Program Work Statements

Environmental Assessment of the Alaskan Continental Shelf

Volume 5 - Chemistry and Microbiology

U. S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration

U. S. DEPARTMENT OF INTERIOR
Bureau of Land Management

CONTENTS - CHEMISTRY AND MICROBIOLOGY

Work Statement # 29

I. Assessment of Potential Interactions of Microorganisms and Pollutants Resulting from Petroleum Development on the outer continental shelf in the Beaufort Sea.

II. Ronald M. Atlas

III. Beaufort Sea - April 1, 1975 - September 30, 1976

IV. FY1975 - 22,880
FY1976 - 72,303

V. Objectives A-26, A-27, B-8, B-9

This study will begin with an examination of existing reports of microorganisms that occur in the Beaufort Sea. Careful attention will be given to reports of potential pathogens and to hydrocarbon degrading microorganisms. Information in the literature on these topics from other marine regions will also be compiled for comparative purposes. OASIS will be used to conduct this literature search.

In order to meet the task objectives samples will have to be collected and processed to enumerate the existing microbial communities. Collections will be seasonal. Processing will require controlled laboratory conditions beginning with the time of collection. Organisms will be characterized by standard morphological and biochemical tests, according the physiological groups or in the case of selected organisms to the genus level. Microbial communities will be characterized by numerical taxonomy and cluster analysis.

Rates of hydrocarbon biodegradation and bioemulsification will be measured in the laboratory using mixed microbial communities in water samples collected from the Beaufort Sea and with selected microorganisms isolated for their hydrocarbon degrading activity by elective culture methods. Some samples will be amended with nutrients to determine in hydrocarbon biodegradation is nutrient limited. Similarly incubation temperatures will be varied to determine whether hydrocarbon biodegradation is temperature limited.

Chemical and physical data at collection sites will have to be obtained from the chemistry groups. Some fish and benthic organisms will be required to determine microorganisms normally associated with such organisms and it is expected that such organisms can easily be supplied by the appropriate groups.

By September 1976 a summary of existing literature and the results of a few cruises should be available. Naturally several years of data colelction will be necessary to establish accurate baseline microbial populations and to enable accurate prediction of the rates of biodegradation and bioemulsification of hydrocarbons by the indigenous microorganisms. Output from a one-year study must thus be viewed as preliminary. The

first year will establish the sampling regime and will produce a list of microorganisms found in the Beaufort Sea samples collected including estimation of population sizes.

Methods

To assess the available literature several commercially available computer search and abstracting services supplied by OASIS will be used. These searches will examine both technical scientific publications and government reports.

Sampling will be done on a semiannual basis at stations along perpendicular sampling transects. The sampling transects should begin at Barrow and extend approximately 50 miles with sampling stations every 10 miles. Sampling on this basis will permit estimation of the fluctuations in the size and composition of the microbial communities in the region. Water and sediment for enumeration of microorganisms will be collected using Nisking samplers and a sediment corer. In addition to enumeration of microorganisms from water and sediment select groups of fish and benthic organisms will be needed to assay for microorganisms that normally are found in association with such organisms. Sampling of such macroorganisms will be left to the appropriately concerned groups. For enumeration of microorganisms replicate samples will be used to assure sampling adequacy.

Viable microorganisms enumerated under several conditions, aerobic, anaerobic, 5C, 20C, 37C, and with various carbon sources will be characterized by morphological and biochemical tests. Results from these numerical taxonomic tests will be subjected to cluster analysis and also will be compared to previously characterized microorganisms. The computer facilities of the National Institute of Health and the cultures of the American Type Culture Collection will be used for these analysis. An interagency agreement will be needed for this service ($500/yr)

With respect to hydrocarbon biodegradation petroleum residues will be recovered by freon extraction and analysed by gas chromatographic and mass spectrophotometric procedures following exposure to microorganisms in Bering Sea water and sediment under a variety of test conditions.

Data will be forwarded on punch cards to NODC for transcription to magnetic tapes and storage NODC (or NOAA) will bear the cost for transcription.

VI. The research will produce a report containg descriptions of microbial communities in the Beaufort Sea with cluster analysis triangles and lists of important genera of bacteria listing frequency of isolation with seasonal and spatial distribution. A table of rates of hydrocarbon biodegradation and bioemulsification under various conditions and a chemical characterization of residual hydrocarbons will be included.

VII. OASIS will be required for conducting the literature survey. A limited number of fish and benthic organism samples maintained under refrigerated conditions will have to be supplied for determination of associated microorganisms. Frozen samples will be supplied to the chemistry group for nutrient analysis. The chemistry group (Dr. Vera Alexander) will be relied on to supply chemical data at the sampling sites including salinity, nutrient concentrations, (P, N, O, organic C) and chemical forms (including NH_4, NO_3, PO_4). Joint sampling sites with Dr. Morita's microbiology group will be established and analysis will be performed on the same samples.

VIII. Any required archival of collected material will be accomplished by submission of a culture to the American Type Culture Collection.

IX. Three months after the beginning of this project the literature search of existing data will be accomplished. Within 3-5 months after the beginning of this project the first sampling should be accomplished. Analysis from this sampling trip should take 4 months. The schedule will be repeated semiannually.

X. Niskin sterile water samplers will be required for sample collection. These will be ordered upon receipt of this grant. Delivery should be within 2 months. Similarly other major equipment, microscope, incubators, gas chromatograph will be ordered immediately upon receipt of the contract and delivery should be within 3 months.

Sampling will be by helicopters which should be equipped with lime and much capability for bottom sampling. Helicopters much be capable of landing at sampling sites. Space will be required at NARL for laboratory work as well as living quarters.

XI. All logistic requirements will be supplied by NOAA and have not been budgeted within this grant. We will require helicopter support meeting the equipment requirements described in item X. Sampling will be done in summer (Aug-Sept) and winter (Feb). In summer '75 8 days of helicopter time will be needed. 1 flight per week along a 100 km transect. Two transects will be used each being sampled on alternate weeks. One transect will be north from the Harrison Bay regions, the other will be perpendicular to the first approximately paralelling the shoreline 20 km offshore. These same transects will be sampled once each in February. In summer '76 4 helicopter support days will be necessary, 1 per week to either sample the same transects or a similar perpendicular pair of transects near Prudhoe Bay.

Onshore labspace will be required at NARL including housing for 3 men for 2 months in summer '75, 2 weeks winter '76 and 1 month summer '76. A laboratory will be required equipped with incubators and an autoclave. If the summer '76 sampling is done near Prudhoe Bay occasional fixed wing flights between Barrow and Prudhoe may be needed. Occasional ground transportation will be necessary.

I. Assessment of Potential Interactions of Microorganisms and Pollutants Resulting from Petroleum Development on the outer continental shelf in the Gulf of Alaska.

II. Ronald M. Atlas

III. Gulf of Alaska - April 1, 1975 - September 30, 1976

IV. FY 1976 - 95,183

V. Objectives A-26, A-27, B-8, B-9

This study will begin with an examination of existing reports of microorganisms that occur in the Gulf of Alaska. Careful attention will be given to reports of potential pathogens and to hydrocarbon degrading microorganisms. Information in the literature on these topics from other marine regions will also be compiled for comparative purposes. OASIS will be used to conduct this literature search.

In order to meet the task objectives samples will have to be collected and processed to enumerate the existing microbial communities. Collections will be seasonal. Processing will require controlled laboratory conditions beginning with the time of collection. Organisms will be characterized by standard morphological and biochemical tests, according to physiological groups or in the case of selected organisms to the genus level. Microbial communities will be characterized by numerical taxonomy and cluster analysis.

Rates of hydrocarbon biodegradation and bioemulsification will be measured in the laboratory using mixed microbial communities in water samples collected from the Gulf of Alaska and with selected microorganisms isolated for their hydrocarbon degrading activity by elective culture methods. Some samples will be amended with nutrients to determine in hydrocarbon biodegradation is nutrient limited. Similarly incubation temperatures will be varied to determine whether hydrocarbon biodegradation is temperature limited.

Chemical and physical data at collection sites will have to be obtained from the chemistry groups. Some fish and bemthic organisms will be required to determine microorganisms normally associated with such organisms and it is expected that such organisms can easily be supplied by the appropriate groups.

By September, 1976 a summary of existing literature and the results of one summer and one winter cruise should be available. Naturally several years of data collection will be necessary to establish accurate baseline microbial populations and to enable accurate prediction of the rates of biodegradation and bioemulsification of hydrocarbons by the indigenous microorganisms. Output from a one-year study must thus be viewed as preliminary. The first year will establish the sampling regimen and will produce a list of microorganisms found in the Gulf of Alaska samples collected including estimation of population sizes.

Methods

To assess the avilable literature several commercially available computer search and abstracting services supplied by OASIS will be used. These searches will examine both technical scientific publications and government reports.

Sampling will be done on a semi-annual basis at stations along sampling transects. The sampling transects should be the same as the existing sampling grid in the Gulf. Sampling on this basis will permit estimation of the fluctuations in the size and composition of the microbial communities in the region. Water and sediment for enumeration of microorganisms will be collected using Niskin samplers and a sediment corer. In addition to enumeration of microorganisms from water and sediment select groups of fish and benthic organisms will be needed to assay for microorganisms that normally are found in association with such organisms. Sampling of such macroorganisms will be left to the appropriately concerned groups. For enumeration of microorganisms replicate samples will be used to assure sampling adequacy.

Viable microorganisms enumerated under several conditions, aerobic, anaerobic, 5C, 20C, 37C, and with various carbon sources will be characterized by many morphological and biochemical tests. Results from these numerical taxonomic tests will be subjected to cluster analysis and also will be compared to previously characterized microorganisms. The computer facilities of the National Institute of Health and the cultures of the American type culture collection will be used for these analysis. An interagency agreement will be needed for this arrangement.($500/yr)

With respect to hydrocarbon biodegradation petroleum residues will be recovered by freon extraction and analyzed by gas chromatographic and mass spectrophotometric procedures following exposure to microorganisms in the Gulf of Alaska water and sediment under a variety of test conditions. Mass spectrometry will be performed by Petroleum Analytical Services (Houston, Texas) to reveal % class composition of petroleum components.

VI. Data will be forwarded on punch card to NOAC for transcription to magnetic tapes and storage. NOAC will bear the costs for transcription.

The research will produce a report containing descriptions of microbial communities in the Gulf of Alaska with cluster analysis triangles and lists of important genera of bacteria listing frequency of isolation with seasonal and spatial distribution. A table of rates of hydrocarbon

biodegradation and bioemulsification order various conditions and a chemical characterization of residual hydrocarbons will be included.

VII. OASIS will be required for conducting the literature survey.

A limited number of fish and benthic organism samples maintained under refrigerated conditions will have to be supplied for determination of associated microorganisms. Frozen samples will be supplied to the chemistry group for nutrient analysis. The chemistry group (Dr. Vera Alexander) will be relied on to supply chemical data at the sampling sites including salinity, nutrient concentrations, (P, N, O, organic C) and chemical forms (including NH_4, NO_3, PO_4, etc.). Joint sampling sites with Dr. Morita's microbiology group will be established and analysis will be performed on the same samples.

VIII. Any required archival of collected material will be accomplished by submission of a culture to the American Type Culture Collection.

IX. Three months after the beginning of this project the literature search of existing data will be accomplished. Within nine months after the beginning of this project the first cruise should be accomplished. Analysis from this cruise should take 3-4 months which should be the time of the next cruise. The schedule will be repeated.

X. Niskin sterile water samplers will be required for sample collection. These will be ordered upon receipt of this grant. Delivery should be within 2 months. Similarly other major equipment, microscope, incubators, gas chromatograph will be ordered immediately upon receipt of the contract and delivery should be within 3 months.

Sampling vessels should be equipped with lime and much capability for bottom sampling. Incubator space will be required. Electric current compatible with incubator autoclaves and pumps will be necessary.

XI. All logistic requirements will be supplied by NOAA and have not been budgeted within the grant. We will require ship space, meeting the equipment requirements described in X, two times per year. The timing of the initial cruise will be determined by the time of receipt of the contract allowing time for the purchase of necessary supplies and equipment. The ship will have to be outfitted with incubators at 5, 20 and 37C. At the point of departure, we will require a shore based facility where media can be prepared and samples processed for shipping. This shore based facility will be needed for 1 week before and 1 week after each cruise. For our purposes, cruises ideally should be short lasting, approximately 10 days.

Revised Alaskan Marine Environmental Assessment Program

Work Statement # 43/44/45

I. Trace Hydrocarbon Analysis in Previously Studied Matrices and Methods Development for: A) Trace Hydrocarbon Analysis in Sea Ice and at the Sea Ice-Water Interface, B) Analysis of Individual High Molecular Weight Aromatic Hydrocarbons

II. Four principal coinvestigators will function as a team:

Stephen N. Chesler, Ph.D.
Barry H. Gump, Ph.D.
Harry S. Hertz, Ph.D.
Willie E. May

Research Chemists
Bioorganic Standards Section
National Bureau of Standards

III. Geographic Areas

Gulf of Alaska and the Southeastern Bering and Beaufort Seas
Inclusive Dates: July 1, 1975 - September 30, 1976

IV. Cost Summary

FY 1976 - $165 K

V. Proposed Research

A. Background and objectives

The objectives of the proposed research are as follows:

1) To serve as a quality assurance laboratory for hydrocarbon analysis in sediments, tissue and water; to participate fully and cooperatively in the first sampling trip made by each laboratory or contractor that undertakes hydrocarbon analyses.

2) To develop sampling methodology for the analysis of hydrocarbons in sea ice and at the sea ice-water interface.

3) To identify major organic compounds present at the sea ice-water interface.

4) To develop methodology for identification and quantitation of individual 3-, 4-, 5-, and 6-membered ring aromatic hydrocarbons at the ng/kg (part per trillion) level.

It would be beneficial to coordinate the sea ice-water work in the Bering Sea with Alexander. Her expertise in microbiology will be necessary in selecting sampling sites and overlap of sampling sites should be valuable.

The NOAA task of primary emphasis in this research is:

A-33--Determination of total content and chemical species of hydrocarbons in the water column, selected marine organisms, sea ice and sea ice-water interface.

The results of the proposed research could have secondary influence on the following NOAA tasks:

A-31--Determine the relationship of living resources to the ice environment.

B-8--Examine the processes which determine the fate of hydrocarbons introduced into the environment.

B-14--Develop means to predict possible interactions between ice and oil and other contaminant discharges.

With the large number of environmental analyses to be performed in the future, the need for quality assurance (i.e., accurate and precise measurements) is great. Clearly, accuracy is far more difficult and costly to achieve than precision, and, indeed, even precision is not easily achieved on an interlaboratory scale. Until such time as standard reference materials are available, a quality control function is essential to assure the comparability of numbers obtained by different laboratories.

It is anticipated that all objectives will be met and all results will be reported to NOAA by September 30, 1976. In meeting

the four objectives listed above, a total of approximately 75 samples will be analyzed. Continuing studies arising from the methods development and continued participation as a quality assurance laboratory would require support beyond FY 76.

B. Methods

Detailed sampling and analysis protocols for previously studied matrices are contained in Appendix I (October, 1974 Progress Report to NOAA). This existing methodology will be used as a technological base for sea ice and polynuclear aromatic hydrocarbon (PAH) research.

VI. Information Products

Quarterly reports to NOAA will contain all quality assurance data and results of methods development research. The primary output from sample analyses will be in the form of chromatograms, numerical data, and associated interpretive discussions. It is anticipated that several publications in the scientific literature will result from the proposed research.

VII. Data or Sample Exchange Interfaces

We anticipate the prime function of this laboratory to be one of quality assurance, which necessitates that this laboratory be the focal point for hydrocarbon baseline sample exchange.

The following program of sample exchange has been agreed upon with the University of Alaska:

1) We will send splits of 50% of the NBS collected samples to Dr. Shaw.
2) Dr. Shaw will send NBS splits of 10% of the University of Alaska collected samples.
3) The laboratory making sample collections will be responsible for bottling, freezing and shipping of frozen samples to the other laboratory.
4) Results of these analyses will be transmitted through the Program Office.

All samples collected for sea ice or PAH analysis will, with prior arrangement, be available for splits.

Use of the EDS OASIS system for current awareness searches is requested. An EDS bibliographic search of previous hydrocarbon baseline studies and trace hydrocarbon methodology would be beneficial. A copy of the EDS literature search on physiological effects of hydrocarbons would be valuable.

Exchange of quarterly reports with other chemical investigators would be beneficial.

VIII. N/A

IX. Schedule

The sampling schedule will be dependent upon that of collaborating laboratories. It is anticipated that sea ice and water samples for methods development will be collected in conjunction with the sampling trips mentioned above, or, if necessary, on independent trips in the summers of 1975 and 1976.

X. Equipment Requirements

Sample collecting equipment for sediment, water, tissue and sea ice will be provided by the National Bureau of Standards. A salinometer and freezer space plus dry ice will be required for each sampling trip.

The following specialized laboratory equipment will be supplied by NBS: gas chromatographs, gas chromatograph-mass spectrometer-computer system, and a liquid chromatograph-spectrofluorimeter (this last item to be purchased with NOAA funds).

XI. Logistics Requirements

The following logistics support will be requested of NOAA for each sampling trip:

1) a helicopter.
2) ship support or other base from which one can sample the sea ice-water interface; this sampling in the Bering Sea is to be performed in conjunction with phytoplankton sampling.
3) limited shore facilities for sample handling and packaging; when samples are to be frozen on site for transport back to NBS, freezer space would be desirable and dry ice will be required.

It is anticipated that each sampling trip would require several days of satisfactory flying weather in the Gulf and at the sea ice-water interface in order to join the sampling parties.

WORK STATEMENT
(Research Unit #47)

I. TITLE

Environmental Assessment of Alaskan Waters - Trace Element Methodology - Inorganic Elements.

II. PRINCIPAL INVESTIGATOR

Dr. Philip D. LaFleur
Chief, Analytical Chemistry Division
National Bureau of Standards
A-309 Chemistry
Washington, D. C. 20234

NOTE: Dr. LaFleur is acting as overall project director. The program is divided into two main parts, organic and inorganic trace elements with the following personnel as project leaders:

Organic

Dr. S. N. Chesler
Dr. H. S. Hertz

Inorganic

Dr. I. L. Barnes
Mr. D. A. Becker

III. GEOGRAPHIC AREA AND INCLUSIVE DATES

Beaufort and Bering Seas

FY-76 July 1, 1975 to September 30, 1976

IV. COST SUMMARY

Salaries and Benefits $76K

V. PROPOSED RESEARCH

A. Background and Objectives

1. The research proposed here is directly related to Task A-33, determine the content of selected trace metals in the water column, suspended particulate matter and bottom sediments. A part of this will include Task A-32, a survey of the available literature (including an evaluation) for data on the concentration and distribution of selected trace elements.

A portion of the acquired data may be used as a part of Task B-11 to characterize chemically sediment influx and deposition and all of the data may be used as a part of Task E-2 to predict possible short and long term environmental effects of possible oil and gas development.

2. We are in the process of examining the records of literally thousands of samples collected in the marine environment and of assessing the potential validity of the data from analyses for trace elements in water and sediments. This examination and evaluation is being conducted as part of the National Environmental Specimen Bank program sponsored jointly by the Environmental Protection Agency and NBS. Until this is complete (September or October, 1975) we do not feel that we can describe the present state of knowledge in this area for it is becoming increasingly evident that much of the present data may be completely invalidated by inadequate or contaminating collection and storage methods. In addition since few if any standards exist in this area it is difficult to determine the accuracy of many of the analytical methods used.

3. To meet the task objective i.e., to provide accurate and precise data on the concentration and distribution of potentially harmful or toxic trace elements it is necessary that:

   a. Samples be collected and stored in a manner known not to increase nor decrease the concentration of trace elements.

   b. The samples must be stored and subsampled in a manner which does not contaminate either positively or negatively.

   c. The analyses must be performed using techniques and methods of proven precision and accuracy.

   These points will be discussed in more detail below.

   The basic information required is the results of precise and accurate analyses of the selected trace elements in the water column, in suspended particulates and in the various components of the sediment samples.

Recent work at NBS by Harrison et al. [1] has shown that commercial water samples may contaminate the samples taken with as little as three to five minutes contact. As a result an all teflon sampler has been designed and tested in the Chesapeake Bay and which is believed not to either add or subtract trace elements. In addition this unit has provisions for immediate field filtration to remove suspended matter so that it may be preserved for subsequent analysis. Extensive research has been conducted into possible contamination by various storage containers (cf Murphy [2]) and for methods to clean these. Work in progress indicates that with proper cleaning and handling water samples with 12 toxic trace elements may be stored and preserved for extended periods.

For sediment samples Brinckman and Iverson have shown that many heavy metals may be preferentially located in oil fractions where they have been chemically converted to volatile organo-metallic compounds. These are nearly completely lost with improper handling and storage. Finally extensive work at NBS has shown that modern analytical techniques when applied to trace elemnt analyses are subject to large and unsuspected errors especially in areas where no or inadequate standards are available.

4. We expect to collect water and sediment samples in cooperation with Dr. D. Burrell, University of Alaska and to be provided additional samples by him. The sampling schedule is described in Section VII below. The analytical work will be completed and the results reported to the Project Office on or before September 30, 1976. These results will be neither released nor discussed with persons outside the Project Office unless otherwise directed by them.

B. Methods

1. As mentioned above an extensive effort is already underway to examine the results of published and unpublished results on the collection, storage and analysis of waters, marine organisms and sediments, muds, etc. The results of this study are expected to apply directely to this study and will be available for use by all interested groups.

2. The group from NBS who are participating in the organic trace analysis effort will sample and preserve water samples and suspended material samples using the teflon sampler mentioned above. This and specially cleaned containers will be provided by NBS. In addition containers will be provided by NBS for sample spits of sediments obtained by other NOAA contractors. Sampling will be at the same points as for the organic trace element sampling. Duplicate and triplicate samples will be taken from selected points.

3. Water and suspended matter samples will be analyzed for Ni, V, Mn, Cd, As, Se, Ba, and Cr by two or more independent methods. The primary analytical methods used will be neutron activation analysis (NAA) and atomic absorption analysis (AA). Variations of the AA procedure i.e. carbon furnace, cold vapor and plasma source will be used where deemed applicable. If and where the results of the analysis do not agree a third independent method, that of isotope dilution spark source mass spectrometry (IDSSMS), will be used.

   Each of these has been used at NBS for the accurate analysis of water samples as reported by Rook and Moody [3].

   Lead will be determined in both these and sediment samples (various fractions) by isotope dilution mass spectrometry (IDMS) which is a definitive analytical technique for small lead concentrations (cf Barnes et al. [4]). A method of separating sediment samples into the various fractions while preserving the possible volatile compounds is now under investigation. The procedure has been demonstrated to work for the elements Hg, Cd, and Sn and is expected to apply to others also. The separated fractions will be analyzed by NAA, AA, IDMS, and IDSSMS as above. Analyses for additional elements may be made after initial examination of the samples.

VI. INFORMATION PRODUCTS

Information obtained will be the concentration of the selected trace elements along with the data of sampling location, time, etc. Also provided will be detailed descriptions of the analytical methods used. Another product will be intercomparison capability for analyses conducted by us and other investigators, particularly Dr. Burrell's group.

VII. SAMPLE EXCHANGE

On the trip(s) made by NBS personnel, 50% of the samples collected will be shared with Dr. Burrell. This is expected to be about 10 samples of each type. For these samples containers will be provided by NBS. Dr. Burrell will provide splits from about 10% of samples taken on subsequent trips. This is expected to be about 15 water and 15 sediment samples. For these, containers will be provided by Dr. Burrell. Dr. Burrell will assume responsibility for storage and shipping costs for those samples collected by him, and NBS will assume responsibility for these samples collected by NBS personnel.

VIII. SAMPLE ARCHIVAL REQUIREMENTS

Samples gathered or provided for this portion of the program will be stored frozen or freeze dried at NBS. Personnel of the Analytical Spectrometry Section will maintain responsibility. The necessary freezer equipment is available.

IX. SCHEDULE

See Appendix

X. EQUIPMENT

Except for sampler and containers to be provided by NBS no equipment is required.

XI. LOGISTICS REQUIREMENTS

None

XIII. REFERENCES

[1] Harrison, S. H., LaFleur, P. D., and Zaller, W. in Accuracy in Trace Analysis, Sampling, Sample Handling, Analysis, P. D. LaFleur, Ed., Proceedings of the 7th Institute for Materials Research Symposium, NBS, Gaithersburg, Maryland, October 7-11, 1974, National Bureau of Standards Special Publication 422, U.S. Government Printing Office, Washington, D. C. 20402 (in process).

[2] Murphy, T. J., ibid.

[3] Rook, H. L. and Moody, J. R., Proceedings of the 2nd International Conference on Nuclear Techniques in Environmental Research, University of Missouri - Columbia, July 1974.

[4] Barnes, I. L., Garner, E. L., Gramlich, J. W., Machlan, L. A., Moody, J. R., Moore, L. J., Murphy, T. J., and Shields, W. R., Isotopic Abundance Ratios and Concentrations of Selected Elements in Some Apollo 15 and Apollo 16 Samples, Proceedings of the Fourth Lunar Science Conference, Geochim. Cosmochim. Acta Suppl. 4, 2, 1197-1207 (1973).

APPENDIX I

Milestones

Inorganic Trace Analysis

	Task	FY-76 Scheduled Completion	Note
1.	Provide special water sampler	June 1975	Completed
2.	Clean and prepare sample containers	June 1975	90% Complete
3.	Receipt of first samples	Aug. 1975	
4.	Receipt of contractor samples	Oct. 1975	
5.	Analyses of water samples	Dec. 1975	
6.	Analyses of suspended material	Jan. 1976	
7.	Analysis of sediment samples	March 1976	Depends on 4
8.	Final report	Sept. 1976	

WORK STATEMENT #153/155

I. TITLE: Distribution of Light Hydrocarbons, C_1-C_4, in the Gulf of Alaska and Southeastern Bering Shelf.

II. CO-PRINCIPAL INVESTIGATORS: Dr. Joel D. Cline
Dr. Richard A. Feely

III. GEOGRAPHICAL AREAS:

NE and NW Gulf of Alaska - 1 July 1975 - 30 September
Southeastern Bering Shelf

IV. COST:

Gulf of Alaska - FY 1975 - 0
FY 1976 - 60.0K

Bering Sea - FY 1975 - 21.3K
FY 1976 - 40.7K

Total Cost: $122,000

May 8, 1975

V. PROPOSED RESEARCH

A. Background and Objectives

Introduction

The development of petroleum resources in the Gulf of Alaska may result in the release of toxic hydrocarbons to the marine environment with possible deleterious effects on the pelagic, benthic, and intertidal biota. Increases in the natural levels of petroleum-derived hydrocarbons are likely to occur from the normal activities associated with exploration, production and transportation of crude and refined products within the region. Thus, it is of environmental significance and necessity that baseline levels of both natural occurring and petroleum-derived hydrocarbons be established prior to the development of fossil fuel resources in the area.

Presently the most toxic fractions of crude oil are recognized as the low boiling point aliphatic and aromatic hydrocarbons (Blumer, 1971). Also associated with these complex fractions are the low molecular weight "light" hydrocarbons, C_1-C_4, including both the saturated and olefinic homologs. While it is generally presumed that these compounds are of lower toxicity than the aforementioned groups (Sackett and Brooks, 1974), they are more soluble and hence are likely to be dispersed by normal mixing processes. Because of their relatively high solubility and low natural abundance, the temporal and spatial distributions of C_1-C_4 hydrocarbons are valuable indicators of petroleum pollution arising from offshore drilling and production platforms, ballast tank discharge, and shipping and transfer operations involving petroleum and petrochemicals (Brooks and Sackett, 1973; Sackett and Brooks, 1974). It is also likely that the distributions of

methane, ethane, propane, and butane may serve as valuable tracers for the relatively soluble, but highly toxic, mono- and polynuclear aromatics commonly associated with crude oil (Blumer, 1971).

The occurrence of light hydrocarbons in the water column may arise from both pertoleum production activities and natural marine sources. Gaseous hydrocarbons may exchange across the sea surface in response to a concentration gradient, diffuse from underlying sediments (Frank et al., 1970), escape in the form of bubbles from natural occurring gas and oil seeps (Link, 1952; Geyer and Sweet, 1973), or be produced by in situ processes (Lamontagne et al., 1973b).

Methane (CH_4) is a significant component of natural gas and is also produced in anoxic sediments by bacterial CO_2 reduction and fermentation reactions (Claypool, 1974). Thus, the presence of excess methane in the water column overlying organic-rich sediments is not an unequivocal indicator of petroleum impact, unless viewed jointly with the distribution of the heavier fractions, C_2-C_4 (Brooks and Sackett, 1973).

Above saturation values of methane, ethylene and propylene also have been observed in the surface layers of open ocean and are believed to be related to biological activity or photochemical reactions involving organic matter (Swinnerton and Lamontagne, 1974; Lamontagne, et al., 1973b). The reactions relating to these processes are poorly understood, however.

Previous Studies

There is apparently little information available on the natural concentration levels of C_1-C_4 hydrocarbon in the Gulf of Alaska or the outer continental shelf region (Rosenberg, 1972). In contrast, a few analyses are available for Cook Inlet, but the concentrations reported for C_1-C_4 hydrocarbons are not usually excessive (Kinney, et al., 1970). The authors also suggest that ele-

vated concentrations found near the forelands may be indicative of seeps. It is assumed that historical exploration in the continental shelf region may have disclosed potential offshore seeps, but this information is not available for our perusal.

Although the data on the distribution of light hydrocarbons in the Gulf of Alaska are nearly non-existent, their distributions in the Gulf of Mexico have been useful in the early detection and identification of various pertoleum development sources (Frank et al., 1970; Brooks et al., 1973). On the basis of the $C_1/(C_2 + C_3)$ ratio observed in surface waters, Brooks, et al. (1973) were able to discern hydrocarbon inputs from offshore platform drilling and production, refining and shipping activity in ports, and open ocean contamination from tankers. Similarly, near bottom profiles of the light hydrocarbons may identify natural seeps and distinguish them from normal sedimentary methane production resulting from bacterial catalysis. In general, $C_1/(C_2 + C_3)$ ratios ≤ 20 are indicative of natural gas seeps, pollution, or natural cracking processes in the sediment column, whereas greater ratios suggest bacterial methane production (Frank, et al., 1970).

As an example the relative distributions of C_1-C_4 fractions in the Gulf Coast region are shown in Figure 1 (Brooks and Sackett, 1973). Particularily striking are the relative increases of propanes and butanes near known hydrocarbon pollution sources. A similar analytical program for light hydrocarbons in the Gulf of Alaska might thus detect sources of hydrocarbon pollution and serve as an environmental early warning system of potential ecological harm arising from the exploration, drilling, production, and transportation of crude oil and products. The analytical procedure to be adopted here (see below) has been routinely applied to the quantitative estimation of dissolved light hydrocarbons in various marine environments (Swinnerton

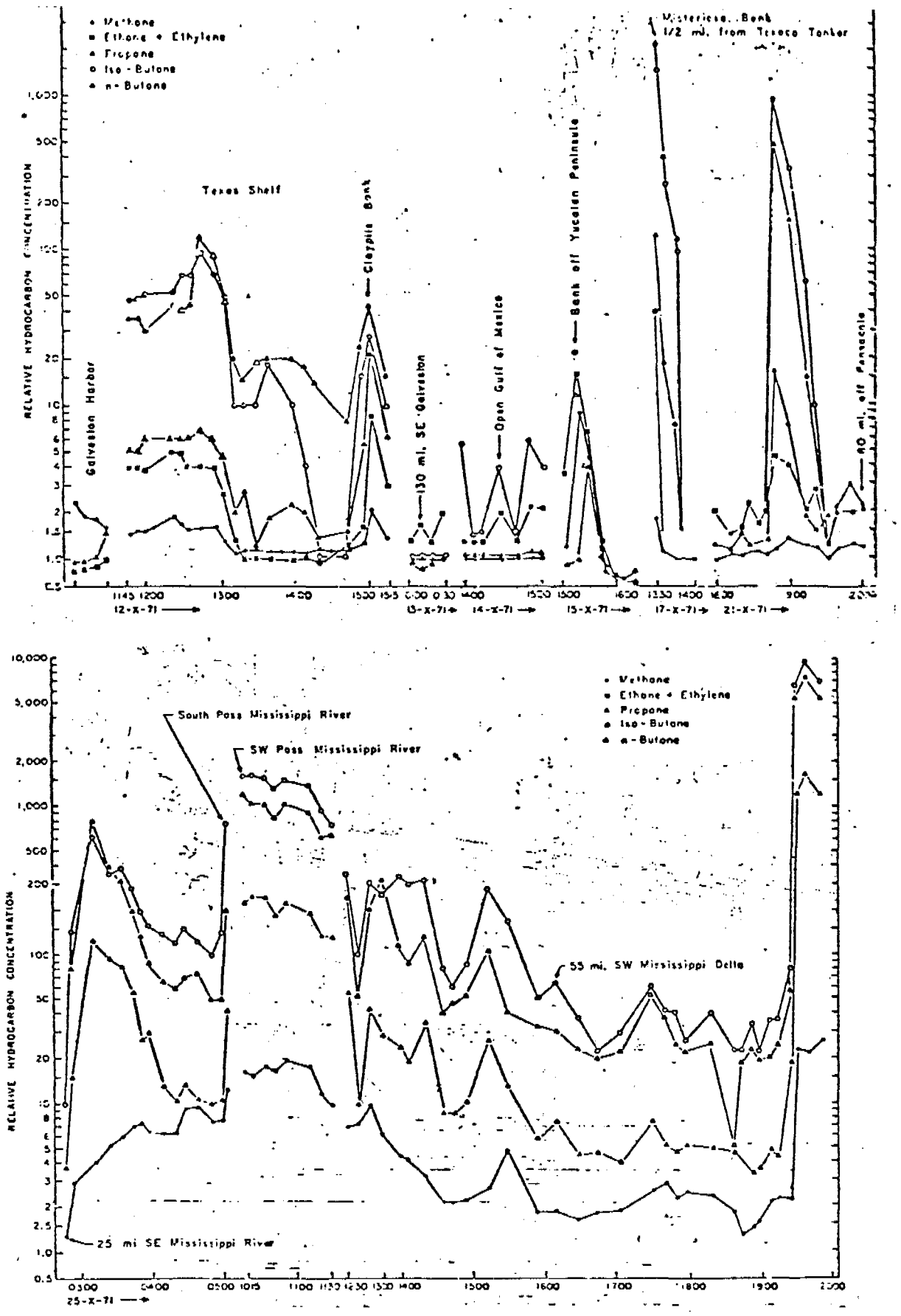

Figure 1. Relative hydrocarbon concentrations in the Gulf of Mexico (after Brooks and Sackett, 1973).

and Linnenbom, 1976; Frank, et al., 1970; Linnenbom and Swinnerton, 1970; Lamontagne, et al., 1973a; Brooks and Sackett, 1973). It is a rapid, precise, field-tested procedure, which can be readily adapted to either discrete or to continuous on-line sampling (Brooks and Sackett, 1973).

Objectives

It is proposed to ascertain the seasonal horizontal and vertical distributions of methane (CH_4), ethane (C_2H_6), propane (C_3H_8), butane (iso, n-C_4H_{10}), ethylene (C_2H_4) and propylene (C_3H_6) in the Gulf of Alaska and Bering Sea lease areas. This work will be in direct response to task A35 and in support of task A33 in the sense that natural petroleum hydrocarbon sources may be identified and subsequently sampled for possible biological impact under ambient conditions.

Our principal goals for the first year are to (1) establish normal baseline concentration levels of light hydrocarbons and (2) to elucidate seasonal variations over the region to the extent that seasonal coverage can be implemented.

Emphasis will be placed on the natural concentration levels of propane (C_3) and butane (C_4), as these components appear to be particularly sensitive to anthropogenic petroleum sources as well as natural gas and oil seeps (Brooks and Sackett, 1973). Attention also will be given to the immediate surface layers and to the establishment of temporal and spatial variations against which future trend assessment studies may be compared.

In the event that natural gas seeps are located (either by this survey or from the historical data bases), an attempt will be made to analyze the waters surrounding the seeps for "light" hydrocarbon concentrations. It is hoped that some flexibility in cruise scheduling can be obtained in order to delineate the dispersion plume. The occurrence and magnitude of the plume will depend primarily on the diffusive and advecting mixing scales and the frequency and effusive flux rates of hydrocarbons from the individual seeps. Composition of gases

emanating from seeps should determine whether they are of recent biological origin or derived from deep-seated petroleum reservoirs.

Because of fiscal and time constraints, no special effort will be mounted to locate gas and oil seeps. If they are found during the normal survey operations, they will be noted and the gases analyzed as described above.

The long-range objective is to provide the criteria for an early warning detection of petroleum-derived hydrocarbons and to establish the feasibility of using light hydrocarbons as dispersion tracers, particularly in reference to near-bottom mixing and resuspension processes. In the event of a spill, it is likely that the C_1-C_4 fraction may be useful in guiding a sampling protocol for the relatively soluble, toxic fractions of crude oil.

This study will be fully coordinated with the ongoing hydrocarbon program under the auspices of Dr. David Shaw at the University of Alaska. Also, if submarine seeps of gas and/or petroleum are uncovered during the implementation of this program, these locations will be communicated to representatives of the Geological Survey, at Menlo Park, and to Dr. David Shaw of the University of Alaska. Our program will be logistically interfaced with the proposed circulation and suspended particulate matter programs at PMEL.

METHODS

Analytical

The analyses of C_1-C_4 hydrocarbons will be carried out by the method of Swinnerton and Linnenbom (1967) as modified by Brooks and Sackett (1973). Briefly, the method is as follows: The hydrocarbons are stripped from 1 liter of seawater with a He carrier and the C_2-C_4 paraffins and olefins are isolated on activated alimina at -78°C while CH_4 is retained on activated charcoal at the same temperature. The extraction system is shown in Figure 2. After a quantitative extraction of all hydrocarbons, the cold traps are warmed and the gases are chromatographed.

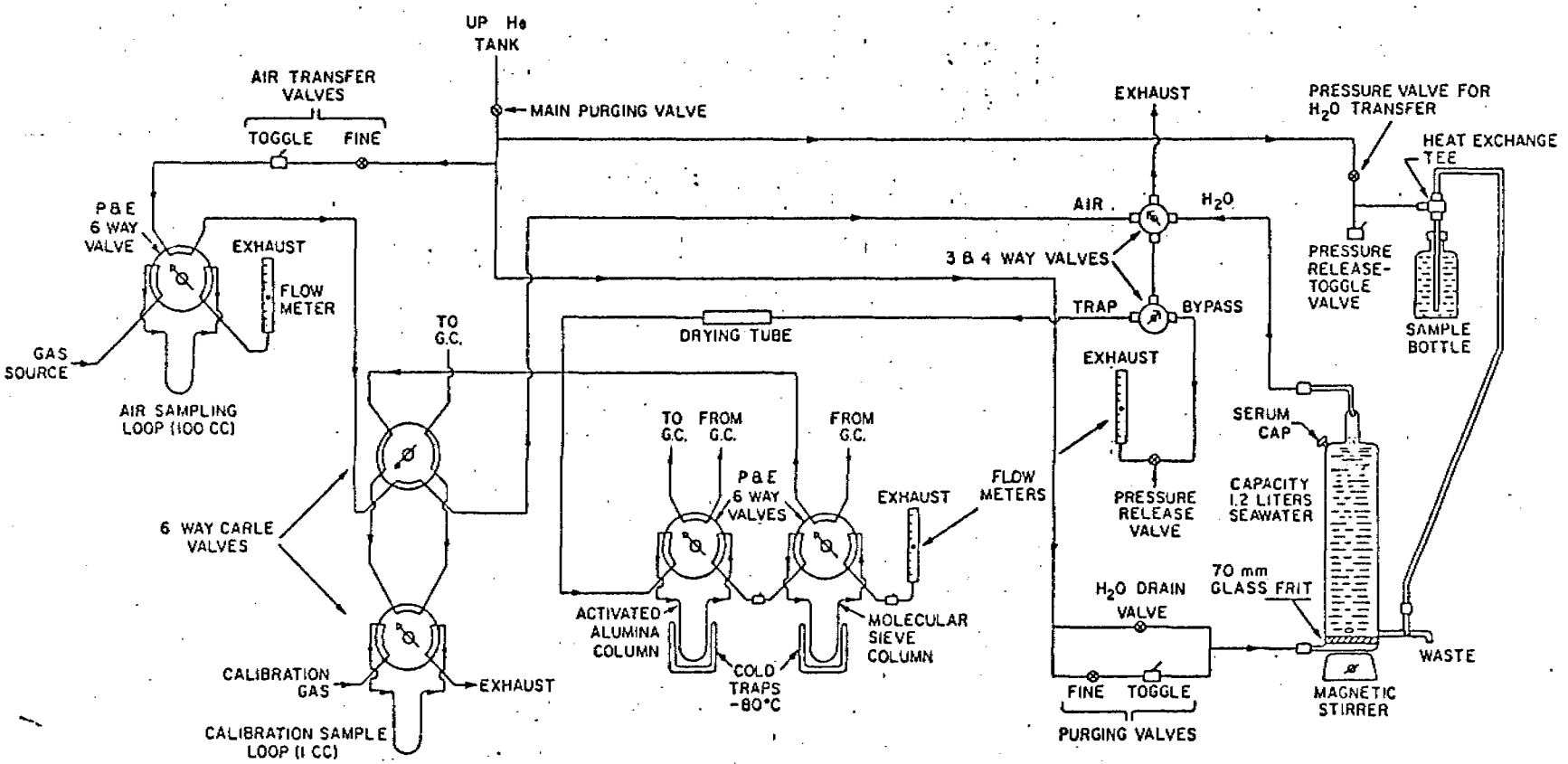

Figure 2. Light hydrocarbon extraction system (after Swinnerton and Linnenbom, 1967).

Analysis time is approximately 30 minutes, using the above stripping system. In order to reduce the analysis time and thus improve spatial coverage of the survey areas, a rapid, quantitative, vacuum extraction system is being developed. This procedure, together with G.C. temperature programming, should reduce the analysis time to approximately 15 minutes.

The reported detection limit of the method is approximately 4.5×10^{-8} ml/liter (STP) with a nominal electrometer sensitivity of 1×10^{-11} amperes (Swinnerton and Linnenbom, 1967). Modern electrometer amplifiers are capable of a tenfold increase in sensitivity over the above working values; however, the detection limit will ultimately depend on the observed signal-to-noise ratio.

The chromatographic system to be used here is the Hewlett Packard 5711 gas chromatograph and 3380 processor. The latter feature provides internal standardization, peak area integration, data processing and for the output of results in both analog and digital form. The processor can also be readily interfaced with magnetic and paper tape readout systems.

Standardization of the analysis will be carried out by the procedures recommended by Swinnerton and Linnenbom (1967) and Brooks and Sackett (1973). To insure maximum precision during all phases of this work, replicate surface samples will be taken during each cruise and sent to the Naval Research Laboratories and Texas A&M University for duplicate analyses. The results of the intercalibration study will be used as internal checks on the precision and accuracy of our observations. It is our intention to continue our close working relationship with Mr. Robert Lamontagne of the Naval Research Laboratory and Dr. James Brooks of Texas A&M University to insure a rapid and smooth implementation of this study.

Samples can be stored for approximately one month, hence sampling and analysis time can be optimized to meet the interdisciplinary requirements of the cruise. Surface mapping requires no station time and can be effected while the ship is underway.

Sampling Strategy (NEGOA)

Sampling for light hydrocarbons in the eastern Gulf of Alaska will be directly coordinated with the suspended particulate matter program of PMEL. Water samples will be acquired with 10-l Niskin® samplers, from which approximately 1.2 l will be required for hydrocarbon analysis. Logistically our program also will be coordinated with the plankton and STD-nephelometry studies at PMEL as well as the hydrocarbon studies at University of Alaska. Because a single, quantitative, hydrocarbon analysis requires about 30 minutes, optimum ship utilization suggests that this study be interfaced with other sampling activities to provide adequate delay in sampling. Short term synoptic measurements are not critical.

The sources of light hydrocarbons in the water column include gas seeps, atmospheric exchange, and biological related mechanisms in the surface layers. With the possible exception of the first of these, no short term spatial or temporal variations are expected. Seasonal coverage will be required to delineate possible biological components. The impact of gas seeps on the overlying water column will depend on the quantity of gas released, emission frequency, depth of water, and mixing scales. Because of the high mixing rates of the shelf region, the sampling protocol outlined below will not be sufficient to identify seep locations.

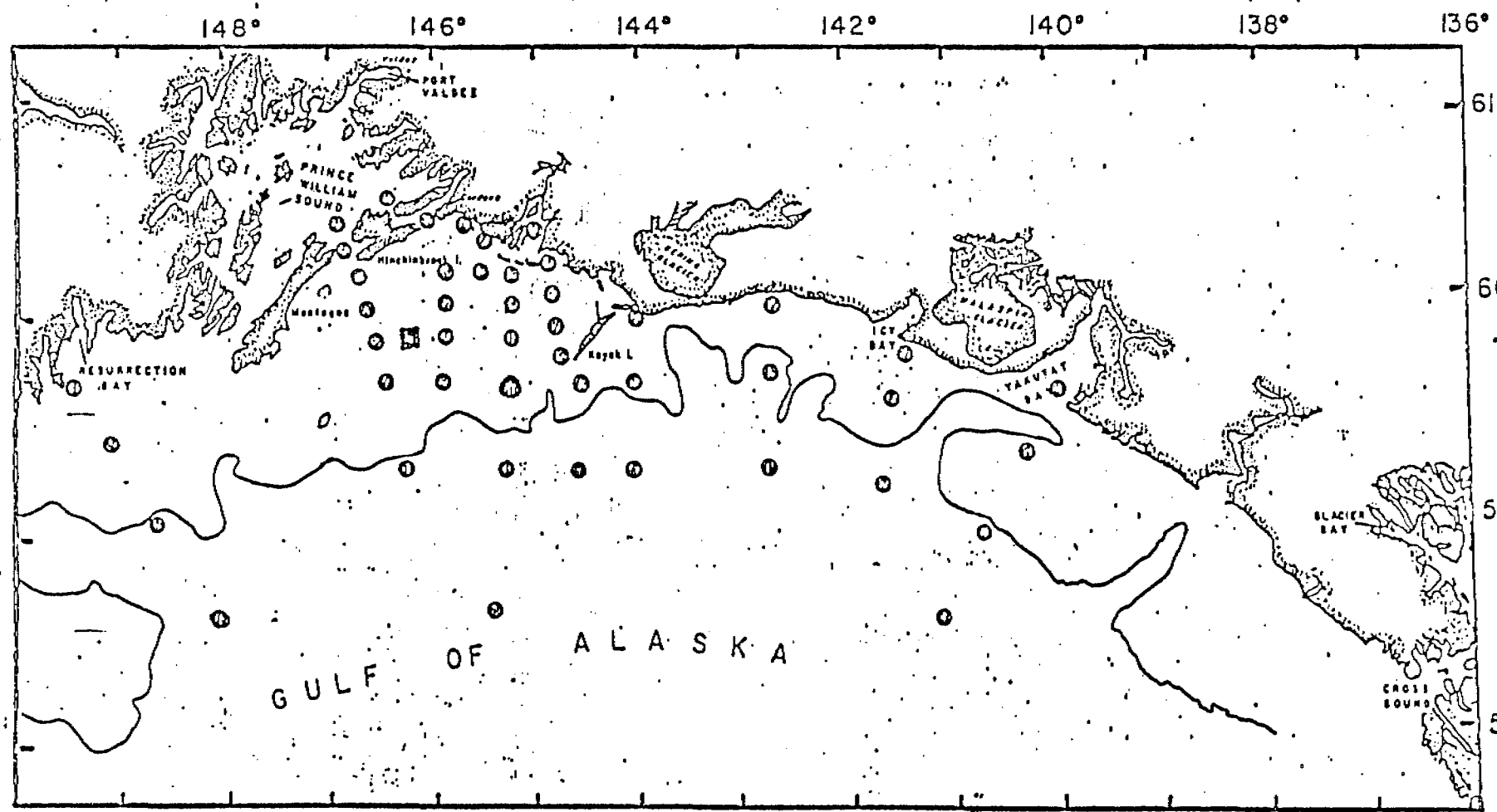

Figure 3. Station locations in the eastern Gulf of Alaska. Approximately one-half of these stations will be occupied in cooperation with the hydrographic and suspended particulate matter programs.

AREA AND PROJECT	JUL	AUG	SEP	OCT	NON	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
NEGOA				⊢	⊣					⊢	⊣		⊢	⊣	
Current meters & nephelometer						H			H						
NWGOA														⊢	⊣
Southeastern Bering Shelf		⊢	⊣								⊢	⊣			
Ship-time Days		14		21		1			1	21		21		21	21

Table 1. Ship time request in the NE and NW Gulf of Alaska and Southeastern Bering Shelf

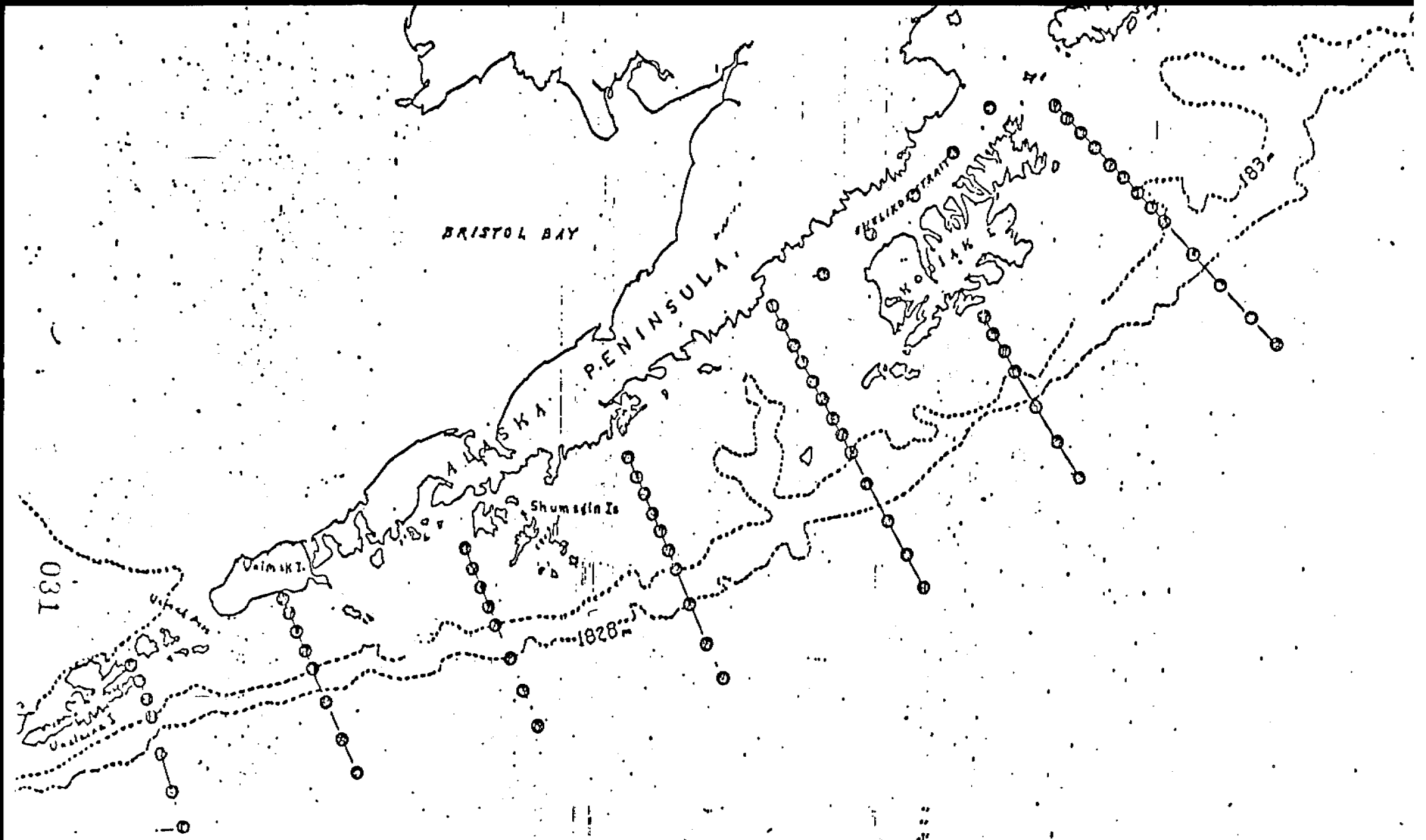

Figure 4. Station-locations in the western Gulf of Alaska.

Of the 48 stations shown in Figure 3, approximately one-half will be sampled in vertical profile for C_1-C_4 hydrocarbons. Additional stations may be added as time permits. To detect and evaluate vertical concentration gradients, water samples will be taken from a minimum of six depths at each station with emphasis on the upper and lower portions of the water column. Surface samples will be taken from the ship's sea chest between stations; the number actually taken will depend on station queueing. We anticipate that at least one surface sample will be taken between stations.

A total of three cruises are scheduled for FY1976 in the eastern Gulf as shown in Table 1.

Sampling Strategy (NWGOA)

The sampling grid for the western Gulf is shown in Figure 4 and represents the S-T-D network of stations proposed in the GAS-MOP program (see Hayes and Schumacher). Because of logistic overlap with other areas, personnel constraints, and the likelihood that dual GC systems will not be available by Fall 1975, sampling has been postponed until Fall of 1976 (see Table 1). Seasonal coverage will continue in Spring and Summer of 1977.

Water sample acquisition is similar to that described above for NEGOA. Approximately one-half of the stations will be sampled in vertical profile; the remaining occupied for surface samples only. Allocation of time for this sub-region is shown in Table 2.

Table 2. Summary of Sampling Protocol

	NEGOA	NWGOA	BERING
Sampling Events	Fall 1975 Spring 1976 Fall 1976	Fall 1976	Fall 1975 Summer 1976
Nominal distance between stations	40 km	20 km	60 km
Number of vertical stations	24	35	32
Number of surface stations	24	35	32
Total number of water samples	168	245	224
Wire time (est.)[1]	12 hrs	18 hrs	16 hrs
Shipboard analysis time	84 hrs (3.5 days)	123 hrs (5.1 days)	112 hrs (4.7 days)
Time between vertical stations[2]	5 hrs	5 hrs	5 hrs
Personnel	2-3	2-3	2-3

[1] Based on the estimate of time required to make a 200 m rosette sampler cast

[2] This will allow sufficient time to complete 6 analyses at each vertical station and at least one surface sample between vertical stations.

Sampling Strategy (Southeastern Bering Shelf)

The proposed sampling grid is shown in Figure 5 and was constructed after the STD grid proposed by Dr. J. Schumacher of PMEL. The stations are spaced every 30 nautical miles or about 60 km. A total of 64 stations are shown, approximately one-half will be sampled in vertical profile (O) for C_1-C_4 hydrocarbons with additional stations added as time and resources permit. Surface samples will be taken at intermediate stations (□).

Tentatively, sampling is scheduled to commence in August of 1975 (Table 1), if equipment needs and lead time requirements can be met in sufficient time (see below). It is anticipated that the initial cruise will focus on surface samples and vertical profiles near the coast in conjunction with the suspended particulate matter programs. The first full scale program for Bristol Bay is scheduled for May-June 1976. Seasonal coverage will continue in FY 1977.

INFORMATION PRODUCTS

The horizontal and vertical distributions of methane, ethane, ethylene, propane, propylene, butane and isobutane will be observed and the reduced data depicted in tabular form as a function of depth. Data will be transcribed onto standard IBM cards in whatever format is convenient for NODC/EDS and submitted to the project office. It is assumed that the cost of data translation to 9-track tape will be borne by the project at no cost to this contract. Ancillary temperature and salinity measurements will be taken from STD casts at each station. From these data, horizontal and vertical distribution maps will be constructed and compared to solubility distributions. Source indicative ratios, such as $C_1/(C_2+C_3)$, will also be tested.

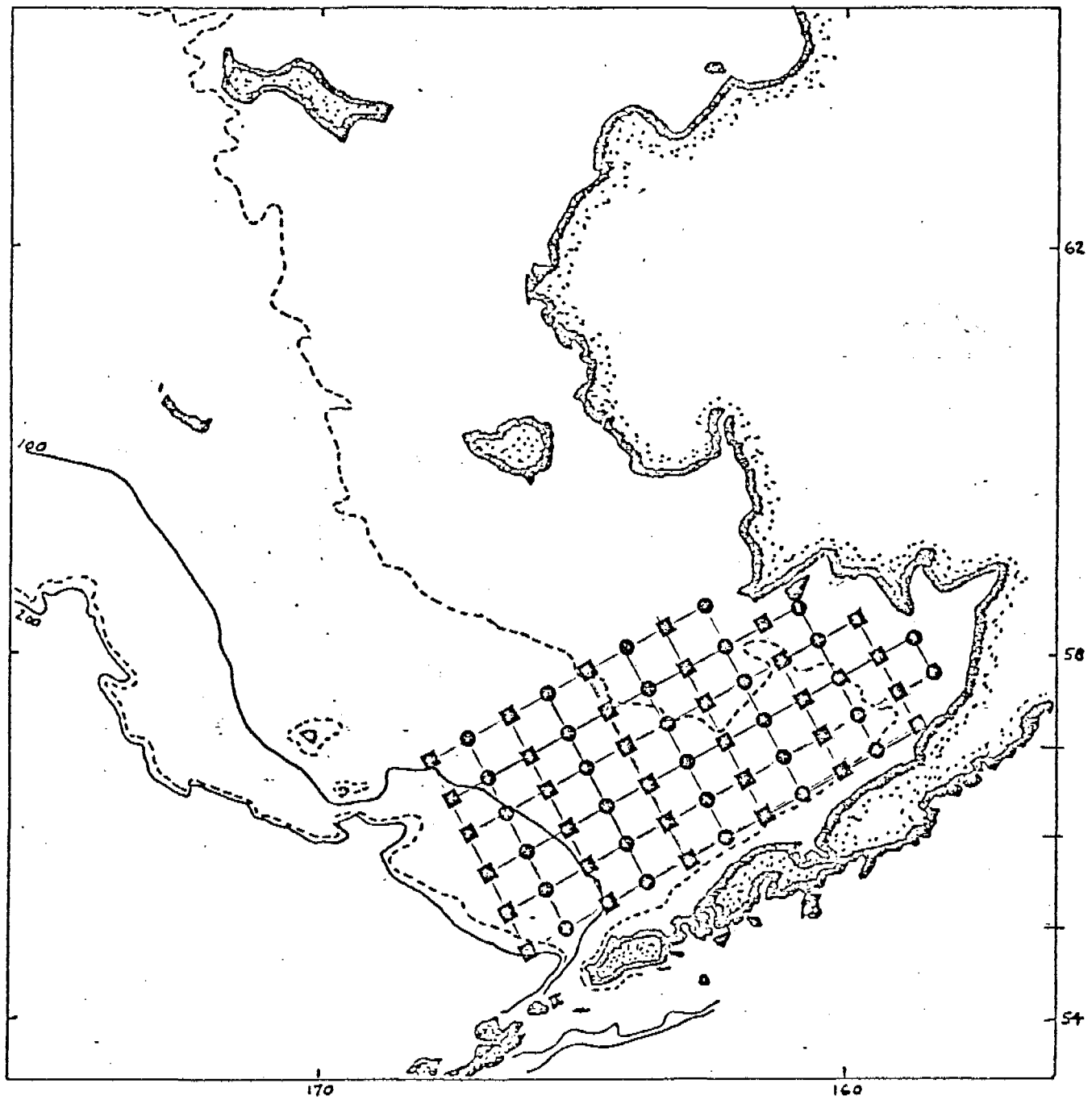

Figure 5. Proposed sampling station grid, Bristol Bay.

DATA INTERFACE

Ancillary data requirements include temperature and salinity, which willbe acquired with an STD system. These data will be obtained at the time of sampling and are required to calculate saturation levels of the individual hydrocarbons. It would also be desirable to obtain productivity measurements to correlate with hydrocarbon levels in the surface layers.

Locations of known gas and oil seeps would be extremely valuable.

SCHEDULE

Water samples will be acquired in conjunction with other PMEL programs and analyzed on board ship. All data, including temperature and salinity, will be processed and available to EDS or NODC within 60 days of the termination of the cruise. Depending on the precise cruise schedule, quarterly reports will be brought up to date with new data as it arrives. As seasonal trends are noted, these data will also be reported.

EQUIPMENT REQUIREMENTS

Special equipment required to carry out these activities include a gas chromatograph, gas extraction system, and data processing unit. The lead time for the GC and DPU system (see Figure 2) can be built from locally available parts in approximately 15 to 30 days. We anticipate that about 1-2 months will be required to bring the analytical system on line after all components are on hand. That is to say, in order to be field operational by late Summer 1975 (Aug-Sep), initial funding must be "in hand" by June 1, 1975.

To cover this eventuality, a complete system is being ordered for the Bering Sea hydrocarbon project under FY 1975 funding, and will be deployed until the second system is purchased and developed.

Appropriate spare parts, such as circuit boards, and backup components will also be requisitioned to minimize component failure in the field.

LOGISTICS REQUIREMENTS

All sampline will be conducted from a conventional oceanographic vessel, capable of handling 10 l rosette array of Niskin® samplers. Analysis must be carried out in a dry, ambient temperature laboratory (20-25°C) equipped with bench space and 110v, 50-60 Hz regulated power. The gas chromatograph requires regulated voltage control and should not be operated from general ship's power for best results. We will provide voltage regulation, but experience dictates that circuits should be used that are not subject to frequent voltage surges.

Approximately 30 ft^2 of bench space is required for the GC, DPU, and gas extraction system. Additional space must be provided for tank gases (large cylinders, 220 ft^3), which provide carrier flow and fuel for the flame ionization detector (FID). A total of six tanks of gas (2 each helium, hydrogen, and air) will be required for each cruise.

The above analytical system will be modularized and shipped by air to points of departure, or remain on board the vessel as future response commitments dictate. The total instrument package will weigh approxiamtely 200 lbs, not including tank gases. These must be purchased locally, or be stockpiled on board ship for later use.

Each cruise will be staffed with at least two (2) chemists to carry out the analysis. We would anticipate that deck sampling could be carried out by a member of the ship's technical staff. Principal investigators and possibly NOAA Corps officers will be used to supplement field activities as needed.

When required, personnel and equipment will be sent by commercial carrier. Lodging facilities must be provided for personnel en route to the ship.

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WORK STATEMENT

I TITLE

Natural distribution of trace heavy metals and environmental background in three Alaskan shelf areas.

II PRINCIPAL INVESTIGATOR:

Dr. David C. Burrell
Associate Professor
Institute of Marine Science
University of Alaska
Fairbanks, Alaska 99701
(907) 479-7768

III GEOGRAPHIC AREA AND INCLUSIVE DATES

Gulf of Alaska	July 1, 1975 - September 30, 1976
Bering and Beaufort Seas	May 1, 1975 - September 30, 1976

IV COST SUMMARY

FY 1975, through June 30, 1975	$48,511
FY 1976-77, July 1, 1975 - September 30, 1976	$413,716

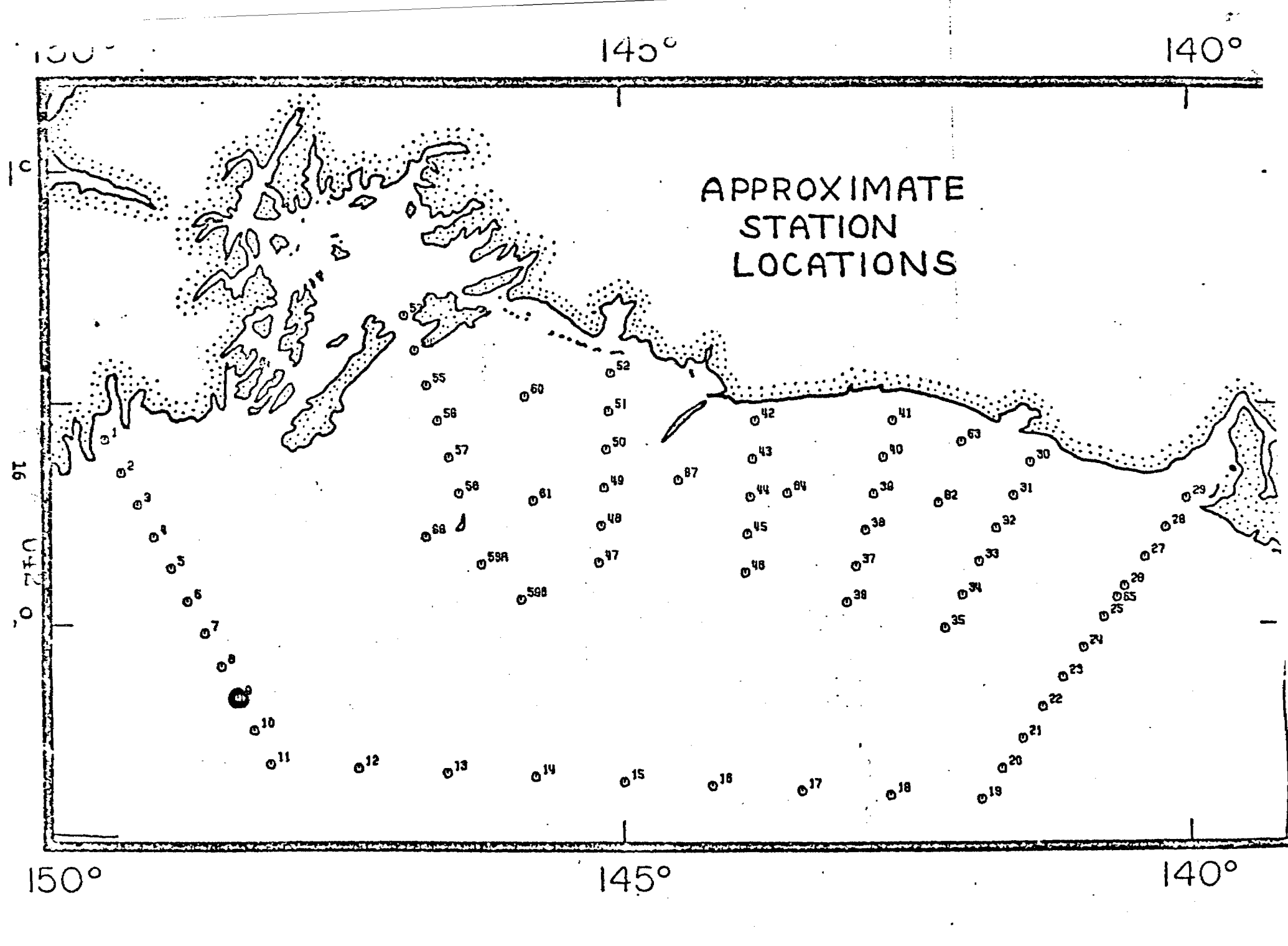
V PROPOSED RESEARCH

A. Background and Objectives

1. Tasks addressed:

Primary objectives: Tasks A32 and A33

Secondary objectives: Tasks B8, B10, B11 and C1 leading towards, but not specifically addressing, B7.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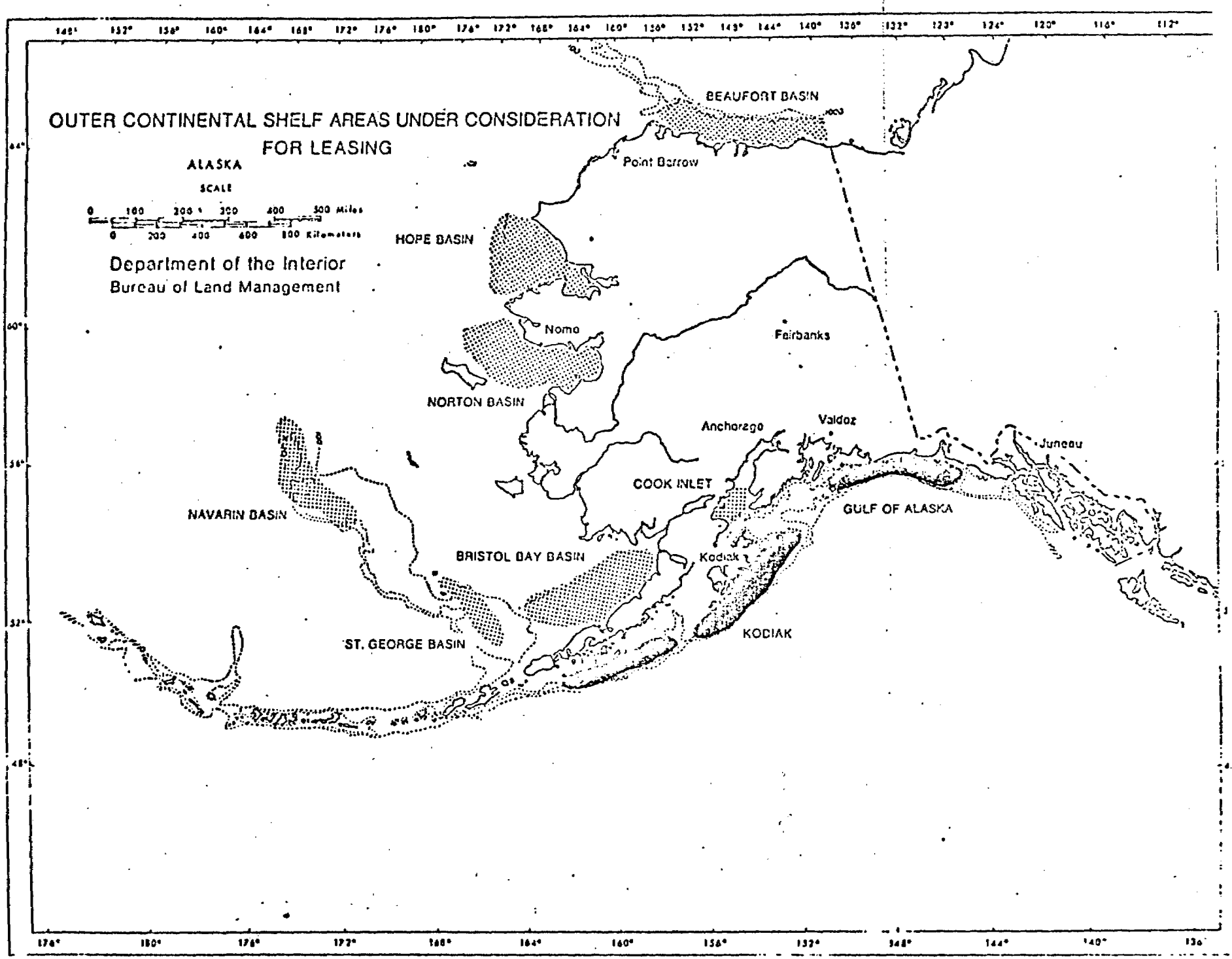
OUTER CONTINENTAL SHELF AREAS UNDER CONSIDERATION FOR LEASING
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Department of the Interior
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Point Barrow
HOPE BASIN
Nome
NORTON BASIN
Fairbanks
Anchorage
Valdez
Juneau
COOK INLET
GULF OF ALASKA
NAVARIN BASIN
BRISTOL BAY BASIN
Kodiak
KODIAK
ST. GEORGE BASIN

2. Objectives:

The primary objective of this program is to characterize the trace metal contents of sea water, sediment and selected indigenous animal and plant species in the three defined study areas; the Gulf of Alaska, the Bristol Bay Basin region of the Bering Sea, and the Beaufort Basin region of the Beaufort Sea as shown on the accompanying index map. These data are required to define the natural baseline conditions existing prior to industrial activity in these areas, against which potential perturbations may be measured. This work will concurrently provide the necessary background information for studies describing the chemical character, pathways and rates of transport between the system components. This latter aspect of the research, which should extend over many years, described in the Workshop Proceedings (Seattle, January 1975), has not been supported through Fiscal 1976 to any extent. Since, however, this work will be required eventually, the current characterization program has been designed in large measure to provide the best framework to enable us to pursue these process studies in subsequent years. Similarly, our assigned resources will not permit laboratory experimentation on the effects of anthropogenic hydrocarbon additions to the natural environment (Task B7). Again, this present program has been planned - in terms of the metals selected for study and the solid phases emphasized - to enable this work to be

speedily initiated once funding is approved.

The sediments are by far the most quantitatively important reservoirs for heavy metals in the marine environment. We believe that, from the point of view of trace metal distributions, the major impact from OCS resource development will result from perturbations of the chemical environment of the surface sediments. Other effects such as mechanical disturbance of sediment (which may itself induce chemical changes) and injection of, for example, formation waters into the seas, are likely to impact more localized areas. These concepts have been discussed many times in previous OCS symposia.

The primary aim in dealing with the sediments should be to characterize the trace metal contents of those fractions which can be extracted from the sediments using various chemical treatments, i.e., inorganic and organic extractants. Such "available" fractions are potentially liable to solubilization as a result of the type of man-induced impacts which are the concern of this OCS study. Unfortunately, it appears that at the present time various factors (such as the need to divert resources to obtain geological data and the large number of elements required to be studied) will necessitate obtaining mostly "whole rock" trace metal data during this initial contract period. This will be the

case for those elements to be determined by neutron activation analysis (see Section V, B4), although "extract" data will be obtained for Cu, Zn, Fe, Mn, Ni, Cd using atomic absorption analysis.

The previously proposed "Sources and migrations..." study has been extended to include the southern Bering Sea and is also incorporated into this proposal. This sub-project (hereafter referred to as the "sediment dynamics" work) is based upon a total analysis of the sediment for a suite of both major and minor constituent elements (see Section V, B4 below). This work will attempt to relate elemental contents and ratios to provenance and transport factors.

Remobilisation of heavy metals from the sediment "sink" would increase contents in solution potentially available for uptake by the biota; hence the emphasis on benthic indicator species in this project. It should be noted that current resources will not permit either determination of the various chemical species of the metals in the various phases, or research on transfer mechanisms to and from the biota. This research must be deferred until subsequent years and presumably will be then financially restricted to selected sub-systems in quite localized areas.

The natural trace metal contents of the sediments, and their

behavior under stress, is, of course, a function of the compositional character of the sediment; of, for example, the particle size distribution, mineralogy and organic content. Our experience on Gulf of Alaska samples to date has been that these ancillary geochemical data have not been forthcoming from other sources; neither does it appear that this situation will be markedly different in the coming year. For the program proposed here, therefore, it seems to be necessary to divert a not inconsiderable part of the allocation from trace metal chemistry *per se* to provide simple size fractionation and clay mineralogy data for many of the samples selected for trace metal study.

The elements to be studied in this program have been selected based on the following criteria:

a) Metals recognized as major pollutants affecting man: Ag, As, Cd, Cu, Hg, Pb, Sb, Se, Zn.

b) Natural petroleum "index" metals: Cr, Ni, V.

c) Indicators of chemical environmental changes to the sediments: Fe, Mn, Co.

It is important to note that this list of elements, chosen on the criteria discussed above, is considerably larger than that generally selected by participants in other continental OCS programs. These latter programs appear to be geared to the concept that only metals likely to be derived directly

from the oil, formation waters or constructional impact (eg. Pb from paint) are of importance. By extending this number of elements to be studied it has, of course, been necessary to very drastically restrict the number of stations, biota species, etc. which can be examined. Each trace metal added to the list involves new demands on processing and analytical techniques, on personnel and equipment. For example, although values for selenium and mercury can be obtained in some phases by neutron activation analysis at no, or little, additional cost, a complete investigation of the distribution of these elements between all phases (sediment fractions, water, biota) has necessitated the services of two additional senior investigators, new equipment demands, etc. It is important that this trade off between elements and the total number of samples likely to be examined be appreciated at this time.

3. Current state of knowledge:

There are a few very scattered series of trace metal values available for these study areas. All relate to a very restricted number of metals in one or two phases or species only, and in isolated localities. Such data, both published and unpublished, available mainly from University of Alaska and various federal agency personnel, will be collected together insofar as is possible and presented in the final report as noted below.

Some background values for grain-size distribution and the clay mineralogy of the sediments exist for shelf areas of both the Bering and Beaufort Seas. Lithological variations in the Beaufort have been described by Carsola (1954), Naidu (1974), Barnes and Reimnitz (1974) and Naidu and Mowatt (1975). Size distribution data for Bering Sea sediments have been summarized by Knebel *et al.* (1974), Nelson *et al.* (1974) and Sharma (1974). No equivalent reports have been published for the Gulf of Alaska. Moll (1970) and Matthews (1973) have described the clay mineral assemblages from bottom sediments of the Chirikov Basin of the north Bering Sea and the Yukon River estuary, respectively. Clay mineral dispersion patterns on the Beaufort Sea shelf have been given by Naidu *et al.* (1971) and Naidu and Mowatt (1974). Transportation and geochemical aspects of the Bering Sea sediments have been addressed by Askren (1972), Sharma (1972, 1974) and Sharma *et al.* (1972). Only preliminary data reports are currently available on the geochemistry of sediment in the Gulf of Alaska study area.

4. Information required:

The primary Task Objectives (A32 and A33) are concerned with a baseline survey of the heavy metal contents of various "reservoir" phases in the three study areas. The samples required to address these objectives are discussed below.

considered below (Section V, B3). In the Bering Sea also we look to this group to establish the most suitable primary station sampling grid in Bristol Bay since it appears that we cannot tie in with a hydrographic network as in the Gulf.

b) We require some cooperative help from the above referenced working group in collecting and identifying the agreed upon benthic species, particularly on cruises specifically designed for trawl and dredge sampling. (See Section VII below)

c) We require equivalent help from the intertidal benthic working group in collecting and identifying suitable biota and in selecting the most suitable taxonomic groups. We must rely totally on personnel from this group to forward sample splits to us since no resources are available to enable any of our personnel to participate on these latter sampling expeditions. (See Section VII)

d) We would appreciate input as noted in (a) and (b) from the biologists working with pelagic fin-fish. However, as noted elsewhere, collection and analysis of these samples is considered second priority to the benthic program. (See Section VII)

e) We would appreciate similar input as noted in (a) and (b) above from the biologists working with planktonic populations. Trace metal contents of the primary

5. Performance:

Task Objective A33 is quite open-ended. To the extent delineated by the sampling plan outlined below, and for the metals listed above, this objective will be completed by September 30, 1976 provided that the essential information, data and samples from the allied programs discussed below are forthcoming on schedule. Data from this Task Objective will provide the required background for Task Objectives B7 and B8. These "process study" objectives have not, however, been specifically funded at this time and no substantive data will be generated by September 30, 1976. Task Objective B10 will be completed as regards particle size characterization and clay mineralogy for the "trace element sample stations" described below. Task Objective B11 will be addressed to the extent of determining the bulk chemistry of some 20-30 samples in both the Gulf of Alaska and Bering Sea. The results of the literature searches (A32 and C1) will also be available by September 30, 1976.

6. Inter-project coordination:

The work discussed in this Work Statement can only be accomplished in cooperation with many other contemporaneous projects as follows:

a) We require information from the open-ocean benthic biologists regarding choice of the best "indicator species" to analyze in all three work areas. This is

producers can be valuable indicators of "availability" in the water column as noted below in Section V, B3. Unfortunately, plankton samples are difficult to process free of contamination and, since mixed species are collected, qualitative and quantitative expert identification is required. Samples of this type must be collected contemporaneously with water column samples.

f) We require samples to be collected for us by the marine mammal biologists. Sub-samples of organs (liver, etc.) are preferable. At the present time the NOAA management personnel have not coordinated the necessary contracts with the mammal biologists. The particular importance of food chains involving many of the marine mammals native to Alaska is noted below (Section V, B3).

g) We require as complete a sedimentological, mineralogical and geochemical description as possible of the sediment samples from which extracts will be taken for trace metal analysis. Since it appears that the geologists are not, for some reason, making collection of this data a top priority it has been necessary in this Work Statement to divert some of the chemistry money to obtain sand-silt-clay ratios for all our "primary" trace metal samples from the Gulf and Beaufort Sea and clay mineralogy data for all three areas. Dr. C. M. Hoskin's geological program in the Bering Sea will provide detailed size fractionation data for this area.

We would, obviously, appreciate any other auxillary geochemical data from any other ongoing geology programs. These data help in interpreting trace metal distributions in the sediment and are not required until late in the program, during the interpretive phase.

h) Information concerning the organic chemical character of the bottom sediment is similarly required. We would hope to have sediment sample collection coordinated with the hydrocarbon working group. Our data would naturally complement the work proposed by Dr. I. R. Kaplan and vice versa.

i) In order to eventually apply the data to be obtained under the auspices of this first year program to Task Objective B8 in subsequent years, we shall require the research results of the physical oceanographic group, and particularly dissolved nutrient data. We have designed our current sampling program in the Gulf of Alaska to conform to the hydrographic grid and this pattern will be maintained wherever possible in subsequent years and field areas.

For this 1975-76 program also, it appears that, in the absence of ship support, it will be necessary to attempt to collect our water and sediment samples on the transects and stations proposed by Dr. K. Aagaard in the Beaufort Sea.

j) The "sediment dynamics" portion of this project in the Gulf of Alaska and Bering Sea will necessitate close cooperation with the work proposed by Drs. Feely and Kline particularly with regard to the geochemistry of the suspended sediment discharged from the Copper and Kuskokwim Rivers. (See also Section XI)

k) It is essential that the work proposed here be closely coordinated with the NBS project "Research and evaluation of trace element methodology for the analysis of water, sediments and marine organisms" particularly with regard to standardization and optimization of analytical techniques, distribution of interlaboratory standards etc. This is considered further below in Section V, B4.

Cooperation between us and the various diverse projects listed above can only be accomplished through the various NOAA management offices.

B. Methods

1. Historical data:

Part of the resources of this project will be assigned to a literature search. This is noted further below in Section VI.

2. Sampling schemes:

a) GULF OF ALASKA

General:

It is intended to continue to tie in to the standard

hydrographic grid shown on the accompanying figure during 1975-1976 as has been our practise during the current contract year. In particular, we have attempted to occupy the "primary" stations at which dissolved nutrient profiles have been determined. Many biological programs have utilized this network also. During the coming contract period some 25-30 standard "trace-metal stations" will be samples, but this will include coverage also of the western extension of the present study area. The exact grid has not yet been precisely formulated by the physical oceanographers but a series of traverses normal to the Aleutian Chain appears to be planned.

Water:

Water column depths will depend upon the water structure; in general this means one surface sample, one below the halocline and another close to the bottom. Replicate filtered samples are drawn from each over-the-side bottle. The latter containers are specially aged and individually monitored bottles reserved exclusively for trace metal sampling. It will be necessary to purchase additional specialized, non-metallic samplers for this enlarged program.

It is not intended to sample on a seasonal basis. The sophisticated analytical techniques required preclude

generating large bodies of data and circulation patterns will not be known in any detail until much later. There are almost no precedents for attempting to monitor potential seasonal fluctuations in open-ocean areas. Such a program would necessitate chemical species differentiation at the very least and is felt to be unjustified as part of the current project.

It has, however, been decided to establish a single "standard station" for the second year program in this area. At this station a more detailed profile will be obtained and exploratory work regarding the desirability of seasonal data can be begun. Preliminary discussions with the physical oceanographers suggest that either Station No. 9 (see chart) or one of the 33-38 block would be most suitable, at least as regards the hydrographic conditions as presently understood.

Sediment:

Sediment samples will be collected once only on the extended hydrographic (physical oceanographic) grid. This is a continuation of the program already commenced this year. All samples to date have been taken contemporaneously with sediment retrieved for the benthic biology and organic chemistry programs. Unfortunately, we have not yet received any complementary sedimentological data from the geological program; nor

does this input appear likely. For the second year's work in this area we shall be forced to obtain simple size fractionation and other geochemical parameters needed to correlate with the determined "available" trace metal contents and whole-rock analysis. It is intended to process samples from some 25-30 localities for trace metals and this ancillary sedimentological information.

For the "sediment dynamics" work, it will probably be necessary to diverge from the "standard grids" to establish transects keyed to the plume discharge area from the Copper River. Sampling here should probably be tied in with the suspended sediment program directed by Dr. Feely. It may also be possible, if resources permit, to obtain total trace metal contents of the suspended material for a few elements.

Biota:

Biota samples, selected based on the criteria briefly outlined below, can only be collected on an "opportunity" basis, and hence we have little control over the temporal and spatial sampling patterns. Wherever possible we require, as explained elsewhere, species which can be dissected, and these can generally only be collected as a second priority on the single purpose trawl/dredge cruises. Coordination with the relevant biological

personnel is discussed below in Section V, B3.

b) BERING SEA

General:

The rationale for the primary and secondary sampling pattern will be essentially as described above: a standard network of some 25-30 stations in the southern Bering Sea (predominantly Bristol Bay) closely coordinated with hydrographic and benthic biological stations, and "special interest" patterns, for example, associated with discharge from the Kuskokwim. Initial establishment of the standard grid has been hindered as compared with the Gulf of Alaska because of a lack of a clearly defined hydrographic grid. We are cooperating, during these planning stages, with the benthic biology program.

Water:

Water samples will initially be collected at one or two depths only on a grid covering the entire study area. Later - as noted above for the Gulf - we shall probably designate a more detailed "standard station".

Sediment:

Sediment samples will be collected on a one time only basis from some 25-30 stations in the primary study area extending out from Bristol Bay with additional coverage adjacent to the Kuskokwim for the "sediment

dynamics" work. We do not believe that seasonal coverage is justified at this stage although, judging from our experiences in the Gulf, several cruises may be required to complete the grid.

It is planned to obtain sediment (20-30 samples) using a special purpose (HAPS design) shallow corer. Sediment will be collected for both whole-rock and extract data and it is hoped to squeeze sub-samples for interstitial water.

Details of the "sediment dynamics" sampling scheme are not known at present. Sampling will be coordinated with the suspended sediment program wherever possible and small boat and/or helicopter support may be required as noted in Section XI.

Biota:

Biota collection poses the same problems as for the Gulf. This is considered below.

c) BEAUFORT SEA

We are logistically limited in this area. The sampling scheme is entirely dictated by cruise tracks of available ice-breakers or some other means as may be devised by the project office. We have tentatively agreed to coordinate with the hydrographic grid design

by Dr. Aagaard provided that sampling equipment can be properly deployed and that no chemical contaminants are introduced.

3. Index species and biota sampling program:

As noted several times previously, we are entirely dependent upon the biological personnel for collecting the majority of samples. The accompanying table indicates the species suggested for initial analysis together with the personnel who have agreed to collect for us (see also Section VII).

"Index species" for a baseline monitoring program may be chosen for a variety of reasons. Some of the criteria used for this Alaskan program are as follows:

a) In keeping with the objective of the overall "impact" program, we believe that biota of importance (or potential importance) to man as a food should be covered. At the same time it is important wherever possible to select species which can be dissected to enable the metal contents of various parts of the organism to be determined separately. We are currently attempting to work with tanner crab in the Gulf and intend to utilize other such species in the Bering Sea.

b) The sediments constitute the major trace metal reservoir. It is important, therefore, to emphasis organisms most intimately associated with this phase. We propose to

Biota species selected and to be collected by

O. C. S. biologists for trace metal program.

	GULF	BERING	BEAUFORT
Fin Fish	Rock sole (1)	Rock sole (1)	
	Pollack (1)	Pollack (1)	[Arctic cod Mesiolotea Amphipods Mysid (4)]
	Salmon (2)	Salmon (2)	
Crustacea	Tanner crab (1)	Tanner crab (1)	
	King crab (1)	King crab (1)	
Gastro	Neptune (1)		
Clams	Scallop (1)	Macoma (1)	
	Mytilus (3)		
	Razor (2)		
Macroalgae	Fucus (3)	Fucus (3)	

Collectors:

(1) N. W. Fisheries Center

(2) Alaska Department of Fish & Game

(3) N. M. F. Auk Bay

(4) O. S. U.

analyze several open-ocean and intertidal benthic species as shown in the table. We shall be at some time interested in looking at those species which ingest sediment. Present experience in the Gulf suggests the suitability of sea cucumber, for example.

c) Most cited "concentration factor" data for metals in organisms over coexisting water contents are fallacious because the animal probably acquires its trace metal burden from lower trophic organisms. Phytoplankton and macro-algae extract directly from the water and the latter particularly are frequently used as sensitive indicators of aquatic pollution (see Burrell, 1975). Kelp will probably be collected by the inter-tidal working group personnel and we shall attempt to collect mixed plankton samples on some standard hydrographic station.

d) Pelagic fin-fish have lowest priority, mostly because any anomalous metal contents may not be easily associated with a specific geographic area. One advantage to this group however is the greater abundance of available comparatory data from other areas; for Pacific Hake for example (Naidu and Cutshall, 1974). We are dependent on biological trawling operations for samples from this category.

e) Marine mammals are an important food source for many people in Alaska, and there has already been some concern regarding trophic level transferals of heavy metals

involving some of these mammals. It is felt to be important to look at a food chain such as clam-walrus; this is short and involves potential transferal from the benthic environment to a human food source.

It seems to be impossible at the present time to design a biota sampling program based on any reasonably cogent scientific criteria for the Beaufort Sea. We feel that all that can probably be accomplished is analysis of any samples which can be obtained for us of virtually any variety so long as the collection and storage treatment has been adequate to prevent contamination or changes in the *in vivo* metal contents and distributions.

4. Analytical Methods:

Because of the large number of metals to be covered in a variety of natural phases, at least four different analytical techniques must be used for this program. This will result in duplication of effort in some cases as listed below. This latter may provide some interlaboratory calibration (see discussion of this topic at the conclusion of this section). Some parts of the proposed analytical methods are still relatively new and inevitably there will be some analytical development work during the course of the project.

a) Cd, Cu, Pb and Zn in sea water by thin-film anodic stripping voltammetry (Burrell and Lee, 1975). This

method is not ideal for Pb and we will attempt to intercalibrate with some lab which specializes in this element.

b) Ag, As, Co, Hg, Fe, Mn, Se, Sb and V in water, biota and total sediment by thermal neutron activation analysis (Robertson and Carpenter, 1974).

c) Additional Cd and Zn analysis in sea water by carbon filament atomic spectrometry (Burrell *et al*, 1973).

d) Se and Cr in sea water, biota and sediment extracts by gas chromatography (sub-program directed by Dr. T. Gosink). Parts of this work will duplicate the work proposed under (b) but different handling techniques and analysis methods are involved.

e) Additional Hg concentrations in biota by ambient temperature flameless atomic absorption (eg. Burrell, 1974).

f) Cu, Pb, Zn, Cd and Ni contents of biota and some interstitial water by furnace atomic absorption. Also these elements, plus Fe and Mn, extracted from sediments by the same analysis procedure (eg. Burrell, 1974).

g) Ni in sea water by conventional flame atomic absorption (eg. Brooks *et al*., 1967).

h) Whole rock sediment analysis for Na, K, Ca, Mg, Mn, Ba, Sr, Co, Ni, Cu and Zn in support of the "sediment dynamics" program (directed by Dr. G. D. Sharma) by

atomic absorption analysis (Riley, 1958).

The accompanying table summarizes the primary instrumental technique to be employed for each metal in the major phases.

Each technique necessitates a specialized pre-analysis treatment as described in previous documentation and in the above cited references. The "sediment extract" procedure to be employed is of particular importance since this treatment defines the fraction analyzed and reported. The various possibilities are discussed at length elsewhere. It is intended to initially use the treatment advocated by Chester and Hughes (1967), mainly because of the widespread use to date which facilitates comparative studies.

Grain size analysis will be restricted to the determination of gravel, sand, silt and clay percentages by standard sieve and pipet analysis. Clay mineral analysis will be by the methods described by Naidu *et al.* (1971) and Mowatt *et al* (1974). Data interpretation will utilize parts of the work of Biscaye (1965) and Pierce and Siegal (1969). Some Co samples total will be run for clay mineral information. Each involves fast and slow scans on glycolated and Mg and K saturated samples; i.e., in excess of 700 diffraction patterns.

Primary Methods of Analysis

	Seawater	Biota	Sed extracts	IW
Silver	NAA	NAA	NAA	
Arsenic	NAA	NAA	NAA	
Cadmium	ASV	FAA	FAA	
Cobalt	NAA	NAA	NAA	
Chromium	GC	GC	GC	
Copper	ASV	FAA	FAA	
Mercury	NAA	FAA	NAA	
Iron	NAA	NAA	FAA	
Manganese	NAA	NAA	FAA	
Nickel	AA	AA	AA	
Lead	ASV	FAA	FAA	
Antimony	NAA	NAA	NAA	
Selenium	GC	GC	GC	
Vanadium	NAA	NAA	NAA	
Zinc	ASV	FAA	FAA	

One facet of the analysis program which should be of prime concern is that of the accuracy and precision of the values generated. This is particularly important in an environmental program of this type since the concentration ranges encountered are frequently at the detection limit of the analysis procedure used, and the matrices are very complex. Each investigator associated with this project is emphasizing different analytical techniques so that inter-calibration is quite difficult. Some duplication is included however, as noted above, and the accompanying table attempts to illustrate a few cases where intra-project comparisons may be possible. However, it is felt that these topics fall within the province of the National Bureau of Standards project.

"Research and evaluation of trace element methodology for the analysis of water, sediments and marine organisms". It is assumed that a close liaison can be maintained with personnel associated with this project. Funds have been allocated to permit some of our analysts to visit the NBS facilities in Washington, D. C., and we hope to benefit from their methodology researches, and to establish and exchange standardization samples.

It is understood that NBS has initially proposed to establish independent analysis programs for Ni, V, Pb,

	NAA	FAA	Low T AA	ASV	GC
WATER COLUMN					
Cadmium		x		x	
Copper				x	
Lead				x	
Selenium	x				x
BIOTA					
Arsenic	x		(x)		(x)
Mercury	x		x		x
Iron	x	x			
Selenium	x				x
SEDIMENT EXTRACTS					
Arsenic	x		(x)		(x)
Cadmium		x		x	
Iron	x	x			
Selenium	x				x
Zinc		x		x	

Mn, Zn, Cd, Hg, As, Se, Cr, Fe, Co and Sn in sea water and for total sediment contents. We have arranged to submit splits of approximately 10% of our samples to this program. This program, as presently constituted, will not benefit the analysis programs concerned with biota and extracts from the sediments.

VI INFORMATION PRODUCTS

Historical data concerning the distribution of the metals of interest to this program within the three study areas will be tabulated and discussed in the final report. Similar information will be provided on the grain size distribution and clay mineralogy of the shelf sediments.

All new data collected under the auspices of this project will be provided in tabulated form with graphical and map representation where appropriate. We cannot accomplish transcription of data to magnetic tape format within the existing budget. It is expected that the project managers will indicate any special format for parts of our data which may be required by other investigators.

The work discussed in this Work Statement, and hence the expected products, is predicated on an April 1, 1975 starting date so that not all the work originally envisaged may now be accomplished prior to September 1976. The effect of this delay will be minimized if funds budgeted for the April through June 1975 period are freely transferable into the following financial year. However, we are now

rapidly approaching the point at which the ability to collect samples will become limiting (see also Section XI).

VII DATA OR SAMPLE EXCHANGE INTERFACES

The major input we shall require from other investigators is help with the selection and collection of organisms for analysis. This is essential and has been discussed in detail above. The following investigators are understood to be the contact personnel for the organizations keyed on the biota collection table given previously:

a) N. W. Fisheries Center - Dr. Pereyra

b) A. D. F. & G. - Dr. Pennoyer

c) NMF Auk Bay - Dr. Zimmerman

d) O. S. U. - Dr. Carey

Input from the geological teams would be useful, particularly as regards additional sedimentological and geochemical descriptions of both the bottom and suspended sediments. As noted above, the water column sampling is to be tied in with the physical oceanographic - nutrient chemistry program. To some extent, hydrographic data will be required contemporaneously with the water sampling for this program so that the best depths can be selected. Any future attempt at interpreting the dynamics of the soluble contents will require nutrient concentration values at the very least.

VIII SAMPLE ARCHIVAL REQUIREMENTS

Storage of samples of all three phases - water, biota and sediment - will be necessary since it is expected that the majority of this

material will be collected during the summer of 1975 and analyzed through the subsequent year. Both sediment and biota samples must be kept frozen without chemical treatment, so that freezer storage space will be at a premium. The storage treatment for water samples is largely a function of the subsequent method of analysis. Samples for Se and Cr must be analyzed immediately on retrieval at sea as noted further below. In general, samples for processing by other procedures may be preserved and retained for reasonable periods of time before analysis.

We have previously pointed out the desirability of retaining samples for periods beyond the lifetime of this present contract period. This would provide the means for generating additional data if such were ever required. No funds have been specifically allocated for this use however.

IX The following work schedule is given in as much detail as seems presently possible given the continuing delay in the initiation dates and the field collection uncertainties.

A. Gulf of Alaska and Bering Sea:

Summer 1975 - Field work completed as far as possible.

Fall through Winter 1975 - Processing and analysis of samples. Literature search.

Summer 1976 - Summation and publication of results. Additional field work as function of any new contractual requirements.

B. Beaufort Sea:

Logistic support in the Beaufort Sea is currently an entirely unknown entity so that no work schedule can be proposed.

X EQUIPMENT REQUIREMENTS

Equipment items required to support this proposed program are listed in the adjacent table in two groups: items needed before and subsequent to the initial field work period (July 1, 1975). It should be noted that much of the analytical work required by this program is quite complex and time consuming. A long lead time is required in order to aquire and set up some of the items, such as the spectrophotometer. This is particularly critical in the case of Se and Cr analysis which must be accomplished immediately on retrival at sea. Equipment (i. e. the listed GLC system) must be ordered several months before the proposed field work.

XI LOGISTIC REQUIREMENTS

A. Initial field work requirements for this program have been granted by the project management as given in the accompanying table. The 7 days Copper River plume cruise is in support of the "sediment dynamics" portion of this work.

EQUIPMENT REQUIREMENTS

Required before July 1, 1975	Required after July 1, 1975
Water sampling bottles	Interstitial water squeezers
Electroanalytical equipment	C analyzer
Atomic spectrometer	Drying overn
Analog recorder	Portable freeze-drier
Analysis bombs	Gravity corer
Drying oven	Bench centrifuge
Hop plate	Chemical balance
GLC system	
Digestion apparatus	

Ship support for trace metal chemistry program tentatively scheduled as of May 7, 1975:

MONTH		GULF	BERING	BEAUFORT
1975	July			
	August	21 days E & W 7 days Copper River Plume		
	September		14 days	
	October			
	November			
	December			
1976	January			
	February			
	March			
	April			
	May			
	June			
	July			
	August			
	September			

B. Additional work may be required on other "major vessel" scheduled cruises in the Gulf and Bering Sea. These requirements are not known until the products of the listed cruises are known.

C. An additional, so far unscheduled, cruise is required in support

of the "sediment dynamics" work on a vessel capable of sampling the outflow region of the Kuskokwim; or helicopter and/or small boat support from some larger vessel. This is the Bering Sea equivalent of the Copper River plume cruise already scheduled. Both these "plume cruises" should be coordinated with suspended sediment project personnel (Feeley).

D. Our primary need in the Beaufort Sea is for a vessel capable of traversing the area and handling large sediment coring devices and water samples.

The three sections proposed by the physical oceanographers (Aagaard) at 142°, 147° and 153° are quite acceptable scientifically so long as the sampling and processing operations can be correctly accomodated.

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WORK STATEMENT #190

I. Title: Baseline study of microbial activity in the Beaufort Sea and Gulf of Alaska and analysis of crude oil degradation by psychrophilic microorganisms

II. Principle Investigator: Richard Y. Morita, Professor of Microbiology and Oceanography, Department of Microbiology, Oregon State University, Corvallis, Oregon, 97331

Co-Investigator: Robert P. Griffiths, Research Associate, Department Of Microbiology, Oregon State University, Corvallis, Oregon, 97331

III. Geographic Area and Inclusive Dates: Beaufort Sea and Gulf of Alaska; May 1, 1975 - September 30, 1976

IV. Cost Summary:

FY 1975	FY 1976
$28,560	$81,440
	600*
	$82,040

*Additional air fare required because of expanded sampling schedule. (See section XII).

V. Proposed Research

A. Background and Objectives:

Our first objective is to determine the relative heterotrophic activity, and population density of microorganisms in the Beaufort Sea and Gulf of Alaska (task number A-27). We propose to conduct field studies during the summers of 1975 and 1976 and the winter of 1976 (February) which will give us preliminary data on the concentration and activity of the natural microorganisms in these waters. To obtain a complete picture of both the temporal and spacial variations in these parameters, studies beyond these sampling periods will have to be undertaken. Water and sediment samples will be taken according to the schedule outlined under section IX of this proposal.

Our second objective is to isolate and characterize psychrophilic hydrocarbon utilizing microorganisms which are present in the Beaufort Sea and Gulf of Alaska (task number B-9). Due to the seawater temperatures encountered in these waters, a basic understanding of the function of these organisms must be obtained before biodegradation of crude oil in the Beaufort Sea and Gulf of Alaska can be understood. Isolates will be taken from enrichment cultures made with sediments and crude oil taken from this region. Once isolated in the field,

these cultures will be transported under low temperature conditions to our laboratory at Oregon State University. It is impossible to determine at this time how much of this basic information we will be able to obtain before September 30, 1976, but certainly preliminary data will be available which will be helpful in the making of management decisions.

Our third objective during these studies is to determine the acute and chronic effects of crude oil on the heterotrophic activity of microorganisms in the Beaufort Sea and Gulf of Alaska (task C-2). These studies will be made in conjuction with culture enrichment studies (isolation of psychrophilic hydrocarbon utilizing microorganisms and, by the end of this study period, should give us a reasonably good evaluation of at least the acute effects of crude oil on heterotrophic activity.

During these studies, we will be pooling our efforts with those of Dr. Atlas and his associates. We will be working as a team analyzing same samples using our respective techniques. This will effectively eliminate unnecessary duplication and produce data that can be directly compared. This approach will produce the most comprehensive picture of microbial function possible under the present budgetary limitations.

In addition to the data collected by our team, we would like to obtain other types of data which can be used in the evaluation of our findings (these are listed under section VII of this proposal). Wherever possible, we would like to obtain these data from the same samples that we use for our heterotrophic potential studies. Nutrient data obtained by Dr. Alexander on the same water samples that we analyze will be used to interpret our data in a more meaningful way. The nutrient analyses will be performed on subsamples that are immediately frozen at the site of collection.

B. Methods

1. Heterotrophic potential studies

The kinetics of soluble organic nutrient uptake by natural microbial populations will be made using the basic method of Wright and Hobbie (1966) as further modified by Hobbie and Crawford (1969). We have had considerable experience with this technique and we feel that it is a powerful tool for measuring <u>in situ</u> microbial activity. By using this technique, the maximum heterotrophic potential (Vmax), natural substrate turnover time (T_t) and the transport constant plus the natural substrate concentration ($K_t + S_n$) can be calculated. In addition, we will be able to calculate the relative mineralization rates for several different organic nutrients. Since this is an extremely sensitive method for assaying microbial activity, it should be very helpful in determining the immediate effects of crude

oil pollution on normal microbial function. This method has already proven helpful in studying the effects of heavy metals on microbial function (Albright and Wilson, 1974; and Morita, unpublished data).

Heterotrophic potential measurements will be made on all sea water and sediment samples taken. In the sea water samples, the percent respiration as well as the kinetic parameters described above will be measured. In the sediment samples, only the mineralization potential will be measured. The method used to analyze the data will be that described by Hobbie and Crawford (1969).

2. Microbial distribution studies

At the present time, there is no one method available which can be used to accurately assess the total number of functional microorganisms present within a given water or sediment sample. By coordinating our studies with those of Dr. Atlas, more than one technique will be used to estimate cell numbers. In addition to the techniques that Dr. Atlas will be employing, we will estimate the total number of bacteria present using a recently developed technique which permits high resolution direct enumeration of marine bacteria (Zimmerman and Meyer-Reil, 1974). Samples for these studies will be fixed with formalin in the field and then further processed in our laboratory at Oregon State.

Classification of hydrocarbon utilizing psychrophilic microorganisms isolated from Arctic waters and sediments will be made using standard biochemical techniques. Those isolates which prove to be particularly versatile in their ability to utilize and alter hydrocarbons will be studied in greater detail.

Physiological studies on the ability of the above isolates to degrade components of crude oil will be made using pure and mixed cultures under simulated Arctic marine conditions. Evaluation of hydrocarbon degradation will be made on the basis of gas chromatographic identification of oxidation products.

During the entire contract period, we will be in close communication with Dr. Atlas to insure that there will be no unnecessary duplication of effort in either the field or home laboratory studies.

3. Studies on the effects of crude oil on microbial activity

To study the acute effects of crude oil on microbial function, we plan to expose sea water and sediment samples to aqueous extracts of local crude oils and measure both the quantitative and qualitative changes in the ability of the natural microbial populations to take up and respire selected ^{14}C labeled organic compounds.

The assessment of the long term effects of crude oil on these populations represents a more difficult task. Any study of this nature that is attempted in an enclosed system over an extended period of time is going to be greatly affected by physical and chemical changes that take place within the reaction vessel itself as a direct result of the biodegradation process. For this reason, we would like to study the long term effects of crude oil on endogenous microbial populations in an "open" system e.g., a controlled oil slick. If there are other investigators who anticipate establishing these experimental conditions, we would like to make qualitative and qualitative measurements of changes in heterotrophic potential during a coordinated investigation.

4. References

Albright, L.J. and E.M. Wilson. 1974. Sub-lethal effects of several metallic salts-organic compounds combinations upon the heterotrophic microflora of a natural water. Water Research 8:101-105.

Hobbie, J.E. and C.C. Crawford. 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. Limnol. Oceanogr. 14:528-532.

Wright, R.T. and J.E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47:447-464.

Zimmermann, R. and L. Meyer-Reil. 1974. A new method for fluorescent staining of bacterial populations on membrane filters. Kieler Meeresforschungen 15:24-27.

VI. Information Products

A. Information obtained from seawater samples

1. Heterotrophic potential data will be reported on studies in which at least one organic compound (glutamate) and in some cases more than one compound have been used. These data will include the maximum velocity of uptake (μmoles x liter^{-1} x hour^{-1}), the mineralization potential (μmoles x liter^{-1} x hour^{-1} CO_2), the turnover time in hours required by the natural population to utilize the natural level of compound present, and a figure which includes both the transport constant and the natural substrate concentration (μmoles x liter^{-1}). In addition, the amount of the material taken up by the cells that is respired as CO_2 will be calculated.

2. The total number of microorganisms per ml of sea water will be reported for all water samples.

B. Information obtained from sediments

Since it is virtually impossible to obtain measurements of the substrate uptake by microorganisms in sediments, we will measure and report only mineralization potential in these samples.

C. Information taken from studies on the effects of crude oil on microbial activity.

The acute effects of crude oil extracts on microbial activity will be reported in terms of alteration in heterotrophic potential using the parameters previously described. We will report temporal changes in function using one substrate and will also report any relative changes which might occur at a given time using several labeled organic substrates. The chronic effects of crude oil pollution on the function of natural microbial populations will be measured and will result in the same type of data described above. The chronic effects studies will be made only if some other group of investigators establish the appropriate experimental conditions.

D. Taxonomical and physiological characterization of psychrophilic hydrocarbon utilizing microorganisms.

Taxonomic determinations to genus level will be made on all cultures that we employ in our physiological studies. This will not duplicate Dr. Atlas's program. If we want to further identify our organisms to the species level, we will send our cultures to Dr. Atals.

During the physiological studies, the effects of salinity, temperature, and inorganic nutrient ions on the degradation of crude oil and of selected individual hydrocarbons will be measured. These data will be reported in graph form.

VII. Data or Sample Exchange Interfaces

We will require the services of OASIS to furnish us with updated bibliography material on reports published on cold environments.

We will also require outside assistance in obtaining data on the inorganic nutrients in our samples. This would include data on the ammonium, nitrate/nitrite and phosphate ion concentrations. These requirements could be fulfilled by the plan suggested at the May 6th meeting in Seattle. This plan proposed that additional funds be made available to Dr. Alexander for this purpose. If these parameters are not made available to us while on station, we would like to obtain them no later than 60 days after the field studies have been terminated.

Our heterotrophic potential data may be helpful to the chemical oceanographers, particularly those who are concerned with variations in dissolved O_2 and CO_2 in the marine environment. It is also possible that the data on the distribution of hydrocarbon utilizing microorganisms will be helpful to those studying hydrocarbon concentrations in sea water since a correlation between these two parameters has been tentatively established.

VIII. Sample Archival Requirements

All data collected in the field will be catalogued by sample number, location, depth and time. We would encourage those making concomitant measurements on the same samples to do the same.

Transposing our data to cards and tapes will be required. We do not have the facilities or funds for punching cards and converting these data into the formate requested for the archives. Raw data will be forwarded to NODC for processing in the formate requested for the archives.

IX. Schedule

1. Field studies during the summer of 1975 (from about August 1 to September 30, 1975)

Initially, water and sediment samples will be taken from one station located just offshore from Point Barrow. During this phase of the study, limits of sampling error as well as the normal variation at a given location will be established. The water samples taken there will also be used to initiate enrichment cultures and will be used in experiments designed to evaluate the immediate impact of crude oil on microbial activity. These studies will then be expanded to include other geographical locations.

During this period, 5 equidistant stations (20 km apart) on a N-S line from Harrison Bay will be sampled every other week. On alternate weeks, we will sample stations on an E-W tract running from Point Barrow to the Harrison Bay tract. This sampling schedule could be modified to take advantage of cooperative sampling with other investigators.

It is impossible at this time to estimate the exact day to day sampling schedule that we will be following. The number and location of sampling will depend on the type of sampling platform that is available to us and the degree to which we will be able to benefit from cooperative sampling with other investigators. Our main requirement will be the time between when the sample is taken and the time it is processed be less than two hours. If there is no room available to process the samples immediately after they are taken then the samples must be returned to shore within two hours. If there is space available (approximately six feet of bench space), then the samples must be taken to Point Barrow for counting within one week of the time they are processed.

We should have all of the raw data for the heterotrophic potential and microbial distribution accumulated within 60 days of when we return from our field sampling period. Most of the heterotrophic

potential data should be available in rough form when we return (on or about September 30).

2. Field studies during February, 1976.

During this period, we will be analyzing samples taken in both the Gulf of Alaska and the Beaufort Sea. In the Gulf of Alaska, we will participate in a two week cruise during which we will collect samples at ten or more stations on the standard sampling grid in this region. After sampling in the Gulf of Alaska, we will spend two weeks at Point Barrow sampling the stations previously described.

3. Field studies during the summer of 1976 (from about July 1 to September 1, 1976)

Our initial sampling efforts will be made in the Gulf of Alaska. At this time we plan a two week cruise during which we will sample the same stations that we sampled during February 1976. After these samples are processed and counted, we will move our equipment to the Beaufort Sea area.

It is impossible to predict at this time what our sampling plan in the Beaufort Sea will be during this period. Our strategy will depend on what we find during our first field study and during our subsequent studies at Oregon State. Our tentative plans initially call for sampling the stations studied during the 1975 season and then extending our sampling area to the east. If an appropriate laboratory facility is made available to us in the area of Prudhoe Bay, we would make this our base of operations during this sampling period. We plan to establish a sampling grid in this region similar to that used near Point Barrow.

By the end of the funding period, we will have most of the raw data collected and reported. However, unless we are given funds to continue this work beyond September 30, 1976, there will not be enough time available to completely analyze and correlate our 1976 data with that reported by other investigatiors.

X. Equipment requirements

We intend to purchase and arrange for the shipment of all permanent equipment listed under "Cost" in section XII of this statement. Some of it will be sent directly to Point Barrow by the manufacturer (i.e. the liquid scintillation counter) and some will be sent by air freight from our laboratory at Oregon State to Point Barrow.

In addition, we plan to borrow the following equipment from the Department of Microbiology at Oregon State University: portable autoclave, drying oven, rotary shaker, micropipettes, vacuum pump, etc.

This equipment plus all of the other supplies that we will require, will be crated and shipped by us from Oregon State. Since there will be very little lead time, we intend to air freight all of this material from Portland, Oregon, to Anchorage and then on to Point Barrow. Since we will be borrowing this equipment, we assume that NOAA will replace it if it is lost or severly damaged during this operation.

It is further assumed that NOAA will supply the necessary special clothing and survival gear that we will require during the field studies. We also assume that ice making and dish washing facilities will also be made available to us. We will also need low temperature incubators and a large capacity autoclave. During our winter sampling period, we will require a device to make one meter holes through the ice.

XI. Logistics Requirements

1. All of the material that we will need during the first season should be in Point Barrow before July 20, 1975. We will need to have a NOAA representative at Point Barrow who will be responsible for receiving and storing these supplies prior to our arrival. Since some of the parcels that we will be shipping to Point Barrow are very heavy, we will require assistance in moving this gear from the airport to the laboratory. Between field trips, the equipment and supplies that we have assembled for these studies will have to be stored at Point Barrow. A sotrage space roughly 8'x8'x10' will be required.

2. We will require food and lodging for three men at Point Barrow (NARL) during the months of August and September, 1975. During this period, we will share the full sized laboratory which will be assigned to Dr. Atlas's group. In February of 1976, we will require the same facilities for two men for a two week period. During the month of Autust, 1976, we will require the same facilities for three men. If the laboratory facilities at Prudhoe Bay are sufficient for sample preparation, we will require occasional lodging for at least two men at Prudhoe Bay. Just before and after our planned 14 day cruise in the Gulf of Alaska (August, 1976) we will require laboratory space on land for media preparation and processing of samples. During all of our field study periods, we will require both dry ice and distilled water. We will also require access to a large autoclave and refrigerator sized cold incubators.

3. We will require air transportation for three people from Corvallis, OR to NARL and back during both summer sampling period. During the winter sampling period, we will require air transportation for one person to the Gulf of Alaska area and then to NARL and back to Corvallis, OR. On the return trips, we will require air shipment

of samples at a temperature of about $0^{\circ}C$ from NARL to Corvallis. We will make these arrangements ourselves.

Assuming that both a helocopter and laboratory facilities are available to us at Prudhoe Bay, we will require weekly air transportation for two three persons and their sampling equipment between NARL and Prudhoe Bay during the month of August, 1976.

4. Assuming that we will have to limit our sampling to helocopters, we will require one day of helocopter time per week (this time will be shared with Dr. Atlas and his group). This will include our eight week sampling period during August and September, 1975, two weeks during February, 1976 and four weeks during August 1976. During the first two sampling periods, we will be sampling out from NARL and during the third period, we will be sampling out from Prudhoe Bay.

Because of the nature of our sampling, we will require a helocopter equipped with accurate navigational ability, a light powered winch and float capability.

5. We will require at least 14 days of ship time (shared with Dr. Atlas) during Feburary, 1976 and another 14 to 20 days ship time during July, 1976.

ALASKA MARINE ENVIRONMENTAL ASSESSMENT PROGRAM
WORK STATEMENT (Research Unit 275/276/294)

I TITLE

Hydrocarbons: Natural Distributions and Dynamics of the Alaskan Outer Continental Shelf

II PRINCIPAL INVESTIAGOR:

Dr. D. G. Shaw
Assistant Professor
Institute of Marine Science
University of Alaska
Fairbanks, Alaska 99701
(907) 479-7723
SS#: 552-66-0166

III GEOGRAPHIC AREA AND INCLUSIVE DATES

Gulf of Alaska, Bering Sea, Beaufort Sea April 1, 1975 through September 30, 1976

IV COST SUMMARY

FY 1975 through June 30, 1975	FY 1976 July 1, 1975-Sept 30, 1976
$63,800	$367,520

V PROPOSED RESEARCH

A Background and Objectives

1. Tasks addressed: The primary emphasis of this proposal is on Task A-33. Secondary emphasis is on Tasks A-25A and B-8.

2. Present state of knowledge: The only substantial body of data on hydrocarbons of the Alaskan OCS is that collected in the first year of this project. The little other data that is available is fragmentary and of unknown quality.

3. Information required: The objective of Task A-33 (as it applies to hydrocarbons) is to sufficiently define the ambient amounts of hydrocarbons in the OCS environments to allow later detection of changes in these amounts caused by oil activities. This overlaps somewhat with task A-25A which requires (in part) the characterization

of critical regions and habitats with respect to hydrocarbon content. Examination of the processes which determine the fate of hydrocarbons (Task B-8) is extremely broad. Only a few critical processes can be considered.

4. Extent to which requirements can be met:

A-33: Hydrocarbons will be assessed in most of the major environmental components in the Gulf of Alaska. At present, it is impossible to be certain what level of detail in individual analyses or in spatial coverage is required since we have only a general notion of what levels of change we may ultimately need to detect. However, our knowledge of what resolution we need should improve as data about biological effects becomes available. In the meantime, we will continue with the fairly light coverage that is imposed by our resource limitations. In the Bering and Beaufort Seas hydrocarbon baseline work will begin, but even by September 1976 will be very sparse. The later start of work in these areas, the more difficult environment and lack of resources combine to produce only a beginning effort in these northern seas.

A-25: Progress in this task cannot be known until critical areas have been identified. When such areas are identified, sampling plans will be modified to give them proper emphasis.

B-8: A critical transport process that we believe we are in a position to make real and rapid progress toward understanding is the transport and sedimentation of hydrocarbons by suspended sediments. In coordination with measurements of ambient levels of hydrocarbons on suspended sediments, a few laboratory measurements of the ability of sediments to sorb hydrocarbons should give a general understanding of this process.

5. Coordination: The analytical work proposed here will be carried out in two laboratories; one in Alaska, the other in California. Close coordination and intercalibration will be maintained between the two. Contact will also be kept with the hydrocarbon analysis group at the National Bureau of Standards. Collection of biological material for this project will be made by other investigators (see Table 1).

B. Methods

1. Use of prior data: Summary and evaluation of existing literature on the distribution of hydrocarbons (Task A-32) is not addressed by this proposal.

2. Sampling Plan

a. Gulf of Alaska: The sampling grid developed for the first year's work will be continued to be used. This standard grid has been extended into Prince William Sound and the western Gulf area. A suite of approximately 20 benthic sediment samples will be collected, principally from the western area and Prince William Sound. Only a few stations in the first year's study area will be reoccuppied for comparative purposes. Approximately 24 surface water stations will be selected from the hydrographic station grid of the entire study area. These will sampled on a quarterly basis. Surface sampling for floating materials will be accomplished whenever surface water samples are taken, weather permitting. Sampling of surtidal benthos (20 samples) will be carried out in conjunction with the biological projects of this program. Particular reliance will be placed on obtaining animals from benthic trawling activities (see Table 1). Suspended sediments will be collected at four locations, two near the mouth of the Copper River and two at or near Hinchinbrook Entrance.

b. Bering Sea: Benthic sediment samples will be collected at approximately 20 stations. These stations will be selected from the standard set of stations being used for hydrography, benthic biology, sedimentology, and trace metal analysis. A single suite of samples will be collected, primarily by grab smapler but occasionally by box corer to obtain sufficient sample for replicate analyses. Sampling of floating material will be conducted on an opportunity basis. Biological sampling will begin in 1976. Analysis of this material will not be accomplished during this contract period.

c. Beaufort Sea: Benthic sediments will be collected along three north-south section lines (20 samples), one east of Barrow, one near Prudhoe Bay, and one near Barter Island during the summer of 1975. Biological sampling will begin in 1976. Analysis of this material will not be accomplished during this contract period.

d. Sampling adequacy: A quantitative expression of sampling adequacy cannot be made for reasons discussed in part Va4.

e. Measure of variance: Early in the project each kind of sampling will be carried out in triplicate to determine the variability due to sampling error. This will be compared to the variabilities observed over space and time.

f. Committment to quality: The numbers of samples to be collected and analyzed quoted above are realistic estimates of what will be accomplished by this proposal. A prudent allowance for unforeseen difficulties has already been made. However, an unexpected development might require substantial method development or might increase the time required for sample collection or analysis. In

such a case we will always opt for a smaller number of quality analyses over a larger volume of questionable results.

3. Criteria for Species Selection: Species to be studied (see Table 1) were selected in consultation with various biologists who have agreed to make the necessary collections.

4. Methods of Analysis: The analytical activities of this project will be divided between the laboratory of D. G. Shaw at the University of Alaska and the laboratory of I. R. Kaplan at UCLA. Shaw will perform analyses of surface water, biota, suspended sediments and floating tar. Kaplan will perform analyses of benthic sediments.

   a. Surface Water. The method presently in use in the Gulf of Alaska will be continued. This procedure has been modeled after that developed for this work at the National Bureau of Standards (Hertz *et al.* 1974). As presently implemented in Alaska, the actual analysis in this technique is carried out by packed column gas chromatography. This is not completely satisfactory and will be used only until GC-MS-data handling system to be purchased for this project becomes available. This is expected to be approximately 8 months after the date of funding.

   b. Biota. The analytical procedure for biota will be essentially that of Madeiros and Farington (1974). This involves saponification, partition into hexane, column chromatography on alumina packed over silica and gas chromatography on packed columns of OV-101. When available, the GC-MC-data handling system will also be used in the biota analyses.

   c. Suspended Sediments. Size fractionation of these sediments will be carried out by a method similar to pipet analysis. Aliquots of

the individual size fractions will be analyzed for hydrocarbons in a manner similar to that described above for biota. Other aliquots of the fractionated sediments will be used in laboratory experiments to measure the uptake and release of petroleum hydrocarbons by these sediments.

d. Floating Tar. A statistical evaluation of the distribution of floating tar in each of the study areas will be made. GC-MS analyses of representative tar samples will be carried out.

e. Benthic Sediments. An aliquot of each sample (ca. 10 g) will be removed for C, N, S and carbonate concentrations using LECO techniques. A second aliquot of each sample (ca. 250 g) will be extracted for organic solvent soluble lipids and hydrocarbons. Subsequent analytical procedures on the organic extract are described below.

The sediment residue from the extraction procedure is then to be extracted with 0.2 N NaOH to recover alkali soluble humic and fulvic acids.

Light hydrocarbon analysis (C_1-C_7). To weighed aliquots of frozen sediment will be added a known volume of cold water in a microblender. The mixture will be agitated and heated to near boiling to transfer the hydrophobic volatile hydrocarbon from the sediment to an overlying gas phase. Aliquots of the gas phase will be removed and analyzed by a gas chromatograph equipped with a SCOT column and temperature programmed from -50°C to 100°C. The light hydrocarbons will be quantitatively measured using a flame ionization detector. This general method has been described by Hunt (1974).

Lipids and hydrocarbons. These components will be removed from the dried powdered sediment by exhaustive extraction using a Virtis Homogenizer with benzene-methanol (3:1 v/v), and fractionated by liquid/solid chromatography on silica gel closely following the methods of Farrington *et al.*, 1972; Aizenshtat *et al.*, 1973; Hunt *et al.*, 1975).

Hexane (1 column pore volume)
- n-alkanes
- branched alkanes
- cycloalkanes

Selected samples will be further fractionated using Urea adduction into an n-alkane fraction and branched cyclic alkane fractions.

20% benzene in heptane (1 column pore volume)
- alkyl benzenes
- single and double ring aromatics

Benzene (excess)
- porphyrins
- condensed ring aromatics

All results will be reported as ppm on dry sediment weight basis.

Fulvic and Humic Acids and Kerogen. After organic solvent extraction, the sediment will be exhaustively extracted with 0.2 N NaOH under nitrogen until the solutions obtained are clear. The alkaline solution will be acidified to precipitate humic acid. This will be washed and treated with HF. The solution containing fulvic acids will be dialysed to remove salts, and the fulvic acids separated by the technique of Brown *et al.*, 1972. The residual kerogen will be purified by treatment with HF to remove minerals.

Stable Carbon Isotopic Compositions. C^{13}/C^{12} analysis will be performed on humic acid and kerogen and the hydrocarbon/lipid fractions, provided quantities of each are sufficient. The

relevant sample is combusted at 1100°C in the presence of oxygen. The CO_2 is analyzed for C^{13}/C^{12} in parts per thousand relative to the PDB standard.

f. Intercalibration. Approximately ten percent of all samples collected will be split for intercalibration analysis at the National Bureau of Standards. Analysis of such samples will be given first priority. Results of these analyses will be transmitted promptly to the project office for comparison.

VI CONTENT AND FORMAT OF RESULTS

This project will provide data on the kinds and amounts of hydrocarbons and related materials present in major components of the Alaskan OCS ecosystems. Products of this project will be:

A. Tabulated and graphical results of hydrocarbon analyses.

B. Interpretation of these results.

C. A magnetic tape record of raw GC-MS data for future manipulation, analysis, and interpretation. This will be available only after a GC-MS-computer system becomes operational (see X below). In the interim analyses will be performed by a GC procedure similar to that presently in use at the University of Alaska for this work.

D. Information broadly defining the importance of suspended sediments as a transporter of petroleum in the Gulf of Alaska.

E. Any computer formated data display other than specified in item 3 will be the responsibility of the project management.

VII INFORMATION EXCHANGE

Rapid exchange of data and ideas with all baseline and biological effects

projects is critical to a continuing refinement of the focus of this work. The program management group will devise and implement lines of communication from investigator to investigator to augment the present lines from investigator to management. Biological samples will be provided to this project by other investigators (see table 1).

VIII SAMPLE ARCHIVAL

Archiving of samples is not addressed by this proposal.

IX SCHEDULE

Sample collection will be carried out following a logistics schedule to be provided by the project management. Sample analysis will begin promptly after collection. Results will be transmitted to management not more than 120 days after those results are obtained. All analyses will be complete by September 30, 1976.

X EQUIPMENT

A gas chromatographic-mass spectrometric (GC-MS) technique will be used for the analysis of samples in this project at the University of Alaska. The procedure will be modeled after that developed by the National Bureau of Standards hydrocarbon group. Obtaining the major instrumentation for this technique and making it operational will require at least 8 months from date of funding.

XI LOGISTICS

A. Gulf of Alaska

1. Bimonthly cruises of the hydrographic grid of the entire study area,

The vessel used in the summer of 1975 should have the ability to operate a box corer.

2. A one week cruise in August 1975 to sample suspended sediment.

B. Bering Sea

One cruise in the summer of 1975 to sample benthic sediments, some box coring will be done.

C. Beaufort Sea

One cruise in the summer of 1975 to sample benthic sediments, some box coring will be done.

Table 1. Biological materials to be collected in the Gulf of Alaska and investigators that will make collections.

Species	Collector
Rock sole	W. Peryra
Pollack	W. Peryra
Tanner Crab	W. Peryra
King Crab	W. Peryra
Weathervane Scallop	W. Peryra
Razor Clam	S. Pennoyer
Salmon	S. Pennoyer

Similar species lists will be prepared for the Bering and Beaufort Seas after the 1975 sampling season, but in time for the 1976 season when biological collections for hydrocarbon analyses are to begin.

REFERENCES

Aizenshtat, Z., M. J. Baedecker, and I. R. Kaplan. 1973. Distribution and diagenesis of organic compounds in JOIDES sediment from Gulf of Mexico and western Atlantic; *Geochim. et Cosmochim. Acta* 37:1881-1898.

Brown, F. S., M. J. Baedecker, A. Nissenbaum and I. R. Kaplan. 1972. Early diagenesis in a reducing fjord, Saanich Inlet, British Columbia--III. Changes in organic constituents of sediments; *Geochim. et Cosmochim. Acta* 36:1185-1203.

Farrington, J. W., C. S. Giam, G. R. Harvey, P. L. Parker and J. Teal. 1972. Analytical techniques for selected organic compounds; *In* Goldberg, E. D. (ed.), Marine Pollution Monitoring: Strategies for a National Program, U.S. Dept. of Commerce, Washington, D.C.

Hunt, G., D. Horton, J. Levine, D. Mayo, D. Donovan, W. Shelley, L. Jiang, R. Crane and R. Johnson. 1975. The evaluation and development of passive tagging procedures for the identification of crude oil spilled on water; Proc. EPA, API, USCG Conf. on Prevention and Control of Oil Pollution.

Hunt, J. M. 1974. Hydrocarbon and kerogen studies. *In* Pimm, A. C. (ed.), Initial Reports of the Deep Sea Drilling Project, Vol. XXII.

ALASKA MARINE ENVIRONMENTAL ASSESSMENT PROGRAM
WORK STATEMENT (Research Unit 278)

I. TITLE: Microbial Release of Soluble Trace Metals from Oil-Impacted Sediments

II. PRINCIPAL INVESTIGATOR:

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University of Alaska
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SS#: 210-26-3269

III. GEOGRAPHIC AREA AND INCLUSIVE DATES: Bering Sea April 1 1975-Sept 30, 1976

IV. COST SUMMARY

FY 1975	FY 1976
through June 30, 1975	July 1, 1975 through Sept 30, 1976
$6,255	$15,170

V. PROPOSED RESEARCH:

A. Background and Objectives

This project is designed primarily to determine through laboratory experimental techniques the potential release of soluble trace metals from oil-impacted sediments. It addresses most of the elements of task B-7 but during the time period proposed here specifically excludes experiemental investigation of the relative importance of various metal species in terms of uptake by the biota. This work will attempt to determine how the addition of oil to sediments would change the trace metal environment and more specifically to determine the distributions of copper, lead, zinc, and possibly cadmium between the dissolved and particulate phases of sediments.

Perturbations of the marine trace metal environment could produce undesirable effects such as toxicity to food chain organisms or accumulation of metals in fish and other commercially harvested foodstuffs. Trace metals may be introduced to the environment from drilling muds, formation water, or petroleum hydrocarbons, but the quantity of trace metals from these sources is small, and severe effects are likely to occur only in localized areas. However, the introduction of petroleum hydrocarbons to sediments may change the rate and nature of microbial activity through stimulation or suppression of natural detritus decomposition or through decomposition of the petroleum hydrocarbons themselves. The reduced inorganic compounds resulting from the anarobic decomposition of natural detritus, particularly the sulfides, play an important role in immobilizing trace metals. Some soluble organic substances released by bacteria and bacterial grazers can complex with trace metals and become more mobile and potentially available for reintroduction into the water column.

The introduction of petroleum hydrocarbons to the sediments thus may alter the rate of release of trace metals from the organic fractions of detritus, may alter the rate of production of reduced inorganic compounds, and may change the rate of production of soluble trace metal complexing agents. Since the abundance of metals is so high in the particulate phase of sediments in comparison with that in the sediment-pore water or the open waters of the continental shelf, changes in sediment processes could result in substantial alteration of the water column trace metals through transport across the water—sediment interface.

This is a relatively small project and is addressed to a series of problems for which there is little background and little routine, pre-established methodology. Therefore the experimental design has been kept simple. If significant trace metal changes result from the additions of oil under these circumstances, it may be desirable to elaborate the work after 30 September 1976--to investigate the effects of oil on various degrees of aging or weathering, to methodically follow the time course of effects, to explain the mechanism which directly causes the observed changes, and to determine the relative importance of various metal species in terms of uptake by the biota.

B. Methods

Samples will be frozen in the field as they are acquired and will be kept frozen until used. Experimental incubation will be done at temperatures similar to the natural environment. Operationally the comparison between systems with and without added hydrocarbons will be divided into studies of oxidized and reduced systems. In reduced systems microbial oxidation will be monitored by following transformations of ^{35}S-labeled sulfate in a technique developed by Jørgensen at the University of Aarhus, Denmark. In oxidized systems oxidation will be followed by the changes in concentration of dissolved oxygen (EPA STORET 00550 and 00560). Distribution of metals between particulate and dissolved forms will be monitored by radionuclide tracer techniques, and organic complexation of trace metals will be assayed by electrochemical titration using anodic stripping voltammetry.

VI. INFORMATION PRODUCTS:

The principal information product of this project will be a statement describing the results of the experimental work on the effects of oil on soluble trace metals in sediments, together with the quantitative supporting data which will include replicate analyses for respiration rate and trace metals in solution for oil-impacted sediments in comparison with non-oil controls.

VII. DATA OR SAMPLE EXCHANGE INTERFACES

The sediment samples to be processed in this work are being acquired in conjunction with the major trace metal investigations project (Dr. David Burrell, principal investigator). No other sample interchanges are anticipated. No data exchanges with other AMEAP projects through FY 1976 are deemed necessary, but the findings of this project should be considered in the design of any future trace metal studies on water, sediments, and biota.

VIII. SAMPLE ARCHIVAL REQUIREMENTS

None

IX. SCHEDULE

As noted above, major sample acquisition will occur during the June 1975 *Discoverer* cruise to the Bering Sea Bristol Bay area. Additional samples principally of eelgrass (*Zostera marina* L.) detritus will be acquired from Izembek Lagoon near Cold Bay before the end of October 1975. Contingent upon the availability of the requested equipment by 1 September 1975, the analytical development work on sediments and detritus under oxidizing conditions, along with preliminary results on oxygen consumption and trace metal distribution, should be available by 1 February 1976, and preliminary results on the sediments under reducing conditions should be available by 1 April 1976. A qualitative statement concerning degree and nature of the effect of oil on sediments under the experimental conditions described above, together with the supporting data in final form, will be available by 30 September 1976, the completion date of this project.

X. EQUIPMENT REQUIREMENTS

The requested equipment items include a refrigerated water bath, a bio-oxidation system, an oxygen meter, and magnetic stirrers. These items must be acquired and made operational before the experimental work can begin.

XI. LOGISTICS REQUIREMENTS

Through coordination within the Institute of Marine Science Dr. Burrell is acquiring most of the necessary samples on the June *Discoverer* cruise. In the remote possibility that this June cruise does not provide the required samples, the sampling activity will be rescheduled for the August *Surveyor* cruise. The Institute of Marine Science will be responsible for arrangements for the Izembek Lagoon/Cold Bay sampling.

(Research Unit #332)

WORK STATEMENT

I. TITLE: Research Unit 332. Determine the incidence and pathology of marine fish diseases in the Gulf of Alaska, Bering Sea, and Beaufort Sea

II. PRINCIPAL INVESTIGATORS: Dr. Bruce B. McCain, University of California, Davis, California, and a Cooperating Investigator at the National Marine Fisheries Service, Northwest Fisheries Center, 2725 Montlake Boulevard East, Seattle, Washington 98112 ((206)442-4806), and Dr. S. R. Wellings, University of California, Davis, California ((916)752-2710)

III. GEOGRAPHIC AREA AND INCLUSIVE DATES: Gulf of Alaska, Bering Sea, and Beaufort Sea. July 1, 1975 to September 30, 1976

IV. COST SUMMARY

FY 1975 through June 30, 1975	FY 1976 July 1, 1975 - Sept. 30, 1976
$0	$50.0

V. PROPOSED RESEARCH

A. Background and objectives

1. Task A-28--Determine by field and literature studies the incidence of diseases presently existing in fish, shellfish, birds, and mammals for use in evaluating future impacts of petroleum-related activity.

2. State of knowledge: Very limited knowledge is presently available concerning the pathology of fish species in or associated with the Gulf of Alaska and Bering and Beaufort Seas. Because our investigation is concerned primarily

with diseases which produce externally visible pathological conditions, this literature review will emphasize diseases of marine fishes in the Northern Hemisphere with obvious symptoms and/or in which the disease-causing organism is readily detected. At least eight different agents or factors are known to be responsible for diseases of these types. They are as follows: (1) bacteria, (2) fungi, (3) protozoa, (4) helminths, (5) copepods, (6) viruses, (7) environmental factors, and (8) genetic anomalies. In addition, tumors may be caused by one or more of the above.

The bacterium, Vibrio anguillarum, is thought to cause red, hemorrhagic skin lesions and in many cases death in several groundfishes, including cod, eels, and pleuronectids (Ljungberg, 1963; Buckman, 1952; Hodgkiss and Shewan, 1950). Other groups of bacteria, such as the pseudomonads and mycobacteria, are reported to cause ulcerated skin, fin erosion, and various other lesions (Zobell and Wells, 1934; Hodgkiss and Shewan, 1950; Mahoney et al., 1973).

Fungi are not known to be a widespread cause of marine fish diseases. They have been shown to cause epizootics among herring in the Northwest Atlantic. The disease results in extensive hyphal growth in the internal organs and muscles. The genus Ichthyophonus is most commonly isolated from fungal disease (Sindermann, 1966).

Protozoan parasites infest a great many bottom-dwelling fish along the Pacific coast of North America (Margolis, 1970). The presence of these organisms does not always result in pathological damage to the host. Myxosporidia, however, are known to cause a condition in fish muscles referred to as "milkiness." Several marine fish species are affected including halibut (Hippoglossus stenolepis), starry flounder (Platichthys stellatus), and hake (Merluccius productus) (Margolis, 1953; Patashnik and Groninger, 1964). In most cases the disease is not obvious in freshly captured fish, but the condition can be readily detected after the affected fish have been refrigerated for 4-24 hrs. During this period, proteolytic enzymes present in the spores are thought to cause foci of softened and liquefied muscle in which cysts containing spores can be macroscopically observed.

Another group of protozoans, the microsporidia, form easily detected cysts or "tumors" in the somatic muscles of many groundfishes. Members of the families Gasterosteidae, Zoarcidae, and Gadidae have been reported with this disease (Weissenberg, 1921; Nigrelli, 1946; Polzanski, 1955).

The helminths, including trematodes, cestodes, and nematodes, produce disease symptoms in marine fish in greatly varying degrees. Trematode species have metacercariae which form cysts under the skin, but are

thought not to cause serious diseases (Wolfgang, 1954). Cestodes have been reported in several fishes in Canadian Pacific Waters (Arai, 1967). The larval cestodes may be found in the musculature, but few gross pathological problems have been observed. Larval nematodes are present in the muscles of a large number of marine species (Scott and Martin, 1957; Templeman et al., 1957). In some cases, pathological damage results from the movement of these larvae to other parts of the fish; for example, the liver may become infested.

Many copepod species attach to the gills and body surfaces of a wide variety of marine fishes in the northeastern Pacific Ocean (Arai, 1969). Some of these parasites are able to invade internal organs, such as the heart (Mann, 1954), and some cause skin ulcerations.

Another type of disease found in Alaskan waters is characterized by the presence of tumors. The two most frequently reported types of neoplasia are epidermal papillomas of pleuronectid fishes, and adenomas and adenocarcinomas of cod. The causes of these tumors are not yet known. Papillomas were identified on large numbers of starry flounder (Platichthys stellatus) and Arctic flounder (Liopsetta glacialis) in waters of the Aleutian Islands in 1886 (Turner, 1886). An incidence of papillomas of about 32% was found in sand sole (Psettichthys melanosticus) in the Northern Hecate Strait of Canada in 1965 (Nigrelli et al., 1965). Recently,

several rock sole (Lepidopsetta bilineata) with papillomas were captured in the Bering Sea by a National Marine Fisheries Service vessel (Johnson, 1974).

Nine species of Pleuronectidae have been reported to have papillomas in Puget Sound, Washington (Miller and Wellings, 1971; McArn and Wellings, 1971; Wellings et al., 1969). The life history and histopathology of the disease have been characterized (Brooks et al., 1969; Wellings et al., 1967). The cause of the disease has been investigated, but no causative agent or factor has yet been determined (McCain, 1974; Wellings, McCain and Miller, 1975).

Pacific cod (Gadus macrocephalus) with adenomas and/or adenocarcinomas associated with the pharynx and gills have been captured in the Bering Sea (Weber, 1975), and in the coastal waters of British Columbia (Levings, 1968; Wellings, 1969). These tumors can become quite large (6x3cm) and are readily recognized. Similar tumors have been found in walleye pollack (Theragra chalcogrammas) (Takahashi, 1929), a fish common in Alaskan waters.

Other types of neoplastic diseases are likely to be found in Alaskan groundfishes. For example, lipo-osteomas, fibro-osteomas, and fibromas have been described in Pacific halibut (Hippoglossus stenolepis) from Alaska (Wellings, 1969).

Few virus-caused diseases have been reported in Alaskan marine fishes. A disease which is present in other marine waters of the Northern Hemisphere is lymphocystis

(Weissenberg, 1965). This disease takes the form of numerous nodules on the body surface. The nodules are composed of giant connective tissue cells. Species affected include many members of the Pleuronectidae family.

Other diseases which may be present in Alaskan waters that have not yet been scrutinized and reported include those caused by environmental and genetic factors. Hopefully, the investigation described in this application will determine the presence of such diseases.

3. Information required: Information required to meet the task objective will include the frequency and pathological characteristics of observed fish diseases, the fish species affected, and the sampling data (location, type of gear, etc.). The pathological properties to be defined will consist of (a) describing the macroscopic characters of the diseased fish, the lesion, and possible associated disease agents; (b) determining the histopathology of the lesion and affected organs and tissues, including blood, and (c) isolating possible disease-causing microbial agents (bacteria, protozoa, viruses, and fungi).

4. Accomplishment of Requirements: The information necessary to meet the objectives of our research will be obtained and reported by September 30, 1976. Because our research will depend upon examination of fish samples captured by other investigators, the quantity and quality of research information will be partially a function of how the fish sampling is performed.

In addition, diseases may be found which cannot be adequately defined during the period supported by this contract. Such a situation may be the result of sub-optimum sample sizes or sampling procedures. Therefore, certain diseases may require additional efforts to more fully characterize them.

5. Related research: We plan to station one or more research personnel aboard sampling vessels and with sampling expeditions during regular sampling trips. Demersal fish in the Bering Sea and Gulf of Alaska will be examined in cooperation with Dr. Walter T. Pereyra's group at the NWFC, Seattle (Research Unit 174).
A specially trained fishery biologist will remain on board the R.V. MILLER FREEMAN for the entire sampling period. Nearshore fishes of the Beaufort Sea will be monitored in cooperation with Gene Reguski of the Alaska Department of Fish and Game. In addition, efforts will be made to work with an as yet to be selected research group that will sample fish populations in Prudhoe Bay for ARCO.

B. Methods

Accomplishment of the above-mentioned tasks will require cooperation between our research group and the agencies performing resource assessment investigations in Alaskan waters. The specially trained fishery biologist stationed aboard vessels during regular sampling trips will examine subsamples of catches of demersal and intertidal fishes for externally visible pathological lesions. Diseased fish will be treated as follows: (a) the lesions will be photographed and examined for macroscopic parasites; (b) a small piece of each lesion will be removed and used for isolation of microorganisms; (c) samples of internal organs will be obtained aseptically and examined for evidence of systemic infections; (d) a sample of blood will be taken for hematological analysis; (e) the weight, length, sex, and species of the fish will be determined; and (f) the entire fish (or excised lesions and major internal organs of fish too large to keep in toto) will be preserved in formalin for later histological examination.

Normal fish of the same species as diseased fish will be examined in the same manner as described above to determine the gross and histological properties of nondiseased animals. Also, a representative number of fish will be stored at 4°C for 4-24 hrs. and reexamined for the presence of liquefied muscle tissues and protozoan cysts.

Upon completion of each sampling expedition, specimens and cultures of microorganisms will be returned to the laboratory at the Northwest Fisheries Center. Each specimen

will be examined for gross pathological anomalies and by histological procedures. Bacterial and fungal cultures will be subjected to standard taxonomic tests and classified into subgeneric groups. Extracts will be prepared from frozen lesions and inoculated onto cell cultures of marine and freshwater fishes in order to isolate possible viruses. We will coordinate work and cooperate with projects on disease at the Northwest Fisheries Center and at the Mid-Atlantic Coastal Fisheries Center, NMFS.

VI. INFORMATION PRODUCTS: The principal Information Products of this undertaking include the following:

1. A catalogue of the following information:
   (a) the types of diseases found;
   (b) the incidence of each type of disease with respect to species and location;
   (c) the species, length, weight, age, and sex of affected fish; and
   (d) the date, geographical location, depth, and type of sampling gear used in the capture of each diseased fish.
2. Each type of disease will be defined by the histological characteristics of disease lesions, blood, and major internal organs, i.e., kidney, liver, spleen, and brain.
3. Microorganisms, including bacteria, viruses, fungi, and animal parasites associated with diseased fish will be isolated and classified into taxonomic groups. Normal fish of the same species and location will be subjected to similar isolation procedures in order to distinguish possible disease-specific organisms from constituents of the fishes' normal flora.

4. All pertinent data contained in the above Task Products will be recorded on computer punchcards for use as baseline information in future assessment of environmental changes.

VII. DATA OR SAMPLE EXCHANGE INTERFACES

As mentioned earlier, the accomplishment of our research objectives is dependent upon cooperation between our group and investigators capturing fish in Alaskan waters. The majority of the fish samples monitored will be examined directly by one of our personnel at the time of capture. In addition to this approach, we plan to familiarize other investigators performing baseline resource assessment studies with the types of fish diseases expected in Alaskan waters. These people will be provided with appropriate fixatives and storage containers for preserving any diseased fish.

Extensive cooperation and interaction will take place between our group and the investigators at the University of Louisville led by Dr. Ronald Atlas with respect to the bacteriology of Alaskan fish diseases. Areas of emphasis will include identification of bacterial pathogens and the taxonomic classification of bacterial isolates.

VIII. SAMPLE ARCHIVAL REQUIREMENTS

No requirements for archiving of samples are foreseen at this time.

IX. SCHEDULE

July 1, 1975 - August 1, 1975: Supplies and equipment will be purchased and a fishery biologist will be trained to perform basic histopathological and microbiological procedures.

August 1, 1975 - September 30, 1976:

1. Fishery biologist and/or principal investigators will accompany other investigators on sampling expeditions according to the schedules of these latter individuals (See XI).
2. The remaining objectives of our research will be accomplished depending upon the types and incidence of fish diseases.

X. EQUIPMENT REQUIREMENTS

No additional special equipment will be required.

XI. LOGISTICS REQUIREMENTS

This Task plans to utilize vessels during biological baseline surveys financed by BLM and MARMAP. Requirements for this Task are to have one specially trained biologist on each cruise. For example, a disease specialist will be on board to collect samples from (1) the groundfish cruises of the R.V. MILLER FREEMAN in the Bering Sea in August and September of 1975 and 1976, (2) in the Gulf of Alaska in January and February of 1976, (3) the MARMAP-funded shrimp cruise in the Gulf of Alaska, September 4 to November 6, 1975, and (4) fishes of the Beaufort Sea will be monitored in cooperation with Gene Reguski of the Alaska Department of Fish and Game. Intertidal fishes of the Gulf of Alaska will be sampled with the help of the Auke Bay Laboratory, NMFS, Juneau, Alaska.

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