by reviewing the results of the hydrocarbon analysis for the control site samples. These samples were collected from locations picked a priori by the PI for each project, and are not expected to contain hydrocarbons characteristic of crude oil, on the basis of independent evidence (such as other chemical analyses, absence of hydrocarbon degrading bacteria, etc.). The PIs will be polled to identify the sample numbers of such control site samples, and then the number of these catalogue will be determined. Catalogues containing at least 5% of these control site samples will be identified, and the control site samples in these catalogues will be examined for evidence of extraneous hydrocarbon contamination. Extraneous hydrocarbon contamination will be considered present if more than 5 hydrocarbon analytes in the following hydrocarbon classes are present at greater than 5 times their respective method detection limits (MDL): fluorenes, dibenzothiophenes, phenanthrenes, chrysenes, and phytane. Catalogues containing at least 5% control site samples, of which one or more control site samples contain extraneous hydrocarbon contamination, are listed as type II suspect catalogues.

C. Final Data Evaluation

The final acceptability of samples in these catalogues will depend on which of six categories the catalogues belong. First, consider catalogues that contain more than 5% control site samples; there are four possibilities. If a catalogue is neither type I nor type II suspect, then all of the samples in that catalogue will be accepted. Alternatively, if a catalogue is both type I and type II suspect, then none of the samples in that catalogue will be accepted. If a catalogue is type I but not type II suspect, then all the identified deviant samples, together with all other samples in the catalogue that do not have replicates, will not be accepted. If a catalogue is type II but not type I suspect, then none of the samples in that catalogue will be accepted.

Second, consider catalogues that contain less than 5% control site samples; there are two possibilities - they may or may not be type I suspect. At a minimum, all the identified dissimilar samples, together with all other samples in the catalogue that do not have replicates, will not be accepted in these type I suspect catalogues.

Samples that are accepted after evaluation using the processes described above will be used for the further statistical tests below.

Hydrocarbon Data Interpretation

There are three main phases to be completed in order to satisfy the objectives of this project after all spurious data have been removed. Each of these phases must be completed for both the sediment and mussel tissue hydrocarbon data. Initially, the hydrocarbon analyses will be resolved into a simple index that describes the amount of oil observed at each site. The second phase provides each PI with a summary of their samples and associated amounts of oil. In addition, patterns in the data will be highlighted and statistically interpreted. Finally, the indices will be used in conjunction with a GEO/SQL database to explore the data across projects, and perform the indicated analyses.

A. Development of Hydrocarbon Index

An index reflecting the amount of oil in a sample will be developed for accepted samples. This index will be the first component score from a principal component analysis (PCA) for the whole data set. Previous work with a small subset of this data has shown the first component score to be highly correlated with hydrocarbons characteristic of crude oil. PCA is an ordination technique where final component scores are derived from the data matrix alone, and no extrinsic values are required. PCA resolves the data set into a space described by k axes (components) so that each axis accounts for progressively smaller amounts of variance.

Ideally, the first few components account for the majority of the variance; and the system can be discussed in terms of the reduced space (For a complete description see Gauch 1982). Scores will be obtained by submitting the data set to a PCA routine in the Statistical Analysis System (SAS) computer system. After the PCA is completed each PI will receive a report listing the samples retained in the data set, the concentration of each of the hydrocarbons, and the component 1 score.

B. Pattern Recognition and Interpretation

After PCA scores are obtained the scores will be mapped using a GEO/SQL mapping system. Map symbols will be developed that reflect various hydrocarbon quantities and the overall component 1 score. Additional symbols will be developed for each project. The symbols will be mapped onto their geographic locations permitting identification of patterns in oil distribution and response variables. PIs will be provided with summaries of each analysis, test results, associated maps and rationale.

C. Identification of Patterns Across Projects

Once the PIs have been provided with summaries of hydrocarbon analysis for their projects we will begin to explore patterns in oil distribution and response across all projects. Response and oiling symbols will be mapped using a GEO/SQL computer system. While each project provides insight into the effects of the Exxon Valdez oil spill, the most complete picture will emerge by combining the results of all contributing projects.

DELIVERABLES

- A. Data - Lists of evaluated samples and associated hydrocarbon indexes will be provided to the PIs who collected the samples.
- B. Maps - Maps of hydrocarbon indexes and summary contamination levels will be produced for each project that collected samples, and for all projects together, for 1989 and 1990 samples.
- C. Reports - A final report will be prepared describing in detail the final procedures and criteria used, the results, and conclusions supported by the results.

SCHEDULES AND PLANNING

Data and Report Submission Schedule

<u>MILESTONE</u>	<u>EXPECTED COMPLETION DATE</u>
Identification of suspect catalogues	Mar. 16 1992
Development of hydrocarbon index	Mar. 31 1992
Pattern recognition and interpretation	Aug. 30 1992
Pattern recognition across projects	Dec. 1 1993
Final Report	Feb. 28 1993

Sample and Data Archival

Data will be archived in the Technical Services #1 database, where additional fields will be added to identify samples associated with suspect catalogues, types of suspect catalogues, and final petroleum hydrocarbon indexes arising from the principal component analysis. Maps will be archived with Technical Services #3.

LITERATURE CITED

- Brodersen, C.C., S.D. Rice, J.W. Short, T.A. Mecklenburg, and J.F. Karinen. 1977. Sensitivity of larval and adult Alaskan shrimp and crabs to acute exposures of the water-soluble fraction of Cook Inlet crude oil. In Proceedings of the 1977 Joint Conference (Prevention, Behavior, Control, Cleanup), Am. Pet. Inst. Wash., D.C. pp. 575-578
- Cheatham, D.L., R.S. McMahon, S.J. Way, J.W. Short, and S.D. Rice. 1977. The relative importance of evaporation and biodegradation, and the effect of lower temperature on the loss of some mononuclear and dinuclear aromatic hydrocarbons from seawater. Environ. Ass. Alas. Cont. Shelf. 12:44-65
- Gauch, H.G. 1982. Multivariate analysis in community ecology. Cambridge University Press. Cambridge U.K. 298 pp.
- Rice, S.D., M.M. Babcock, C.C. Brodersen, M.G. Carls, J.A. Gharrett, S. Korn, A. Moles and J.W. Short. 1986. Lethal and sublethal effects of the watersoluble fraction of Cook Inlet crude oil on Pacific herring (Clupea harengus pallasii) reproduction. U. S. Dept. Comm., NOAA, OCSEAP Final Report 63(1989):423-490
- Rice, S.D., R.E. Thomas, and J.W. Short. 1977. Effect of petroleum hydrocarbons on breathing and coughing rates and hydrocarbon uptake-depuration in pink salmon fry. In: Vernberg, F. John, Anthony Calabrese, Frederick P. Thurberg, and Winona B. Vernberg, (eds.), Physiological Responses of Marine Biota to Pollutants. Academic Press. N. Y. pp. 259-277
- Rice, S.D., J.W. Short and J.F. Karinen. 1977. Comparative oil toxicity and comparative animal sensitivity. In: Wolfe, Douglas A., (ed), Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press. N. Y. pp. 78-94.
- Rice, S.D., J.W. Short, C.C. Brodersen, T.A. Mecklenburg, D.A. Moles, C.J. Misch, D.L. Cheatham, and J.F. Karinen. 1976. Acute Toxicity and Uptake-Depuration Studies with Cook Inlet Crude Oil, Prudhoe Bay Crude Oil, No. 2 Fuel Oil and Several Subarctic Marine Organisms. NWFC Processed Report, National Marine Fisheries Service. Seattle. 90 p.
- Rice, S.D., J.W. Short, and J.F. Karinen. 1976. Toxicity of Cook Inlet crude oil and No. 2 fuel oil to several Alaskan marine fishes and invertebrates. In Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment Proc. Symp., American University, Washington, D. C., American Institute of Biological Sciences. Washington, D.C. pp. 394-406

Rice, S.D., D.A. Moles, and J.W. Short. 1975. The effect of Prudhoe Bay crude oil on survival and growth of eggs, alevins, and fry of pink salmon, Oncorhynchus gorbuscha. In: Proceedings of the 1975 Joint Conference on Prevention and Control of Oil Pollution Am. Pet. Inst. Wash., D.C. pp. 503-507

Short, J.W., S.D. Rice, and D.L. Cheatham. 1976. Comparison of two methods for oil and grease determination. In: Hood, D. W., and D. C. Burrell, (ed.), Assessment of the Arctic Marine Environment. Institute of Marine Science, University of Alaska, Fairbanks, Ak., pp. 451-462

BUDGET (\$K)

Salaries	\$ 118.6
Travel	1.8
Contracts	40.0
Supplies	9.6
Equipment	15.0
Subtotal	<u>\$ 185.0</u>
General Administration	20.6
Total	<u>\$ 205.6</u>