Environmental Assessment of the Alaskan Continental Shelf

Annual Reports of Principal Investigators for the year ending March 1981

Volume IV: Effects of Contaminants

U.S. DEPARTMENT OF COMMERCE
National Oceanic & Atmospheric Administration
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The facts, conclusions and issues appearing in these reports are based on interim results of an Alaskan environmental studies program managed by the Outer Continental Shelf Environmental Assessment Program (OCSEAP) of the National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce, and primarily funded by the Bureau of Land Management (BLM), U.S. Department of Interior, through interagency agreement.

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ANNUAL REPORT

Assessment of Potential Interactions of Microorganisms and Pollutants Resulting from Petroleum Development on the Outer Continental Shelf of Alaska

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Outer Continental Shelf Energy Assessment Program
National Oceanic and Atmospheric Administration

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Consultants:	Micah Krichevsky Elaine Krichevsky

I. Objectives

A. To determine the distribution of microbiological populations in ice covered waters of the Bering and Beaufort Seas, and to assess the potential interactions in such areas of microorganisms and pollutants produced by petroleum development on the outer continental shelf. Specifically:

1) To determine distribution and densities of microbial populations in sediment and water samples collected from USCG icebreakers.

2) To determine the potential rates of biodegradation of petroleum hydrocarbons in these water and sediment samples.

B. To examine the potential rates of degradation of various classes of petroleum hydrocarbons within the regions in the Bering Sea into which hydrocarbon contaminants may be transported as a result of petroleum development in the St. George and North Aleutian Shelf lease areas. Specifically:

1) To measure microbial hydrocarbon degradation potentials.

2) To examine the distribution of microbial populations.

C. To determine the distribution of microbiological populations in Norton Sound and to assess the potential interactions of microorganisms and petroleum hydrocarbons. Specifically:

1) To determine if a natural oil seepage in Norton Sound has altered the distribution of microbial populations.

II. Introduction - Scope of Work

This study is a continuation of an effort to characterize microbial populations and the ability of microorganisms to biodegrade petroleum hydrocarbons in proposed Alaskan OCS oil and gas lease areas. The approach has

been to determine the distribution and population levels of several microbiological groups, e.g. hydrocarbon degraders within a geographic area, and to examine potential rates of hydrocarbon biodegradation.

Analyses were performed to follow the chemical changes in crude oil as it undergoes biotic (biodegradation) and abiotic (physical and chemical) weathering in experimentally oiled sediment in the Beaufort Sea.

III. Current State of Knowledge

The state of knowledge concerning microbial populations and hydrocarbon biodegradation in Alaskan OCS areas has been summarized in previous annual reports and in reports of synthesis meetings. New information developed from this project is described below.

IV. Study Areas

Studies were conducted in Beaufort Sea and Bering Sea regions.

V. Methods

Part of the NOAA OCSEAP research included sampling conducted in ice covered waters around Alaska. During August 1978, April 1979, and May and June, 1980 our laboratory participated in sample collection cruises on the USCGC Northwind, Polar Sea, and Polar Star respectively. During these cruises samples were collected and initial processing was done onboard the vessels preparatory to final analysis in the laboratory at Louisville.

Another part of the OCSEAP research involved an integrated chemistry and microbiology program in Norton Sound aimed at assessing the impact of a natural gas seepage. A cruise was conducted aboard the NOAA Discoverer in Norton Sound during August 1979.

During August-September 1980 a cruise was conducted along the North Aleutian Shelf and in the St. George basin aboard the NOAA Surveyor. This cruise was part of an integrated program concerning transport processes in these OCS regions.

Surface water samples were collected with sterile Niskin butterfly samplers (General Oceanics). Sediment samples were collected with either Smith MacIntyre or VanVeen bottom dredgers, a Sutar-VanVeen box corer, or a Kahl mud snapper for shallow water samples. All samples were processed within 2 hr. of collection.

The procedure done included: direct counts of microorganisms, viable counts of microorganisms, Most-Probable-Number (MPN) of hydrocarbon degrading microorganisms, biodegradation potential of hexadecane, pristane, 9-methy-anthracene, and/or benzanthracene by microorganisms, and denitrification potentials of sediment microorganisms. The methods and results of the denitrification studies were detailed in a previous report.

The direct count samples were prepared by adding 5 ml of either sea-water or a 10^{-2} dilution of sediment to 5 ml of 5% formalin in duplicate. Upon return of the samples to the laboratory the samples were mixed with acridine orange and filtered through Nuclepore filters (0.22 μm) which had been stained with irgalan black. The filters were examined under an Olympus Vanox epifluorescent microscope. If necessary, the smaples were diluted until a countable sample was achieved. Twenty fields per sample were counted and averaged. The numbers of microorganisms per milliliter of seawater or per gram dry weight sediment were calculated.

Viable microorganisms in seawater and sediment were enumerated by preparing serial dilutions of water and sediments in sterile Rila marine salts dilution blanks and plating on Marine Agar (Difco). Seawater (1 ml) was also filtered through 0.45 μm Millipore filters and placed on Marine Agar plates. Dilutions for seawater were 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Dilutions for sediment were 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. After the plates were inoculated by spreading with sterile glass rods, they were

incubated 21 d at 4°C. After incubation the colonies were counted and numbers of microorganisms per ml of seawater or sediment were calculated.

The most probable number of hydrocarbon degrading microorganisms was determined by a three tube MPN procedure. Seawater and serial dilutions of seawater and serial dilutions of sediment were added to sterile 30 ml serum vials that contained 5 ml of sterile Bushnell Haas broth in Rila marine salts. Each vial was amended with 30 µl crude oil spiked with ^{14}C labelled hexadecane. Prudhoe Bay crude oil and Cook Inlet crude oil were used as carriers for the radiolabelled hexadecane. Sets of three vials were inoculated with 0.5 ml of the appropriate dilutions of either seawater or sediments. The vials were sealed by applying silicone rubber cement to the rubber serum stoppers. After incubation for 21 d at 5°C, 0.5 ml of saturated KOH was added to each vial to stop biological activity. The radioactive $^{14}CO_2$ produced was measured. Three drops of methyl red were added to each vial. The $^{14}CO_2$ was released by adding 6NHCl and passing air through each vial into scintillation vials containing 10 ml of Oxifluor-CO_2 (New England Nuclear). Radioactivity in each vial was counted in a Beckman 100 LSC scintillation counter. Vials with 100 or more counts above background were considered positive. After scoring the vials positive or negative, the MPN of hydrocarbon degraders per ml or g of sediment were calculated from standard MPN tables.

The potential for biodegradation of hexdecane, pristane, 9-methyl-anthracene or benzanthracene was assessed. Sterile 30 ml serum vials with 5 ml of Bushnell Haas broth in Rila were inoculated with 0.5 ml of seawater or 0.5 ml of a 10^{-2} dilution of sediment. In the North Aleutian Transport studies replicate biodegradation potentials were run using 5 ml of Rila marine mix instead of Bushnell Haas broth. Sets of three vials were inoculated. Each radiolabelled substrate was dissolved in a carrier of either

Prudhoe crude or Cook Inlet crude oil. Each vial received 30 µl of ^{14}C labelled crude oil. After inoculation, all vials were sealed with silicone rubber cement and incubated for 21 d at 5°C. Biological activity was stopped by adding 0.5 ml of saturated KOH to each vial. Radioactive CO_2 was measured as described above for the MPN procedure. The percent of radiolabelled substrate degraded was calculated by after subtracting background. The counts of $^{14}CO_2$ were divided by the total counts of ^{14}C labelled compound added to each vial to yield percent mineralized during the incubation period.

VI. Results

Icebreaker Cruises

Station locations are shown in Fig. 1. The water depths at each location are shown in Fig. 2.

The direct counts of microorganisms in Bering Sea water and sediment (Polar Star and Polar Sea cruises) are shown in Figs. 3 and 4 respectively. Over all the areas sampled total numbers of microorganisms ranged from 1×10^4 to 6.8×10^5 per ml in water. In general populations were lower in the Navarin Basin than in the northern Bering Sea. Counts were also up to two times higher in 1980 than in 1979 in the same area of the northern Bering Sea.

Total counts of microorganisms in sediment ranged from 1×10^8 to 6.9×10^9 per gram sediment. In contrast to the total counts found in water, the total counts in sediment were generally comparable between the 1979 and 1980 samples.

The water and sediment viable plate counts are shown in Figs. 5 and 6 respectively. The viable microorganisms in water ranged from 1×10 to 1.5×10^4 per ml. Viable counts were generally two orders of magnitude less than total counts. Viable microorganisms in the Northern Bering Sea were

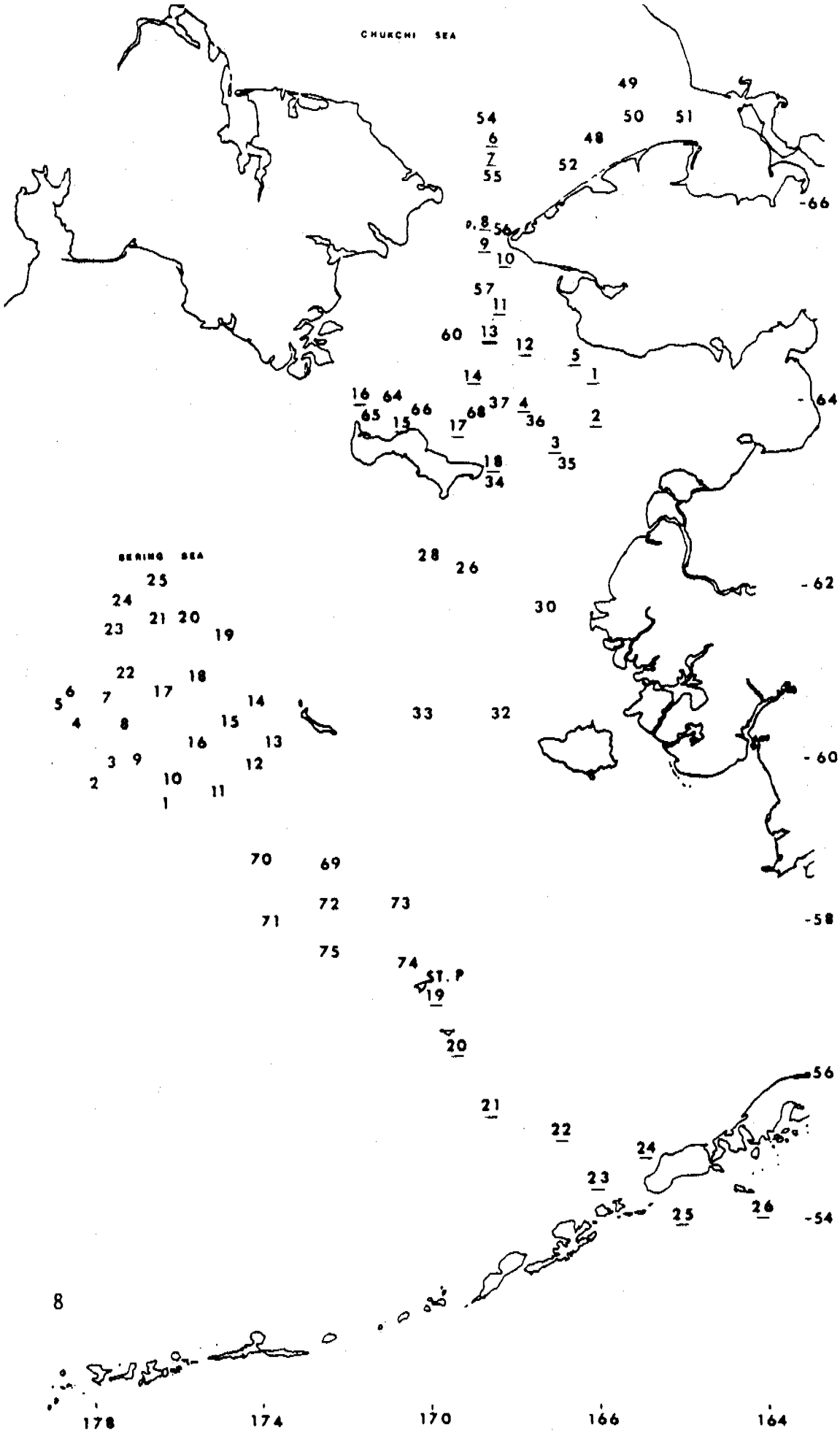


Fig. 1 Station locations for Polar Sea and Polar Star cruises. Polar Sea stations are underlined.

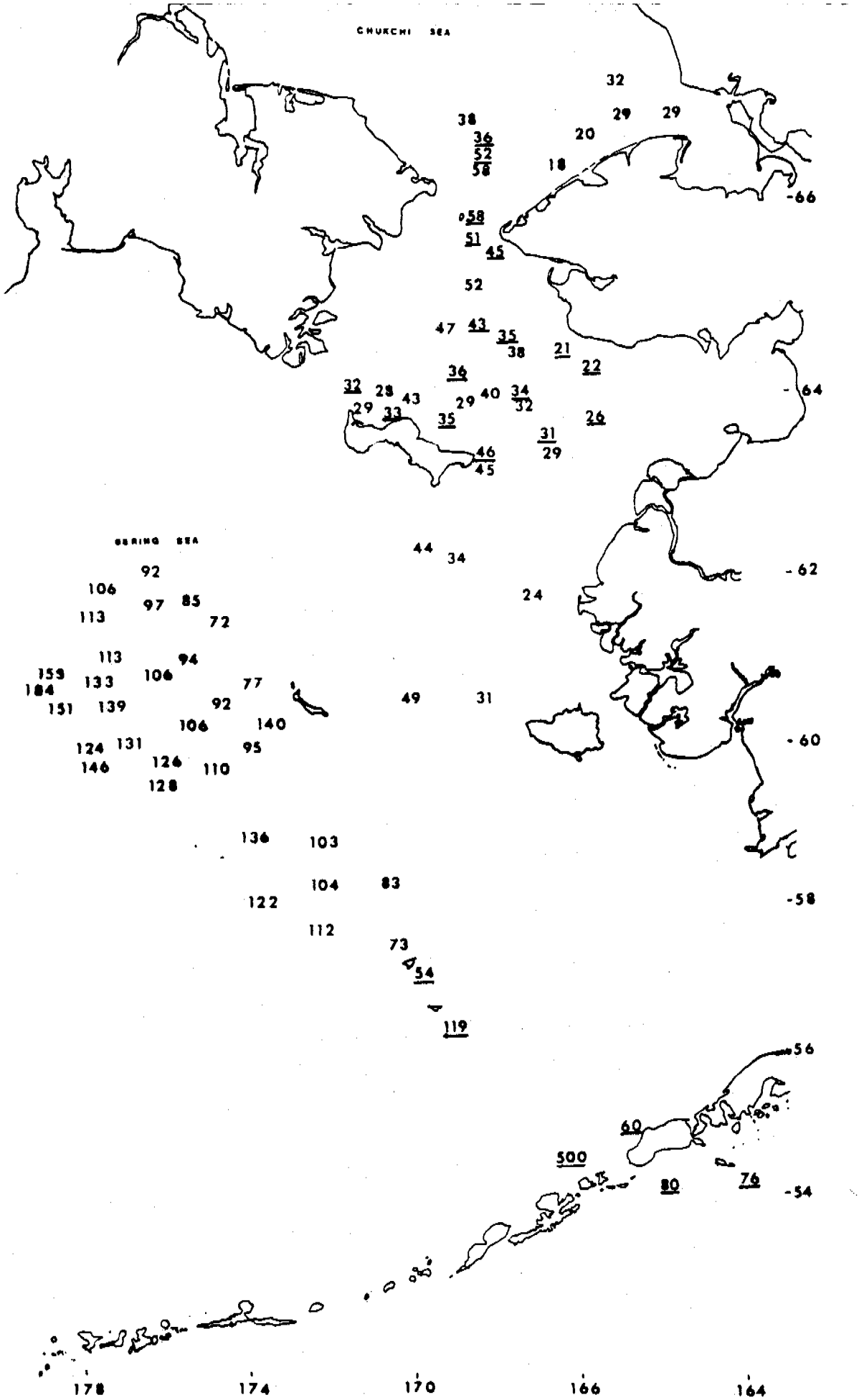


Fig. 2 Depth of water column at each sampling site.

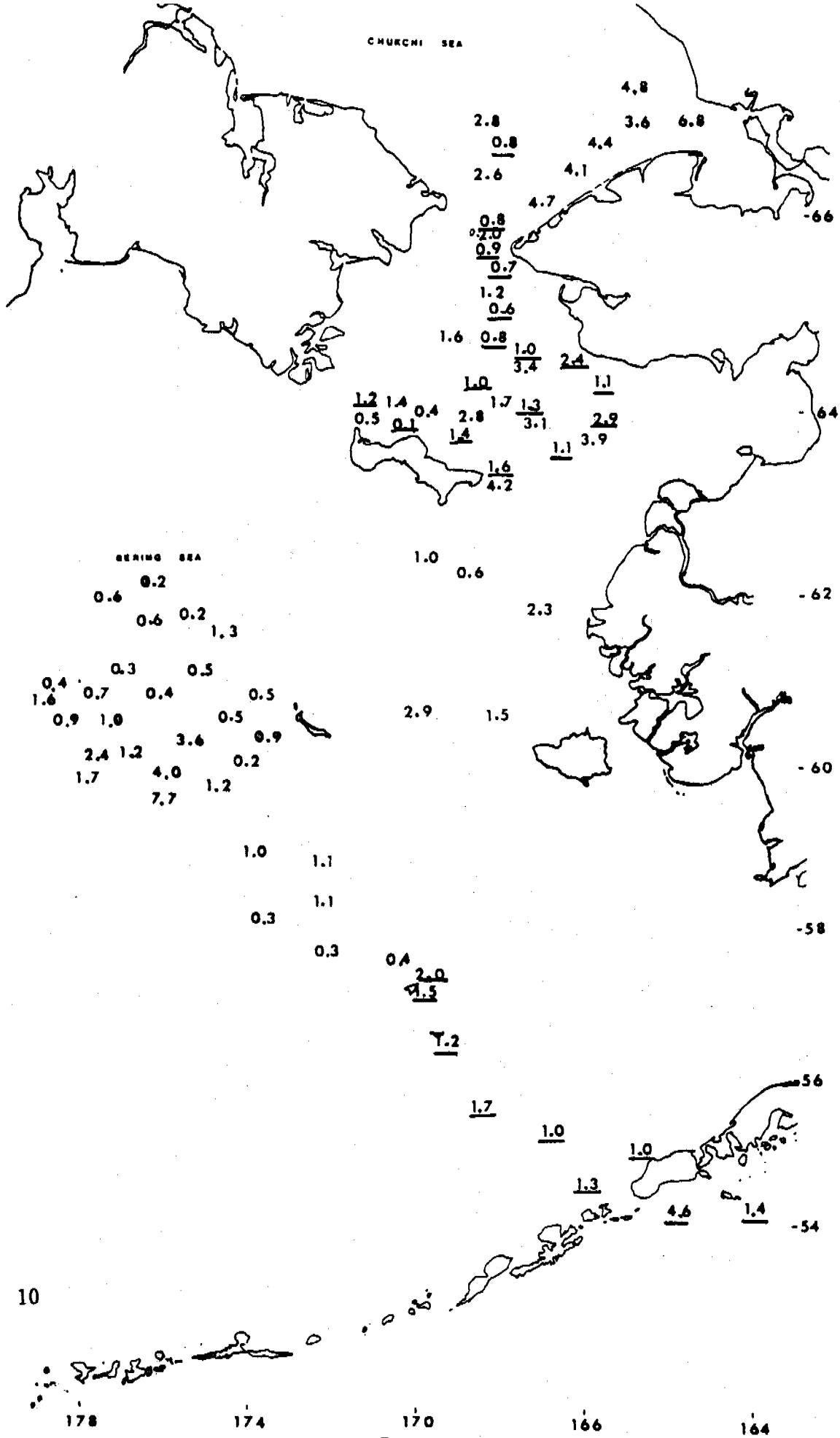


Fig 3 Direct counts x 10^5 -Water samples

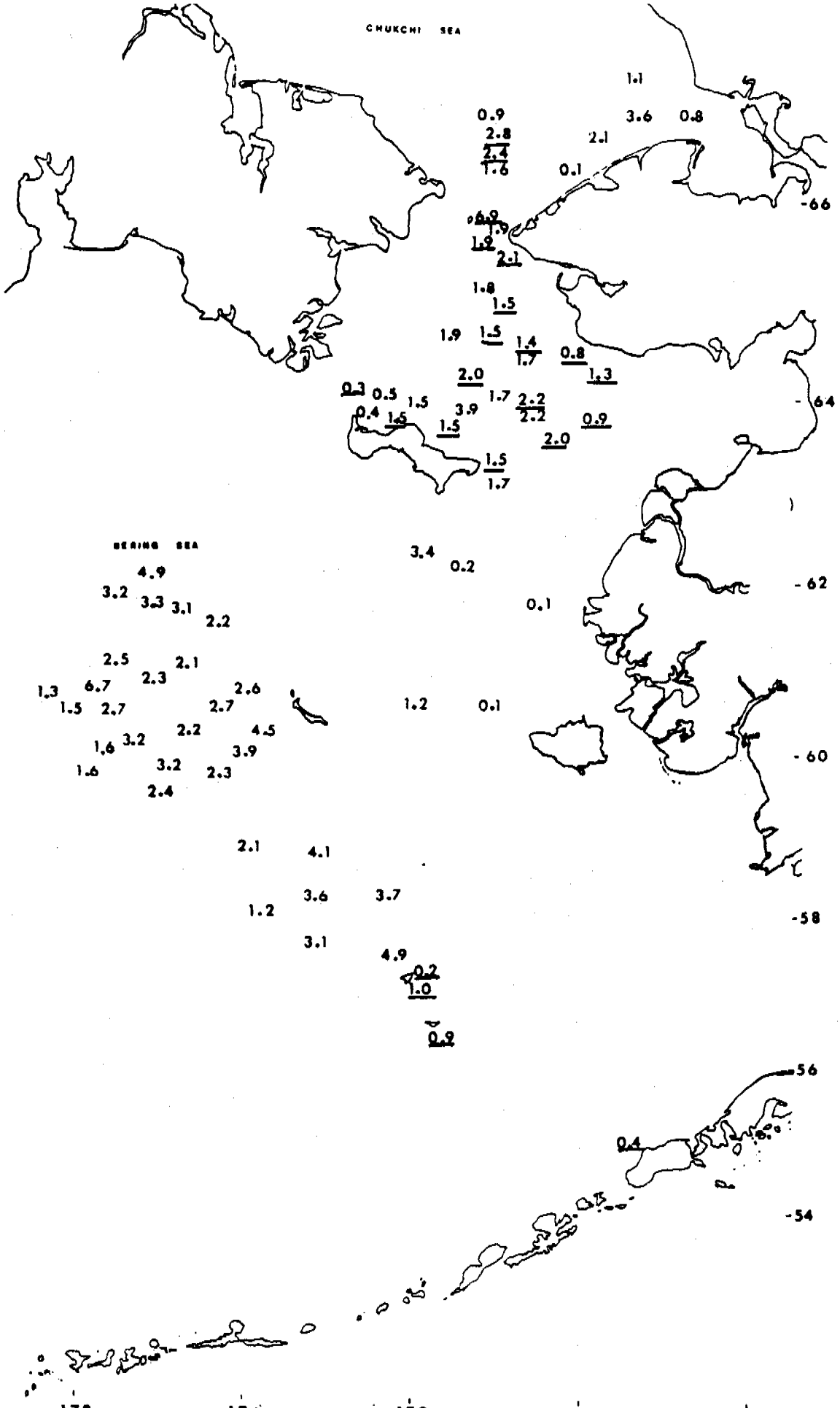


Fig 4. Direct counts x 10^9 -Sediment samples

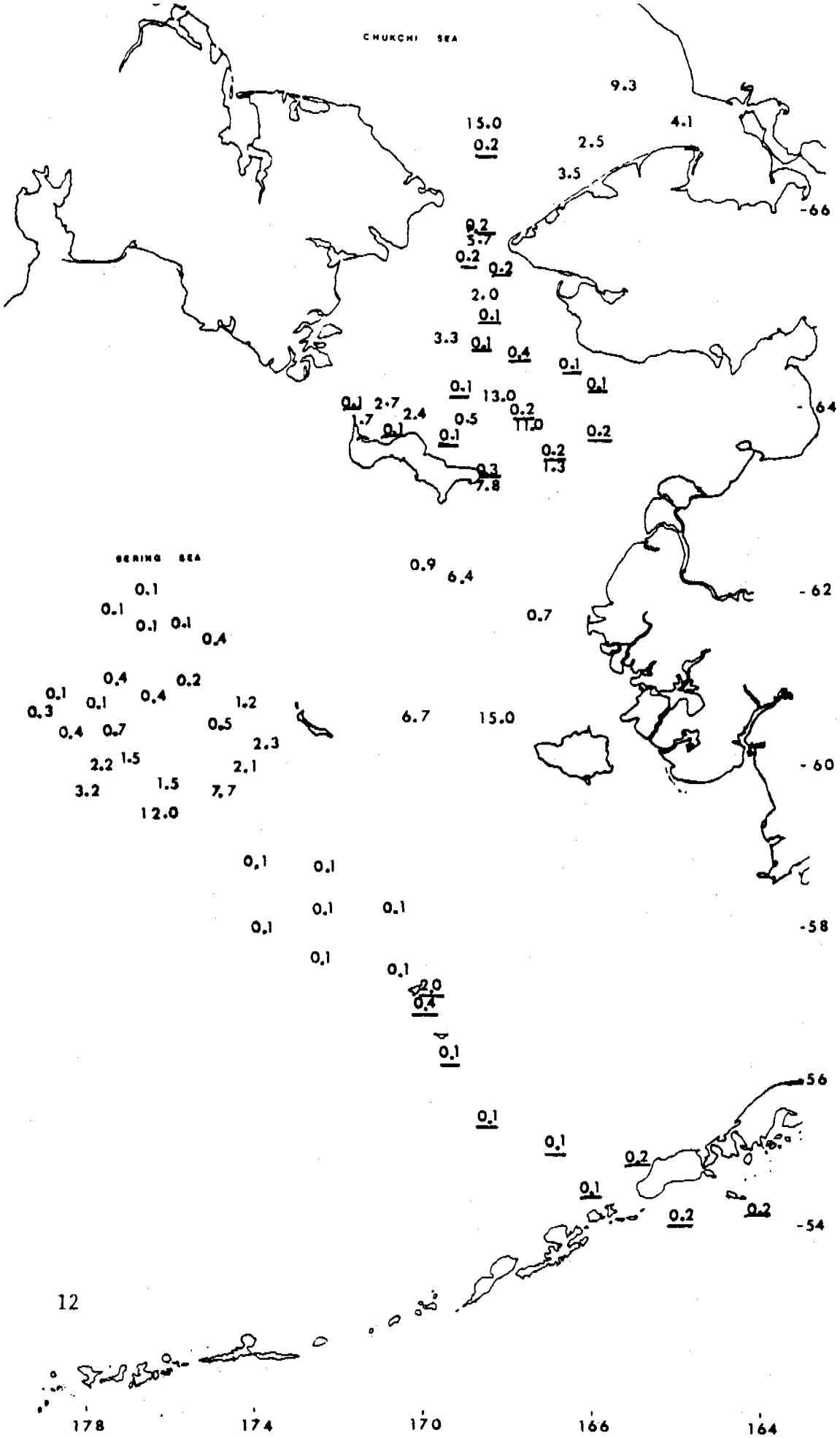


Fig 5 Viable counts x 10^6 -Water samples

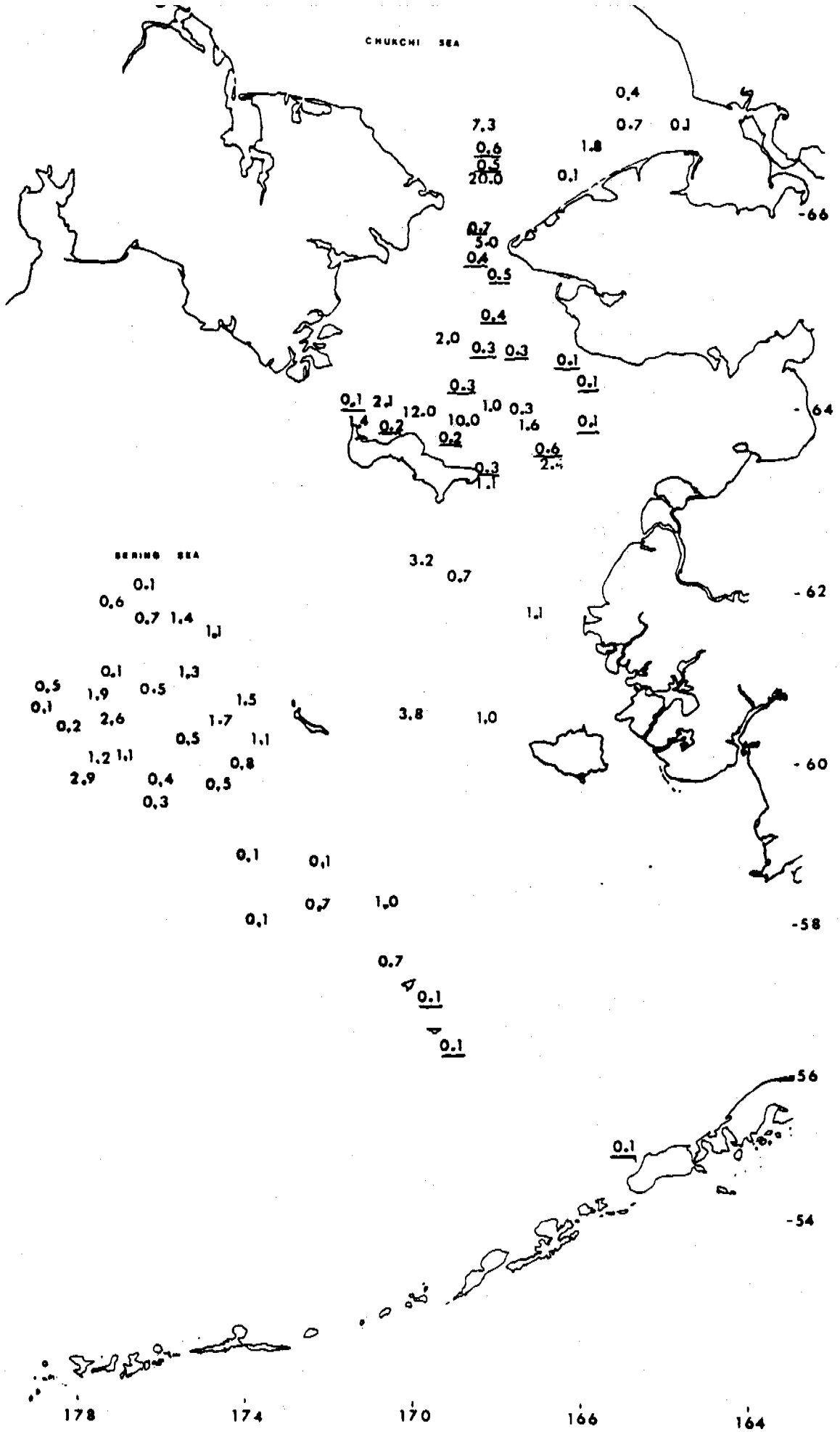


Fig 6 Viable counts x 10^6 -Sediment samples

present in higher numbers in 1980 than in 1979. In the Navarin Basin numbers of viable microorganisms ranged from 1 x 10^2 to 1.2 x 10^4 per ml.

Viable counts found in sediment ranged from 1 x 10^5 to 2.0 x 10^7 per gram dry wt. As with other results there was a difference between 1979 and 1980 samples in the Northern Bering. In 1979 viable counts ranged from 1 x 10^5 to 7 x 10^5 per gram, in 1980 viable counts were from 1.0 x 10^5 to 2.0 x 10^7 per gram.

Determination of the MPN of hydrocarbon degrading microorganisms (Figs. 7 and 8) revealed some surprising results. The 1979 MPN values ranged from 2 x 10^{-1} to 2.4 x 10^1 per ml in water. In 1980 the only two stations that had positive allowing for an estimate of the MPN for hydrocarbon degraders and these had less than 30 organisms per ml. The Navarin Basin was devoid of hydrocarbon degrading microorganisms in all samples, i.e. concentrations of less than 0.3/ml.

The MPN of hydrocarbon degraders found in sediments was also unusual. In 1979 the range of sediment hydrocarbon degraders was from 3 x 10^0 to 1.5 x 10^3 per grams. All samples of sediment from 1980 had less than 3 x 10^2 hydrocarbon degraders per gram. The sediments of the Navarin Basin were essentially devoid of hydrocarbon degrading microorganisms.

The potential for biodegradation of several classes of hydrocarbons was assessed in both 1979 and 1980.

Biodegradation of n-alkanes represented by hexadecane in water and sediments is shown in Figs. 9 and 10. The values shown are the mean percent of total radiolabel added, which was converted to CO_2 during the assay. The water samples from the 1980 cruise were all negative. In 1979 the percent of hexadecane degraded ranged from 0.9 to 2.4 for water samples.

In the 1979 sediment samples the percent of ^{14}C-hexadecane degraded ranged from 0.9 to 2.7. Four sediment samples from the 1980 cruise showed

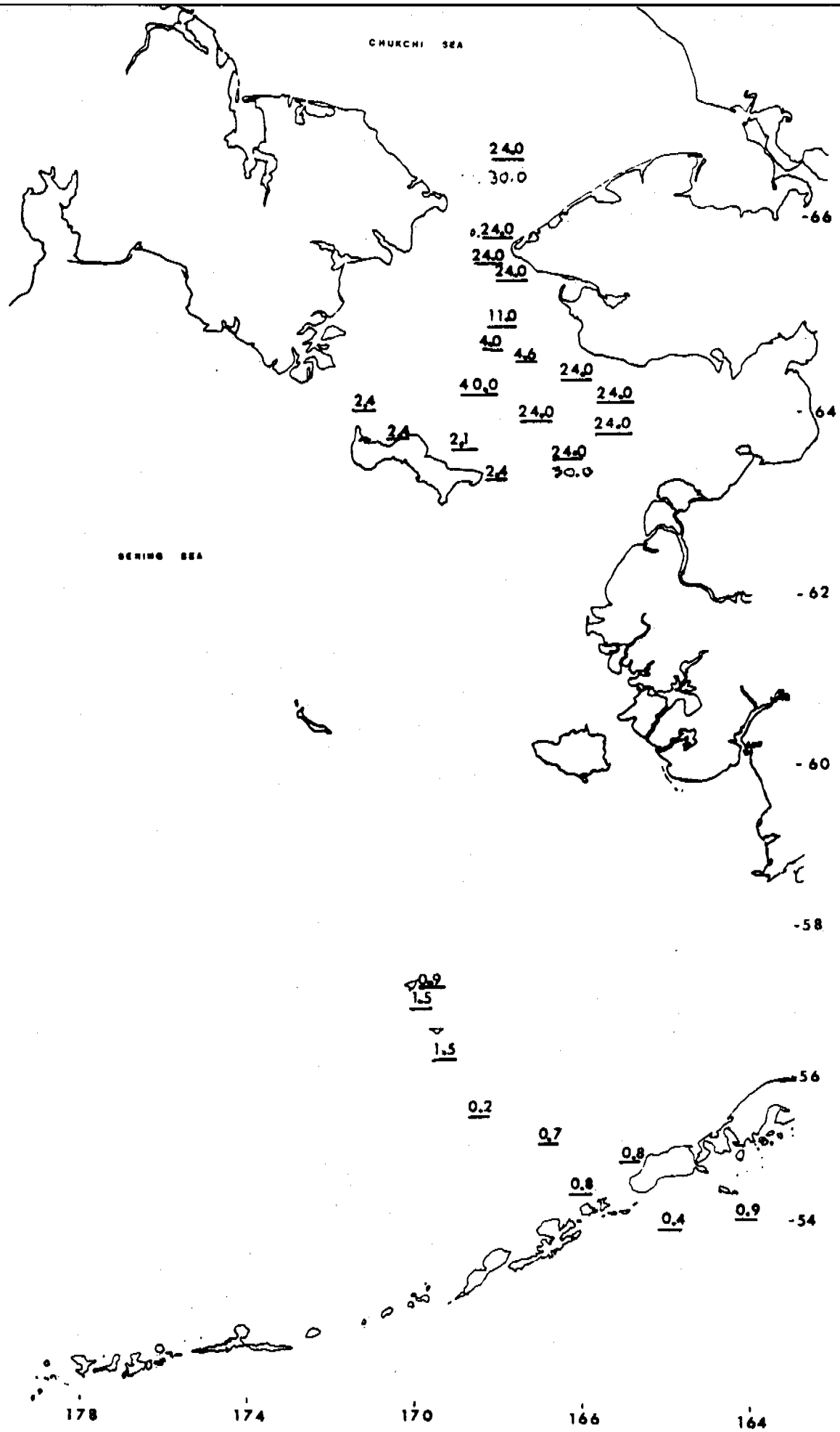


Fig 7 MPN of hydrocarbon utilizers - Water samples

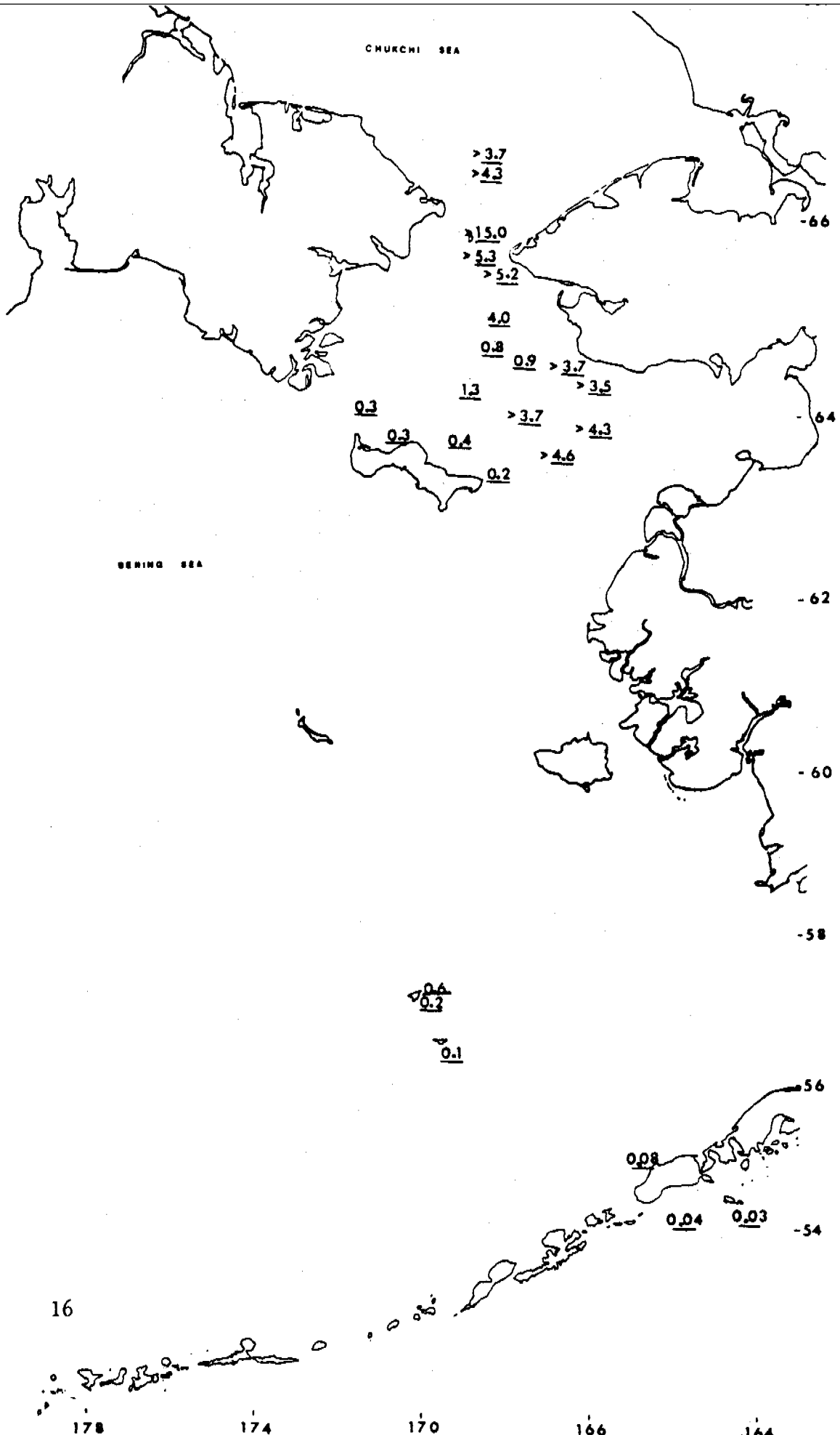


Fig 8 MPN of hydrocarbon utilizers x 10^2 -Sediment samples -

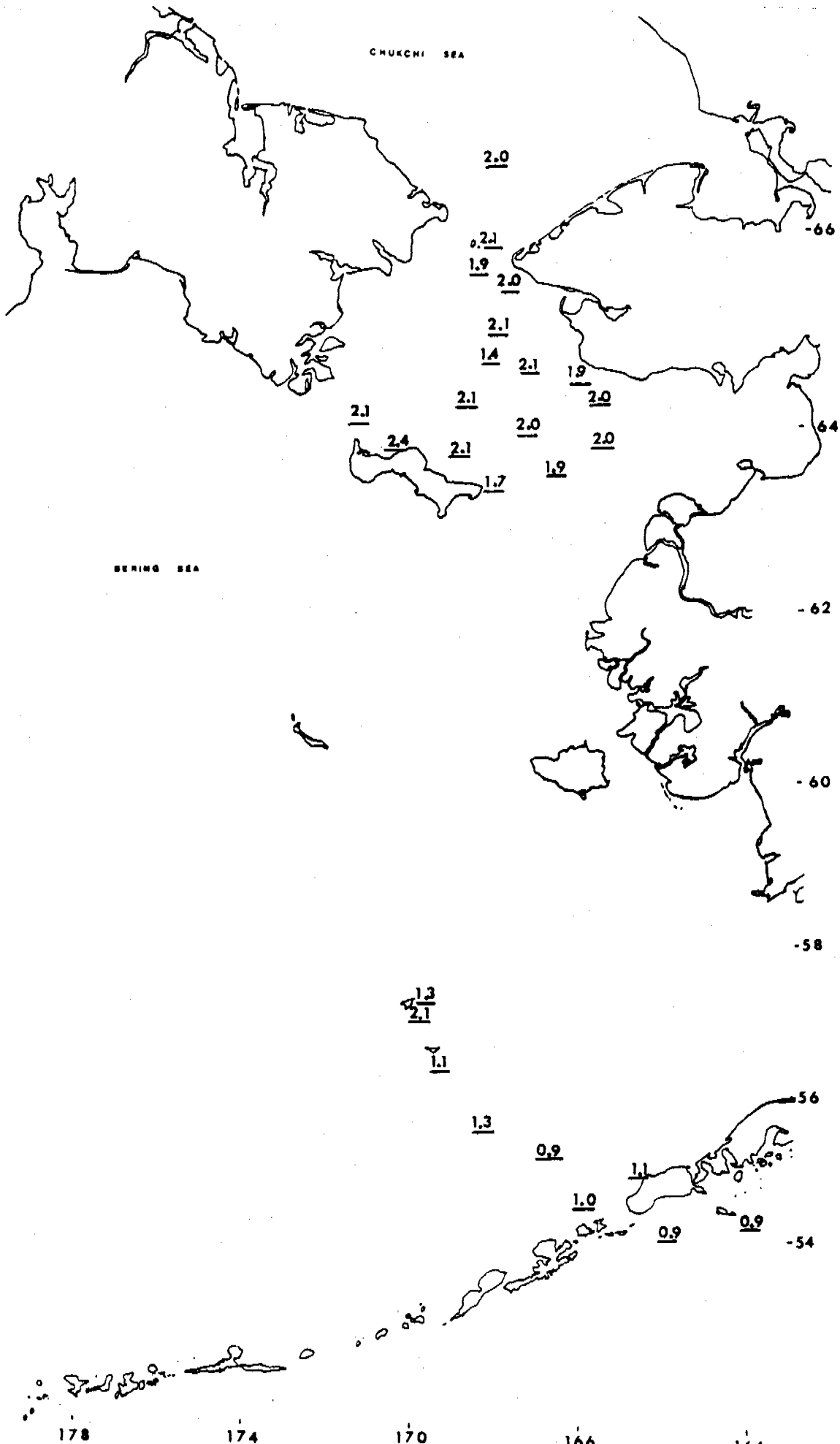


Fig 9 Biodegradation potentials for hexadecane- Water samples- Numbers represent % conversion to CO_2 - All other sites had 0%

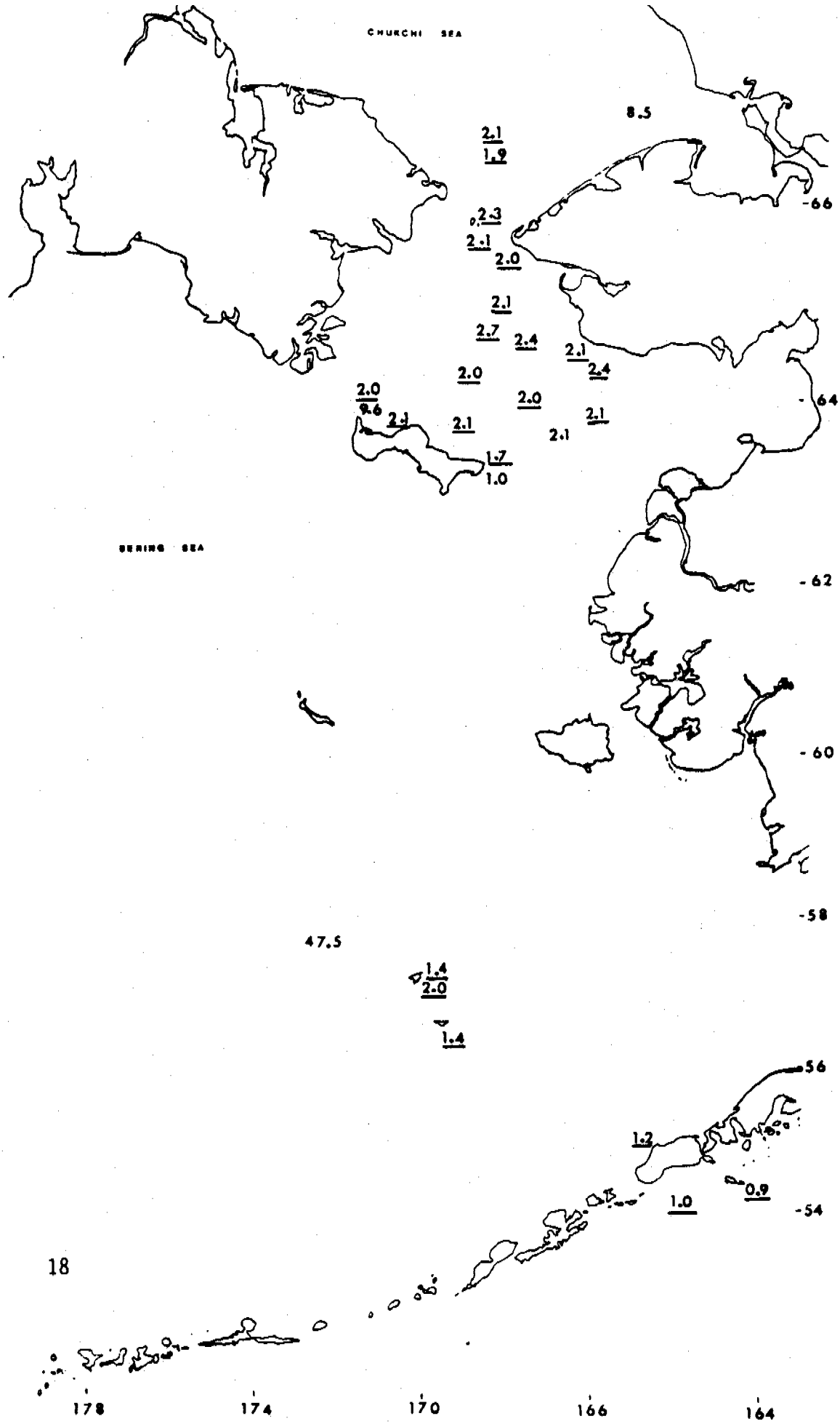


Fig 10 Biodegradation potentials for hexadecane- Sediment samples- Numbers represent % conversion to CO_2 - All other sites had 0%

positive degradation of hexadecane. The four positive values were 8.5, 9.6, 1.0, and 47.5. The one high value was found in sediments collected near St. Paul Island. The Navarin Basin samples did not show positive hexadecane biodegradation potentials.

Branched chain alkanes, represented by pristane, were less susceptible to biodegradation than hexadecane (Figs. 11 and 12). All water samples from the 1980 cruise were negative for biodegradation of pristane. Fewer water samples from the 1979 cruise demonstrated biodegradation of pristane than hexadecane; the range of the biodegradation potentials of pristane was from 0.1 to 0.3%.

The biodegradation of pristane by sediment organisms followed a pattern similar to that found in water. Fewer sediments demonstrated biodegradation of pristane than hexadecane. Only one sediment sample from the 1980 cruise was positive for biodegradation of pristane. The biodegradation potentials of pristane for the 1979 sediment samples ranged from 0.1 to 0.3%.

Biodegradation of alkylated three ring aromatic compounds represented by 9-mehtylanthracene was very limited (Fig. 13). Only one sediment sample from either the 1979 or 1980 cruise demonstrated degradation of 9-methyl-anthracene. The sample was collected on the beach of St. Paul Island. No water samples from either cruise demonstrated biodegradation of 9-methyl-anthracene.

No biodegradation of benzanthracene was found in either water or sediment from the 1980 cruise. Biodegradation of benzanthracene was found in some water and sediment from the 1979 cruise (Figs. 14 and 15). The range of percent biodegradation of benzanthracene in water samples was from 0.2 to 0.3. In sediment the range was from 0.2 to 0.6 percent. Only four water samples and five sediment samples were found to possess the ability to degrade benzanthracene.

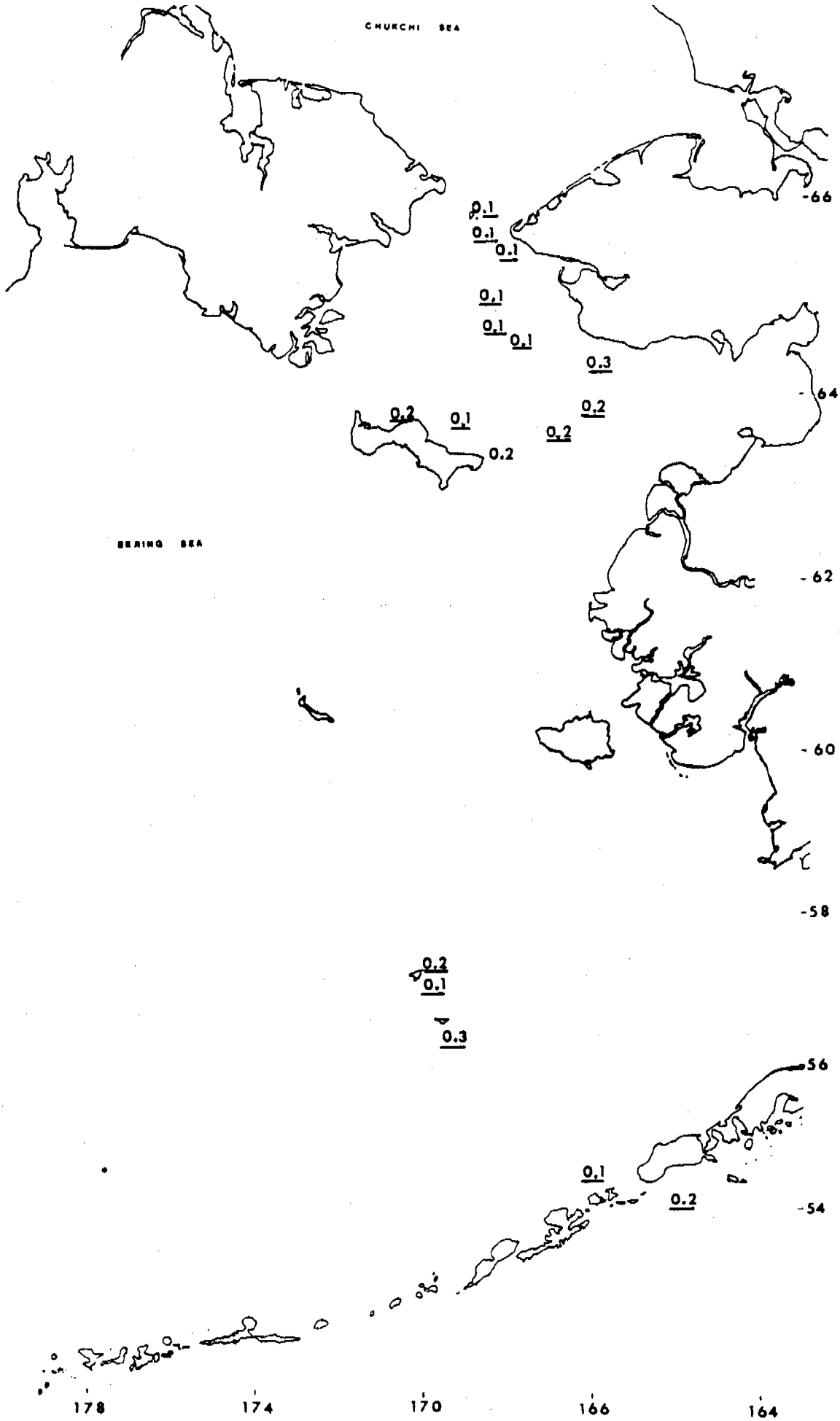


Fig II Biodegradation potentials for pristane - Water samples

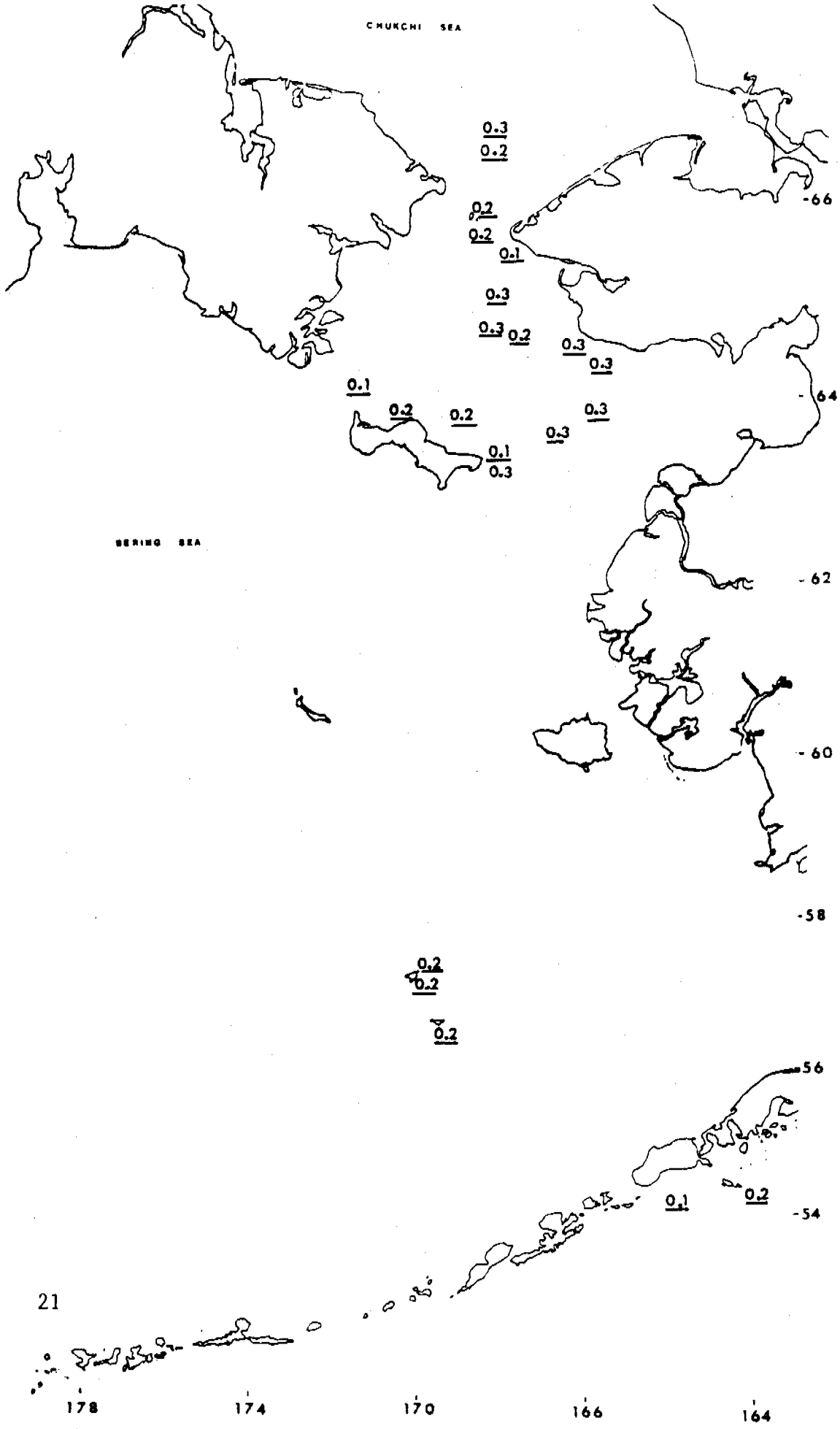


Fig 12 Biodegradation potentials for pristane- Sediment samples

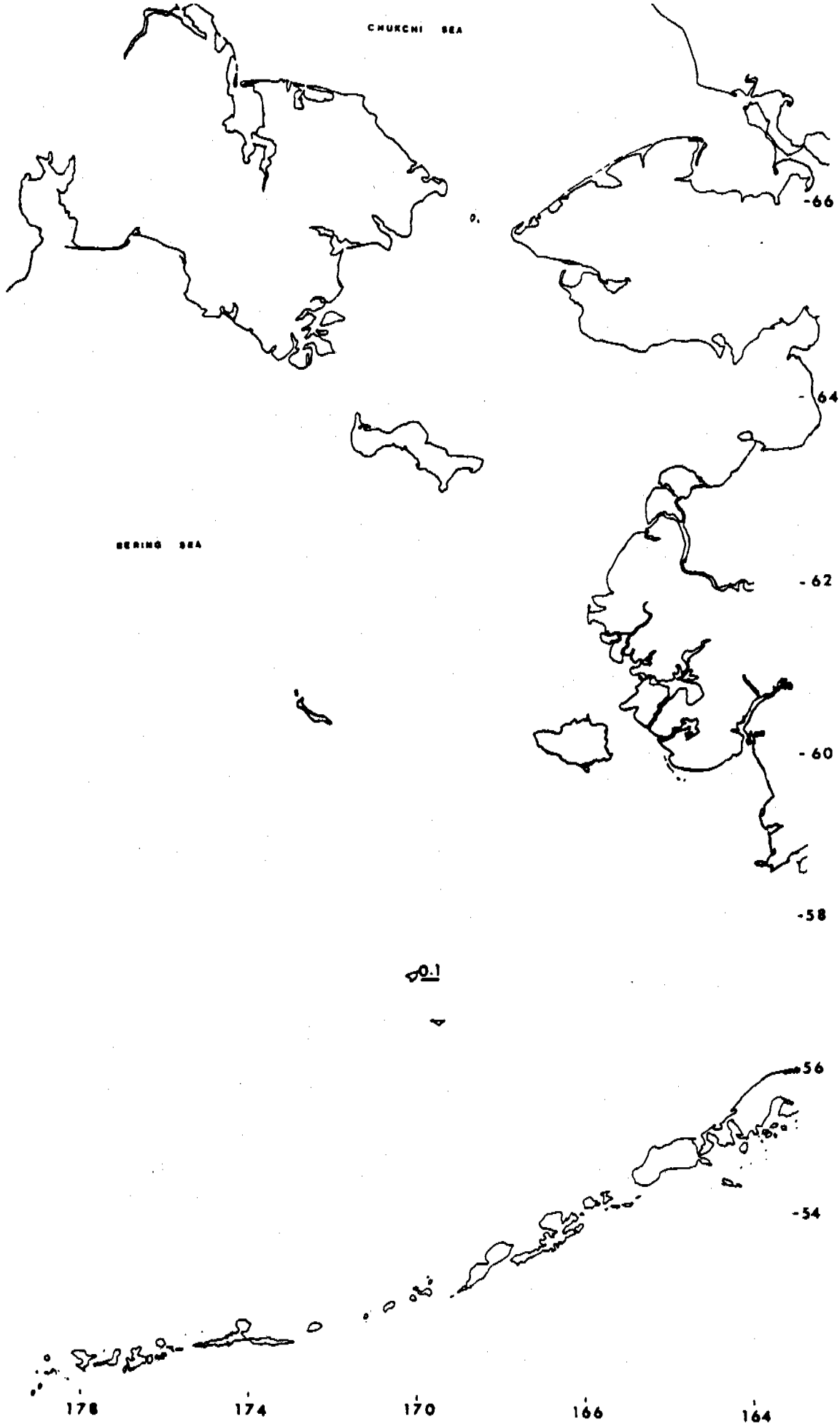


Fig 13 Biodegradation Potentials for 9 methylanthracene - Sediment samples- Numbers shown represent % conversion to CO_2 - All other sites had 0%

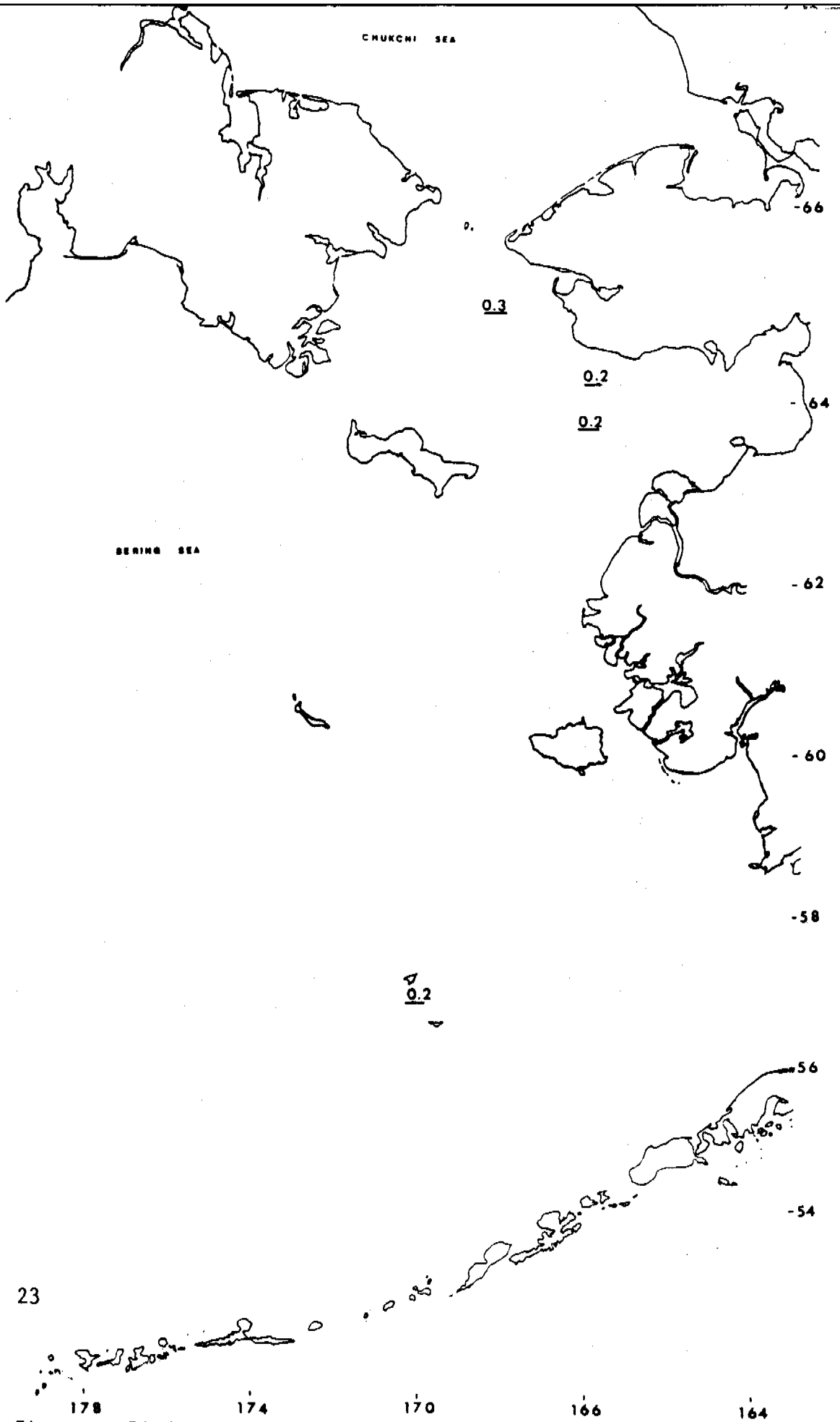


Fig 14 Biodegradation potentials for benzanthracene - Water samples- Numbers shown represent % conversion to CO_2 - All other sites showed 0%

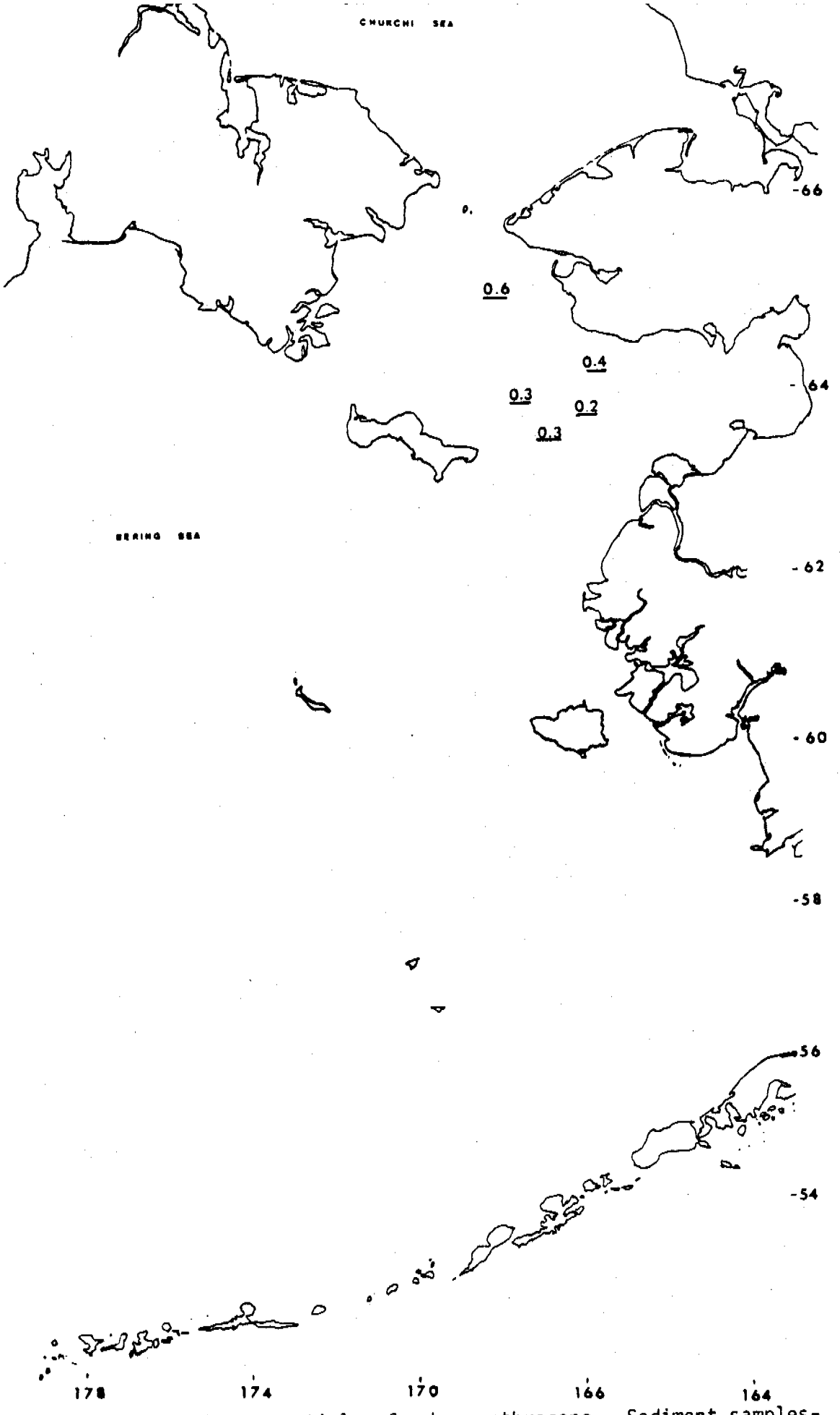


Fig 15. Biodegradation potentials for benzanthracene- Sediment samples- Numbers shown represent % conversion to CO_2 - All other sites showed 0%

Over all the degradation of petroleum hydrocarbons followed the pattern hexadecane > pristane >> benzanthracene >> 9-methylanthracene.

Samples were collected in the Beaufort Sea in August, 1978. Station numbers and locations are shown in Fig. 16. Water depth at each station are shown is Fig. 17. Salinity data collected is shown in Fig. 18. Salinity of the surface waters ranged from 10.3 to 29.3 parts per thousand. Water temperatures Fig. 19 ranged from -2 to $6^{o}C$.

Total counts of microorganisms in water and sediment are shown in Figs. 20 and 21. Total populations of organisms in water ranged from 2.2×10^{5} to 3.1×10^{9} per gram in sediment.

Viable microorganisms were enumerated by counting on Marine agar (Figs. 22 and 23). Water samples yielded from 7×10^{1} to 1.8×10^{4} organisms per ml. Most of the higher viable populations of microorganisms were found off the mouths of rivers, in shallow water. The viable microorganisms recovered from sediments ranged from 1×10^{5} to 3×10^{7} per gram. As with water samples most of the higher populations were found in near shore sediments, especially at the mouths of rivers.

The MPN of hydrocarbon degraders found in the waters of the Beaufort Sea varied across three orders of magnitude (Figs. 24 and 25). The range found was from 1 to 2400 per ml in water. Similar variation was observed in the MPN of hydrocarbon degraders found in sediments. The range of populations of hydrocarbon degraders was from 1 to 2.4×10^{5} per gram sediment. There was little if any, association of hydrocarbon degraders with proximity to the mouths of rivers.

The percent biodegradation of hexadecane by water and sediment microorganisms are shown in Figs. 26 and 27. Biodegradation of hexadecane was relatively low with only one water sample exhibiting more than 1% biodegradation of hexadecane. Many of the values observed were less than 0.1%

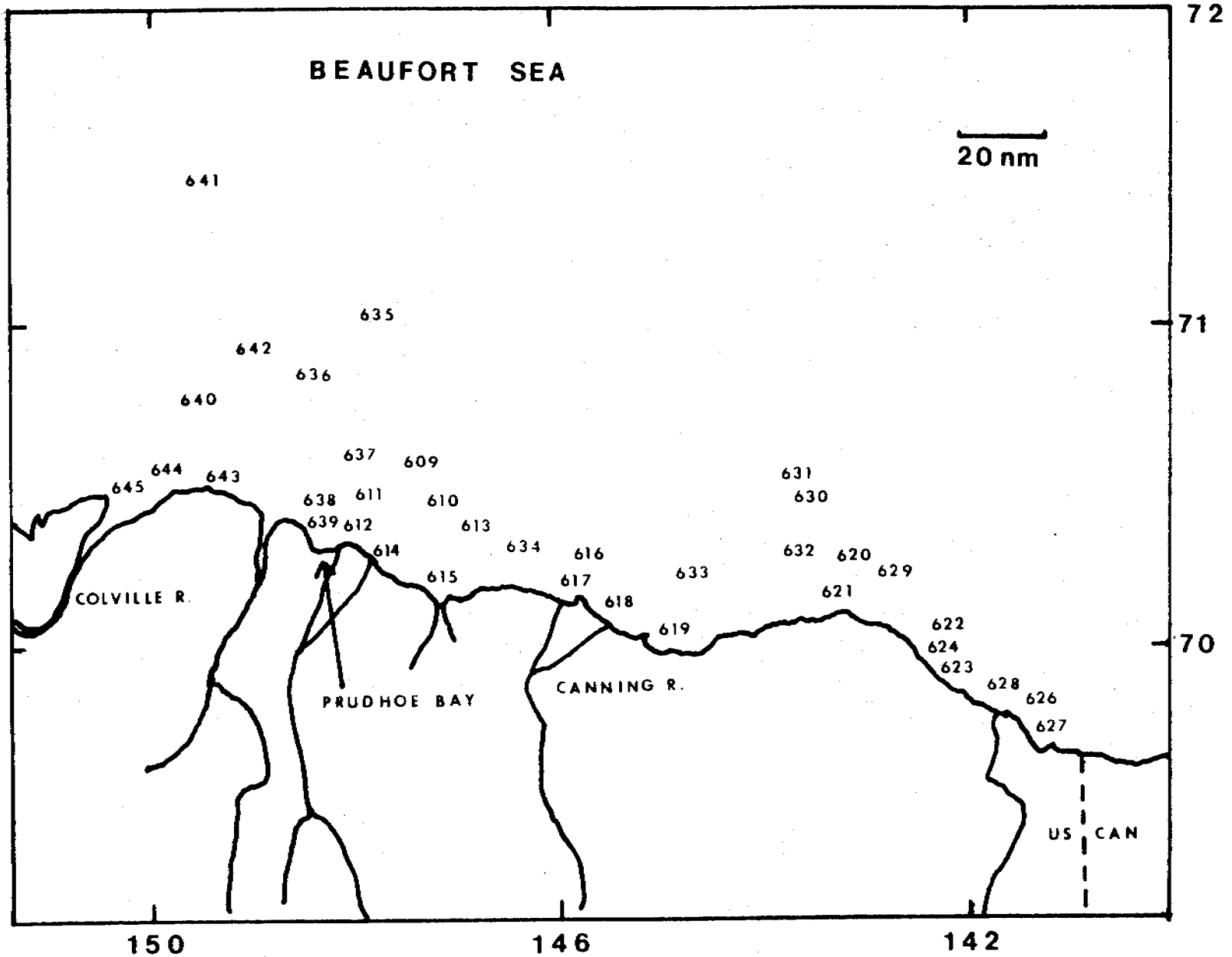


Fig 16 Station locations

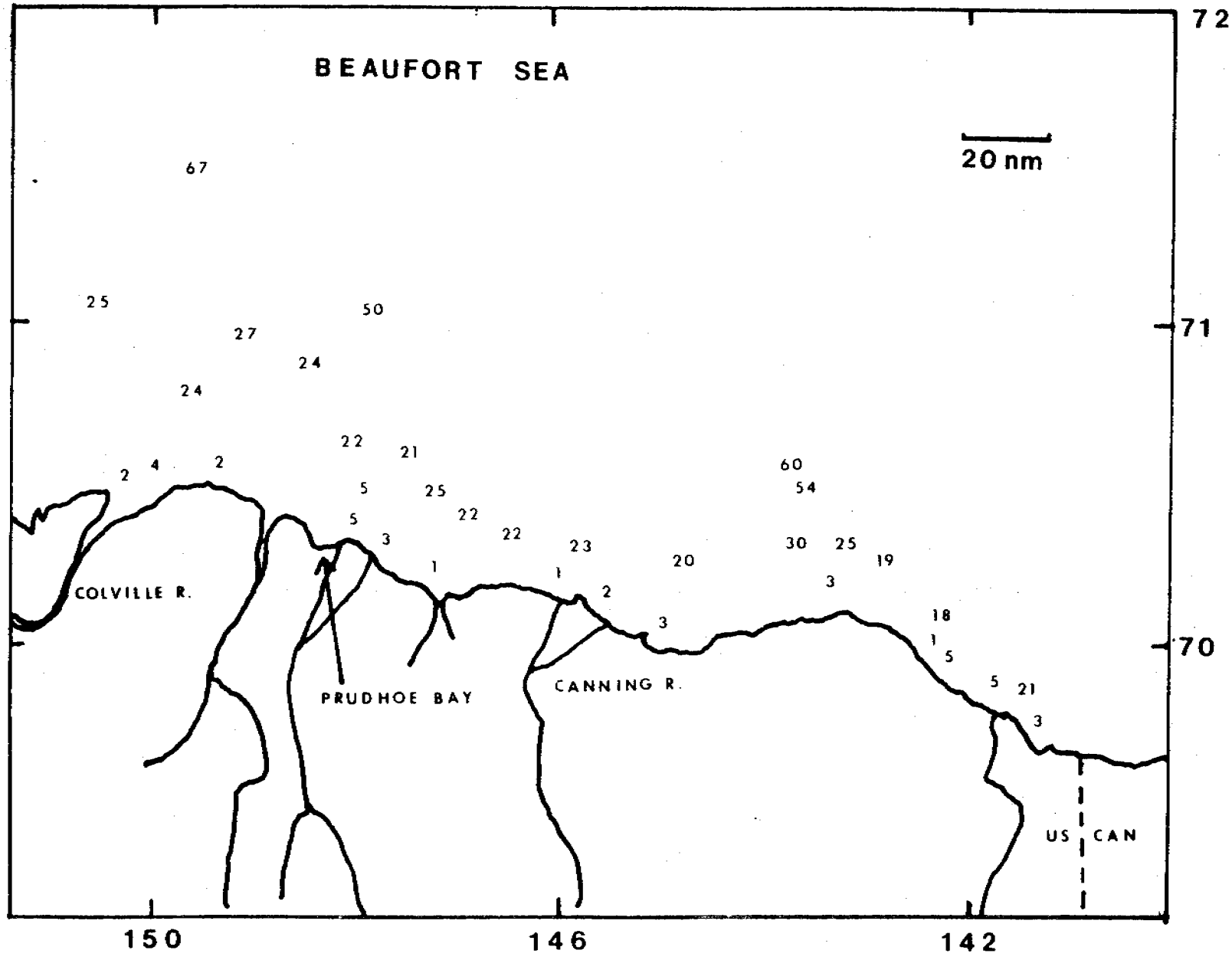


Fig 17 Water depth in meters

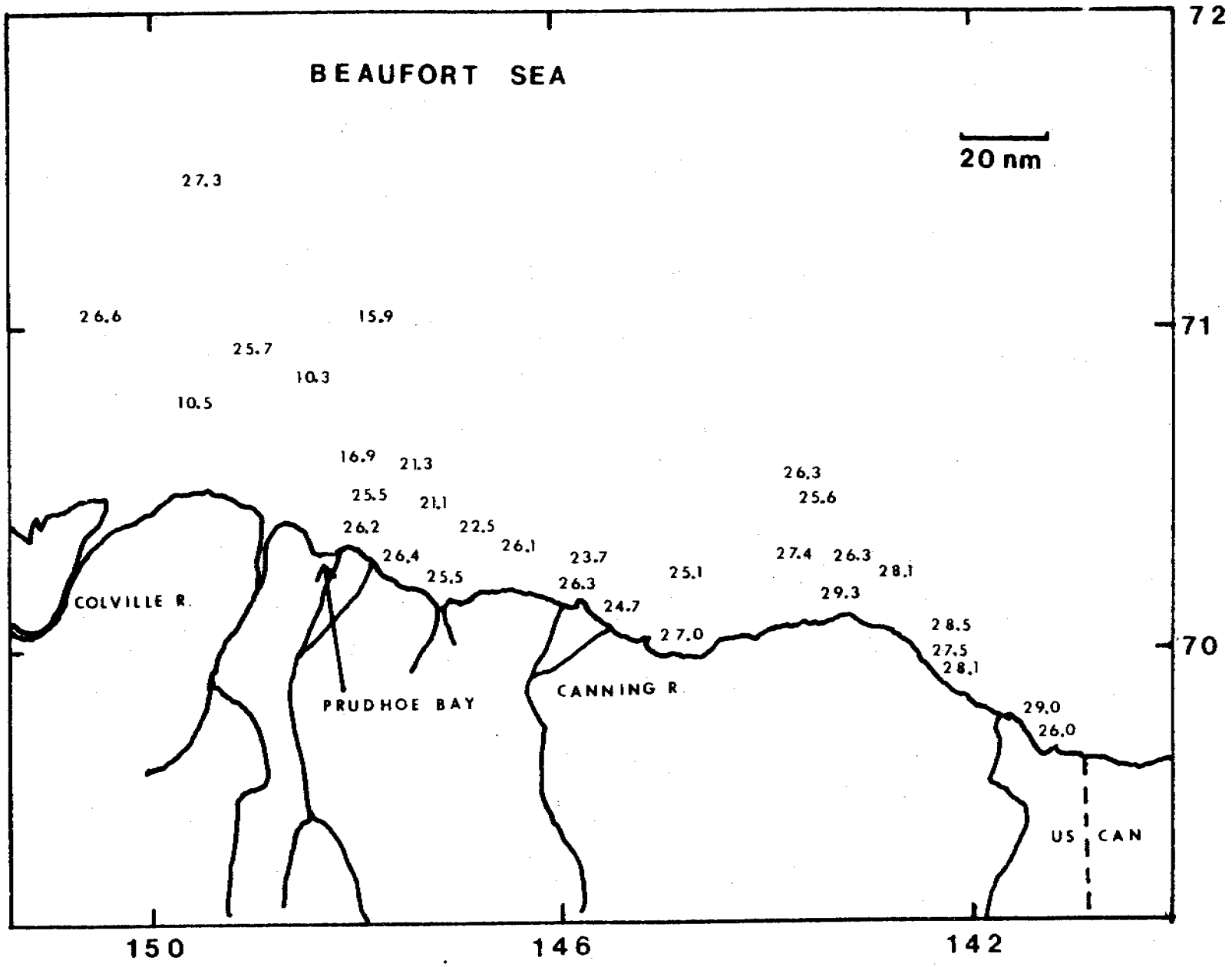


Fig 18 Salinities

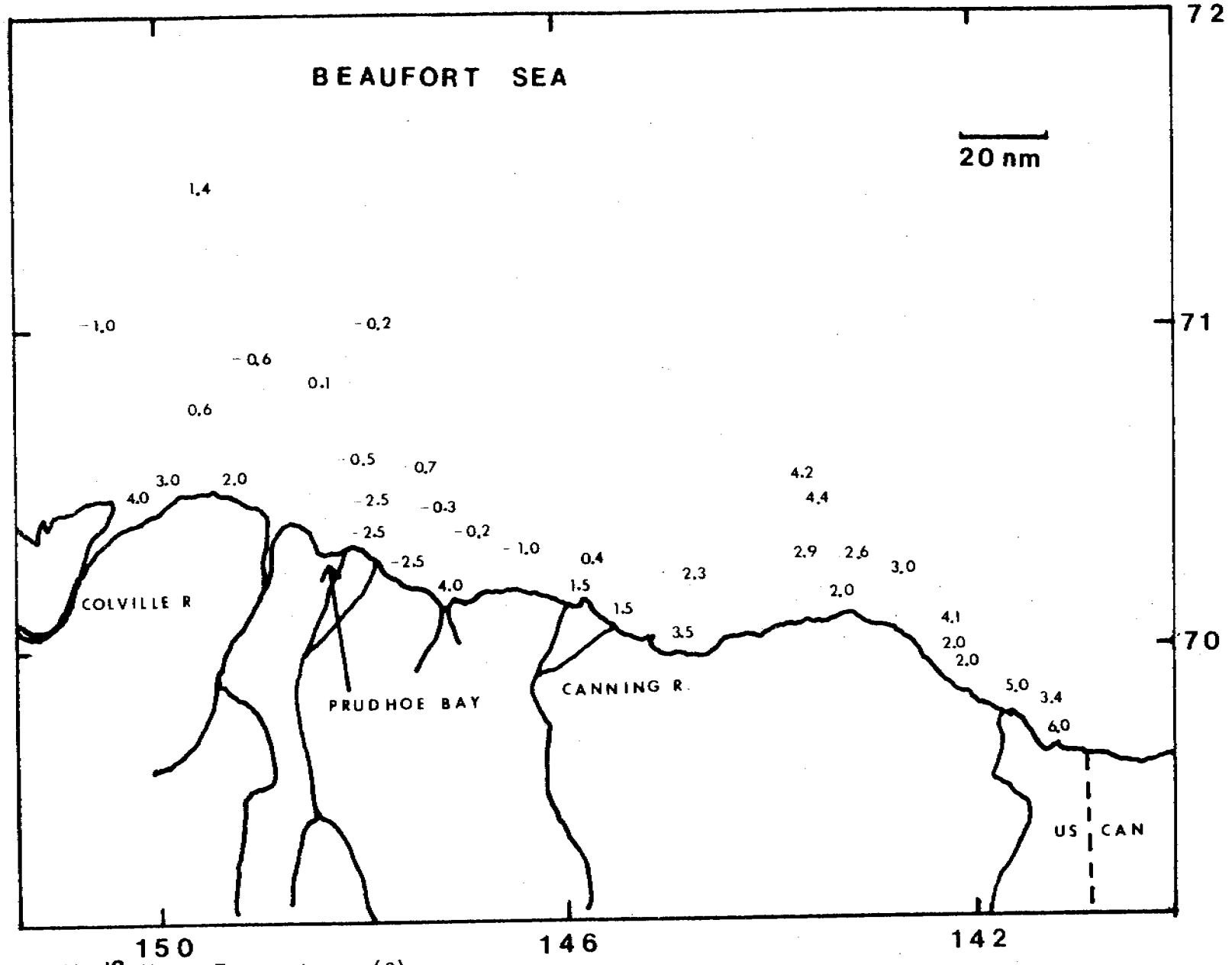


Fig 19 Water Temperatures (C)

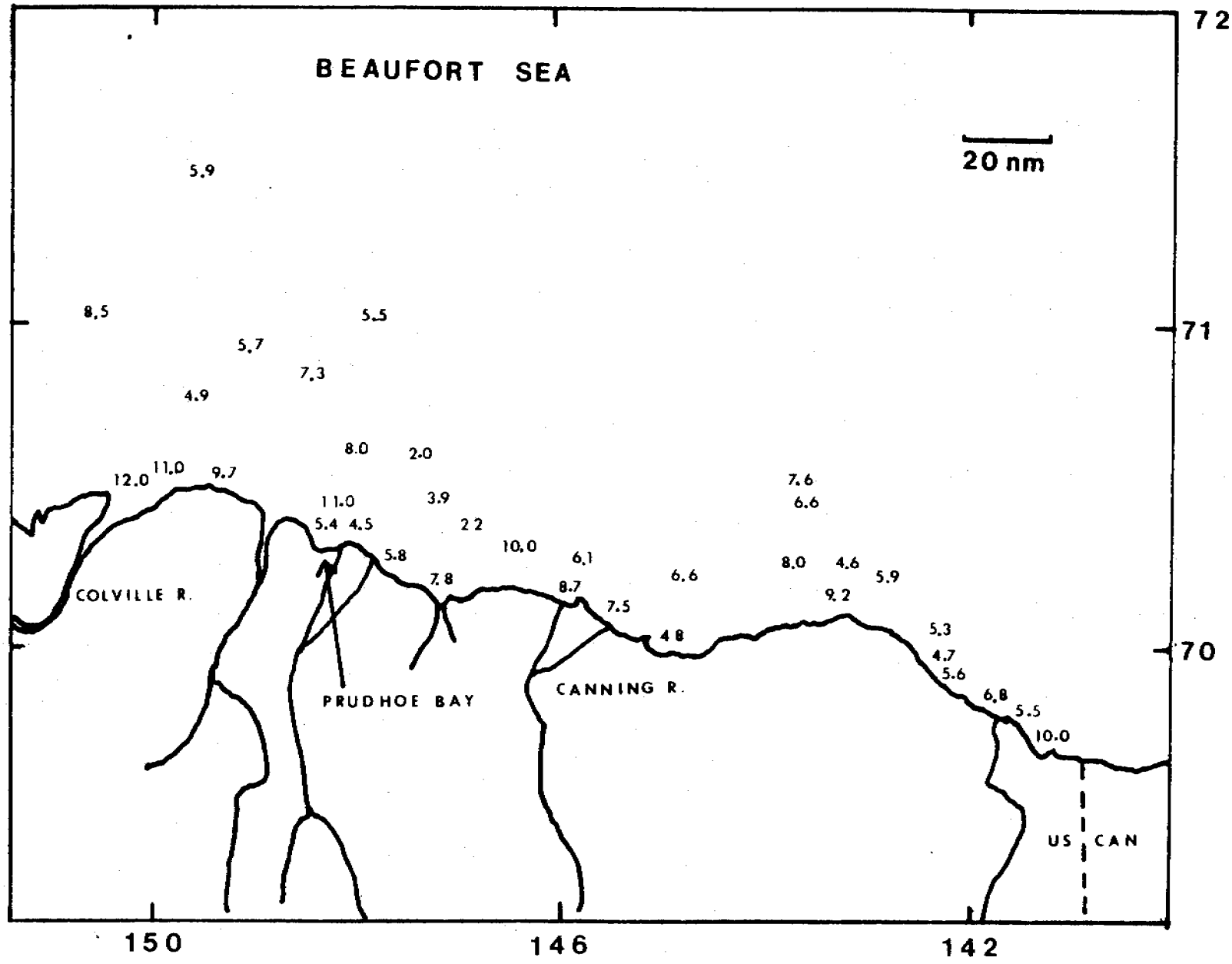


Fig 20 Direct counts x 10^5 -water samples

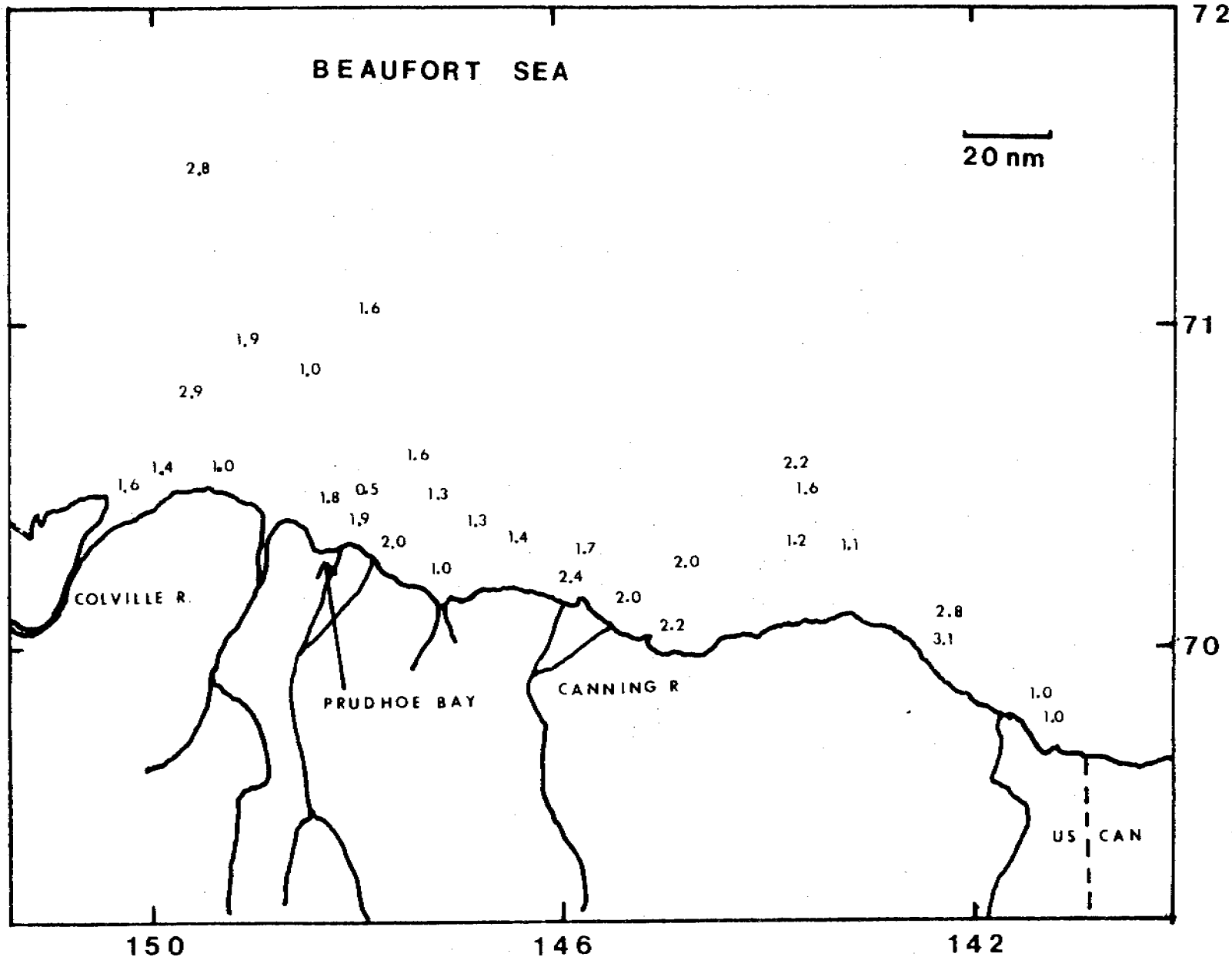


Fig 21 Direct counts x 10^9 per gram dry wt. - Sediment samples

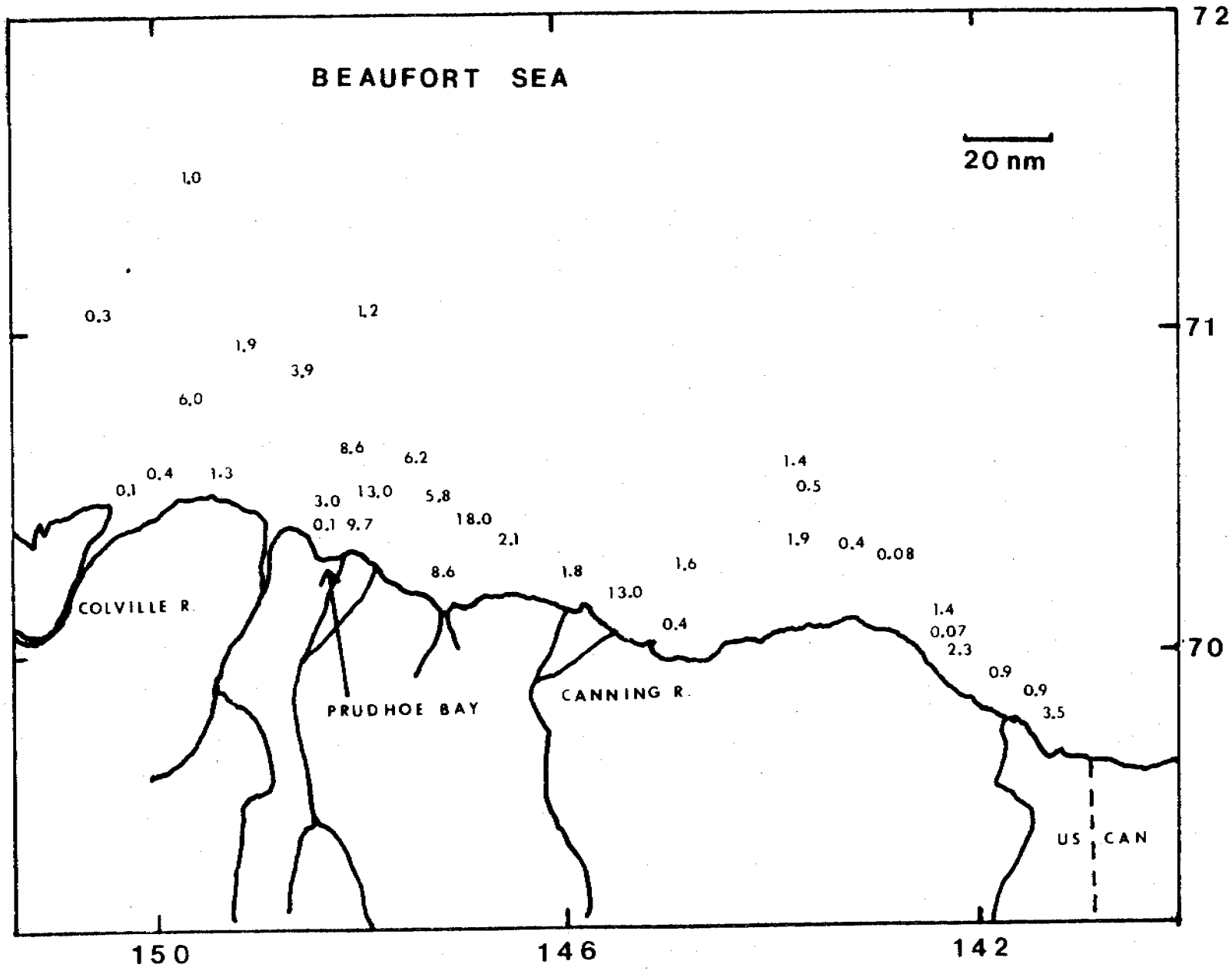


Fig 22 Viable counts x 10^3 - Water samples

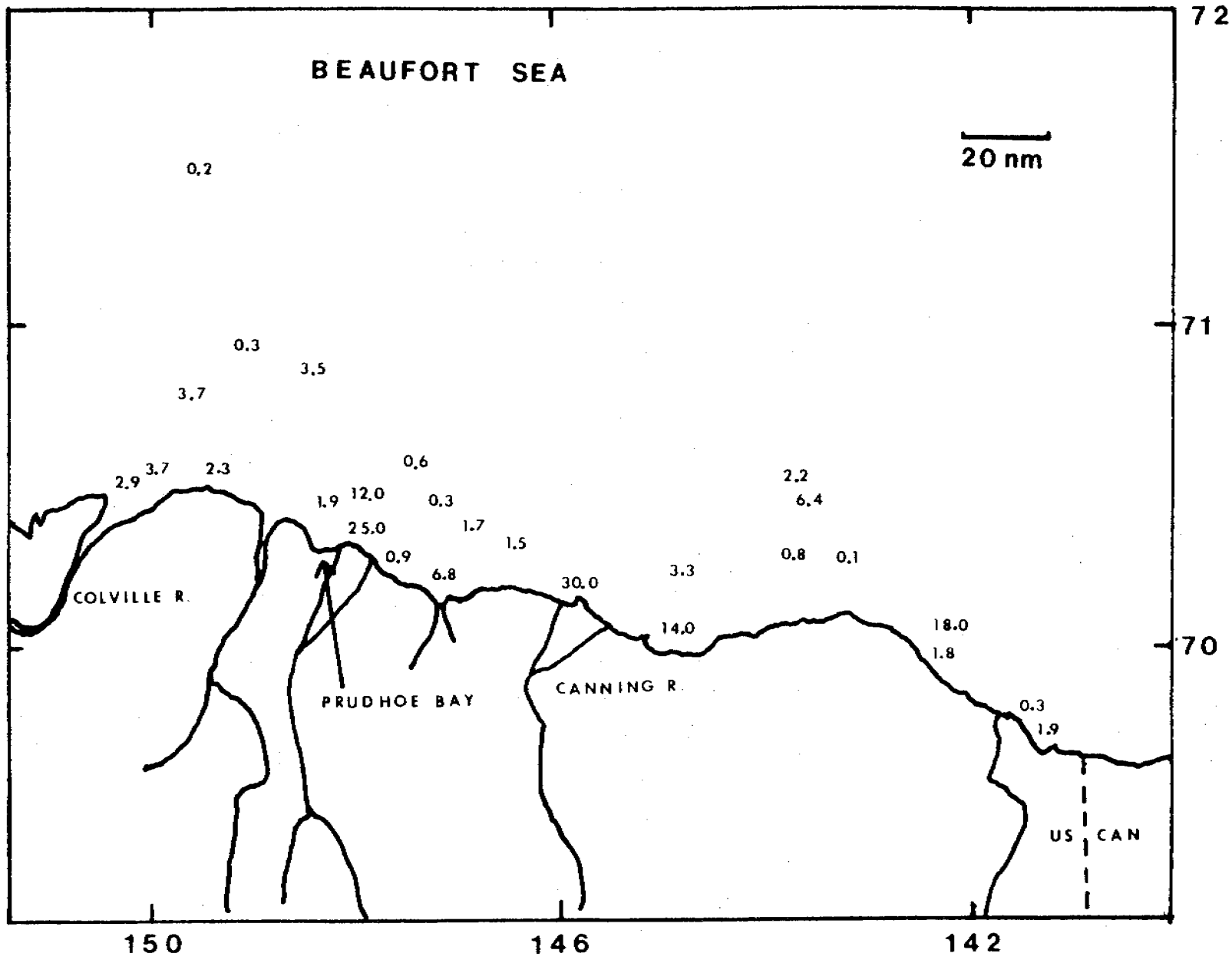


Fig 23 Viable counts x 10^6 - Sediment samples

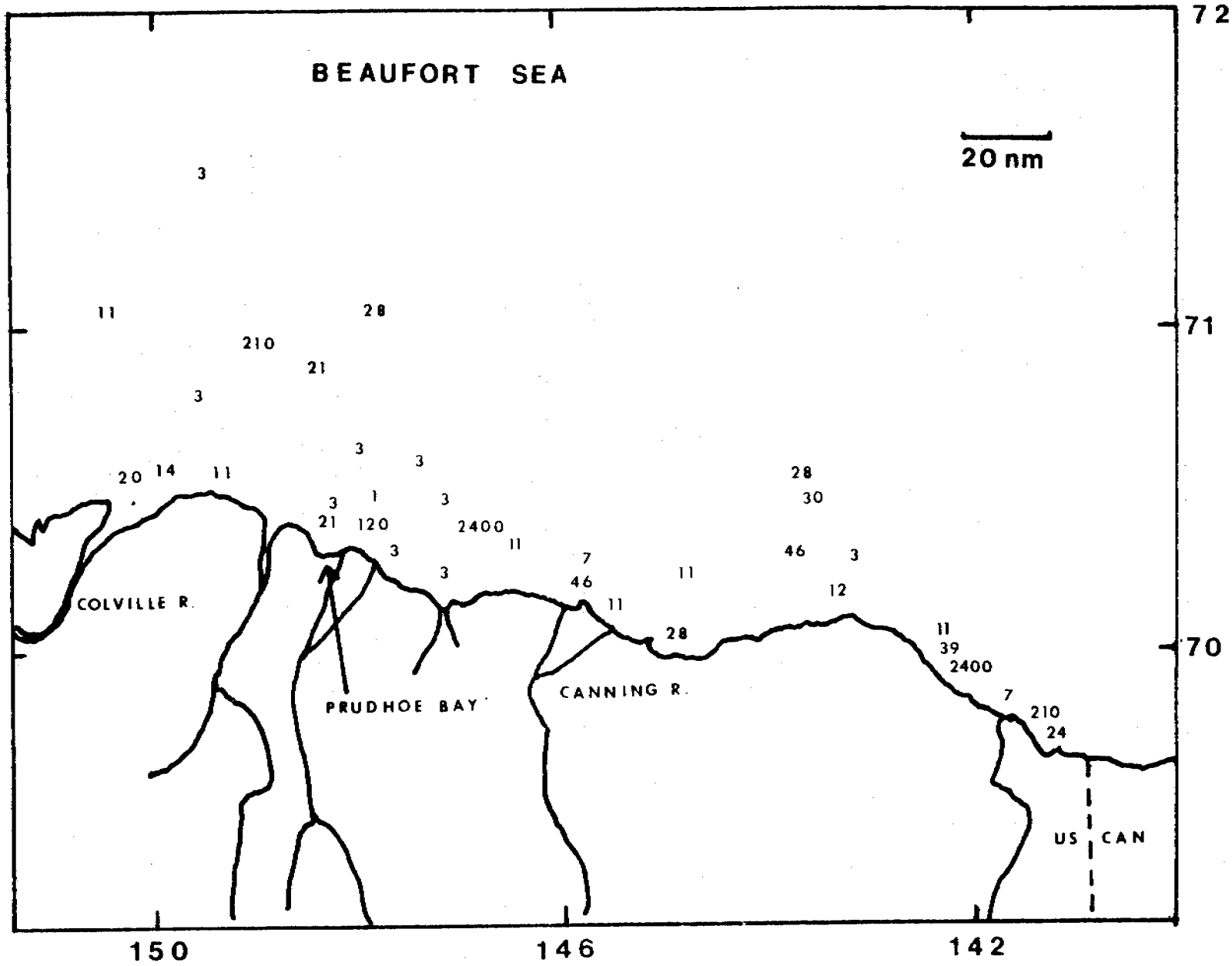


Fig 14 MPN of hydrocarbon degraders - Water samples

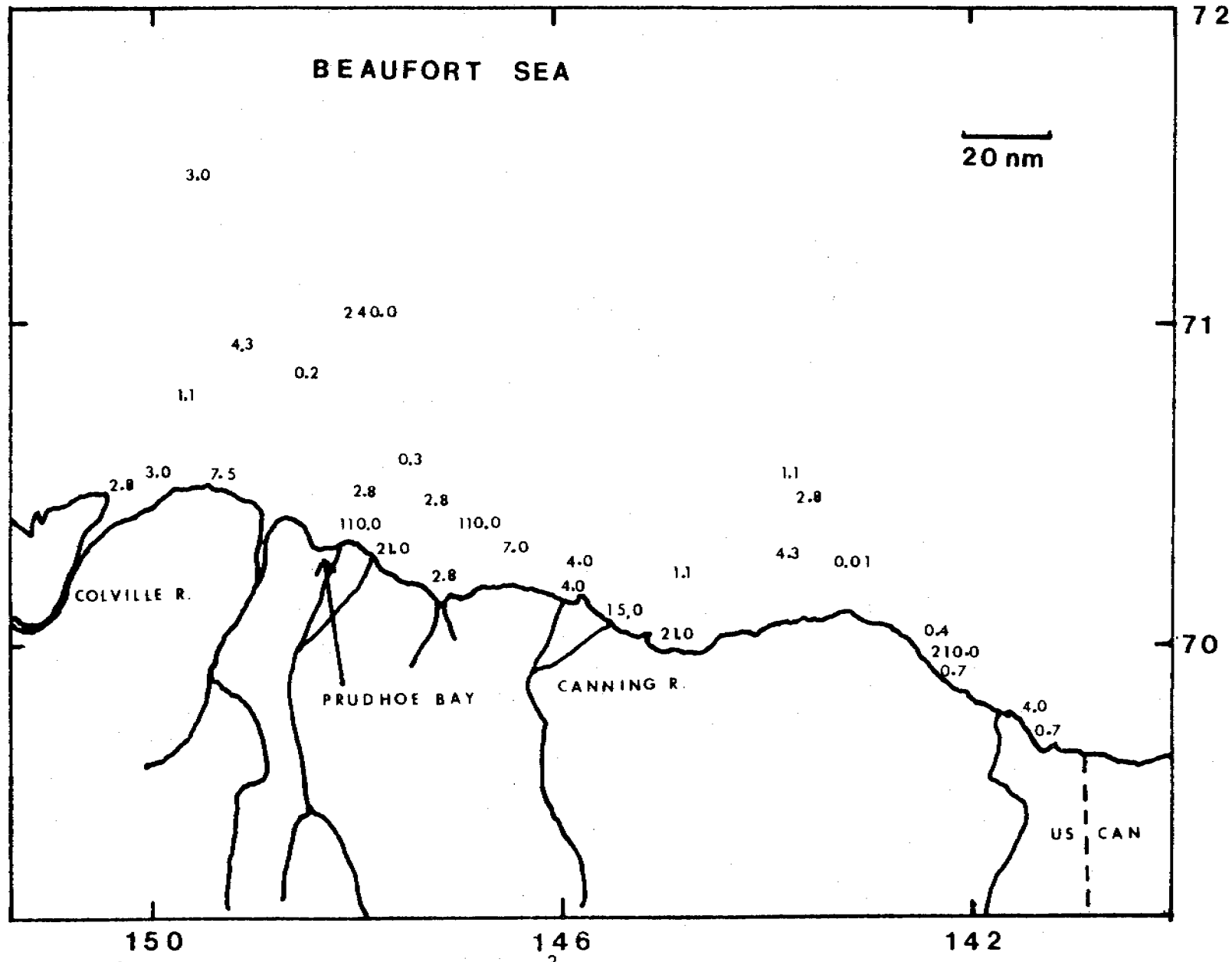


Fig 25 MPN of hydrocarbon degraders x 10^3 - Sediment samples

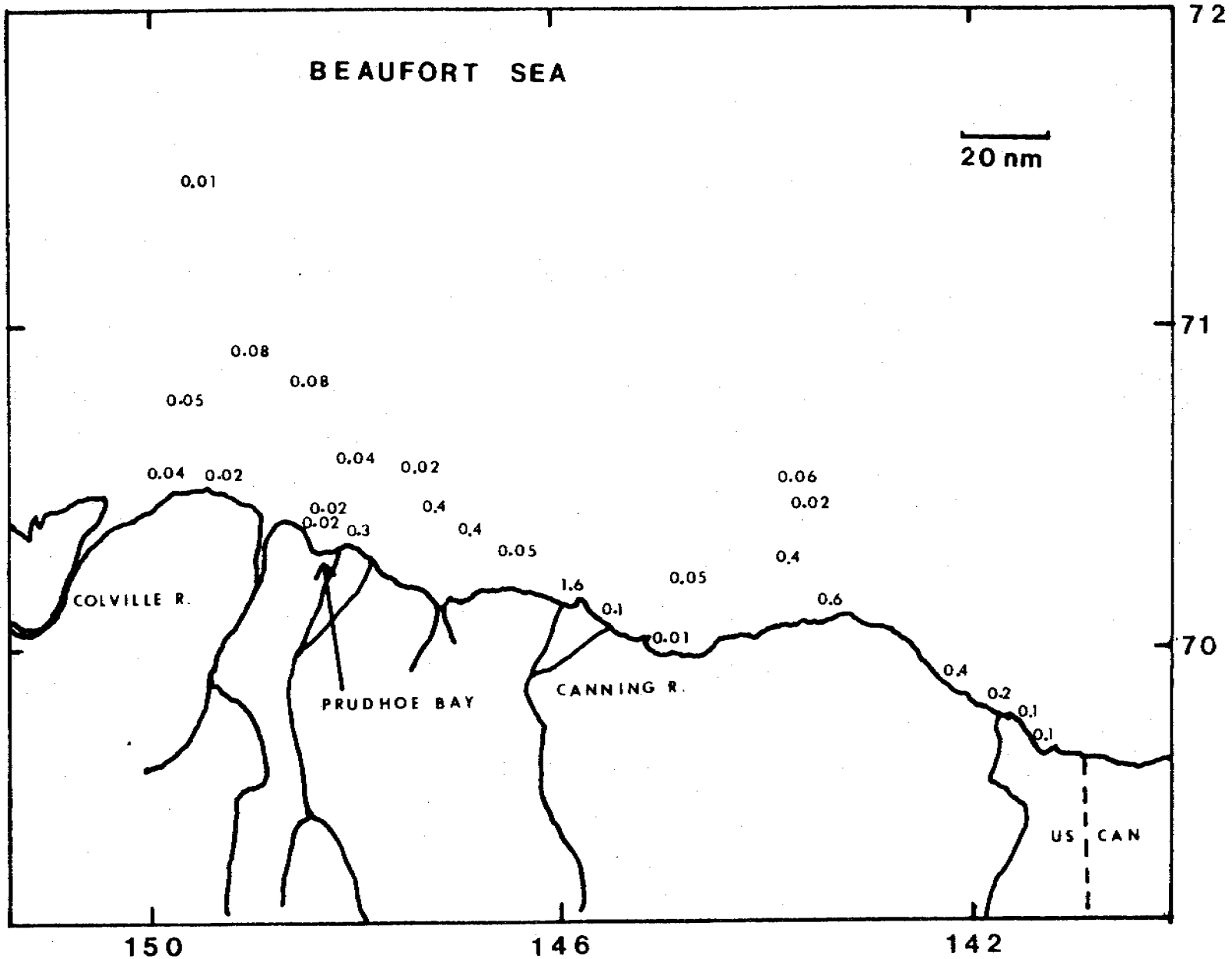


Fig 16 Biodegradation potentials for hexadecane - Water samples

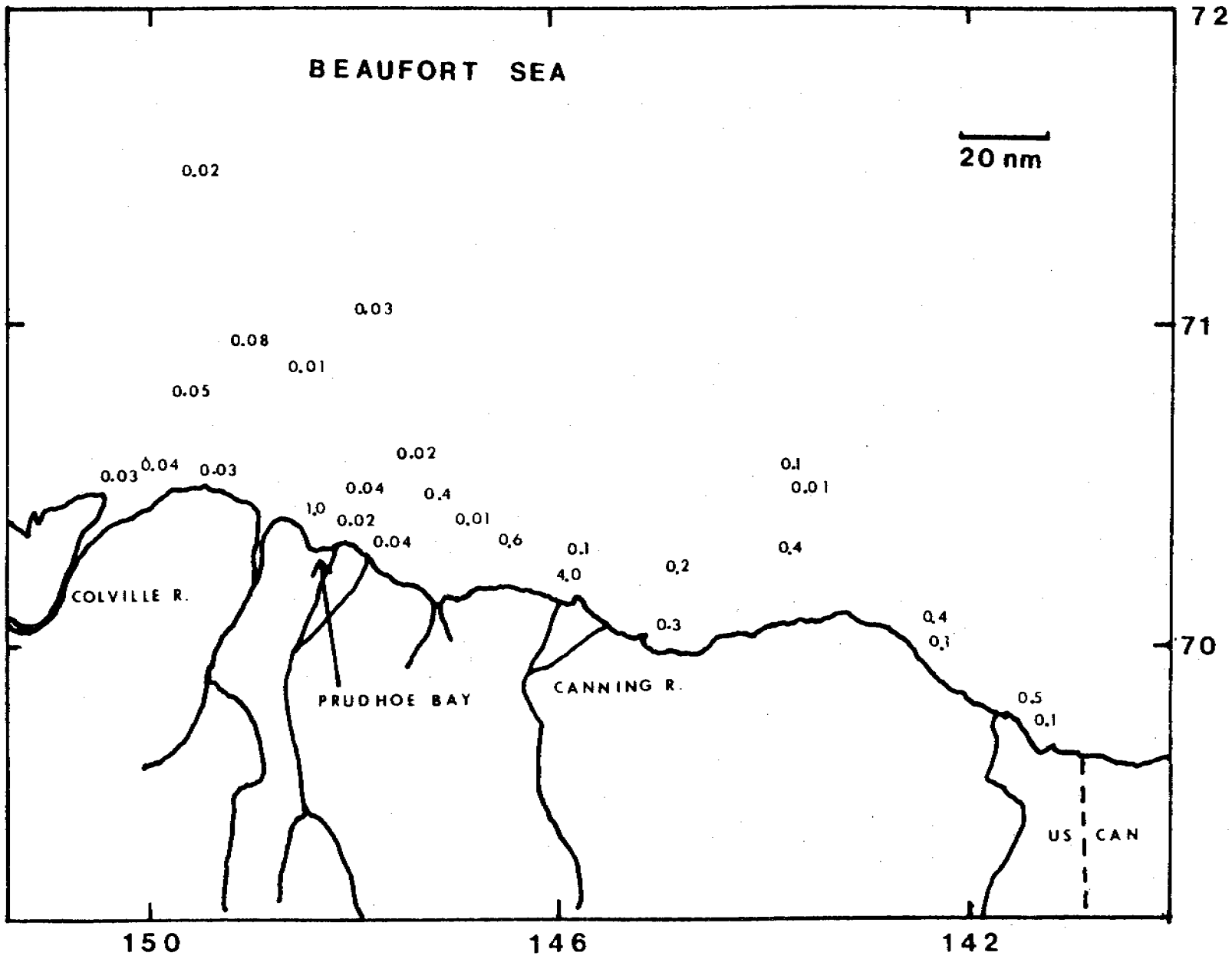


Fig 17 Biodegradation potentials for hexadecane - Sediment samples

biodegradation of hexadecane in water. Only two sediment samples exhibited more than 1% biodegradation of hexadecane. Most sediment samples showing greater than 0.1% biodegradation were near shore stations and most offshore stations and most offshore stations had less than 0.1% biodegradation of hexadecane.

Biodegradation of pristane in water samples was shown by fewer stations than biodegradation of hexadecane (Fig. 28). No water sample exhibited more than 1% biodegradation of pristane. Fewer sediment samples than water samples exhibited biodegradation of pristane (Fig. 29). Most sediment exhibited less than 0.1% degradation of pristane. Ten stations exhibited more than 0.1% degradation of pristane by sediment organisms. Only one sediment exhibited 1% or more degradation of pristane in the period of the experiment.

Biodegradation of benzanthracene by water and sediment organisms is shown in Figs. 30 and 31. Degradation of benzanthracene was found in fewer water samples than either pristane or hexadecane. Four water samples exhibited 0.1% or greater biodegradation of benzathracene. Most of the water samples exhibiting biodegradation of benzanthracene showed 0.1% or less degradation of benzanthracene. In sediment samples higher degradative capcity for benzanthracene was found. The range of percent biodegradation of benzanthracene was from 0.01 to 0.8%. Most sediment samples exhibited less than 0.1% biodegradation of benzanthracene. Six sediment samples exhibited biodegradation of benzanthracene of 0.1% or more.

Overall the potential for biodegradation of petroleum hydrocarbons followed the pattern: hexadecane > pristane > benzanthracene. Greater degradative activity was usually found in sediments than in waters.

NASTE

Samples were collected on the North Aleutian Shelf and St. George Basin during August-September 1980. Station numbers and locations are shown in

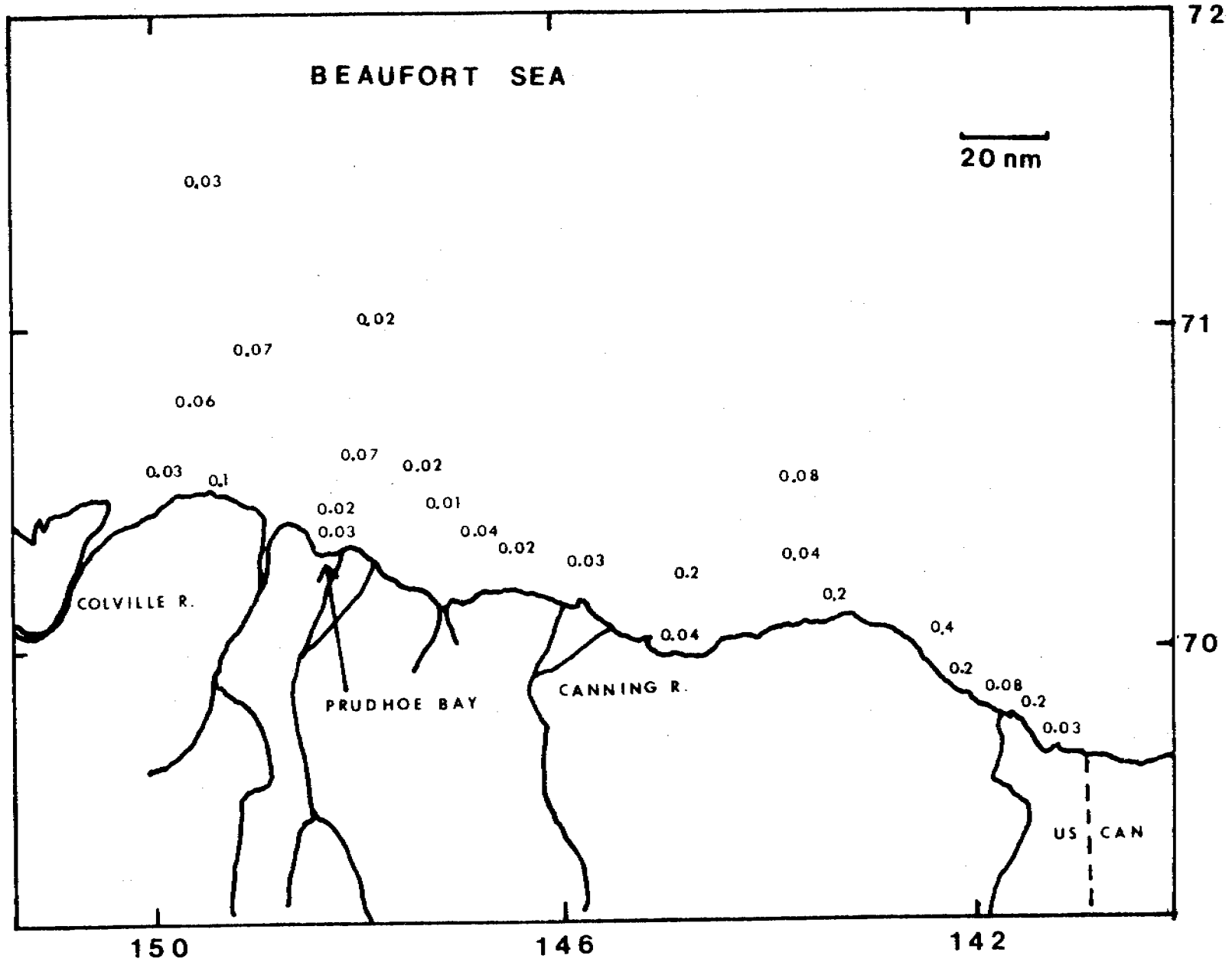


Fig 28 Biodegradation potentials for pristane - Water samples

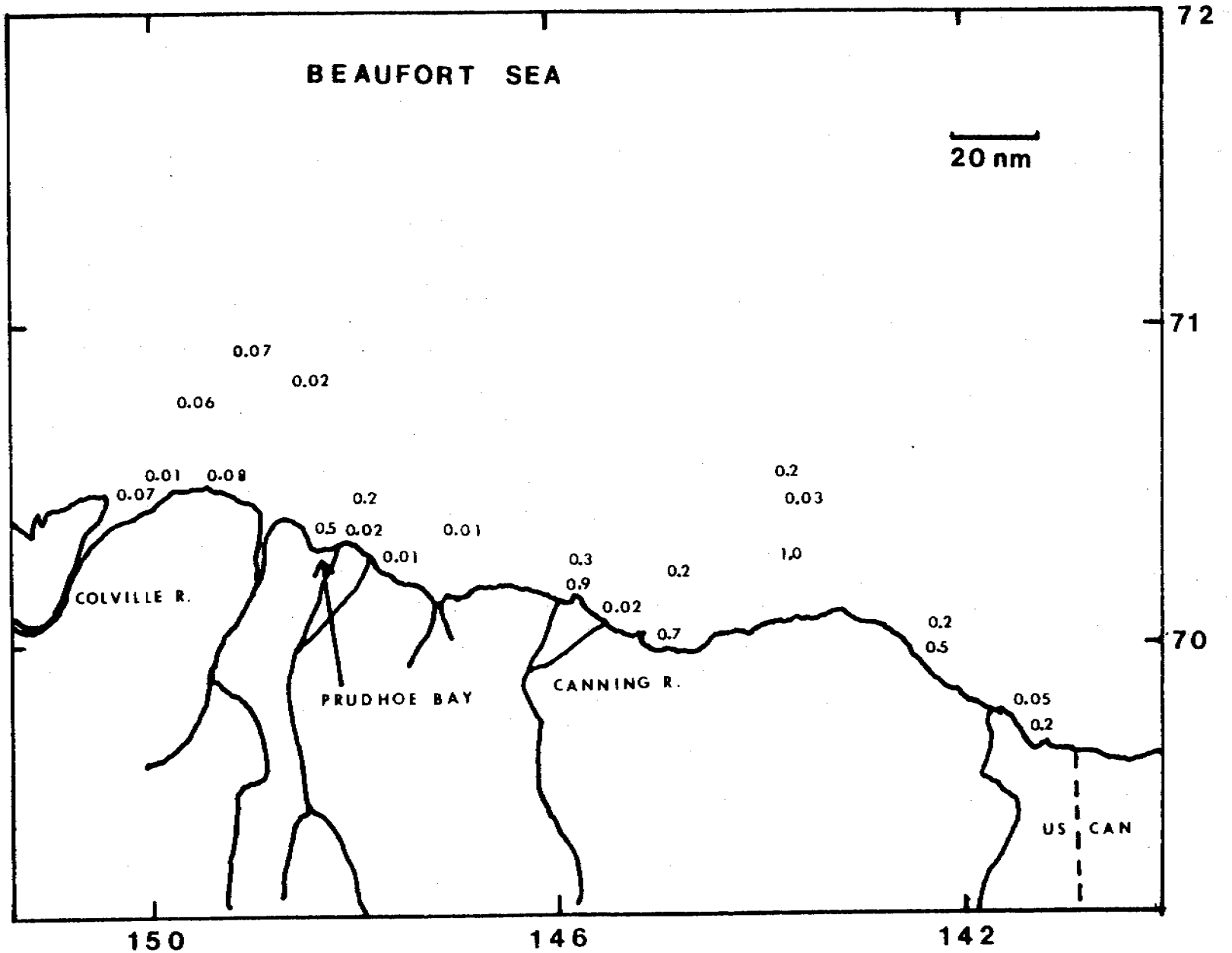


Fig 19 Biodegradation potentials for pristane - sediment samples

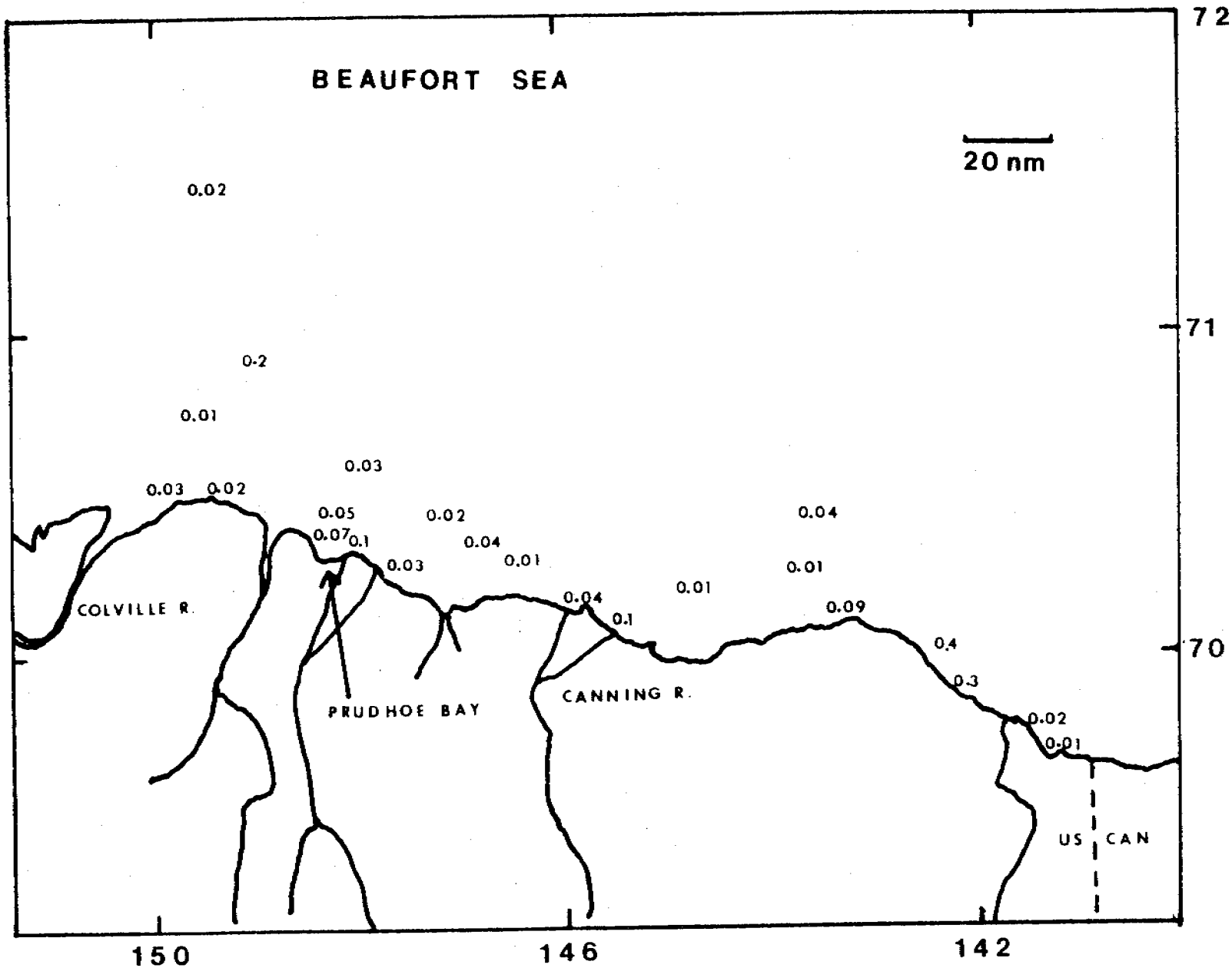


Fig 30 Biodegradation potentials for benzanthracene - Water samples

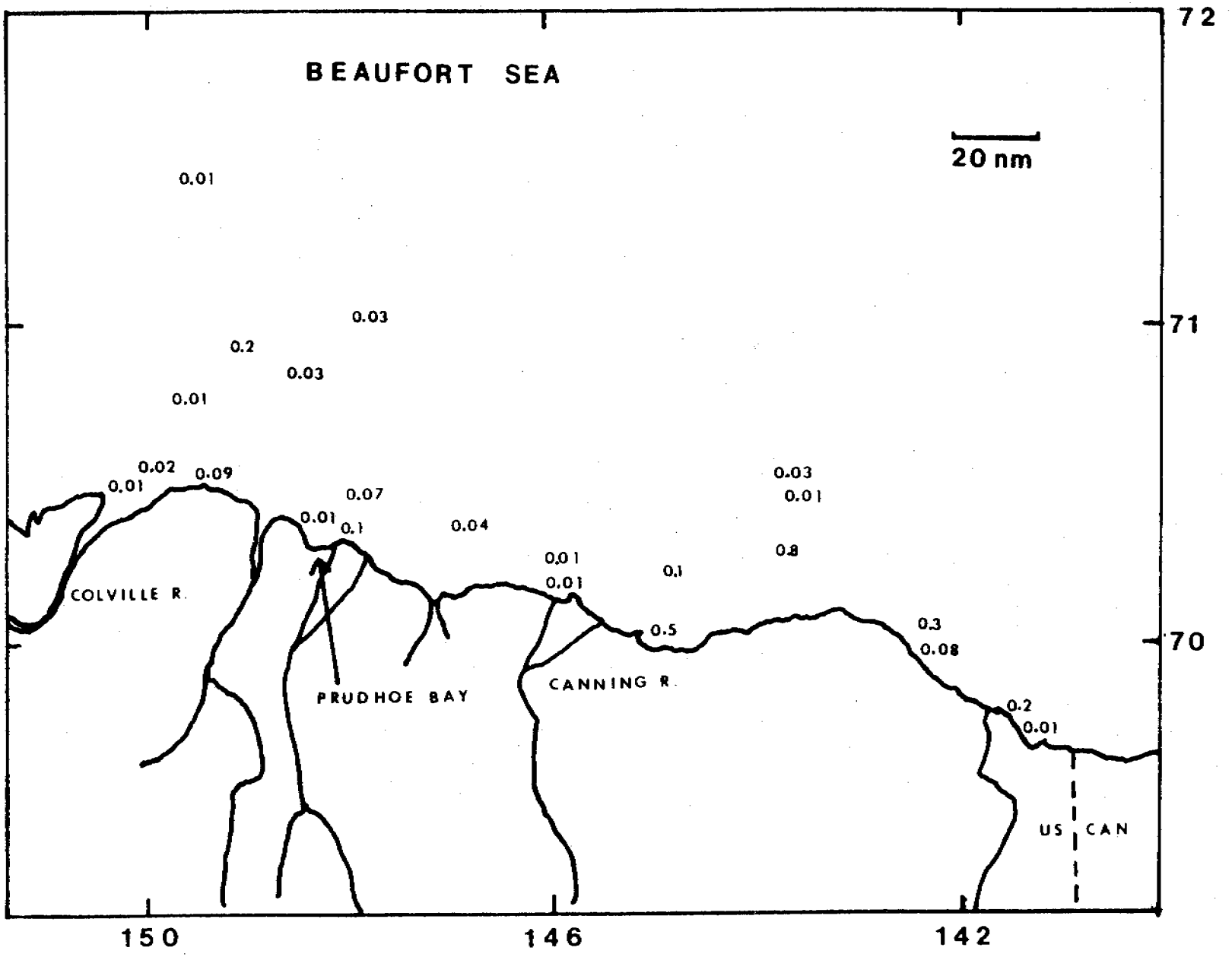


Fig 31 Biodegradation potentials for benzanthracene - Sediment samples

Figs. 32 and 33. Total counts of microorganisms for surface waters, mid waters (five meters above the bottom), bottom waters (< 1 meter above the bottom), and sediments are shown in Figs. 34-39. Total microorganisms in water samples were of the order of 10^5 per ml. No stratification of total microbial populations was evident, nor was there any significant patterns of geographic distribution along the Aleutian Shelf.

Total sediment populations varied from 3×10^6 to 9.8×10^9 per gram dry weight sediment. Higher microbial populations were found in sediments of the St. George Basin than of the North Aleutian Shelf. Near shore sediment populations and those nearest Bristol Bay appeared to be lower than offshore sediment populations of the North Aleutian Shelf.

The enumeration of hydrocarbon utilizers showed strikingly low populations of microorganisms capable of metabolizing hydrocarbons in these regions (Figs. 40 and 41). Only two water samples yielded any positive indications of the presence of hydrocarbon utilizers. All other water samples contained less than 300 hydrocarbon utilizers per liter. All sediment samples contained less than 30 hydrocarbon utilizers per gram dry weight.

The biodegradation potentials with and without inorganic nutrients added showed mineralization of less than 0.1% of added hydrocarbon in all cases.

Norton Sound

The total numbers of microorganisms (direct counts) and the numbers of viable microorganisms (plate counts) are shown in Figures 42-45. While direct counts were somewhat higher in surface water near the mouth of the Yukon (Fig. 42) the direct counts in sediment did not reflect any elevation due to the influx of the Yukon River (Fig. 43). Total numbers of microorganisms showed only limited variability throughout Norton Sound and were actually rather uniform, of the order 10^4/ml in surface water and 10^7/g dry wt. in sediment. There was greater variability in the viable counts, particularly

Fig 32 1980 August-September NASTE cruise stations

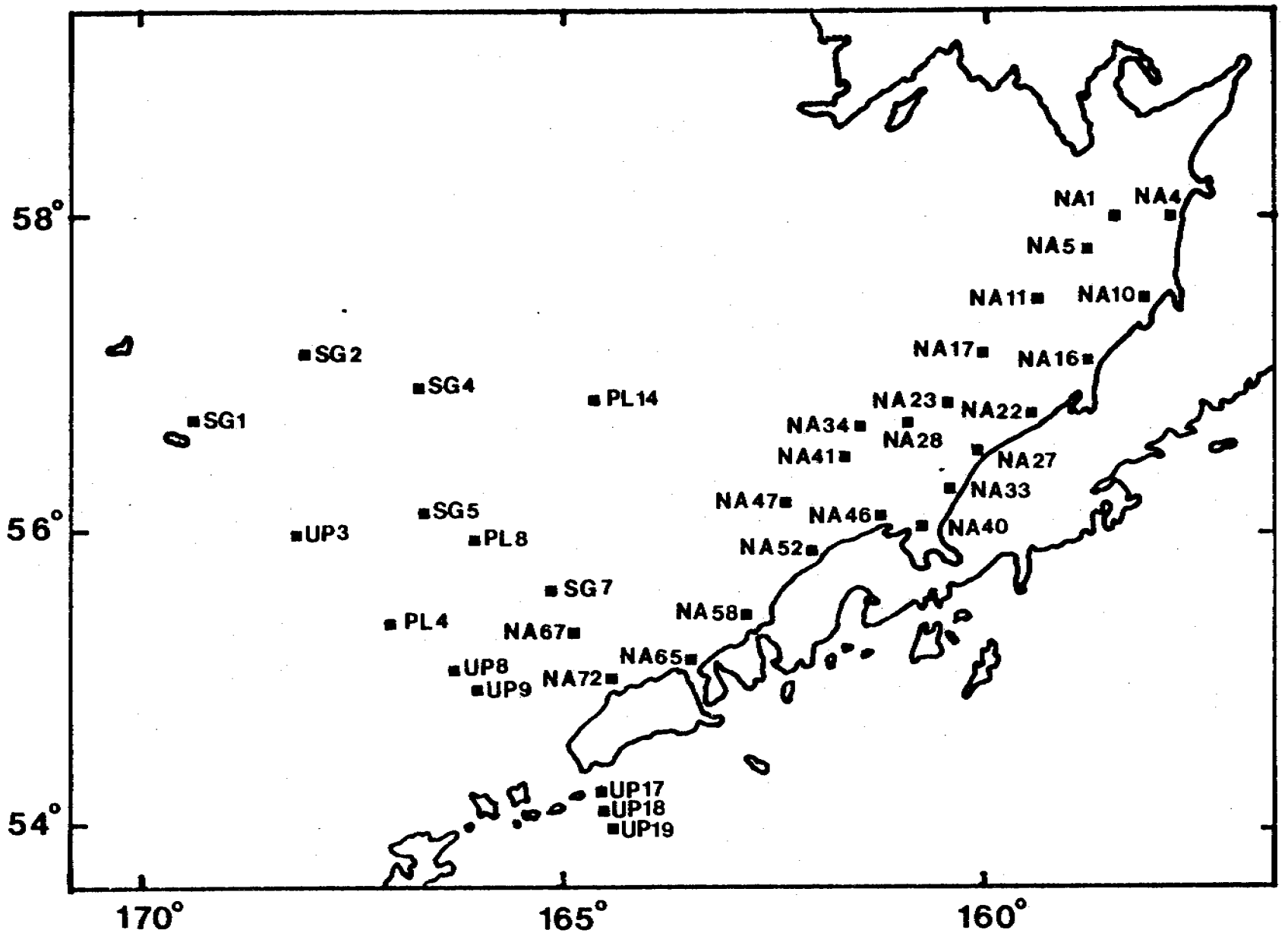


Fig 33 1980 August-September NASTE Cruise Stations

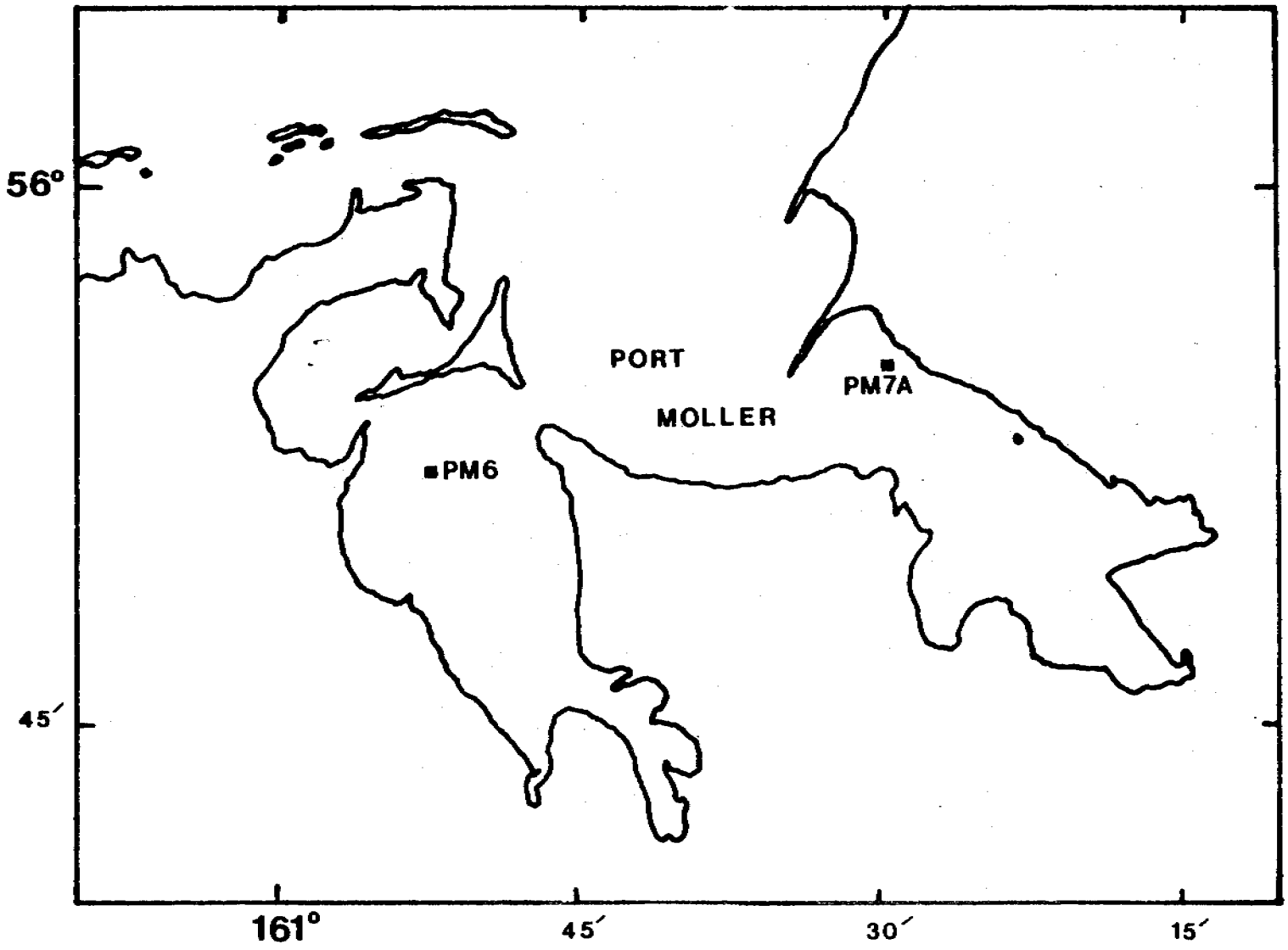


Fig 34 Surface water, direct counts, August-September 1980 cruise, (number per ml x 10^4)

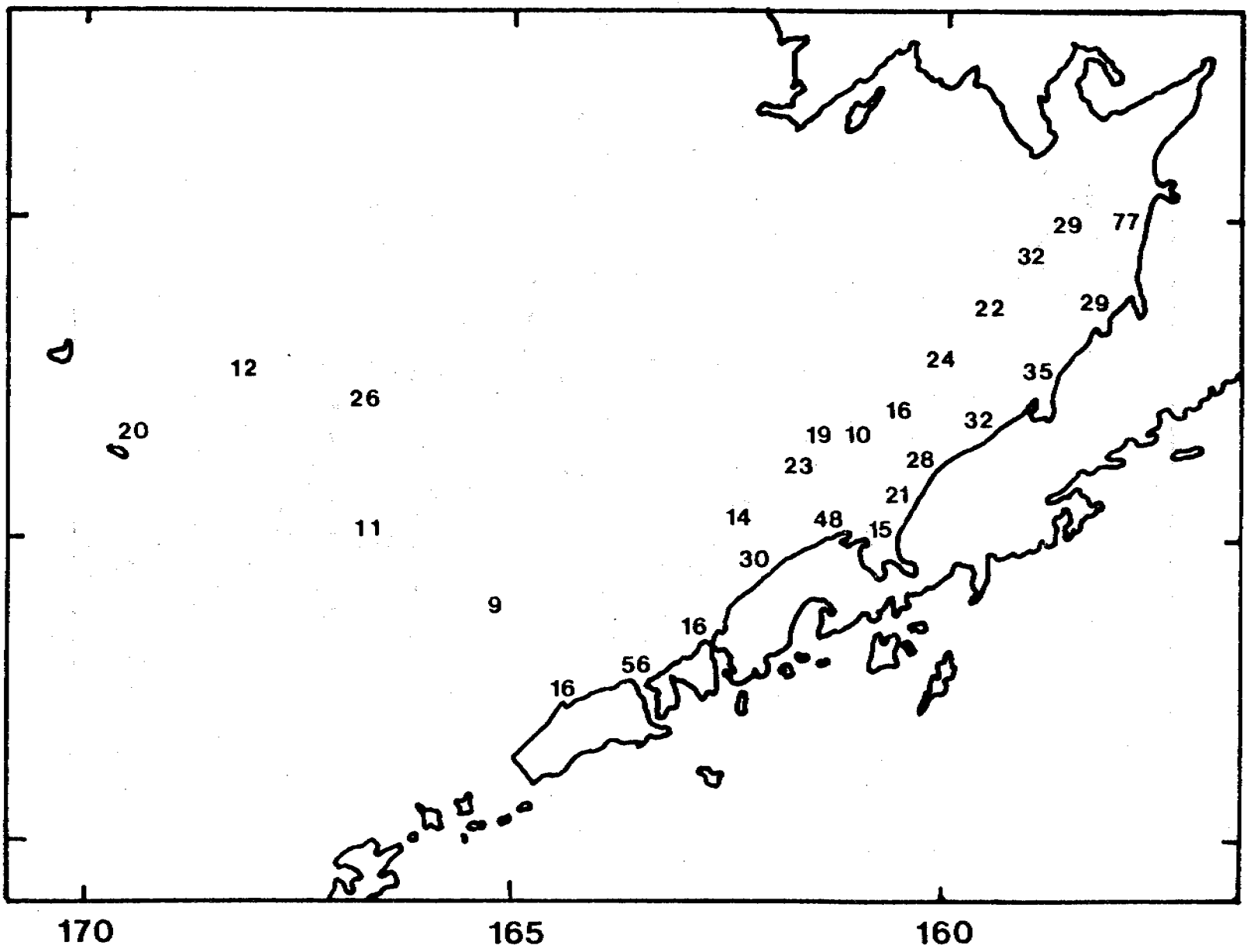


Fig 35 Surface water, direct counts, August-September 1980 cruise, (number per ml x 10^5)

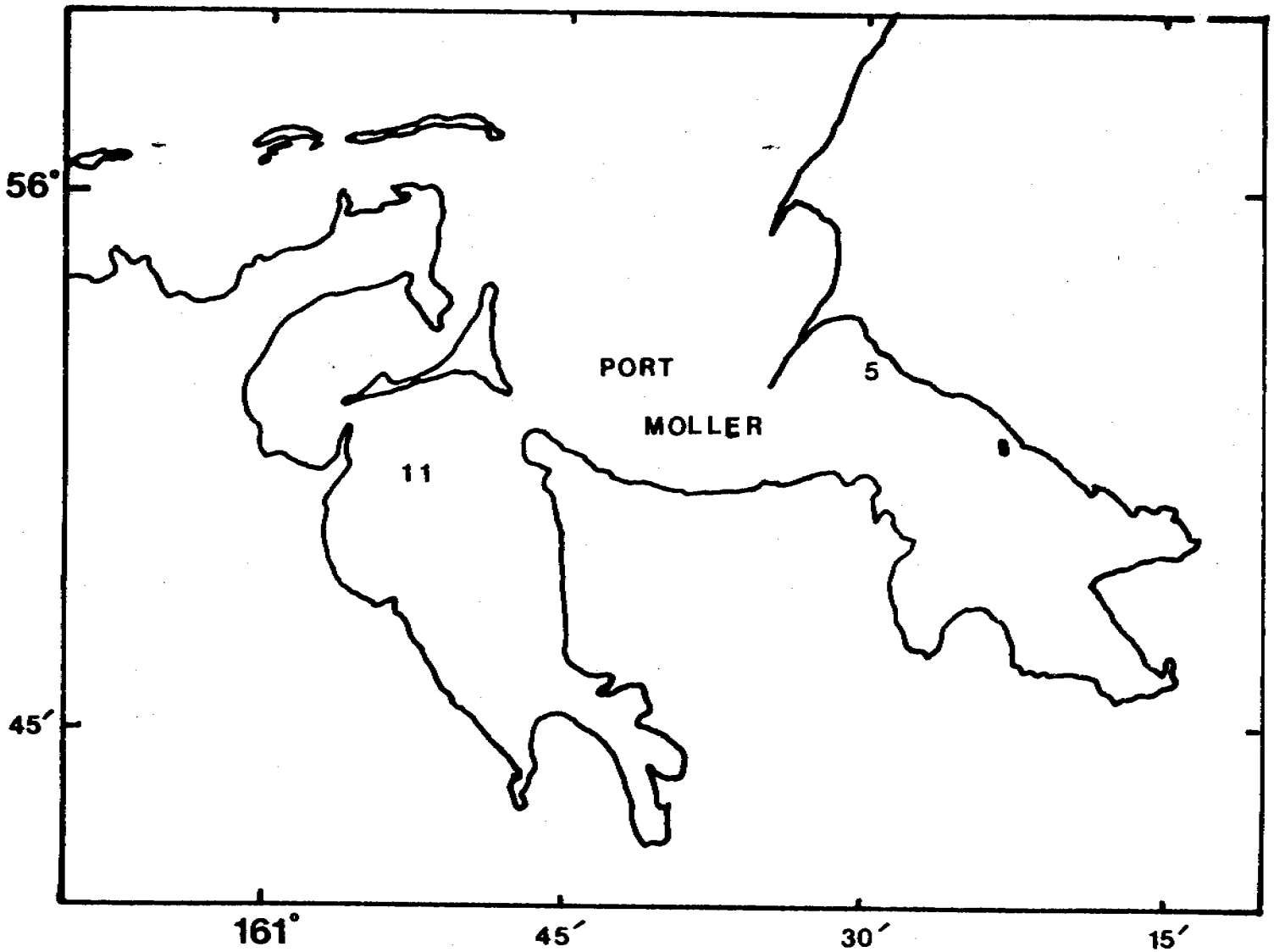


Fig 36

Mid water, direct counts, August-September

1980 cruise, (number per ml x 10^4)

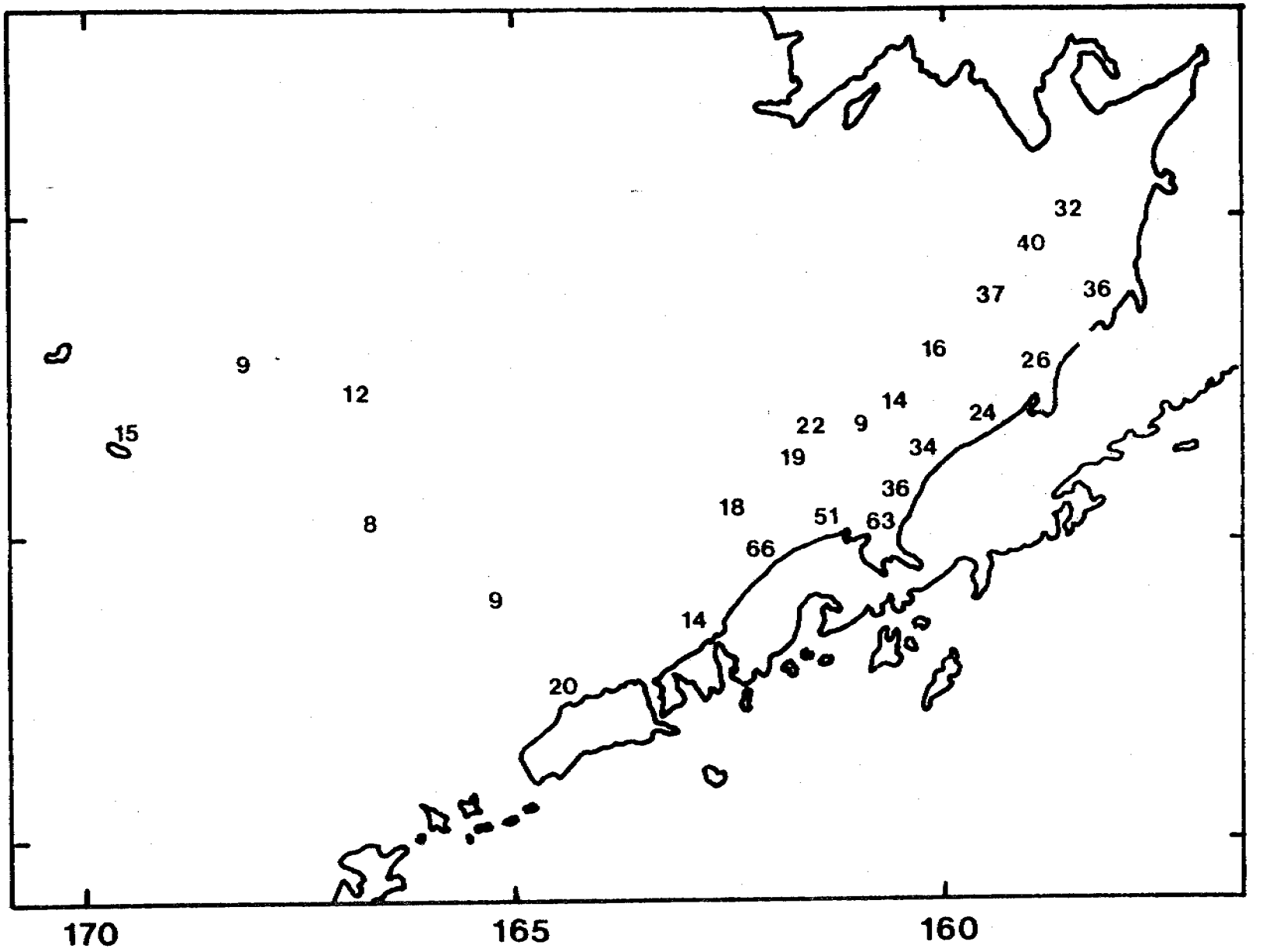


Fig 37

Bottom water, direct counts, August-September

1980 cruise, (number per ml x 10^5)

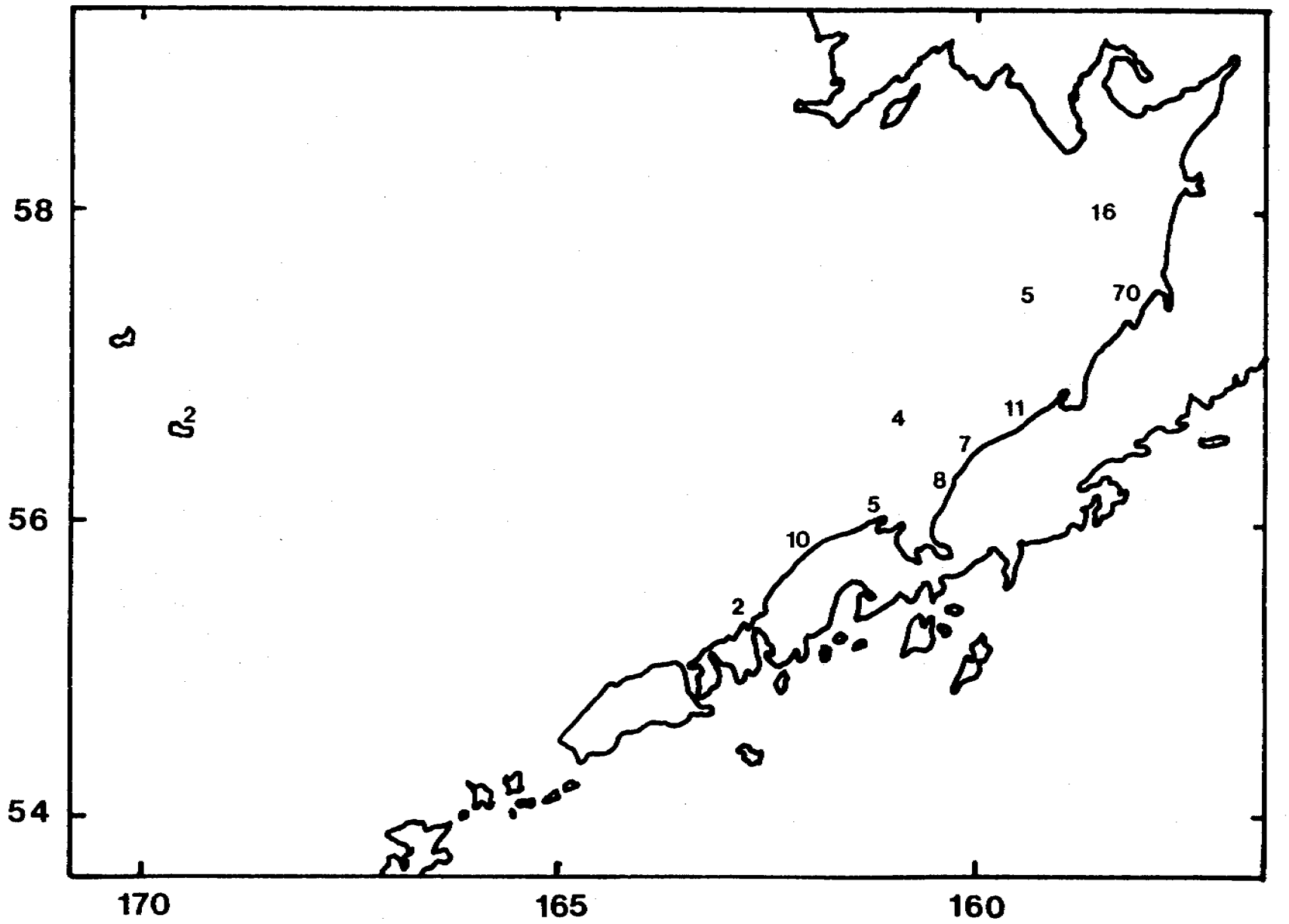


Fig 38 Sediment, direct counts, August-September 1980 cruise, (number per gram dry weight x 10^6)

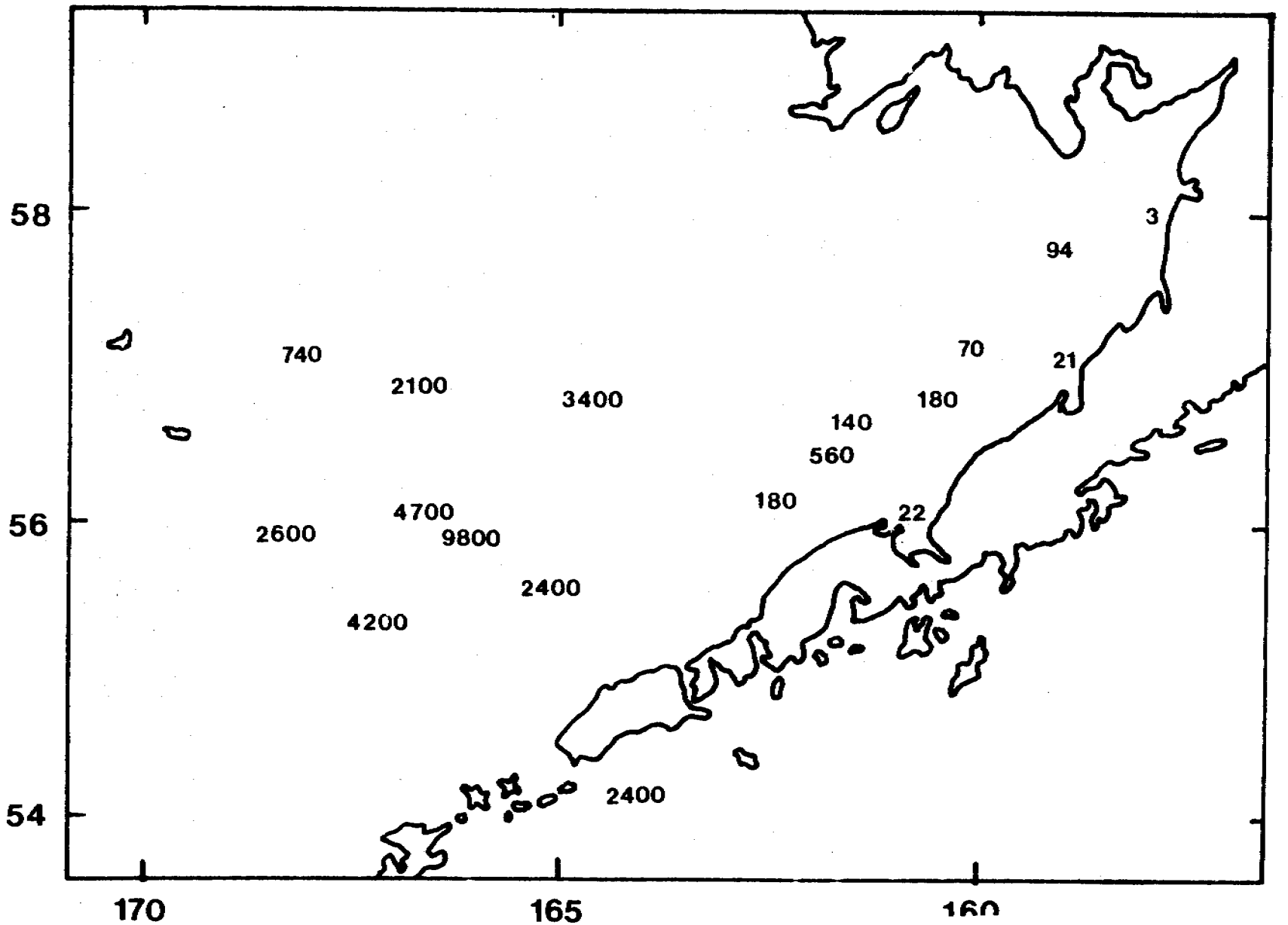
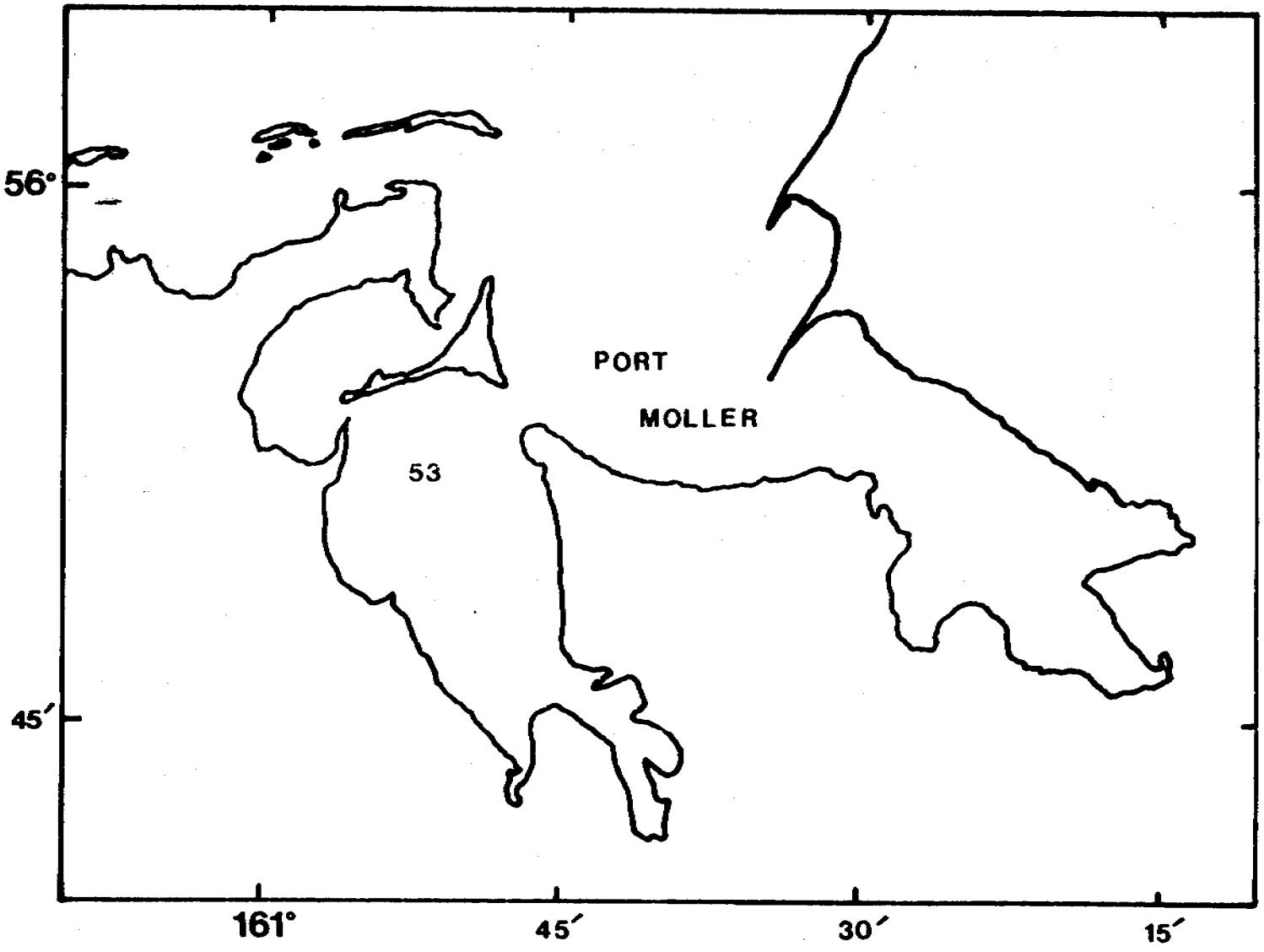


Fig 39 Sediment direct counts, August-September 1980 cruise, (number per gram dry weight x 10^6)



Fg 40 Most Probable Number of Hydrocarbon utilizers, surface water, August-September

1980 cruise ■ equals < 0.3/ml

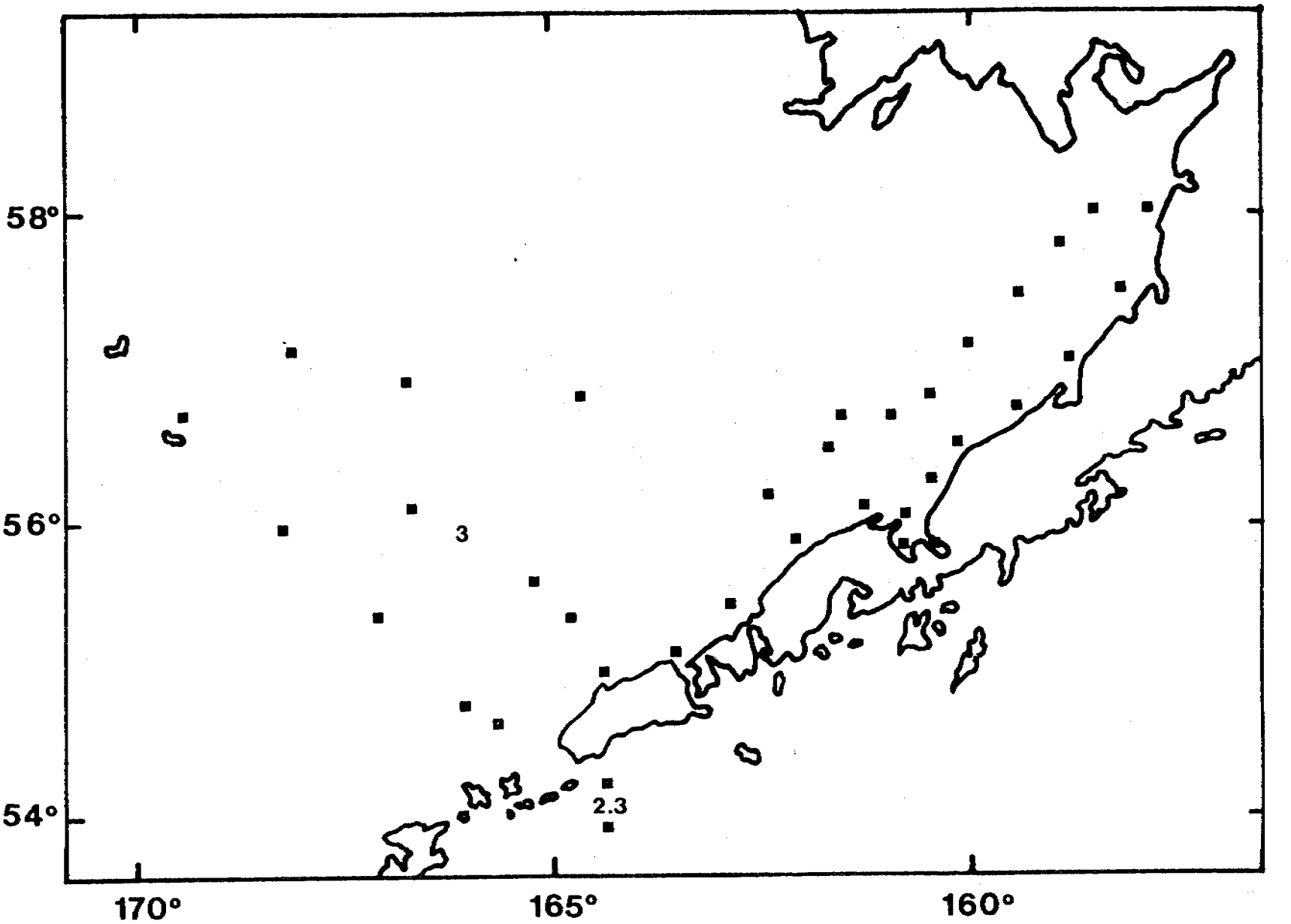
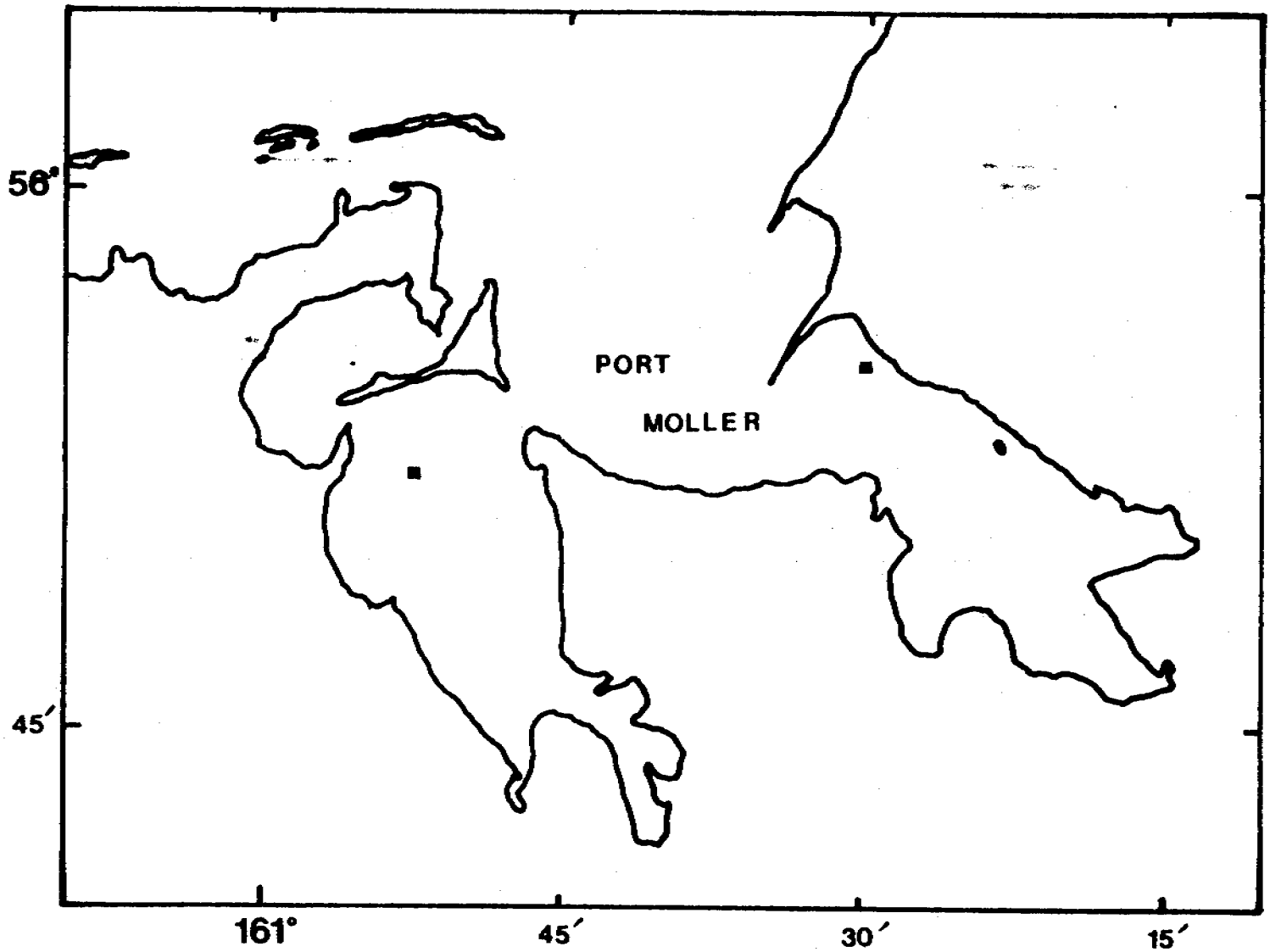


Fig 41 Most Probable Number of Hydrocarbon utilizers, surface water,

August-September 1980 cruise ■ equals < 0.3/ml



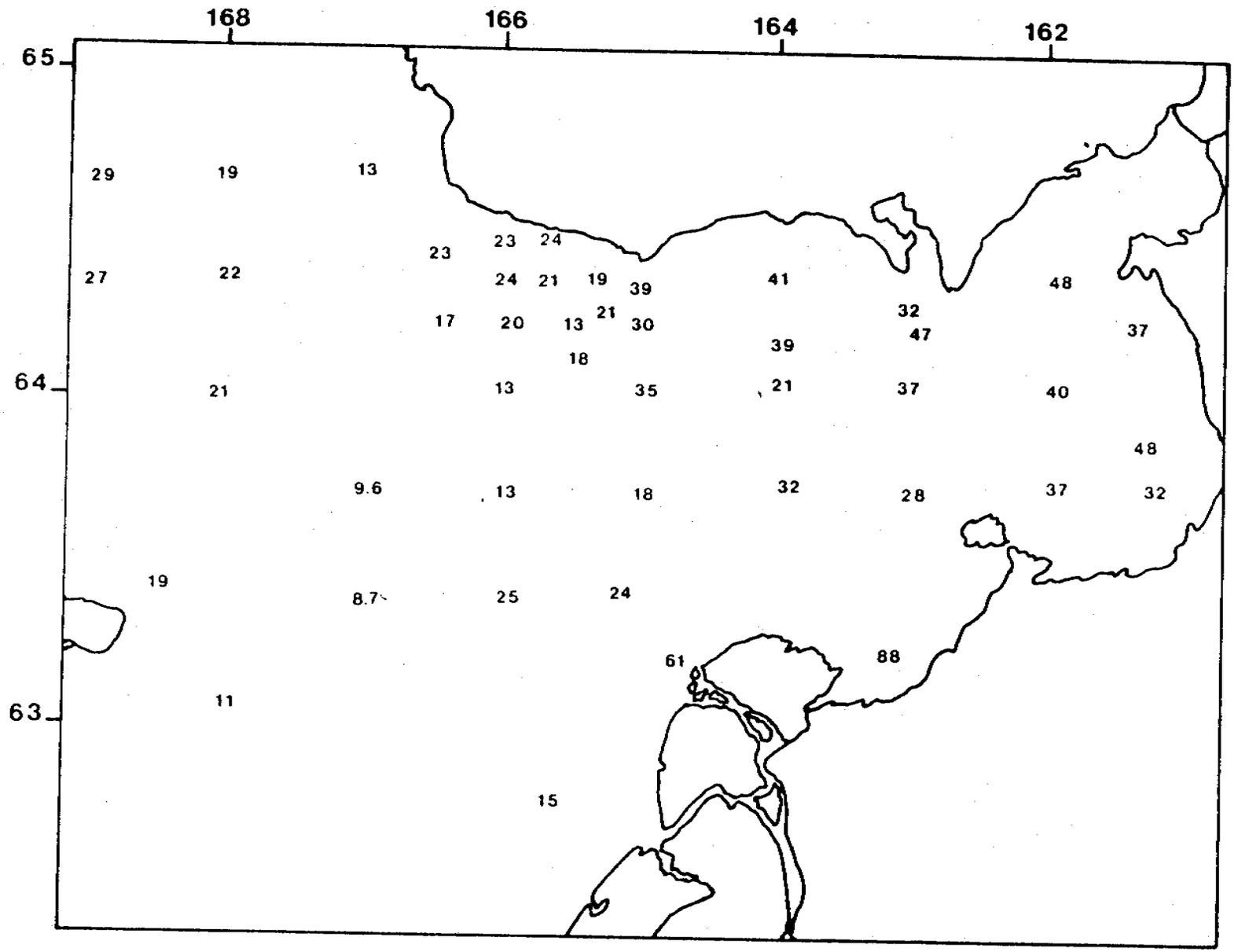


Fig. 42. Total numbers of microorganisms x 10^4 per ml water.

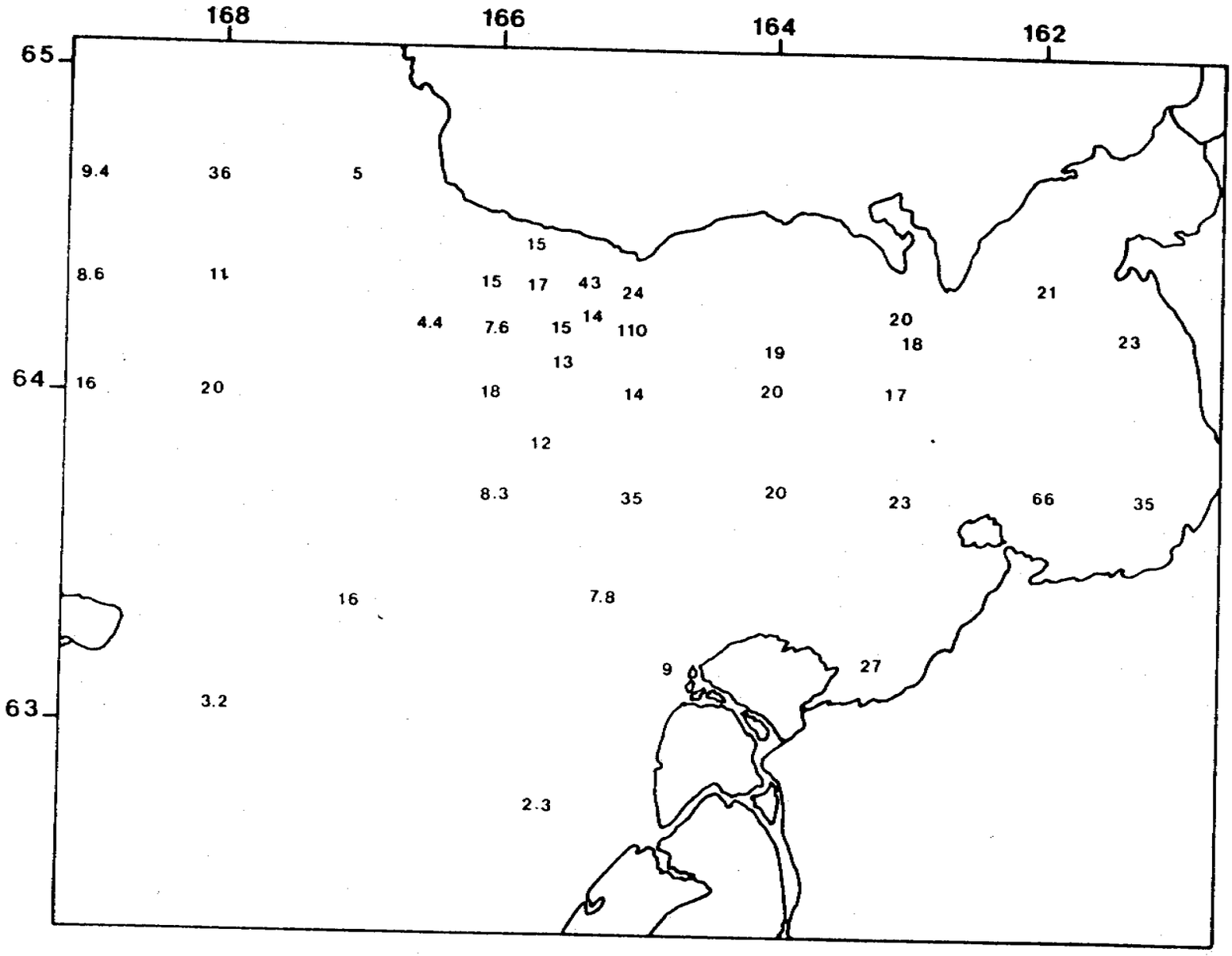


Fig. 43. Total numbers of microorganisms x 10^7 per g dry wt sediment.

in water (Fig. 44). However, here again the input of the Yukon River did not result in a pattern of elevated viable microbial counts. Similarly, neither the total nor the viable counts of microorganisms indicated any significant alteration in the vicinity of the reported natural hydrocarbon seepage in Norton Sound.

The counts of hydrocarbon utilizers within Norton Sound, likewise, did not show any elevation in numbers in the vicinity of the gas seepage (Fig. 45). Some higher counts were found at the inner end of the Sound which may reflect inputs of terrestrially derived hydrocarbon. Overall the numbers of hydrocarbon utilizers are not indicative of areas of petroleum hydrocarbon contamination.

The data represented in Figure 46 is for sediment. In surface waters, counts of hydrocarbon utilizers were uniformly low, i.e. less tha 1/10 ml which was the limit of dectection for the procedures used in this study. The rates of hydrocarbon utilization determined in these studies were likewise very low, of the order less than 1% hydrocarbon converted to CO_2 during 3 weeks incubation at $5^{o}C$.

VII. Discussion

The data on the distribution of hydrocarbon utilizing microorganisms suggests that the areas of the Bering Sea examined are pristine and free from any recent historical exposure to petroleum hydrocarbons. This conclusion includes the Norton Sound region in the vicinity of the gas seepage. Similarly, there is no evidence that methane output from Port Moller has caused an increased presence of petroleum hydrocarbon utilizers. Terrestrial inputs, such as from the Yukon River do not appear to have resulted in a substantial enrichment of hydrocarbon utilizers.

Likewise, there does not appear to be a building of hydrocarbon degrading microorganisms in areas of the Bering Sea during ice cover, as might occur

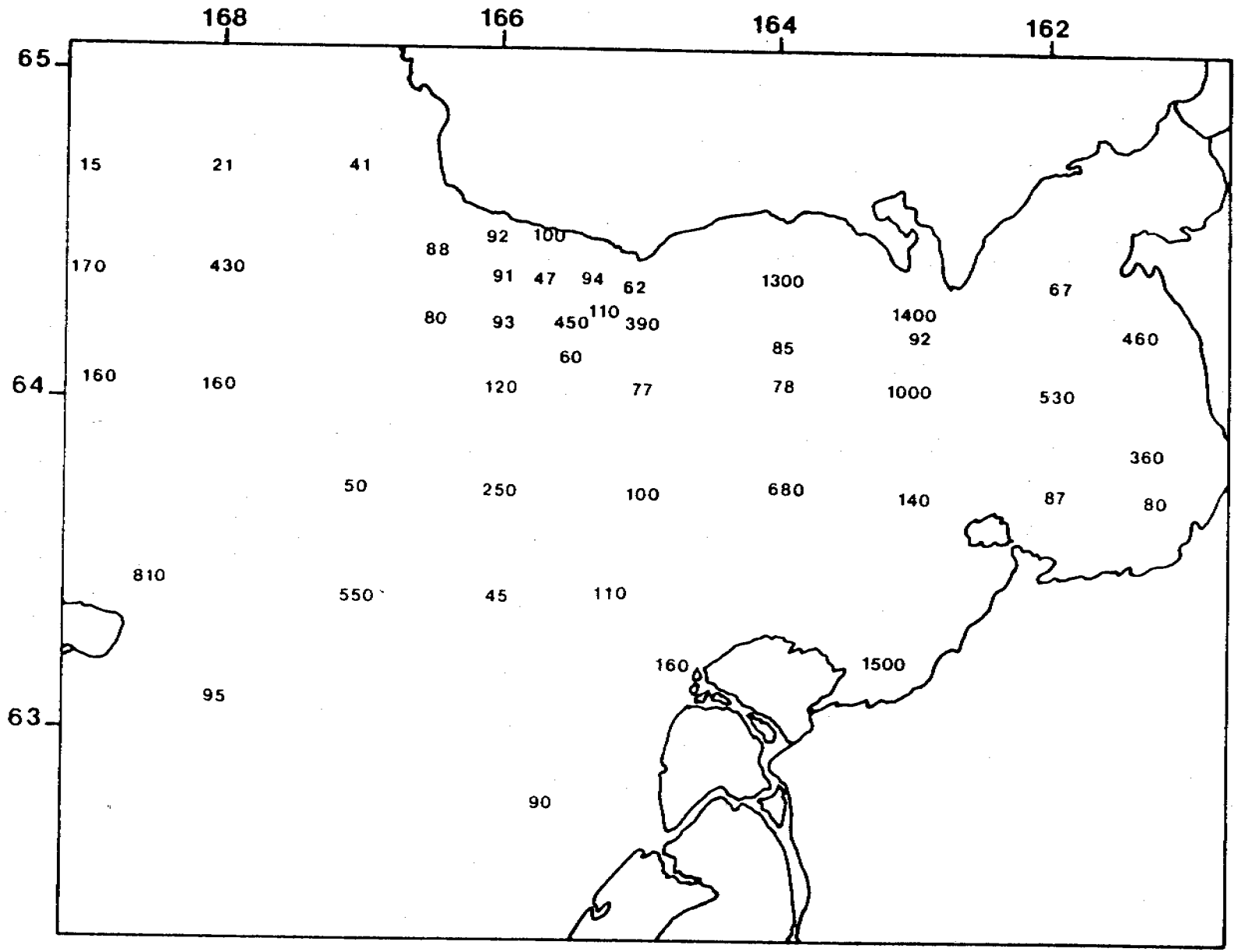


Fig. 49. Viable numbers of microorganisms per ml water.

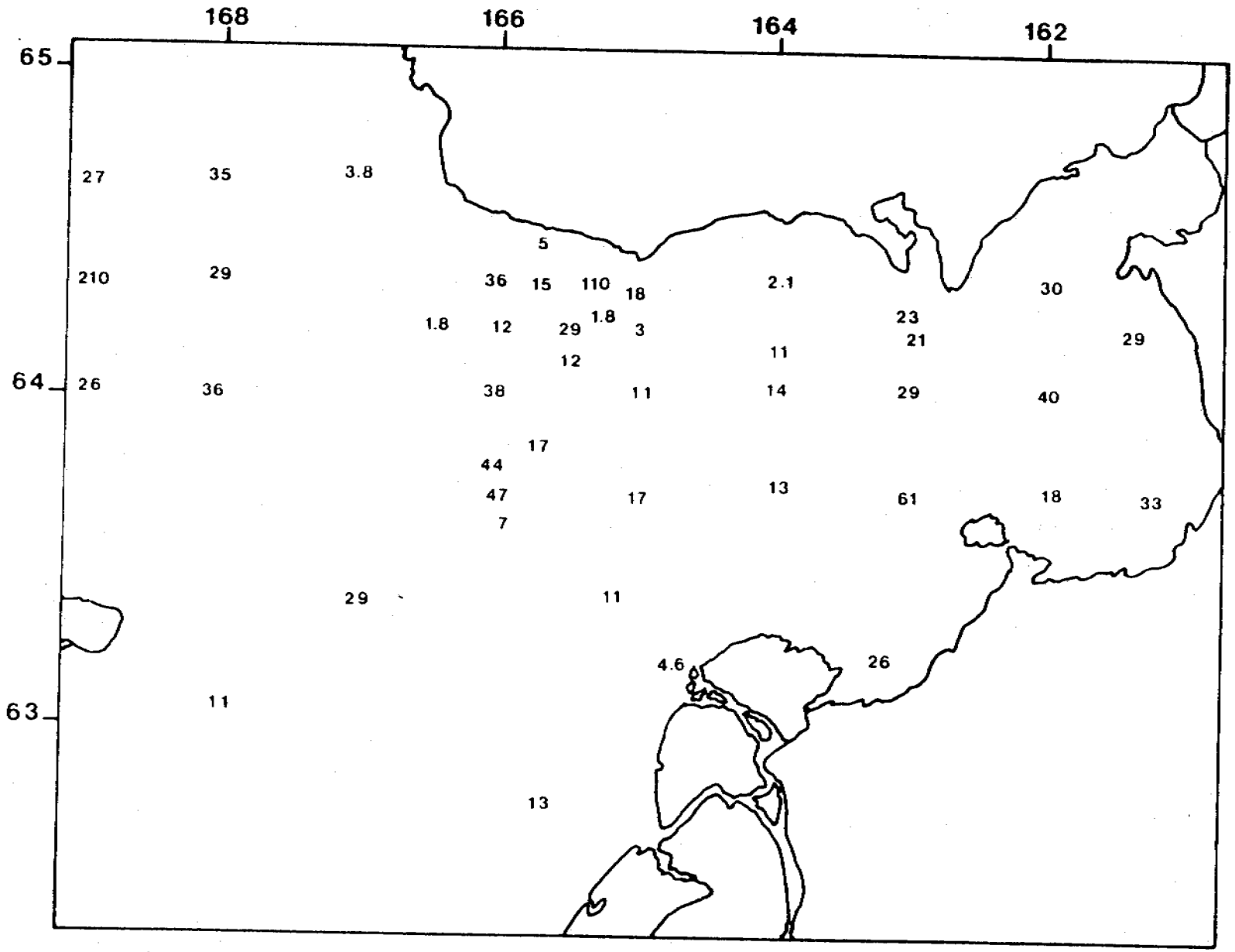


Fig. 45. Viable numbers of microorganisms x 10^5 per g dry wt sediment.

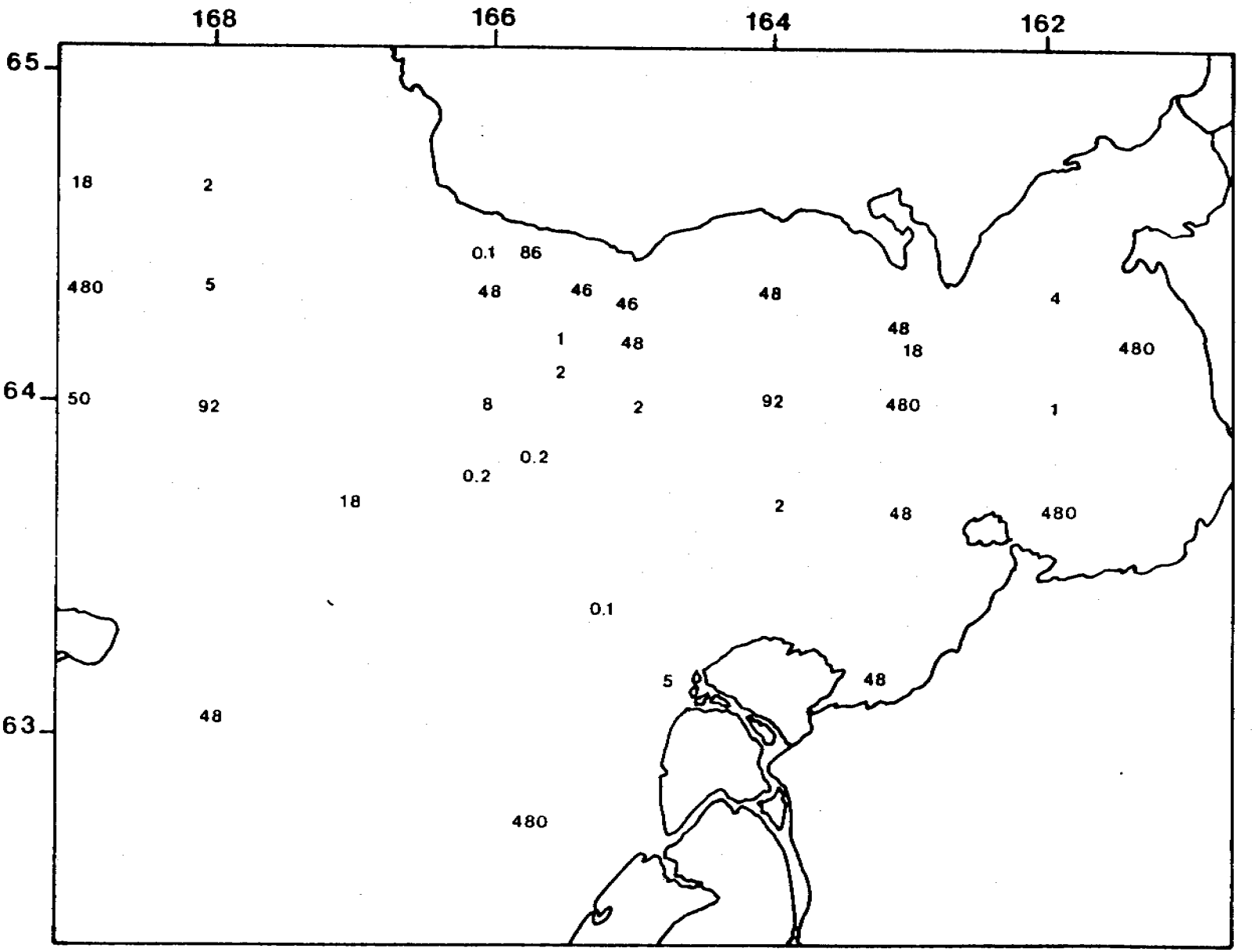


Fig. 46. Most Probable Numbers of hydrocarbon utilizers per g dry wt sediment.

if oil films were accumulating amid ice flows.

In contrast to the Bering Sea some near shore samples from the Beaufort Sea showed higher numbers of hydrocarbon utilizers indicative of some prior exposure to hydrocarbons. Such prior exposure is likely due to natural sources rather than as a result of human activities.

The biodegradation potentials were low in almost all cases. In fact, in almost all Bering Sea samples we failed to detect any biodegradative activities by the microbial populations in the samples examined. Preliminary results from a cruise conducted during February 1981, in the North Aleutian Shelf and St. George Basin regions indicate that hydrocarbon degraders are present, but at low levels. When an adequate sample size is used to insure inclusion of hydrocarbon degrading microorganisms, then rather high biodegradation potentials are observed with Bering Sea samples. The results indicate spatial and temporal variations in the size of the indigenous hydrocarbon degrading populations which can result in significantly different measurements of hydrocarbon biodegradation potentials. Despite the results of the present study which indicate a potential total lack of hydrocarbon degrading activity in areas such as the Navarin Basin we have not concluded that hydrocarbon biodegradation will not occur in these regions. In fact, results of the 1981 cruise may support a hypothesis that the low levels of hydrocarbon utilizers in the Bering Sea are capable of responding to a hydrocarbon contamination yielding greater rates of hydrocarbon biodegradation than in the Beaufort Sea. Until the validity of this hypothesis can be futher examined, we must suggest that great caution be employed in leasing basin regions of the Bering Sea for petroleum development. Our results, including the preliminary 1981 data, suggest that conservation of petroleum hydrocarbon should be employed in transport models for the time frame up to 240 hours following any petroleum spillage.

LETHAL AND SUBLETHAL EFFECTS ON SELECTED ALASKAN MARINE SPECIES AFTER ACUTE AND LONG-TERM EXPOSURE TO OIL AND OIL COMPONENTS

by

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Sid Korn

and

John F. Karinen

Research Unit 72

Submitted as the Annual Report, Contract #R7120822
for the period October 1, 1979 to September 30, 1980

OUTER CONTINENTAL SHELF ENERGY ASSESSMENT PROGRAM
Sponsored by
U. S. Department of the Interior
Bureau of Land Management

April 1, 1981

Northwest and Alaska Fisheries Center Auke Bay Laboratory
National Marine Fisheries Service, NOAA
P.O. Box 155, Auke Bay, AK 99821

Table of Contents

Introduction

This report consists of an Annual Report that summarizes status and programs for FY 80 studies, followed by a Quarterly Report summarizing status of FY 81 studies. The OCSEAP research by ABL addressed the general question, "What are the effects of petroleum hydrocarbons on subarctic animals?" All of our studies are applied laboratory studies, where we expose a variety of marine species and life stages, to determine survival and effects of short and long-term exposures to water-soluble factions of oil. Our methodology includes flow-through exposures and quantitation of individual hydrocarbons by gas chromatography.

We had two major objectives in FY 80: (1) to determine the vulnerability of pink salmon alevins exposed to oil in a simulated intertidal spawning environment. (2) to synthesize and publish results of previous OCSEAP research.

Midway through the fiscal year, OCSEAP contracted with us to conduct additional studies of drilling-mud toxicity to crab and shimp larvae. Although additional funds were provided, they were insufficent (with available personnel) to complete all of the original objectives plus the drilling mud experiments. We fully met the data collection requirements of the pink salmon alevin exposures in a simulated intertidal environment and completed the drilling mud study, but were forced to diminish the effort and progress on the manuscript-publication objective.

The drilling-mud studies are not covered in this report because the final report has already been submitted in January 1981.

Progress and accomplishments.

Objective I: Determine the vulnerablity of pink salmon alevins exposed to oil WSF in a simulated intertidal spawning environment.

Background Information: Many pink salmon stocks in Alaska spawn in the lower reaches of small streams, an area that is intermittently covered with seawater during twice-daily high tides. If the seawater were contaminated with oil, developing pink salmon alevins would be exposed to two potential stresses: intermittent seawater and intermittent oil. Our experimental design had four exposure conditions which separated the factors of intermittent versus continuous oil exposure and continuous freshwater versus intermittent seawater exposure. Potentially, the combination of oil and seawater might be stressful and toxic to alevins; however, intermittent exposures with a clean water phase, might not lead to a significant tissue buildup of hydrocarbons and with resultant harmful effects.

Relevance of study to oil pollution:

The pink salmon is an important commercial species that often utilizes the intertidal zone of freshwater streams for spawning and egg/larval development. Pink salmon typically spawn in the late summer and fry emerge from the intertidal substrate the following spring. It is important to assess the potential effect of oil pollution because of the extended incubation period in the intertidal spawning gravels. Earlier studies by Korn and Rice (1981) have demonstrated that salmon eggs and alevins take up large quantities of hydrocarbons slowly, so a potential exists for lethal effects of chronic low level oil exposures. Information generated in this research will be used by managers to assess the hazard of petroleum coming ashore and impacting intertidal salmon spawning habitats.

Current State of Knowledge:

The sensitivity of pink salmon earlylife stages to short-term oil exposures and the uptake-depuration of oil hydrocarbons is known from previous experiments using continuous exposures (Korn and Rice 1981). In the current study, we determined the sensitivity of pink salmon early-life stages to oil using a simulated intertidal exposure cycle, and included long-term but intermittent oil exposures which are more realistic in terms of what actually might occur in the environment.

Progress:

Pink salmon were tested to determine vulnerability to four different exposure schedules of the water soluble fraction of Cook Inlet Oil. The experiments and anaylses have been completed, including analyses of tissue samples for hydrocarbon content. A paper for publication has been drafted.

Methods:

Pink salmon eggs from the Auke Creek hatchery were incubated in Heath trays until the alevin stage. Early and late alevins were transferred to the test containers as required.

The 30-day exposures with early alevins were started within 5 days of hatching. The second test with late alevins, began 60 days after hatching. Wild fry in the natural environment emerge from the gravel, about 90 days after hatching having depleted their yolk reserves, and begin feeding.

Each series of tests consisted of three concentrations: 0.5, 1.5 & 2.4 ppm total aromatics, plus a control. The exposure containers were 4-liter glass jars wrapped with tape to exclude light. A layer of pea gravel covered the bottom of the jar, and 150 pink salmon egg alevins were added. Toxicant was delivered to the bottom of the jar at the rate of (400 ml/m by long-stemmed glass funnels held in place by perfurated plixiglass lids through which excess toxicant overflowed.

For each series of tests (early alevins, late alevins), there were four simultaneous continuous-flow treatments to test the effect of intermittent salinity and intermittent crude oil WSF on pink salmon alevins:

Treatment 1 Continuous oil WSF-100% freshwater: continuous exposure to crude oil WSF for 30 days in freshwater.

Treatment 2 Continuous oil WSF-intermittent seawater: continuous exposure to crude oil WSF for 30 days in a simulated intertidal environment (3 h seawater out of every 12-h exposure).

Treatment 3 Intermittent oil WSF-100% freshwater: exposure to crude oil WSF for only 3 h out of every 12 h. The remaining 9 hrs of the cycle was clean freshwater.

Treatment 4 Intermittent oil WSF-intermittent seawater: exposure to crude oil WSF for 3 h out of every 12 h, during the seawater phase only. The non-oil exposure period of 9 hrs was clean freshwater.

The different concentrations of oil were delivered to the exposure bottles with a continuous-flow generation apparatus. Cook Inlet water-soluble stock solution was generated by dripping water through a constantly replenished 15-cm layer of oil atop a 2-m column of water. Two manifolds served each test container: one delivered stock toxicant at a given flow rate, and the other delivered dilution water. The concentrations of 10 aromatic hydrocarbons (benzene; toluene; Ortho; meta and par-xylenes; ethylbenzene; naphthalene; 1 and 2 methylnaphthalenes and 2-6 dimethynaphthalenes) in the WSF of crude oil were measured daily by gas chromatography. We estimated the concentration of total aromatic hydrocarbons in the solution by summing the concentration of the ten hydrocarbons.

Two oil-exposure devices were operated simultaneously: one for freshwater and the other for saltwater. Exposure bottles were moved by hand from one to the other, or to clean water as needed. Dosing concentrations were stable over the 30-day period.

During each test, survival was assessed and 10 alevins were removed periodically for tissue hydrocarbon analysis. At the end of the exposure periods, 30 alevins were removed from each jar and preserved in 5% buffered formalin for measurement of weights and lengths. Bam's K_D values (an index of fry development) were calculated using the following formula: $K_D = \frac{10\ (\text{weight})^{+.33}}{\text{Length}}$ Differences in K_D values were evaluated using a three-way analysis of variance.

Results and Discussion:

Survival

Fish continuously exposed to the top dose (2.4 ppm total aromatic hydrocarbons) died in 7-16 days; the late alevins died sooner than the early alevins and the alevins in the intermittent seawater exposures usually died before their freshwater counterparts. The late alevins receiving the top dose of 2.4 ppm in the intermittent oil exposure died in the exposures with intermittent seawater, but not in 100% freshwater exposures. No other groups experienced greater than 5% mortality--the same as in the controls, Seawater-oil exposure was apparently more stressful, because the first deaths occured in the intertidal high doses. The early alevins continuously exposed took longer to die than the late alevins, probably because of larger quantities of yolk that absorbed and retained the hydrocarbons in semi-isolation from the developing tissues.

Size and developement of Surviving Fry

At the end of the 30 day exposures, we measured the length and weight of individual fry. From these measurements, a developmental index K_D (Bams) was calculated and was used to compare the effects of the various treatments. Higher K_D values denote more girth and weight relative to length, and indicate an earlier development stage. As the alevin develops and absorbs yolk the K_D drops, demonstrating an increased length relative to weight.

The size (K_D) of fry was affected by both salinity and oil exposures. However, early alevins were affected less than late alevins probably because the developmental rate is a little slower at that stage, and effects take longer to become evident.

In all cases, alevins exposed to oil were smaller than the controls, and alevins exposed to seawater were smaller than the freshwater counterparts. Both treatments appear stressful enough to cause increased energy consumption and consequently, the fry were smaller. In general, there was little difference between continuous and intermittent oil exposures. The smallest fry were those exposed continuously to the highest oil dose with an intermittent salinity exposure.

The completeness of absorption of the yolk by fry when they leave the streambed bears directly on their survival. Fry with a large amount of yolk (large K_D) have not attained their maximum potential size, are relatively poor swimmers, and are therefore more vulnerable to predators.

Hydrocarbon Accumulations:

Concentrations of hydrocarbons in tissues were affected by length of exposure, stage of the alevins, salinity and continuous or intermittent exposure.

In general, early and late alevins accumulated similar concentrations of aromatic hydrocarbons, but the pattern of uptake was much different. The yolk rich early alevins were slow to accumulate hydrocarbons and reach maximum concentrations at the end of the 30 day exposures. Hydrocarbon concentrations in tissues of late alevins peaat about 12 days of exposure and then dropped toward control levels by 30 days. At 30 days, the late alevins contained 5-50% of the peak tissue concentration depending on treatment. The late alevins consummed yolk rapidly, and hydrocarbons sequestered in the lipid-rich yolk became available for metabolism or excretion when the yolk was diminished.

Exposures to intermittent salinity resulted in consistently higher tissue concentrations, often reaching 2-3 times higher. This is significant, since the oil exposures were equal between the freshwater and intermittent seawater groups, and the seawater exposures were only 25% of the time. The seawater exposures apparently enhance the uptake or retention of hydrocarbons.

Groups exposed to intermittent oil accumulated aromatic hydrocarbons, but the levels were much lower than for groups exposed continuously to oil, and appeared to approach equilibruim concentrations by 10 days of exposure.

Interpretaion of Results

Final interpretation of results will be made in a manuscript that will be completed in 1981. Preliminary conclusions are as follows:

(1) Survival of early and late alevins was reduced by exposure to 2.6 ppm total aromatic hydrocarbons in continuous oil-exposures. There were mortalities in the alevins exposed intermittently to oil during the seawater exposures, but there were no mortalities in groups exposed intermittently to oil in 100% freshwater.

(2) Bam's K_D factors were significantly affected by seawater and oil exposure. The intermittent oil-seawater exposure reduced K_D values to the same level as continuous oil exposure.

(3) Highest concentrations of hydrocarbons accumulated in continuous oil-intermittent seawater exposures, followed by continuous oil-freshwater exposures. Intermittent oil exposures caused little accumulation, apparently because the hydrocarbons taken up during the intermittent exposure were not retained in the yolk material and were depurated during the clean water-phase of the simulated tidal cycle.

(4) Effects of oil exposure on survival and size were at relatively high levels (1.6-2.4 ppm), and intermittent oil exposure (3 h twice per day) had less effect than continuous exposure. Therefore, pink salmon alevins in the intertidal habitat are not especially sensitive to marine oil pollution. However, alevins in the intertidal habitat receiving a continuous exposure may be more vulnerable to the combined stress of seawater and oil.

Objective II: Synthesis and publication of previous OCSEAP research in scientific journals.

Progress: We have published results of most OCSEAP research done in the previous years, but a few papers remain to be completed.
As noted earlier, progress on old manuscripts in 1980 was less than originally anticipated, due to presidence given to undertaking the research on drilling mud toxicity to larvae, midway in the fiscal year. The status of manuscripts is as follows:

Karinen, J.F. 1980. Petroluem in the deep sea environment: potential for damage to biota. Environmental international 3: 135-144.

Abstract:

Information on the fate, persistence and biological impact of petroleum hydrocarbons in shallow marine environments, coupled with recent data on hydrocarbons in offshore sediments and the biology of deep sea organisms, have provided new perspectives on the potential impact of oil on the deep sea environment. A review of literature on petroleum hydrocarbons in deep sea sediments, machanisms for transport of petroleum to the deep sea floor, interaction of petroleum hydrocarbons and particulate matter, and the physiology and metabolism of deep sea fish and crustaceans has resulted in the following conclusions:

1. Hydrocarbons of apparent anthropogenic origin are accumulating in bottom sediments of coastal margins and in deeper offshore waters at unknown rates.
2. Several mechanisms exist for the rapid transport of petroleum hydrocarbons to the deep sea floor.
3. Petroleum hydrocarbons are intimately associated with particulate matter in the sea, behaving much the same as natural biogenic material and having the potential to modify natural processes.
4. The unique physiology of deep water life forms increases the potential for adverse impact of petroleum hydrocarbons on the deep sea environment.
5. There is a need to determine trends of temporal and spatial deposition of hydrocarbons in deep sea sediments and to evaluate the biological impact of this introduction of xenobiotic compounds.

Thomas, R.E., and S.D. Rice (1981), Excretion of labeled toluene and napthalene by gills vs. gut of Dolly Varden trout. Symposium Proceeding: Marine Pollution and Physiology. (In Press)

Abstract:

Dolly Varden char were fed gelatin capsules containing ^{14}C toluene or ^{14}C napthalene. Fish were held in a container which utilized a double rubber dam around the fish midsection to isolate the gills from fecal discharge. Fish were catheterized to collect urine. Samples of water from the gill chamber, fecal chamber, urine, and fish tissues were analysed by scintillation counting. The percent matabolites in the above samples was also determined using the method of Roubal et al., 1977.

The gills were the most important pathway for excretion of carbon-14 from ^{14}C-napthalene. Most of the carbon-14 excreted by the gills was still attached to the parent compound. About 10% of the excreted carbon-14 appeared in the cloacal chamber, mostly as metabolites. Less than 1% of the total carbon-14 was excreted in the urine, predominantly as metabolites.

Tissues retained a significant amount of carbon-14 labelled compound at 24h. Although muscle contained large amounts of carbon-14 because of its mass, the gall bladder had the highest specific activity. The brain also retained significant quantities of carbon-14.

^{14}C-toluene was excreted and metabolized more than ^{14}C-napthalene, while more ^{14}C-napthalene was retained in the tissues. A lower percentage of the carbon-14 was recovered in ^{14}C-naphthalene metabolites than in ^{14}C-toluene metabolites.

Seawater and freshwater Dolly Varden char excreted similar amounts of carbon-14; however, the percentage of metabolites in the excretions and tissues of seawater fish was lower than the percentage of metabolites in excretions and tissues of freshwater fish. For example, we recovered greater amounts of carbon-14 with a lower percentage of metabolites from the brain-spinal cord of seawater fish than from the brain-spinal cord of freshwater fish--possibly explaining why seawater Dolly Varden are more sensitive to aromatic hydrocarbons and the water-soluble fraction of oil than freshwater Dolly Varden.

Rice, S.D., D.L. Cheatham, D. Brown, and S. Korn. The toxicity, uptake, and availability of phenol, substituted phenols, naphthols, and heterocyclic compounds from WSF of crude oil and No. 2 fuel oil. (Drafted, in Lab review).

Abstract:

The possible contribution of phenol and p-cresol to the toxicity of oil WSF was determined by: analyses of the concentration of phenolic compounds by gas ahromotography in two crude oil WSF's; determination of toxicity (static bioassays) of phenol and cresol to shrimp and pink salmon's and determining the uptake and depuration of phenol and cresol in shrimp and pink salmon.

Lauren, D. and S.D. Rice. Uptake, depuration, metabolism, and excretion of naphthalene by the purple shore crab, Hemigrapsus nudus. (In Lab Review.)

Abstract:

Adult male shore crabs, Hemigrapsus nudus, were statically exposed to ^{14}C naphthalene (4 ppm) for 12 h, followed by up to 156 h of depuration. Hemolymph, heart, cardiac and pyloric stomachs, muscle, thoracic ganglion, digestive gland, and gills were taken at various time intervals and analyzed for biomagnification, depuration, and the accumulation of naphthalene metabolites. Biomagnification was rapid, and by 12 h, the digestive gland had the highest concentration of carbon-14 (105X water); other tissues were less than 15X. Total body burdens of a theoretical standard animal at 12 h followed the order: digestive gland > muscle > hemolymph > all other tissues.

Depuration was biphasic, rapid at first, but slowed by 12 h. After 156 h depuration, the distribution of naphthalene in a standard animal followed the order: digestive gland > muscle > gills > hemolymph > stomach > the other tissues.

The distribution of the total naphthalene matabolites present in individual tissues of a standard animal after 168 h followed the order: gills > muscles > hemplymph > digestive gland > stomach > antennal glands.

No significant difference in depuration rate was found between control animals injected with naphthalene and those with their nephropores and anus blocked, so it was concluded that the gills are the major route of naphthalene depuration. TLC separation of extracts of depurated naphthalene indicated that less than 10% of the total naphthalene depurated was composed of metabolites.

In vitro aromatic-hydrocarbon-hydroxylase (AHH) activity towards diphenyloxazole (PPO) was assayed spectrophometrically on 15,000 xg tissue supernatants. No significant activity was detected in the digestive gland, muscle, and stomach of male or female crabs. For both sexes, gill specific activity was the highest and no significant difference existed between the sexes. Female antennal-gland specific activity was approximately twice that of male crabs. However, even the highest gill activity was only about 35% that of fish liver determined in concurrent assays.

Phenol, cresol, and some C_2, C_3 substituted phenols were detected in sub ppm concentrations of water soluble fractions of two crude oils and No. 2 fuel oil. Naphthol and substituted naphthols were not detected. We conclude that phenol and cresol do not contribute greatly to the toxicity of oil and fuel oil WSF. This is due to the low concentration of phenols in oil WSF (0.013 - 0.092 ppm), the relatively low toxicity (96h TLM = 3-10 ppm) compared to important aromatic-hydrocarbon components of oil, and the relatively low accumulation and rapid depuration. In comparison, several mono- and di-aromatic hydrocarbons are both more toxic and more available in WSF's of oil and fuel oil than phenol and cresol. However, the possiblity of synergistic effects between phenolic compounds and other oil components is unknown. This could affect the contribution of phenolic compounds to oil toxicity.

Carls, M. and S.D. Rice. Effects of drilling muds on larval marine organisms. (In Lab Review).

Abstract:

We tested effects of six water-based drilling muds obtained from Prudhoe Bay and Cook Inlet, Alaska, on six species of crustacean larvae: King crab, Tanner crab, Dungeness crab, coonstripe shrimp, dock shrimp, and kelp shrimp. Drilling muds were toxic to crustacean larvae, with the level of toxicity dependent on mud composition and species. However, the toxicity was quite low, since the toxic concentrations of the most toxic mud (used Cook Inlet mud) ranged from 0.5 to 9.4 parts per thousand. Crustacean larvae are approximately an order of magnitude more sensitive to drilling mud concentrations reported in the literature for adult crustaceans and fish.

Drilling muds were tested in toto and after separation of the solid and liquid phases by centrifugtion, which allowed comparison of the particulate and chemical toxicities. WSF toxicities ranged from 9 to 376 ppt for king crab and coonstripe shrimp larvae. The WSF's of drilling muds were stable in solution, but the particulate fraction settled out of the water quickly. Particulate matter, primarily bentonite and barite, caused roughly 80% ($\pm$16%) of the observed toxicity. Drilling muds do not appear to be a dramatic threat to larval crustaceans because the toxicity of drilling muds are generally low, slow to exert their effect, and the length of potential contact in the environment would be short.

It was concluded that the biomagnification and depuration of naphthalene are controlled by ventillation/perfusion-assisted diffusion and hydrophobic attraction of the petroleum hydrocarbon for cellular lipids, and that the major route of elimination is the gill; the antennal glands and alimentary tract play only minor roles, as does metabolism of naphthalene.

Carls, M. Sensitivity of arctic marine organisms to crude oil and naphthalene. (In lab Review).

Abstract:

Increasing offshore oil exploration and production in nearshore Arctic waters has increased the probability of oil spills in this environment. Animals in this habitat may experience broad extremes of temperature, salinity, and light. A question suggested by the possibility of an oil spill in the Arctic is: Are animals which are adapted to a wide range of environmental parameters also resistant to unaccustomed stresses like oil?

Five species of circumpolar benthic organisms were tested in flowthrough bioassays at several temperatures with Cook Inlet Crude Oil WSF's or with naphthalene. The species were: Amphipods Anonyx nugax, Boekosimus nanseni, and Gammaracanthus loricatus; Arctic cod (Boregadus saida); and an Arctic marine sculpin (Oncocottus hexacornis).

Crude oil median lethal concentrations (LC50's) ranged from 1.6 to 3.8 ppm total aromatics. Naphthalene LC50's ranged from 1.35 to 3.35 ppm.

Linear or simple curvilinear relationships between exposure temperatures and LC50's were not found. Differences between "temperate" and "arctic" animal sensitivity were not apparent from comparisons with the literature.

In conclusion, adapation to a broad range of naturally occurring environmental factors does not guarantee resistance to abnormal stress factors such as petroleum hydrocarbons.

Quarterly Progress Report for RU 72 (1 Jan - 1 Apr 81)

SCOPE OF PROJECT

This project was undertaken to determine the sensitivities of Coonstripe shrimp and Pacific herring to oil hydrocarbons in water by testing the most sensitive life stages of each species. The Coonstripe (Paudalis hypsinetus) was chosen as a representative S.E. Alaskan shrimp because it is an important commercial species, of the genus that includes most of the Alaskan commercial shrimp species, and because its larvae are easier than most to rear in the laboratory. The Pacific herring was chosen for its importance both as a commercial species itself and its importance as a major food source of other commercial species.

SPECIFIC OBJECTIVES FOR 1981

SHRIMP: Our objectives were to test the longterm sensitivity of Coonstripe shrimp at three life stages: gravid adult, egg, and larvae. Specifically, the gravid females and the eggs they bore were exposed to several continuous-flow concentrations of Cook Inlet crude oil WSF for either 4 days or 30 days in the last month before the eggs hatched. They were then returned to uncontaminated water until hatching was complete. Survival of exposure for the adults was defined as being in apparently normal health after the end of exposure, and for the eggs was defined as hatching into swimming larvae after exposure. Larvae (previously unexposed) were bioassayed in continuous-flow WSF exposures at several points in their first month of development, with exposure times ranging from 29m to 30 days, and short exposures done during both intermolt periods and during molting itself. Because crustacean larvae are known to be prone to die slowly even after fatal exposure to oil hydrocarbons, all exposed larvae were held for several days after exposure for continued observation.

HERRING: Our objectives were to test the sensitivity of Pacific herring at 2 life stages: egg and yolk-sack larvae. Both were exposed for different periods of time from 2-16 days then returned to uncontaminated water. Survival of exposure for eggs was defined as hatching into swimming larvae after exposure, and for larvae it was defined as normal appearances and behavior after exposure.

PROGRESS (Jan.1 - Mar. 31)

Plans have been finalized, adult gravid shrimp collected, and most apparatus assembled. The shrimp have been caught in shrimp pots set and hauled by hand from small boats in Auke Bay. Collected shrimp are being held in running sea water and fed on chopped herring and shellfish. The necessary glass crude oil WSF generators have been build and are operating. The WSF delivery and dilution manifolds feeding the 19 l. jars that will hold the egg bearing adults during exposure and the cooling system for the jars, are assembled. The large and complex exposure and culture system for the larvae and herring eggs is under construction. Besides the WSF manifolds and cooling system, this apparatus requires a pump system to raise and lower the water level in the exposure containers to insure solution exchange through the nylon screen bottoms of the glass tubes that will hold the larvae, and a viewing system to make observations of the tiny creatures possible. Cultures of rotifers to feed the herring larvae (and of algae to feed the rotifers) have been established.

Exposures will begin about April 1, and the experiments are on schedule.

Problems encountered

Set up of a continuous flow device suitable for exposing almost transparant larvae, without loosing them in the volume of the exposure, and though the device has been completed and tested without larvae and appears to work. This has consummed more labor than anticipated and more technical design than anticipated.

Funding for RU 72 was received in mid February, and at 28% decrease from the original proposal submission. Late and decreased funding has forced us to diminish the effort in manuscript synthesis. Drafted manuscripts have been worked on, but the spring research effort is imminent and progress after Apr. 1 may be low. On top of these problems, a separate but related research effort for OMPA has not been finalized at this time. Administrative effort to negotiate the OMPA contract has diminished progress on manuscripts.

SUBLETHAL EFFECTS OF PETROLEUM HYDROCARBONS AND TRACE METALS, INCLUDING BIOTRANSFORMATIONS, AS REFLECTED BY MORPHOLOGICAL, CHEMICAL, PHYSIOLOGICAL, PATHOLOGICAL, AND BEHAVIORAL INDICES

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I. SUMMARY OF OBJECTIVES, CONCLUSIONS, AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

A. Summary of Objectives

The overall objective of this program is to assess potential effects of petroleum and petroleum-related operations on marine organisms indigenous to Alaskan waters. Several principal objectives were addressed by this research unit (OCSEAP RU73) during the contract period. These were:

1) Complete analyses of previously collected data and provide information concerning effects of petroleum on embryo and larval development of chum salmon (Oncorhynchus keta), flatfish (Pleuronectidae), and surf smelt (Hypomesus pretiosus), and the ability of the organisms to survive in a petroleum-contaminated environment.

2) Complete analysis of the effects of exposure to petroleum-impacted sediments on disease resistance of flatfish .

3) Assess the effects of petroleum on predator-prey relationships in salmon.

4a) Continue studies on the uptake of specific aromatic hydrocarbons (3-, 4-, and 5-ring) in an Alaskan species of flatfish exposed to these compounds in the sediment or by way of their diet.

4b) Evaluate the potential of specific petroleum hydrocarbons and their oxidized products for interacting with critical cellular constituents of a key Alaskan species of flatfish.

5) Determine the relative toxicities of weathered petroleum components in relation to fresh petroleum hydrocarbons.

B. Summary of Conclusions

The conclusions of this program are summarized according to disciplinary areas. Several aspects of the studies have been completed and others are continuing.

Behavior

Exposure of chum salmon fry to the seawater-accomodated fraction (SWAF) of Cook Inlet crude oil (CICO) at an average concentration of 350 ug/l total hydrocarbons for periods of 24, 48, 72, and 96 h resulted in statistically significantly different (greater at 24, 48, and 72 h and less at 96 h) consumption of the oil-exposed prey by coho predators compared to the consumption of non-oil-exposed controls.

Similarly, exposure of coho salmon (O. kisutch) predators to the SWAF of CICO for 10 and 17 days at an average of 343 ug/l total hydrocarbons was correlated with statistically significant alteration (reduction) in the

numbers of salmonid fry prey eaten by the oil-exposed predators compared to the numbers of prey eaten by non-oil-exposed controls. For these experiments, chemical analysis suggested that it was not the parent petroleum hydrocarbons that were primarily affecting the coho's predatory behavior, but rather metabolites of the hydrocarbons.

Chemistry

The results show that English sole (Parophrys vetulus) exposed to benzo[a]pyrene (B[a]P) in oil-contaminated sediment take up and readily metabolize the hydrocarbon. A number of mutagenic and carcinogenic metabolites were identified in the liver; some of the toxic compounds were bound to cellular macromolecules (e.g. DNA and protein).

Further, B[a]P tends to remain largely unconverted in sediment and thus can be available for continued uptake by benthic organisms. Continued uptake, greater retention, and more extensive metabolism of B[a]P than naphthalene (NPH) by benthic fish indicate that, although B[a]P is a minor component of petroleum, its derivatives can be bioconcentrated in tissues of demersal organisms. The substantial bioconversion of B[a]P in fish liver very probably explains why B[a]P is usually not detected in fish tissues even when considerable concentrations of B[a]P are detected in the environment of the fish.

Pathology

Juvenile English sole exposed to CICO-impacted sediments for up to 7 days, and juvenile starry flounder (Platichthys stellatus) exposed to Prudhoe Bay crude oil (PBCO)-impacted sediment for up to 6 wk showed no changes in disease resistance as measured by laboratory challenge with the pathogenic bacterium Vibrio anguillarum. Also, adult rock sole (Lepidopsetta bilineata) exposed to PBCO-impacted sediments for 2 wk did not show an altered ability to resist bacterial infection.

Physiology

Exposure of embryos and larvae of chum salmon, English sole, sand sole (Psettichthys melanostictus), and surf smelt to the SWAF of weathered crude oil at concentrations of 100 to 500 ppb typically induced in all species either high embryo mortality or larval mortality, gross abnormalities, and pathological changes. Exposure of smelt embryos to hydrocarbon concentrations less than 100 ppb resulted in cellular damage to retinal and neural tissue and severely reduced larval survival. Laboratory conditions under which the experiments were conducted were designed to simulate natural conditions in which embryogenesis occurs; thus, it is predicted that the ability of embryos and larvae studied to survive similar oil exposure in the natural environment is similar to that observed.

C. Implications With Respect to OCS Oil and Gas Development

Findings from this program have clear implications with respect to petroleum effects on aquatic species and consequently to OCS oil and gas development. Most of the studies were designed as laboratory experiments with emphasis, as much as feasible, on oil exposures of marine organisms in flowing seawater tanks. Controlled studies with experimental designs of the types reported here are indispensible parts of a total program directed at understanding effects of petroleum on the marine environment. It is primarily through controlled experiments such as these that petroleum can, with a high degree of accuracy, be identified as causing specific effects. In addition, through these types of studies, the relative susceptibility or resistance of important species to petroleum components can be established, as can the precise nature of petroleum-induced abnormalities. The laboratory results can then be used to design directed field studies, to formulate hypotheses for testing petroleum impacts on marine species and ecosystems, and as a basis for developing best estimates of petroleum impacts on the environment.

Implication of studies conducted in this period for each disciplinary area are presented below.

Behavior

Chum salmon and pink salmon (O. gorbuscha) fry spend several months in coastal estuaries and near shore before going to sea. At this life stage they are extremely vulnerable to predation by other salmonid fishes. The predator-prey studies conducted were designed to determine if oil-exposed fry experience increased predation and, conversely, if oil-exposed predators capture fewer prey. In both instances the results have been that oil has such effects in laboratory exposures.

Chemistry

Polynuclear aromatic hydrocarbons (PAHs), such as B[a]P, upon entry into the marine environment can persist in sediment and are thus available for continual uptake by demersal fish. B[a]P is rapidly and extensively metabolized in flatfish into a number of mutagenic and carcinogenic compounds. The rate of metabolism and retention time of metabolites by flatfish are considerably greater for B[a]P than for NPH. Although B[a]P is a minor component of crude oil, these factors raise serious concerns regarding B[a]P and other high molecular weight PAHs in the marine environment.

These findings serve to point out that, in the assessment of marine pollution, considerable bias may arise from determining only the parent PAHs in marine animals.

Pathology

Exposure of juvenile and adult flatfish species to crude oil-impacted sediments did not demonstrably alter disease resistance. This does not, however, necessarily rule out the possibility that under conditions of additional stress, such as stress induced by multiple contaminants including crude oil, or stress from starvation plus crude oil, that susceptibility to bacterial infection might be increased.

Physiology

Early developmental stages of salmon, flatfish, and smelt exposed to the SWAF of weathered crude oil all showed similar effects, with reduced survival of embryos and larvae in each species. The spawning strategy is different for each of the three species; however the effects observed could be expected to occur in each of the species in marine and estuarine environments contaminated by petroleum hydrocarbons at the environmentally attainable concentrations used in the experiments.

II. INTRODUCTION

A. General Nature and Scope of Study

The responses of marine organisms to environmental contaminants are reflected in a number of changes detectable at organismic levels as well as at organic, tissular, cellular, subcellular, and molecular levels. The general scope of this study is to detect petroleum-related effects at various levels in marine species and evaluate their implications for survival and well-being of the animals.

B. Specific Objectives

In the interdisciplinary approach used in this study, there is a series of objectives to evaluate the effects of petroleum on marine organisms. The specific objectives of research performed during the current reporting period of April 1, 1980 to March 31, 1981, for individual disciplines are as follows:

Behavior

1) Determine the effects of CICO on avoidance of predation by salmonid prey.

2) Determine the effects of CICO on predation of salmonid prey by salmonid predators.

Chemistry

Determine the concentrations of specific hydrocarbons and metabolites in water, sediment, and tissues of exposed organisms in order to correlate their presence with biological effects.

Pathology

Assess the effects of petroleum hydrocarbons on host defense mechanisms and disease resistance of selected species of flatfish.

Physiology

Address the following with respect to studies on early developmental stages of chum salmon, flatfish, and surf smelt:

1) Effects of petroleum on embryos and larvae as determined from gross structural and ultrastructural measurements.

2) Evaluate the ability of embryos and larvae to survive oil exposure in the natural environment.

C. Relevance to Problems of Petroleum Development

When petroleum is transported in or obtained from coastal or offshore areas, inevitably petroleum hydrocarbons and associated trace metals escape into the marine environment. These materials, at some level, have a potential for producing critical damage to marine resources. This damage by crude oil components can take several forms (Blumer, M., Testimony before Subcommittee on Air and Water Pollution, Senate Comm. on Public Works, Machias, Maine, 8 Sept. 1970):

1. Direct kill of organisms through coating and asphyxiation, through contact poisoning, or through exposure to water-soluble toxic components of oil at some distance in space and time from the accident.

2. Destruction of the generally more sensitive juvenile forms of organisms.

3. Incorporation of sublethal amounts of oil and oil products into organisms resulting in reduced resistance to infection and other stresses and in failure to reproduce.

4. Destruciton of the food sources of higher species.

5. Exposure to long-term poisons, e.g., carcinogens.

6. Low-level effects that may interrupt any of the numerous events necessary for the feeding, migration, and propagation of marine species and for the survival of those species which stand higher in the marine food web.

7. Contamination of marine food resources to make them unfit for human consumption.

Studies of OCSEAP RU 73 are largely concerned with indirect, long-term effects of petroleum such as those detailed in items 2, 3, 5, and 6. These effects are much more difficult to detect and evaluate than those related to acute exposures, but may over a period of time have even greater impact on marine biota.

III. CURRENT STATE OF KNOWLEDGE

Behavior

Evaluation of the effects of petroleum on the indigenous marine fauna of Alaska's continental shelf regions are of continuing importance because of existing and proposed oil exploration activities in these areas. In reviews of the acute toxicity and sublethal biological effects of petroleum on arctic and subarctic marine organisms, Craddock (1977) and Patten (1977) presented evidence of lethargy, loss of appetite, and alterations in schooling behavior associated with exposure to various seawater-soluble fractions of petroleum. However, there were no studies reported on the influence of petroleum on predator-prey behavior of fishes, which has been described as a sensitive indicator of perturbed environmental conditions (Goodyear 1972, Hatfield and Anderson 1972, Sylvester 1972, Coutant et al. 1974, Yocum and Edsall 1974, Sullivan et al. 1978, Woltering et al. 1978). The purpose of the present studies were to determine the influence of crude oil in seawater on salmonid predator-prey interactions. Coho salmon were chosen as predators; this species has been identified as a primary predator of juvenile salmonids in seawater (Parker 1971).

Chemistry

Aromatic hydrocarbons enter the marine environment from a number of sources. A large portion of these compounds eventually settle in sediments. Benthic fish can accumulate aromatic hydrocarbons via the food chain and via interstitial water into which a small proportion of these compounds, which are bound to or associated with sediment, are slowly and continuously released. Recent analyses of sediment in the Pacific Northwest (Malins et al. 1980a) demonstrate the presence of a number of diaromatic and polyaromatic hydrocarbons, such as NPH and B[a]P. Analyses of tissues of benthic invertebrates from these areas reveal a variety of aromatic hydrocarbons including NPH and B[a]P in more than half of the animals examined. Interestingly, less than 5% of the livers of fish sampled from these areas contained detectable concentrations of B[a]P, whereas more than 50% of these samples contained detectable levels of NPH. McCain et al. (1978) reported that English sole exposed to sediment containing 1% PBCO sequestered NPH and its alkylated derivatives in tissues (e.g., liver, muscle, and skin) but phenanthrene (PHN), though present in sediment, was not detected in the fish. In these studies (McCain et al. 1978; Malins et al. 1980a), only the concentrations of hydrocarbons were measured; therefore, it was not possible to assess whether the PAHs (PHN and B[a]P) were less bioavailable to the fish or were more extensively metabolized by the fish than were the diaromatic hydrocarbons.

Studies on uptake of PAHs, such as B[a]P, by fish are few (Lee et al. 1972; Gerhart and Carlson 1978) and virtually no information is available on the extent of metabolism of B[a]P by fish. However, recent studies (Varanasi and Gmur 1980; Varanasi et al. 1980a) demonstrate that liver enzymes of pleuronectid fish extensively convert B[a]P *in vitro* into phenols and dihydrodiols. Some of these metabolites are cytotoxic, mutagenic, and carcinogenic in mammals; therefore it is important to determine both the

degree of bioconcentration and the extent of metabolism of PAHs in a demersal fish and to characterize the metabolites formed by the liver in vivo.

Pathology

There is considerable experimental evidence indicating that petroleum hydrocarbons and associated trace metals affect host defense mechanisms in various mammals (Kripke and Weiss 1970; Hemphill et al. 1971; Jones et al. 1971; Filkins and Buchanan 1973; Koller 1973; Koller and Kovacic 1974; Stjernsward 1974; Cook et al. 1975: Koller et al. 1975; Koller and Roan 1980), birds (Vengris and Mare 1974) and fish (Robom and Nitkowski 1974). Since disease is the result of complex interaction among the host, the pathogen, and the environment, any envronmental perturbation which compromises host defense can precipitate an outbreak of disease. Studies conducted over the past several years under OCSEAP RU 73 have addressed the potential of various exposures to crude oil to adversely impact disease resistance in selected flatfish species, salmonids, and spot shrimp (Pandalus platyceros). The results of the flatfish investigations and of preliminary investigations on effects of dispersants are summarized in this report.

Physiology

There is increasing evidence that crude oil may particularly affect the early development stages of marine fishes. Lonning (1977) demonstrated that embryos of cod (Gadus morhua), plaice (Pleuronectes platessa), and flounder (Platicthys flesus) exposed to crude oil for even short periods (1-15 h) developed deformed notochords, and severe abnormalities in the head region, and changes in melanophore distribution of emerging larvae. Particularly sensitive stages were those at cleavage and gastrulation, hatching, and again after yolk resorption. Smith and Cameron (1979) observed that newly hatched Pacific herring (Clupea harengus pallasi) larvae exposed to PBCO were unable to swim and maintain themselves in the water column; abnormalities of the mouth, pectoral fins, and branchiostegal membranes were also observed. Linden (1975) found that petroleum hydrocarbons disrupted lipid membranes and primordial fin formation of Baltic herring (Clupea harengus membras) larvae. Mazmanidi and Bazhashvili (1975) exposed eggs of the Black Sea flounder (Platichthys luscus) at various stages of development to the water-soluble fraction of crude oil at concentrations of 2.5 to 0.025 ppm. All concentrations greater than 0.025 ppm were found to be toxic. Eggs exposed in the gastrulation stage died immediately and most embryos exposed at more advanced stages hatched but perished soon afterwards. Surviving larvae exhibited scoliosis, reduced activity and rate of yolk absorption, and abnormalities in heart rate and pigment configuration. Several studies have been reported concerning the effect of petroleum on salmon embryos and alevins (Rice et al. 1975; Kuhnhold and Busch 1977/1978); however, these investigations have largely been focused on acute toxicity of oil and on the uptake of specific hydrocarbons. No published information has been found detailing effects of weathered crude oil on embryonic and larval development of salmonids, flatfish, or smelt.

IV. STUDY AREA

All experiments were conducted either in laboratories or in fish-holding facilities at the Northwest and Alaska Fisheries Center (NWAFC), Seattle, and at the NWAFC's saltwater field station at Mukilteo, Washington, on representative subarctic marine and anadromous species.

V. SOURCES, METHODS, AND RATIONALE OF THE DATA COLLECTION

Behavior

Oil-exposed prey experiments: The effect of the SWAF of crude oil on salmonid prey was studied with chum salmon fry obtained from the Washington State Department of Fisheries salmon-rearing facility at Hoodsport, Washington. They averaged 5.4 cm total length when testing started early in June and 6.6 cm at the end of testing in late July. The predators used were 2 year old juvenile coho salmon from the Washington State Fisheries Department hatchery at Issaquah, Washington. They were obtained as fertile eggs, hatched and reared in fresh water at the NWAFC, Seattle, to an age of 1 year and then in seawater at the Mukilteo Facility to an age of 2-plus years and an average length of 35.0 cm.

The experimental design was to expose test fry to the SWAF of CICO for periods of 24, 48, 72, and 96 h while holding control fry under similar conditions for an identical length of time. An equal number of test and control fry were then introduced simultaneously into an observation tank containing hungry predators. After approximately one-half of the fry were consumed the predation was halted and the numbers of test and control prey remaining were determined. The resulting data were evaluated by chi-square analysis (Coutant 1973).

A few days prior to the start of an experiment, test and control fry were distinctively marked by cold-branding (Fujihara and Nakatani 1967). The coho salmon were trained to feed on chum fry for at least one month before testing. The predator cohos were always starved 48 h before being used in the study.

The SWAF of crude oil was produced by a flow-through apparatus similar to that described by Nunes and Benville (1978). Crude oil was continuously pumped (1 ml/min) onto the surface of seawater in a large glass carboy. A flow of 4 l/min of seawater was directed onto a dispersion plate and dripped through the layer of oil where the soluble fractions were extracted. The resulting product was siphoned into the exposure tank and diluted with an equal quantity of fresh seawater. Water samples collected from the exposure tanks were analyzed by gas chromatography (GC) (MacLeod et al. 1976) at the National Analytical Facility (NAF), Seattle.

The water quality (temperature, pH, and dissolved oxygen) which were monitored every other day in the test exposure tanks and the control tanks did not differ appreciably. The greatest difference was in the water temperature which was approximately 0.5°C higher in the test tanks than in control tanks

due to heating the solubilizer. Ten test and ten control fish were introduced to the predator tank simultaneously. Fifty percent predation usually took place in a few seconds to a few minutes but was never allowed to extend beyond 2 h.

Oil-exposed predator experiments: Fish were obtained from the following sources: adult coho salmon from a stock reared at the NWAFC, Seattle, and rainbow trout (Salmo gairdneri) fry from Trout Lodge, Tacoma, Washington. The trout fry were 4.6±0.5 cm ($\bar{X}$±S.D.), and the coho predators were 35.2±5.0 cm in fork length. The trout fry were maintained (fed to satiation) on a diet of Oregon Moist pellets (OMP). The coho predators were maintained on OMP until four months prior to the experiments, from which time they were fed exclusively on live chum salmon or rainbow trout fry. Rainbow trout, a species which may be anadromous as steelhead trout, were selected as prey for the studies primarily as a result of their availability. Although natural predation by coho salmon on Salmo gairdneri in seawater is unlikely, we concluded after preliminary trials that this combination provided a viable experimental design for a laboratory evaluation of the effects of petroleum on the predatory behavior of coho salmon.

The coho predators were exposed to the SWAF of CICO which was also prepared using the solubilizer similar to the one described by Nunes and Benville (1978). Water samples were collected twice weekly from the exposure tank during the experimental period. These samples were analyzed for total hydrocarbon content by GC at the NAF, Seattle. Dissolved oxygen concentrations, pH, and temperature were also determined twice weekly for water in the exposure and control tanks (APHA Standard Methods 1975).

Two rectangular fiberglass tanks (118x42x44 cm, capacity of 170 l) were used as the exposure and control tanks. Circular fiberglass tanks (1.2 m diameter, 55 cm deep, capacity of 950 l) were used for the predation tests. Seawater inflow rates to the circular tanks provided for a 95% exchange within 24 h.

The coho salmon predators were randomly divided into two groups of 21 fish each. One group was designated as the control while the other 21 fish, the test group, were exposed to the SWAF of CICO. All test fish were exposed (together) to the oil for a period of 17 days, with predatory evaluations occurring after 3, 10, and 17 days of exposure. Each of the test groups was further divided into subgroups of 3 fish each for the predator evaluations, so that there were 7 replicates for each group at each of the 3 evaluation periods. Each fish was freeze-branded (Fujihara and Naktani 1967) for identification and maintained in the same subgroup throughout the study, with the following exceptions: three mortalities occurred in the control group (one fish each from Subgroups 1, 3, and 6), just prior to the final testing period. The 2 surviving fish from Subgroup 1 were redistributed to Subgroups 3 and 6 to maintain the subgroup sizes of 3, which were essential to stimulate feeding behavior. The predators were not fed between evaluation periods.

The predator-prey interactions were evaulated in the following manner: predators were transferred from the control or exposure tanks to the predator evaluation tanks at 4 p.m. on the day prior to testing. At 9 a.m. on the day

of testing 10 rainbow trout fry were transferred without acclimation into a tank containing one of the 7 predator subgroups (3 adult coho salmon), and then the number of prey surviving after 10 minutes were recorded. Surviving prey were discarded. Predators were returned to their respective control or oil-exposure tanks immediately after the predator-prey evaluations. At the termination of the experiment, 3 control (Subgroup 4 in Figure 6) and 6 oil-exposed fish (Subgroups 1 and 6) were selected for analysis of brains and livers for hydrocarbons because of their opposing behavioral responses in the predator-prey evaluation.

Water quality for both the control and exposure tanks was as follows, ($\bar{X}\pm$S.D.): salinity, 26.5±1.3 °/oo; temperature, 13.4±0.7°C; pH, 7.6±0.1; and dissolved oxygen 6.8±1.1 mg/l. In the exposure tanks the SWSF concentrations ranged from 230-530 ug/l ($\bar{X}\pm$S.D.=343±93).

Chemistry

English sole (62±22 g) trawl-caught in Puget Sound were kept in flowing seawater (28 °/oo and 12.0±0.5°C) and fed a diet of minced clams for two weeks. The feeding was stopped three days prior to the initiation of experiments.

Preparation of oil-contaminated sediment was identical to that described by Malins et al. (1978) except that in the present study ^{3}H-B[a]P and ^{14}C-NPH were dissolved in PBCO prior to mixing with the sediment. The oil-contaminated sediment was placed in a 17 l glass tank where it was washed in flowing seawater for 24 h (day 1). The water temperature was 12.0±0.5°C., salinity was 28 °/oo, and the flow rate was 20 l/day.

Six fish were placed in the experimental tank for a 24-h exposure on day 2, three fish were sampled on day 3, and the remaining fish were placed on clean sediment in flowing seawater for 24 h. Immediately after the first six fish were removed on day 3, five additional fish were placed in the experimental tank. These fish were exposed to the oiled sediment for 168 h before sampling on day 10.

Samples of wet sediment, unfiltered and filtered sediment-associated water (SAW), and samples of gill, skin, muscle, blood, liver, bile, stomach, and intestine were analyzed for total radioactivity (^{3}H and ^{14}C) using liquid scintillation spectrometry (Varanasi and Gmur 1981). Ethyl acetate extracts of the sediment, SAW, and bile before and after enzymatic hydrolysis (Varanasi and Gmur 1981), were analyzed for parent B[a]P and its metabolites by thin-layer chromatography (TLC). Assessments of NPH and total NPH metabolites in sediment, SAW, bile, and liver were made by a solvent partitioning method using hexane and sodium hydroxide (Roubal et al. 1978; Varanasi et al. 1979). TLC was used to examine individual classes of metabolites in ethyl acetate extracts of tissue samples (Varanasi and Gmur 1980). Ethyl acetate-soluble metabolites of B[a]P from liver (homogenized in isotonic saline) were analyzed by a newly developed technique using two dimensional TLC as outlined in Figure 1 (Varanasi and Gmur 1981). Four Solvent systems were employed for TLC analyses: Solvent system A (Toluene:Ethanol, 9:1,v/v); Solvent system B (Plate was developed up to 6 cm in ethyl acetate and then redeveloped in the same direction with toluene: ethanol, 100:3,v/v); Solvent system C (upper phase of concentrated ammonium hydroxide:water:n butanol, 10:50:40,v/v/v) and Solvent system D (hexane: diethyl/ether, 95:5,v/v).

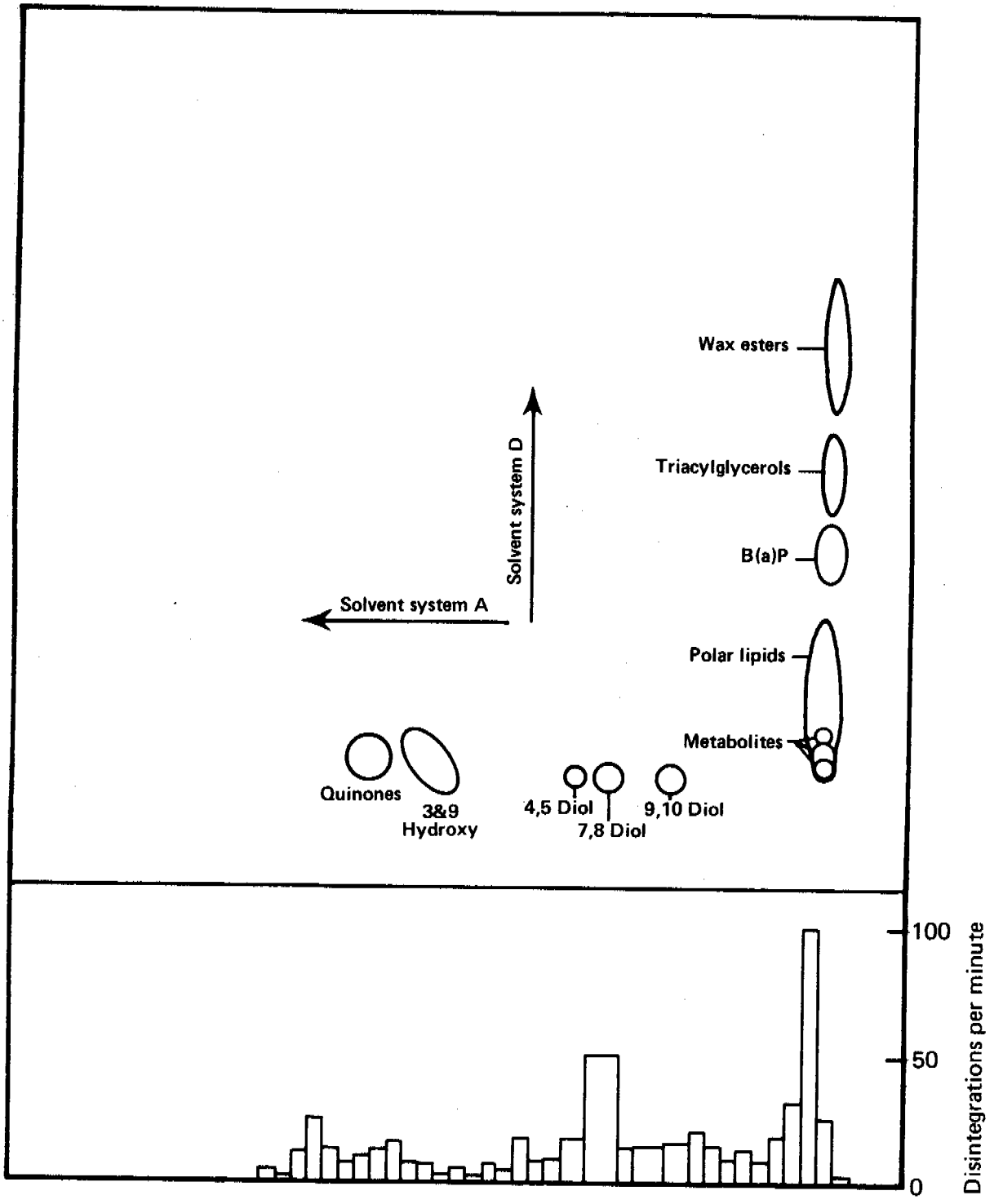

Figure 1. Two-dimensional TLC of liver extracts. Liver lipids and ^{3}H-B[a]P were separated from polar lipids and B[a]P metabolites by developing the plate in one direction with the solvent system D with appropriate standards. The plate was then turned 90° and redeveloped in the solvent system A. The resulting profile of B[a]P-derived radioactivity is from one fish from the 168 hr exposure group.

The aqueous phase from the ethyl acetate extraction of the liver homogenate was centrifuged (500xg, 5 min), the liquid was removed, and the pelleted liver protein was extracted with acetone and diethyl ether. The protein was desiccated under vacuum, then solubilized to determine the radioactivity that was not extractable.

Pathology

The effects of petroleum hydrocarbons on disease resistance of selected marine fish and shellfish were evaluated under OCSEAP RU 73/74 over the past 5 years. The methods used to determine the effects of exposure to PBCO-impacted sediment on disease resistance of selected flatfish species have been previously reported (OCSEAP Annual Report RU 73-1979). During FY1981, laboratory experiments were conducted to determine the effects of petroleum-impacted sediments on disease resistance of juvenile English sole and a preliminary study was conducted on the effect of an oil-dispersant on disease resistance of coho salmon.

Juvenile English sole (avg. wt. = 11.6 g) were exposed to oil-contaminated sediment in flow-through seawater aquaria containing a 5 cm layer of uncontaminated sediment or sediment containing 1% (vol:vol) fresh CICO. Disease resistance was assessed after exposures of 24 and 168 h by determining the ability of oil-exposed and non-exposed fish to survive a laboratory challenge with the marine fish and shellfish pathogen Vibrio anguillarum. Samples of sediment, interface water (water collected 2 cm above sediment) and liver tissue were collected at the beginning of exposure and at each sample point for analysis of petroleum hydrocarbons.

Disease resistance was quantitated by the calculation of an LD_{50} value as described by Reed and Muench (1938). Only those dead fish from which V. anguillarum was reisolated were used in these calculations.

Studies were also initiated to evaluate the effects of oil dispersant on disease resistance of coho salmon. In preliminary assays, coho salmon (avg. wt. 28.5 g) were exposed for 30 min to 30 ppm (vol:vol) Corexit 9527 in seawater containing various concentrations of V. anguillarum. Control fish were similarly exposed in seawater containing bacteria only. Fish were then transferred to individual 30 l aquaria containing uncontaminated seawater and mortality was monitored for 10 days. All dead fish were examined by bacteriological culture techniques and death was considered to be due to V. anguillarum only when the bacterium was reisolated in pure culture.

Physiology

The experimental design for exposure of early developmental stages of salmon, flatfish, and smelt to petroleum was dependent largely upon the fishes' spawning characteristics and the conditions under which embryogenesis occurs. A brief description of the reproductive cycle of each species follows.

Chum salmon frequently spawn in tidal areas at the mouths of streams (Bakkala 1970; Neave 1966). Eggs are deposited in redds and then covered with gravel as a result of upstream redd digging and stream flow. Subsequently, at least one high tide a day inundates the redds with salt and brackish water;

the length of exposure to water of high salinity depending upon redd location. After approximately 50-90 days (development rate is a function of water temperature) the eggs hatch, and the chum salmon alevins remain in the gravel for another 30-50 days before emergence and migration to saltwater.

Surf smelt are found along the Pacific coast from California to the Aleutian Islands and have a life history similar to the commercially and ecologically important capelin (Mallotos villosus). Surf smelt spawn intertidally on coarse sand-pea gravel beaches, the adhesive eggs being submerged intermittently at high tide until the embryos hatch as well-developed larvae. Duration of embryonic development (8 to 30 days) is dependent upon both water and air temperature (Yap-Chiongco 1941; Loosanoff 1937; Pentilla 1978), and hatching is promoted by wave agitation at completion of embryogenesis (Misitano 1977).

Sand sole and English sole are pleuronectid flatfish with pelagic eggs and larvae. Eggs of most pleuronectid flatfish are released on or near the bottom and after about one day, depending upon depth of spawning, the eggs rise to float near the water's surface (Alderdice and Forrester 1971; Ketchen 1956). Eggs hatch after approxiamtely one week and the larvae emerge relatively undeveloped.

Weathered oil was prepared in a wave machine that subjected fresh PBCO or CICO to mixing with seawater by wave action, exposure to sunlight, and loss of volatile components through evaporative processes (Fig. 2). A paddle hinged at the bottom was attached to an electric motor which produced a steady wave periodicity of 48 per min. At the opposite end from the paddle an artificial beach was added to dampen waves and simulate water-accomodated oil passing down through the gravel of a chum salmon spawning redd. Coarse gravel (80% from 1 to 5 cm in diameter, remainder finer) was spread 25 cm deep over a perforated pipe. Over this gravel beach was placed a removable baffle which isolated the gravel from the oil during the weathering process thus preventing oil from coming in contact with the "beach" before weathering was completed.

Fresh PBCO or CICO were layered on the water surface (91 μ l/cm^2) in the wave machine resulting in an initial oil concentration of 4,000 ppm (volume of oil to volume of water). During the weathering process, there was a continuous flow of salt water (27-30 $^{o}/_{oo}$) through the wave machine with a water replacement time of once per hour. Water overflow was removed 25 cm beneath the water's surface via an adjustable standpipe in order to maintain the surface oil slick. After 30-48 h of flow-through operation and wave action, the oil was defined as weathered.[1] For experiments with chum salmon embryos and alevins the salinity

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[1]Weathering of PBCO and CICO is defined here as a loss of volatiles, generally those hydrocarbons with boiling points less than 210°C and representative of the naphtha fraction. Presumably the principal processes in this loss are a combination of dissolution and evaporation. The term water-accomodated oil, or seawater-accommodated fraction (SWAF), denotes water removed from under an oil slick. In water samples collected, there was often a Tyndall effect indicating that along with the soluble petroleum hydrocarbon components, there were some oil particles dispersed in the water column.

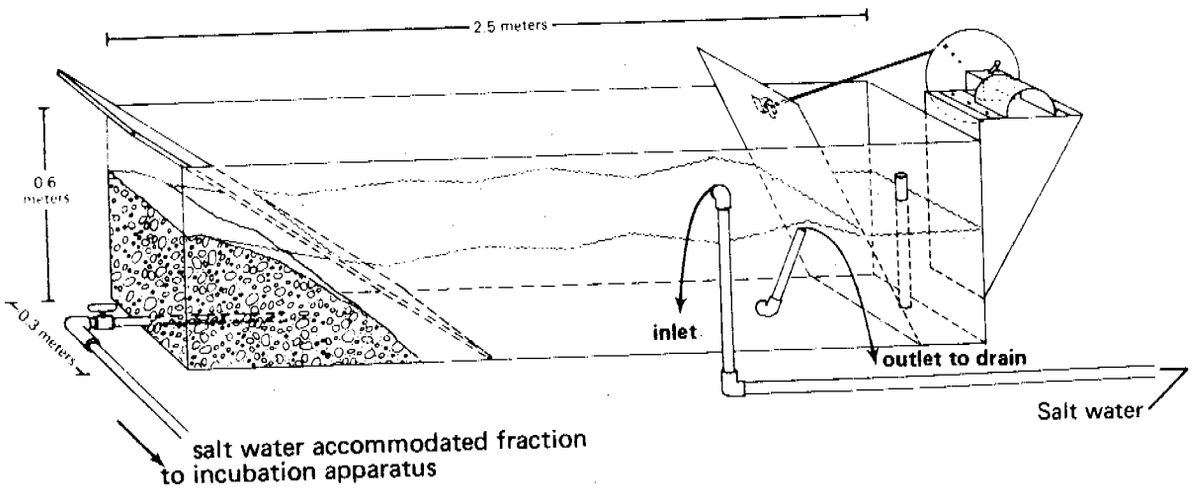

Figure 2. Apparatus for weathering of crude oil.

of the incoming water was then reduced to 16-24 °/oo and the baffle removed, allowing the water-accommodated oil to come in contact with the gravel. The oil-contaminated water was then drawn off through the gravel for treatment of embryos and alevins. Once a week the gravel and wave machine were emptied and cleaned, the cleaned gravel was replaced and fresh oil was added, and the process was repeated.

In experiments with flatfish and surf smelt embryos the gravel in the wave machine was replaced with an inclined plane similar to the removable baffle shown in Figure 2, and the perforated pipe for SWAF delivery extended through the incline. Also, salinity was not reduced after oil weathering, nor was the machine cleaned and oil replaced during the course of each experiment.

Chum salmon eggs were obtained from the U.S. Fish and Wildlife Service National Fish Hatchery at Quilcene, Washington. Immediately after fertilization the eggs were transported to the Mukilteo Laboratory. Subsequent sampling from control groups indicated that 2.6% of the salmon eggs were not fertilized.

Spawning English sole and sand sole were obtained by trawling in Puget Sound and the eggs fertilized immediately on board the trawling vessel. Fertilization success and subsequent viability in flatfish eggs was high, approximately 90% as indicated by cell cap formation. Flatfish eggs used in each experiment were taken from a single female to reduce variations in viability.

Surf smelt eggs were collected from either the intertidal area of Hood Canal, Puget Sound, or from spawning females from Hood Canal. All surf smelt eggs were visually checked at the initiation of each experiment and only those with viable embryos were used in the tests.

One day after fertilization, approximately 70 chum salmon eggs were placed in each of 56 (30 x 75 cm) glass cylinders. Both ends of the cylinder were covered with teflon netting. A glass tray was divided longitudinally into eight troughs and small pea gravel was layered 2.5 cm deep on the bottom. Seven cylinders of eggs were placed horizontally into each trough (Fig. 3).

Eggs were exposed 3 h per day to oil-contaminated brackish water (16-24 °/oo, and 4.5 to 10.2°C ambient temperature) at a flow rate of 400 ml/min per trough. For 21 h per day the eggs received fresh dechlorinated water (5.5° to 10.5°C ambient temperature) at the same flow rate for a water replacement of 8 times per hour. Exposure to oil-contaminated water occurred 4 days per week. On the other 3 days, eggs and alevins received uncontaminated brackish water for 3 h per day. The oil exposure conditions (by group) designated for each of the 8 troughs is expressed diagrammatically in Figure 4.

Twenty-four h after fertilization (early cell-cap stage) flatfish eggs were introduced into 1,000 and 2,000 ml separatory funnels containing the SWAF of weathered PBCO obtained from the wave machine. During oil weathering (48 h duration), the salt water temperature was 7.8° to 9°C. The funnels were attached to an air supply through the bottom and the water bubbled slowly, thus creating a current in the funnel keeping the eggs in suspension. Funnels were submerged in a water bath with ambient (10°C) flowing seawater.

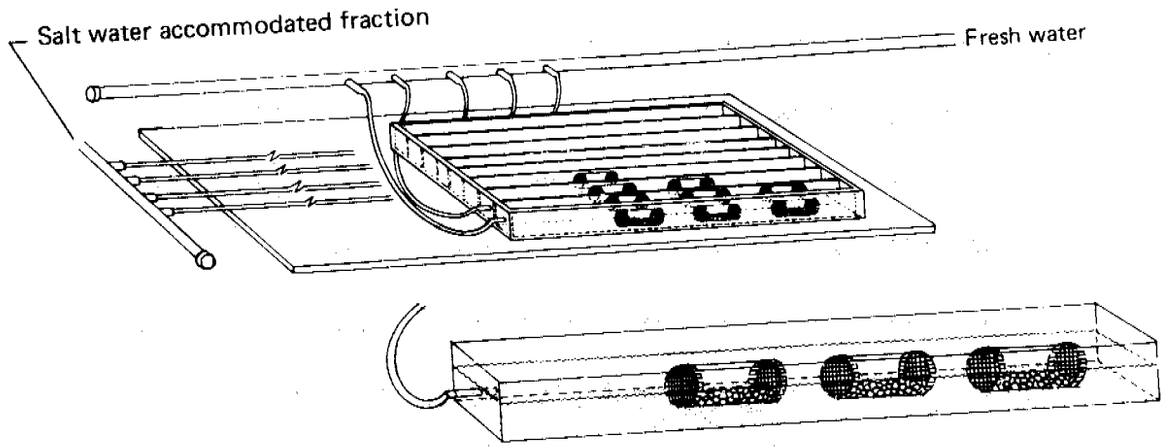

Figure 3. Chum salmon egg incubation apparatus with delivery of brackish SWAF of weathered PBCO from the wave generator (Fig. 2) for 3 h/day and delivery of fresh water for 21 h/day.

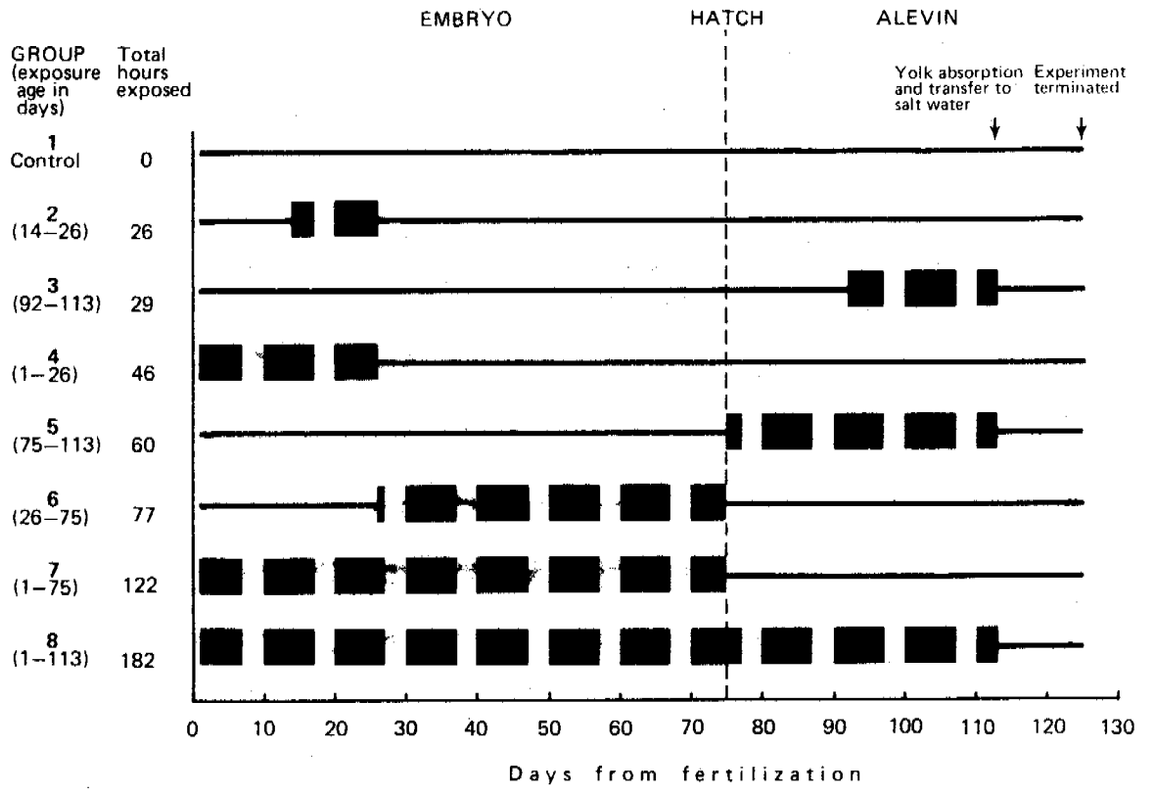

Figure 4. Exposure groups of chum salmon embryos and alevins showing duration and stage of exposure.

At the end of each test, flatfish eggs and larvae were examined and categorized using the following nomenclature:

Nondeveloped eggs: Two types of eggs were indistinguishable as to the cause of nondevelopment: (1) nonviable eggs which were not successfully fertilized, and (2) embryos which died in early cell division as a result of oil exposure or natural failure (embryo not formed).

Normal embryo: Embryo transparent, with regular heart contractions.

Abnormal or dead embryo: Opaque, often scoliosis evident, and intermittent or no heart contraction.

Normal larvae: Regular heart contractions, notochord straight, finfold continuous, digestive tract complete, and pigmentation complete.

Abnormal larvae: Regular heart contractions, slight curvature of the notochord and entire body, generally a lateral curvature of up to 45°.

Grossly abnormal larvae: Regular heart contraction, but with body curvature exceeding 45° (some with notochord curvature of 180° and double 180° curvatures), finfold deformed, digestive tract incomplete, pigmentation not in patches but scattered, lying motionless on bottom, and opaque.

Dead larvae: No heart contraction, generally opaque, and usually contorted.

Exposures of surf smelt eggs to the SWAF of CICO were repeated once in the month of November and once in December, at ambient water temperatures of 8.8° to 11.5°C and air temperatures ranging from -2.2° to 16.7°C. Four to six days after fertilization, 400 to 500 eggs were placed in each of four square-sided baskets (7 x 14 x 14 cm with 570 um mesh teflon netting on the bottom) filled with 2.5 cm of fine gravel (Figure 5). The baskets were submerged 3 h per day throughout the incubation period in the SWAF of weathered CICO with a flow rate of 750 ml/min to each basket. The SWAF from the wave generator was introduced into a diluter and three concentrations used in exposure: (1) undiluted (100%) SWAF from the wave machine, (2) 1/2 diluted (50%) SWAF, and (3) 1/4 diluted (25%) SWAF.

During the 16 week exposure period of chum salmon embryos and alevins to the SWAF of weathered PBCO, water samples were collected daily, 4 days per week, at intervals representing 30, 54, 72, and 96 h of weathering. Of these samples, 34 were selected as being characteristic of the duration of exposure and hours of weathering, and were analyzed for petroleum hydrocarbons.

Flatfish embryos were exposed to the SWAF of PBCO which was first weathered for 48 h in the wave machine. Water samples for chemical analysis were taken at initiaiton of the tests, after 3 days of exposure, and again after hatching (day 8). Water samples were collected by transferring the contents of the incubation funnel into a beaker. The water was then siphoned from the beaker through a screened cylinder to prevent passage of embryos and/or larvae. For samples taken in midincubation, the SWAF was renewed with a portion of the original SWAF which had been been refrigerated in a sealed glass bottle with a teflon-lined lid.

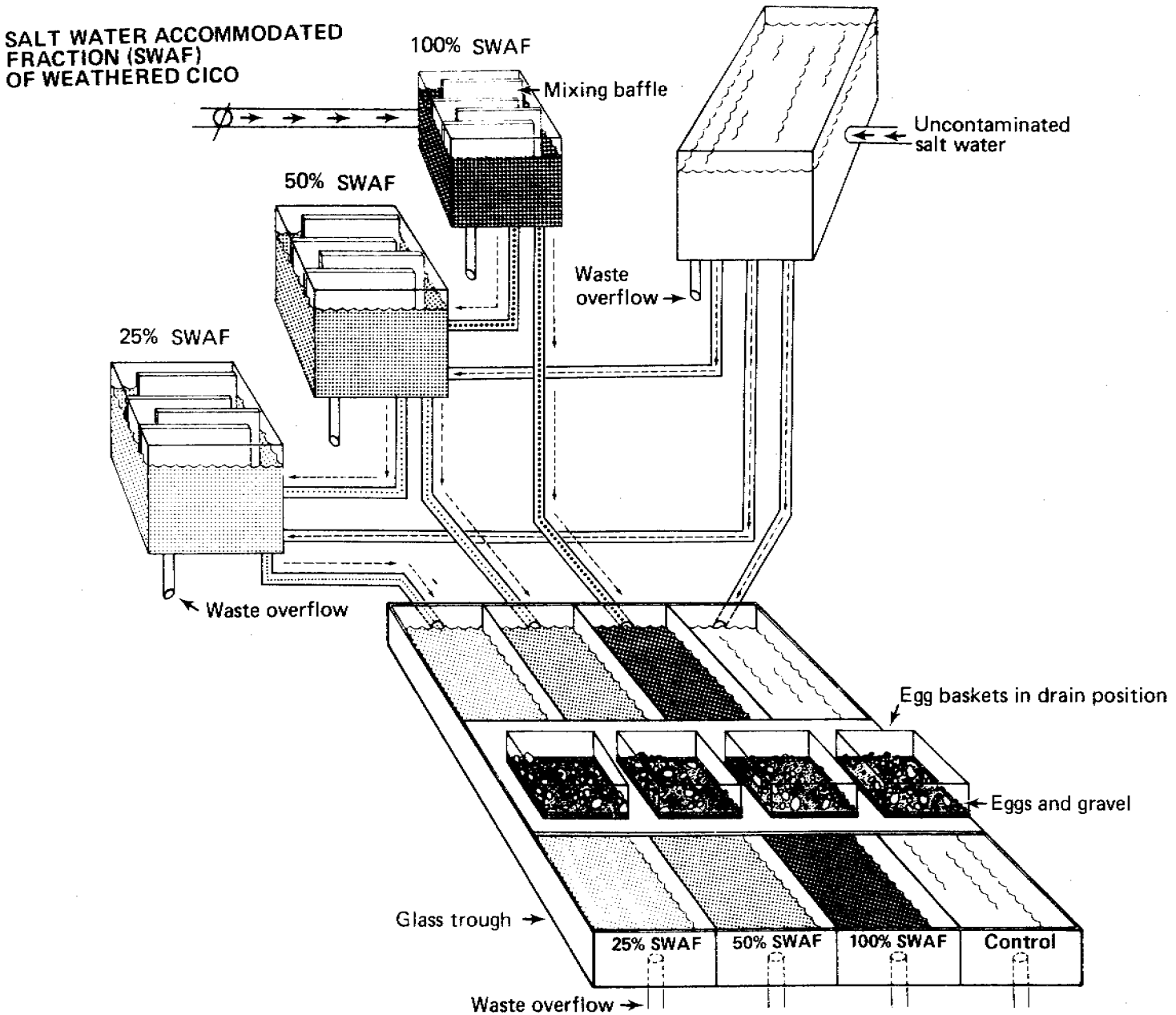

Figure 5. Surf smelt embryo exposure apparatus with delivery of the SWAF of weathered CICO from the wave generator (Fig. 2).

In experiments concerned with the early developmental stages of surf smelt, CICO was continuously weathered throughout both of the 3-week tests. Water samples for chemical analysis were collected daily from the trough containing undiluted SWAF, and every 4 days from all troughs. Thirty-five representative samples were analyzed for petroleum hydrocarbons, and another 10 water samples were analyzed for the concentration of hydrocarbons present in the 50% and 25% dilution.

Water samples were collected in 315-ml glass bottles containing 3 ml of concentrated hydrochloric acid and 12 ml CH_2Cl_2. Immediately after collection the samples were shaken for 3 min to partition most of the oil into the CH_2Cl_2 and the samples stored at 2°C. At the time of processing, recovery standards were added and the water samples extracted three times with a total of 24 ml of CH_2Cl_2. The extract was dried with Na_2SO_4 and reduced to 0.8 ml in a concentrator tube. A GC internal standard (10 ug hexamethylbenzene in CH_2Cl_2) was added and the extracts analyzed by glass capillary-gas chromatography. Identities of hydrocarbon components were confirmed by mass spectral analysis (for description of equipment and characteristics, see Malins et al. 1980a). The limit of detection for individual aromatic compounds was 1 ppb. Total hydrocarbon concentration was determined from the total area under the chromatogram.

In all experiments, the concentration of extractable material in control water averaged 2.2 ($\pm$1.1 S.D., N=10) ppb; none of this extracted material was identified as being of petroleum origin. The hydrocarbon concentrations presented are not corrected for extraction efficiency which averaged 67% ($\pm$9 S.D., N=34), 80% ($\pm$6 S.D., N=16), and 84% ($\pm$6 S.D., N=45) for data associated with experiments on salmon, flatfish, and smelt, respectively.

Surf smelt embryos were analyzed for petroleum hydrocarbons using methods of tissue extraction and analysis as described by Malins et al. (1980a). Data were analyzed using chi-square tests, robust locally weighted regression (Cleveland 1979), and predictive sample reuse (Geisser and Eddy 1979). The latter analysis is designed to determine within- and between-group differences, and to select the correct model $\geq$95% of the time.

Embryos and larvae of salmon, flatfish, and smelt were randomly sampled throughout each experiment or at the termination of an experiment, and fixed in a solution containing 0.7% glutaraldehyde, 3% formalin, 0.5% acrolein, and 0.1 M sodium cacodylate buffer (pH 7.4) with 0.02% $CaCl_2.H_2O$, 5.5% sucrose. Embryos were removed from the chorion and post-fixed in 1% osmium tetroxide in buffer. The tissues were dehydrated with ethanol, embedded in Spurr medium (Spurr 1969), and sectioned with a glass or diamond knife. Semi-thin sections (1.0 mm) were stained with Richardson's mixture or with a modified periodic acid-Schiff (PAS) reagent (Nevalainen et al. 1972) for light microscopy. Depending on the plane of the section, tissues from all organs of the embryos could usually be examined. Thin sections (80nm) were triple-stained with lead citrate, uranyl acetate, and again with lead citrate for transmission electron microscopy (TEM).

Samples for scanning electron microscopy (SEM) were fixed in the same manner as for TEM but without osmium tetroxide. Fixed embryos were removed

from their egg envelopes, dehydrated, critically point dried in freon, sputter-coated with gold-palladium, and examined with a scanning electron microscope.

VI. RESULTS

Behavior

Oil-exposed prey experiments: The SWAF concentrations to which the fry were exposed averaged 0.35 ppm and ranged from 0.13 to 0.62 ppm. Mortality of oil-exposed fish was greater than that of control fish during the exposure; approximately 5% compared to <1%.

Statistically significantly different numbers of oil-exposed prey than of control fish were consumed by predators. Of 280 exposed fry only 85 survived predation, whereas of 280 control fry, 161 survived predation. The hypothesis that the survival of prey chum fry exposed to the predators is independent of exposure to the SWAF of CICO was tested by chi-square analysis and rejected in every case: 24 h, $P<0.01$; 48 h, $P<0.01$; 72 h, $P<0.001$; 96 h, $P<0.05$; entire experiment, $P<<0.001$.

A greater number of oil-exposed fish than controls were consumed by predators at 24, 48, and 72 h. However, significantly fewer oil-exposed fish were consumed than controls at 96 h.

Oil-exposed predator experiments: Many of the coho predators exposed to the SWAF of CICO (X=343 ug/l) began to show behavioral modifications by the tenth day of exposure. In general, the oil-exposed predators appeared lethargic and showed little or no interest in the prey presented to them (these predators were designated as noneaters). However, one of the oil-exposed subgroups (number 6, designated as eaters) demonstrated none of these behavioral modifications and continued to feed at rates comparable with those of the unexposed predators. We have also observed in another experiment similar behavioral responses with eater and noneater groups in coho predators exposed to No. 2 fuel oil.

Figure 6 depicts the numbers of rainbow trout fry consumed during 10 min of exposure to the control or oil-exposed predators at three time intervals. An initial Yates X^2 evaluation showed a significant difference in prey consumption between the control and oil-exposed predator subgroups ($X^2 = 45.0$, $P \leq 0.005$). To determine within- and between-group differences with the small sample sizes, the data were subjected to predictive sample reuse analysis (Geisser and Eddy 1979). With a sample size of seven, this test is designed to select the correct model >95% of the time. The data were used to select one of two models; Model 1 (test populations were equivalent) or Model 2 (test populations were unequal). Model 1 ($=$) was selected on the basis of this test for the control observatons at 3, 10, and 17 days, as it was for the comparison tests of control and oil-exposed fish after 3 days of exposure. However, Model 2 ($\neq$) was selected for the control and oil-exposed fish comparison tests after 10 and 17 days of exposure. Model 1 ($=$) was also selected for the comparison of the 10 and 17 days oil-exposed predators. Even with the bias of Subgroup 6 toward the control values, our results clearly indicate that there was reduced predation by the adult coho salmon exposed to the SWAF of CICO for periods of 10 days or longer.

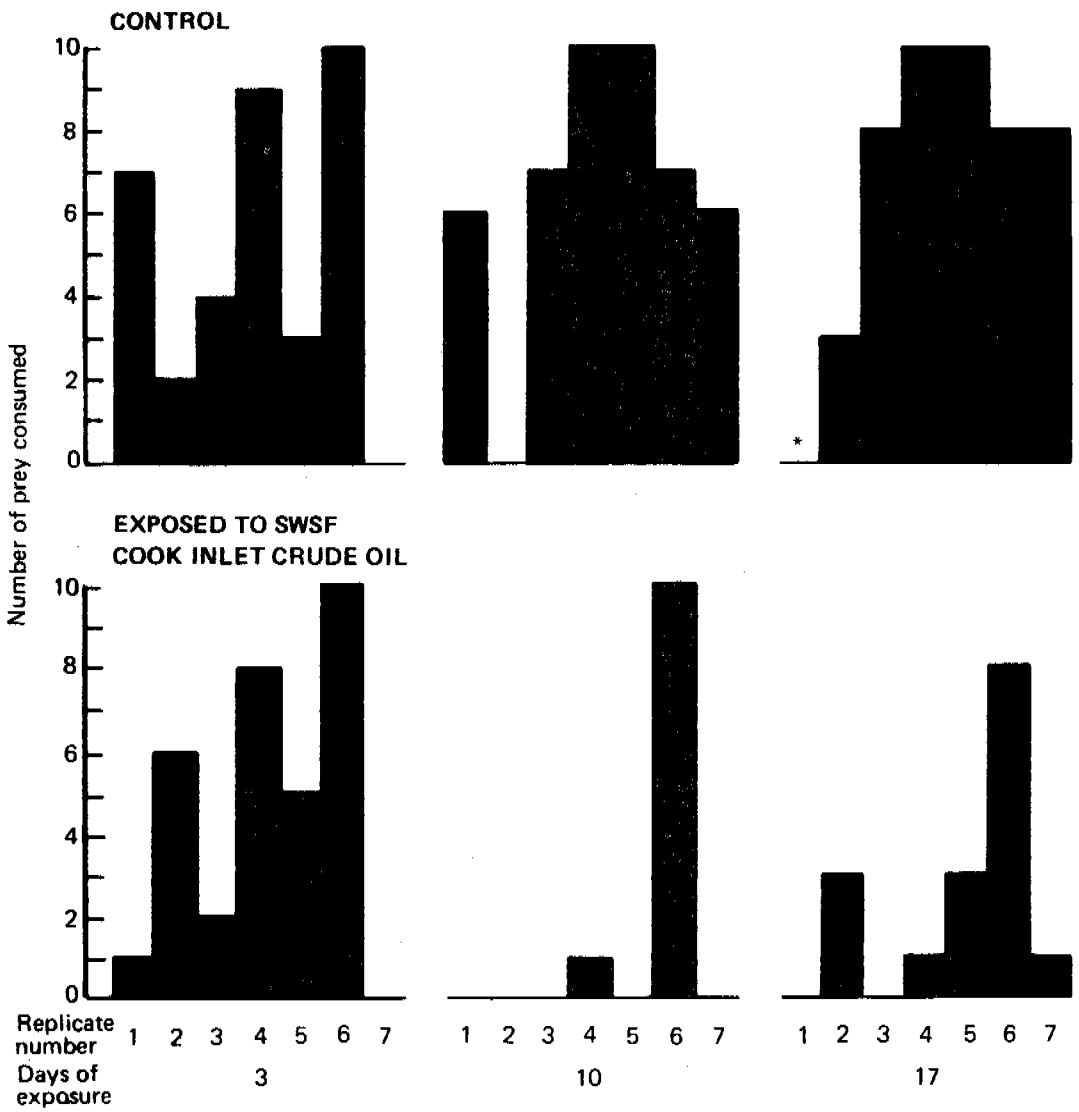

Figure 6. Ten rainbow trout fry were offered to each of the seven (3 fish) replicate control and oil-exposed coho salmon predator subgroups after 3, 10, and 17 days of oil exposure. The histograms represent the number of prey consumed by each of the control and oil-exposed subgroups at the three sampling periods. Each numbered subgroup represents the same three predators throughout the experiment, except for the exception for controls on day 17 noted in V. (Methods). (* = no data due to mortalities. Other absences of a bar represent no prey consumed.)

Concentrations of all hydrocarbons detected by GC in both liver and brain of the oil-exposed fish were higher in the eater subgroup than in the noneater group. All three fish from the eater subgroup (number 6) had higher levels of detected hydrocarbons in both liver and brain than did any of the three fish from the noneater subgroup (number 1). The highest concentrations detected were those of naphthalenic compounds (naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene). Biphenyl and 1, 2, 2, 4-tetramethylbenzene concentrations were also in excess of 100 ng/g in both livers and brains. All of the above hydrocarbons showed a high degree of parallelism between concentrations in the brains and livers of the oil-exposed fish. These results are similar to those found for naphthalene in rainbow trout by Collier et al. (1980). Benzothiophene and n-propylbenzene were also detected at levels in excess of 100 ng/g in the livers, but not in the brains, of the eater subgroup.

Chemistry

The concentrations of B[a]P in sediment did not change markedly over a period of ten days (Table 1); however, a trend of declining NPH concentrations was evident. Chromatographic analyses of B[a]P-derived radioactivity (^{3}H) from sediment and SAW revealed that B[a]P remained largely (>93%) in the form of parent hydrocarbon throughout the experiment.

Analyses of NPH-derived (^{14}C) radioactivity in sediment also showed that no more than 7% of the total radioactivity was present as total metabolites in sediment throughout the exposure (Table 1). For the first three days, the radioactivity (^{14}C) in the SAW was primarily due to NPH (Figure 7). However, samples of the water from days 4 to 10 contained an average 16% of the total radioactivity (^{14}C) in the form of metabolites of NPH (Table 1).

Radioactivity derived from both B[a]P and NPH was detected in tissues and body fluids of English sole within 24 h after the fish were placed on the sediment containing 1% PBCO with ^{3}H-B[a]P and ^{14}C-NPH (Table 2).

From 24 to 168 h of exposure, B[a]P-derived radioactivity increased significantly ($P<0.05$) in liver (5 fold), and bile (16 fold), whereas NPH-derived radioactivity decreased for all tissues and fluids except for gill and bile. An increase of NPH-derived radioactivity occurred in the bile (3 fold) and gill (4 fold) of these fish (Table 2).

Following 24 h of exposure to oil-contaminated sediment, fish placed (for 24 h) on "clean" sediment showed a significant decrease ($P<0.05$) in NPH-derived radioactivity in most tissues and fluids except for bile (Table 2); no significant change occurred in levels of B[a]P-derived radio-activity in most tissues. A seven-fold increase was observed, however, in B[a]P-derived radioactivity in bile and a small decline was observed for muscle.

Chromatographic analyses of B[a]P-derved radioactivity in bile (Figure 8) and liver (Figure 1) of fish at 24 h revealed that 2% or less of the total radioactivity in these samples was due to unconverted B[a]P metabolites (quinones, phenols, diols, and the more polar nonconjugates). TLC analyses, both before and after, enzymatic hydrolysis showed these nonconjugates to be present in the bile along with their glucuronide (51%) and sulfate (15%) conjugates (Table 3).

Table 1. Concentrations of B[a]P- and NPH-derived radioactivity in sediment, sediment-associated water[a] (SAW) and the water column.

Day	Event	Sediment		Sediment-associated Water (SAW)				Water Column			
		B[a]P	NPH	B[a]P		NPH		B[a]P		NPH	
				unfiltered	filtered	unfiltered	filtered	unfiltered	filtered	unfiltered	filtered
		pmoles of hydrocarbon equivalents/gm of sample									
1	Sediment was placed in aquarium	2200±400[97][b]	14000±3000[94]	--[c]	--	--	--	--	--	--	--
2	Begin 24 hr exposure	1700±300[96]	13000±2000[93]	190	10.6	960	238[93]	15	0.7	110	20
3	Remove 24 hr exposed fish for sampling and depuration experiment; and place 168 hr exposure fish on oil-contaminated sediment	1300±100	7200±600	250	7.4[97]	420	124[94]	4	0.3	72	38
4	Continue 168 hr exposure of fish to oil-contaminated sediment	2500±600[96]	3200±700[94]	190	7.0[96]	--	212[84]	3	--	130	--
7		990±150[93]	960±140[94]	--	6.0[96]	470	361[86]	--	--	--	--
9		1400±200	600±100[96]	130	6.1	--	--	2	0.1	--	36
10	Remove fish for sampling	2300±340[96]	2500±400[97]	--	14.2	--	317[81]	--	--	--	--

[a] Sediment and SAW samples were taken from 2 cm below the sediment/water interface as described in the text. SAW was decanted off from the sediment and analyzed before and after filtration. Three portions of sediment were analyzed to get average concentrations of tritium and carbon-14 in each sample. Concentration values (Mean ± S.D.) for sediment are based on dry weight. Samples of water at the air/water interface (water column) were also analyzed after filtration.

[b] Values in brackets are percentages of total radioactivity present as the parent hydrocarbon.

[c] Not done

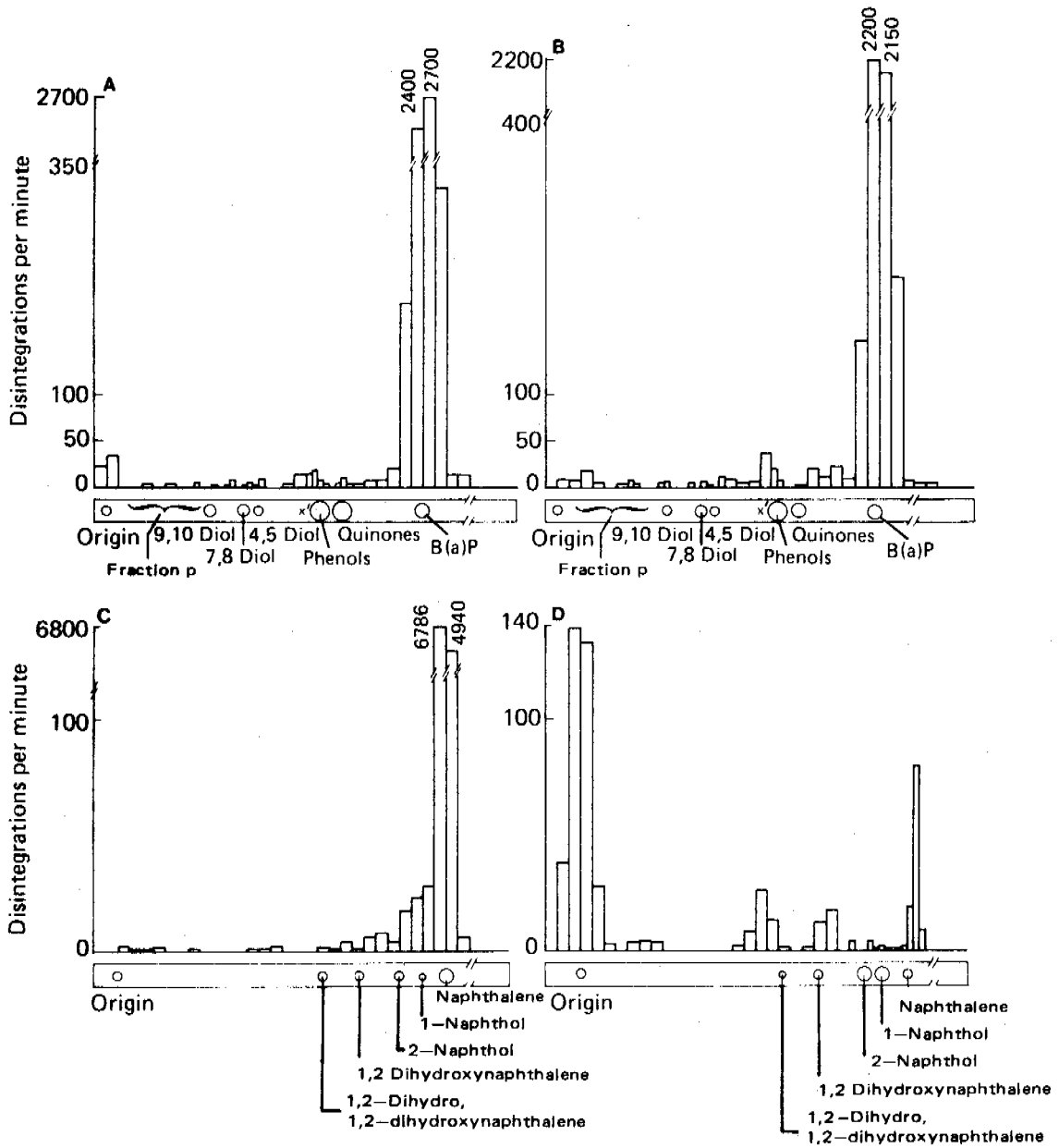

Figure 7. Representative chromatograms (TLC) of B[a]P-derived radioactivity (^{3}H) in (A) sediment and (B) SAW (solvent system A); NPH-derived radioactivity (^{14}C) in (C) sediment and (D) SAW (solvent system B). Abbreviations are P = metabolites with R_f values between origin and B[a]P-9, 10-dihydrodial; 9,10 diol = B[a]P 9,10-dihydrodriol; 7,8 diol = B[a]P 7,8-dihydrodiol; 4,5 diol = B[a]P 4,5-dihydrodiol; phenols = 3- and 9- hydroxy B[a]P.

Table 2. Hydrocarbon-derived radioactivity[a] in tissues of English sole exposed to ^{3}H-B[a]P and ^{14}C-NPH in sediment containing 1% Prudhoe Bay crude oil.

	Gill	Blood	Liver	Skin	Muscle	Bile	Stomach	Intestine
				24 hr Exposure				
B[a]P	0.390 ±0.049	0.39 ±0.11	0.35 ±0.13	0.140 ±0.067	0.044 ±0.001	8.3 ±3.1	0.22 ±0.14	0.380 ±0.040
NPH	14.4 ± 4.5	7.8 ±1.8	66 ±11	3.3 ±1.5	8.9 ±2.3	330 ±123	19.2 ± 4.9	20.4 ± 5.8
				168 hr Exposure				
B[a]P	0.55[b] ±0.10	0.67 ±0.38	1.60[b] ±0.60	0.140 ±0.016	0.036[b] ±0.003	130[b] ± 15	0.30 ±0.18	0.45 ±0.17
NPH	55 ±42	3.3[b] ±0.9	23[b] ± 8	1.5 ±1.0	1.2[b] ±0.4	980[b] ±300	5.2[b] ±0.9	6.1[b] ±2.8
				24 hr Depuration after 24 hr Exposure				
B[a]P	0.51[b] ±0.10	0.460 ±0.052	0.66 ±0.21	0.100 ±0.011	0.032[b] ±0.004	58 ±32	0.138 ±0.015	0.28 ±0.12
NPH	5.3 ±1.0	2.5[b] ±0.6	19[b] ± 9	2.9 ±2.3	2.3[b] ±0.9	1600[b] ± 600	3.4[b] ±1.0	6.3[b] ±0.6

[a] Values are expressed as nmoles of hydrocarbon equivalents per g of dry wt. (Mean ± S.D.)

[b] Significantly different ($p<0.05$) from corresponding values at 24 hr after the PAH exposure.

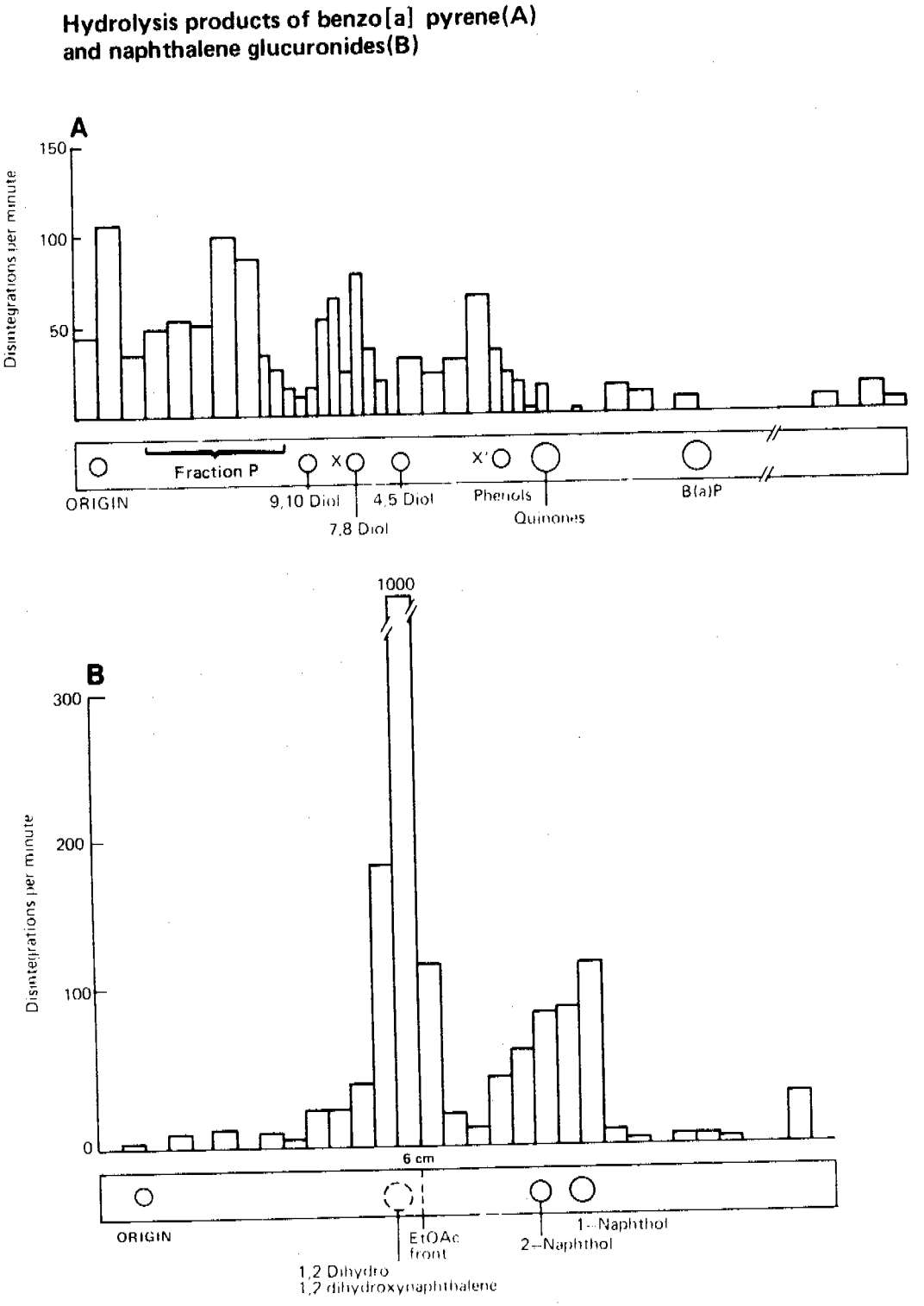

Figure 8. Thin-layer chromatograms of (A) metabolites of ^{3}H-B[a]P released after hydrolysis of aqueous phase of bile with β -glucuronidase (solvent system A) and (B) metabolites of ^{14}C-NPH released after hydrolysis of the glucuronide fraction (previously isolated by TLC using solvent system C) with β-glucuronidase (solvent system B) (for abbreviation see Figure 7).

Table 3. Proportions of B[a]P and its metabolites in liver and bile of English sole exposed simultaneously to ^{3}H-B[a]P and ^{14}C-NPH in sediment.

	Liver		Bile		
	Before enzymatic hydrolysis		Before enzymatic hydrolysis	After treatment of aqueous phase with β-glucuronidase	After treatment of aqueous phase with aryl sulfatase
	24 hr[a]	168 hr[b]	24 hr[b]		
	% of total radioactivity				
Uncoverted B[a]P	2	0.8±0.1	2±3	0.3±0.3	1.1±0.5
Ethyl acetate extractable metabolites	16	19±2.6	14±10	51±3.6	15±3.4
Radioactivity in aqueous phase	82[c]	80[c]±2.6	84±12[d]	49±3.6	84±2.8
	% of total metabolites in ethyl acetate				
Origin		40.8±2.0	42.7±2.9	12.5±1.9	16.9±4.6
Fraction P		20.6±3.8	13.2±1.6	27.9±9.4	12.7±5.8
9,10-diol		2.4±0.8	2.9±1.9	6.8±4.8	5.9±2.4
X		2.3±0.6	9.7±4.0	12.4±2.3	5.7±6.1
7,8-diol		5.9±0.4	4.5±0.3	12.2±4.2	8.3±1.6
4,5-diol		2.5±0.4	3.6±2.2	1.4±2.4	6.5±2.6
X'		--[e]	--	5.6±1.4	--
Phenols		5.8±1.5	1.9±1.6	8.6±1.0	10.2±1.7
Quinones		11.6±1.6	3.6±1.8	2.1±1.3	9.0±2.5
Unclassified		31.0±6.3	18.0±4.6	10.3±5.7	25.0±6.9

[a] Three livers were pooled and one set of values obtained.

[b] Each value is the mean of three individual values ± S.D.

[c] 41 and 35±2%, respectively, of the radioactivity in the aqueous phase at 24 and 168 hr was unextractable.

[d] Treatment with glucuronidase and sulfatase released a total of 66% of the radioactivity into ethyl acetate. The remaining radioactivity is assumed to be due to glutathione conjugates.

[e] not detected

Examination of the aqueous phase remaining after ethyl acetate extraction of liver showed that greater than one-third of the radioactivity in the aqueous phase was bound to cellular macromolecules in fish liver at both 24 and 168 h after the B[a]P exposure (Table 3).

Analyses of NPH-derived radioactivity in the liver of fish at 24 and 168 h revealed that 85% and 21%, respectively, of the total radioactivity was due to unconverted NPH (Table 4). Radioactivity (^{14}C) in bile was due primarily to metabolites (99%) of which glucuronides were the major component (88%). Hydrolysis of the glucuronide fraction revealed the presence of a large proportion (70%) of the 1,2-dihydro 1,2-dihydroxynaphthalene and a much smaller proportion (11%) of 1-naphthol (Figure 8, Table 4).

Pathology

The results of disease resistance tests with flatfish exposed to PBCO are shown in Table 5. The LD_{50} doses of *V. anguillarum* for juvenile starry flounder maintained for 2 and 6 wks on oil-impacted sediment did not differ substantially from those of the non-exposed controls. Also, there was no evidence of altered disease resistance in adult rock sole exposed for 2 wk to PBCO-impacted sediment.

The results of further testing of the effect of petroleum hydrocarbons on disease resistance of juvenile flatfish are shown in Table 6. English sole exposed to CICO-impacted sediment for 2 wk showed no demonstrable alteration in the ability to resist a laboratory bacterial challenge. Accompanying analyses of sediment, water, and liver tissue documented both the availability and uptake of petroleum hydrocarbons by the exposed fish.

Testing was also initiated to evaluate the effects of exposures to oil-dispersant on disease resistance. The results of preliminary assays suggested that simultaneous exposure of juvenile coho salmon to the oil-dispersant Corexit 9527 and virulent *V. anguillarum* induced a greater rate of infection and subsequent mortality than that which occurred in fish similarly exposed to bacteria only (Table 7).

Physiology

In experiments concerning the effects of petroleum hydrocarbons on embryos and larvae, PBCO was employed in tests with salmon and flatfish, and CICO in tests with smelt. A list of the major components in each crude oil as determined gravimetrically is given in Table 8. A more detailed composition of aliphatic and aromatic fractions of PBCO and CICO, as determined by glass-capillary GC, is given by MacLeod et al. (1980). The major differences which exist between these two oils (Table 8) are in the naphtha and saturate fractions. In our experiments the low molecular weight saturates through $n\text{-}C_{11}$ and most of the naptha fraction of both PBCO and CICO disappear from the SWAF in the first 30-48 h of weathering. The result of weathering on the hydrocarbon content of water-accommodated CICO is shown in Figures 9A-D. Generally, as weathering of CICO progresses, there is a rapid initial loss of both akyl-substituted benzenes and low molecular weight alkanes. With increasing time the naphthalenes disappear from the SWAF, and after over 500 h of weathering the dominant hydrocarbons are compounds less volatile than

Table 4. Proportions of NPH and its metabolites in liver and bile of English sole exposed simultaneously to ^{14}C-NPH and ^{3}H-B[a]P in sediment.

	Liver		Bile[a]		
	24 hr[b]	168 hr	Before enzymatic hydrolysis	After hydrolysis of glucuronide fraction[c] 168 hr	After hydrolysis of sulfate/glucoside fraction[d]
			% of total radioactivity		
Unconverted naphthalene	85	21±11	n.d.[e]	n.d.	n.d.
Total metabolites	15[f]	79±11[f]	100	100	100
			% of total metabolites		
Glucuronides			88	n.d.	n.a.[g]
Mercapturic acids			2	n.a.	n.a.
Sulfate/glucosides			3	n.a.	4.0
1,2 dihydrodiol			0.3	70.0	73.0
1-naphthol			n.d.	11.0	3.0
2-naphthol			n.d.	8.0	2.0
Unclassified			7	11.0	18.0

[a] Three samples of bile from fish exposed for 168 hr were pooled to get sufficient radioactivity for quantitation of metabolite classes.

[b] There was not sufficient radioactivity in metabolite fraction to allow quantitation of individual classes.

[c] Glucuronide fraction was isolated by TLC of the bile extract and then hydrolyzed with β-glucuronidase.

[d] Sulfate/glucoside fraction was isolated and then treated with aryl sulfatase.

[e] not detected

[f] This value includes one-third of the total radioactivity which was not extractable by solvents.

[g] not applicable

Table 5. LD_{50} values for oil-exposed and control starry flounder and rock sole challenged with virulent *Vibrio anguillarum*.

	LD_{50} dose of *V. anguillarum*	
	2 week exposure	6 week exposure
Starry flounder		
oil-exposed (1,800 ppm in sediment)	1.1×10^7	1.2×10^7
control	5.6×10^7	6.0×10^7
Rock sole		
oil-exposed (2,500 ppm in sediment)	1.5×10^7	N.T.[a]
control	3.8×10^7	N.T.

[a] N.T. = not tested

Table 6. Result of disease resistance tests on Cook Inlet crude oil-exposed and non-exposed juvenile English sole and related petroleum hydrocarbon analyses.

Sediment condition during fish exposure	Duration of exposure (h)	LD_{50}[a]	Total petroleum hydrocarbons (ppm)		
			Sediment	Water[b]	Liver Tissue
Oil-contaminated	0	----	461.0	2,900	----
Control	0	----	0.3	0.002	----
Oil-contaminated	24	7.0×10^5	295.0	0.180	4.2
Control	24	1.2×10^6	0.3	0.004	1.7
Oil-contaminated	168	2.9×10^6	282.0	0.037	8.0
Control	168	4.0×10^6	0.3	0.009	1.0

[a] Number of microorganisms that killed 50% of the test animals; the dashes indicate not tested.

[b] Water was collected 2 cm above the sediment-water interface.

Table 7. Percent mortality among juvenile coho salmon exposed for 30 min to various concentrations of V. anguillarum and 30 ppm Corexit 9527, alone and in combination. Mortality was monitored for 10 days after exposure.

Treatment	Mortality (%)
10^5 bacteria + Corexit	27 (8/30)[a/]
10^5 bacteria only	3 (1/30)
10^4 bacteria + Corexit	17 (5/30)
10^4 bacteria only	3 (1/30)
Corexit only	0 (0/30)

a/ Significantly different than control of bacteria only (P=0.05)

Table 8. Comparison of major petroleum fractions of fresh PBCO and CICO. Analysis determined gravimetrically, and each fraction expressed as percent of total.

Fraction	CICO[b]	PBCO[c]
Naphtha[a]	27.9	18.6
Saturates	39.5	48.9
Aromatics	19.7	19.4
Polars	11.5	13.8
Insolubles	2.3	1.4
	100.9	102.1

[a] Naphtha fraction includes saturates and aromatics boiling at less than 210°C.

[b] Personal communication, Paul Robisch, NWAFC.

[c] Malins et al. (1978).

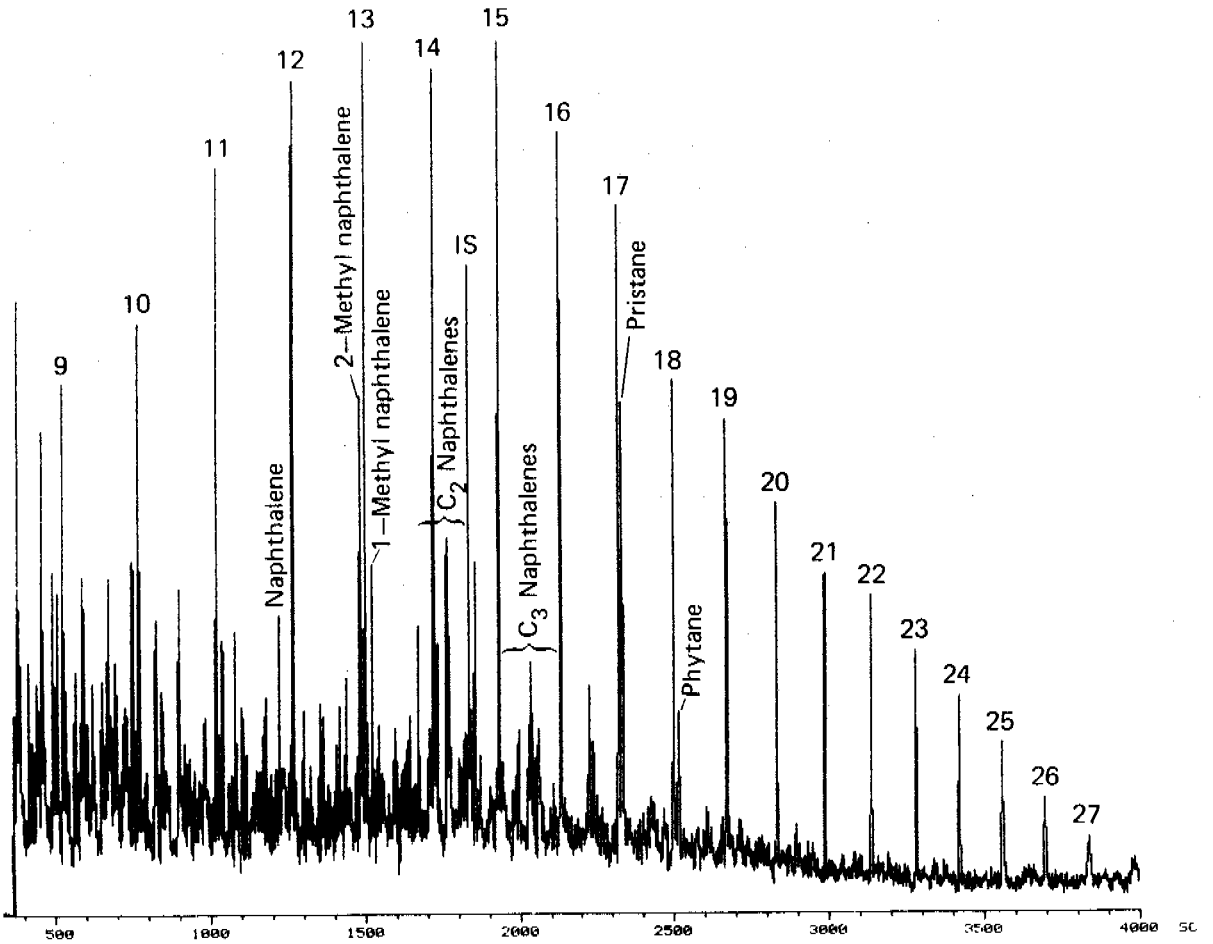

Figure 9A. Gas chromatogram of fresh Cook Inlet crude oil (CICO), normalized to n-pentadecane.

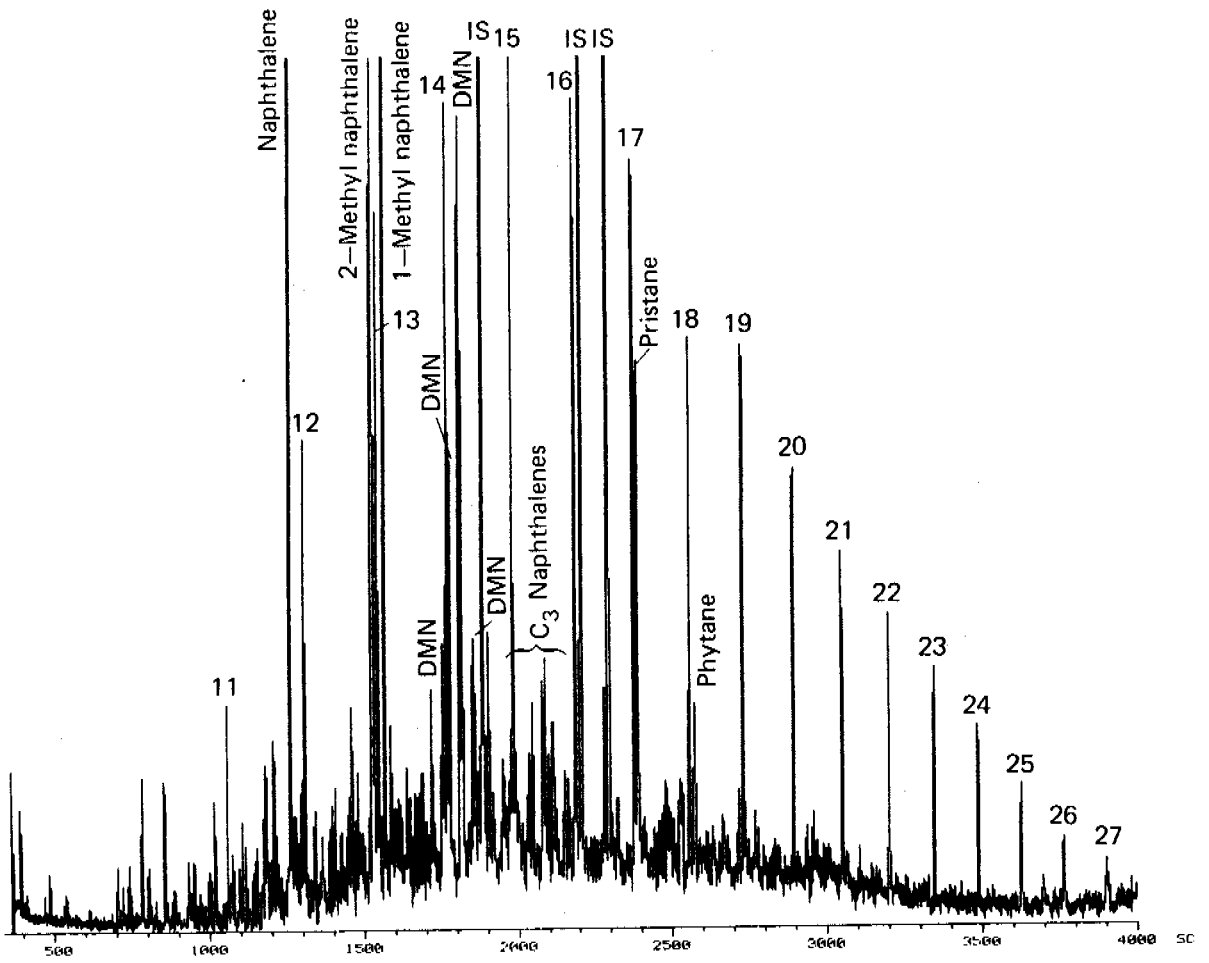

Figure 9B. Gas chromatogram of salt water-accommodated CICO, normalized to n-pentadecane; weathered 48 h.

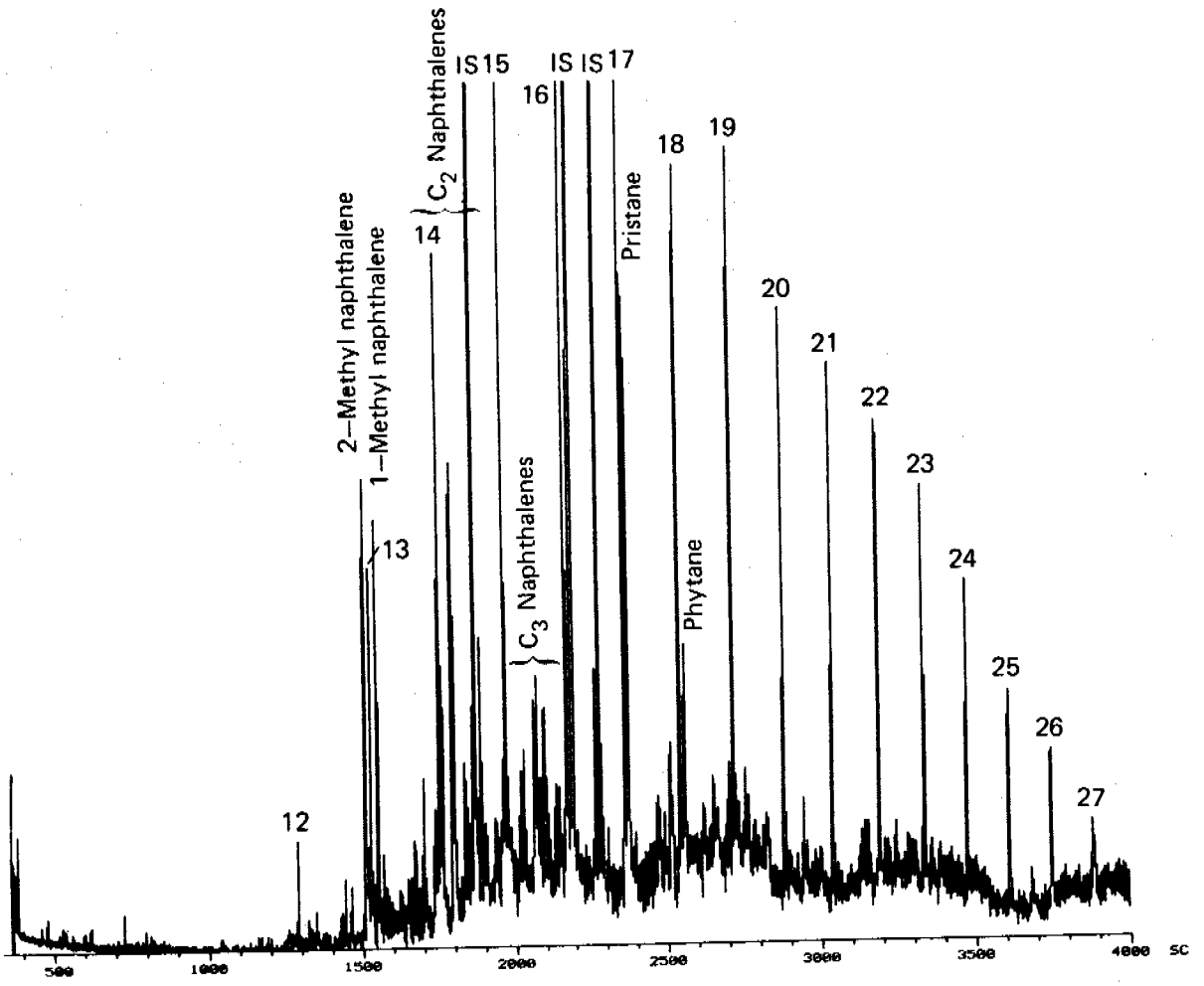

Figure 9C. Gas chromatogram of salt water-accommodated CICO, normalized to n-pentadecane; weathered 192 h.

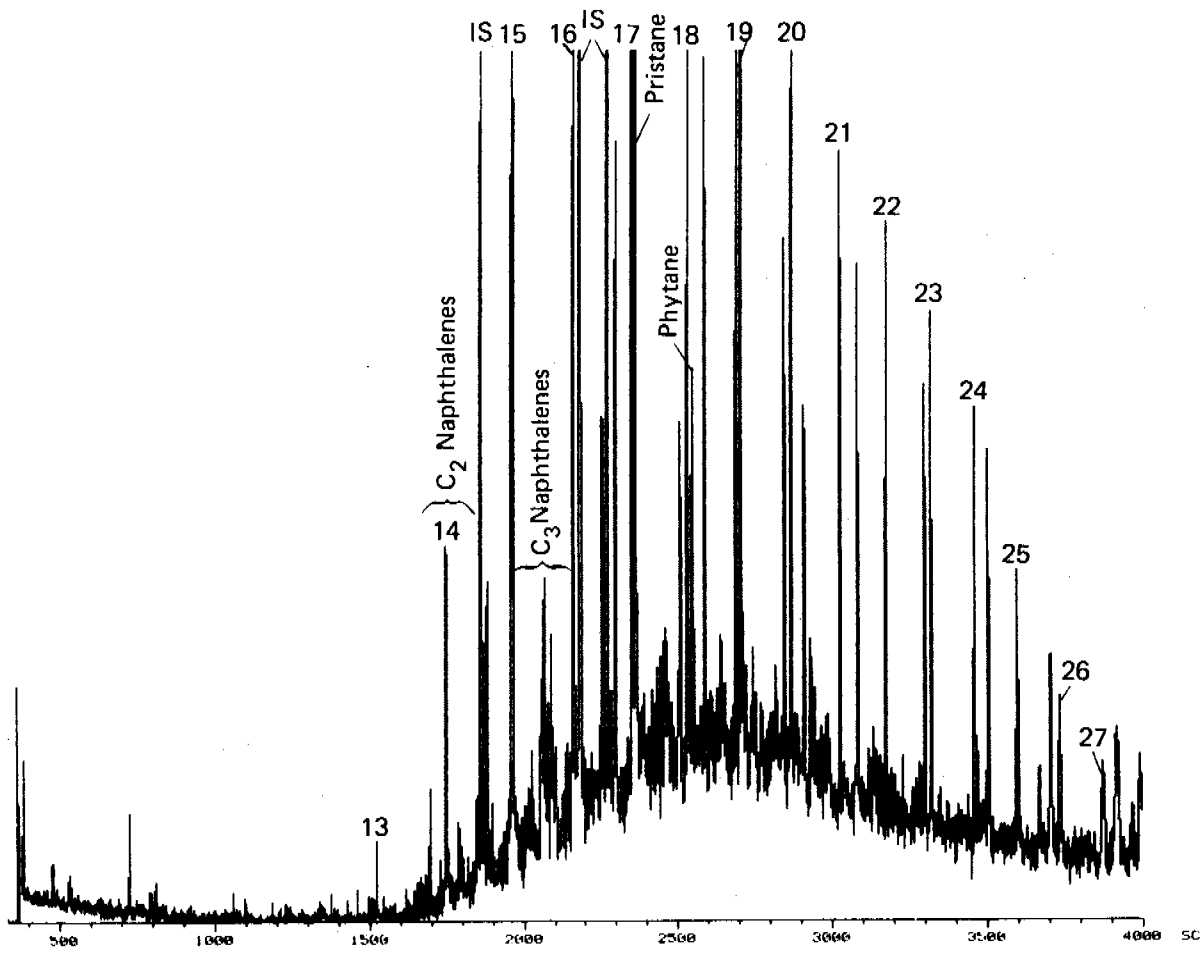

Figure 9D. Gas chromatogram of salt water-accommodated CICO, normalized to n-pentadecane; weathered 528 h.

n-C_{14}. PBCO has the same general pattern of weathering as CICO with the more volatile benzenes and naphthalene compounds disappearing with time (Figure 10).

Chum salmon embryos and alevins were exposed to the SWAF of weathered PBCO for an average of 2.8 h per day, 4 days a week. Each week, for 16 weeks, the oil weathering process and exposure regime was repeated. The concentration of petroleum hydrocarbons remained relatively constant for the first 3 days of exposure and then dropped on day 4 (Table 9).

Flatfish embryos were exposed to the SWAF of PBCO beginning one day after fertilization at which time the oil had weathered 48 h. A water sample taken from the rearing funnel containing embryos 72 h later (mid-incubation) indicated almost total loss of hydrocarbons (Figure 11). At mid-incubation the SWAF was renewed with a portion of the original SWAF. The hydrocarbon concentrations in the water at the start of oil exposure, half-way through incubation, and at time of hatching, are given in Table 10.

Experiments in which surf smelt embryos were exposed to the SWAF of weathered CICO were replicated, and the hydrocarbon concentrations found in water samples taken throughout each of the two tests are shown in Figure 12. Eggs collected in November were first exposed to the SWAF 4 days after fertilization. The undiluted hydrocarbon concentration, as measured by GC analysis, averaged 324 ($\pm$125 SD) ppb for the first 7 days of exposure (4 to 10 days post-fertilization), and then dropped sharply to a relatively constant average concentration of 77 ($\pm$64 SD) ppb. The overall undiluted hydrocarbon concentration throughout the 23 days of embryo exposure averaged 173 ($\pm$152 SD) ppb. The SWAF was also mixed directly with uncontaminated seawater to give diluted hydrocarbon concentrations, calculated from water flow as 53% and 25% of the original SWAF. This results in estimated average hydrocarbon concentrations for these dilutions of 92 and 43 ppb, respectively. Chemcal analysis of 6 water samples showed an average hydrocarbon concentration of 66$\pm$8 ppb for the 53% dilution, and 38$\pm$30 ppb for the 25% dilution. Dilution concentrations related to effects presented in the text are based on calculated values. The water flows measured for dilution of the original SWAF are considered accurate, and chemical analysis of the undiluted SWAF is more comprehensive than the few samples analyzed from diluted waters.

For surf smelt exposed to weathered CICO in December the general trend of hydrocarbon concentration with time is similar to the November test (see smoothed data, Figure 12). After the oil had weathered for 8 days (12 days post-fertilization) the SWAF concentration in December dropped from an average of 298$\pm$148 ppb to an average concentration of 56$\pm$32 ppb. The overall hydrocarbon concentration for the December test was 113$\pm$128. The calculated dilutions of the orignial SWAF for the December test were 48% and 23% (54 and 26 ppb, respectively). Chemical analysis of 4 water samples taken from these dilution resulted in average hydrocarbon concentrations of 78$\pm$62 and 47$\pm$37 ppb, respectively.

Mortality of chum salmon embryos from oil-treated and control groups was evaluated at time of hatching (Table 11). Embryos treated for the full duration of development of 75 days (122 h exposure) showed a significant increase in mortality over controls ($P=0.01$).

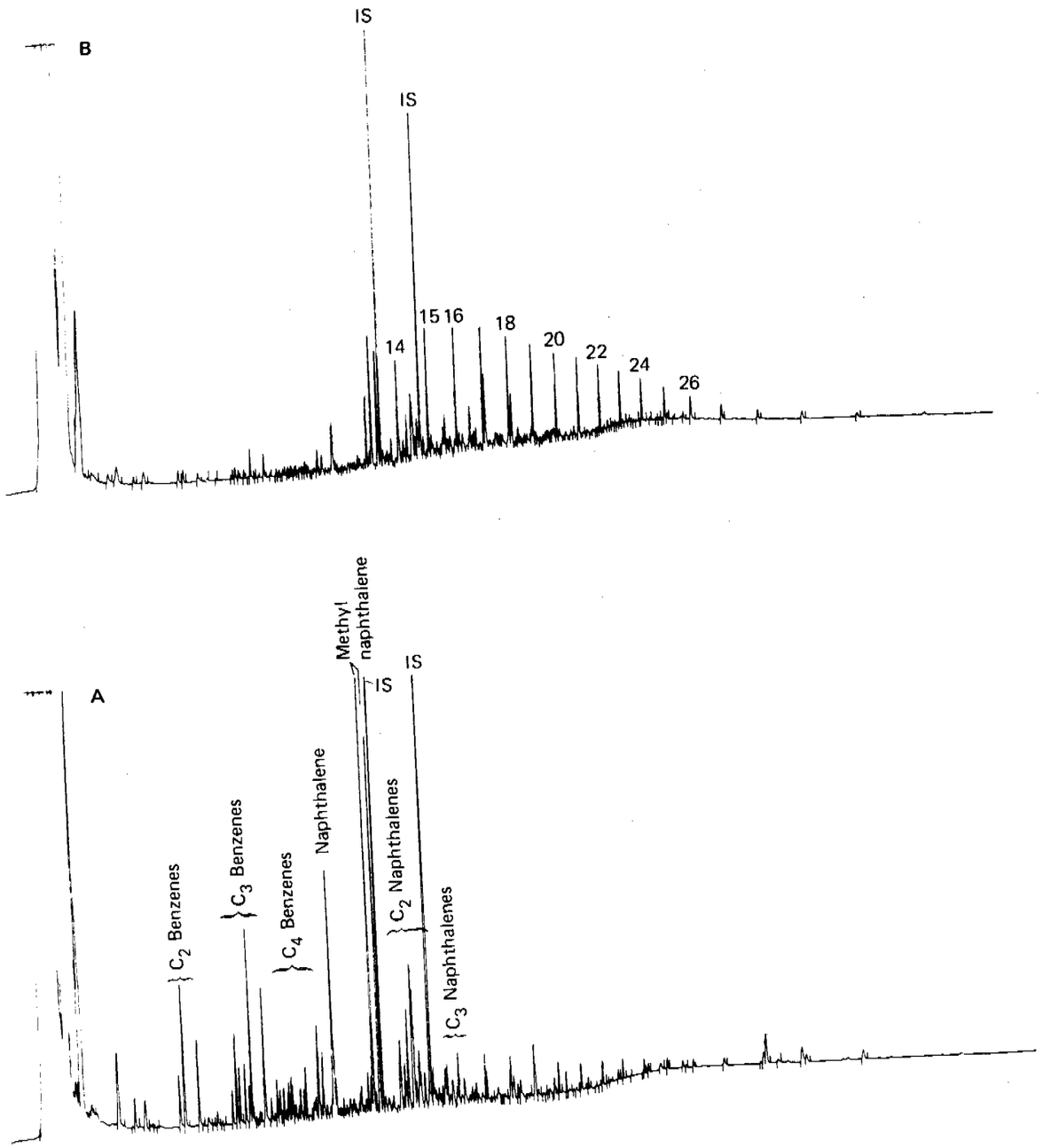

Figure 10. Gas chromatograms of salt water accommodated Prudhoe Bay crude oil (PBCO) A. After weathering for 30 h. and B. of salt water accommodated after weathering for 54 h.

Table 9. Petroleum hydrocarbon concentration in the SWAF of PBCO during exposure of chum salmon embryos and alevins.

Day of exposure	Hours oil weathered	Number of samples	Total hydrocarbon concentration in ppb (± SD)
1	30	10	731±538
2	54	10	421±477
3	78	9	445±510
4	102	5	68±31
	Average	34	467±500

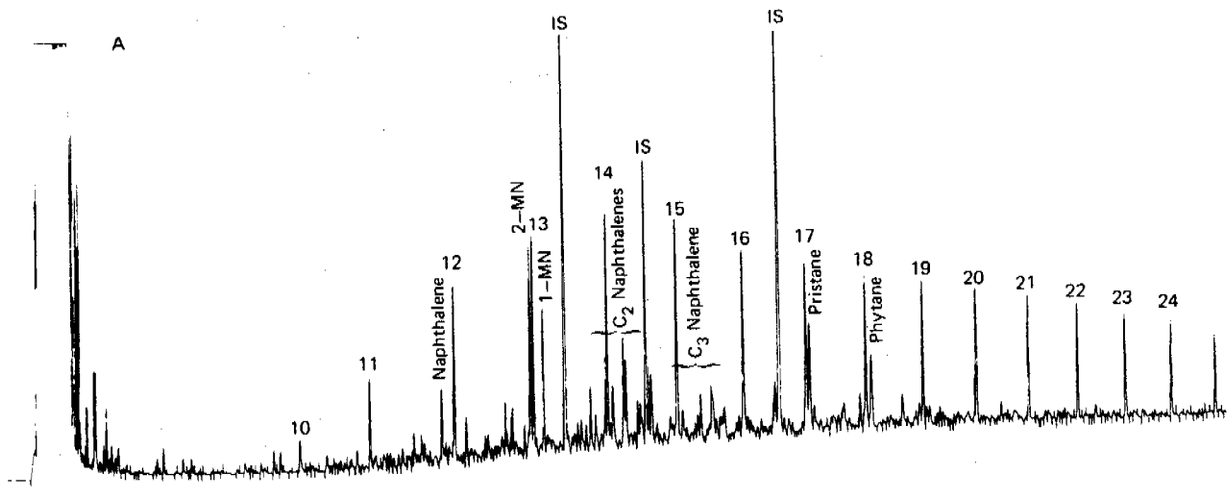

Figure 11A. Chromatogram of the SWAF of PBCO used in exposure of flatfish embryos. 48 h weathered PBCO sampled at initiation of experiment.

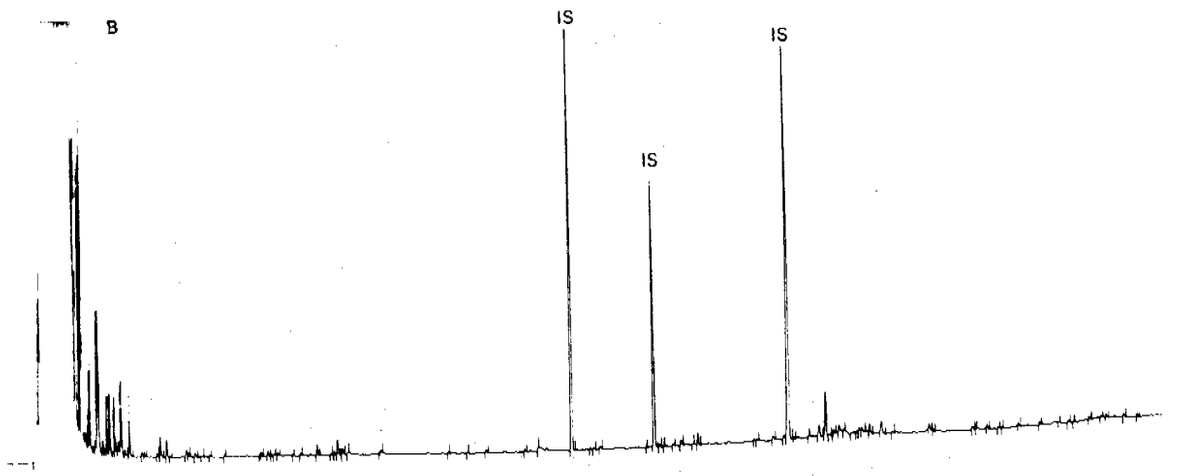

Figure 11B. Chromatogram of the SWAF of PBCO used in exposure of flatfish embryos. Same SWAF as 11A, sampled half-way through incubation 72 h later.

Table 10. Concentration of petroleum hydrocarbons in the SWAF of weathered PBCO to which English and sand sole embryos were exposed as related to days post-fertilization.

Species and treatment	Number of samples	Total hydrocarbon concentration (ppb) Days post-fertilization 1	4	8	$\bar{X}$
Sand sole					
Control	3	2	4	13	6
Test 1	4	430	25(193)[a]	11	164
Test 2	4	200	9(91)[a]	15	79
English sole					
Control	4	4	10(2)[a]	4	5
Test	4	278	60(184)[a]	8	133

[a] Hydrocarbon concentrations of replacement portions of original SWAF, or of control water, are in parentheses.

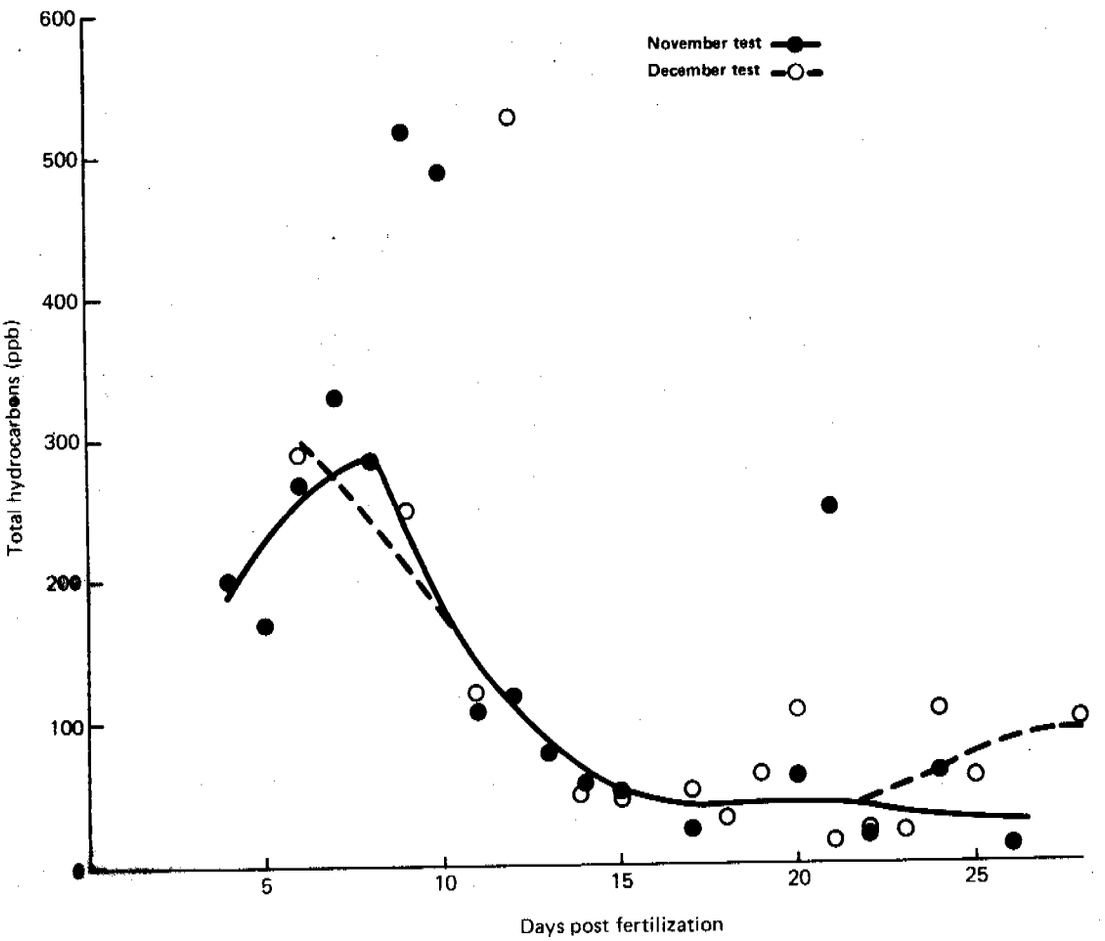

Figure 12. Total petroleum hydrocarbons in the SWAF of weathered CICO as related to time of surf smelt embryo exposure following fertilization. Circles represent hydrocarbon concentration in water samples collected from the undiluted SWAF during each test (Nov. and Dec.). Corresponding solid and dashed lines represent smoothed data calculated by method of Cleveland (1979).

Table 11. Percent mortality of chum salmon embryos at time of hatching (75 days post-fertilization), and percent of viable eggs hatched on days 76 and 82.

Group(s)[a] (exposure age in days)	Total exposure (h)	Percent[b] mortality (±range)	Cumulative % hatch of viable eggs on days 76	82
1,3,5(control)	0	24(±10)	15	70
2 (14-26)	26	28	60	95
4 (1-26)	46	25	95	99
6 (26-75)	77	19	80	98
7,8 (1-75)	122	42(±21)	50	96

[a] See Figure 4.

[b] Data adjusted for 2.6% of eggs which were unfertilized.

Table 12. Gross morphological abnormalities observed in chum salmon alevins 12 days after hatching (87 days post-fertilization).

Group	Exposure period (days post-fert.)	Hours exposed	Sample size	% Abnormalities[a]		
				Yolk sac malformation	Cephalic dome	Kyphosis
1	(Control)	0	204	0	0	0
2	(14-26)	26	15	0	9	0
3	(1-26)	46	19	16	16	0
4	(75-87)	21	10	10	33	0
5	(26-75)	77	15	0	33	0
6	(1-75)	122	6	33	33	0
8	(1-87)	143	16	61	44	11

[a] Pictorial description of yolk sac and cephalic abnormalities are shown in the 1980 OCSEAP Annual Report (Malins et al. 1980b).

Table 13. Fin ray counts of chum salmon alevins 16 days after hatching (91 days post-fertilization), and corresponding exposure conditions.

Group	Exposure period (days post-fert.)	Hours exposed	$\bar{X}$ Number of fin rays with calcification[a]		
			Caudal	Anal	Dorsal
1	(Control)	0	17	7.8	8.3
2	(14-26)	26	18.7	11.2	9.2
4	(1-26)	46	12.5	8.7	6.3
5	(75-91)	29	10.0	1.2	0
6	(26-75)	77	20.1	12.7	10.9
7	(1-75)	122	15.0	11.7	6.0
8	(1-91)	151	5.0	3.5	0

[a] Following staining with alizarin red S.

Effects of the SWAF of weathered PBCO on English and sand sole embryos and larvae are given in Table 14, and summarized in Figure 14. (For nomenclature of categories, see Section V, Sources, Methods, and Rationale of Data Collection).

The single test with English sole eggs indicates that exposure to an average hydrocarbon concentration of 133 ppb results in a high percentage hatching, but all larvae were either abnormal or dead.

In repeated control tests with sand sole, an average of 90% (±2%, range) of the eggs hatched into normal larvae. At a SWAF concentration of 164 ppb embryos developed and hatched, however, two-thirds of the larvae were deformed; the most common abnormality was scoliosis. Severe secondary abnormalities were observed in some larvae: shortened length, pigment scattered rather than in patches, finfold deformed, incomplete digestive tracts, and inactive larvae lying on the bottom of the containers. Exposure to the SWAF at a concentration of 79 ppb resulted in an average of 79% (±2%, range) normal larvae and only 10% (±1%) deformed larvae.

An accounting of all surf smelt embryos and larvae in the two replicate experiments was conducted when hatching of live control embryos was apparently complete. These data are presented in Tables 15 and 16, along with the hydrocarbon exposure concentrations. A majority of the eggs were unaccounted for at the end of both experiments, and as no intact embryos could be lost from the incubation baskets, this loss is attributed to early embryo death and subsequent embryo disintegration. In controls this averaged 49% (±3%, range).

In both experiments the control embryos and those exposed to the lowest hydrocarbon concentration produced an equal number of live larvae (controls 44±4%; the 26 and 43 ppb exposure groups 43±1%). However, eggs exposed to the two higher hydrocarbon concentrations produced few live larvae with most dying in the embryonic stage (89±9% as dead embryos or disintegrated eggs, as compared with 52±3% for controls).

Cumulative hatching rates for both experiments are shown in Figures 15 and 16. Control larvae in the December experiment hatched approximately 6 days later than those in the November experiment, but the total percent hatching were similar. At an average hydrocarbon concentration of 26 ppb (December experiment) there was an apparent acceleration in hatching similar to that observed for the chum embryos exposed to the SWAF of weathered PBCO.

Samples of developing embryos were taken at two intervals during the December experiment. On day 14 post-fertilization, mortality in control embryos was greater than in those exposed to oil, however, in embryos sampled one week later (approximately two-thirds of the way through incubation), this was reversed. An appropriate statistical model (Geisser and Eddy 1979) indicated that the percent abnormal and dead embryos in the control group and those exposed to 26 ppb petroleum hydrocarbons were similar, but differed from embryos exposed to 54 and 113 ppb. These latter two in turn differed from each other.

A comprehensive cytopathological study was conducted on surf smelt embryos sampled from the two higher oil exposure concentrations of the

Table 14. Test conditions and results of English and sand sole embryos exposed to the SWAF of weathered PBCO. Data were collected at time of hatching (end of 8 day exposure) and are reported in percent of total eggs introduced.

Species and hydrocarbon concentration (ppb)	Eggs		Embryo		Larvae			
	Number of eggs introduced	Non-developed (%)	Normal (%)	Abnormal or dead (%)	Normal (%)	Abnormal (%)	Grossly abnormal (%)	Dead (%)
English sole								
Control	348	13.5	0.9	0	74.7	5.2	0	5.7
133	372	22.0	0	3.0	0	0	64.5	10.5
Sand sole								
Control[a]	1469	7.4	0.1	0	89.7	1.8	0	1.0
164	365	15.6	0	4.9	0	43.6	22.2	13.7
79[a]	968	5.5	0.4	0.2	78.9	8.9	1.6	4.5

[a] Tests repeated and data pooled.

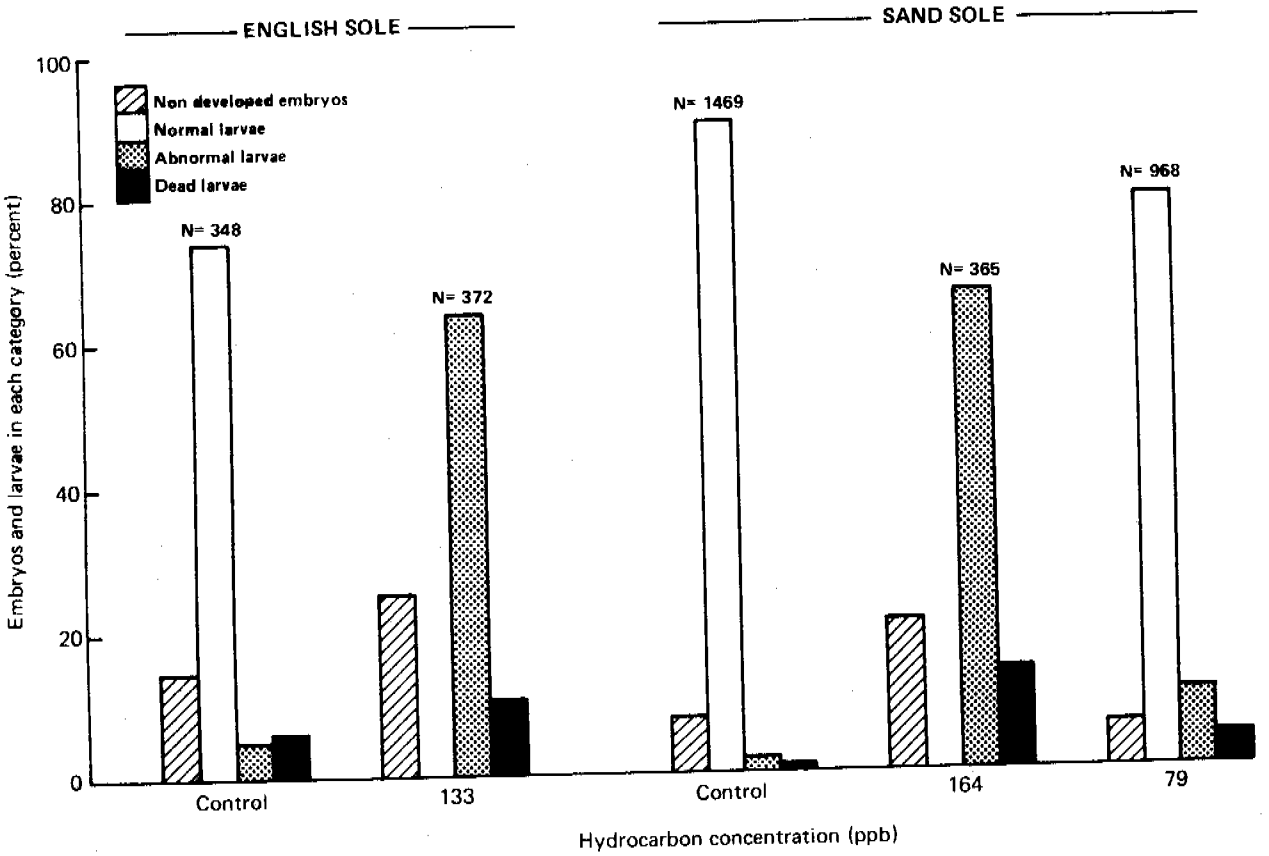

Figure 14. Hydrocarbon concentration of SWAF of PBCO and percent English and sand sole in each category at termination of tests. Data from Table 14 (% eggs and embryos, and abnormal larvae categories combined).

Table 15. Results of November experiment with surf smelt embryos exposed to the SWAF of weathered CICO for a total of 57 h ($\bar{X}$=2.5 h/day). Data compiled at completion of hatching in controls, and reported in percent of total embryos introduced.

Hydrocarbon concentration (ppb)	Embryos				Larvae	
	Initial no. (N)	Alive (%)	Dead (%)	Disintegrated (%)	Alive[a] (%)	Dead (%)
Control	441	3.2	3.6	51.9	40.6	0.7
43	427	0	3.0	52.1	42.2	2.7
92	419	9.6	13.8	66.1	9.1	1.4
173	425	1.7	47.1	50.5	0.7	0

[a] Live larvae were collected daily immediately after hatching, and the percent represented is cumulative.

Table 16. Results of December experiment with surf smelt embryos exposed to the SWAF of weathered CICO for a total of 63 h ($\bar{X}$=2.9 h/day). Data compiled at completion of hatching in controls, and reported in percent of total embryos introduced.

Hydrocarbon concentration (ppb)	Embryos				Larvae	
	Initial No. (N)	Alive (%)	Dead (%)	Disintegrated (%)	Alive[a] (%)	Dead (%)
Control	461	0	2.2	46.2	47.7	3.9
26	458	0.7	12.9	28.6	43.2	14.6
54	455	4.0	12.8	71.1	5.9	6.2
113	446	0.7	4.9	86.8	4.0	3.6

[a] Live larvae were collected daily and held in uncontaminated seawater to evaluate post-exposure survival; the percent represented is cumulative.

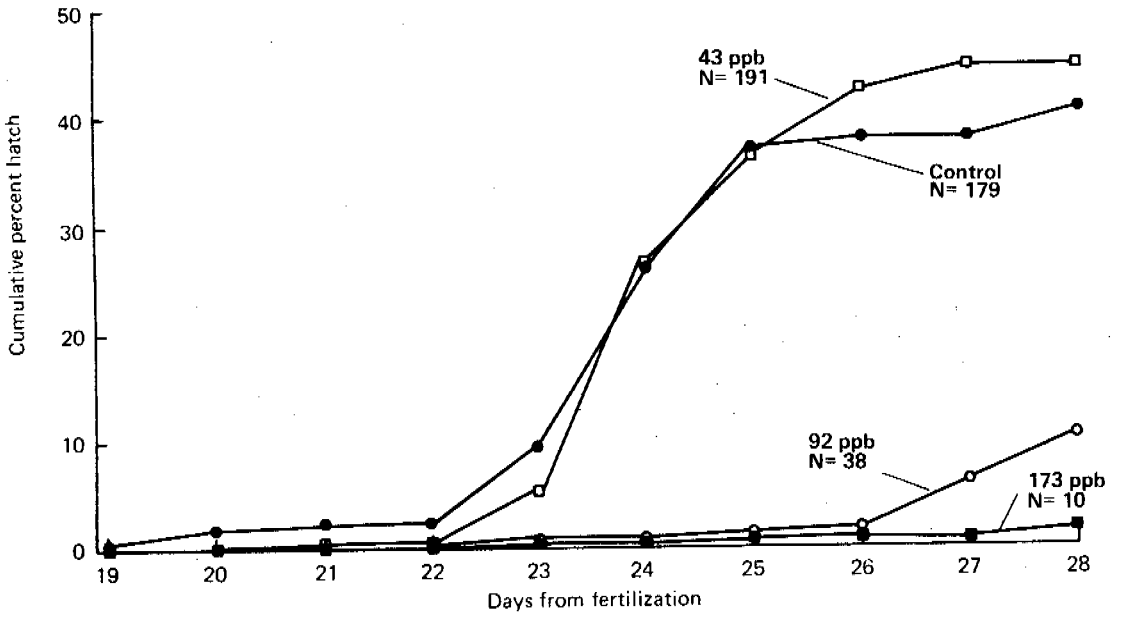

Figure 15. Cumulative percent hatching by days post-fertilization for surf smelt embryos exposed to 3 different concentrations of the SWAF of weathered CICO for a total of 57 h (November experiment). Average hydrocarbon concentration for undiluted SWAF (173 ppb) was determined by GC analysis. The two lower SWAF concentrations (92 and 43 ppb) were calculated from proportional dilutions of the undiluted SWAF.

FIGURE LEGENDS

Figures 17-22. Light micrographs of the eye and brain from control and exposed (113 ppb CICO) surf smelt embryos. (X 2,000).

(g) ganglion cell
(h) horizontal cell
(i) inner segment of receptor cell
(ipl) internal plexiform layer
(o) outer segment of receptor cell
(p) pigment epithelium
(rn) retinal neuron

Figure 17. Normal eye in 28-day-old control embryo.

Figure 18. Brain tissue of same animal as Figure 17 with normal neuronal cells.

Figure 19. Dark staining necrotic neurons (arrows) in the eye of 21-day-old exposed embryo. Receptor cells appear normal but the melanosomes of the pigment epithelium are more dispersed than in the controls.

Figure 20. Necrotic cells (arrows) in brain of same animal as Figure 19.

Figure 21. Numerous necrotic cells in eye of 28-day-old exposed embryo. Retinal cells are hypertrophied and vacuolated (arrow).

Figure 22. Brain from same animal as Figure 21 with many necrotic neurons.

Figures 23-27. Transmission electron micrographs and diagram of the eye from 28-day-old smelt embryos.

(e) ellipsoid region of inner segment of receptor cell
(h) horizontal cell
(m) myoid region of inner segment of receptor cell
(n) nucleus of receptor cell
(o) outer segment of receptor cell
(rn) retinal neuron
(s) synaptic junctional complex

Figure 23. Retinal cells of an unexposed embryo (X 5,000). Insert: enlargement of a synaptic junction (X 18,000).

Figure 24. Exposed embryo (113 ppb CICO). Vesiculation is evident in the myoid regions (*) of the receptor cells and necrotic neurons are present (arrow). (X 5,000). Insert: synaptic junction appears normal (X 18,000).

December experiment. This section will focus on pathological changes in surf smelt followed by changes observed in embryos and larvae of sand sole and chum slamon.

Sections of entire control (Figures 17, 18, 23, 26) and oil-exposed (Figures 19, 20, 21, 22, 24, 25, 27) surf smelt embryos were examined with light and electron microscopy, and all tissues appeared normal except for necrotic cells in the brain and eye tissues of embryos exposed to 54 and 113 ppb of the SWAF of weathered CICO (Table 17). In both the brain and eye of oil-exposed embryos necrotic neurons appeared intensely blue-colored after application of Richardson's stain. These neurons also stained intensely with PAS reagents (which stain carbohydrates), whereas no PAS reaction was evident in neuronal tissues of control embryos. Ultrastructurally, necrotic neurons were easily identified by their electron-dense nuclei and cytoplasm. In some nuclei, the heterochromatin was clumped in a central mass; in others, the heterochromatin was also condensed but distributed along the inside of the nuclear membrane. The cytoplasm of many of the necrotic cells was uniformly granular. However, some had clusters of autolysosomes which contained fragments of organelles such as mitochondria (Figure 25).

The retinal receptor cells of smelt embryos sampled 21 days after fertilization were normal, but the retinal receptors in 75% to 80% of the embryos sampled 6 days later had lesions localized in the ellipsoid and myoid regions of the inner segment. The most severe damage was in the myoid region (Figure 24, 27). In contrast, the tightly stacked membranes of the outer segment were normal or only slightly disorganized, and the basal portion of the cell, which contains the nucleus, also appeared normal. In some embryos, the cytoplasm of the inner segment was filled with clear vacuoles. In damaged receptor cells, the mitochondria were in various stages of lysis. Commonly, the outer mitochondrial membrane was intact but the cristae were disrupted and vacuous areas were evident in the matrix.

The olfactory area of 35 surf smelt embryos from the November experiment was examined by SEM for condition of the cilia. Of 16 embryos exposed to hydrocarbon concentrations of 92 and 173 ppb, the olfactory cilia in 50% of the embryos were reduced in size and numbers (Hawkes 1980); 8 embryos exposed to 42 ppb total hydrocarbons had normal appearing cilia, comparable to 11 controls.

SEM examination of 5 sand sole larvae exposed to 164 ppb of the SWAF of weathered PBCO during embryogenesis revealed 4 in which the length of the olfactory cilia was severely shortened and the epidermal microrigdes were absent from the surface of keratinocytes surrounding the olfactory epithelium. In addition, some of these keratinocytes were rounded and protruded from the skin surface, a possible indication of cellular hypertrophy.

TEM examination of sections from 8 sand sole larvae (4 exposed to 164 ppb as embryos, and 4 controls) revealed, in oil-exposed larvae, ultrastructural changes in the mitochondria of epidermal cells. Although the sample size was too small to definitely relate observed change to oil exposure, the abnormal mitochondria showed classic hydropic changes, a reduction in cristae and a

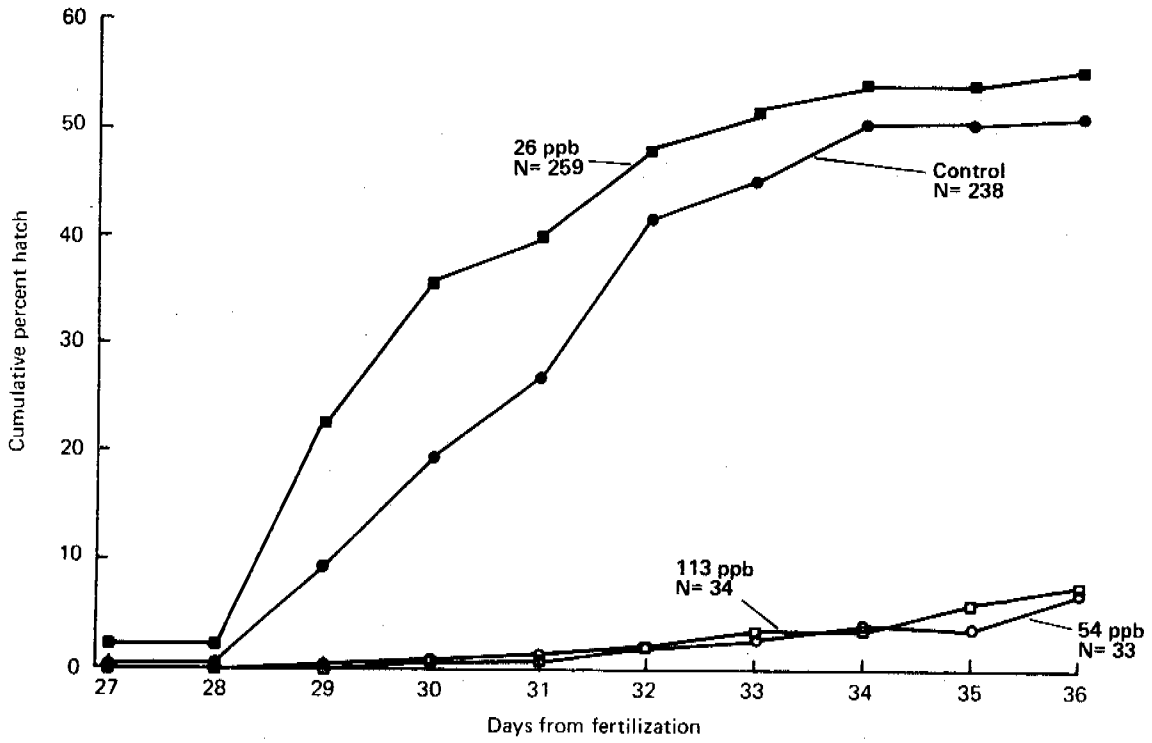

Figure 16. Cumulative percent hatching by days post-fertilization for surf smelt embryos exposed to 3 different concentrations of the SWAF of weathered CICO for a total of 63 h (December experiment). Average hydrocarbon concentration for undiluted SWAF (113 ppb) was determined by GC analysis. The two lower SWAF concentrations (54 and 26 ppb) were calculated from proportional dilutions of the undiluted SWAF.

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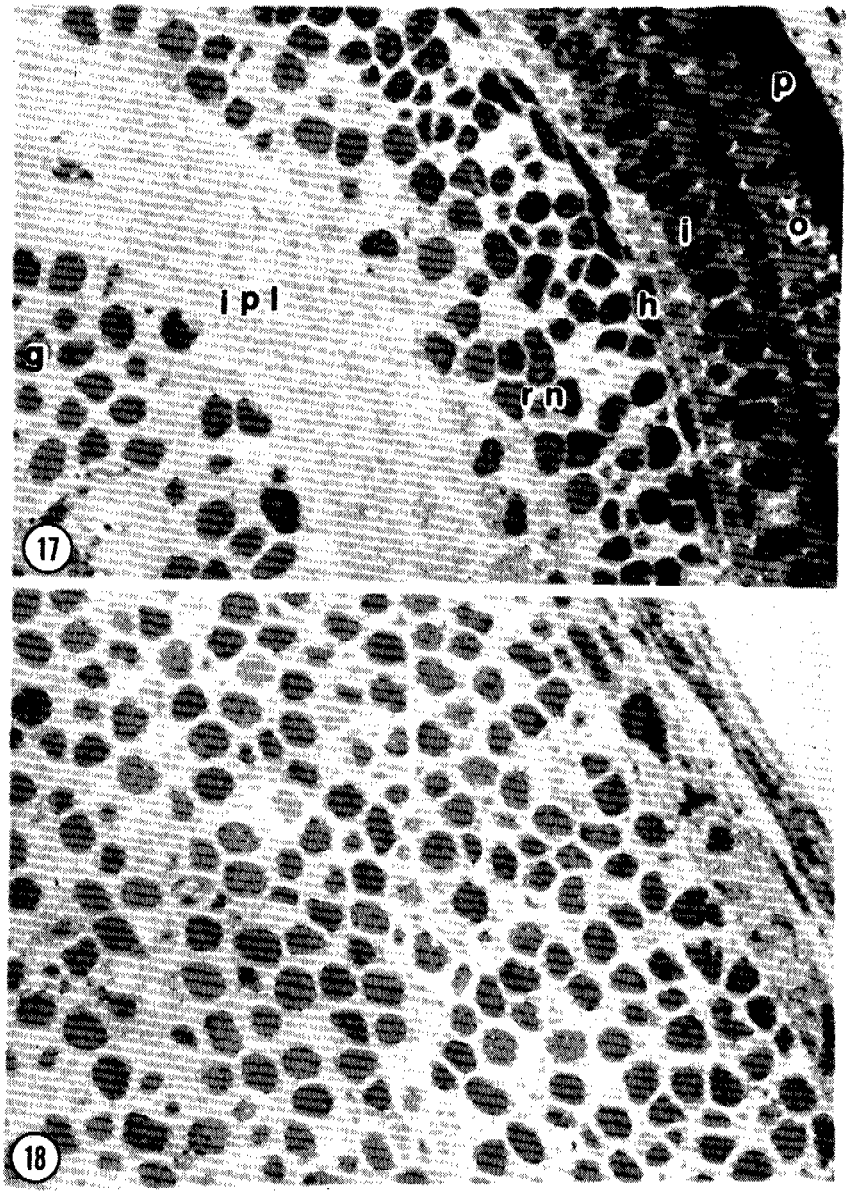
Figure 25. Necrotic neuron containing secondary lysosomes (arrow) in the eye of an exposed embryo (54 ppb CICO). (X 25,000).

Figure 26. Enlargement of receptor cells in a control embryo. Note both the Golgi complexes (*) and the endoplasmic reticulum (arrow) of the myoid region of the inner segment. (X 16,500).

Figure 27. Receptor cells of exposed embryo (113 ppb CICO). The outer segments show little, if any, damage, but both the myoid and ellipsoid regions of the inner segments are vacuolated (arrows). (X 16,500).



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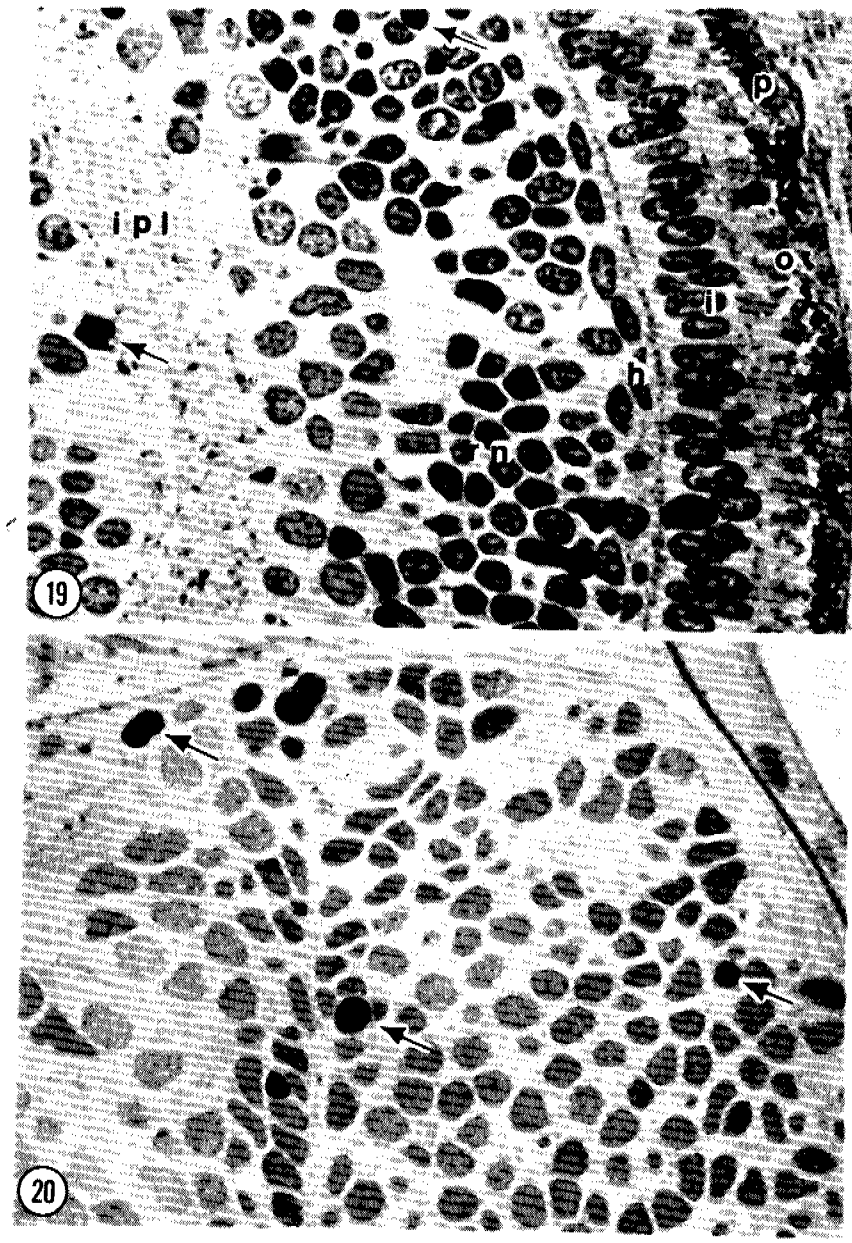

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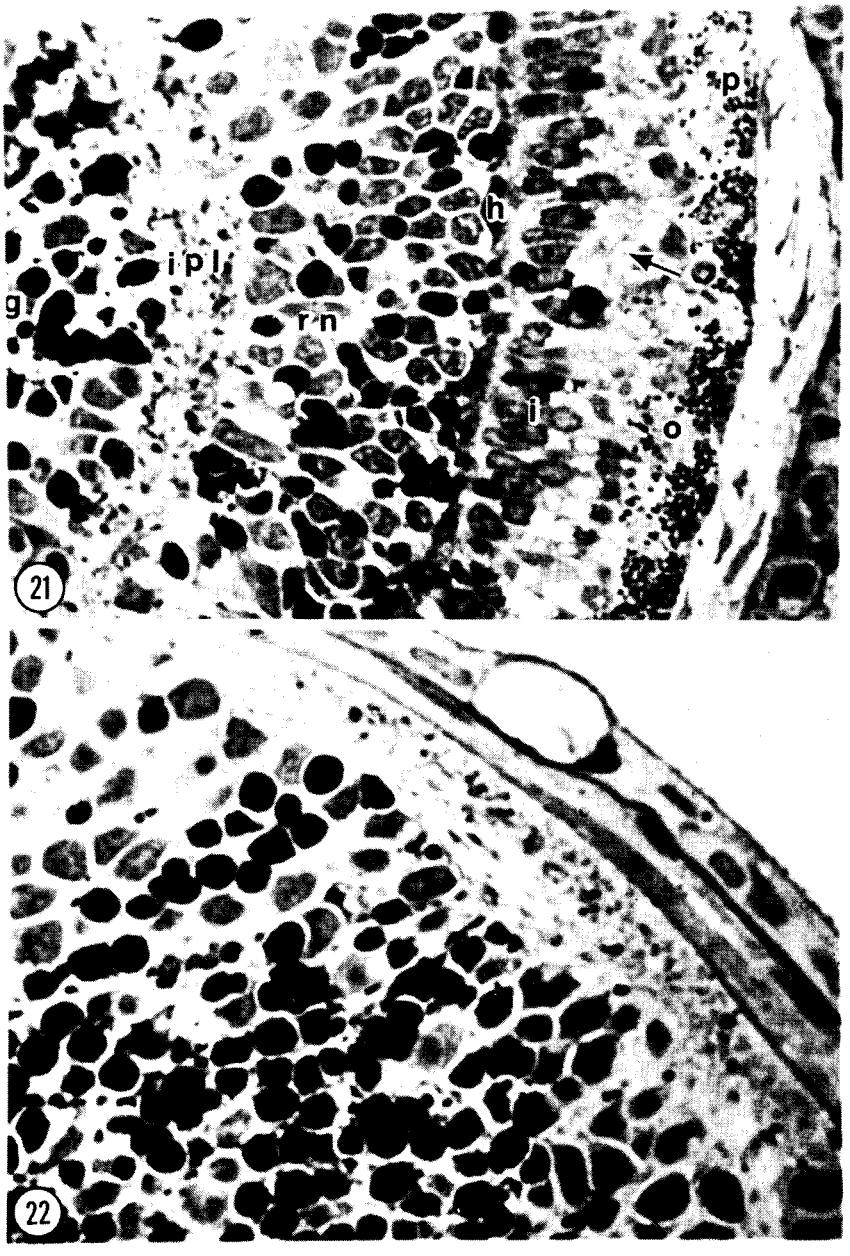

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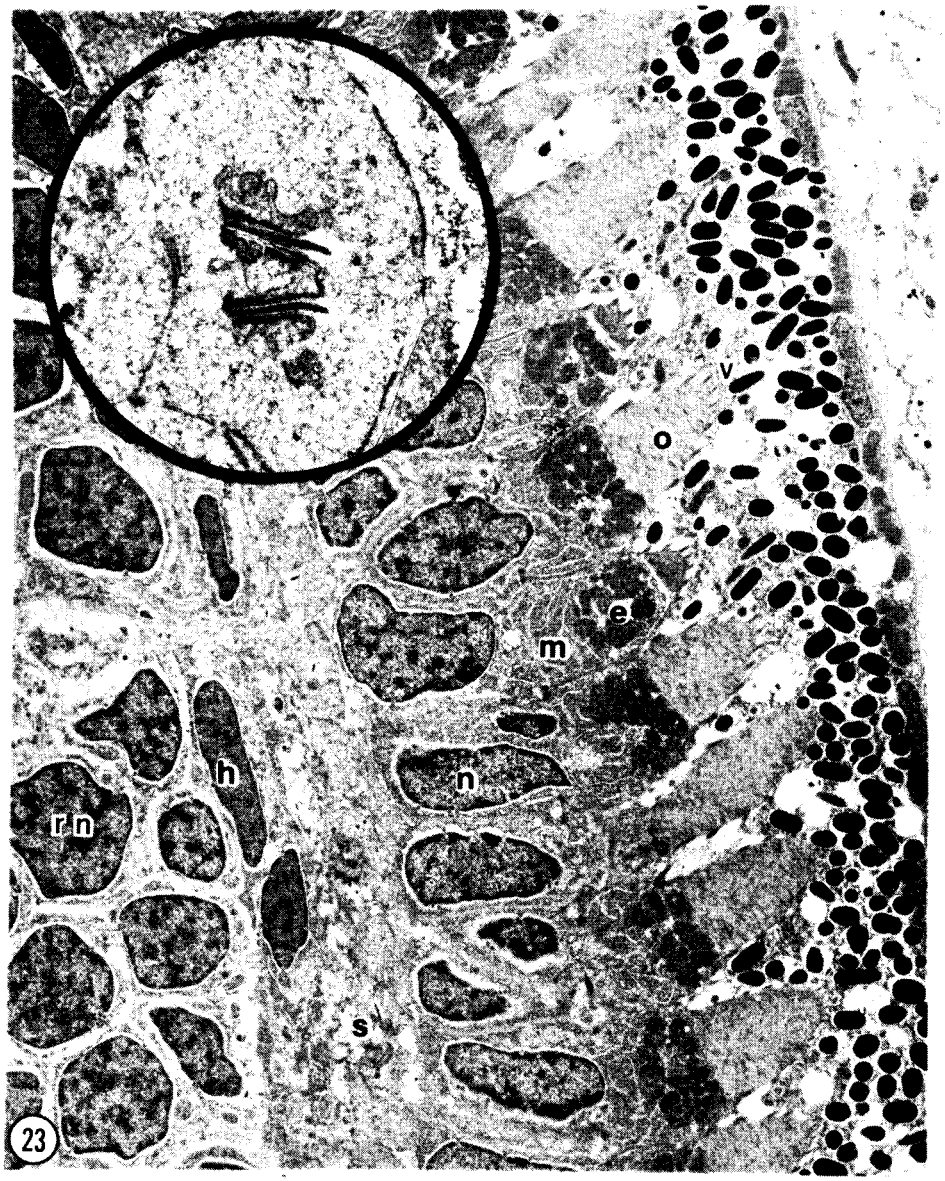


Figure 23. Retinal cells of an unexposed embryo (X 5,000). Insert: enlargement of a synaptic junction (X 18,000).

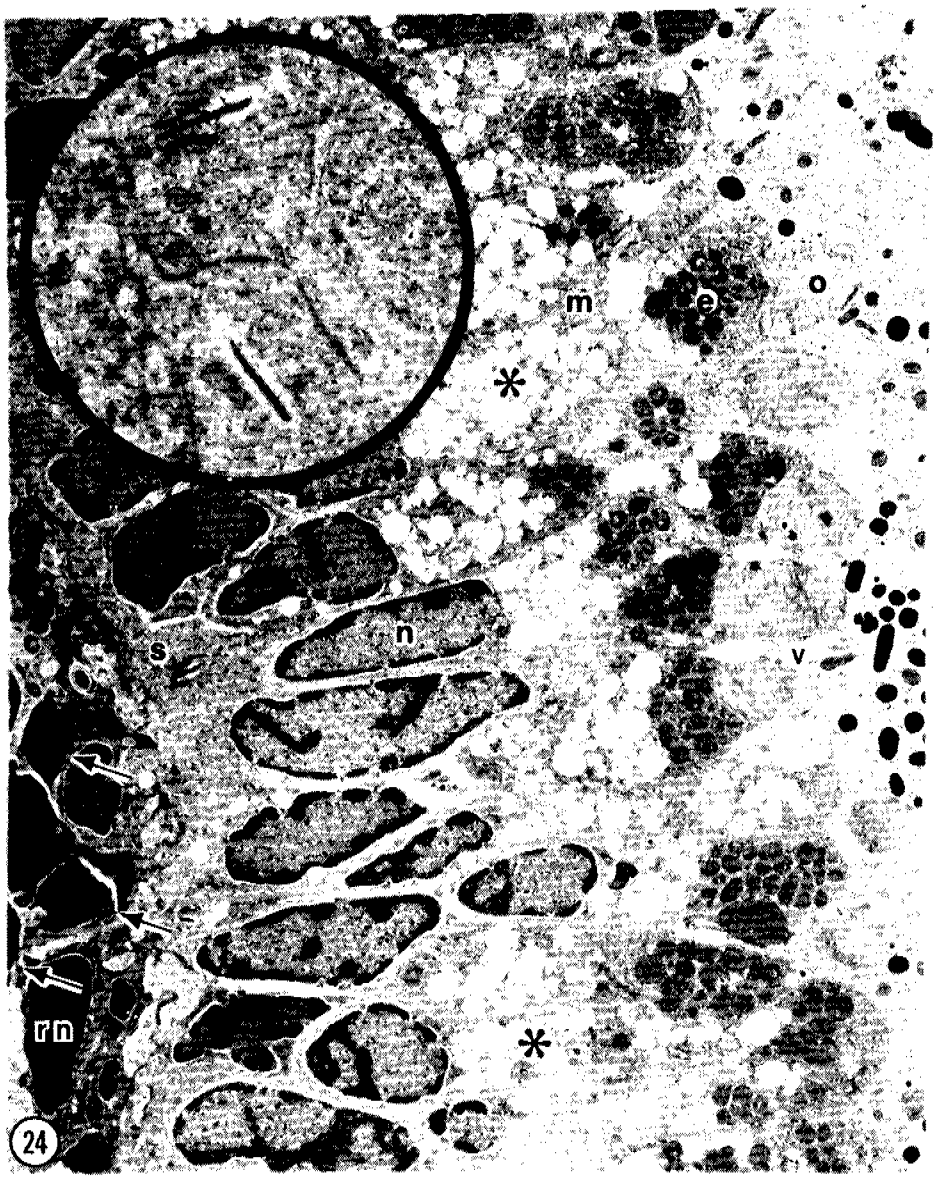


Figure 24. Exposed embryo (113 ppb CICO). Vesiculation is evident in the myoid regions (*) of the receptor cells and necrotic neurons are present (arrow). (X 5,000). Insert: synaptic junction appears normal (X 18,000).

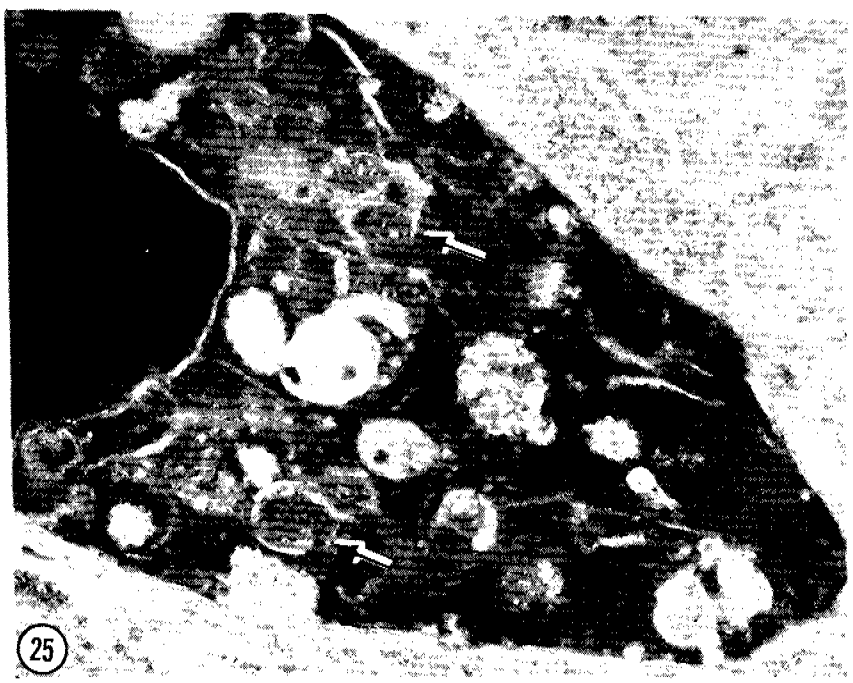
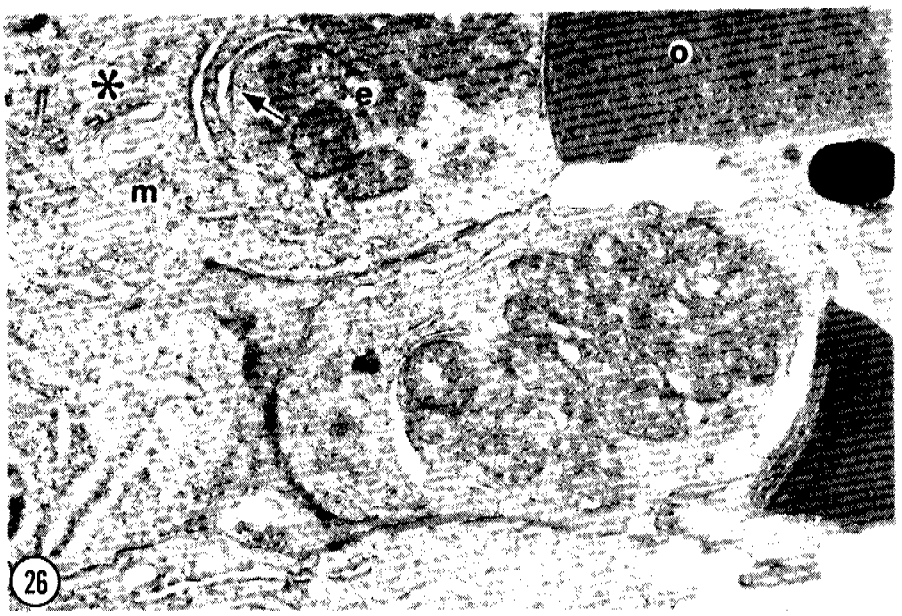


Figure 25. Necrotic neuron containing secondary lysosomes (arrow) in the eye of an exposed embryo (54 ppb CICO). (X 25,000).



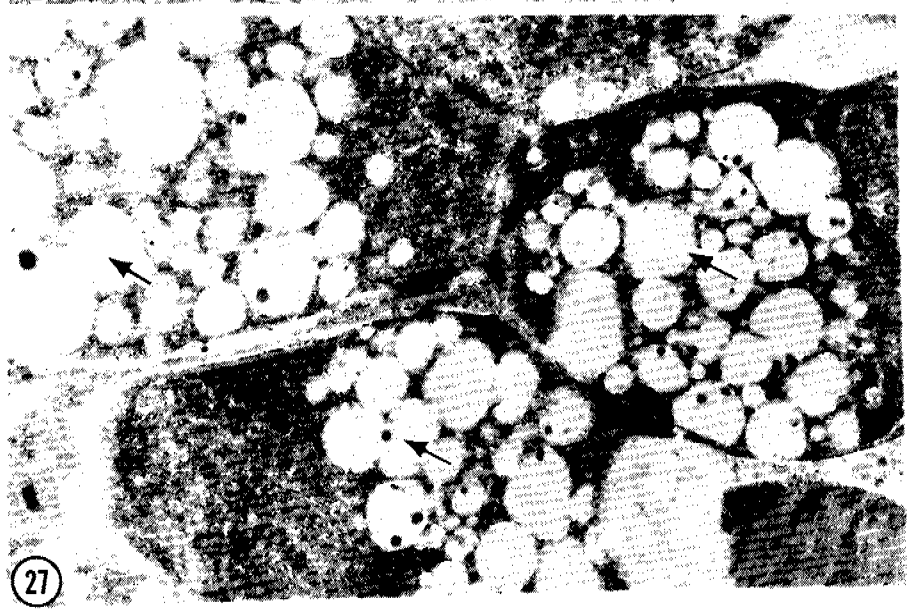


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Figure 27. Receptor cells of exposed embryo (113 ppb CICO). The outer segments show little, if any, damage, but both the myoid and ellipsoid regions of the inner segments are vacuolated (arrows). (X 16,500).

Table 17. Percent surf smelt embryos sampled 21 and 27 days after fertilization (45 and 63 h oil exposure) with cellular abnormalities of the eye and brain.

Hydrocarbon concentration (ppb)	Cellular abnormalities											
	21 days post-fertilization						27 days post-fertilization					
	Vacuolated retinal receptor cells		Necrotic retinal neurons		Necrotic forebrain neurons		Vacuolated retinal receptor cells		Necrotic retinal neurons		Necrotic forebrain neurons	
	(%)	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)	(N)
Control	0	(4)	0	(4)	0	(5)	0	(4)	0	(4)	0	(5)
54	0	(6)	83	(6)	72	(7)	80	(5)	80	(5)	80	(5)
113	0	(4)	75	(4)	80	(5)	75	(4)	80	(5)	66	(6)

reduction in the electron density of the matrix. Separation between the outer and inner mitochondrial membranes was quite pronounced, and breaks in both membranes were observed. Other organelles such as the nuclei, Golgi assemblies, and microtubules were normal. SEM and TEM micrographs of olfactory and epidermal pathology are given in the 1980 OCSEAP Annual Report of RU-73 by Malins et al. (1980b).

Larvae of sand sole exposed to 164 ppb of the SWAF of weathered PBCO during embryogenesis also showed retinal and brain pathological changes similar to that observed in surf smelt. Of 3 control larvae examined, all brain and eye tissues were normal; however, 100% (7 out of 7) of the oil-exposed larvae exhibited necrotic cells in these same tissues.

Gross morphological observations of chum salmon alevins revealed possible cephalic abnormalities in all oil-exposed groups (Table 12). However, in 5 alevins (3 oil-exposed, 2 control) processed and examined with both light and TEM microscopy there was no evidence of neural damage.

Five chum salmon alevins exposed to oil continuously from fertilization through yolk absorption and 3 controls were processed for SEM with particular attention focused on the surface morphology of the skin and ciliated olfactory cells. No differences were evident between the oil-exposed and control alevins.

VII. DISCUSSION

Behavior

Oil-exposed prey experiments: Results of this study were that exposure of chum salmon fry to low levels of SWAF of CICO for periods of 24 through 72 h increased their consumption by coho predators. These low level exposures caused few if any detected behavioral differences, but they resulted in very significant differences in survival of predation. The longest exposure period, 96 h, did not cause the greatest loss to predation as might be expected.

Oil-exposed predator experiments: These studies have shown that exposure to SWAF of CICO can also significantly impair the capturing of prey by coho salmon predators. An interesting observation associated with that behavioral difference was that levels of the parent hydrocarbons were markedly higher in the tissues of the oil-exposed eater subgroup than in those of the oil-exposed noneater subgroup. Brain and liver hydrocarbon concentration differences between the eater and noneater subgroups suggest differential uptake, excretion and/or metabolism of these chemicals. Differential mixed function oxidase induction could be a possible explanation.

Since the eater subgroup had much higher tissue concentrations of the parent hydrocarbons in both brain and liver than the noneaters, it appears unlikely that the parent hydrocarbons were the xenobiotics primarily influencing feeding behavior. The lower concentrations of the parent hydrocarbons in the noneater subgroup suggest, as indicated, that the metabolic products of the crude oil may have been responsible for the cessation of feeding. Our preliminary results are in apparent contrast with reported findings that

preliminary results are in apparent contrast with reported findings that acute neurotoxic effects (Savolainen 1977) and behavioral changes (Dixit and Anderson 1977) were related to accumulation of the parent compounds.

Chemistry

Chromatographic analyses showed that B[a]P in sediment and SAW remained primarily unchanged over a period of several days. NPH concentrations in sediment declined steadily, probably because NPH is more susceptible to microbial degradation (Herbes and Schwall 1979; Lee 1977) and is more soluble in water compared to B[a]P. English sole exposed simultaneously to B[a]P and NPH in sediment accumulated these hydrocarbons in various tissues to levels greater than those present in the SAW. A substantial percentage of NPH-derived radioactivity in the liver of fish was in the form of the parent hydrocarbon, whereas most of the B[a]P-derived radioactivity in the liver was in the form of metabolites. It may be argued that the presence of other hydrocarbons in PBCO may have influenced the extent of the metabolism of both B[a]P and NPH; however, feeding studies conducted at this laboratory have shown that when fish were exposed to NPH or B[a]P, singly or in the presence of other hydrocarbons, the liver metabolized B[a]P more extensively than NPH. These results probably explain why B[a]P is detected in very low concentrations or not detected at all in liver of fish sampled from areas containing considerable concentrations of B[a]P (Veldre et al. 1979; Malins et al. 1980a).

Certain metabolites known to be toxic to mammals were detected in liver and bile of English sole (Varanasi and Gmur 1981, 1981a). Studies with mammals have demonstrated that 7,8-dihydrodiol of B[a]P is further metabolized to 7,8-dihydroxy 9,10-epoxy 7,8,9,10-tetrahydrobenzo[a]pyrene which is kwown to bind covalently with DNA (Sims et al. 1974). The diolepoxide is hydrolyzed to 7,8,9,10-tetrahydro 7,8,9,10-tetrahydroxybenzo[a]pyrene. Preliminary analyses of polar metabolites by HPLC (Figure 28) revealed the presence of tetrols in the bile of English sole indicating that the diolepoxide, which is implicated as the ultimate carcinogen of B[a]P, was produced in the liver (Varanasi and Gmur 1981a).

Metabolites of NPH in the bile of English sole were the same as those in bile of starry flounder and rock sole exposed to dietary NPH (Varanasi et al. 1979, 1980b). The glucuronide of 1,2-dihydro 1,2-dihydroxynaphthalene was the major metabolite in the bile of English sole; pleuronectid fish tend to produce a greater proportion of glucuronide conjugates than sulfate conjugates. It appears that as with hamster embryo cells (Baird et al. 1977) and rat hepatocytes (Burke et al. 1978), glucuronidation is the major detoxification pathway in pleuronectidae.

Pathology

The results of a series of tests to assess the effects of petroleum hydrocarbons on disease resistance of juvenile and adult flatfish failed to identify a marked impairment. Whether or not a lowered host resistance could occur under other stress and exposure conditions and to different diseases cannot be decisively predicted from the present data. These data do strengthen the concept, however, that petroleum alone does not produce impaired disease resistance.

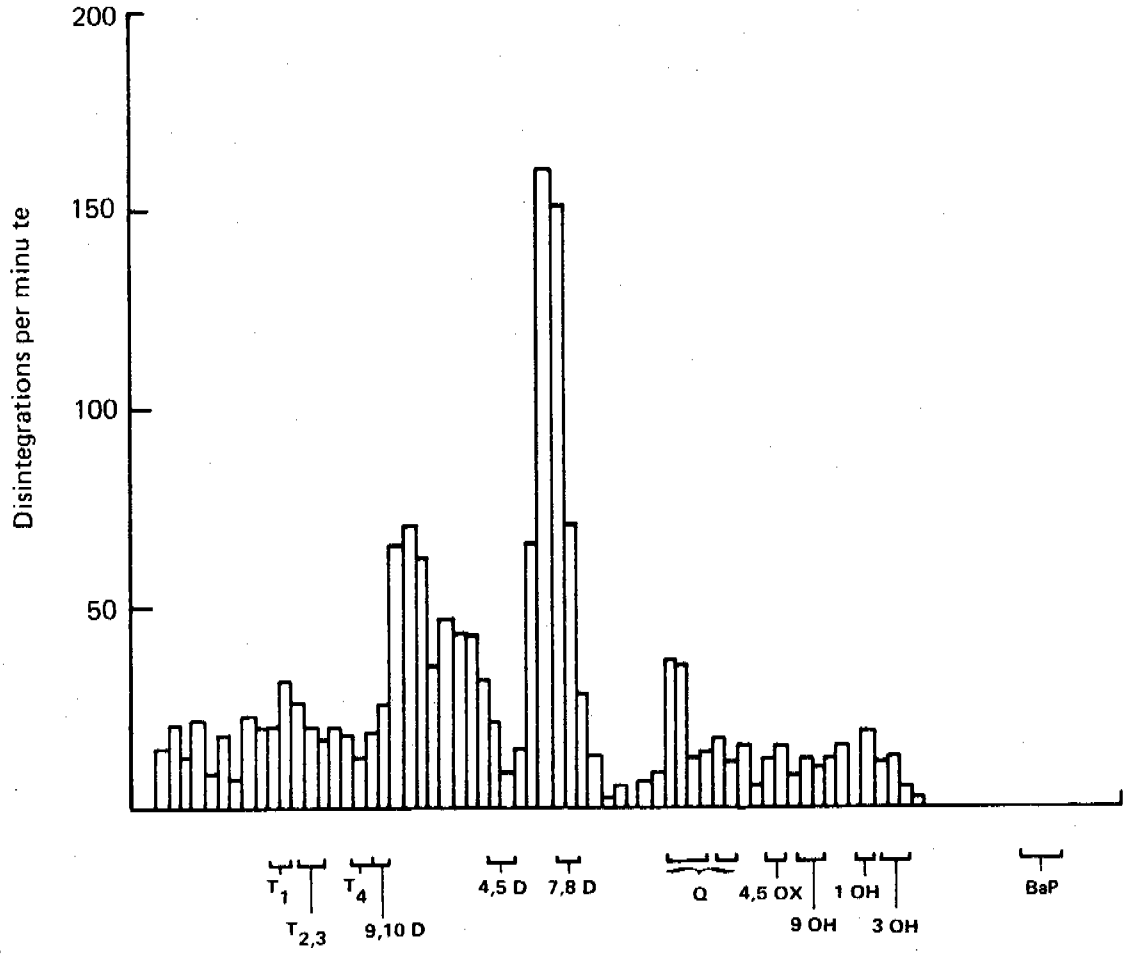

Figure 28. High-pressure liquid chromatography of ethyl acetate soluble metabolites released after the treatment of aqueous phase of bile from English sole with β-glucuronidase. Metabolites were separated by fractions collected at 15 sec. intervals as described earlier (Varanasi et al. 1980). Abbreviations: T_1, $T_{2,3}$ and T_4 - tetrahydro tetrahydroxy B[a]P; 9,10 D - B[a]P 9, 10-dihydrodiol; 4,5 D - B[a]P 4,5-dihydrodiol; 7,8 D - B[a]P 7, 8 dihydrodiol; Q - quinones; 4,5 ox - B[a]P 4,5 oxide; 1 OH, 3 OH and 9 OH - 1-, 3- and 9-hydroxy B[a]P

In contrast, preliminary assays with a representative petroleum dispersant (Corexit 9527) suggested the potential for an adverse impact of oil-dispersant mixtures on disease resistance.

Physiology

Embryos and larvae of three species of commercially important North Pacific fishes (salmon, flatfish, and smelt) were exposed to the SWAF of weathered crude oil. Each of these species has a different spawning strategy, and for each the experimental design duplicated as closely as possible, under laboratory conditions, the natural environment in which embryogenesis occurs. Flow-through systems were considered the most realistic method of oil exposure and were used in experiments with salmon and smelt. For pelagic flatfish eggs, a flow-through system presented technical difficulties, thus, a static-renewal method of oil exposure was developed. Because we chose to simulate the natural environment (i.e., as opposed to an experimental design using a common exposure regime which did not consider differences in species spawning characteristics) there was virtually only one similarity among the various experiments -- the use of the SWAF of weathered crude oil for exposure.

Our definition of the SWAF of weathered oil is based on routine chemical analysis of the water-accomodated oil which indicated a progressive disappearance of the more volatile hydrocarbon compounds with time. This definition does not preclude the possible production of oxidized products resulting from photo-oxidization or microbial action, both of which may be important factors contributing to the severe detrimental effects observed.

In all our experiments, there was some mortality in the controls, ranging from a low of 10% for sand sole to a high of 50% for embryos of surf smelt. These mortalities are attributed, in part, to stresses induced by simulating natural environmental conditions. For example, chum salmon embryos and alevins were subjected daily to wide fluctuations in salinity, and surf smelt embryos were subject to desiccation and thermal stress. Thus, the effects of petroleum hydrocarbons we observed are most likely the result of a combination of chemical, physical, and biological factors, and not petroleum acting alone.

VIII. CONCLUSIONS

Behavior

Exposure of chum salmon fry to the SWAF of CICO at an average concentration of 350 ug/l total hydrocarbons for periods of 24, 48, 72, and 96 h resulted in significantly different (greater at 24, 48, and 72 h and less at 96 h) consumption of the oil-exposed prey by coho predators compared to the consumption of non-oil-exposed controls.

Similarly, exposure of coho salmon predators to the SWAF of CICO for 10 and 17 days at an average of 343 µg/L total hydrocarbons was correlated with significant alteration (reduction) in the numbers of salmonid fry prey eaten by the exposed predators compared to the numbers of fry eaten by non-oil-exposed control predators. Concentrations of all hydrocarbons detected by GC in both

liver and brain of analyzed oil-exposed predators were higher in the eater subgroup than in the noneater subgroup. This suggests that the parent hydrocarbons were probably not the compounds primarily influencing the feeding behavior. Instead, metabolites of the crude oil may have been responsible for the cessation of feeding.

Chemistry

English sole, exposed to B[a]P in oil-contaminated sediment, take up and extensively metabolize the hydrocarbon to a number of mutagenic and carcinogenic compounds, some of which bind to cellular macromolecules of liver. The polynuclear aromatic hydrocarbons, such as B[a]P, (which is a minor component of crude petroleum, but present in higher concentrations in combustion products of petroleum) tend to remain largely unchanged in sediment and can be available for continued uptake by benthic organisms. The substantial bioconversion of B[a]P in fish liver probably is the reason why B[a]P is usually not detected in high concentrations in fish tissues even when considerable concentrations of B[a]P are detected in the environment of the fish.

Pathology

Laboratory studies evaluating the effects of petroleum hydrocarbons on immunocompetence and disease resistance of juvenile and adult flatfish failed to demonstrate a marked impairment. Preliminary assays did, however, suggest petroleum dispersants may enhance the incidence of infectious disease.

Physiology

The principal objective of the studies on early developmental stages of marine fishes was to complete analysis of previously collected data and provide information concerning the effects of petroleum on embryo and larval development.

Chum salmon embryos and alevins were exposed 4 h per day in a flow-through system to an average of 470 ppb of the SWAF of slightly weathered PBCO. Survival of embryos exposed during either the first third or last two-thirds of their development was similar to controls, however, continuous exposure throughout development significantly reduced embryo survival. The primary effect of oil exposure during embryonic development occurred in alevins in the first 10 to 15 days after hatching. For embryos and alevins exposed continuously, only 20% of those hatching survived through yolk sac absorption; 85% of the control alevins survived during a comparable period. Oil exposure of newly hatched alevins not previously subjected to oil contamination during embryogenesis resulted in mortality double that of controls.

Flatfish eggs were exposed to the SWAF of slightly weathered PBCO using a static-replacement regime with the SWAF being renewed at mid-incubation. At average hydrocarbon concentrations of 130 to 165 ppb embryo survival and percent hatching were high, but all hatched larvae were either abnormal or dead. Embryo incubation at a concentration of 80 ppb resulted in percentages of normal larvae comparable to those of controls.

In replicate experiments, surf smelt embryos were exposed in a flow-through system to the SWAF of weathered CICO for 3 h per day during embryonic development. At the two highest hydrocarbon concentrations (113 and 173 ppb) less than 10% of the embryos hatched into normal larvae. At the lowest hydrocarbon concentrations (26 and 43 ppb), 43% of the exposed embryos hatched into apparently normal larvae -- a percentage similar with that of controls. The hatched larvae were held in uncontaminated water and the survivors were enumerated 10 days later. Over 42% of the control larvae survived, whereas, survival was less than 9% for larvae exposed to the lowest concentrations of weathered oil as embryos.

In addition to the gross effects, there were also cytopathological changes, particularly evident in surf smelt embryos and in larvae of sand sole exposed to the SWAF of weathered crude oil during embryogenesis. Sand sole larvae and surf smelt embryos both exhibited disruption of olfactory cilia and necrosis of eye and brain neurons. No cytopathological changes were evident in oil-exposed embryos or alevins of chum salmon.

The average hydrocarbon concentrations to which the embryos and larvae were exposed spanned a range of approximately one order of magnitude (500 ppb to slightly less than 50 ppb). Over this range of hydrocarbon concentrations a variety of effects were observed, including ultrastructural changes in apparently viable embryos and larvae, gross abnormalities, and direct mortality. It was concluded that the hydrocarbon concentrations and the effects induced could be expected to occur in a petroleum-contaminated environment.

IX. NEED FOR FURTHER STUDY

An important question for consideration has been raised by the predator-prey studies. This concerns the clear possibility that metabolites of petroleum may be more profoundly influencing certain types of behavior in fish than parent petroleum hydrocarbons. This possibility should be further examined using a similar experimental design as was used for the previous predation studies and more extensive state-of-the-art analytical chemistry for both hydrocarbons and metabolites. Because the latter are not readily detectable by the routine methods used for analysis of hydrocarbons, these metabolites may be present in fish in high concentrations and causing severe behavioral impairment without either their presence or their potential for harm being recognized.

Preliminary studies showing altered toxicity of petroleum hydrocarbons after photooxidation on early life stages demonstrate a need for in-depth investigation in this little known but potentially important area.

X. SUMMARY OF FOURTH QUARTER OPERATION

Ship or Laboratory Activities

Ship or Field Trip Schedule

Several trips were made by small boat and car to various areas of Puget Sound to collect animals for testing. Reseasrch activities were conducted at the NWAFC, Seattle, and the Mukilteo Facility of the NWAFC.

Scientific Party

The scientific party consists of scientists and technical personnel in several major study areas. The overall supervision of the research is carried out by Dr. Donald C. Malins, Principal Investigator, Director of the Environmental Conservation (EC) Division, NWAFC, Seattle, Wash. The scientific party also consists of the following persons from the EC Division, NWAFC:

Sin-Lam Chan, PhD., Supervisory Research Chemist, Principal Investigator, Assistant Director of Environmental Conservation Division.

Harold O. Hodgins, PhD., Supervisory Fishery Research Biologist; Principal Investigator and overall supervisor of pathological, behavioral, and physiological studies.

Usha Varanasi, PhD., Supervisory Research Chemist; Principal Investigator, and overall supervisor of biochemical effects of hydrocarbons and metabolites studies.

Bruce B. McCain, PhD., Supervisory Microbiologist; Principal Investigator, in charge of work on petroleum in sediments and their effect on flatfish pathology.

Douglas D. Weber, M.S., Fishery Research Biologist; Principal Investigator, egg and larval studies.

Donald W. Brown, M.S., Supervisory Chemist; Principal Investigator; Assistant Manager of NOAA National Analytical Facility (NAF).

Paul A. Robisch, Research Chemist; oxidized petroleum toxicity studies.

Herbert R. Sanborn, M.S., Oceanographer; oxidized petroleum toxicity studies.

David A. Misitano, M.S., Research Biologist; eggs and larvae.

Joyce W. Hawkes, PhD., Cell Biologist; electron microscopy, developmental biology and pathology.

Carla Stehr, Fishery Research Biologist; developmental biology.

Dennis J. Gmur, M.S., Research Chemist; hydrocarbon and metabolite analyses and effects.

Mark S. Myers, Fishery Research Biologist; histopathology.

Tom Hom, Chemist; hydrocarbon and metabolite analyses and effects.

Marc Nishimoto, Aide; hydrocarbon metabolite analyses.

Gloria D. Sergneri, Administrative Officer.

Marci Worlund, Clerk-Typist.

Methods

The research is conducted primarily through laboratory studies. Except for slight modifications the methods used have been described earlier in this report or in previous OCSEAP reports.

Photooxidation of Prudhoe Bay Crude Oil

The heavy black polar materials were removed from Prudhoe Bay Crude Oil using the ASTM method D-2007-65T. The remaining oil containing the paraffin and aromatic fractions was subject to photooxidation.

Two glass trays (19 x 30 cm) containing 100 ml of seawater (filtered and sterilized) were placed in a water bath maintained at 12°C. A solution consisting of 5.0 g of the oil dissolved in 30 ml of pentane was poured on the surface of the water in each tray. Tray No. 1 was covered with aluminum foil. Tray No. 2 was exposed to ultraviolet (UV) light for 120 h. The oil in Tray No. 1 (control), which had been shielded from the UV light, had noy changed visibly after the 120 h. The light-exposed oil in Tray No. 2 (test), however, had turned a dark yellow-brown. The oil floating on the surface of the water in each tray was separated from the underlying water. Extractions of portions of the underlying water with methylene chloride:diethyl ether (70:30, v/v) showed that the control water contained 5.75 μg of organic material per gram of water and test water contained 73.6 μg of organic material gram of water. The extracts from the water were subjected to infrared analysis using a Perkin-Elmer model 681. The results show no absorption in the carbonyl region for the extracts from the control water, but significant absorption in the carbonyl region for the extracts from the test (irradiated) water, indicating the presence of photooxidation products.

Sample Localities/Ship or Tracklines - N/A

XI. AUXILIARY MATERIAL

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ORAL PRESENTATIONS

The following presentations were made at the Fate and Effects of Petroleum Program Review sponsored by the Interagency Committee on Ocean Pollution Research, Development and Monitoring held Sept. 21-23, 1980 in Boulder, CO:

Dr. Donald C. Malins
Overview of NWAFC/NMFS Oil Pollution Research

Dr. William MacLeod, Jr.
The Nature and Extent of Petroleum Pollution

Dr. Harold O. Hodgins
Effects of Crude Oil

Dr. Usha Varanasi
Effects of Petroleum Components

Drs. Donalc C. Malins and Sin-Lam Chan participated in the Northern Sound Synthesis Meeting in Anchorage, AK, on October 27-28, 1980. Malins gave a presentation on "Toxicity of Oil in Marine Habitats".

Dr. U. VARANASI presented a paper entitled "Metabolism of Benzo[a]pyrene and naphthalene in vivo by flatfish" at the Fifth International Symposium on Polynuclear Aromatic Hydrocarbons at Battelle Columbus Laboratories, Columbus, Ohio, Oct. 29, 1980.

DR. DONALD C. MALINS and ROBERT C. CLARK, JR. presented testimony on effects of petroleum on marine biota at the Energy Site Evaluation Council hearings on the Northern Tier pipeline in Olympia, WA on March 24, 1981.

A series of presentations were made at an Environmental Conservation Division/NWAFC Program Review which contained large segments relating to analysis, fate, disposition and effects of petroleum. Presentations were made by all OCSEAP Principal Investigators plus other staff members on April 7-8, 1981 at NWAFC, Seattle. An invited peer review panel composed of recognized authorities on marine pollutant problems was chaired by Dr. Douglas A. Wolfe of NOAA. A written evaluation and critique is being prepared currently by the panel.

BAFFIN ISLAND OIL SPILL EXPERIMENT
CHEMISTRY COMPONENT
FINAL REPORT ON BASELINE YEAR ACTIVITIES

VOLUME 1:

Field Work

Environmental Chemistry

Hydrocarbon Infrared Data

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Seakem Oceanography Ltd.
20 February, 1981

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1. INTRODUCTION

The following report summarizes the activities of Seakem Oceanography Ltd. for the baseline year of the Baffin Island Oil Spill Project. Our role, in partnership with Energy Resources Co., Cambridge, Mass., was to provide a broad spectrum of chemical services which can be divided into four categories: a field program, environmental chemistry analyses, hydrocarbon baseline analyses, and hydrocarbon analyses for the shoreline oil spill plots. A summary of the tasks performed, and the company responsible for each, is given in Table 1.1. This volume summarizes only the Seakem portion of the contract.

TABLE 1.1

CHEMISTRY COMPONENT TASKS

CATEGORY	TASKS	RESPONSIBLE COMPANY
Field Program	set-up of field laboratory; sampling; sub-sampling; preliminary sample handling; preservation, storage, and transport of samples	Seakem
Environmental Chemistry	Water Analyses: pH, DO, NO_3, PO_4, N, Chl, SS (organic), SS (iunorganic), POC, DOC Sediment Analyses: TOC, NO_3, PO_4, N, Pb-210 Beach Analyses: TOC	Seakem
Hydrocarbon Baseline Study	Water: IR, UV/F, GC/MS Sediment: IR, UV/F, GC/MS Beach: IR, UV/F, GC/MS Tissue: GC/MS	ERCO: UV/F, GC/MS Seakem: IR
Shoreline Oil Plots	GC/MS Total Hydrocarbons	ERCO Seakem

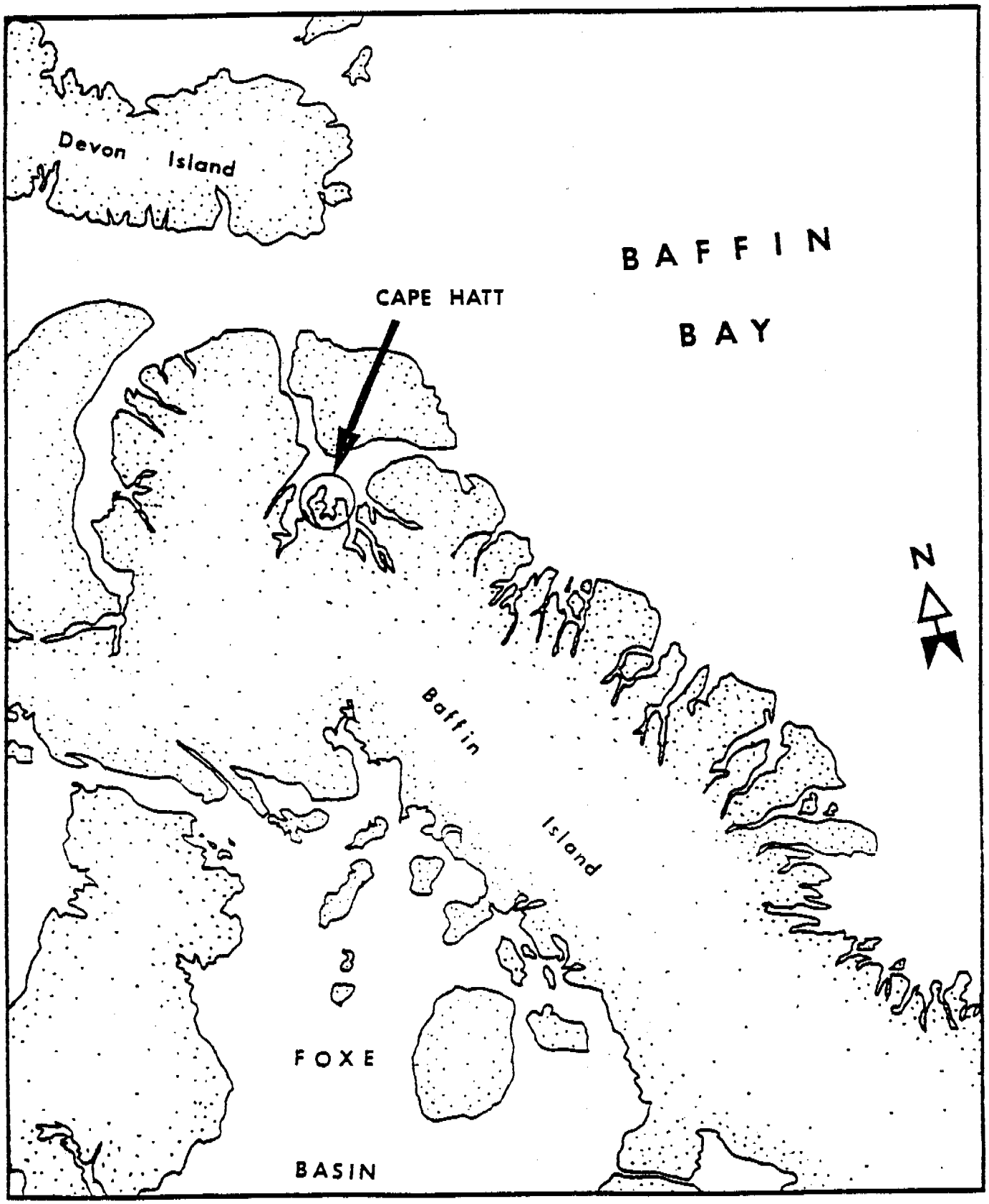

FIGURE 1.1: Location of Cape Hatt, Baffin Island.

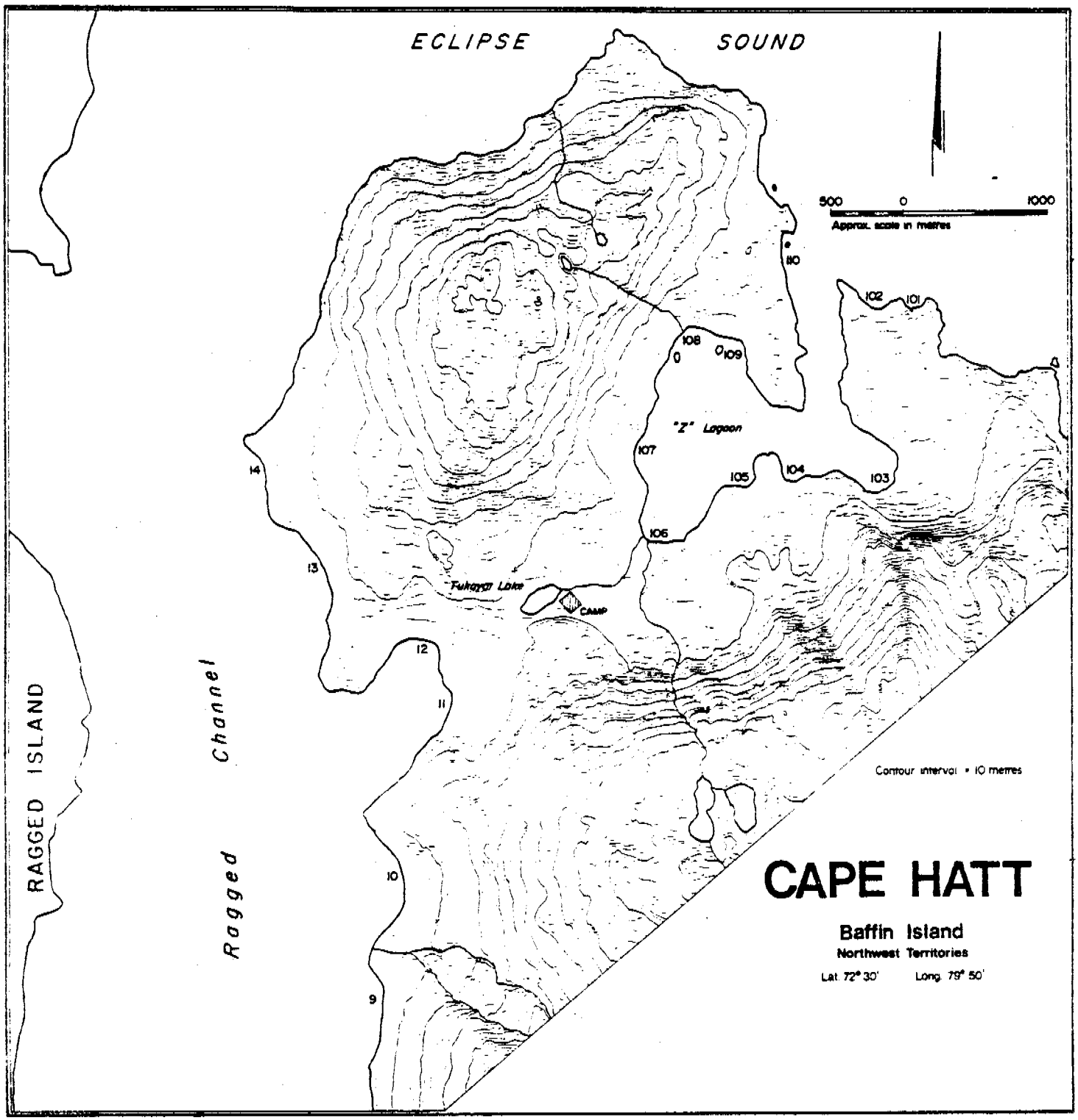

FIGURE 1.2: The Cape Hatt Site, showing the numbering of the experimental bays.

2. FIELD WORK

Chemists conducted sampling at the Cape Hatt field site during the following periods:-

5 - 23 June, 1980	(D.R. Green)
11 August - 4 September	(B. Fowler)
2 - 21 September	(D.R. Green)

We sampled for environmental chemistry analyses, for baseline hydrocarbon analyses, and for measurements of the oil budget on the oiled beach plots. Tables summarizing all of the samples taken, and maps showing approximate location of the sampling sites follow.

Various filtration and extraction work-up steps were done at the Cape Hatt laboratory, then samples were shipped to Seakem Oceanography Ltd and to Energy Resources Co. Ltd., Cambridge, Mass., for analysis.

2.1 Environmental Chemistry

2.1.1 Water Samples

In June, all sampling was conducted through holes in the ice. Sampling stations were established in 12-14 m water in bays 13, 10 and 9 in Ragged Channel at 12-14 m. Ice thickness varied from 1.5 to 2m. Water samples were taken with a Niskin 5L water sampler at 1, 5, and 10 m under the ice. The microbiology sampling team took water samples first, then the environmental chemistry samples were taken. O_2 and pH samples were drawn first, carefully avoiding the introduction of air bubbles, then nutrient and total organic carbon samples were drawn into test tubes. The remaining water was poured into a 4L bottle and filtered for chlorophyll, particulate organic carbon, and suspended solids in the field laboratory immediately after returning to camp.

We sampled in the morning every second day beginning 6 June, 1980. Two stations were sampled in each bay on each sampling day: a total of six stations (H1 to H6) sampled as follows:-

Location	Station ID	Sampling Dates
Bay 13	H1, H2	6, 12, 18 June
Bay 10	H3, H4	8, 14, 20 June
Bay 9	H5, H6	10, 16, 22 June

For comparison and out of general interest, some extra samples were collected from the bottom of the ice by divers, and from melt pools during the period 19 to 22 June.

The same pattern of sampling was followed in the August -September sampling period, except the sampling in Bay 13 was shifted to Bay 11. The sampling pattern was as follows:-

Location	Station ID	Sampling Dates
Bay 11	H1, H2	11, 19, 28 Aug 5, 12 Sept
Bay 10	H3, H4	13, 21, 30 Aug 7, 14 Sept
Bay 9	H5, H6	15, 23 Aug 1, 9, 16 Sept

Sampling was done from a zodiac at depths of 1, 5, and 10 m at each station. (Note that these depths do not correspond to the June sampling depths, which were measured from the ice bottom). The approximate locations of the sampling stations are shown in Figure 2.1.

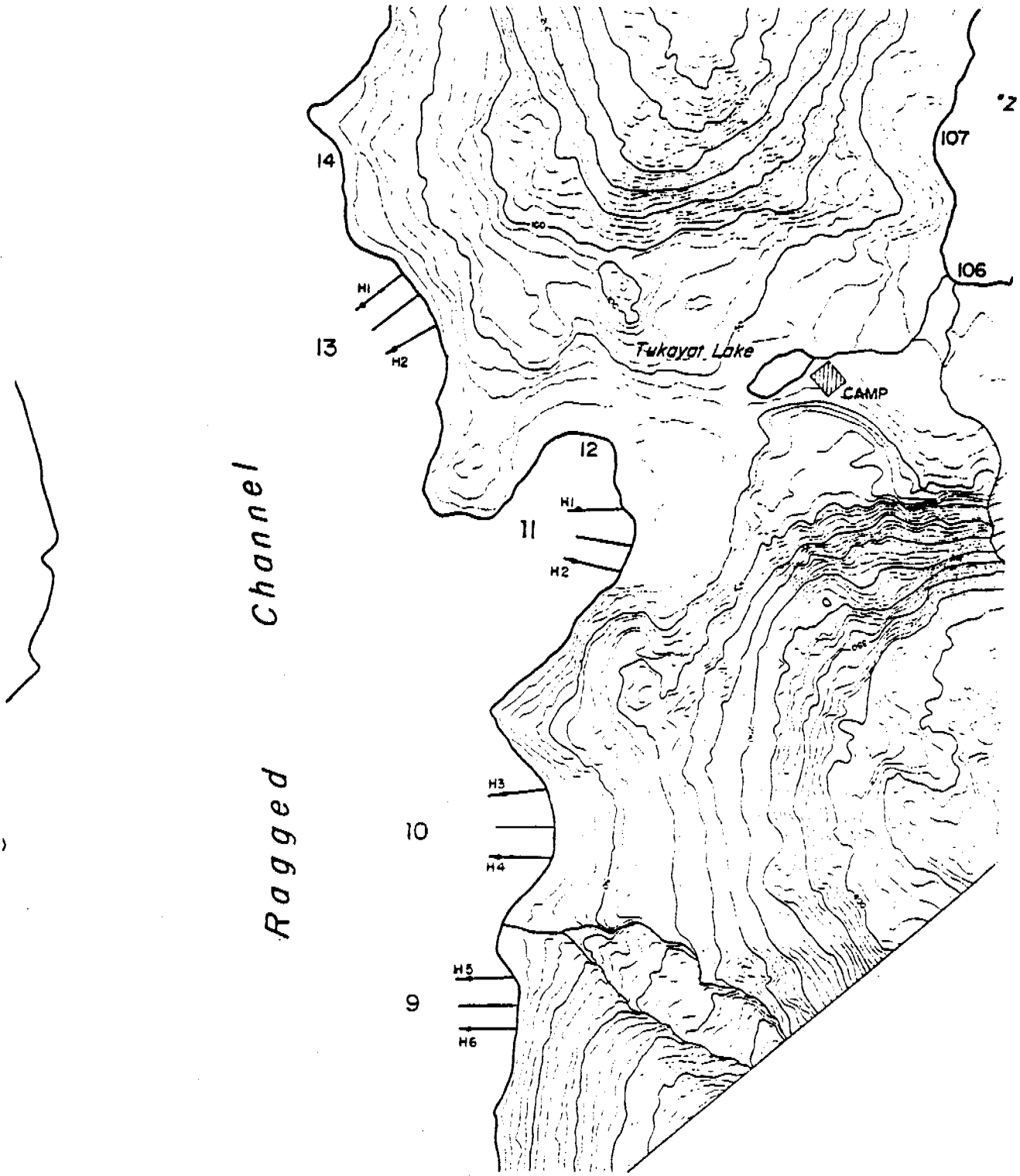

FIGURE 2.1: Locations of Environmental Chemistry water sampling stations. Note: The positions of H1 and H2 were changed from Bay 13 in June to Bay 11 in August/September.

2.1.2 Sediment Samples

In June, sediment samples for environmental chemistry purposes were collected with a Peterson grab sampler through the ice upon completion of water sampling. Because of ice-rafted rock it was often difficult to get the grab sampler to work properly. The jaws usually closed on a rock, allowing the sediment to escape. Samples were frequently missed, or were too small to press sufficient interstitial water for all of the analyses. The suite of samples was augmented with samples collected by divers through the ice holes in each bay.

In August, the same problems with the grab sampler were encountered operating from a Zodiac, so most of the samples were collected by divers, and most from 7 m depth instead of 13 m, to ensure that they came from within the 1981 experimental area.

Table 2.1 summarizes all of the sediment samples collected expressly for environmental chemistry purposes. Subsamples were also drawn from some of the baseline hydrocarbon cores for environmental chemistry and these are summarized separately in Table 2.3.

TABLE 2.1
SEDIMENT SAMPLES
ENVIRONMENTAL CHEMISTRY

Date	Location	I.D	Depth	Sampling Method	Comments
07 June	Bay 13	H2	14.5 m	grab	
08 June	Bay 10	H4	15 m	grab	
10 June	Bay 9	H5	15 m	grab	limited sample
12 June	Bay 13	H2	15 m	grab	
14 June	Bay 10	H4	15 m	grab	limited sample
16 June	Bay 9	H5	15 m	grab	limited sample
20 June	Bay 13	dive hole	12 m	diver	
21 June	Bay 10	dive hole	12 m	diver	
22 June	Bay 9	dive hole	12 m	diver	
19 Aug	Bay 11	H1	12 m	grab	
21 Aug	Bay 10	H3	11 m	grab	
		H4	12 m	grab	limited sample
23 Aug	Bay 9	H5	15 m	grab	limited sample
31 Aug	Bay 10	H3	10 m	diver	
		H4	10 m	diver	
02 Sept	Bay 9	H5	7 m	diver	
		H6	7 m	diver	
02 Sept	Bay 11	H1	7 m	diver	
		H2	7 m	diver	
06 Sept	Bay 11	H1	7 m	diver	
		H2	7 m	diver	
07 Sept	Bay 10	H3	7 m	diver	
		H4	7 m	diver	
10 Sept	Bay 9	H5	7 m	diver	
		H6	7 m	diver	
13 Sept	Bay 11	H1	7 m	diver	
		H2	7 m	diver	
14 Sept	Bay 10	H3	7 m	diver	
		H4	7 m	diver	
15 Sept	Bay 9	H5	7 m	diver	
		H6	7 m	diver	

2.2 Hydrocarbon Baseline Samples

2.2.1 Water Samples

In June, 4L water samples were taken with a National Bureau of Standards water sampler (see Figure 2.2) through the same ice holes as those used for environmental chemistry sampling. The samples were extracted in the field laboratory with 3 x 75 mL Freon 113. Extraction was done in the sampler containers by shaking for 3 minutes in a paint shaker. The three extracts were combined, then divided in half, half for analysis by ERCO (scanning UV- fluorescence) and half by Seakem Oceanography Ltd (I.R.). In addition, three 4 L water samples from Bay 9 (H5, depths 1, 5, 10 m below ice) were collected but not extracted and delivered directly to ERCO for extraction in the laboratory.

In August - September, some large volume water samples were collected from Ragged Channel. The apparatus used is shown in Figure 2.3. Problems with the generator, the pumping system, and interference by ice limited the number of samples which could be taken in this manner, so the remainder were taken with a National Bureau of Standards sampler in the same manner as in June.

The sampling location and descriptions of the samples are summarized in Table 2.2.

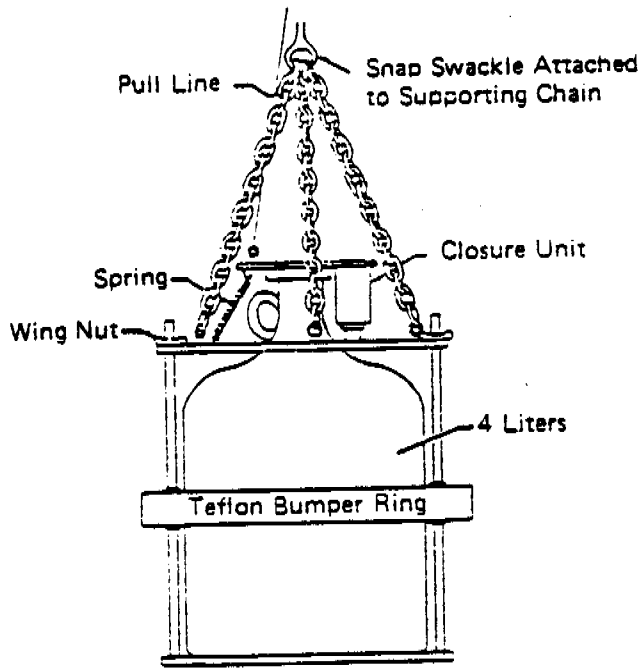

FIGURE 2.2: The National Bureau of Standards water sampler used for obtaining baseline hydrocarbon samples for IR and UV/F analyses.

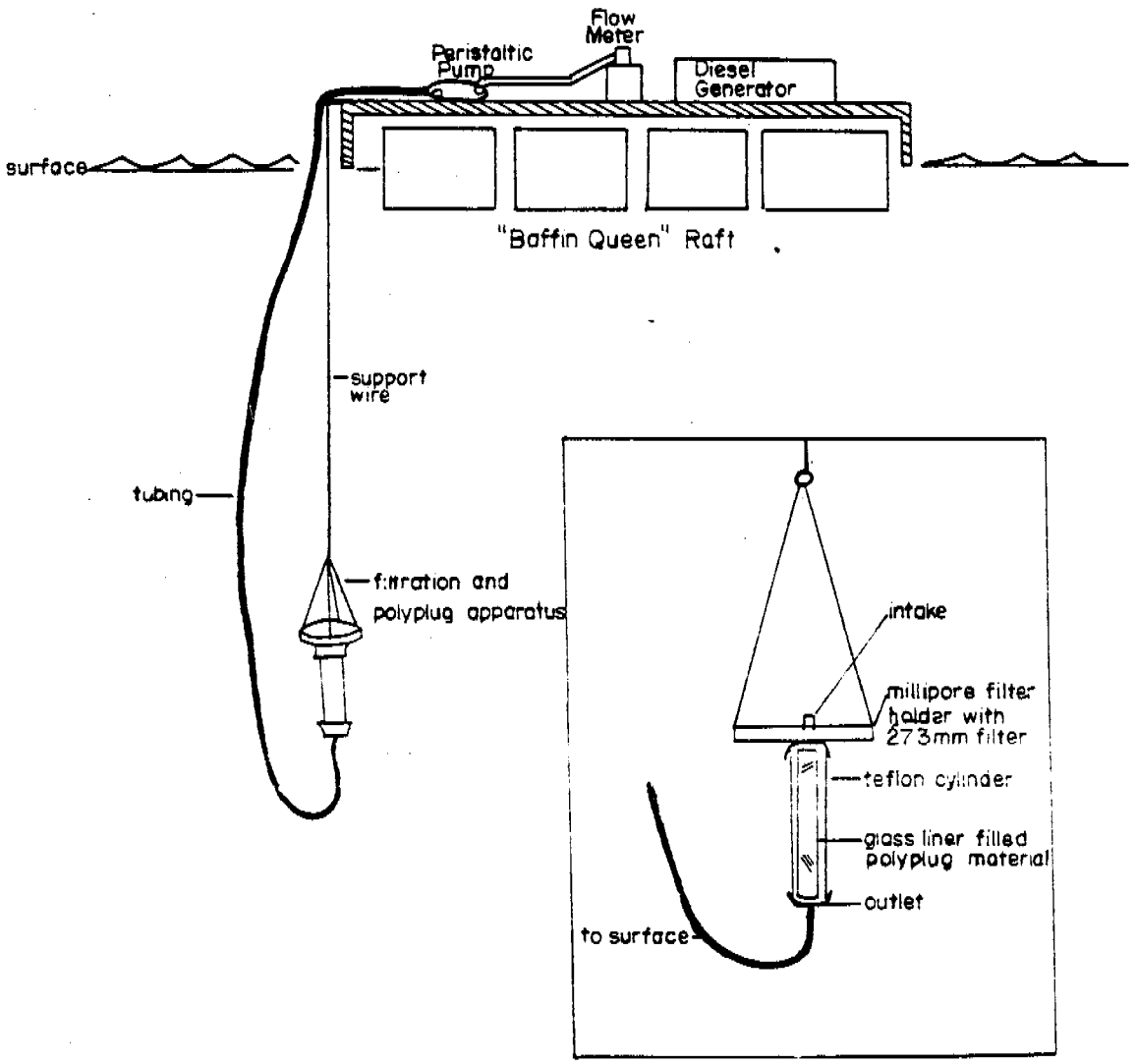

FIGURE 2.3: The large volume water sampler used for obtaining baseline hydrocarbon samples for gc/ms analyses.

TABLE 2.2

WATER SAMPLES
HYDROCARBON BASELINE STUDY

Date	Location	Depth (m)	Vol (approx)	Type of sampler	Type of analysis
14 June	Bay 9	1,5,10	4L	NBS	IR,UV/F
	Bay 10	1,5,10	4L	NBS	IR,UV/F
	Bay 11	1,5,10	4L	NBS	IR,UV/F
22 June	Bay 9	1,5,10	4L	NBS	not extracted
26 Aug	Bay 9	1,5,10	4L	NBS	IR,UV/F
	Bay 10	1,5,10	4L	NBS	IR,UV/F
	Bay 11	1,5,10	4L	NBS	IR,UV/F
20 Sept	Bay 9	1,5	4L	NBS	IR,UV/F
19 Sept	Bay 10	1,5,10	4L	NBS	IR,UV/F
18 Sept	Bay 11	1,5,10	4L	NBS	IR,UV/F
20 Sept	Bay 103	1,5	4L	NBS	IR,UV/F
07 Sept	Bay 10	1 m	210L	LVWS	GC/MS
19 Sept	Bay 10	1.5	20L	NBS	GC/MS
11 Sept	Bay 11	8 m	130L	LVWS	GC/MS
17 Sept	Bay 11	1,5 m	20L	NBS	GC/MS
20 Sept	Z-lagoon	1,5 m	20L	NBS	GC/MS

NOTE: 1. NBS = National Bureau of Standards sampler

2. LVWS = Large volume water sampler (Risebrough and de Lappe type).

2.2.2 Hydrocarbon Baseline Sediment Samples

In June, 23 core and 16 grab samples were collected from Ragged Channel, Z-lagoon, and Eclipse Sound, primarily by B. Barrie and J.M. Sempels. The cores were stored and shipped frozen in their plexiglass liners, thawed and subsampled in the ERCO Hydrocarbon Laboratory by D.R. Green, then re-frozen until analyzed. The grab samples were kept frozen until analyzed. After subsampling, some portion of thirteen cores remained for future analysis, in addition to three undisturbed cores.

In September (11-13th), another set of samples was collected for hydrocarbon baseline determinations. These samples were collected by divers with a polycarbonate tube into whirlpac bags which were sealed underwater. Six samples were collected from each of the three experimental bays in Ragged Channel, for a total of eighteen. Each sample was subdivided into four subsamples for IR, UV/F, GC/MS, and TOC (total organic carbon) analyses. The subsamples for hydrocarbon analyses were stored in solvent rinsed tins and jars, the TOC samples in Whirlpac bags. All were stored frozen until analysed. All of these samples are recorded in Table 2.3, and the sample locations are shown in Figures 2.4 and 2.5.

TABLE 2.3

SEDIMENT SAMPLES
HYDROCARBON BASELINE STUDY
JUNE, 1980

Location	Transect	Depth	Type	Size	I.D.	Subsampling
Ragged Channel						
Bay 9	N	7.4	C	23 cm	cc 18	IR & TOC surface
		18.5	C	39 cm	cc 17	GCMS - surface
	C	7.4	C	30 cm	cc 12	GCMS - surface
		14.3	C	38 cm	cc 13	Pb 210
	S	3.9	C	400 g	cc 14	IR & TOC
		14.0	C	26 cm	cc 15	IR & TOC-3 depths
		18.2	C	35 cm	cc 16	GCMS-3 depths
Bay 10	N	1.8	C	50 cm	cc 9	preserved
		11.9	C	43 cm	cc 10	GCMS - surface
	C	6.1	C	30 cm	cc 7	preserved
		10.4	C	43 cm	cc 8	IR & TOC-3 depths
		22.1	GRAB	400 g	GS 1	IR & TOC-surface
	S	5.1	C	26 cm	cc 11	GCMS - surface
		10.1.	GRAB	400 g	GS 2	IR & TOC-surface
		15.8	C	400 g	GS 3	preserved
Bay 13	N	2.7	C	32 cm	cc 2	IR & TOC-surface
		13.4	C	35 cm	cc 1	IR & TOC- 3x
	C	11.4	C	16 cm	cc 4	GCMS - surface
		16.0	C	45 cm	cc 3	PB 210
	S	3.6	C	19 cm	cc 5	GCMS - surface
		14.5	C	21.5 cm	cc 6	IR & TOC-surface
Eclipse Sound						
Bay 102		14.8	C	10 cm	cc 19	preserved
Z-lagoon						
Mid-lagoon		15.9	C	50 cm	Z-lagoon cc 21	Pb210, IR, TOC-3x
Bay 103		7.6	GRAB	800 g	GS 43	preserved
		10.1	GRAB	800 g	GS 39	preserved
		10.2	GRAB	800 g	GS 38	IR & TOC-surface
		10.6	GRAB	800 g	GS 37	preserved
Bay 104		6.5	GRAB	800 g	GS 27	preserved
		10.0	GRAB	800 g	GS 32	preserved

Location	Transect	Depth	Type	Size	I.D.	Subsampling
Bay 105		4.1	GRAB	1600 g	GS 4	IR & TOC-surface
			GRAB	800 g	GS 16	preserved
		9.2	C	50 cm	cc 20	GCMS - surface
Bay 106		5.1	GRAB	800 g	GS 60	preserved
		5.3	GRAB	800 g	GS 54	preserved
Bay 108			GRAB	800 g	GS 20	preserved
		12.4	C	50 cm	cc 22	preserved
Bay 109		3.3	GRAB	800 g	GS 52	preserved
		3.6	GRAB	800 g	GS 48	IR & TOC-surface
		10.2	GRAB	800 g	GS 45	preserved

SEDIMENT SAMPLES
HYDROCARBON BASELINE STUDY
SEPTEMBER, 1980

Location	Transect	Depth	Type	Size	I.D.	Subsampling
Bay 9	N	2-3	DIVER	300 g	9, N, 2-3 m	IR,UV/F,GC,TOC
		6-7	DIVER	300 g	9, N, 6-7 m	IR,UV/F,GC,TOC
	C	2-3	DIVER	300 g	9, C, 2-3 m	IR,UV/F,GC,TOC
		6-7	DIVER	300 g	9, C, 6-7 m	IR,UV/F,GC,TOC
	S	2-3	DIVER	300 g	9, S, 2-3 m	IR,UV/F,GC,TOC
		6-7	DIVER	300 g	9, S, 6-7 m	IR,UV/F,GC,TOC
Bay 10	N	2-3	DIVER	300 g	10, N, 2-3 m	IR,UV/F,GC,TOC
		6-7	DIVER	300 g	10, N, 6-7 m	IR,UV/F,GC,TOC
	C	2-3	DIVER	300 g	10, C, 2-3 m	IR,UV/F,GC,TOC
		6-7	DIVER	300 g	10, C, 6-7 m	IR,UV/F,GC,TOC
	S	2-3	DIVER	300 g	10, S, 2-3 m	IR,UV/F,GC,TOC
		6-7	DIVER	300 g	10, S, 6-7 m	IR,UV/F,GC,TOC
Bay 11	N	2-3	DIVER	300 g	11, N, 2-3 m	IR,UV/F,GC,TOC
		6-7	DIVER	300 g	11, N, 6-7 m	IR,UV/F,GC,TOC
	C	2-3	DIVER	300 g	11, C, 2-3 m	IR,UV/F,GC,TOC
		6-7	DIVER	300 g	11, C, 6-7 m	IR,UV/F,GC,TOC
	S	2-3	DIVER	300 g	11, S, 2-3 m	IR,UV/F,GC,TOC
		6-7	DIVER	300 g	11, S, 6-7 m	IR,UV/F,GC,TOC

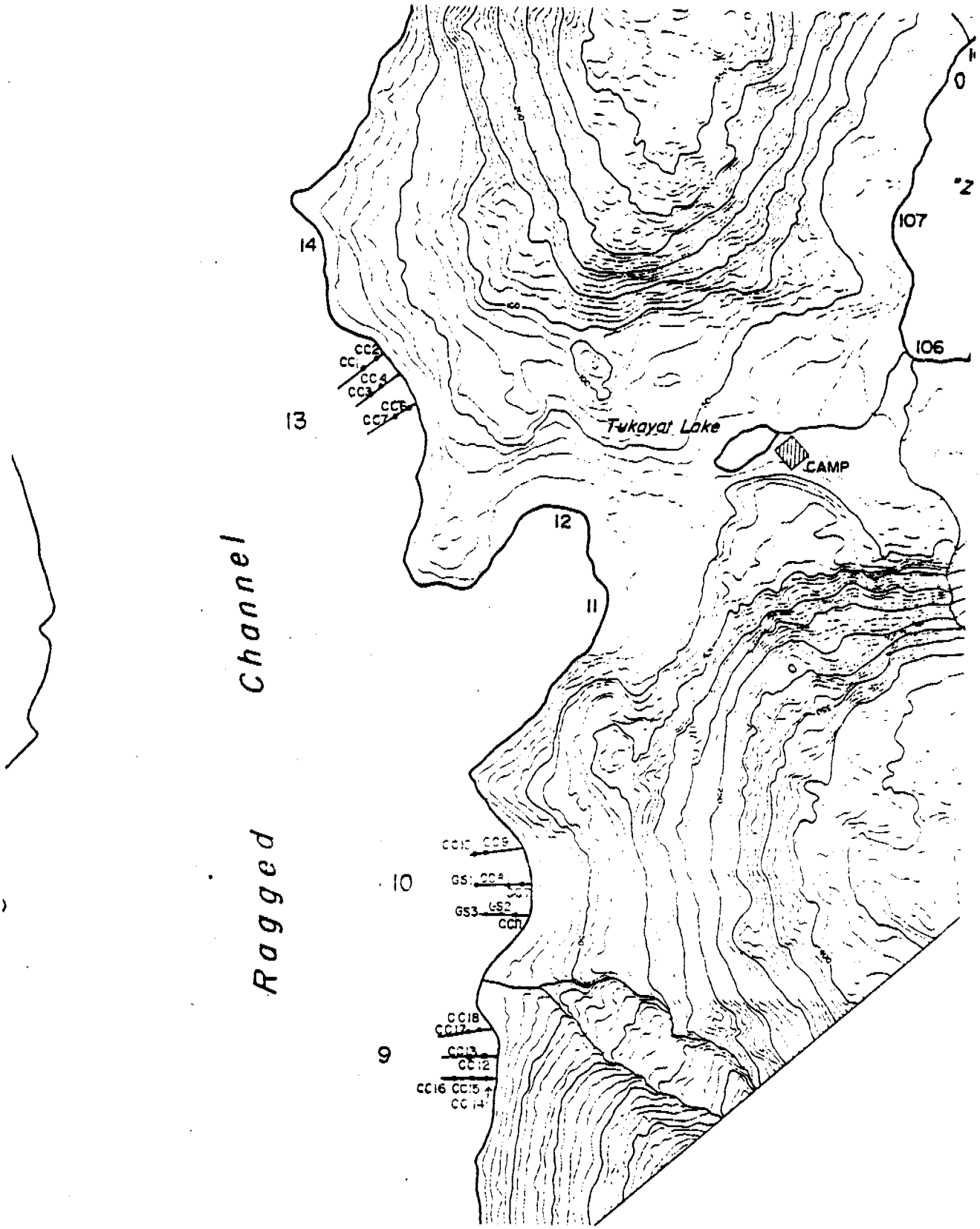

FIGURE 2.4: Locations of hydrocarbon baseline sediment samples taken from Ragged Channel, June, 1980.

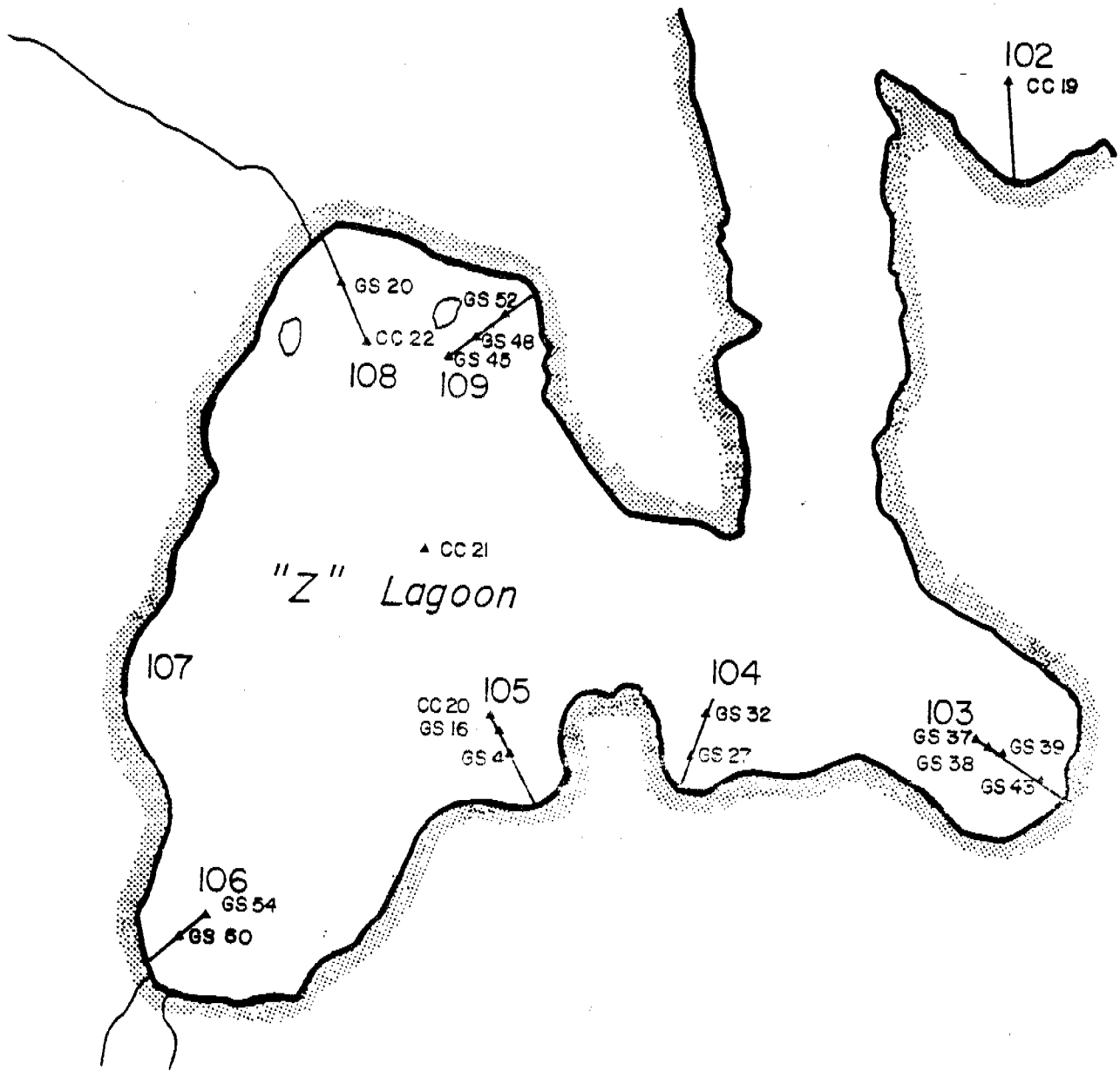

FIGURE 2.5: Locations of hydrocarbon baseline sediment samples taken from Z-Lagoon, June, 1980.

2.2.3 Hydrocarbon Baseline Beach Samples

A suite of samples was collected from along the beaches of the experimental bays in Ragged Channel. They were taken in triplicate for IR, UV/F, and GC/MS analysis from the high and low tide lines at each of three transects in the three experimental bays. (In addition, a set of samples was taken prior to the oiling experiments in Z-lagoon: these are summarized separately in the following section). These beach samples were collected with a trowel and consisted of about 200 grams of surface material for each sample. The samples were stored in solvent-rinsed tins and jars, and frozen until analyzed. All sampling was done on 22 August, 1980. This set of samples is summarized in Table 2.4.

TABLE 2.4

BEACH SAMPLES
HYDROCARBON BASELINE STUDY

Bay	Transect	Location	I.D.	Analyses
Bay 9	N	H	9-N-H	IR, UV/F, GCMS
		L	9-N-L	IR, UV/F, GCMS
	C	H	9-C-H	IR, UV/F, GCMS
		L	9-C-L	IR, UV/F, GCMS
	S	H	9-S-H	IR, UV/F, GCMS
		L	9-S-L	IR, UV/F, GCMS
Bay 10	N	H	10-N-H	IR, UV/F, GCMS
		L	10-N-L	IR, UV/F, GCMS
	C	H	10-C-H	IR, UV/F, GCMS
		L	10-C-L	IR, UV/F, GCMS
	S	H	10-S-H	IR, UV/F, GCMS
		L	10-S-L	IR, UV/F, GCMS
Bay 11	N	H	11-N-H	IR, UV/F, GCMS
		L	11-N-L	IR, UV/F, GCMS
	C	H	11-C-H	IR, UV/F, GCMS
		L	11-C-L	IR, UV/F, GCMS
	S	H	11-S-H	IR, UV/F, GCMS
		L	11-S-L	IR, UV/F, GCMS

NOTES:
1. H refers to high tide line, L to low tide line
2. UV/F and GC/MS samples delivered to ERCO
   IR samples to Seakem Oceanography Ltd.
3. Sampling date 22nd August, 1980

2.2.4 Hydrocarbon Baseline Tissue Samples

In June two sets of clam (Mya truncata) samples were collected by divers for baseline hydrocarbon and histopathology analysis. In September, a wide variety of organisms were collected for baseline hydrocarbon analysis only. The hydrocarbon samples were stored frozen; the histopathology samples were preserved in an alcohol solution. The samples are listed in Table 2.5.

TABLE 2.5

TISSUE SAMPLES
HYDROCARBON BASELINE STUDY

Sampling Period	Type of Organism	Species	Bay 9	Bay 10	Bay 11	Bay 13	Z-Lagoon	Comments
June	Clam	Mya truncata	1	1		1		for histo-pathology
	Clam	Mya truncata	1	1		1		
Sept.	Clam	Mya truncata	5	3	5		4	
		Serripes groen-landica	2		1		1	
	Starfish	Leptasterias polaris	6	3	5		3	
	Sea Urchin	Strongylocentrotus droebachiensis	4	2	6		4	
	Sea Cucumber	Psolus fabricii	1		3		1	
		Psolus sp.	3					
	Seaweed	Fucus resiculosus	1	1	1			
		Agarum sp.		1				
		Laminaria saccharina		1			1	
	Sculpin	Myoxocephalus scorpius	1				1	
	Tunicate	Rhizomolgula globularis	1	1	1		1	for vanadium analysis

Notes:

1. For smaller organisms (e.g. Mya) each sample consists of at least 10 individuals.
2. For larger organisms, each sample consists of at least 100 g tissue.

2.3. Shoreline Experiment

The shoreline experiment was conducted primarily by Woodward-Clyde Consultants, and is reviewed only briefly here. Four pairs of plots were oiled for experimental purposes: two intertidal sets, one low energy (Bay 103) and one high energy (Bay 102); and two corresponding backshore sets of plots. Each paired set of plots consisted of a 4 x 10 m test area to which 2 barrels of crude oil were applied either as 100% aged crude, or as a 50% aged crude/water emulsion, giving a 1 - 2 cm thick layer of crude. A summary of the test plots is given in Table 2.6.

Samples were taken from each test plot at various times following oil application and analyzed for total hydrocarbon content. Additional samples were taken from each test plot for analysis by GC/MS to determine the weathering characteristics of the various fractions of the oil. Table 2.7 shows the sampling scheme, which can be summarized as follows:

2.3.1 Total Hydrocarbon Samples

One 4 cm core sample was taken from each plot before the spill to measure background oil content. After the spill, samples were taken from each test plot: immediately after the spill, and at 2, 4, and 8 days after the spill. The post-spill samples were taken on each plot in 9 locations: 3 in each of the upper, middle, and lower sections of the plot which were mixed to provide one composite for each of the three sections. These samples consisted of a surface (0 - 2 cm) component and sub-surface component (4 - 8 cm).

2.3.2 GC/MS Samples

A single composite surface sample was taken from each test plot on days 1, 2, 4, 8, and 16 following oil application for GC/MS analysis.

2.3.3 Water Samples

A suite of water samples was also taken for IR and UV/F analysis in conjunction with the shoreline experiment. These are summarized in Table 2.8.

TABLE 2.6

SUMMARY OF SHORELINE EXPERIMENT TEST PLOTS

Test Plot I.D.	Test Area (m^2)	Location	Site Description	Type of Oil Spilled	Spill Date
H1	40	Bay 102	Upper intertidal open coast, high energy	aged crude	23 Aug
H2	40	Bay 102	"	50% water/oil emulsion	23 Aug
L1	40	Bay 103	Upper intertidal Z-lagoon, low energy	aged crude	21 Aug
L2	40	Bay 103	"	50% water/oil emulsion	22 Aug
LT1	40	Crude Oil Point	Control plot, backshore area	aged crude	20 Aug
LT2	40	Crude Oil Point	"	50% water/oil emulsion	20 Aug
HT1	4	Bay 102	Control plot, backshore area	aged crude	23 Aug
HT2	4	Bay 102	"	50% water/oil emulsion	23 Aug

TABLE 2.7

SUMMARY OF SAMPLING SCHEME FOR OILED PLOTS

Test Plot	Location in Plot	Before Test	Immediately After Spill	1 Day	2 Days		4 Days		8 Days		16 Days
H-1	Upper Mid Lower	IR, UV/F GC/MS	1 A,B 2 A,B 3 A,B	GC 1	4 A,B 5 A,B 6 A,B	GC2	37 A,B 38 A,B 39 A,B	GC 3	55 A,B 56 A,B 57 A,B	GC 4	GC 5
H-2	Upper Mid Lower	IR, UV/F GC/MS	7 A,B 8 A,B 9 A,B	GC 6	10 A,B 11 A,B 12 A,B	GC 7	40 A,B 41 A,B 42 A,B	GC 8	58 A,B 59 A,B 60 A,B	GC 9	GC 10
L-1	Upper Mid Lower	IR,UV/F GC/MS	13 A,B 14 A,B 15 A,B	GC 11	16 A,B 17 A,B 18 A,B	GC 12	43 A,B 44 A,B 45 A,B	GC 13	61 A,B 62 A,B 63 A,B	GC 14	GC 15
L-2	Upper Mid Lower	IR,UV/F GC/MS	19 A,B 20 A,B 21 A,B	GC 16	22 A,B 23 A,B 24 A,B	GC 17	46 A,B 47 A,B 48 A,B	GC 18	64 A,B 65 A,B 66 A,B	GC 19	GC 20
LT-1	Upper Mid Lower	IR,UV/F GC/MS	23 A,B 26 A,B 27 A,B	GC 21	28 A,B 29 A,B 30 A,B	GC 22	49 A,B	GC 23	67 A,B	GC 24	GC 25
LT-2	Upper Mid Lower	IR,UV/F GC/MS	31 A,B 32 A,B 33 A,B	GC 26	34 A,B 35 A,B 36 A,B	GC 27	52 A,B	GC 28	70 A,B	GC 29	GC 30

TABLE 2.7

SUMMARY OF SAMPLING SCHEME FOR OILED PLOTS

Test Plot	Location in Plot	Before Test	Immediately After Spill	1 Day	2 Days		4 Days		8 Days		16 Days
HT-1			201 A,B	GC 40	203 A,B	GC 42	205 A,B	GC 44	207 A,B	GC 46	GC 48
HT-2			202 A,B	GC 41	204 A,B	GC 43	206 A,B	GC 45	208 A,B	GC 47	GC 49

NOTES:
1. A denotes a surface (0-2 cm) sample
2. B denotes a sub-surface (4-8 cm) sample
3. Numbered samples are for IR (total hydrocarbon) analysis
4. See text for description of how samples were collected.

TABLE 2.8

WATER SAMPLES
SHORELINE EXPERIMENT

DATE	LOCATION	TEST PLOT	DEPTH	ANALYSIS	COMMENTS
18 Aug	Bay 102	H1 & H2	1,4 m	IR, UV/F	prespill
20 Aug	Bay 103	L1 & L2	1,5 m	IR, UV/F	pre-spill
20 Aug	Crude Oil Pt.	LT1 &LT2	1,10 m	IR, UV/F	pre-spill
21 Aug	Bay 103	L1	1,10 m	IR, UV/F	post-spill
20 Sept	Bay 103	L1 & L2	1,5 m	IR, UV/F	post-spill
20 Sept	Crude Oil Pt.	LT1 & LT2	1,5 m	GC/MS	post-spill

3. METHODS

Each of the methods used for the various environmental chemistry and hydrocarbon determinations are outlined below together with comments on intercalibrations or other verification of the methods where appropriate.

3.1 Environmental Chemistry: Water Analyses

3.1.1 Temperature and Salinity

These parameters are the domain of the physical oceanographic component of the B.I.O.S. project. However, to aid in interpretation of results, an effort was made to provide temperature and salinity readings at each of the stations and depths at which samples were collected. Three different methods of temperature measurement were used: a mercury thermometer placed in the Niskin sampler after recovery, a YSI Model 33 salinity-temperature meter, and an Applied Microsystems Model CTD-12 instrument. When quoting results, the order of preference for choosing readings was thermometer, CTD-12, YSI. For salinity, salinometer data provided by the Arctic Biological Station was used.

3.1.2 Oxygen

Two methods were used for measuring oxygen in seawater: a YSI oxygen probe and the Winkler titration method. The YSI oxygen probe was used directly in the Niskin sampler out in the field. The Winkler titration method was used for twenty-four samples as a check on the YSI probe. The Winkler titration is the standard oceanographic method for determination of oxygen and has a precision of at least 0.05 mg/L. The oxygen probe is considerably less precise. The probe is calibrated by measuring the atmospheric partial pressure of oxygen, which introduces variability, and the condition of the membrane is also a significant variable. Nevertheless, the two methods gave excellent agreement with a relative standard deviation of only 3.4%.

3.1.3 pH

pH was measured with a Sargent pH probe. Buffered standards of 4.02 and 7.40 pH were used before and after each set of samples to calibrate the probe. Determinations were made on subsamples in the field laboratory at room temperature. The probe was given about 5 minutes to stabilize before each reading.

3.1.4 Reactive Nitrate and Phosphate

20 mL nutrient samples were run on an auto-analyzer using standard procedures. The Technicon auto-analyzer and the operator were the same as used for thousands of analyses for the Patricia Bay Institute of Oceanography. The method has been extensively checked and intercalibrated with other laboratories over a period of several years.

3.1.5 Suspended Solids

Approximately 1.5L water samples were filtered through pre-baked, pre-weighed 47 mm GF/C filters. The filters were dried, weighed, ashed, and re-weighed. The difference between the dry weight and filter tare gives the total suspended solids. The difference between the ashed and dry weights is a measure of the organic suspended solids.

3.1.6 Dissolved Organic Carbon

5 mL subsamples of filtered water were added to precombusted glass ampoules, acidified with phosphoric acid, and purged with nitrogen to remove inorganic carbonate. The organics were then persulphate oxidized to carbon dioxide with heating to 130°C in a sealed ampoule. The carbon dioxide produced was measured in an Oceanography International total carbon infra-red gas analyzer. Analyses were done in quadruplicate with d-glucose standards. This method is similar to that described by Menzel and Vaccaro, 1964.

The results of this method were compared with the Arctic Biological Station results on duplicate Cape Hatt samples. Satisfactory agreement between the two methods was obtained.

3.1.7 Particulate Organic Carbon

Approximately 1.5L water were filtered through a pre-baked GF/C filter. The wet oxidation procedure described by Copin-Montegut and Copin-Montegut, 1973, was used. The method involves the addition of phosphoric acid to drive off chlorides, acid-dichromate oxidation to oxidize the available carbon, then back-titration with ferrous ammonium sulphate (diphenylamine indicator) to determine the amount of dichromate used in the oxidation of the carbon.

These results were compared with those of the Arctic Biological Station on similar samples from Cape Hatt, and agreement was not satisfactory. In looking for the source of the error, we determined that co-oxidation of chloride ion was a problem. An attempt was made to correct for chloride interference as per the graph in Figure 3.1. This gave good agreement for June samples, but serious discrepancies for August - September. An intercalibration between three laboratories was arranged, which showed satisfactory agreement between the Arctic Biological Station and the Patricia Bay Institute of Oceanography, with the Seakem titration method anomalously high (see Table 3.1). The intercalibration results were confusing, since the Seakem titration results from Cape Hatt were generally too low. Rather than pursue the matter further, the titration method was dropped as being too prone to interference, and the Arctic Biological Station results were adopted. Their method employs wet (persulphate) oxidation in a sealed ampoule, catalytic conversion of the carbon dioxide produced to methane, and determination of the methane using a gas chromatograph with a flame ionization detector.

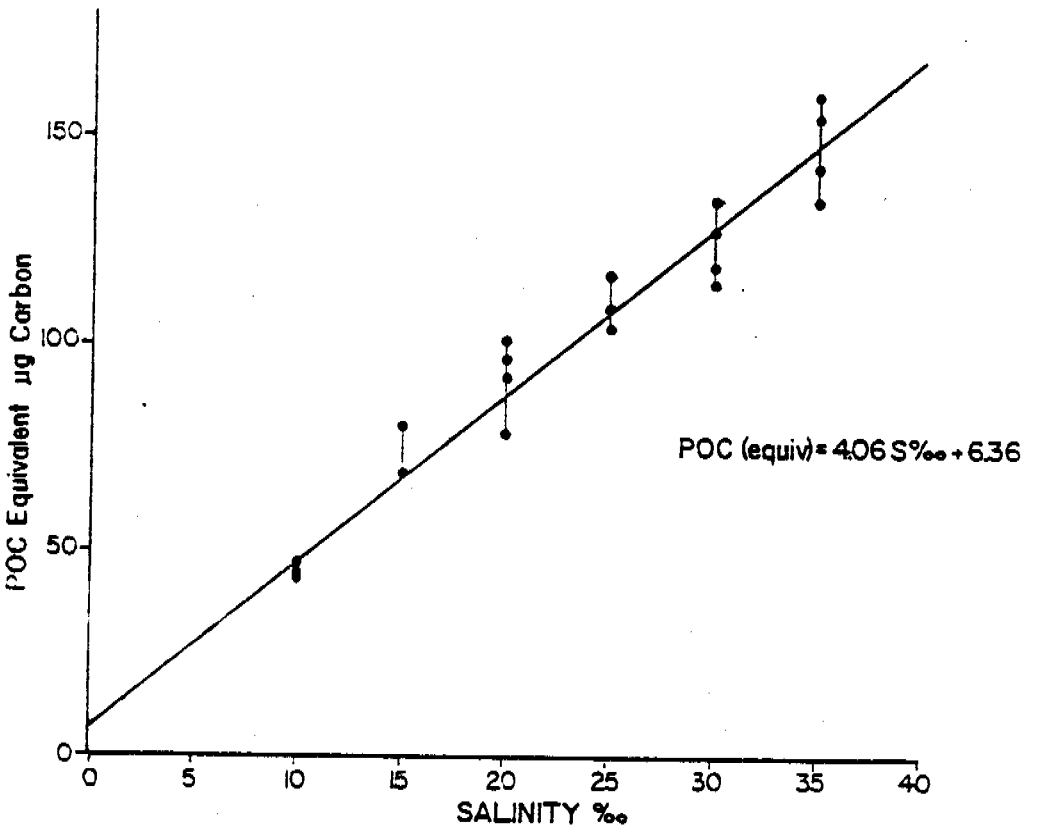

FIGURE 3.1: Graph showing the interference caused by salt in the determination of particulate organic carbon by the titration method. (Carbon-free water of varying salinity was filtered through GF/C filter papers. POC determinations on the filter papers gave the responses shown.)

TABLE 3.1

INTERCALIBRATION STUDIES

a) Chlorophyll

Laboratory	SAMPLE A		SAMPLE B	
	Chl. a $\mu g.L^{-1}$	Phaeo $\mu g.L^{-1}$	Chl. a $\mu g.L^{-1}$	Phaeo $\mu g.L^{-1}$
Seakem (immediate analysis)	1.14 ±0.09	1.38 ±0.11	1.00 ±0.06	0.85 ±0.06
Seakem (stored)	1.06 ±0.10	1.43 ±0.10	0.86 ±0.06	0.78 ±0.06
Institute of Ocean Sciences (stored)	0.79 ±0.06	1.65 ±0.12	0.74 ±0.06	1.13 ±0.08
Bedford Institute (stored)	1.79 ±0.09	1.45 ±0.11	1.81 ±0.14	0.56 ±0.08

Notes: Values are averages and standard deviations of 10 determinations.

b) Particulate Organic Carbon

Laboratory	SAMPLE A $\mu g.L^{-1}$	SAMPLE B $\mu g.L^{-1}$
Seakem[2]	98.7 ± 22.2	93.2 ± 21.0
Arctic Biological Station[3]	70.3 ± 74.7	42.8 ± 8.5
Institute of Ocean Sciences[4]	46.7 ± 19.8	33.1 ± 2.8

Notes:

1. Values are averages and standard deviations of 6 determinations.
2. Wet oxidation, back titration method, corrected for chloride interference.
3. Wet oxidation, catalytic conversion of CO_2 to methane, gas chromatograph FID detector
4. Dry oxidation, measurement of CO_2 with a gas chromatograph thermal conductivity detector.

3.1.8 Chlorophyll a and Phaeopigments

500 - 1000mL water samples were filtered through GF/C filters. The filters were stored frozen, ground in the laboratory, and extracted into acetone. The acetone slurry was filtered, and the chlorophyll content determined fluorometrically. The method is essentially that of Strickland and Parsons, 1972. Phaeopigments were also determined by the addition of acid and remeasurement of the fluorescence.

To check the validity of the chlorophyll determinations, the Turner fluorometer was recalibrated, and an intercalibration exercise was conducted with the Bedford Institute of Oceanography and the Patricia Bay Institute of Oceanography. The results of this intercalibratrion are given in Table 3.1. The Seakem analyses fall between the two sets of data from the oceanographic institutes, and are in reasonably close agreement with the Patricia Bay Institute of Ocean Sciences. Communication is continuing to determine why the Bedford results differ so markedly.

3.1.9 Total Nitrogen

The method used was that of Koroleff, 1976. In his review of methods available for determining total nitrogen in seawater, he points out that: "The determination of total and organic nitrogen is one of the most difficult tasks in marine chemistry". We concur with his comment after spending two weeks experimenting with his method in an attempt to get meaningful measurements of total nitrogen. After correcting blank problems by improved purification of the persulphate oxidant (triple recrystallization) and more careful regulation of autoclaving temperatures, the values obtained for total nitrogen were erratic and anomalously low, often less than the nitrate values.

In July, 1980, Solorzano and Sharp published a paper that identified various problems with the Koroleff method. They began their paper with the comment:

"Our understanding of dissolved organic nitrogen in the sea is poor largely because of analytical inadequacies. The determination has been hampered by technical difficulties due to uncertainly of the chemical structures of most of the organic components, the inability of existing methods to quantify some of the nitrogen compounds, and the lack of a simple, reliable, and inexpensive method for routine use".

They identified the following problems with the method:

a) Clogging of Cd-Cu nitrate reduction columns due to a precipitate released from the boro-silicate glass vessels by the oxidizing, alkaline conditions. Teflon vessels avoided the problem.

b) Insufficient base: The critical pH for complete oxidation of nitrogen compounds was found to be 10. Koroleff's method resulted in lower pH's leading to incomplete recoveries (~75%). 1.5M NaOH was recommended instead of 0.12 M as used by Koroleff.

c) Dilution factor: The dilution factor in the Koroleff procedure is about 5x which increases the scatter of the results. Dilution by 1.32x was recommended.

d) Standard: Urea instead of EDTA was recommended.

Unfortunately this paper arrived after the June analyses were complete. Analyses of seawater for total nitrogen was discontinued after the June sampling period, since the level of interest in the measurements did not warrant further experimentation with the method.

3.2 Environmental Chemistry: Sediment Analyses

3.2.1 Total Organic Carbon

1 - 5 g subsamples were required. The samples were dried, oxidized with a known amount of potassium dichromate, and the dichromate back-titrated with ferrous ammonium sulphate. This is essentially the well-known Walkley-Black method, to which there are many references including Gaudette, 1974.

The method was checked for interference by chloride ion. The chloride concentration in the sediments was measured and ranged between 2 and 10°/oo for most samples, resulting in a chloride correction of 1 to 5% to the total organic carbon determinations. This correction has not been applied to the results reported in the next section since it is relatively small, not very significant next to the subsampling errors, and not usually considered in other marine applications.

The August-September samples were analyzed in triplicate to give an estimate of the variability between subsamples.

3.2.2 Interstitial Nitrate and Phosphate

Sufficient sediment was pressed in a Reeburgh-type sediment press to produce 10-20 mL interstitial water. These samples were then analyzed by standard autoanalyzer methods, although it was found necessary to add two drops of concentrated HCl to the samples to dissolve iron precipitates.

3.2.3 Total Nitrogen

Total nitrogen in six sediment samples from the June sampling period were determined by the standard micro-Kjehdahl method (Black et al., 1965).

3.2.4 Lead-210

These samples which are used to 'date' the sediments, were analyzed under subcontract by CEP Inc., Santa Fe, New Mexico, who used the 'bismuth ingrow' technique (Koide et al., 1972).

3.3 Hydrocarbon Baseline Study

3.3.1 Water Samples: IR Analyses

Water samples were extracted in the field laboratory with 3 x 75 mL Freon 113. The extractions were done in the sample containers by shaking for 3 minutes in a paint shaker. The extracts were roto-evaporated to near dryness, made up to a known volume, and the $-CH_2-$ stretching peak at 2930 cm^{-1} measured with a Perkin-Elmer model 457 grating infra-red spectrophotometer. The peaks were quantified by comparison with a standard curve for Lagomedio crude.

3.3.2 Sediment and Beach Samples: IR Analyses

Approximately 60 g wet sediment samples were dried and extracted for 5 minutes with 3 x 40 mL Freon 113, in an ultrasonic bath. The solvent was recovered by filtration, roto-evaporated to near dryness, and made up to a known volume. The $-CH_2-$ stretch peak at 2930 cm^{-1} was measured and compared to a standard curve for Lagomedio crude. This determination was referred to as a measure of total extractables.

Because of the high organic content of the sediment relative to water, an additional columning step was necessary to remove the polar hydrocarbons. Florisil mini-columns consisting of 0.70 g of 5% deactivated Florisil in disposable pipettes were made up. The sample was added to the column as 200 µL concentrate, eluted with 2 bed volumes (1.8 mL) Freon, and the peak at 2930 cm^{-1} remeasured to determine the hydrocarbon content.

3.4 Shoreline Experiment

3.4.1 Total Hydrocarbons

Total hydrocarbon content of oiled sediment samples taken from the beach plots were determined as follows: 50 g subsamples were dried, extracted with 3 x 40 mL Freon by ultrasonification, the Freon recovered by filtration and roto-evaporated to dryness, and the extracted hydrocarbons determined both gravimetrically and by infrared spectrophotometry. The two methods correlated extremely well, with a correlation coefficient of 0.991.

4. RESULTS

4.1 Environmental Chemistry: Water Samples

The results of all of the environmental chemistry water samples are summarized in Table 4.1. Some additional melt pool and under-ice samples were collected out of general interest, and analyses of these samples are presented in Table 4.2 for comparison purposes.

TABLE 4.1: ENVIRONMENTAL CHEMISTRY: WATER ANALYSES

	Date	Location	Depth m	Temp. °C	Salinity °/oo	Dissolved Oxygen mg.L⁻¹	pH	Reactive Nitrate µg.at.L⁻¹	Reactive Phosphate µg.at.L⁻¹	Suspended Solids (organic) mg.L⁻¹	Suspended Solids (inorganic) mg.L⁻¹	Dissolved Organic C mg.L⁻¹	Particulate Organic C µg.L⁻¹	Chloro-phyll a µg.L⁻¹	Phaeo-pigment µg.L⁻¹
1	06/06/80	H1	1	-1.7	32.8		7.7	7.8	1.31	-	-	1.13	20	0.03	0.02
2			5	-1.7	33.1		7.7	8.3	1.28	-	-	1.23	20	0.02	0.02
3			10	-1.7	32.8		7.7	8.0	1.33	-	-	1.44	10	0.02	0.03
4		H2	1	-1.7	32.8		7.7	7.8	1.36	-	-	1.49	10	0.03	0.02
5			5	-1.7	33.0		7.7	7.8	1.30	-	-	1.56	10	0.05	0.04
6			10	-1.7	33.0		7.7	8.1	1.31	-	-	1.50	20	0.02	0.03
7	08/06/80	H3	1	-1.7	32.7		7.7	7.9	1.31	-	-	1.47	80	0.04	0.03
8			5	-1.7	32.7		7.6	7.7	1.52	-	-	1.03	40	0.02	0.04
9			10	-1.7	32.7		7.7	9.2	1.72	-	-	1.15	80	0.01	0.03
10		H4	1	-1.7	32.7		7.6	7.5	1.31	-	-	1.24	20	0.02	0.03
11			5	-1.7	32.7		7.7	7.8	1.29	-	-	0.92	10	0.02	0.03
12			10	-1.7	32.7		7.7	8.2	1.35	-	-	1.38	10	0.02	0.03
13	10/06/80	H5	1		32.6		7.6	7.9	1.32	-	-	1.28	50	0.03	0.04
14			5		32.7		7.6	8.0	1.32	-	-	1.28	10	0.04	0.03
15			10		32.7		7.7	8.2	1.31	-	-	1.03	50	0.06	0.04
16		H6	1		32.8		7.7	8.5	1.29	-	-	1.04	30	0.05	0.04
17			5		32.8		7.6	8.3	1.31	-	-	1.23	40	0.04	0.03
18			10		32.8		7.7	8.3	1.28	-	-	1.34	30	0.03	0.03

#	Date	Location	Depth m	Temp. °C	Salinity °/oo	Dissolved Oxygen mg.L^{-1}	pH	Reactive Nitrate µg.at.L^{-1}	Reactive Phosphate µg.at.L^{-1}	Suspended Solids (organic) mg.L^{-1}	Suspended Solids (inorganic) mg.L^{-1}	Dissolved Organic C mg.L^{-1}	Particulate Organic C µg.L^{-1}	Chloro-phyll a µg.L^{-1}	Phaeo-pigment µg.L^{-1}
19	12/06/80	H1	1	-1.7	32.7		7.9	8.1	1.35	-	-	1.12	60	0.04	0.04
20			5	-1.8	32.8		8.0	8.0	1.33	-	-	1.32	30	0.04	0.04
21			10	-1.8	32.7		8.0	8.0	1.32	-	-	1.17	40	0.03	0.03
22		H2	1	-1.7	32.4		8.0	8.2	1.29	-	-	1.26	10	0.03	0.03
23			5	-1.7	32.8		8.0	8.0	1.33	-	-	1.28	30	0.03	0.02
24			10	-1.7	32.7*		8.0	8.0	1.28	-	-	1.26	10	0.02	0.02
25	14/06/80	H3	1	-1.5	32.4		7.8	7.8	1.21	-	-	-	130	0.05	0.05
26			5	-1.7	32.7		7.8	7.9	1.29	-	-	-	50	0.04	0.02
27			10	-1.7	32.8		7.8	6.9	1.32	-	-	1.00	30	0.04	0.04
28		H4	1	-0.7	29.9		7.8	7.0	1.31	-	-	1.67	110	0.07	0.10
29			5	-1.8	32.4		7.8	7.6	1.26	-	-	1.01	20	0.04	0.05
30			10	-1.7	32.8		7.8	7.9	1.28	-	-	0.99	20	0.03	0.03
31	16/06/80	H5	1	-0.9	30.6		7.9	7.4	1.24	0.72	3.08	1.24	100	0.06	0.08
32			5	-1.7	32.5		7.9	8.0	1.33	0.39	1.72	1.04	10	0.03	0.03
33			10	-1.7	32.8		7.9	8.0	1.31	0.27	1.20	1.12	20	0.02	0.03
34		H6	1	-1.2	32.0		8.0	7.0	1.15	0.56	1.98	-	70	0.15	0.09
35			5	-1.8	32.7		8.0	8.2	1.31	0.44	1.20	0.83	10	0.03	0.02
36			10	-1.8	32.8		8.0	8.1	1.31	0.37	1.48		80	0.03	0.03

* indicates salinity value taken from YSI field instrument.

#	Date	Location	Depth m	Temp. °C	Salinity °/oo	Dissolved Oxygen mg.L^{-1}	pH	Reactive Nitrate µg.at.L^{-1}	Reactive Phosphate µg.at.L^{-1}	Suspended Solids (organic) mg.L^{-1}	Suspended Solids (inorganic) mg.L^{-1}	Dissolved Organic C mg.L^{-1}	Particulate Organic C µg.L^{-1}	Chloro-phyll a µg.L^{-1}	Phae pigm µg.L
37	18/06/80	H1	1	-1.1	31.4		7.6	7.6	1.27	-	-	1.53		0.08	0.0
38			5	-1.7	32.5		7.6	8.0	1.31	-	-	1.35		0.07	0.0
39			10	-1.7	32.5		7.6	8.0	1.33	-	-	2.05		0.03	0.0
40		H2	1				7.6	7.8	1.31	-	-	1.96		0.18	0.0
41			5				7.6	8.0	1.30	-	-	1.89		0.09	0.0
42			10				7.6	8.0	1.33	-	-	2.04		0.05	0.0
43	20/06/80	H3	1				7.8	7.9	1.19	0.52	2.64	1.16		0.05	0.0
44			5				7.7	8.0	1.30	0.40	1.38	1.03		0.06	0.0
45			10				7.7	8.0	1.32	0.57	2.11	1.16		0.06	0.0
46		H4	1				7.8	7.7	1.24	0.39	1.62	1.15		0.04	0.0
47			5				7.8	7.8	1.35	0.37	1.67	0.90		0.03	0.0
48			10				7.8	8.0	1.27	0.29	1.36	1.16		0.03	0.0
49	22/06/80	H5	1				-	6.8	1.10	1.09	2.58	1.08		0.21	0.0
50			5				-	7.8	1.28	0.30	0.36	1.09		0.15	0.0
51			10				-	8.2	1.27	0.49	1.02	1.10		0.12	0.0
52	11/08/80	H1	1	4.8	14.0		-	0.5	0.29	0.46	1.10	2.64	150	0.10	0.0
53			5	3.2	22.5		-	0.0	0.52	0.51	0.69	1.60	110	0.26	0.1
54			10	1.3	30.0		-	0.1	0.77	0.74	1.30	1.87	210	0.31	0.2

#	Date	Location	Depth m	Temp. °C	Salinity °/oo	Dissolved Oxygen mg.L^{-1}	pH	Reactive Nitrate µg.at.L^{-1}	Reactive Phosphate µg.at:L^{-1}	Suspended Solids (organic) mg.L^{-1}	Suspended Solids (inorganic) mg.L^{-1}	Dissolved Organic C mg.L^{-1}	Particulate Organic C µg.L^{-1}	Chloro-phyll a µg.L^{-1}	Phaeo pigmer µg.L^{-}
55		H2	1	4.6	14.5		-	0.2	0.28	0.76	2.38	1.57	100	0.09	0.06
56			5	3.1	23.4		-	0.0	0.44	0.50	0.88	1.60	130	0.20	0.14
57			10	1.2	30.3		-	0.1	0.72	0.69	0.88	2.03	240	0.56	0.25
58	13/08/80	H3	1	4.6	18.1	10.4	7.8	0.2	0.36	0.83	1.66	2.33	140	0.27	0.24
59			5	3.9	22.0	10.5	7.7	0.7	0.47	0.52	0.62	1.98	150	0.34	0.35
60			10	2.2	27.2	11.7	7.6	0.3	0.64	0.67	0.59	1.81	260	0.54	0.38
61		H4	1	4.5	17.6	10.5	7.7	0.2	0.37	0.61	0.87	1.58	190	0.24	0.20
62			5	3.9	21.3	10.3	7.7	0.7	0.47	0.77	1.59	1.64	140	0.33	0.28
63			10	2.6	26.5	10.4	7.8	0.3	0.65	0.76	0.87	3.33	210	0.50	0.34
64	15/08/80	H5	1	3.9	16.0	12.3	8.0	1.0	0.34	0.56	0.59	2.07	130	0.14	0.10
65			5	2.5	25.7	12.7	7.8	0.3	0.58	0.61	0.61	1.59	190	0.38	0.35
66			10	1.8	29.9	13.8	7.3	0.2	0.73	0.52	-	1.62	210	0.55	0.44
67		H6	1	4.4	15.7	12.1	7.7	0.9	0.31	0.42	0.27	1.50	280	0.25	0.15
68			5	2.8	24.9	11.6	7.8	0.7	0.51	0.52	0.67	1.30	130	0.33	0.26
69			10	1.8	29.9	12.4	7.4	0.1	0.74	0.53	1.10	2.99	190	0.40	0.42
70	19/08/80	H1	1	3.8	22.6	11.0	7.6	0.7	0.49	0.53	0.54	1.73	150	0.57	0.51
71			5	3.2	24.3	12.3	7.5	-	-	0.56	0.92	2.33	190	0.74	0.79
72			10	2.3	28.8	13.0	7.5	0.2	0.74	0.49	-	2.92	160	0.60	0.59

#	Date	Location	Depth m	Temp. °C	Salinity °/oo	Dissolved Oxygen mg.L⁻¹	pH	Reactive Nitrate µg.at.L⁻¹	Reactive Phosphate µg.at.L⁻¹	Suspended Solids (organic) mg.L⁻¹	Suspended Solids (inorganic) mg.L⁻¹	Dissolved Organic C mg.L⁻¹	Particulate Organic C µg.L⁻¹	Chloro-phyll a µg.L⁻¹	Phaeo-pigment µg.L⁻¹
73		H2	1	3.8	23.4	12.6	7.6	0.3	0.48	0.67	1.02	1.61	170	0.60	0.60
74			5	3.2	24.3	13.0	7.4	0.0	0.52	0.76	1.03	3.17	210	0.72	0.71
75			10	2.4	29.0	12.8	7.4	0.1	0.71	0.65	0.91	1.77	210	0.24	0.24
76	21/08/80	H3	1	3.0	24.1	12.10*	7.7	0.1	0.44	0.64	1.24	2.54	150	0.15	0.16
77			5	2.4	26.6	12.27*	7.8	0.1	-	0.68	0.80	2.14	160	0.46	0.61
78			10	2.2	30.4	11.88*	7.8	0.2	0.84	0.58	0.91	2.96	170	0.39	0.53
79		H4	1	3.0	24.1	11.96*	7.7	0.1	0.39	0.62	0.79	4.14	150	0.27	0.23
80			5	2.4	26.4	12.18*	7.8	0.1	0.56	0.63	0.65	3.77	150	0.38	0.38
81			10	2.4	30.7	12.45*	7.8	0.2	0.78	0.63	0.77	-	140	0.43	0.56
82	23/08/80	H5	1	4.8	23.7	11.6	7.8	0.0	0.52	0.57	0.64	2.33	170	0.28	0.24
83			5	3.8	25.6	11.5	7.8	0.0	0.54	0.60	0.61	2.40	160	0.41	0.41
84			10	2.7	29.4	11.8	7.8	4.5	0.69	0.63	0.62	4.62	150	0.56	0.56
85		H6	1	4.1	24.5	11.7	7.7	-	-	0.66	0.73	3.29	180	0.21	0.23
86			5	3.7	25.2	11.7	7.8	0.1	0.50	0.51	0.58	1.63	230	0.46	0.39
87			10	3.0	29.0	11.9	7.8	0.1	0.68	0.64	0.92	2.29	210	0.48	0.60
88	28/08/80	H1	1	3.8	25.4	11.48*	7.9	0.0	0.53	0.46	0.69	3.39	140	0.42	0.53
89			5	3.3	26.4	11.79*	7.9	0.0	0.59	0.55	0.74	2.13	110	0.49	0.67
90			10	2.6	30.5	11.97*	7.9	0.5	0.81	0.48	0.68	2.11	100	0.46	0.66

* indicates oxygen analyses were done by Winkler titration (remainder by probe).

#	Date	Location	Depth m	Temp. °C	Salinity °/oo	Dissolved Oxygen mg.L-1	pH	Reactive Nitrate µg.at.L-1	Reactive Phosphate µg.at.L-1	Suspended Solids (organic) mg.L-1	Suspended Solids (inorganic) mg.L-1	Dissolved Organic C mg.L-1	Particulate Organic C µg.L-1	Chloro-phyll a µg.L-1	Phaeo-pigment µg.L-1
91		H2	1	3.8	25.5	11.49*	7.9	0.1	0.54	0.61	1.30	2.61	130	0.44	0.54
92			5	3.0	26.6	11.71*	7.9	0.1	0.48	0.56	1.04	2.63	180	0.54	0.59
93			10	2.5	29.8	11.88*	7.9	-	-	0.46	0.69	4.09	120	0.48	0.69
94	30/08/80	H3	1	4.1	21.0	11.80*	7.9	0.1	0.38	0.49	0.88	2.39	190	0.29	0.31
95			5	3.8	24.0	11.70*	7.9	0.0	-	0.43	-	2.98	210	0.43	0.55
96			10	3.4	27.1	11.54*	7.8	0.2	0.58	0.50	0.49	2.72	170	0.59	0.77
97		H4	1	4.2	21.1	11.78*	7.7	0.0	0.31	0.49	0.67		140	0.32	0.39
98			5	3.9	23.6	11.68*	7.9	0.0	0.44	0.57	1.11	2.75	140	0.45	0.55
99			10	3.4	27.4	11.54*	7.9	0.1	0.48	0.51	0.70	2.25	120	0.39	0.51
100	01/09/80	H5	1	4.6	22.8	11.80*	7.8	0.0	0.37	0.55	0.75	2.37	160	0.29	0.26
101			5	4.2	23.1	11.82*	7.8	0.0	0.49	0.57	0.72	2.42	240	0.37	0.50
102			10	3.3	27.0	11.76*	7.8	0.0	0.58	0.53	0.69	2.11	160	0.22	0.33
103		H6	1	4.6	19.6	11.85*	7.8	0.0	0.32	0.63	0.91	2.97	140	0.03	0.06
104			5	4.0	24.0	11.81*	7.8	0.2	0.50	0.62	1.03	3.56	190	0.42	0.66
105			10	3.3	27.4	11.71*	7.8	0.0	0.56	0.61	0.48	2.21	200	0.32	0.48
106	05/09/80	H1	1	4.5	20.1	-	7.7	0.0	0.36	0.51	1.14	2.08	150	0.26	0.20
107			5	3.2	26.8	-	7.8	0.0	0.62	0.50	1.09	2.50	170	0.21	0.30
108			10	2.0	30.5	-		0.3	0.76	0.52	1.31	1.97	170	0.52	0.79

* indicates oxygen analyses were done by Winkler titration (remainder by probe).

#	Date	Location	Depth m	Temp. °C	Salinity °/oo	Dissolved Oxygen ppm	pH	Reactive Nitrate µg.at.L⁻¹	Reactive Phosphate µg.at.L⁻¹	Suspended Solids (organic) mg.L⁻¹	Suspended Solids (inorganic) mg.L⁻¹	Dissolved Organic C mg.L⁻¹	Particulate Organic C µg.L⁻¹	Chloro-phyll a µg.L⁻¹	Phaeo-pigment µg.L⁻¹
109		H2	1	4.5	19.9	-	7.9	0.0	0.38	0.52	1.10	1.70	-	0.31	0.23
110			5	3.2	26.8	-	7.9	0.0	0.62	0.48	0.79	2.74	100	0.22	0.39
111			10	2.1	30.4	-	7.9	0.3	0.77	0.59	1.02	2.26	230	0.51	1.00
112	07/09/80	H3	1	4.4	19.5	11.5	-	0.1	0.33	0.57	1.41	1.64	140	0.30	0.40
113			5	3.9	24.8	11.4	-	0.2	0.60	0.51	1.07	2.28	120	0.39	0.58
114			10	2.0	30.8	11.6	-	0.1	0.74	0.60	1.23	3.94	160	0.87	0.84
115		H4	1	4.5	19.3	11.4	-	0.1	0.33	0.48	0.81	2.07	120	0.33	0.19
116			5	3.3	25.8	11.3	-	0.2	0.60	0.64	1.16	3.03	140	0.39	0.50
117			10	2.0	30.8	11.9	-	0.1	0.71	0.62	1.19	2.84	200	0.66	0.60
118	09/09/80	H5	1	3.2	25.5	10.6	7.8	0.2	0.53	0.58	0.84	1.64	120	0.62	0.42
119			5	3.1	25.6	10.5	7.9	0.2	0.54	0.58	0.73	1.85	140	0.57	0.50
120			10	2.5	30.6	11.0	7.9	0.4	0.75	0.77	0.86	1.84	210	2.28	0.85
121		H6	1	3.2	25.5	11.2	8.0	0.2	0.62	0.53	0.66	1.79	130	0.67	0.50
122			5	3.0	25.5	11.2	8.0	0.2	0.54	0.43	0.66	1.75	110	0.57	0.31
123			10	2.0	30.6	11.2	8.0	0.2	0.79	0.65	0.70	1.79	250	2.06	0.49
124	12/09/80	H1	1	2.4	27.6	-	7.6	0.0	0.58	0.68	1.17	2.58	150	0.51	0.36
125			5	2.5	27.9	-	7.7	0.0	0.59	0.70	1.56	2.73	170	0.53	0.37
126			10	2.2	28.7	-	7.7	0.0	0.60	0.69	1.33	1.99	200	0.53	0.37

#	Date	Location	Depth m	Temp. °C	Salinity ‰	Dissolved Oxygen mg.L-1	pH	Reactive Nitrate µg.at.L-1	Reactive Phosphate µg.at.L-1	Suspended Solids (organic) mg.L-1	Suspended Solids (inorganic) mg.L-1	Dissolved Organic C mg.L-1	Particulate Organic C µg.L-1	Chloro-phyll a µg.L-1	Phaeo-pigment µg.L-1
127		H2	1	-	27.7	-	7.8	0.0	0.62	0.59	1.46	2.92	130	0.44	0.39
128			5	-	27.7	-	7.8	0.1	0.55	0.66	1.52	2.99	180	0.45	0.40
129			10	-	28.7	-	7.8	0.0	0.54	0.55	1.20	2.41	150	0.53	0.40
130	14/09/80	H3	1	1.1	30.1	-	-	0.3	0.62	0.64	1.19	1.83	120	0.61	0.37
131			5	1.1	30.1	-	-	1.1	0.63	0.56	1.13	1.73	130	0.69	0.28
132			10	1.2	30.2	-	-	1.5	0.64	0.58	1.09	2.12	140	0.65	0.37
133		H4	1	0.8	30.0	-	-	0.4	0.63	0.51	1.04	4.52	160	0.67	0.38
134			5	1.0	30.1	-	-	1.7	0.65	0.49	0.74	2.23	120	0.72	0.36
135			10	1.0	30.2	-	-	0.7	0.66	0.53	1.15	1.84	180	0.60	0.32
136	16/09/80	H5	1	-0.1	30.0	-	7.7	0.3	0.63	0.56	0.93	1.47	120	0.72	0.26
137			5	-0.1	30.0	-	7.5	0.6	0.60	0.58	0.94	-	80	0.50	0.31
138			10	-0.1	30.0	-	7.6	2.1	0.60	0.71	0.88	1.72	160	0.48	0.28
139		H6	1	-0.1	30.0	-	7.6	0.1	0.60	0.65	0.57	2.47	100	0.57	0.30
140			5	-0.2	30.0	-	7.6	0.2	0.60	0.62	0.58	1.71	210	0.61	0.24
141			10	-0.2	30.0	-	7.6	1.8	0.61	0.59	0.83	1.78	180	0.68	0.23

TABLE 4.2

ENVIRONMENTAL CHEMISTRY: ICE BOTTOM AND MELT POOL SAMPLES

a) Chlorophyll a

Date	Location	Description	Chl a $\mu g.L^{-1}$	Phaeo-pigment $\mu g.L^{-1}$
19/06/80	Bay 13	ice bottom (#1A)	6.92	2.36
19/06/80	Bay 13	ice bottom (#1B)	5.19	2.65
19/06/80	Bay 13	ice bottom (#2A)	14.59	6.95
19/06/80	Bay 13	ice bottom (#2B)	9.61	10.57
19/06/80	Bay 13	ice bottom (#3A)	13.78	10.24
19/06/80	Bay 13	ice bottom (#3B)	11.60	11.50
21/06/80	Bay 9	ice bottom (#1)	1.48	0.07
21/06/80	Bay 9	ice bottom (#2A)	14.70	2.71
21/06/80	Bay 9	ice bottom (#2B)	12.19	1.96
21/06/80	Bay 10	ice bottom (#1A)	0.56	0.55
21/06/80	Bay 10	ice bottom (#1B)	0.86	0.54
21/06/80	Bay 10	ice bottom (#2)	0.34	0.06
21/06/80	Bay 10	ice bottom (#3)	0.46	0.10
22/06/80	Bay 11	melt pool (#1)	0.44	0.00
22/06/80	Bay 11	melt pool (#2)	0.34	0.07

b) Nutrients

Date	Location	Description	Nitrate $\mu g.L^{-1}$	Phosphate $\mu g.L^{-1}$
21/06/80	Bay 9	ice bottom (#1)	6.8	1.28
21/06/80	Bay 9	ice bottom (#2)	6.8	1.38
21/06/80	Bay 10	ice bottom (#1)	6.0	0.89
21/06/80	Bay 10	ice bottom (#2)	4.6	0.62
21/06/80	Bay 10	ice bottom (#3)	5.4	1.03
22/06/80	Bay 11	melt pool (#1)	3.0	0.24
		melt pool (#2)	2.4	0.23

4.2 Environmental Chemistry: Sediment Samples

4.2.1 Total Organic Carbon

The total organic carbon analyses of sediment and beach samples are summarized in Table 4.3.

4.2.2 Interstitial Nitrate and Phosphate

The results of interstitial water nutrient analyses are presented in Table 4.4. The core samples (June only) were, of necessity, frozen, thawed for sub-sampling at ERCO laboratories, the sub-samples refrozen, then thawed and pressed in the Seakem laboratories. In view of the repeated thawing and freezing, these data should be treated with caution.

4.2.3 Total Nitrogen Analyses

The total nitrogen contents of six sediment samples from the June sampling period are presented in Table 4.5.

4.2.4 Lead-210 Analyses

Three cores were selected for dating of the sediments at Cape Hatt. They were analysed for their lead-210 content, which can provide an estimate of the age of sediments up to about 100 years. The results are presented in Table 4.6.

TABLE 4.3

TOTAL ORGANIC CARBON ANALYSES
SEDIMENT SAMPLES
JUNE SAMPLING

Date	Location	Sample I.D.		TOC % dry weight
25/05/80	Bay 13	cc 1	0- 3 cm	0.55
			9-11 cm	0.68
			14-17 cm	0.67
25/05/80		cc 2	0- 2 cm	0.33
28/05/80		cc 6	3- 7 cm	0.73
30/05/80	Bay 10	cc 8	2- 4 cm	0.57
			10-12 cm	0.59
			16-18 cm	0.49
31/05/80		GS-1		0.61
03/06/80		GS-2		0.96
06/06/80	Bay 9	cc 14		0.31
06/06/80		cc 15	0- 2 cm	0.72
			8-10 cm	0.71
			15-17 cm	0.68
18/06/80		cc 18	0- 2 cm	0.70
11/06/80	Z-Lagoon	cc 21	0- 4 cm	0.55
			10-14 cm	0.55
			19-22 cm	0.58
			19-22 cm	0.49
13/06/80	Bay 103	GS-38		0.41
11/06/80	Bay 105	GS-4		1.02
14/06/80	Bay 109	GS-48		1.28
07/06/80	Bay 13	H2		0.93
08/06/80	Bay 10	H4		0.67
10/06/80	Bay 9	H5		0.77
12/06/80	Bay 13	H2		0.83
14/06/80	Bay 10	H4		0.76
16/06/80	Bay 9	H5		0.59
20/06/80	Bay 13	Dive Hole		0.76

Note: Sample cc 1, 0-3 cm was analyzed five times with a mean of 0.55%, standard deviation 0.03%.

TABLE 4.3 (continued)

TOTAL ORGANIC CARBON ANALYSES
SEDIMENT SAMPLES
AUGUST/SEPTEMBER SAMPLING

Date	Location	Sample I.D.	Depth	TOC	
				% dry weight $\bar{x}$	σ
21/08/80	Bay 10	H 3	11 m	2.15	0.06
		H 4	12 m	0.66	0.02
23/08/80	Bay 9	H 5	15 m	0.63	0.01
		H 6		0.68	0.01
02/09/80	Bay 11	H 1	7 m	0.42	0.08
		H 2	7 m	0.50	0.04
31/08/80	Bay 10	H 3	10 m	0.48	0.15
		H 4	10 m	0.82	0.07
02/09/80	Bay 9	H 5	7 m	0.30	0.01
		H 6	7 m	0.35	0.02
06/09/80	Bay 11	H 1	7 m	0.64	0.04
		H 2	7 m	0.71	0.04
07/09/80	Bay 10	H 3	7 m	0.29	0.01
		H 4	7 m	0.39	0.03
10/09/80	Bay 9	H 5	7 m	0.56	0.07
		H 6	7 m	0.37	0.02
13/09/80	Bay 11	H 1	7 m	0.89	0.11
		H 2	7 m	0.70	0.06
14/09/80	Bay 10	H 3	7 m	0.42	0.04
		H 4	7 m	0.54	0.02
15/09/80	Bay 9	H 5	7 m	0.27	0.03
		H 6	7 m	0.70	0.04

Note: Values are the mean and standard deviation of three replicate determinations

Date	Location	Transect	Depth	TOC	
				% dry weight	
				$\bar{x}$	σ
12/09/80	Bay 9	S	2-3 m	0.29	0.03
			6-7 m	0.29	0.04
		C	2-3 m	0.45	0.04
			6-7 m	0.36	0.04
		N	2-3 m	0.26	0.01
			6-7 m	0.53	0.01
13/09/80	Bay 10	S	2-3 m	0.14	0.01
			6-7 m	0.42	0.02
		C	2-3 m	0.18	0.01
			6-7 m	0.35	0.01
		N	2-3 m	0.31	0.02
			6-7 m	0.34	0.02
11/09/80	Bay 11	S	2-3 m	0.20	0.07
			6-7 m	0.43	0.07
		C	2-3 m	0.13	0.05
			6-7 m	0.51	0.03
		N	2-3 m	0.17	0.05
			6-7 m	0.43	0.09

Note: Values are the mean and standard deviation of three replicate determinations.

TABLE 4.3 (continued)

TOTAL ORGANIC CARBON ANALYSES
BEACH SAMPLES
AUGUST/SEPTEMBER SAMPLING

Date	Location	Transect	Beach Position	TOC % dry weight $\bar{x}$	TOC % dry weight σ
22/08/80	Bay 9	S	L	0.05	0.01
			H	0.07	0.02
		C	L	0.07	0.02
			H	0.04	0.01
		N	L	0.04	0.01
			H	0.058	0.001
22/08/80	Bay 10	S	L	0.022	0.003
			H	0.039	0.004
		C	L	0.29	0.03
			H	0.029	0.006
		N	L	0.20	0.03
			H	0.037	0.007
22/08/80	Bay 11	S	L	0.036	0.009
			H	0.147	0.009
		C	L	0.041	0.002
			H	0.033	0.01
		N	L	0.030	0.006
			H	0.079	0.002

Note: Values are the mean and standard deviation of three replicate determinations.

TABLE 4.4

NUTRIENT ANALYSES
INTERSTITIAL WATER SAMPLES

Sampling Date	Location	I.D.		Nitrate ug.at.L^{-1}	Phosphate ug.at.L^{-1}	Comments
07/06/80	Bay 13	H2		44	740	grab
08/06/80	Bay 10	H4		13	660	
10/06/80	Bay 9	H5		17	21	
12/06/80	Bay 13	H2		7.5	9.3	grab
20/06/80	Bay 13	dive hole		3.4	3.7	diver
21/06/80	Bay 10	dive hole		2.1	2.0	
22/06/80	Bay 9	dive hole		5.2	1.4	
25/05/80	Bay 13	cc 1	7- 9 cm	480	101	frozen core sample
			12-14 cm	48	17	
			17-19 cm	4.4	5.4	
25/05/80		cc 2	4- 6 cm	13	2.0	frozen core sample
28/05/80		cc 6	0- 3 cm	6.9	36	frozen core sample
30/05/80	Bay 10	cc 8	2- 4 cm	24	17	frozen core sample
			10-12 cm	1.7	8.0	
			16-18 cm	153	7.5	
06/06/80	Bay 9	cc 15	2- 4 cm	12.7	7.2	frozen core sample
			10-12 cm	56	13.7	
			19-21 cm	2.0	14.9	
23/08/80	Bay 9	H6		9.0	6.5	grab
02/08/80	Bay 11	H1		1.8	10.2	
		H2		5.5	8.6	
31/08/80	Bay 10	H3		2.4	7.4	
		H4		3.1	12.0	
02/08/80	Bay 9	H5		2.5	9.0	
		H6		1.1	20.1	

Sampling Date	Location	I.D.	Nitrate ug.at.L^{-1}	Phosphate ug.at.L^{-1}	Comments
06/09/80	Bay 11	N, 7 m	0.9	18.2	diver
		S, 7 m	1.6	24.5	
07/09/80	Bay 10	N 7 m	0.6	12.5	
		S, 7 m	0.5	13.0	
10/09/80	Bay 9	N, 7 m	0.5	13.4	
		S, 7 m	0.3	14.3	
		S, 7 m	0.3	15.5	
13/09/80	Bay 11	N, 7 m	1.9	27.7	diver
		S, 7 m	2.5	34.8	
14/09/80	Bay 10	N, 7 m	6.0	9.1	
		S, 7 m	8.8	20.0	
15/09/80	Bay 9	N, 7 m	4.0	11.6	
		S, 7 m	0.6	52.3	

TABLE 4.5

TOTAL NITROGEN ANALYSES
SEDIMENT SAMPLES

Sampling Date	Location	L.D.	% Total Nitrogen Mean	(Duplicates)
07/06/80	Bay 13	H2	0.21%	(0.21, 0.21)
08/06/80	Bay 10	H4	0.12%	(0.12, 0.12)
10/06/80	Bay 9	H5	0.11%	(0.11, 0.11)
12/06/80	Bay 13	H2	0.17%	(0.17, 0.17)
14/06/80	Bay 10	H4	0.10%	(0.10, 0.10)
16/06/80	Bay 9	H5	0.17%	(0.175, 0.17)

Note: Sediment samples for total nitrogen analyses were collected in the June sampling period only.

TABLE 4.6

LEAD-210 ANALYSES
CORE SAMPLES

Sampling Date	Bay	Core	Depth in Core	Lead-210 pCi/g
26/05/80	13	cc 3	0- 4 cm	1.4 ± 0.2
			7-11 cm	0.0 ± 0.1
			14-19 cm	0.3 ± 0.1
			21-25 cm	0.8 ± 0.2
			28-32 cm	0.2 ± 0.1
			35-39 cm	0.2 ± 0.1
			42-44 cm	0.3 ± 0.1
04/06/80	9	cc 13	0- 4 cm	0.0 ± 0.1
			7-10 cm	0.4 ± 0.1
			10-12 cm	1.0 ± 0.3
			14-16 cm	0.0 ± 0.1
			21-24 cm	0.0 ± 0.1
			28-30 cm	1.1 ± 0.3
			36-38 cm	0.0 ± 0.1
11/06/80	Z-Lagoon	cc 21	0- 2 cm	0.4 ± 0.1
			4- 7 cm	0.6 ± 0.1
			7- 9 cm	0.0 ± 0.1
			11-13 cm	0.4 ± 0.1
			14-16 cm	0.3 ± 0.1
			20-22 cm	0.6 ± 0.1
			25-27 cm	0.5 ± 0.1
			30-32 cm	0.6 ± 0.1
			36-38 cm	0.4 ± 0.1

Note: Analyses performed by Controls for Environmental Pollution, Inc., Santa Fe, New Mexico.

4.3 Hydrocarbon Baseline Study

4.3.1 Water Samples IR Analyses

The results of the infra-red analyses of seawater samples are given in Table 4.7.

4.3.2 Sediment Samples IR Analyses

The results of the IR analyses of sediment samples are given in Table 4.8. Both total extractable organics and non-polar hydrocarbons are reported (see Methods section for explanation).

4.3.3 Beach Samples IR Analyses

The results of the IR analyses of beach samples are given in Table 4.9.

TABLE 4.7

WATER SAMPLES: IR ANALYSES
HYDROCARBON BASELINE STUDY

Date	Location	Depth	Total Hydrocarbons µg.L-1	Comments
14/06/80	Bay 9	1 m 5 m 10 m	D.L. D.L. D.L.	June sampling
14/06/80	Bay 10	1 m 5 m 10 m	D.L. D.L. 126	
14/06/80	Bay 13	1 m 5 m 10 m	D.L. D.L. D.L.	
26/08/80	Bay 9	1 m 5 m 10 m	D.L. D.L. D.L.	August sampling
26/08/80	Bay 10	1 m 5 m 10 m	26 D.L. D.L.	
26/08/80	Bay 11	1 m 5 m	D.L. D.L.	
20/09/80	Bay 9	1 m 5 m	D.L. D.L.	Sept. sampling
19/09/80	Bay 10	1 m 5 m 10 m	D.L. D.L. 80	
18/09/80	Bay 11	1 m 5 m 10 m	D.L. 72 1138	 non-polar material

Date	Location	Depth	Total Hydrocarbons $\mu g.L^{-1}$	Comments
18/08/80	Bay 102	1 m 4 m	D.L. 62	prespill
18/08/80	Bay 103	1 m 7 m	D.L. D.L.	prespill
21/08/80	Bay 103	1 m 7 m	D.L. D.L.	prespill
20/09/80	Bay 103	1 m 5 m	150 D.L.	
20/09/80	Z-Lagoon (middle)	1 m 10 m	D.L. D.L.	

Note: 1. Units are in micrograms of Lagomedio crude oil equivalents per litre of sea water.

2. D.L. means the response is below the detection limit of 13 $\mu g.L^{-1}$.

TABLE 4.8

SEDIMENT SAMPLES: IR ANALYSES
HYDROCARBON BASELINE STUDY

Sampling Date	Bay	Transect	Sample I.D.		Water Content	Total Extractable Organics	Total Hydro-carbons
					%	$\mu g.g^{-1}$	$\mu g.g^{-1}$
06/06/80	9	S	cc 14		26.3	3.52	0.37
06/06/80		S	cc 15	0- 3 cm	24.3	4.48	0.65
				9-11 cm	30.6	2.44	0.14
				17-19 cm	27.7	2.59	0.41
18/06/80		N	cc 18	1- 3 cm	20.9	5.38	0.18
30/05/80	10	C	cc 8	1- 3 cm	28.7	4.79	1.66
				8-10 cm	29.9	1.87	0.16
				14-16 cm	19.8	1.42	0.14
31/05/80		C	GS 1		45.4	11.11	3.11
03/06/80		S	GS 2		72.5	11.43	1.35
25/05/80	13	C	cc 1	3- 7 cm	27.2	3.04	0.50
				11-13 cm	29.0	3.81	1.58
				16-18 cm	27.6	1.69	0.68
25/05/80		C	cc 2	1- 3 cm	13.7	1.66	0.14
28/05/80		S	cc 6	3- 7 cm	39.4	4.62	1.90
11/06/80	Z-lagoon	middle	cc 21	0- 4 cm	31.1	3.82	1.58
				10-14 cm	41.3	1.69	0.37
				19-22 cm	41.1	1.45	0.50
13/06/80	103		GS 38		44.1	5.58	1.72
11/06/80	105		GS 4		66.8	12.52	3.82
14/06/80	109		GS 48		52.5	6.92	1.18
12/09/80	9	S	9, S,	2-3 m	26.2	35.9	2.0
			9, S,	6-7 m	23.3	7.1	0.6
		C	9, C,	2-3 m	23.2	23.0	D.L.
			9, C,	6-7 m	25.8	22.4	0.9
		N	9, N,	2-3 m	21.5	9.8	D.L.
			9, N,	6-7 m	30.0	22.7	1.5

Sampling Date	Bay	Transect	Sample I.D.		Water Content	Total Extractable Organics	Total Hydro-carbons
					%	$\mu g.g^{-1}$	$\mu g.g^{-1}$
13/09/80	10	S	10, S,	2-3 m	21.1	15.0	0.4
			10, S,	6-7 m	27.3	12.9	2.6
		C	10, C,	2-3 m	17.7	7.2	0.3
			10, C,	6-7 m	24.9	9.4	1.2
		N	10, N,	2-3 m	21.6	24.5	D.L.
			10, N,	6-7 m	23.8	7.3	0.6
11/09/80	11	S	11, S,	2-3 m	32.1	18.6	0.3
			11, S,	6-7 m	24.1	10.9	1.5
		C	11, C,	6-7 m	32.5	14.4	1.4
		N	11, N,	2-3 m	22.4	26.7	D.L.
			11, N,	6-7 m	14.4	11.8	1.4

Notes:

1. Units are in micrograms Lagomedio crude oil equivalents per gram of dry sediment.
2. D.L. means the response is below the detection limit of approximately 0.3 $\mu g.g^{-1}$.
3. For September samples, Freon extracts had a strong sulphide smell. June samples did not.

TABLE 4.9

BEACH SAMPLES: IR ANALYSES
HYDROCARBON BASELINE STUDY

Date	Location	Sample I.D.	Water Content	Total Extractable Organics	Total Hydro-carbons
			%	ug.g^{-1}	ug.g^{-1}
22/08/80	Bay 9	9-S-L	7.4	0.69	0.37
		9-S-H	2.3	0.64	DL
		9-C-L	10.1	0.38	DL
		9-C-H	2.2	1.18	0.48
		9-N-L	7.0	1.00	1.00
		9-N-H	4.1	0.73	0.39
22/08/80	Bay 10	10-S-L	8.4	2.99	2.30
		10-S-H	13.4	0.33	DL
		10-C-L	11.9	1.26	0.60
		10-C-H	7.8	0.32	0.32
		10-N-L	12.0	0.60	0.44
		10-N-H	9.1	0.41	DL
22/08/80	Bay 11	11-S-L	13.7	0.69	0.29
		11-S-H	12.9	1.37	0.42
		11-C-L	5.5	0.67	DL
		11-C-H	14.1	0.89	0.89
		11-N-L	10.8	1.06	-
		11-N-H	14.0	1.22	DL
20/08/80	crude oil point	T-1 prespill		0.43	0.27
		T-2 prespill		2.47	1.77
17/08/80	Bay 102	prespill		DL	DL
17/08/80	Bay 103	prespill		1.29	0.48

Notes:

1. Sample ID's give bay, transect and indicate high (H) or low (L) tide mark at which sample was collected.
2. Concentrations are in micrograms of Lagomedio crude oil equivalents per gram of dried sediment.
3. D.L. means response is below detection limit of approximately 0.25 µg.g.

4.4 Shoreline Experiment

4.4.1 Total Hydrocarbons

The total hydrocarbon concentrations in samples taken from the oiled beach plots are presented in Table 4.10.

TABLE 4.10:

HYDROCARBON ANALYSES OF BEACH SAMPLES FROM OILED BEACH PLOTS		HIGH WAVE ACTION				LOW WAVE ACTION				BACKSHORE PLOT (Z LAGOON)				BACKSHORE PLOT (ECLIPSE SOUND)			
		AGED		EMULSIFIED		AGED		EMULSIFIED		AGED		EMULSIFIED		AGED		EMULSIFIED	
		% OIL IN SEDIMENT				% OIL IN SEDIMENT				% OIL IN SEDIMENT				% OIL IN SEDIMENT			
		Surface	Sub-Surface	Surface	Sub-Surface	Surface	Sub-Surface	Surface	Sub-Surface	Surface	Sub-Surface	Surface	Sub-Surface	Surface	Sub-Surface	Surface	Sub-Surface
IMMEDIATELY FOLLOWING TEST	UPPER	2.04	1.04	1.59	2.80	0.67	0.88	0.19	0.05	2.24	1.65	0.94	0.91				
	MIDDLE	1.16	1.49	0.19	0.13	0.87	1.30	0.45	0.22	5.37	1.88	1.27	0.95	4.62	2.94	5.10	0.17
	LOWER	7.74	1.16	2.32	0.22	3.60	2.46	0.37	no sample	4.43	3.43	1.73	2.75				
2 DAYS AFTER TEST	UPPER	0.001	0.056	0.001	0.002	0.46	0.80	0.021	0.011	5.17	1.68	2.82	1.08				
	MIDDLE	0.019	0.88	0.002	0.001	0.47	0.09	0.032	0.006	3.79	1.70	1.20	2.31	4.96	1.52	10.2	0.46
	LOWER	0.001	0.055	0.001	0.001	0.61	0.69	0.014	0.005	8.53	5.62	1.90	4.74				
4 DAYS AFTER TEST	UPPER	0.007	0.18	0.010	0.11	0.45	0.77	0.008	0.002								
	MIDDLE	0.005	1.62	0.001	0.003	0.25	0.94	0.034	0.001	3.38	3.50	1.27	1.33	5.42	3.27	2.89	6.15
	LOWER	0.016	0.22	0.001	0.001	0.47	0.47	0.006	0.001								
8 DAYS AFTER TEST	UPPER	0.037	2.74	0.005	no sample	0.57	1.26	0.037	0.002								
	MIDDLE	0.32	0.010	0.0003	0.0004	0.77	1.83	0.001	0.016	6.58	1.71	6.00	5.81	4.04	4.77	5.80	0.050
	LOWER	0.001	0.26	0.0009	0.0008	0.60	1.08	0.001	0.005								

TABLE 4.11: MOISTURE CONTENT OF BEACH SAMPLES, BIOS SHORELINE EXPERIMENT

		MOISTURE CONTENT (% by weight)							
		Day 0		Day 1		Day 4		Day 8	
Plot	Tran	A	B	A	B	A	B	A	B
H1	U	1.8	2.2	1.1	1.5	1.8	1.5	1.4	4.8
	M	1.9	2.3	1.5	1.7	3.2	2.2	2.9	4.2
	L	3.9	1.9	1.6	2.0	3.9	1.5	1.9	2.5
H2	U	3.8	8.1	3.2	1.5	3.9	1.6	3.6	-
	M	0.8	1.0	2.9	1.4	5.8	3.6	4.0	4.1
	L	5.7	1.9	2.9	1.6	7.1	1.8	6.1	3.0
L-1	U	0.1	0.6	1.0	1.0	1.0	1.1	0.8	3.1
	M	0.6	2.2	1.5	7.6	0.8	5.6	1.3	3.2
	L	2.6	4.6	5.9	2.8	3.2	9.2	1.9	4.4
L-2	U	3.5	10.7	5.8	8.2	7.2*	9.7*	4.1	10.5
	M	5.7	12.2	4.0	14.1	10.9*	9.0*	19.0	10.4
	L	9.1	-	16.4	10.8	12.7*	10.3	19.9	9.0
LT-1	U	1.6	3.1	3.2	2.2	3.7	5.4	6.9	3.2
	M	2.0	-	2.0	2.1	-	-	-	-
	L	1.5	3.2	3.8	3.7	-	-	-	-
LT-2	U	1.1	1.9	2.6	1.2	2.3	2.4	8.9	8.5
	M	2.7	3.5	1.9	3.7	-	-	-	-
	L	3.0	6.3	2.3	7.5	-	-	-	-
HT-1		3.0	2.9	3.6	4.1	3.3*	3.2*	7.7	6.5
HT-2		14.0	5.1	15.9	10.3	5.9	13.8	10.4	8.0

Notes:

1. Moisture content = % weight loss upon drying sediment at 40°C for 16 hours.
2. A = surface sample (0-2 cm). B = subsurface sample (4-8 cm).
3. Plot Identification:
   H = high energy intertidal plot
   L = low energy intertidal plot
   T - backshore test plot
4. Tran = transect:
   U = upper transect
   M = mid transect
   L = lower transect
5. * samples collected by Woodward-Clyde Consultants (remainder by Seakem)

Atlantic deep water has 15-20 $\mu g.at.L^{-1}$ nitrate, 0.5-1.5 $\mu g.at.L^{-1}$ phosphate (Sverdrup et al., 1942).

The surface (1 m) concentrations of both nutrients began to decrease on 16 June, and by 22 June were each about 15% lower than the 10 m concentrations (See Figures 5.3 and 5.4 for graphs).

Upon returning to Cape Hatt on 11 August, nitrate at all three sampling depths had been virtually exhausted. Phosphate had been 75% depleted at the surface (1 m), and about 50% depleted at 10 m. Nitrate stayed at near zero levels at all three depths until 12 September, when some recovery at 5 and 10 m was evident. Phosphate maintained its depth stratification, with low values at the surface and higher values at 10 m, until about 9 September, when surface concentrations began to recover. The average values for the August/September period were:

nitrate nitrogen		0.28 ± 0.41 $\mu g.at.L^{-1}$ (85 values, all depths)
phosphate phosphorus	1 m	0.44 ± 0.12 $\mu g.at.L^{-1}$ (30 values)
	5 m	0.55 ± 0.06 $\mu g.at.L^{-1}$ (27 values)
	10 m	0.69 ± 0.09 $\mu g.at.L^{-1}$ (29 values)

Apollonio, 1976, in a three-year study of Jones Sound (350 km north of Cape Hatt near Devon Island) obtained a remarkably similar set of data : high nitrate (6-11 $\mu g.at.L^{-1}$) until late June, with rapid depletion to near zero in July through August; phosphate in June 1.2 - 1.4 $\mu g.at.L^{-1}$, declining in July to 0.2-0.6 $\mu g.at.L^{-1}$, at the surface, 0.40 to 0.75 $\mu g.at.L^{-1}$ at 5-10 m. The similarity of these data (also chlorophyll data) to those at Cape Hatt is striking, and implies that the environmental observations from the experimental site are more broadly applicable to this region of the Arctic.

The depletion of nitrate from the water column was undoubtedly due to the July bloom of phytoplankton which would have occurred just before and during ice break-up. The expected utilization ratio of nitrate to phosphate is 16:1 in the open ocean (Redfield et al., 1963). The apparent nitrate utilization at Cape Hatt was circa 8 $\mu g.at.L^{-1}$ versus phosphate 0.75 $\mu g.at.L^{-1}$, or a ratio of 11:1. Nitrate was in short supply, and presumably was the limiting nutrient in the ecosystem.

5.1.5 Suspended Solids

The suspended solids data is obtained by filtration and weighing of the filter paper, as described in the Methods section. Distinguishing between organic and inorganic suspended solids relies upon driving the organic matter from the filter by heating to 500°C. Organic material begins to ignite at 200°C and is completely ignited at 550°C. Inorganic compounds will also decompose with heating. Specifically, $CaCO_3$ will calcine to CaO, but this process does not occur until 700 - 800°C (Dean, 1974). There is obviously some room for uncertainty in the determination since the crossover from organic to inorganic decomposition is unlikely to be clearcut. Some further error is inherent in the method due to tightly bound water being driven off with the organic compounds, leading to systematically high organic suspended solids determinations. These intrinsic limitations of the method should be borne in mind when considering the results.

The June filtrations were done with a screw-closure type apparatus that was awkward to use. The tendency for this apparatus to leak decreased the precision of the June analyses, as well as perhaps introducing a positive systematic error due to salt residue on the fringes of the filter paper. The problem was overcome in the August/September sampling periods, and this later data must be considered of higher quality.

No trends were apparent in the August/September data set, either with depth or time (see graphs in Figures 5.5 and 5.6). The averages were as follows:

organic suspended solids	0.58 ± 0.09 mg.L^{-1} (90 values)
inorganic suspended solids	0.93 ± 0.32 mg.L^{-1} (86 values)
total suspended solids	$1.5 + 0.4$ mg.L^{-1} (86 values)

By way of comparison, deep Atlantic water suspended solids are in the range 0-0.1 mg.L^{-1} (Riley and Chester, 1971, p. 287) but surface waters are much higher and more variable. The author was unable to locate any other suspended solids data from the eastern Arctic. The western Arctic is quite different because of the massive influx of Mackenzie River silt.

The ratio of organic to total suspended solids is significant. For a healthy plankton population the percentage of organic matter is usually 50-60% of the total

5. DISCUSSION

5.1 Environmental Chemistry: Water Analyses

5.1.1 Temperature and Salinity

The water column in June was monotonic at approximately -1.7°C and 32.7 °/oo salinity, except for a thin lens of fresh water that gradually developed under the ice cover as the ice melted.

Upon returning 11 August, 1980, the surface waters (1 m) had warmed to approximately 4.5 °C, and the deep water (10 m) to 1.8 °C. Fresh water run-off had brought salinities down to about 15 °/oo at 1 m. During the first week in September the fresh water run-off quite abruptly stopped, and the water column rapidly cooled as air temperatures dropped below freezing and storms mixed the water column. By 16 September, the last sampling date, water temperatures had fallen below zero at all three sampling depths, and salinity was fairly uniform with depth at 30 °/oo. Graphs of the temperature and salinity of the water column over the summer period are shown in Figures 5.1 and 5.2.

5.1.2 Dissolved Oxygen

The dissolved oxygen concentrations in the water column were uniformly high. Considering only the more precise Winkler titration analyses, they averaged 11.83 ± 0.24 $mg.L^{-1}$ (24 values). Considering as well the YSI oxygen probe determinations, they averaged 11.71 ± 0.68 $mg.L^{-1}$ (60 values). (Uncertainties are always one standard deviation.)

The saturation levels of oxygen in sea water depend on the salinity and temperature of the water. For the range of conditions at Cape Hatt, theoretical saturation levels are:

for 4 °C, 16 °/oo	:	11.84 $mg.L^{-1}$
for 1.8°C, 30 °/oo	:	11.36 $mg.L^{-1}$

(note: 1 $mg.L^{-1}$ = 1.43 $mL.L^{-1}$)

The percent oxygen saturation at the Cape Hatt stations varied from 85% to 120%, with most values in the 100-110% range. These saturation-plus oxygen conditions have been widely reported in Arctic surface waters, including Frobisher Bay (Arctic Biological Station data), Jones Sound (Apollonio, 1976), the Amundsen Gulf (MacDonald et al., 1978), the Beaufort Sea (Wong et al., 1980), and the North Alaskan Shelf (Hufford, 1974).

5.1.3 pH

Measured values varied from 7.4 to 8.0. The mean value was 7.75 ± 0.15 (119 values) with no discernible trends with time or depth.

The pH of sea water in the open ocean rarely falls outside the range of 7.8 - 8.2. In surface and coastal waters, some diurnal variation in pH is expected due to CO_2 production and consumption by biological processes. Variations of 0.4 (winter) to 0.8 (summer) units over a 24 hour period are possible, with the maximum occurring during the day (Riley and Chester, 1971). Seasonal variations also occur, for the same reasons. Finally, a decrease in pH due to fresh water run-off would be expected at the surface.

None of these effects could be detected in the data from Cape Hatt. The diurnal variation, if present, was missed by sampling at the same time each day. The seasonal and freshwater effects were presumably too small to show through the 'noise' of the instrument scatter, and must have been less than about 0.1 pH units.

5.1.4 Reactive Nitrate and Phosphate

The analytical methods for nitrate and phosphate are probably the most reliable of the environmental chemistry methods. The precision of the autoanalyzer method is excellent, and the use of Sagami nutrient standards, which are distributed worldwide, ensures the method's accuracy. (The nitrate determination, it should be noted, actually gives a measure of nitrate plus nitrite. However, nitrite in well-oxygenated surface waters is usually undetectable.)

In June, the nutrient concentrations were relatively high, with nitrate nitrogen averaging 7.9 ± 0.4 $\mu g.at.L^{-1}$ (51 values, all depths), and phosphate phosphorus averaging 1.31 ± 0.08 $\mu g.at.L^{-1}$ (51 values, all depths). For comparison,

suspended solids. Less than about 20% organic material indicates that resuspended sediment, detritus and/or silt are dominant in the water column. At Cape Hatt, for the August/September period, the suspended solids averaged 39% organic material.

5.1.6 Dissolved Organic Carbon

Dissolved organic carbon (DOC) was determined by wet oxidation with infrared detection of the CO_2 generated. A parallel set of analyses was performed by the Arctic Biological Station, again with wet oxidation, but with detection as methane by gas chromatograph with an FID detector. The two sets of data agreed well. Both methods may give systemically low (approximately 15%) results due to incomplete oxidation of refractory organic material in the wet oxidation step (Gershey, 1979).

The DOC levels in June were relatively constant over time and depth at 1.27 $\pm$ 0.29 $mgC.L^{-1}$ (47 values, all depths). The Arctic Biological Station values averaged 1.51 $\pm$ 0.23 (36 values).

In August/September there was much more scatter in the data, with variations up to a factor of two occurring between stations in the same bay at the same time. Trends with depth and time are obscured by this scatter, which is apparently a real phenomenon due to the near shore and near bottom sampling locations. The Arctic Biological Station showed a very similar degree of scatter. The Seakem average for the August/September period was 2.35 $\pm$ 0.74 $mgC.L^{-1}$ (87 values); the Arctic Biological Station average was 2.15 $\pm$ 0.79 (90 values).

The data indicates an increase of circa 1 $mgC.L^{-1}$ over the June DOC concentrations. This increase can be attributed in part to the July plankton bloom. The utilization of 8 $\mu g.at.L^{-1}$ nitrate in the water column by the bloom should be associated with the fixing of circa 55 $\mu g.at.L^{-1}$ carbon, or approximately 0.7 $mgC.L^{-1}$. During the bloom the DOC is produced by phytoplankton as an extracellular exudate. After the bloom, decomposition of dead plankton contributes to the DOC levels in the water column. Additional DOC is contributed by fresh water run-off.

By way of comparison, open ocean DOC concentrations generally vary between the relatively narrow limits of 0.3 to 1.2 $mgC.L^{-1}$ (Rily and Chester, 1971, p. 200). In nearshore areas considerably higher values are common. For instance, Parsons (1979) reports monthly averages of 3 $mgC.L^{-1}$ for the summer months in the Strait of Georgia.

5.1.7 Particulate Organic Carbon

The analyses for particulate organic carbon (POC) were those of the Arctic Biological Station. The Seakem analyses, which used a titration method, were discarded as being too prone to inteference. The Arctic Biological Station method uses wet oxidation and determines the carbon as methane on a gas chromatograph with FID detector. There are many available methods for determining particulate organic carbon: this one is fairly unique. The use of the very sensitive FID detector allows a very small volume of water to be filtered. In fact, the precision of the method is probably limited by the small volume filtered (100 mL versus at least one litre for most methods).

The method may have some systematic errors. Gershey et al. (1979) estimate that wet oxidation gives values that are about 15% low due to incomplete oxidation of refractory organic matter. Another possible problem is absorption of dissolved organic carbon on the filters.

In June POC values were very low. For 5 and 10 m depths, the average was 28 ± 21 $\mu g.C.L^{-1}$ (24 values). At 1 m depth higher values were obtained, particularly towards the end of the month, giving an average 57.5 ± 41 $\mu gC.L^{-1}$. The higher values were presumably due to the increasing productivity immediately under the ice towards the end of June.

In August and September the POC jumped to an average of 162 ± 41 $\mu gC.L^{-1}$ (88 values). The 10 m samples tended to be slightly higher than the shallower samples, possibly due to resuspended sediments in the more turbulent open water conditions of late summer.

The August/September POC values averaged 28% of the organic suspended solids. 'Organic suspended solids' is a measure of the total weight of organic matter in the water, whereas POC is a measure of the weight of carbon alone. If all of the organic material in the water column was carbohydrate, the proportion of POC to total organic material would be expected to be 40%. The 28% value indicates the presence of sulphur, chlorine, organo-metals, and other components of the organic matter that lower the percentage of carbon by weight.

The ratio of POC to chlorophyll a is instructive. Ratios of less than about 200 occur in a healthy phytoplankton population. Ratios of over about 500 indicate most of the suspended material is dead detritus, resuspended sediment, or particulate material from a terrogenous source (Holm-Hansen, 1969; Tanoue and Handa, 1979). The ratio in June was 760, indicating primarily non-living material. In August and September the ratio had improved to 340 suggesting a reasonably healthy phytoplankton population was prominent in the water column.

5.1.8 Chlorophyll a and Phaeopigments

The methods for determining chlorophyll, although very widely used, are not without their pitfalls. The fluorometric method used here is a recent technique, largely displacing the absorption method that was prevalent 5-10 years ago. The fluorometric method is favoured because it is quicker and more sensitive. The spectrophotometric technique can, however, distinguish between the various chlorophyll pigments, providing information on their relative concentrations. Both methods suffer from calibration problems. Chlorophyll as a pure compound is not stable, so reliable standards are not available. The result is that the precision of the methods is good, but the accuracy is circa ± 15%.

The June values were extremely low, averaging 0.05 ± 0.04 $\mu g.L^{-1}$ (51 values, all depths). There is no variation with depth, but some indication that concentrations were just beginning to increase on the last day of sampling. The large bloom that undoubtedly occurred in July during and after ice break-up was missed. From comparison with other data, the peak value probably was in the range of 8-15 $\mu g.L^{-1}$ (Apollonio, 1976).

In August and September, concentrations averaged 0.48 ± 0.31 $\mu g.L^{-1}$ (90 values) and were fairly constant with time and depth, other than a peak at 10 m on 9

September for which there is no readily apparent explanation. These results are very similar to the data reported by Apollonio, 1976, from Allen Bay who reported average concentrations for August of 0.7 $\mu g.L^{-1}$ (69 values). (The chlorophyll concentrations are graphed in Figure 5.9.).

Table 4.1 also reports phaeopigment concentrations which are useful in assessing the condition of the phytoplankton stock. In June the phaeopigment averaged 46% of the total pigment (chlorophyll plus phaeopigment) indicating a senescent phytoplankton population. On the last sampling day in June, however, when the spring bloom was apparently about to start, the percentage phaeopigment dropped abruptly to 15%, indicating a rapidly growing phytoplankton population. In August the average was 47% $\pm$ 10% (excepting a drop to 23% for the 9 September chlorophyll peak) again indicating a fairly senescent phytoplankton population.

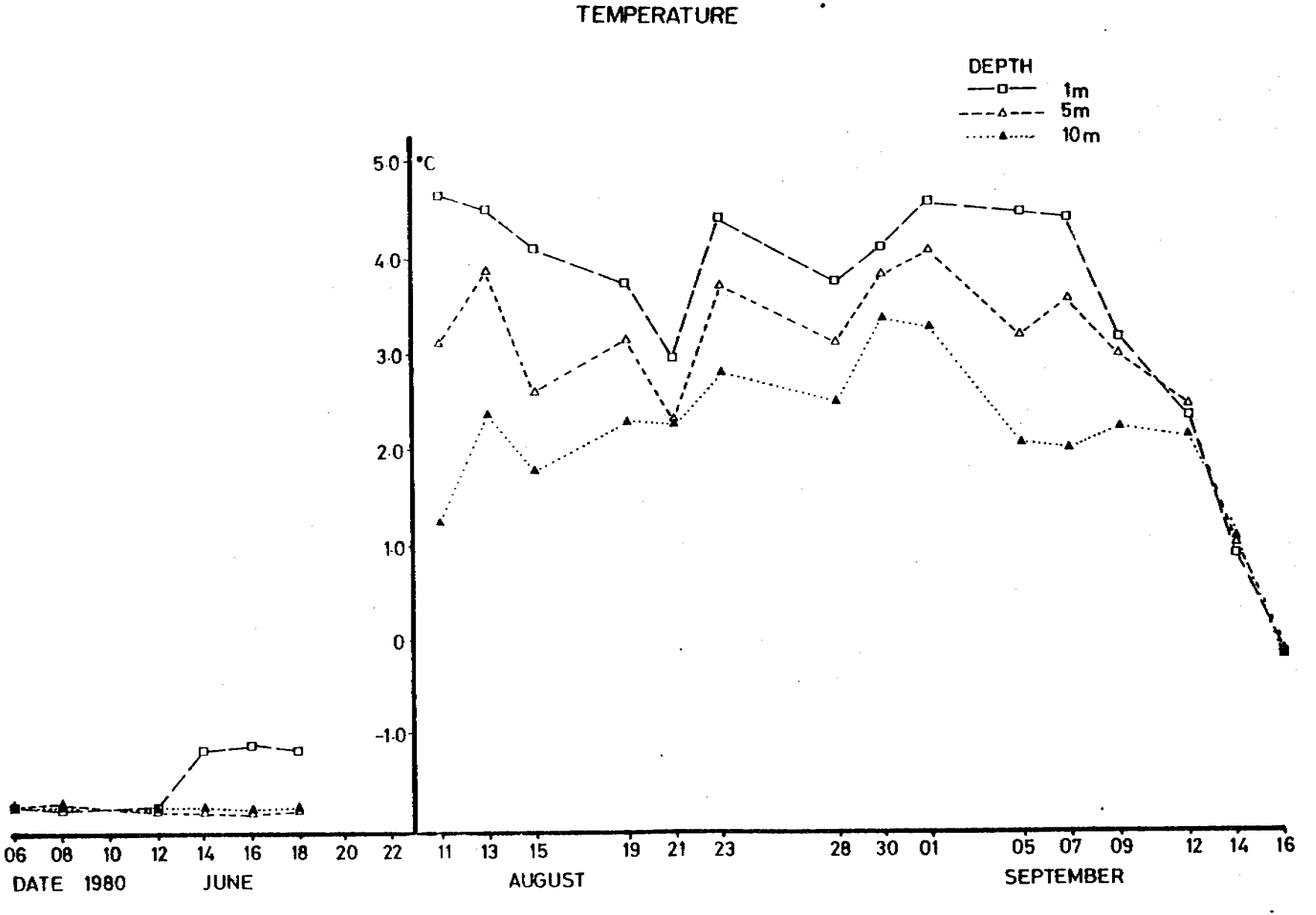

Figure 5.1: Seawater Temperature in Ragged Channel, Summer, 1980

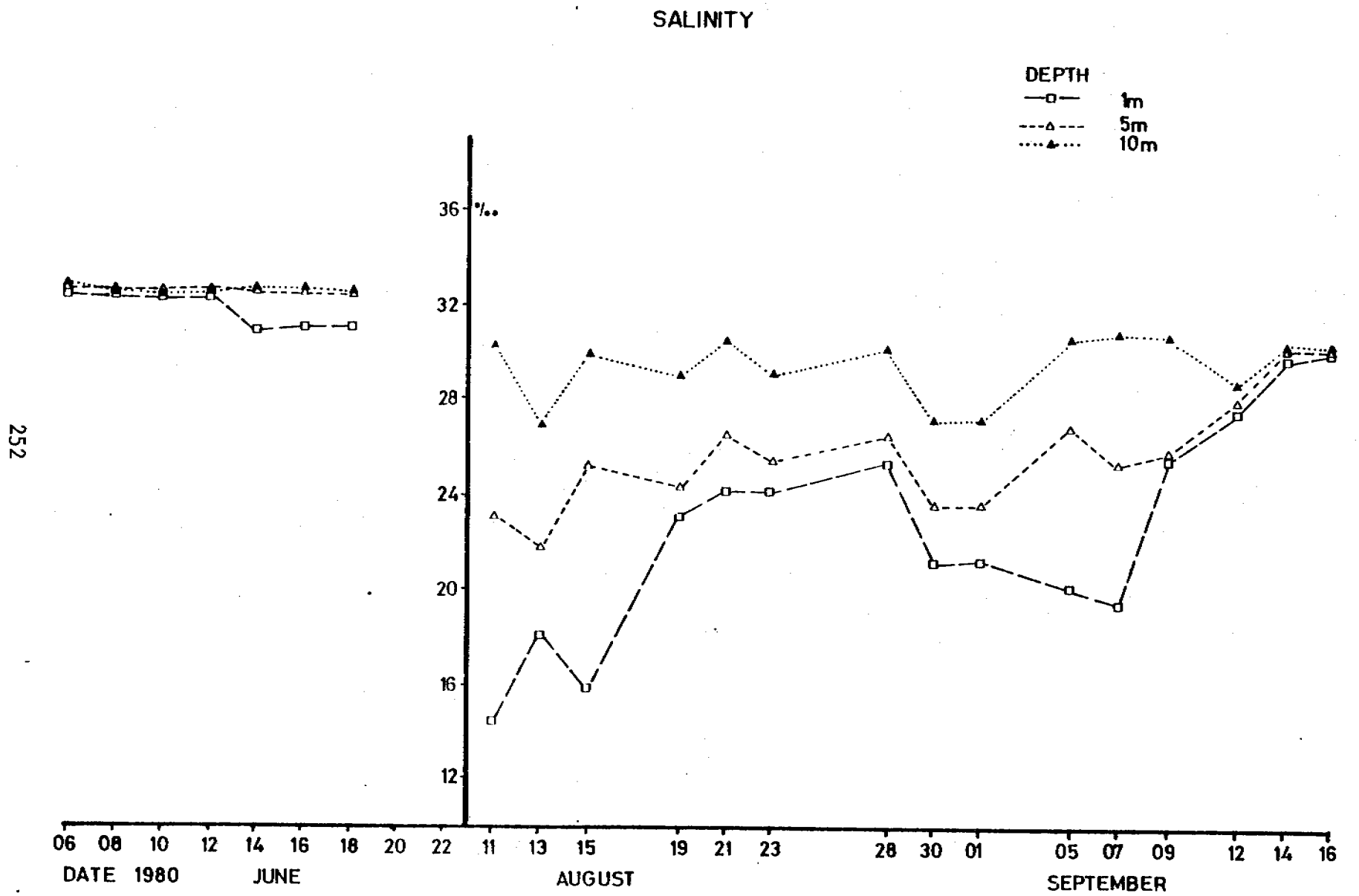

Figure 5.2: Seawater Salinity in Ragged Channel, Summer, 1980.

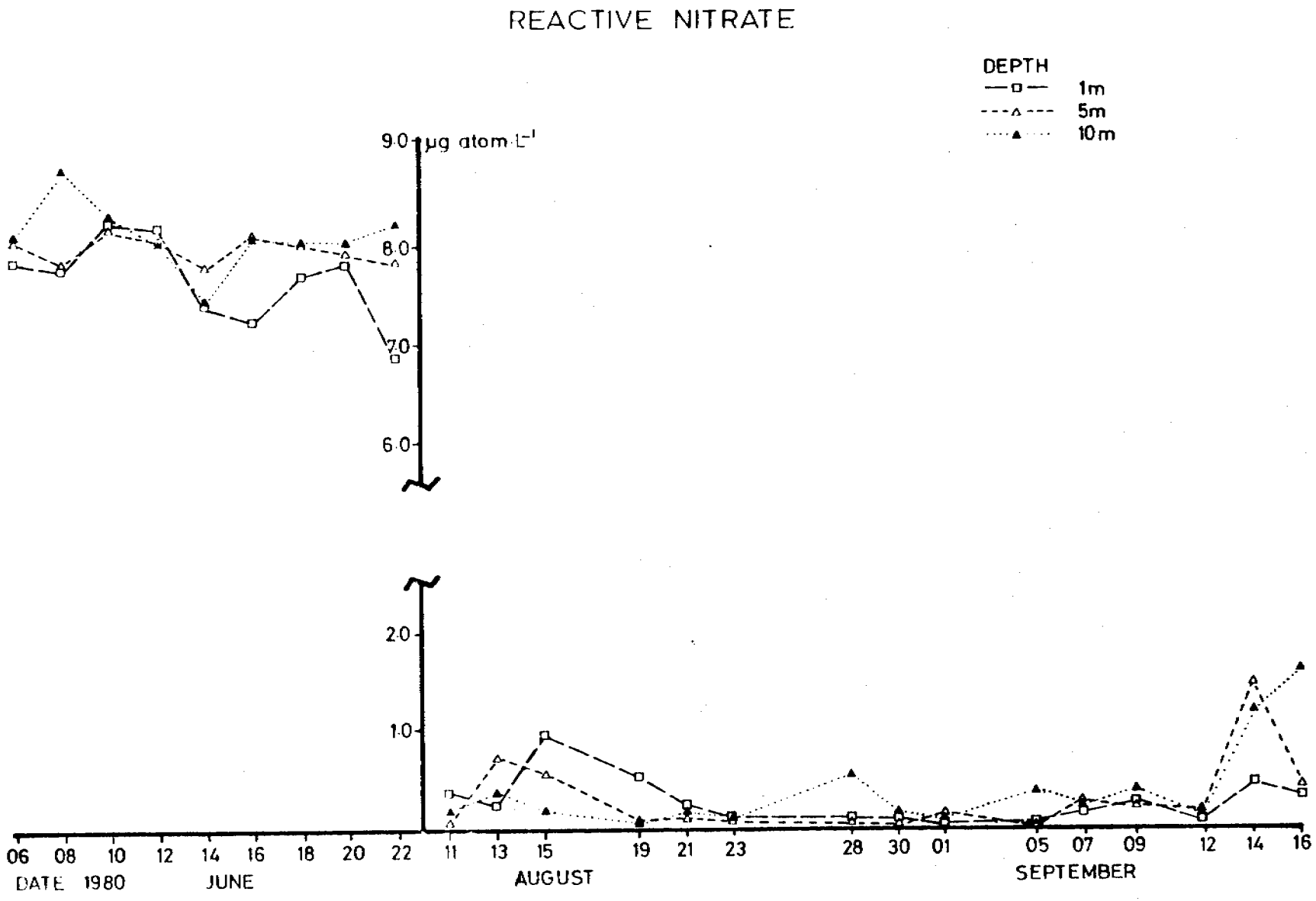

Figure 5.3: Seawater Nitrate in Ragged Channel, Summer, 1980

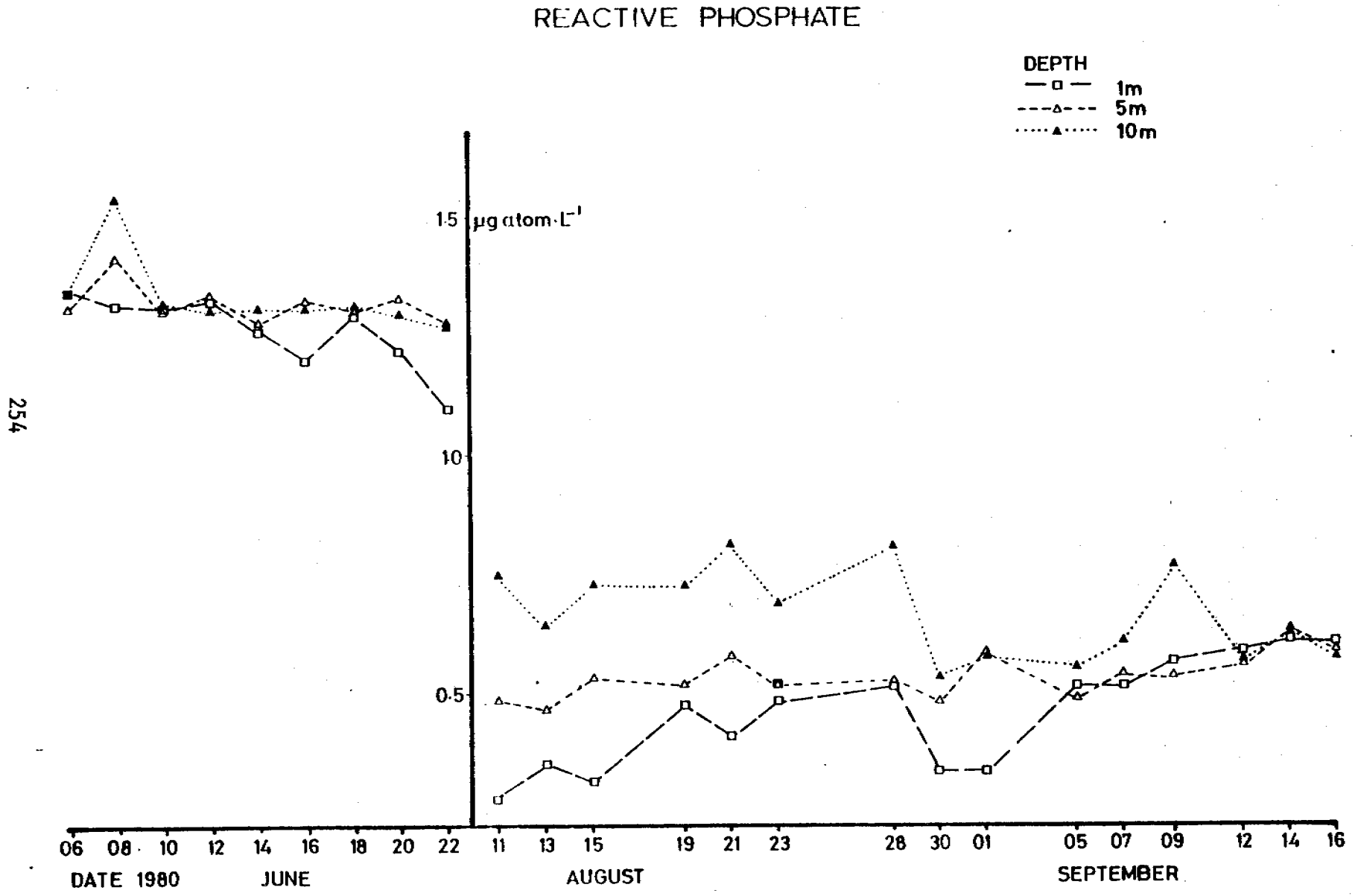

Figure 5.4: Seawater Phosphate in Ragged Channel, Summer, 1980.

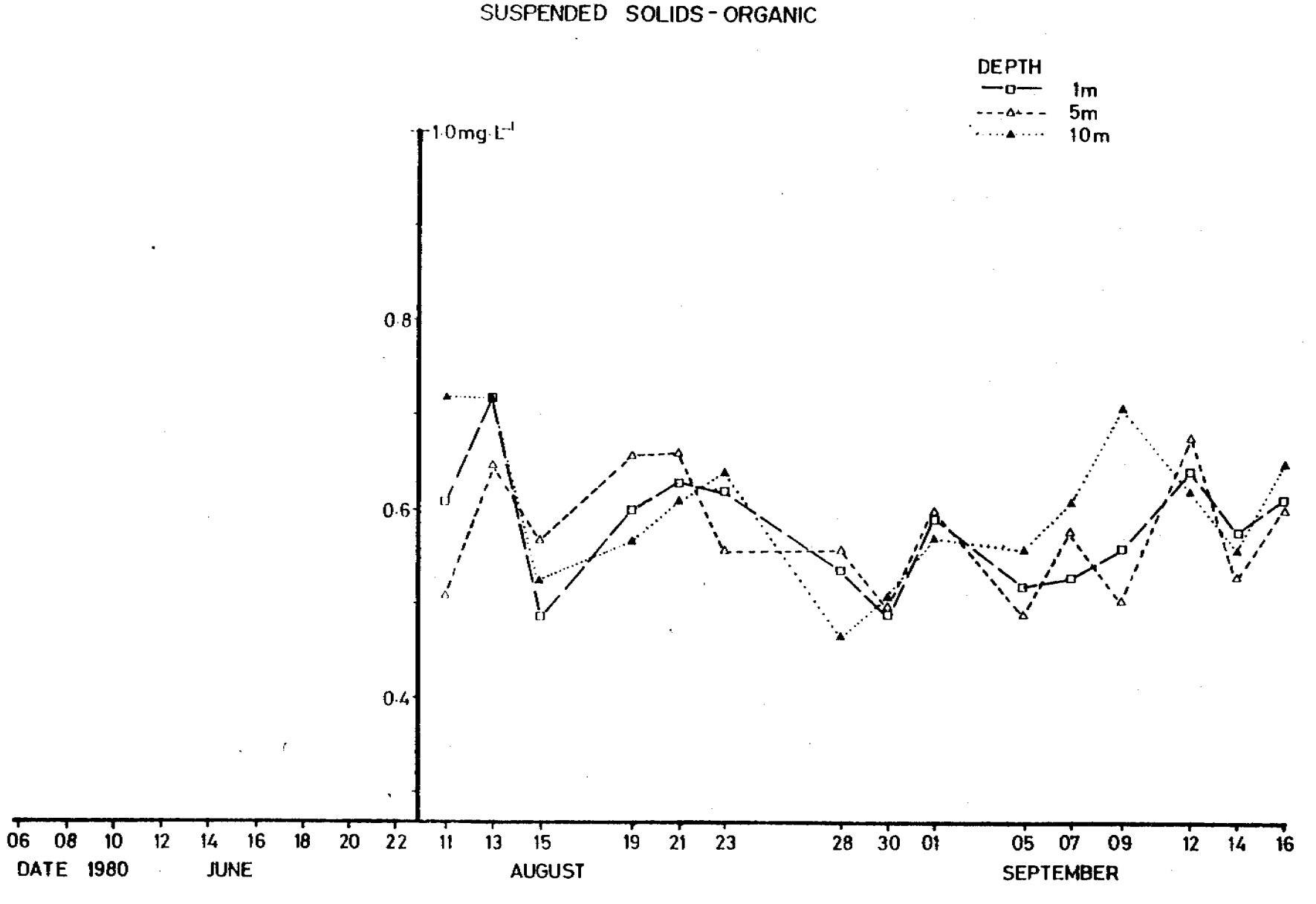

Figure 5.5: Organic Suspended Solids in Ragged Channel, Summer, 1980.

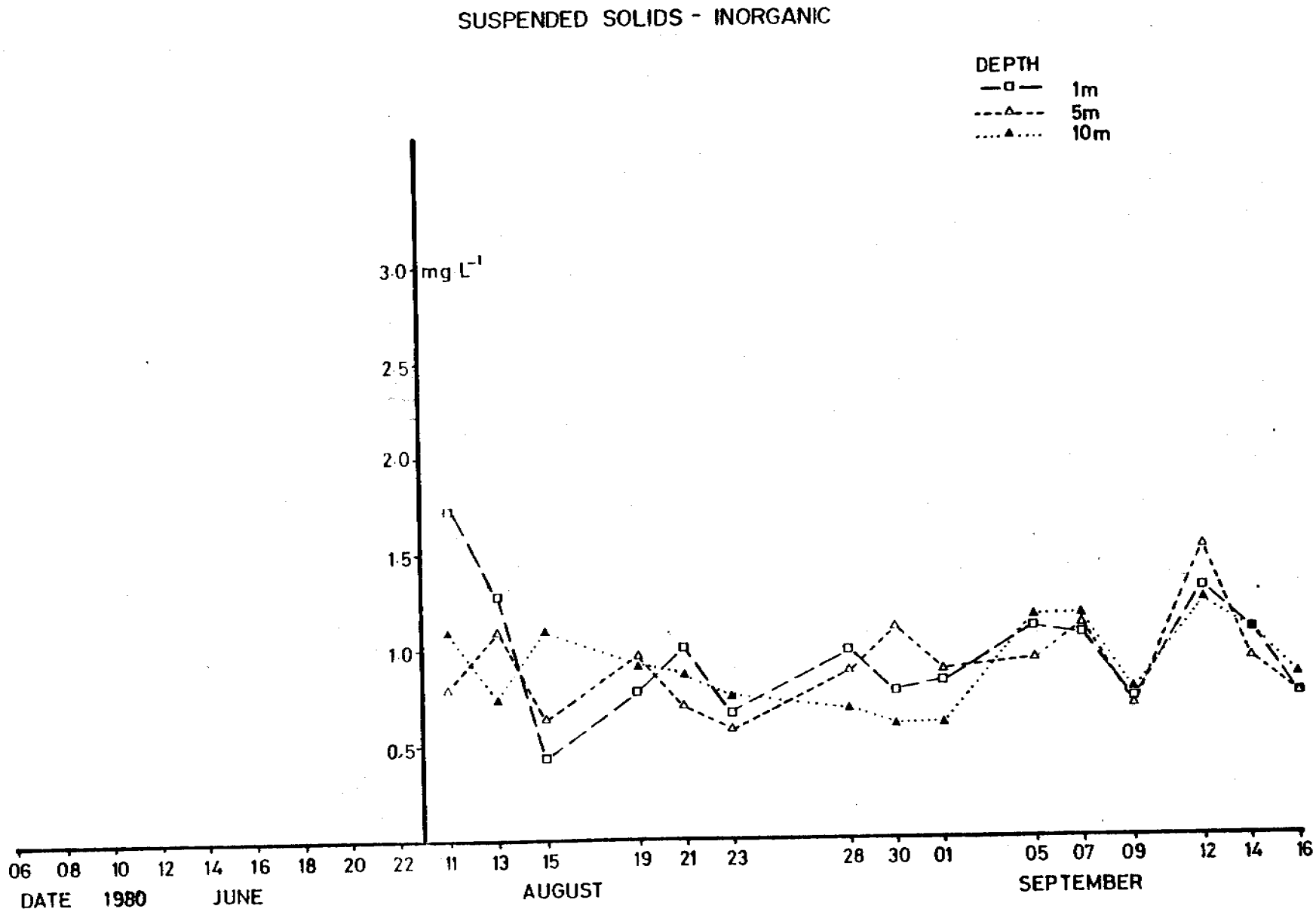

Figure 5.6: Inorganic Suspended Solids in Ragged Channel, Summer, 1980.

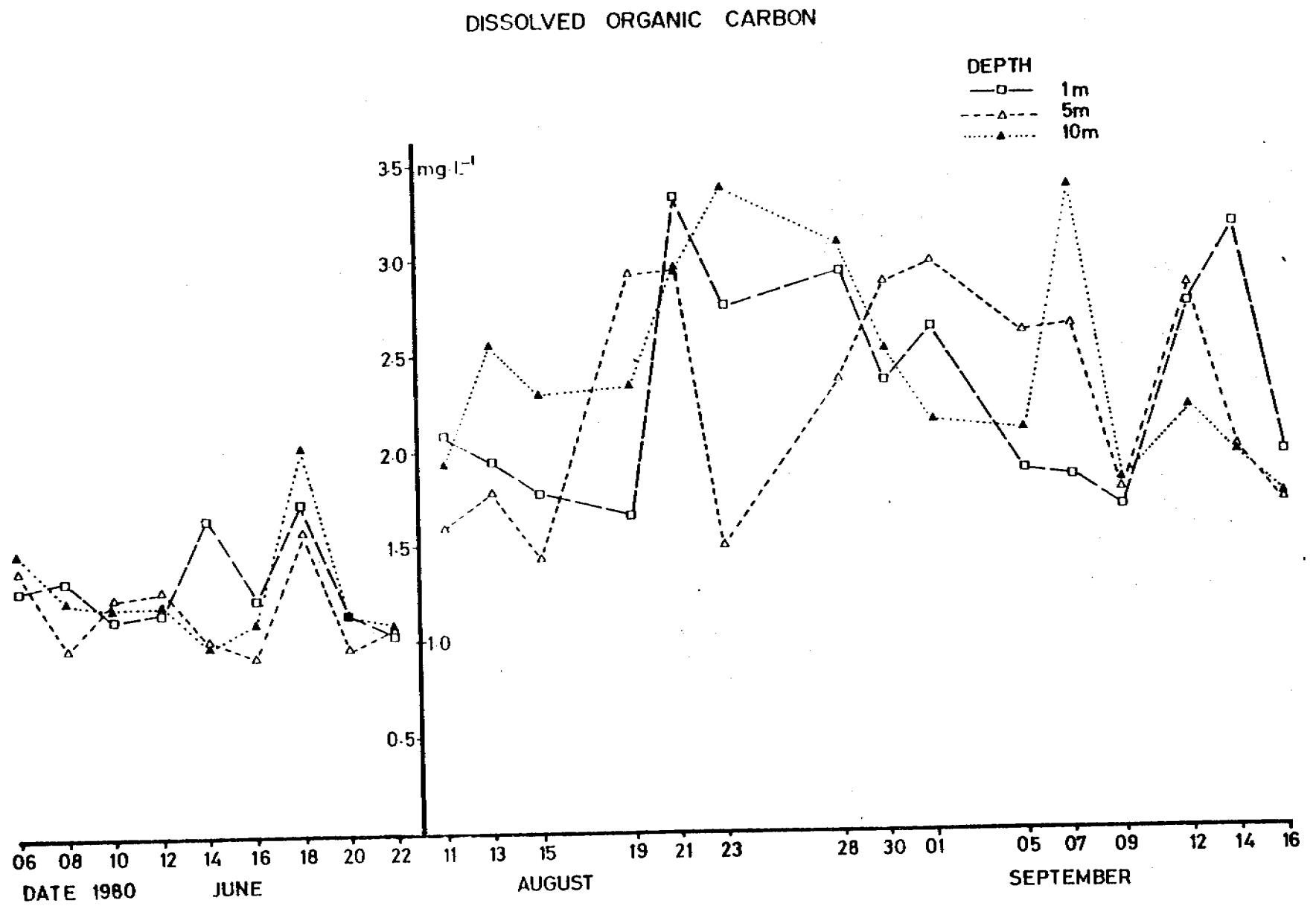

Figure 5.7: Dissolved Organic Carbon in Ragged Channel, Summer, 1980.

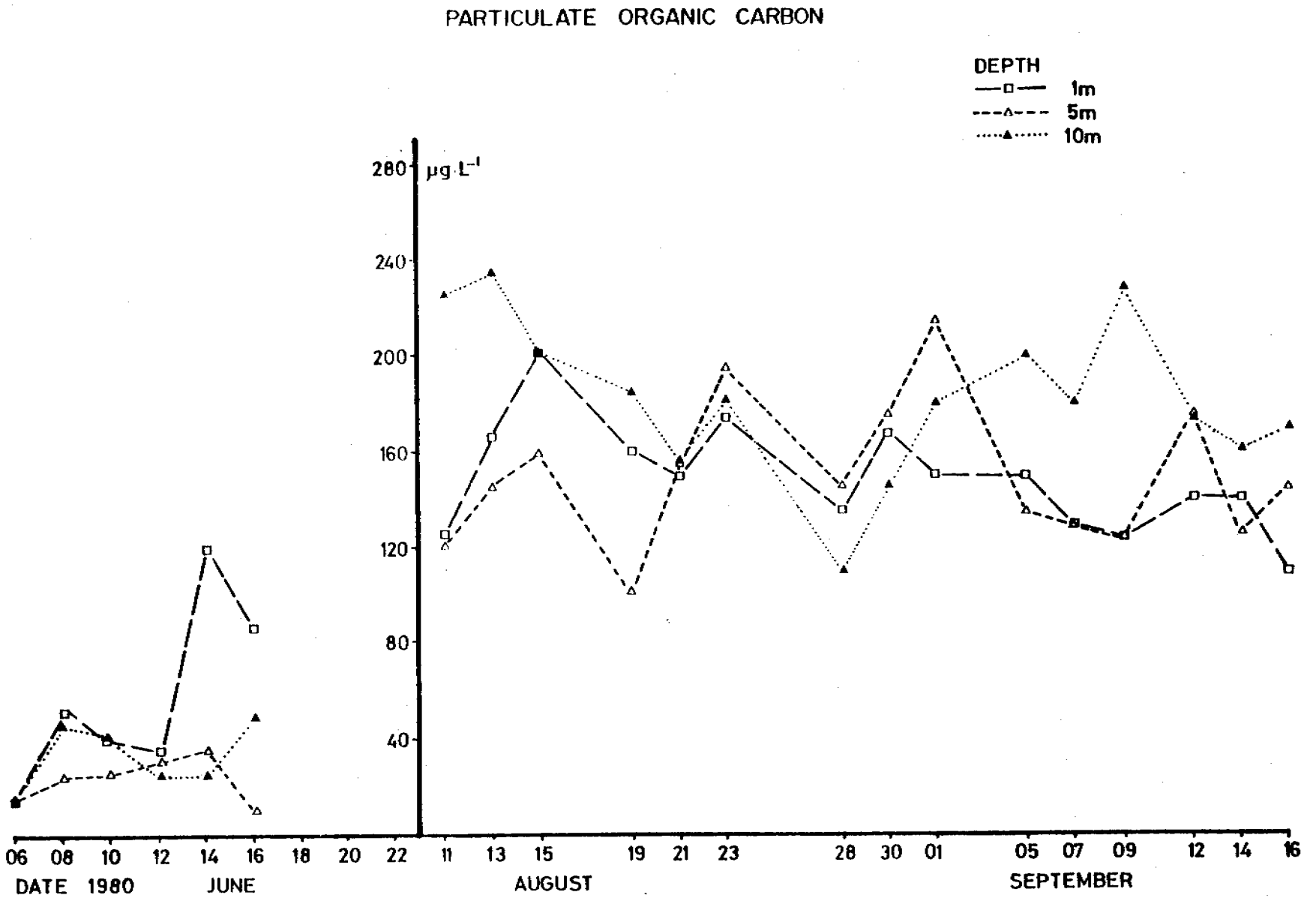

Figure 5.8: Particulate Organic Carbon in Ragged Channel, Summer, 1980.

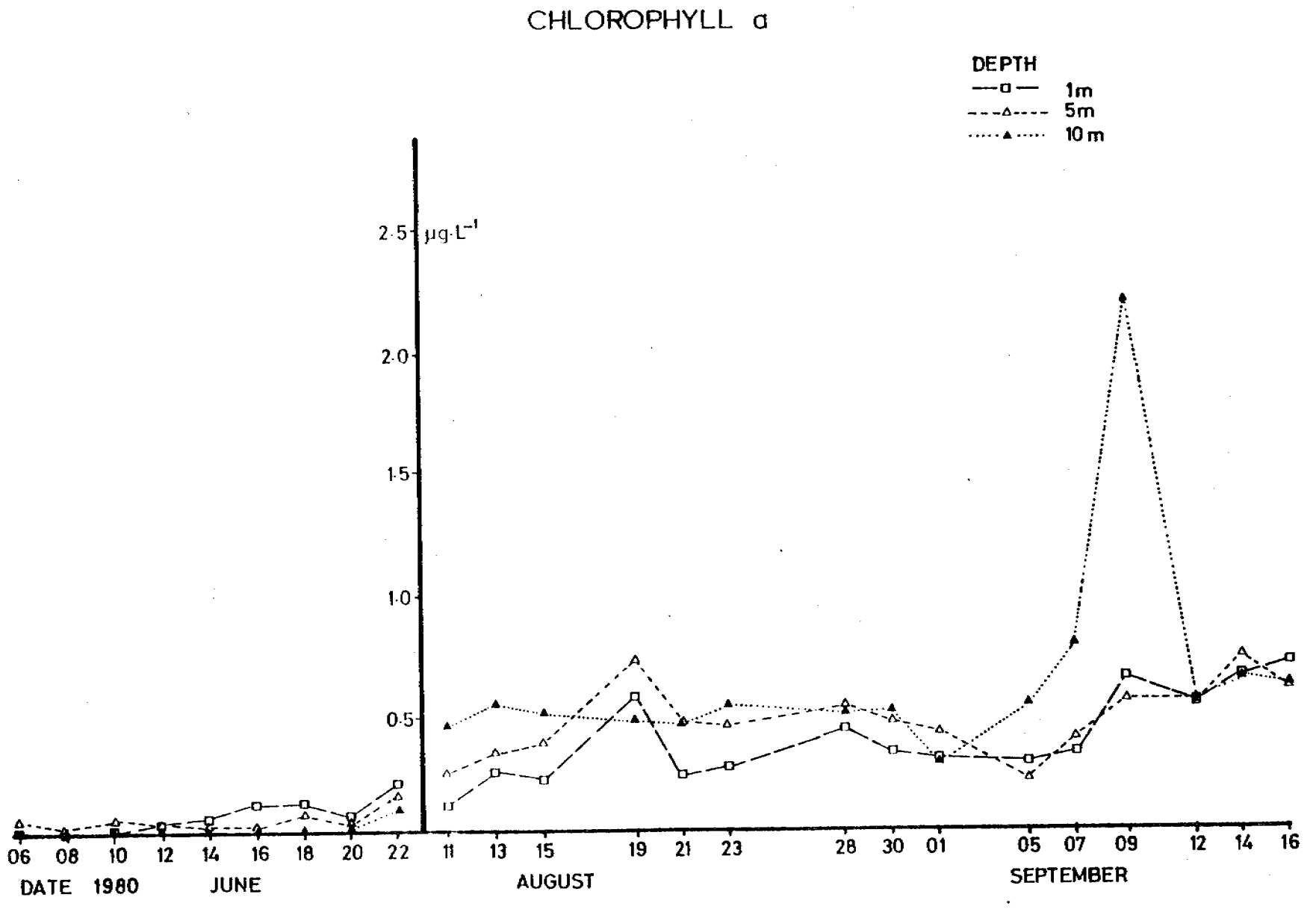

Figure 5.9: Chlorophyll a in Ragged Channel, Summer, 1980.

5.2 Environmental Chemistry: Sediment Analyses

5.2.1 Total Organic Carbon

The method used for total organic carbon (TOC) is the same wet oxidation and back-titration technique that Seakem attempted for particulate organic carbon. However, the levels of organic carbon are far higher in the sediment than in the water column, and interferences are proportionately less of a problem. Nevertheless a check was made by determining the salinity for all of the samples and determining the size of the chloride interference which results. The method was found to report systematically high TOC due to this inteference, with the systematic error averaging about +5% (1-10% spread). The method also suffers from the assumption that the redox state of the sedimentary organic scatter is the same as in the sucrose standards. Finally, oxidation of the more refractory organic carbon in the sediments is probably not complete, resulting in values that are systematically low by about 15% (Gersey et al., 1979). Although the method suffers from a variety of systematic errors, the precision of replicate samples was found to be good. Despite the inhomogeneity of the Cape Hatt sediments, triplicate analyses gave relative standard deviations that averaged 10%.

The TOC values do not show any variation with depth in the core samples, suggesting limited bacterial activity at depth. Neither was there any discernible change in the surface samples over the summer sampling period. The average value of all of the samples was 0.60 $\pm$ 0.28% carbon by weight (52 samples). This excludes the series of shallow (2-3 m) samples collected in August/September which were significantly lower than the 6-7 m or 13-15 m samples, averaging 0.24 $\pm$ 0.10% carbon by weight (9 samples). The beach samples were different again, and extremely low in organic carbon, averaging 0.07 $\pm$ 0.07% (18 samples).

By way of comparison, open ocean sediments average 0.3% organic carbon (Riley and Chester, 1971, p. 213).

5.2.2 Interstitial Nutrients

The measurement of interstitial nutrients in marine sediments is not a routine type of analysis. There are a variety of sampling and analytical problems which have been reported in the literature. In reducing sediments, high phosphate

concentrations of several hundred μg at L^{-1} can exist a few centimeters from the sediment-water interface. This rapid gradient in nutrient concentrations with depth requires careful sampling. Another problem is contamination of the interstitial water with overlying sea water after sampling. There are virtually as many sampling methods as sets of results reported in the literature. These include samplers which extract the interstitial water from the sediment in situ, such as the syringe type (Sayles et al., 1976) and the perforated cup type (Zimmermann et al., 1978) as well as the more conventional corers, grabs and diver-held samplers.

There are further complications in pressing water from sediment samples. The temperature of pressing has been found to have an effect on silica and pH results (Fanning and Pilson, 1971). Pressing sediments in air has been shown to decrease phosphate concentrations, due to formation of iron precipitates. An inert atmosphere is recommended (Bray et al., 1973). Freezing of the interstitial water often causes formation of magnesium- or iron- precipitates (Martens et al., 1978) which must then be redissolved with acid prior to analysis.

The samples collected for this data set were obtained in three different ways. The first set were collected by grab samplers, the second by corers, and the third by divers. The grab sampling method was perhaps the most reliable. The diver sampling may have resulted in some contamination of the pore waters with overlying sea water. The core samples had to be frozen, thawed and refrozen prior to squeezing and analysis, so degradation of these samples, perhaps by precipitation of phosphate, may have been a problem.

The results from the cores were so variable that it is difficult to comment. The expected pattern for phosphate with depth in core samples is for deep samples to be enriched, containing 50 to 500 μgat L^{-1} depending on the organic content and redox potential of the sediment (e.g. Murray et al., 1978). The enrichment of PO_4 is due to regeneration from organic matter by oxidation reactions in the sediment. Nitrate, however, is usually zero at depth, because once oxygen is used up, nitrate becomes the electron acceptor for the oxidizing process in the sediments, and it is reduced to ammonia or nitrogen (Bender et al., 1977). Most sediments contain no nitrate a few centimeters below the water interface. The erratic results from the core samples at Cape Hatt suggests that the samples did not survive the refreezing steps in their handling, or that other problems occurred in the analyses.

The grab- and diver- collected samples of the surface sediment at the microbiology stations gave more consistent results. Excluding the first set of three results from June, which were very high and appear to be contaminated, the averages of the remaining 24 samples are:

Nitrate - nitrogen	3.0 ± 2.7	μg at L^{-1}	(24 values)
Phosphate - phosphorus	14.9 ± 11.3	μg at L^{-1}	(24 values)

A third of these samples were collected by grab sampling, the remainder by divers. There was no significant difference between the two sets of results. In June only the phosphate was enriched in the interstitial water relative to the bottom water. In August - September both nutrients were an order of magnitude higher in the interstitial water than in the bottom water, presumably due to mineralization of organic matter under oxygenated conditions in the surface sediments.

5.2.3 Total Nitrogen

The total nitrogen levels in the sediment averaged 0.15 ± 0.04% by weight. The interstitial nitrate - nitrogen expressed in the same units, averages 0.0000042% by weight, and so does not contribute significantly. Total organic carbon in these samples averages 0.60% carbon by weight. The atomic ratio of carbon : nitrogen is therefore 4.7 : 1.

In plankton the average carbon - nitrogen ratio is about 6.6 : 1 (Redfield et al., 1963). Presumably then, most of the nitrogen is bound up in detritus, with perhaps some additional nitrogen contributed by ammonia from decaying sediments at depth.

5.2.4 Lead-210 Dating

Lead-210 dating can be used to establish the geochronology of marine sediments up to about 100 years. Three cores were collected from Cape Hatt for dating purposes and subsampled at seven to nine depths each (0 - 45 cm). The results for lead-210 determinations are all in the range of 0 - 1.4 pCi/g which are background

levels. There is no sign of any gradient with depth. By way of comparison, a core from Santa Barbara basin showed a gradient from 6.9 pCi/g at the surface to background levels of 1.0 pCi/g at 24 cm (Koide et al., 1972). The lack of such a gradient in the Cape Hatt cores implies that the lead-210 in the surface samples has already decayed to the background levels of the deeper samples. The sedimentation rates must therefore be very slow, and for the amount of reworking of the sediment by biological activity and ice scouring, must be sufficient to mix freshly deposited lead-210 rich sediments down into the sub-surface, destroying any surface accumulations.

5.3 Hydrocarbon Baseline Study

5.3.1 Water Samples, IR Analyses

Most of the infrared determinations on the water samples were below the detection limit of 13 $\mu g.L^{-1}$ (relative to a weathered Lagomedio crude oil standard). The exceptions (7 out of 35) may be due to natural organic matter. Most of the UV/fluorescence determinations on the same samples by ERCO (see volume 2) are also below the detection limit of that method of 3 $\mu g.L^{-1}$. The four exceptions are not the same samples that are high by IR analyses. This implies that the high IR values are not due to petroleum hydrocarbons which usually contain fluorescent aromatic material and therefore should show a UV/fluorescence response.

5.3.2 Sediment Samples, IR Analyses

Both the total extractable organics and the non-polar hydrocarbons were measured in the sediment samples. The total extractable organics of the June samples were significantly lower than for the September samples: 4.6 ± 3.4 $\mu g.g^{-1}$ versus 16.5 ± 8.3 $\mu.g^{-1}$ for the later period. This difference is perhaps due to the fresh detritus deposited in the late summer. The non-polar hydrocarbon levels were, however, consistent between the two sampling periods: 1.1 ± 1.0 $\mu g.g^{-1}$ (21 values) in June versus 0.86 ± 0.78 $\mu g.g^{-1}$ (17 values) in September. This measure of non-polar hydrocarbons should give a reliable baseline against which to compare the sediment after the experimental oil spill.

5.3.3 Beach Samples, IR Analyses

The total extractable organics are much lower for beach samples than for sediment samples, averaging about 10% of the sediment concentrations (0.94 ± 0.68 $\mu g.g^{-1}$, 21 values). The trend parallels the total organic carbon results: beach TOC levels were 12% of levels in the sediment. The non-polar hydrocarbon contents were more consistent, averaging circa 50% of levels in the sediment (0.52 ± 0.57 $\mu g.g^{-1}$, 21 values)

(Note: all IR determinations are expressed in weathered Lagomedio crude oil equivalents per gram of dried sediment.)

5.4 Shoreline Experiment

5.4.1 Total Hydrocarbons, IR Analyses

The significance of the total hydrocarbon measurements made for the shoreline oil spill experiments is discussed fully by the Woodward-Clyde B.I.O.S. report. It should be noted that some improvement is required in the sampling procedures to obtain a better measure of the mass balance of oil in the plots because of the high 'intra-plot' variability. Larger samples, more fully integrated across the plots, will be taken in the future.

6. CONCLUSIONS

The environmental chemistry data presented here describes the biochemical characteristics of the water column, and to a lesser extent the sediment, quite thoroughly, ranking Cape Hatt among the better characterized marine systems in the Arctic. Where comparative data is available (e.g. nutrients, chlorophyll, oxygen) it suggests that Cape Hatt is quite typical of the eastern Arctic nearshore environment.

The hydrocarbon data presented here will provide a good baseline against which to follow the fate of the experimental oil spills, as is discussed in more detail in Volume 2 of this report.

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BAFFIN ISLAND OIL SPILL PROJECT - CHEMISTRY COMPONENT

VOLUME 2

Final Report
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March 1981

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Foreward

Volume 2 of the BIOS - Chemistry Component final report includes all aspects of the organic chemistry analytical components of the program undertaken by ERCO (Energy Resources Co. Inc., Cambridge, Massachusetts, U.S.A.). The infrared (IR) analyses performed on seawater and sediments were generated by Seakem Oceanography Ltd. and thus appear in Volume 1 of this report.

SECTION ONE

INTRODUCTION

1.1 Project Goals

The chemistry component of the Baffin Island oil spill project (BIOS) involved two basic tasks during the first year of the project: (1) to chemically characterize the marine environment of the Ragged Channel bays prior to the experimental oil spills (i.e., the Nearshore Study baseline), and (2) to perform chemical measurements of the oiled shoreline plots to determine the concentration and composition of residual oil in these experimental spills (i.e., the Shoreline Experiment). The undertaking of these tasks required specifically tailored sampling and analytical protocols designed to create the chemical foundation for a multiyear examination of the chemical fates and biological assimilation of the spilled oils.

The specific goals of the analytical chemistry (hydrocarbons) segment are stated in Table 1-1.

1.2 Technical Plan

The analytical plan employed in the study involved the types of samples indicated in Table 1-2 and the types of analyses shown in Table 1-3. The rationale for each type of analysis is presented in detail in Section Two of this report. It should be stated that the overall plan was to blend analytical techniques of varying sophistication and resolution to best enable the program's goals to be achieved within

TABLE 1-1

HYDROCARBON CEHMISTRY (YEAR 1) GOALS

1. To characterize the unweathered, weathered crude, and crude/dispersant mixtures

2. Establish baseline levels and compositions of hydrocarbon compounds in seawater, sediment, and animal tissues

3. To utilize a combination of non-specific screening and sophisticated chemical techniques to investigate the pre-spill biogeochemical environment

4. To evaluate the analytical combination in terms of its use in post-spill investigations

5. To investigate the detailed chemical weathering of spilled oil in the shoreline study

6. To research the fate of minor, but persistent classes of marker compounds - establish baseline levels and obtain initial results on spilled oil

TABLE 1-2

CHEMISTRY COMPONENT - TYPES OF SAMPLES ANALYZED

Sample Type	Nearshore - Baseline	Shoreline - Weathering
Seawater (pre-spill)	X	X
Sediment (offshore)	X	
Sediment (beach)	X	X
Oiled sediment (beach)		X
Tissues	X	
Crude oil	X	X

TABLE 1-3

ANALYTICAL CHEMISTRY MATRIX

	UV/F	SILICIC ACID CHROMA-TOGRAPHY	CAPIL-LARY GC	CAPIL-LARY GC / HO-PANES	CAPIL-LARY GC / AZA-ARENES	CAPIL-LARY GC / AROMATIC H.C.	PHYS-ICAL PROP-ERTIES	TRACE METALS
Crude oils	x	x	x	x	x	x	x	x
Seawater	x	x	x			x		
Sediment (offshore baseline)	x	x	x	x	x	x		
Sediment (beach baseline)	x	x	x	x	x	x		
Sediment (oiled beach)		x	x	x	x	x		
Tissues		x	x	x		x		

the budgetary constraints. We have employed such blends successfully in the past (Fiest and Boehm, 1981; Boehm and Fiest, 1981a, 1981b; Boehm et al., 1981a).

1.3 Background

1.3.1 Pollutant Compounds in the Arctic

Although an abundance of data is not readily available, several studies have been undertaken in recent years to determine levels of organic pollutants, most notably petroleum hydrocarbons (PHC), in remote and/or undeveloped arctic marine environments. A general chemical picture emerges of an environment with very low levels of hydrocarbons, but one that is not free from "contaminants" distributed on a global basis by natural and anthropogenic processes.

Wong et al. (1976), Shaw et al. (1979), Shaw and Baker (1978), and Johansen et al. (1977) have investigated petroleum hydrocarbon pollutant distributions in the offshore Beaufort Sea, the nearshore Beaufort Sea, the Port Valdez nearshore environment and the West Greenland coast respectively. There is little indication in any of these studies of inputs of chronic petroleum related inputs of hydrocarbons, although Shaw et al. (1979) suspect that fossil-fuel-related arenes (aromatic hydrocarbons) from coal outcrops or natural seeps are sources for low levels of sedimentary arenes found at several locations.

Long-range transport of polycyclic aromatic hydrocarbons (PAH = arenes) from pyrolytic sources (i.e., combustion of fossil fuels) are probable sources for observed distributions of low levels of PAH found in the Arctic (Wong et al.,

1976; Shaw et al., 1979) and elsewhere on a global scale (Laflamme and Hites, 1978; Lunde and Bjorseth, 1977).

Some PAH compounds are also produced diagenetically (i.e., after deposition of precursors in the sediment) in surface sediments and may therefore not be related to any pollutant sources. Wakeham et al. (1980), Aizenshtat (1973), and Simoneit (1977 a, 1977b), among others, describe the diagenetic production of PAH compounds including the more commonly encountered retene (1-methyl-7-isopropylphenanthrene) and perylene, and other compounds (e.g., alkylphenanthrenes) that have pollutant sources as well.

Little evidence exists for the input of saturated petroleum hydrcarbons in any arctic environment studied in sufficient quantities to mask natural saturated hydrocarbon profiles consisting of marine and tenigenous biogenic compounds. Alkane compositions suggest biogenic sources (Shaw et al., 1979) as well.

1.3.2 Weathering of Petroleum in the Marine Environment

"Weathering" of oil at sea pertains to that collective set of processes which alter the chemical composition of petroleum mixture through evaporation, dissolution, photochemical oxidation, microbial degradation, and auto-oxidation. The physical processes mediating the chemical changes are mixing, emulsification, and sorption (NAS, 1975). A schematic diagram of the processes of weathering of oil is shown in Figure 1-1.

Incorporation of petroleum in the sediment usually results in accelerated weathering of oil in oxygenated substrate mainly through microbial degradation (Teal et al.,

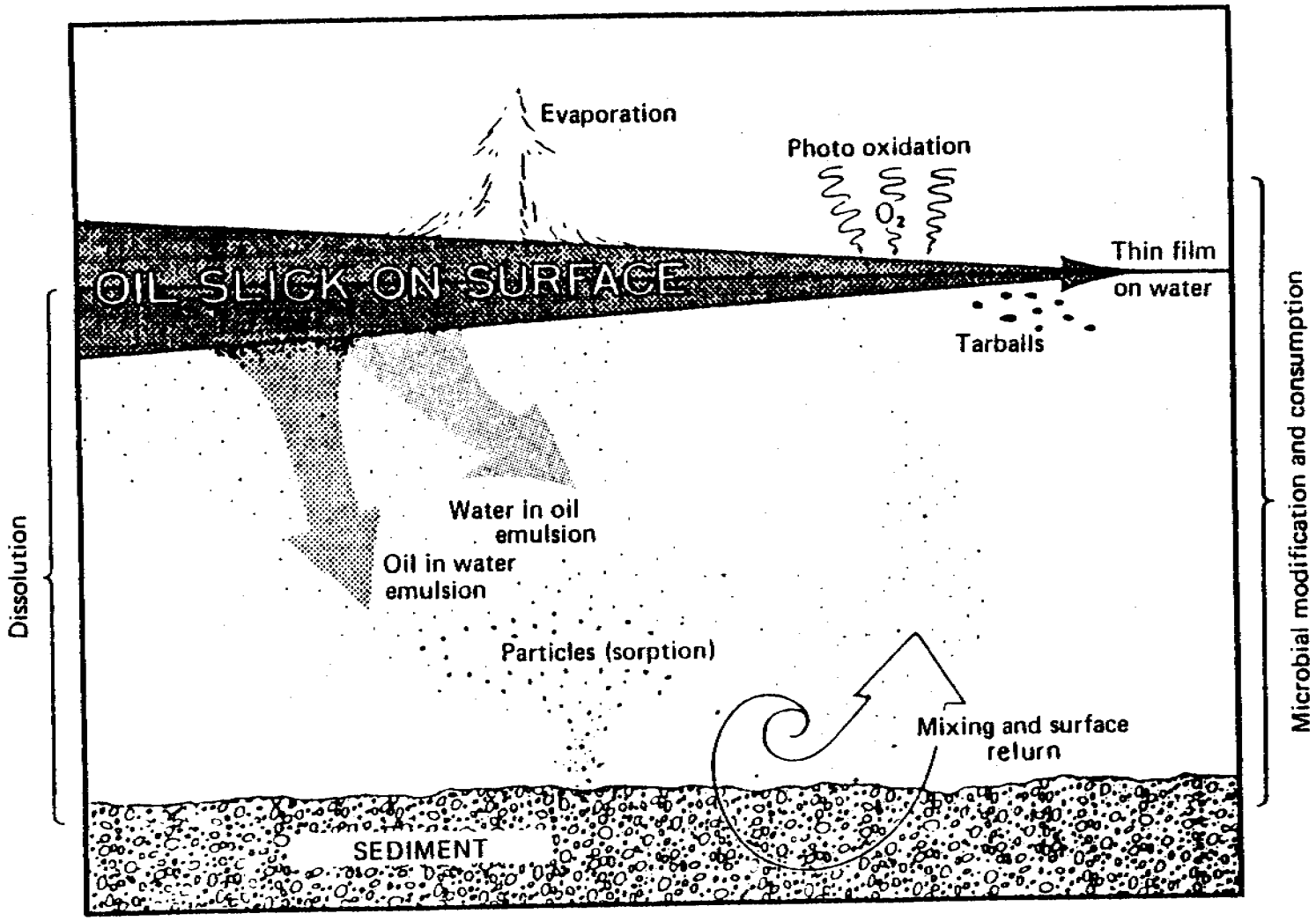


Figure 1.1. Schematic of the transport processes affecting spilled oil in the marine environment.

1978; Cretney et al., 1978; Keizer et al., 1978; Beslier et al., 1981; Atlas et al., 1981; Boehm and Fiest, 1981b). Boehm et al. (1981b) have conducted a comprehensive study of how Amoco Cadiz oil changed markedly in its composition with time after deposition in intertidal sediments (Figure 1-2). Oil buried beneath the aerobic zone is subject to little or very slow anaerobic degradation (Ward and Boehm, unpublished data). Oil may be transported to the benthos by several processes illustrated in Figure 1-3. In the case of chemical dispersion of oil, the magnitude of incorporation of oil into the benthos after dispersion is unknown. Therefore, oil transported to the benthos in small to moderate quantities can be expected to lose much of its obvious fingerprint if the hydrocarbons are available to microorganisms. The paraffinic fraction can first be altered by oxidation and isomerization, followed next by the aromatic fraction. Oil which has been highly weathered requires study by sophisticated and extensive analytical procedures prior to successful characterization. Pelagic tar balls are notorious exceptions to this rule, maintaining characteristic paraffinic patterns for considerable periods of time (Butler et al., 1973).

The use of molecular marker compounds for the long-term identification and detection of oil residues have been used previously. These compound classes are more resistant to environmental degradation than the commonly used fingerprintable material (i.e., alkanes). Of particular interest have been pentacyclic triterpanes (Dastillung and Albrecht, 1976; Boehm et al., 1981b; Atlas et al., 1981) alkylated phenanthrenes and dibenzothiophenes (Boehm et al., 1981b, Teal et al., 1978) and azaarene compounds (heterocyclic nitrogen aromatic compounds) (Jewell, 1980). Use of these markers requires their characterization in the source material, the pre-spill environment, and the post-spill contaminated samples.

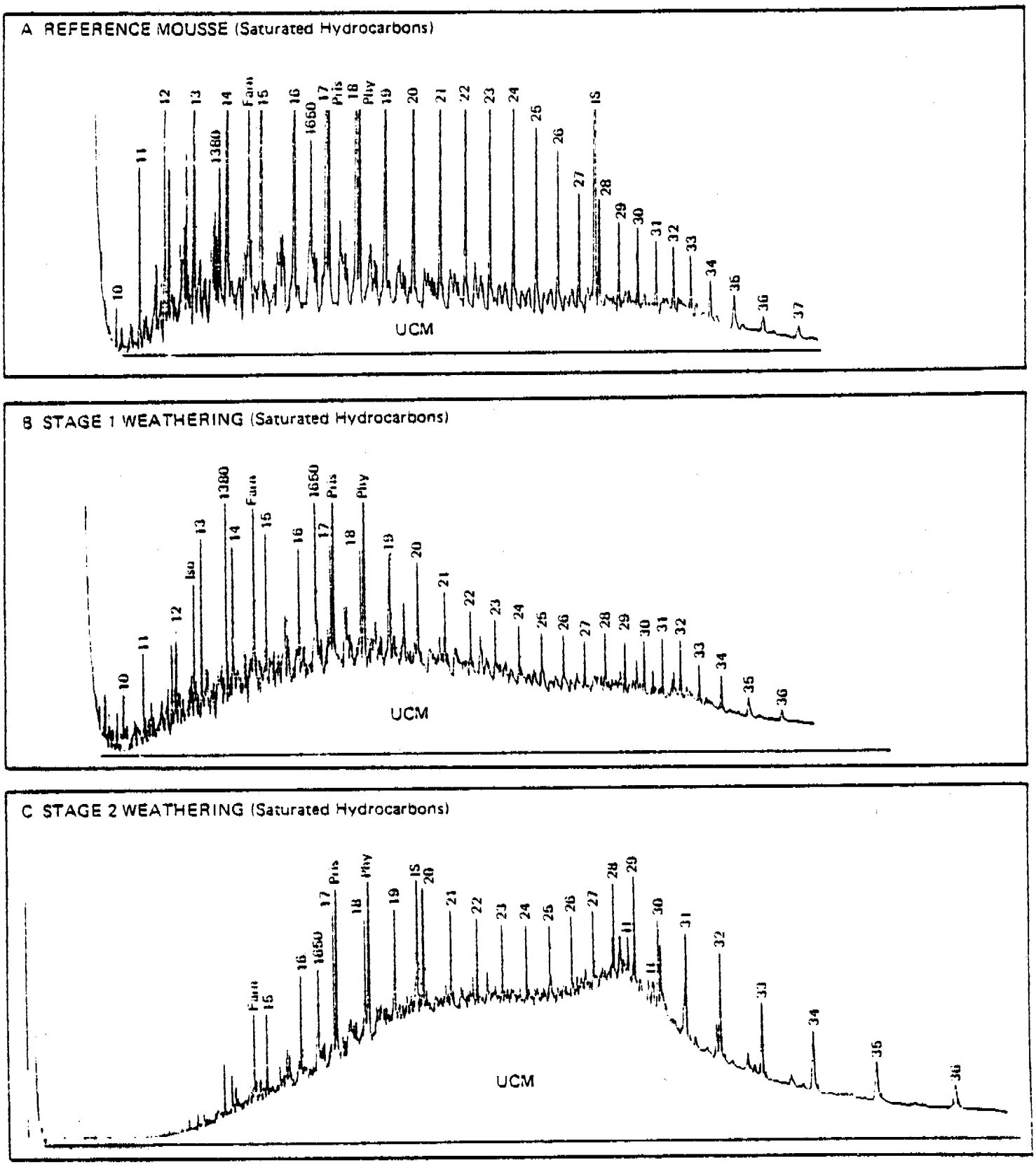


Figure 1.2. Weathering patterns of saturated hydrocarbons in *Amoco Cadiz* oil (from Boehm et al., 1981b).

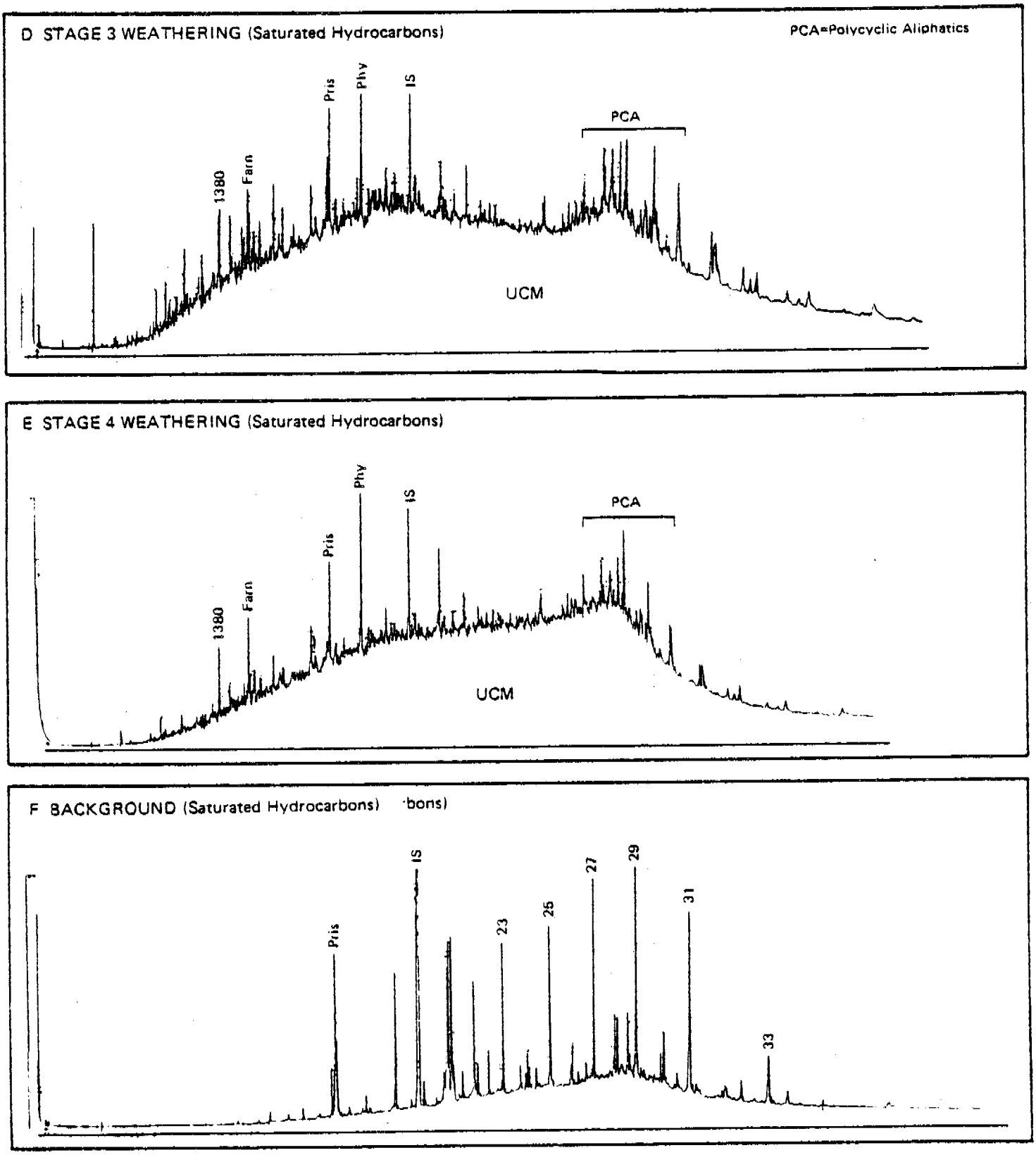


Figure 1.2. (Continued).

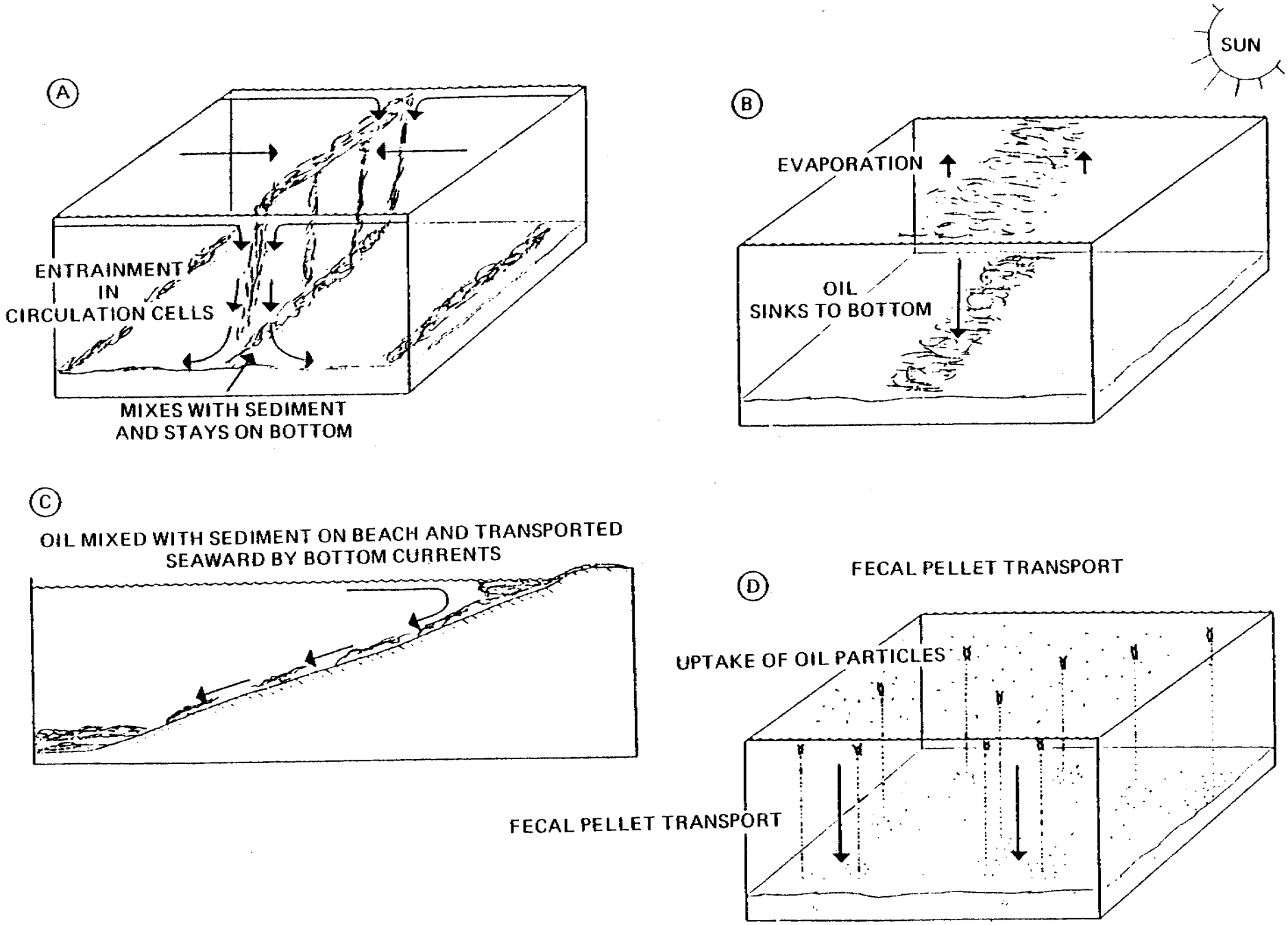


Figure 1.3. Hypothesized methods by which oil may be caused to sink and remain on the bottom.

SECTION TWO

SAMPLING AND ANALYTICAL METHODS

2.1 Sampling

Samples of seawater, offshore sediments, beach sediments (baseline), beach sediments (oiled test plots), and animal tissues were obtained from stations within the bays shown in Figures 2-1 and 2-2. Details of the sampling locations and sampling methods are given in Volume 1 of this report.

2.2 Analytical Methods

The choice of analytical methods used in this program (Table 1.3) was inspired by a need to generate a cost-effective set of data usable to two groups: (1) those requiring information on the presence and approxinate concentrations of petroleum hydrocarbons in samples and (2) those requiring detailed information on the composition of the hydrocarbon assemblage and the concentration of individual petroleum hydrocarbon components and marker compounds (e.g., Figure 2-3). Three analytical methods were employed sequentially: (1) UV/fluorescence-synchronous scan (UV/F), (2) glass capillary gas chromatography (GC^2), and (3) glass capillary gas chromatographic mass spectrometry (GC^2/MS) (Figure 2-4).

In recent years, UV/F spectra of environmental samples obtained when emission and excitation wavelengths are simultaneously scanned have yielded important, useful, compositional information on extracts of environmental samples (John and

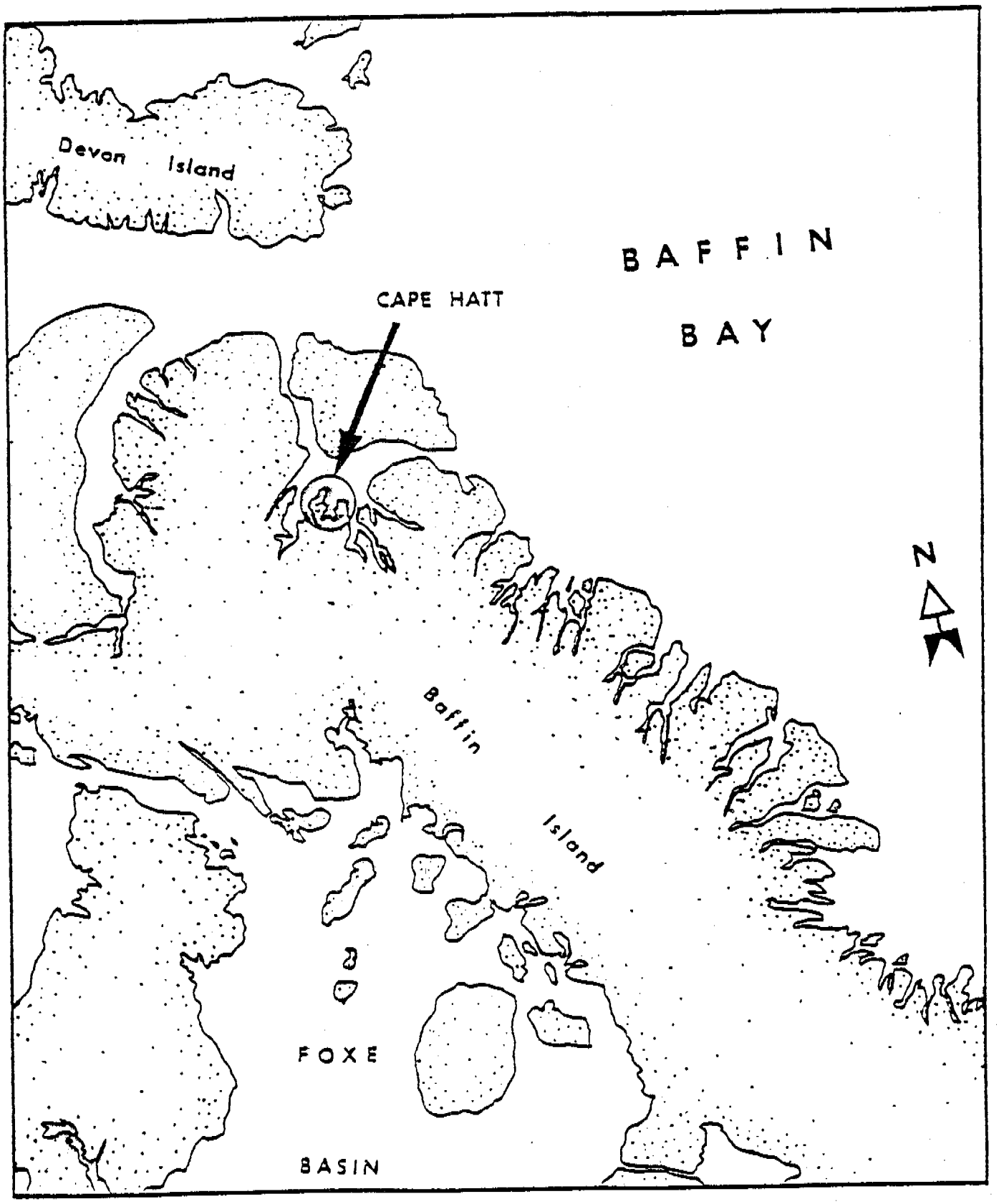


Figure 2.1. Location of Cape Hatt, Baffin Island.

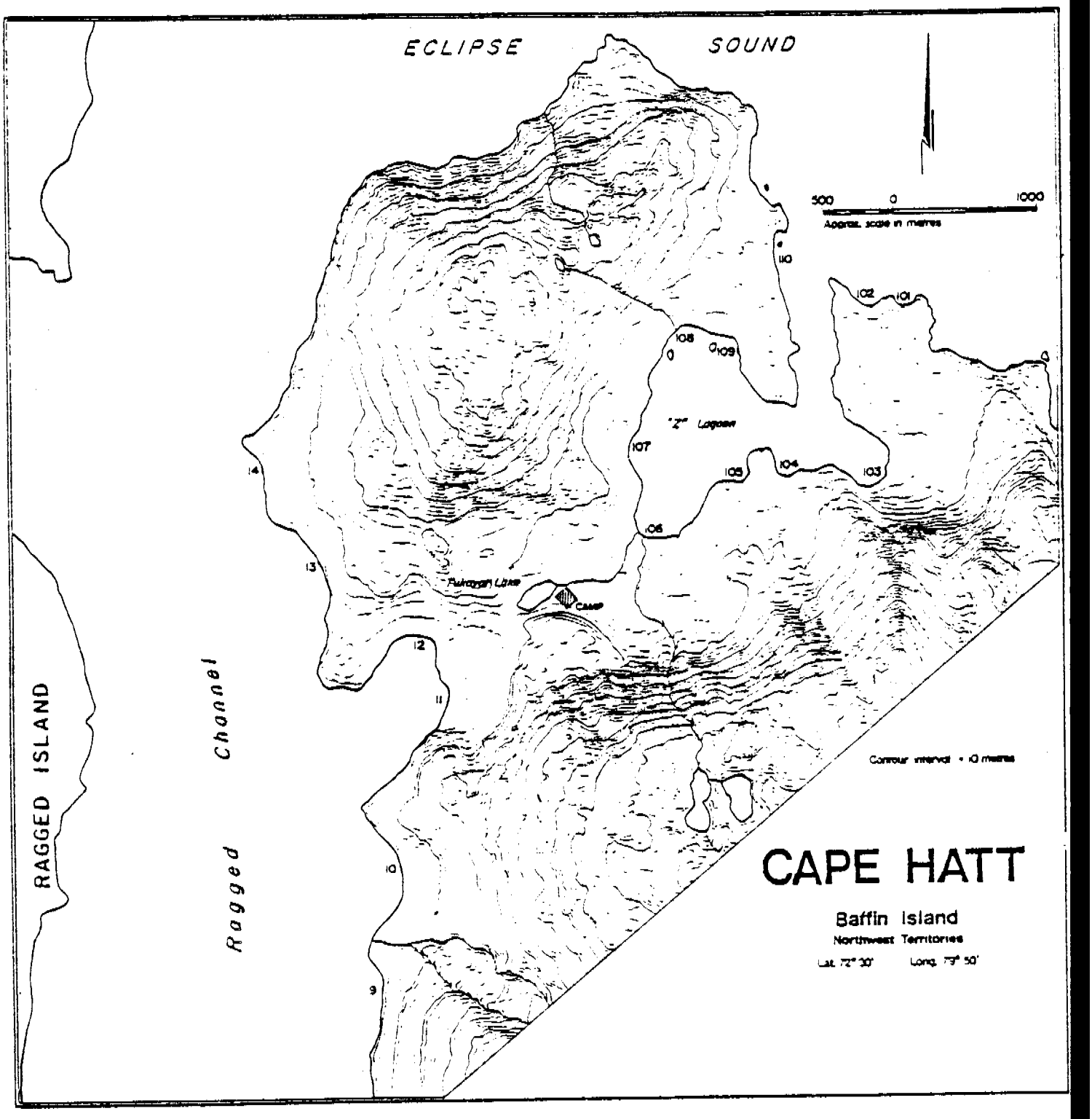
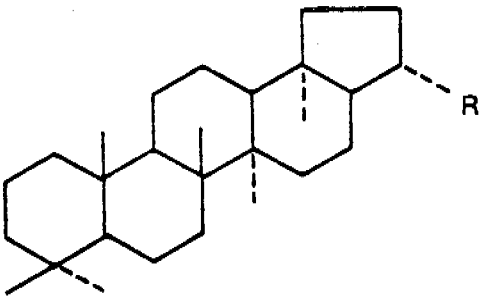


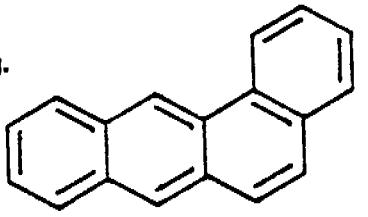
Figure 2.2. The Cape Hatt Site, Showing the Numbering of the Experimental Bays.

Pentacyclic Triterpanes—Marker Compounds



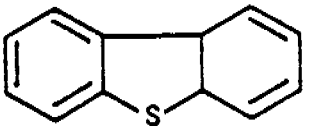
Aromatic Hydrocarbons—Toxicants, Carcinogens

e.g.

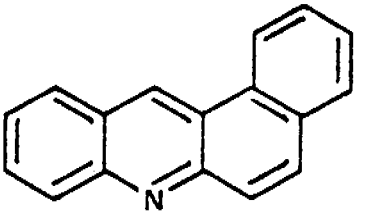


Aromatic Heterocyclics—Marker Compounds, Carcinogens

e.g.



(aromatic sulfur compounds)



(azaarenes)

Figure 2.3. Typical molecular structures of petroleum marker compounds analyzed by GC^2/MS.

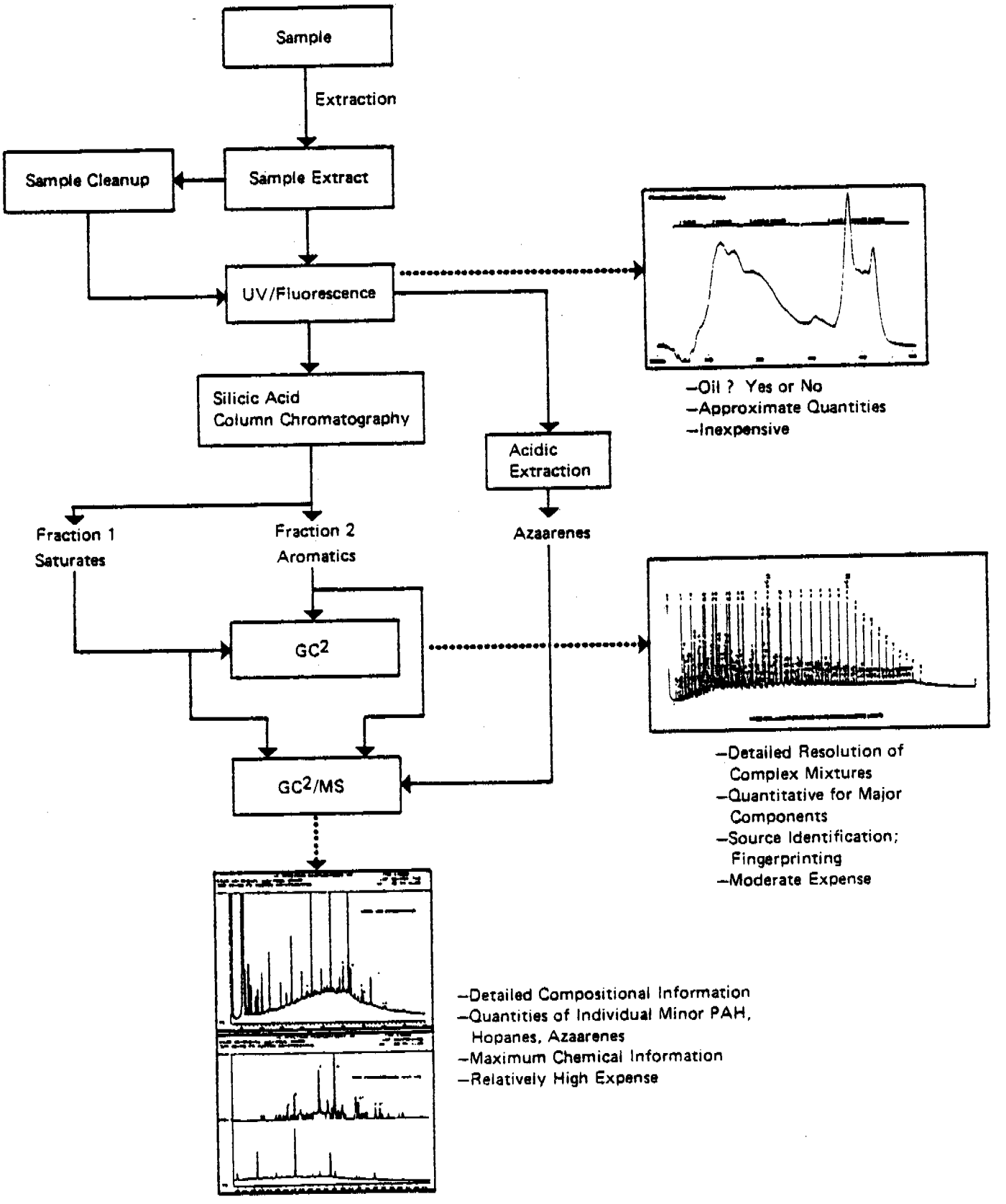


Figure 2.4. Schematic of Analytical Strategy.

Soutar, 1976; Wakeham, 1977; Gordon et al., 1976; Lloyd, 1971; VoDinh, 1978; Fiest and Boehm, 1981; Boehm and Fiest, 1981b). By appropriate selection of the solvent system and the offset of the excitation and emission monochronometers, aromatic (fluorescing) compounds in a mixture are resolved into distinct aromatic ring classes. By choosing an offset of 25 nm, 1-, 2-, 3-, 4-, and 5-ring aromatic compounds are resolved into discrete fluorescent bands (Lloyd, 1971). The wavelength bands are for benzenes, 280-290 nm; naphthalenes, 310-330; 3- and 4-ring compounds, 340-380 nm; and 5-ring compounds, >450 (Figure 2-5). The technique is quite useful for examining the relative weathering of oil in environmental samples and for comparing pre-spill and post-spill samples to determine if oil is present. Accurate quantitative information on hydrocarbon content is more difficult to obtain due to the specificity of the method for fluorescing (aromatic) compounds and the exclusion of, for example, saturates. If differential weathering affects the saturated and aromatic fractions then the use of a "spilled oil standard" is inappropriate unless corrected. Baseline measurements are more difficult to quantify unless the method is cross-calibrated with quantitatively more rigorous methods (e.g., microgravimetry and GC^2). The results obtained yield no information on individual component concentrations and on marker compounds. The method is widely used as a relatively inexpensive screening tool where extensive sample preparation is not involved.

If one desires information on the source of hydrocarbons, the concentrations of compound groups (i.e., saturated and aromatic hydrocarbons) and on concentrations and ratios of saturated hydrocarbons then GC^2 is employed. The method results in more detailed information by virtue of separating complex mixtures into individual components (e.g., Section One,

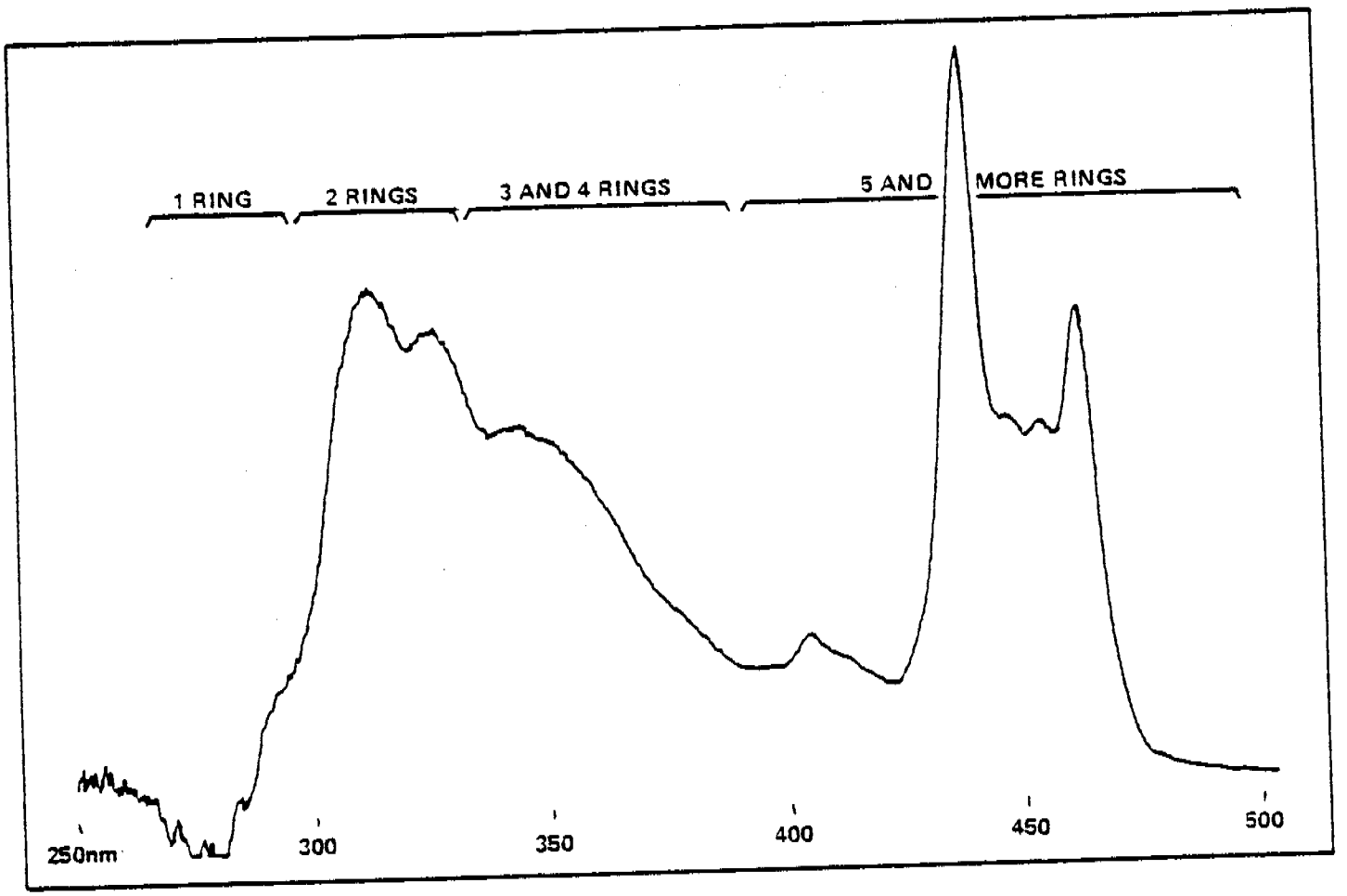


Figure 2.5. Synchronous Spectrofluorometry Spectrum of a Sediment Extract Showing the Resolution of Ring Classes and Perylene in the Right–Hand Side of the Spectrum.

Figure 1-2) thus yielding information on baseline compositions, petroleum weathering patterns, source identifications, and on differential uptake by marine organisms. The method is moderately expensive, requiring sample cleanup, fractionation and a mechanism for handling a large quantity of data. Most major components can be identified and quantified by this method.

GC^2/MS/computer is employed on sample fractions where the definitive identification and quantification of minor compounds (PAH, hopanes, azaarenes) is required. The method is of critical importance in baseline studies to identify and determine concentrations of specific trace level organics. In post-spill studies, the method yields concentrations of individual pollutant toxicants and carcinogens to relate to biological studies. Low-level molecular marker compounds are identified and quantified by GC^2/MS as well. The method is more expensive than GC^2 but yields the maximum amount of analytical information.

A summary of the specific analytical methods used in this project is presented in Table 2-1 for the sake of brevity.

Only the tissue analytical method deserves further mention. The steam distillation technique was adapted from that of Veith and Kiwus (1977). To examine the method in more detail we undertook a short study to determine the absolute recoveries of the range of compounds of interest. This included:

1. Analysis of BIOS tissue samples by the steam distillation and aqueous digestion (Warner, 1976; Boehm et al., 1981a) procedure.

TABLE 2-1

SUMMARY OF METHODS USED IN THIS ANALYTICAL PROGRAM

SAMPLE TYPE	ANALYSIS	METHOD SUMMARY	REFERENCES
Seawater	Synchronous UV/F	Freon extraction; analysis of unfractionated extract	Wakeham, 1977; Gordon et al., 1976; Vo-Dinh, 1978; Lloyd, 1971
Seawater	GC^2	Temperature-programmed capillary analysis; SE52 fused silica columns; internal standard quantification; GC^2 and gravimetric analysis of f_1 and f_2 silicic acid column eluates; computation of individual component levels and key diagnostic parameters	Boehm, 1980; Cram & Young, 1980; Boehm & Fiest, 1981a
Seawater	GC^2/MS	GC/MS/computer system (HP5985); quantification by mass fragmentography	Boehm et al., 1981a, 1981b
Sediments	Synchronous UV/F	Azeotropic room temperature extraction; analysis of whole extract	Wakeham, 1977; Boehm & Fiest, 1981a; Boehm et al., 1981b; Boehm et al., 1979;
Sediments	GC^2	(see GC^2 for seawater)	Farrington et al., 1976; Boehm et al., 1981b; Boehm & Fiest, 1981b

TABLE 2-1 (Cont.)

SAMPLE TYPE	ANALYSIS	METHOD SUMMARY	REFERENCES
Sediments	GC^2/MS	Computer search for 1- to 5-ring aromatics; pentacyclic triterpanes; azaarenes	Teal et al., 1978; Farrington, 1980; Pym et al., 1975 Ensminger et al., 1974; Overton et al., 1981; Boehm et al., 1981b
Tissues	GC^2, GC^2/MS	Steam distillation; isolation of extracted distillate; silicic acid fractionation; GC^2, GC^2/MS	Ackman & Noble, 1973; Veith & Kiwus, 1977; this report; Boehm et al., 1981a; Clark, 1974; Warner et al., 1980
Oils	Physical measure-ments	Absolute viscosity; interfacial tension; density	ASTM, D455; ASTM, D971
Oils	Chemical character-ization (GC^2, GC^2/MS)	Saturates, aromatics, azaarenes, triterpanes	Overton et al., 1981 Boehm & Fiest, 1981a; Pym et al., 1975
Oils	Trace metals	High-temperature ashing; ICAP analysis	Leone & Church, 1976

TABLE 2-2

STEAM DISTILLATION RECOVERY/EFFICIENCY - MIXED SATURATED/AROMATIC STANDARD

COMPONENT	% RECOVERY
$n\text{-}C_{10}$	35
$n\text{-}C_{11}$	43
Naphthalene	91
$n\text{-}C_{12}$	47
$n\text{-}C_{13}$	56
$n\text{-}C_{14}$	80
Hexamethyl benzene	103
$n\text{-}C_{15}$	106
$n\text{-}C_{16}$	117
$n\text{-}C_{17}$	115
Pristane	105
Phenanthrene	113
Anthracene	105
$n\text{-}C_{18}$	112
Phytane	110
$n\text{-}C_{19}$	98
$n\text{-}C_{20}$	96
$n\text{-}C_{21}$	76
$n\text{-}C_{22}$	75
$n\text{-}C_{23}$	85
$n\text{-}C_{24}$	80
$n\text{-}C_{25}$	89
Chrysene	38
$n\text{-}C_{26}$	98
$n\text{-}C_{28}$	102
Perylene	56
$n\text{-}C_{29}$	100
$n\text{-}C_{30}$	101
$n\text{-}C_{31}$	108
$n\text{-}C_{32}$	110
Benzoperylene	85

2. Determining absolute recoveries of a complex mixture of standards.

3. The steam distillation of an actual polluted tissue extract to determine recoveries of a "real world" pollutant assemblage.

Approach 1 was not undertaken after it became apparent that a large intrinsic variation hydrocarbon composition existed in the animals (see Section 3.2.6). A tabulation of the absolute recoveries of the complex standard mixture is given in Table 2-2.

Although the recoveries of several of the compounds are low, they are no lower than those achieved by other techniques. That is, the light saturates and aromatics ($<n\text{-}C_{14}$) are subject to procedural losses in most methods geared for "high-molecular-weight" hydrocarbon analysis. Figure 2-6 illustrates that a complex aromatic hydrocarbon extract from an Amoco Cadiz polluted oyster consisting of alkylated naphthalenes, phenanthrenes, and dibenzothiophenes was quantitatively recovered after its steam distillation.

Thus, the steam distillation method used is quite satisfactory for use in this project.

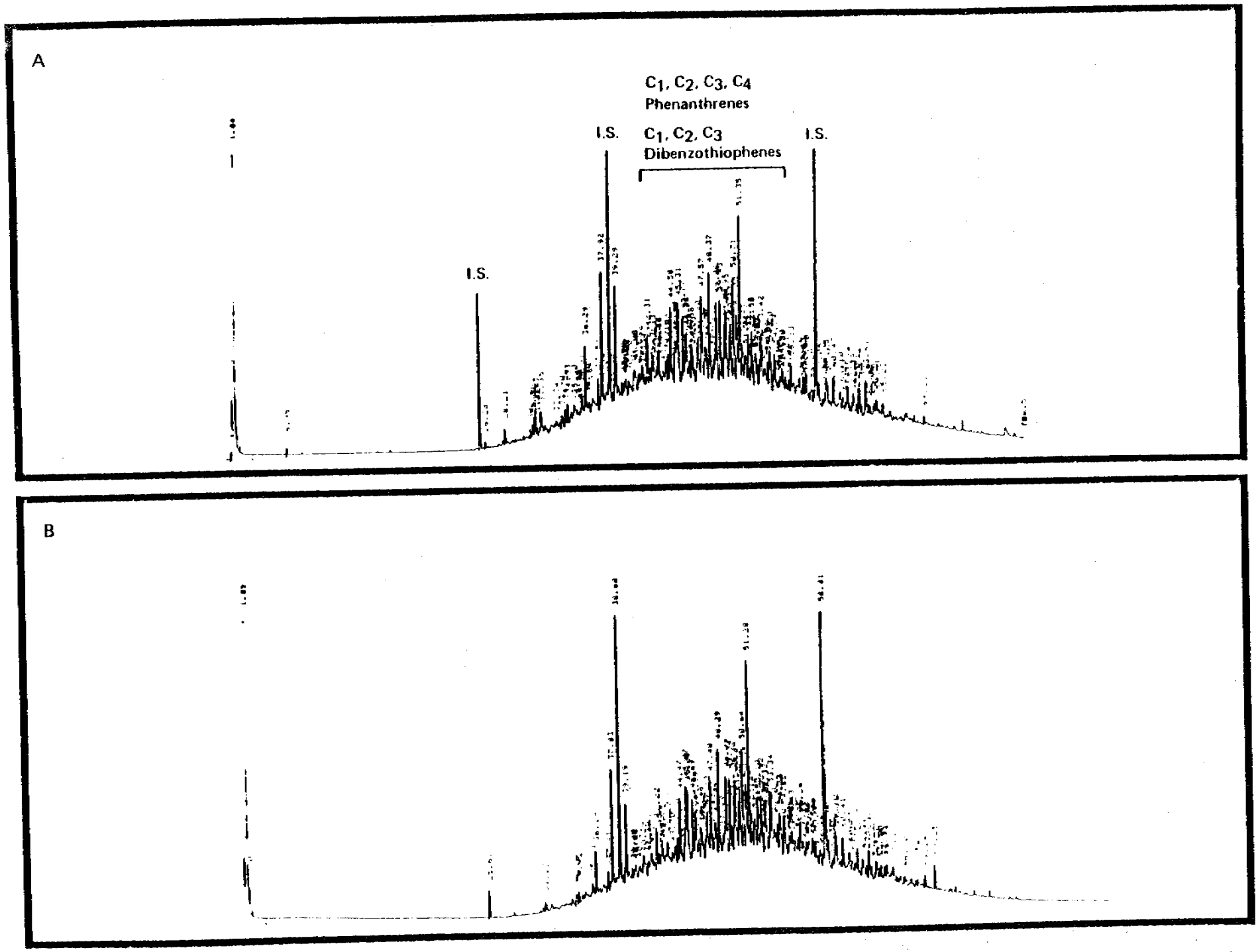


Figure 2.6. Steam Distillation Recovery of Polluted Tissue Aromatic Extract, A–Aromatics (Original); B–Aromatics (After Steam Distillation).

SECTION THREE

RESULTS

3.1 Oil Characteristizations

3.1.1 Gross Composition

The quantitative breakdown between saturated, aromatic polar (NSO), and residual (asphaltene) fractions of the fresh and aged Lagomedio crude oil and a 10:1 oil/corexit mixture are presented in Table 3-1.

3.1.2 Saturated Hydrocarbons

The saturated hydrocarbons of the Lagomedio crude oil include n-alkanes in the boiling range of n-C_9 to n-C_{34} (Figure 3-1). Approximately 70% of the fresh, unweathered crude elutes prior to n-C_{15} compared to 50% for the weathered (or aged) oil. The comparative GC[2] traces are shown in Figure 3-1 with the major normal alkane and branched alkane (isoprenoid) components labelled.

Several other important parameters are presented in Table 3-2. Note how the artificial aging of the crude has influenced the saturate composition through the boiling range n-C_9 to pristane. The changing saturated hydrocarbon weathering ratio (SHWR) is a measure of the evaporative weathering process. The alkane to isoprenoid ratio (ALK/ISO) quantifies the relative composition of the more easily biodegraded n-alkanes to the less readily degraded isoprenoids.

TABLE 3-1

GROSS CHEMICAL CHARACTERIZATIONS OF LAGOMEDIO CRUDE OIL AND OIL/COREXIT 9527 MIXTURE

SAMPLE	% SATURATES[a]	% AROMATICS[a]	% POLARS[a]	% RESIDUAB	% ASPHALTENES[b]
Fresh (unweathered) oil	59.1	35.2	6.3	0	1.2
Aged oil	58.8	30.0	14.8	0	2.5
Aged: dispersant (10:1)	44.2	27.5	24.7	3.6	ND

[a]Determined from silicic acid column chromatographic fractionation; f_1 = hexane eluate; f_2 = hexane:methylene chloride (60:40) eluate; f_3 = methanol eluate; residual = material not eluting off column.

[b]Asphaltenes = pentane-insoluble material. Note: asphaltenes may elute in both f_2 and f_3 fractions.

ND = not determined

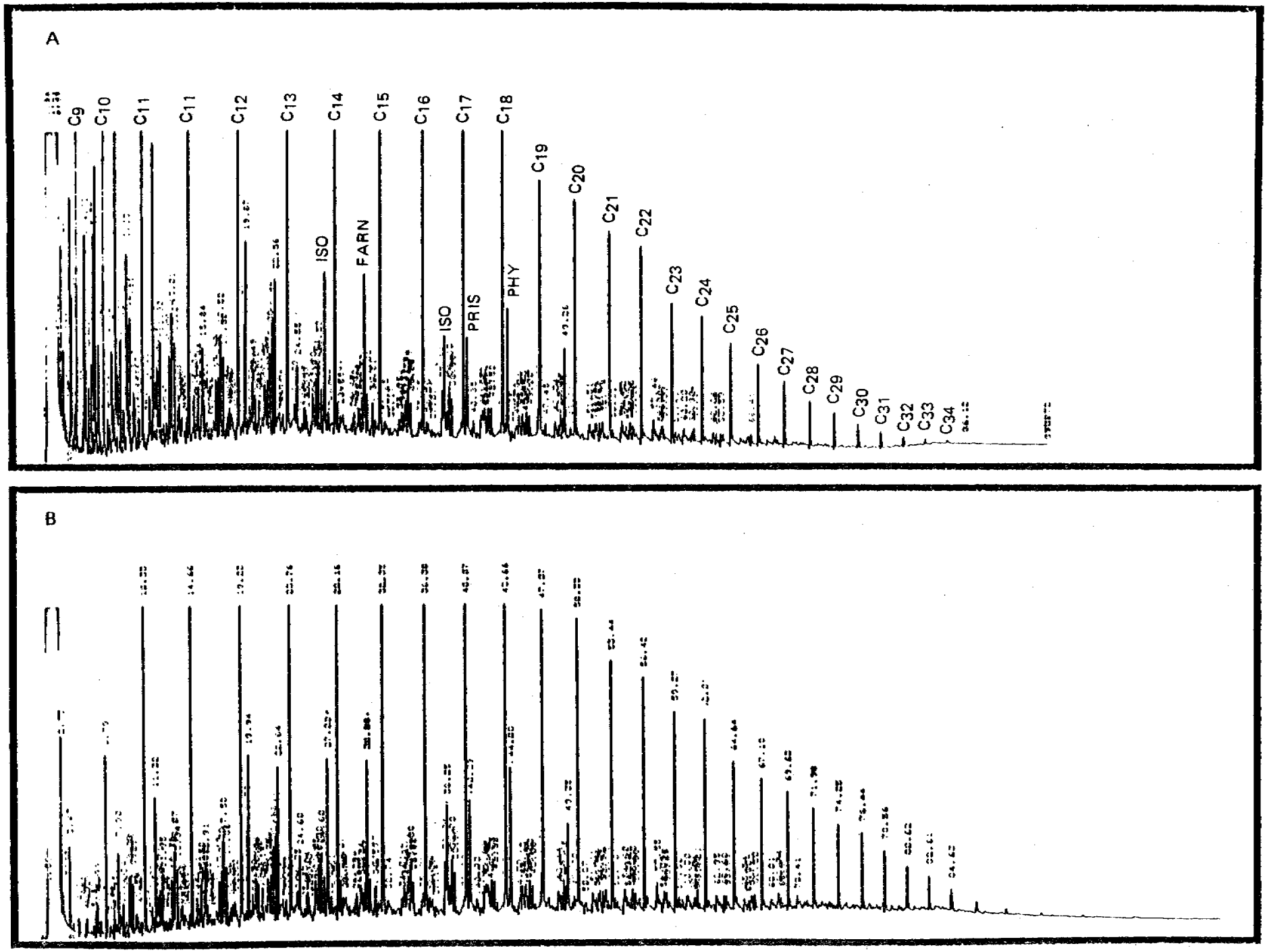


Figure 3.1. GC^2 Traces of Saturated Hydrocarbons of A—Unweathered Lagomedio Crude Oil, B—Aged Oil.

TABLE 3-2

SATURATED AND AROMATIC HYDROCARBON PARAMETERS OF LAGOMEDIO CRUDE OIL[a]

	FRESH OIL	AGED OIL
Saturates		
SHWR	2.87	2.28
ALK/ISO	2.36	2.50
PRIS/PHY	0.85	0.74
PRIS/n-C_{17}	0.51	0.38
PHY/n-C_{18}	0.61	0.62
Aromatics		
AWR	4.29	3.47

[a]Key:

$$\text{SHWR} = \frac{(\Sigma \text{ n-alkanes; } C_{10}\text{-}C_{25})}{(\Sigma \text{ n-alkanes; } C_{17}\text{-}C_{25})}$$

$$\text{AWR} = \frac{\text{(Alkyl Benzenes + Naphthalenes + Fluorenes + Phenanthrenes + Dibenzothiophenes)}}{\text{Phenanthrenes + Dibenzothiophenes}}$$

$$\text{ALK/ISO} = \frac{(\Sigma \text{ alkanes; } C_{14}\text{-}C_{18})}{(\Sigma \text{ 5 isoprenoids; in n-}C_{13} \text{ boiling range})}$$

PRIS = pristane

PHY = phytane

A graphical comparison of the saturated hydrocarbon composition is shown in Figure 3-2. Note from this presentation how significant compositional changes appear throughout the boiling range.

3.1.3 Pentacyclic Triterpanes

GC^2/MS analysis of the hopane-type pentacyclic triterpanes reveals small quantities of four compounds (Figure 3-3): Compound C (norhopane; $C_{29}H_{50}$), Compound D ($C_{30}H_{52}$); Compounds E, E' (homohopanes $C_{31}H_{54}$) and a pattern of 12 secondary peaks of unknown structure.

3.1.4 Aromatic Hydrocarbons (UV/F)

UV/F analysis of several dilutions of the aged Lagomedio crude is presented in Figure 3-4. Major quantities of 2-, 3-, and 4-ring aromatics are apparent with lesser quantities of the 5-ring compounds.

3.1.5 Aromatic Hydrocarbons

GC^2/MS analysis of the Lagomedio crude indicate that compounds from alkyl benzenes to the benzopyrenes are detected in the aged and fresh crudes. The GC^2/MS data is presented in several different ways: (1) a semi-log plot of aromatic hydrocarbon concentrations (Figure 3-5) of the aged oil, (2) comparative GC^2 traces of the fresh and aged crude oil aromatic fraction (Figure 3-6), (3) a comparative plot of the aromatic compositions normalized to trimethyl dibenzothiophene (Figure 3-7), and (4) GC^2/MS mass fragmentograms (Appendix A).

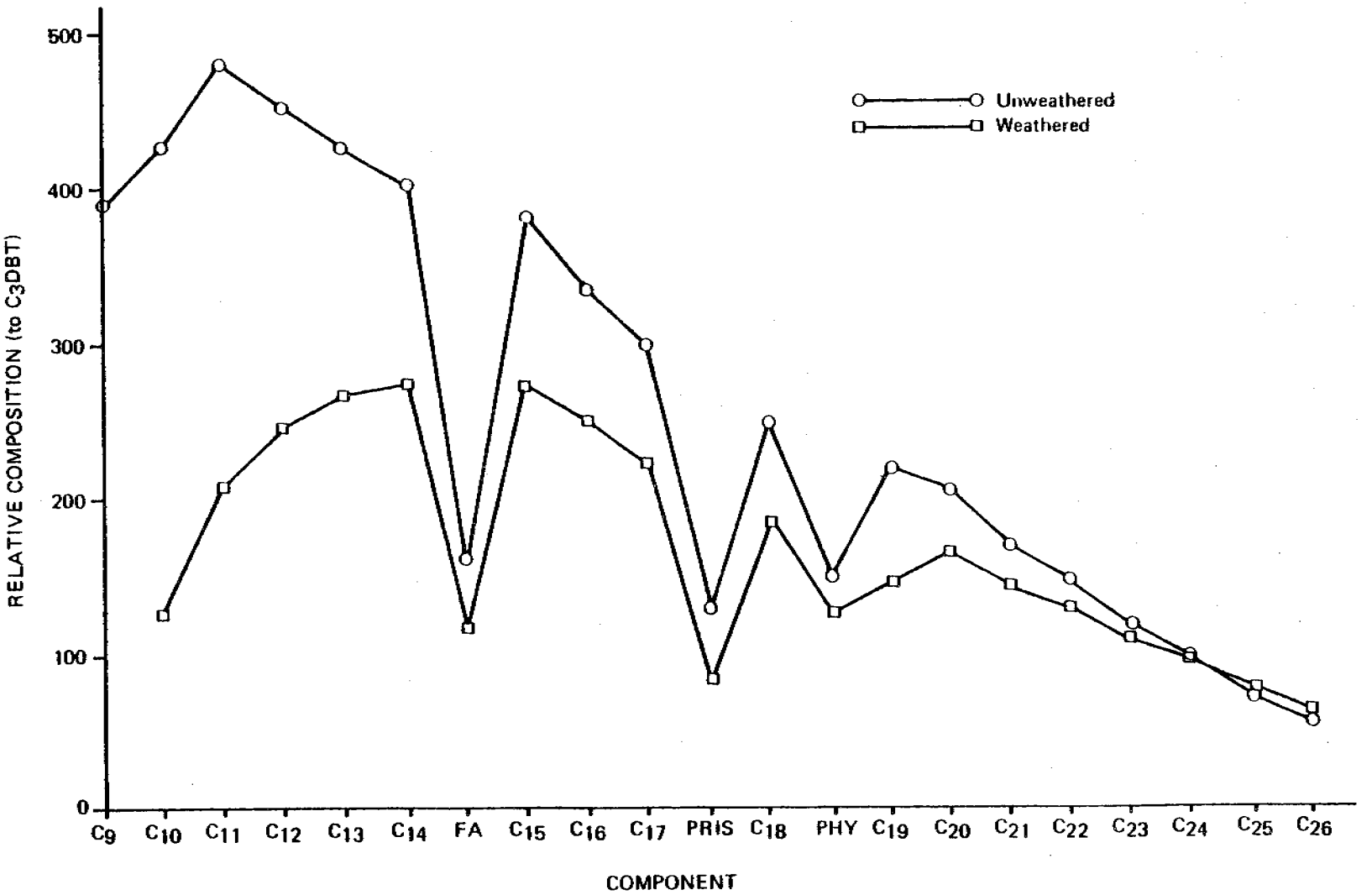


Figure 3.2. Saturated Hydrocarbon Composition of Weathered vs. Unweathered Lagomedio Crude Oil.

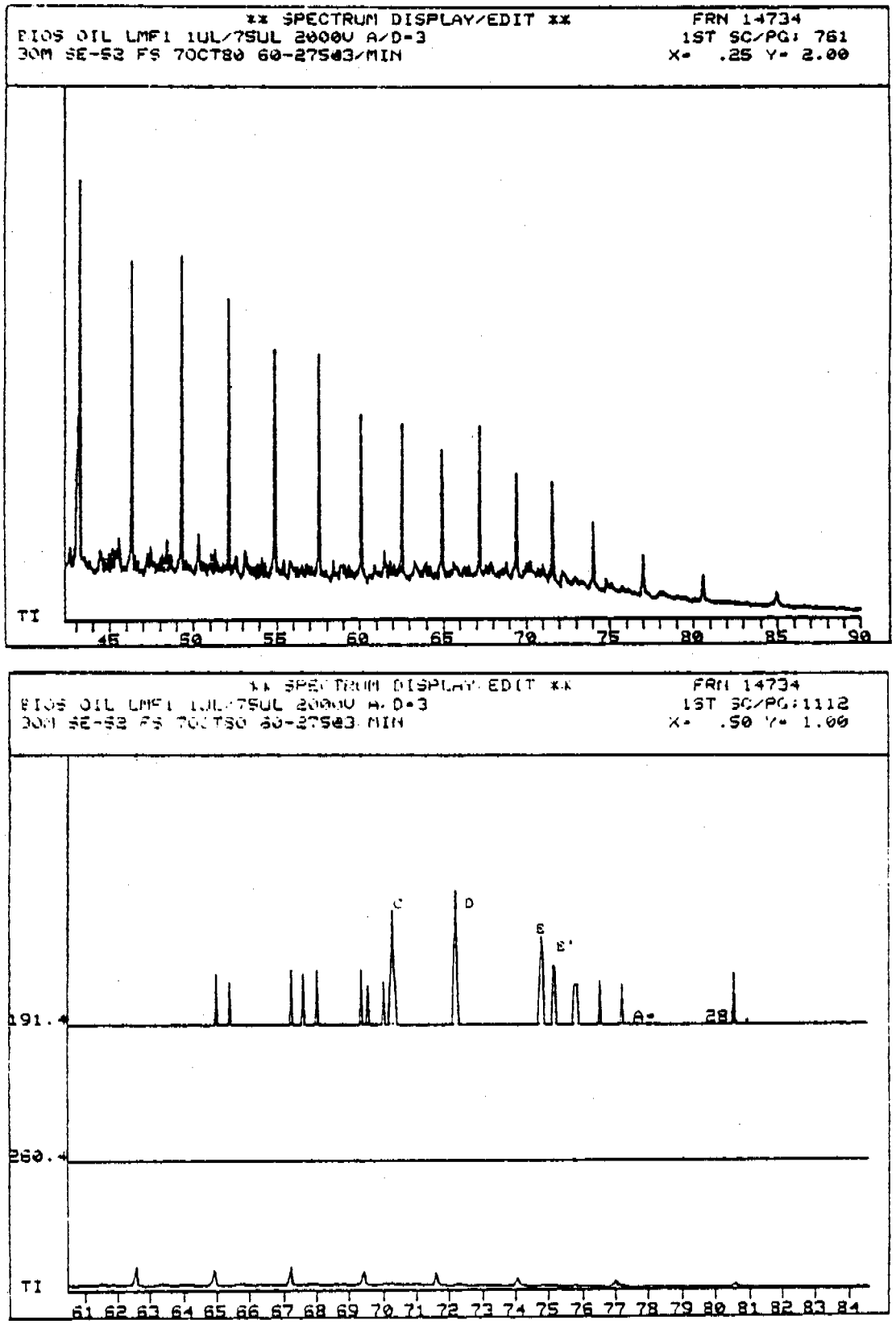


Figure 3.3. Bios Oil—Hopane Analysis (GC^2/MS).

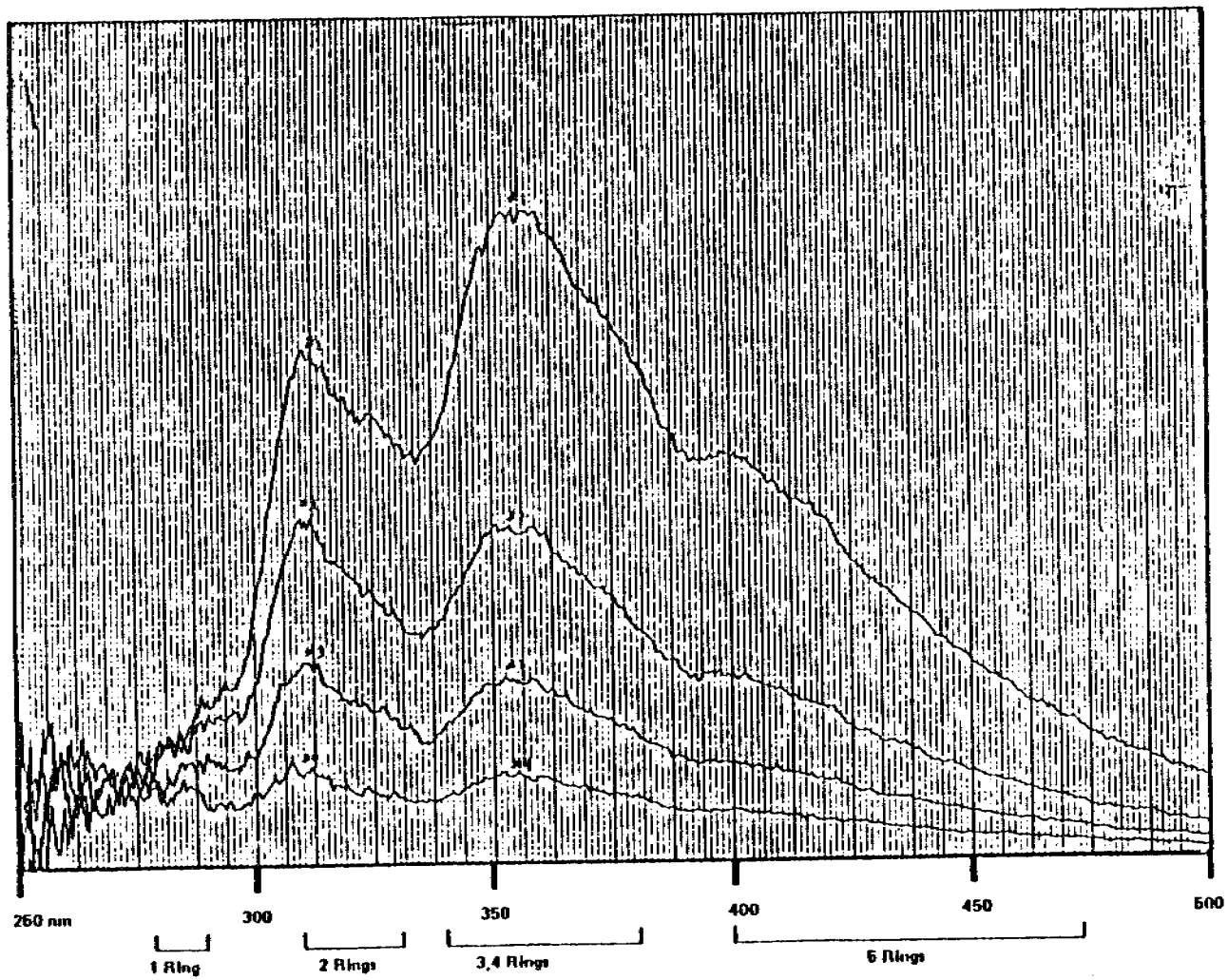


Figure 3.4. UV/Fluorescence (Synchronous Scan) of Lagomedio Crude.

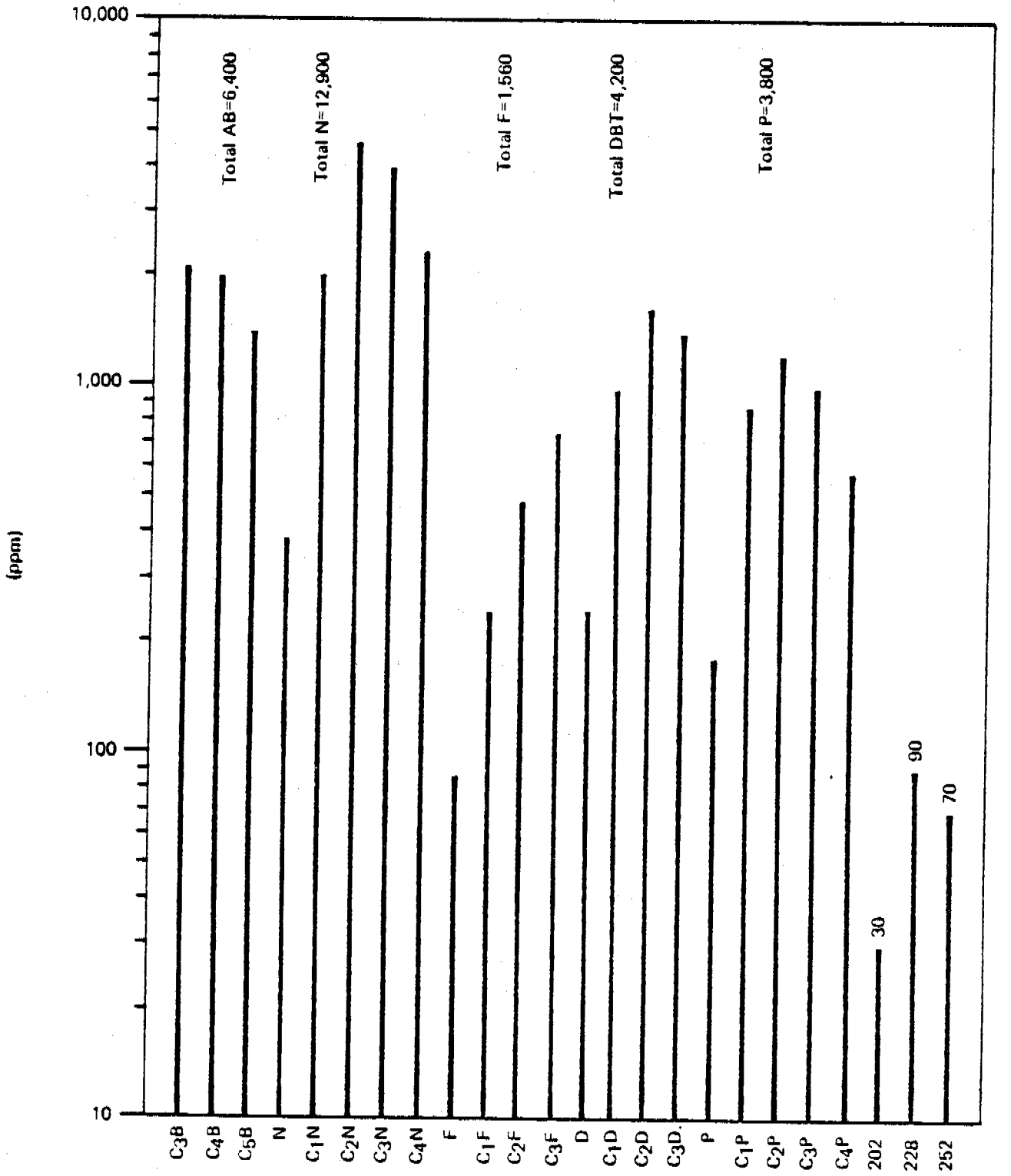
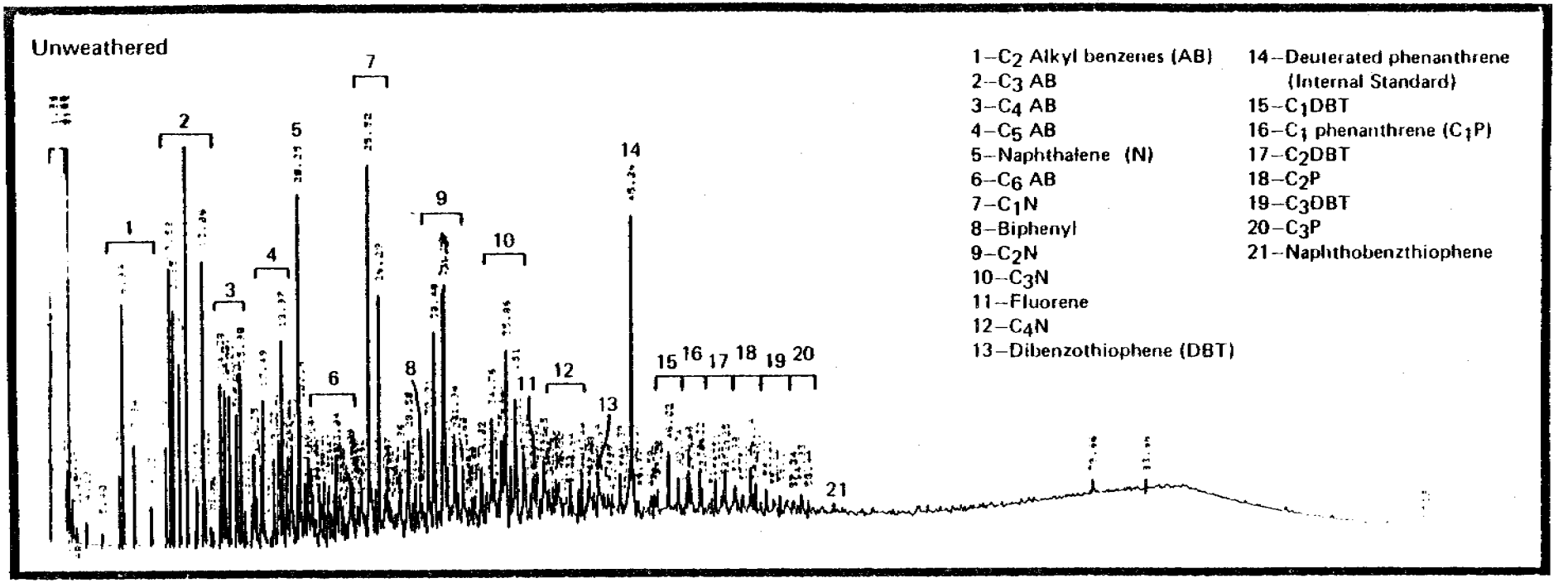


Figure 3.5. PAH Composition of Weathered Lagomedio Crude Oil (ppm).

(B=Benzene, N=Naphthalene, F=Fluorene, D=Dibenzothiophene, P=Phenanthrene, 202=Fluoranthene/Pyrene, 228=Chrysene, 252=Benzopyrenes)



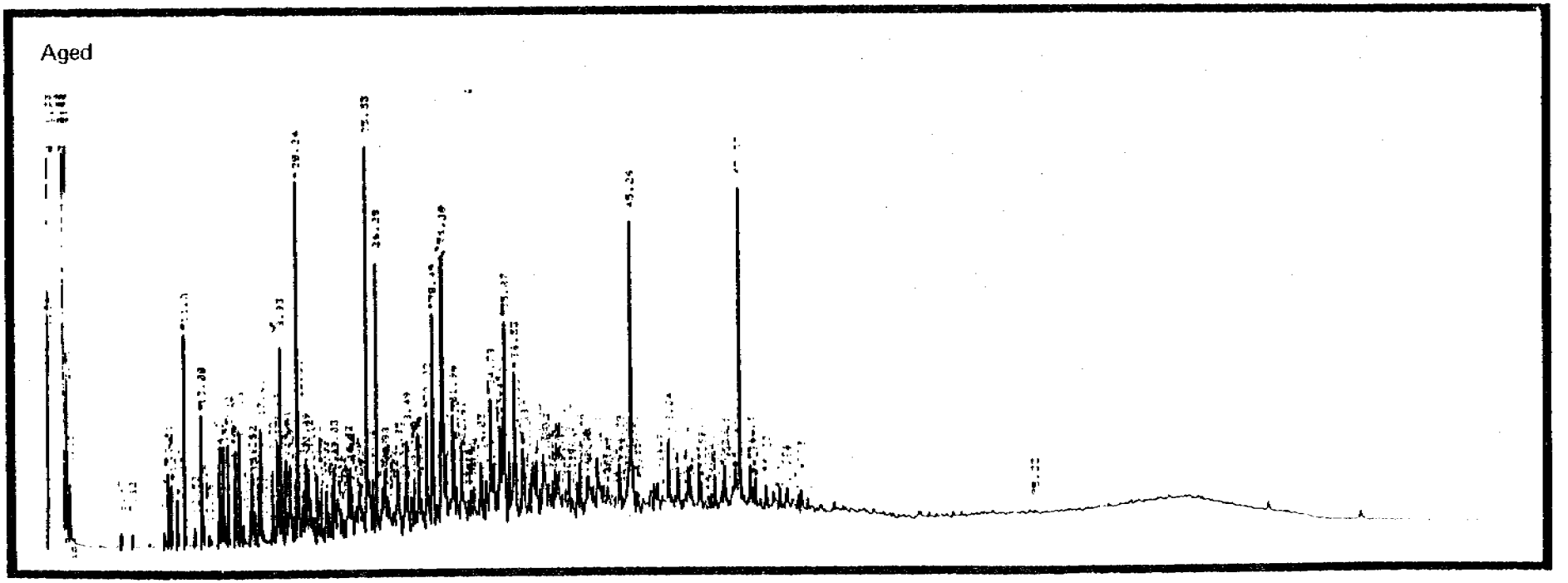


Figure 3.6. GC2 Traces of Aromatic Hydrocarbons in Lagomedio Crude Oil.

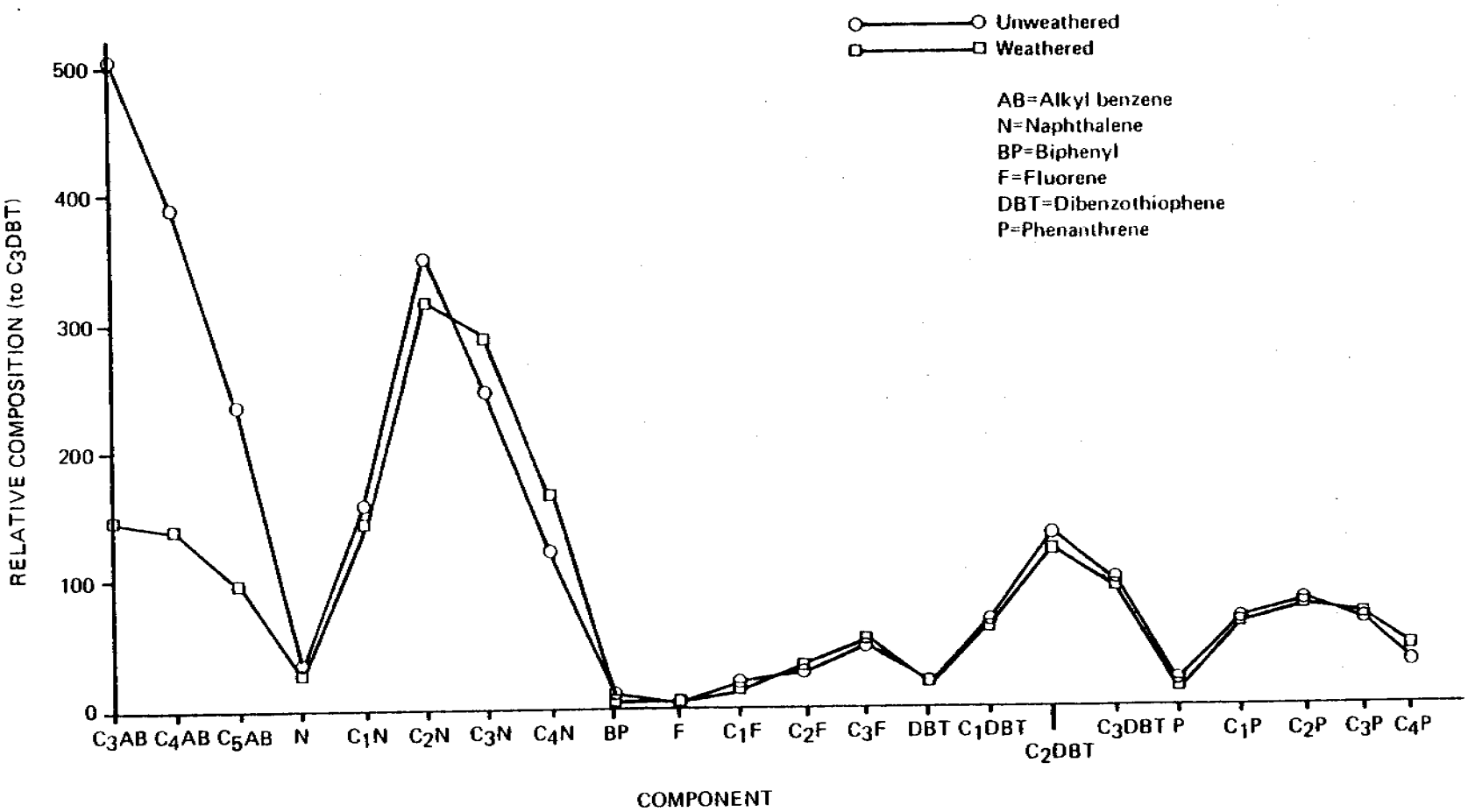


Figure 3.7. Aromatic Hydrocarbon Composition of Weathered vs. Unweathered Lagomedio Crude Oil.

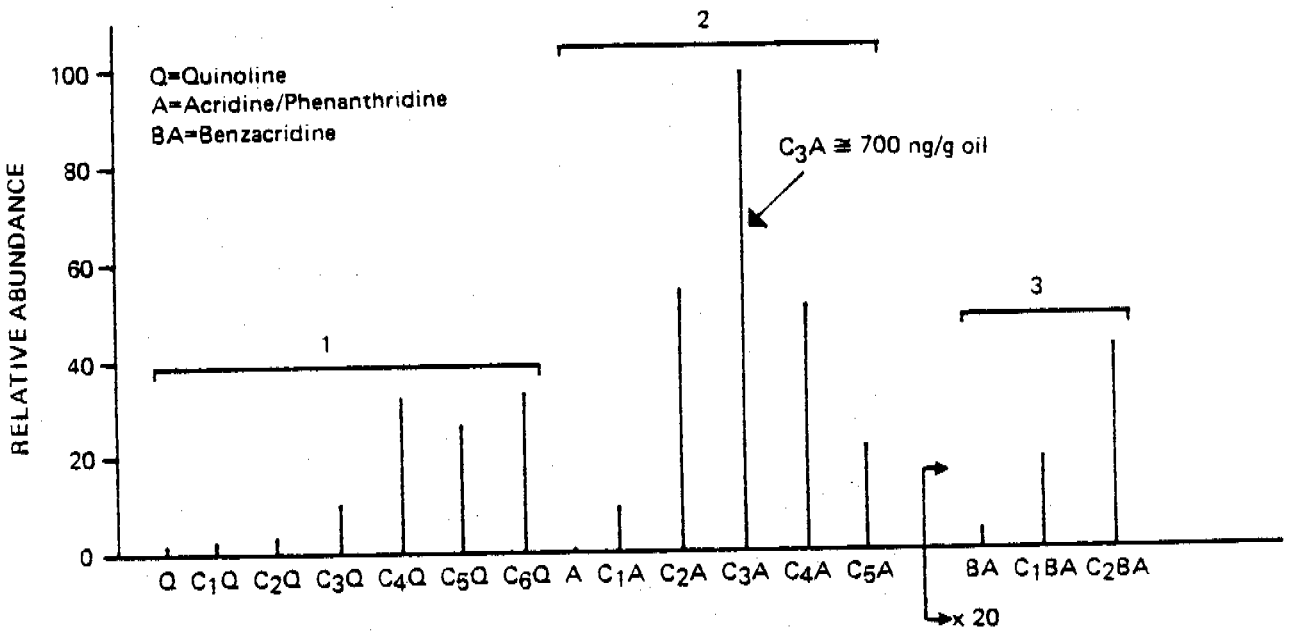
In addition, a key compositional parameter, one sensitive to artificial and post-spill weathering, the aromatic weathering ratio (AWR), is presented in Table 3-2. The crude can be classified as a light crude being heavily dominated by naphthalene and alkyl benzene compounds. The dibenzothiophenes (heterocyclic aromatic sulfur compounds) are the third most abundant group followed by the 3-ring phenanthrenes and fluorenes. Lesser quantities of the 4- and 5-ring polycyclic aromatics are present.

3.1.6 Azaarenes

Acidic extractions of crude oils followed by GC^2/MS analyses of the acidic fraction yielded clean azaarene analysis. Note that the azaarenes are far less abundant than their aromatic hydrocarbon cousins (e.g., trimethyl phenanthrenes $\sim$ 1,000 µg/g oil; trimethyl acridines/phenanthridines $\sim$ 700 ng/g oil). The azarene composition of the aged crude is identical to that from the fresh oil. The complete GC^2/MS analysis of the azaarenes is presented in Appendix B. The relative abundance of the major azaarenes is shown in Figure 3-8. The major components of the azaarene assemblage are the C_3 (trimethyl) acridines (m/e 221) whose mass fragmentogram (Figure 3-9) reveals at least six isomers of the 3-ring azaarenes.

3.1.7 Physical Measurements

Measurements of absolute kinematic viscosity (ASTM method D445), interfacial (oil/seawater) tension (ASTM method D971), and density were performed at -5°C, 0°C, and +5°C for three oils: aged Lagomedio crude; aged Lagomedio crude:Corexit 9527



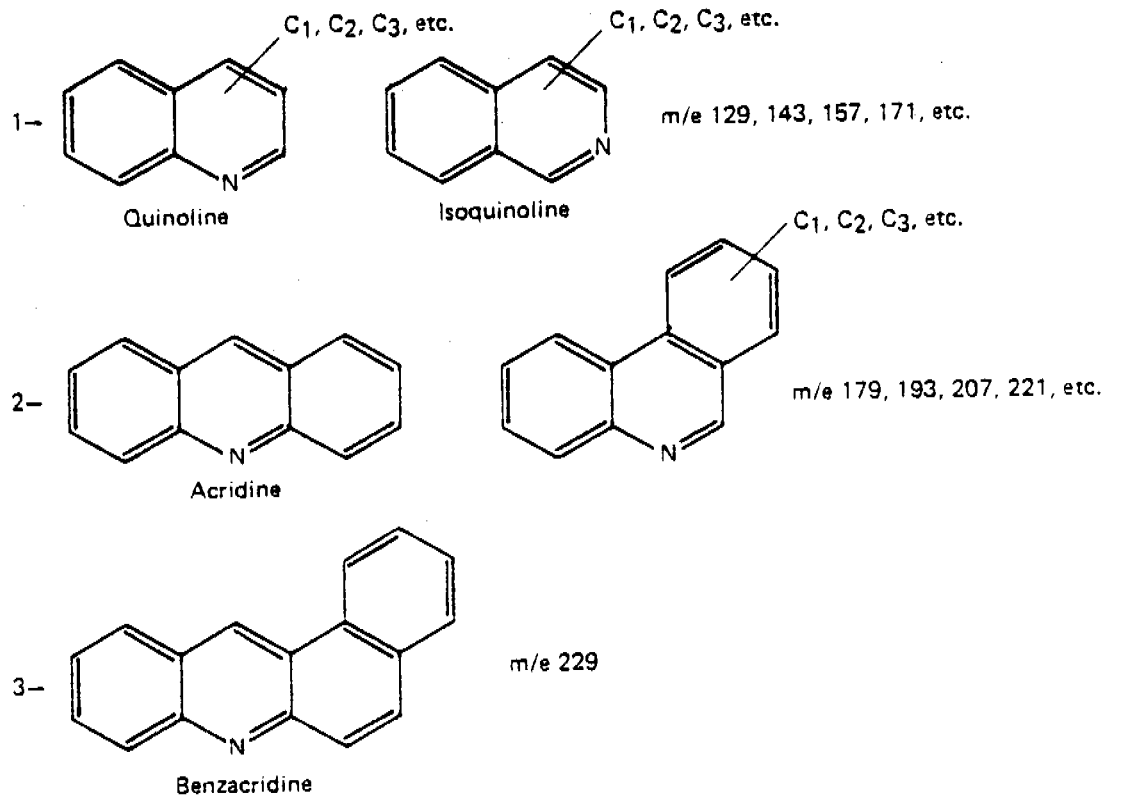


Figure 3.8. Azaarene composition of Lagomedio crude oil.

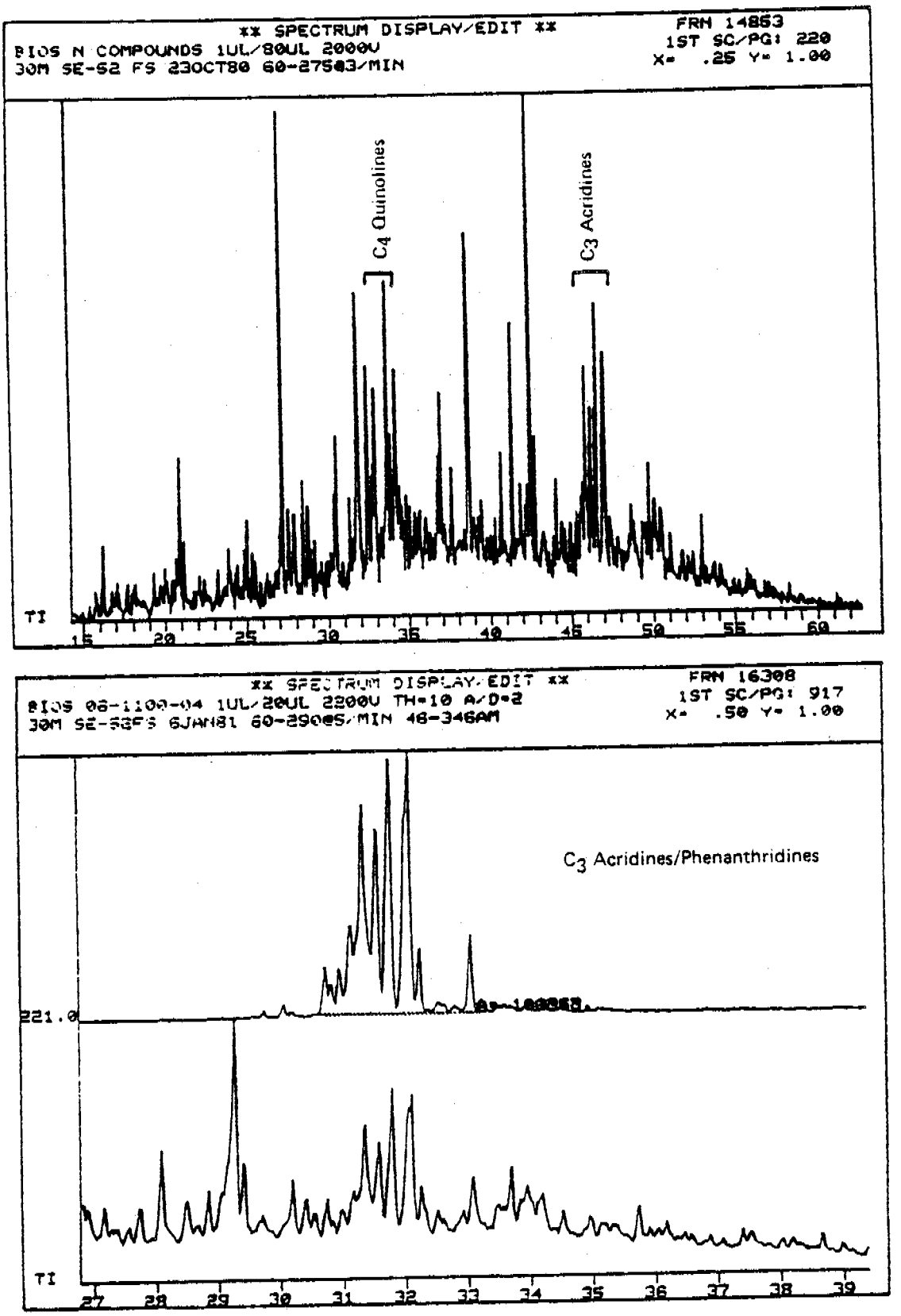


Figure 3.9. Lagomedio Crude Oil: Total Ion Chromatogram (A) and Mass Chromatogram of Trimethyl Acridines/Phenanthridines (m/e = 221) (B).

(10:1); aged Lagomedio crude:Corexit 9527 (1:1). The results are presented in Tables 3-3, 3-4, and 3-5.

As the note in Table 3-3 indicates, significant wax precipitation precluded the obtaining of absolute viscosity measurements. The viscosity values reported in the note (Table 3-3) were found to be both time dependent and influenced by the capillary size of the viscometer used. The samples are non-Newtonian at the temperatures in question and their viscosities cannot be determined by capillary viscometry. Apparent viscosity data, if required, may be determined by the use of the Brookfield viscometer.

The data reported for the 1:1 sample and at 5°C for the crude oil and 10:1 mix samples were obtained under conditions under which wax formation was minimal and under which the samples behaved in a Newtonian manner.

These results have great bearing on the behavior of the oil under the proposed spill conditions. Wax formation is to be expected and must be dealt with both in the diffuser system and in the sampling scheme.

3.1.8 Trace Metal Composition

The trace metal composition of the aged Lagomedio crude oil is given in Table 3-6. The analyses are presented in comparison to another Venezuelan crude, a Kuwait crude, and a Louisiana crude. Of greatest interest are the high nickel and vanadium (12.4 ppm and 135 ppm, respectively) contents of the Lagomedio crude.

TABLE 3-3

ABSOLUTE VISCOSITY OF CRUDE OIL AND OIL/DISPERSANT MIXTURES
(centistokes)

	AT -5°C	AT 0°C	AT +5°C
Lagomedio crude	Note[a]	Note[a]	154.1
Lagomedio crude:Corexit 9527 (10:1)	Note[a]	Note[a]	120.0
Lagomedio crude:Corexit 9527 (1:1)	218.0	144.6	100.3

[a]The samples appeared to precipitate waxy components at 0° C and -5° C. These prevented determination of the viscosity of the sample by clogging the orifice of the viscometer. The viscosities determined in the second section of the reverse flow viscometers used for the determinations were invariably higher than those determined in the first section.

Viscosity (centistokes)

At 0° C		At -5° C	
1st Section	2nd Section	1st Section	2nd Section
1,420	2,640	1,629	3,351
880	1,288	9,801	20,960

TABLE 3-4

INTERFACIAL TENSION OF CRUDE OIL AND OIL/DISPERSANT MIXTURES VERSUS STANDARD SEAWATER (35 o/oo) (dynes/cm)

	AT -5°C	AT 0°C	AT +5°C
Lagomedio crude	ND[a]	16.7	19.8
Lagomedio crude:Corexit 9527 (10:1)	1.7	1.3	3.4
Lagomedio crude:Corexit 9527 (1:1)	1.3	1.3	2.0

[a]Not determined.

TABLE 3-5

DENSITY OF CRUDE OIL AND OIL/DISPERSANT MIXTURES (g/cm^3)

	AT -5°C	AT 0°C	AT +5°C
Lagomedio crude	0.8990	0.8958	0.8923
Lagomedio crude:Corexit 9527 (10:1)	0.9118	0.9082	0.9045
Lagomedio crude:Corexit 9527 (1:1)	0.9621	0.9586	0.9551

TABLE 3-6

SUMMARY OF TRACE METAL ANALYSIS OF OILS
(ppm)

SAMPLE	Ni	V	Al[a]	Ba	Be	Cd	Co
Aged Venezuelan Crude	12.4	135	2.5	0.06	<0.01	<0.02	0.05
Venezuelan Crude[b]	8.1 ±0.5	125 ±13	--[c]	--[c]	--[c]	--[c]	0.094 ±0.022
Kuwait Standard Oil (API)							
ERCO Analysis	7.5	24	0.96	0.05	<0.01	<0.02	0.05
Certified Value	7.7	16.8	--[d]	--[d]	--[d]	--[d]	--[d]
Louisiana Standard Oil (API)							
ERCO Analysis	1.3	0.73	0.58	0.11	<0.01	<0.02	0.10
Certified Value	1.4	0.67	--[d]	--[d]	--[d]	--[d]	--[d]
Blank	<0.2	<0.1	1.1	<0.02	<0.01	<0.02	<0.02

[a]Values are blank corrected.
[b]Leone and Church (1976), p. 42.
[c]Not reported.
[d]Not certified.

TABLE 3-6 (Cont.)

SAMPLE	Cr	Cu	Fe[a]	Mn	Pb	Ti[a]	Zn[a]
Aged Venezuelan Crude	<0.5	<0.05	1.9	<0.05	<0.5	0.05	1.5
Venezuelan Crude[b]	0.04 ±0.05	0	0.76 ±0.10	0.007 ±0.002	--[c]	--[c]	--[c]
Kuwait Standard Oil (API)							
ERCO Analysis	<0.5	0.10	1.1	<0.05	<0.5	0.29	<1.0
Certified Value	--[d]	--[d]	--[d]	--[d]	--[d]	--[d]	--[d]
Louisiana Standard Oil (API)							
ERCO Analysis	<0.5	0.06	3.9	<0.05	<0.5	0.13	<1.0
Certified Value	--[d]	--[d]	--[d]	--[d]	--[d]	--[d]	--[d]
Blank	<0.5	<0.05	2.4	<0.05	<0.5	0.04	3.2

[a]Values are blank corrected.
[b]Leone and Church (1976), p. 42.
[c]Not reported.
[d]Not certified.

3.2 Hydrocarbons Baseline Studies

3.2.1 Seawater Samples - UV/F Analyses

Forty-nine (49) 4-liter water samples, obtained by using an NBS-type drop sampler (see Volume 1) were extracted three times with 75 ml Freon 113. One-half of the resultant unfractionated extract representing 2 liters of seawater was analyzed by synchronous scanning spectrofluorometry (UV/F).

3.2.1(a) June Samples

The UV/F spectra of the June water samples showed very low levels of fluorescent material, not detectable above a significant freon blank (Table 3-7). The values presented in Table 3-7 were obtained by quantifying with respect to a No. 2 fuel oil standard (Figure 3-10A) and by subtracting the blank value. Thus, we relied mainly on quantitation of the 312-nm peak in the spectrum. The spectral characters of most of the water samples were quite similar (e.g., Figure 3-11), exhibiting a single main peak at 312 nm, presumably associated with the blank.

3.2.1(b) August/September Samples

A similar, but larger, suite of samples and field blanks were collected during the August/September sampling. The results in Table 3-8 indicate for the most part nondetectable (<3 µg/l) levels of petroleum-type hydrocarbons. Four samples, however, did contain detectable petrogenic material (Figure 3-10B). The concentrations in these samples ranged from 3 to 86 µg/l. All of these samples were pre-spill

TABLE 3-7

UV/FLUORESCENCE RESULTS OF JUNE WATER SAMPLES

	SAMPLE	COLLECTION DATE	SAMPLING DEPTH (m)	µg/l[a]
Bay 9	H5 (extract)	6/14/80	1	ND[b]
Bay 9	H5 (extract)	6/14/80	5	ND
Bay 9	H5 (extract)	6/14/80	10	ND
Bay 10	H3 (extract)	6/14/80	1	ND
Bay 10	H3 (extract)	6/14/80	5	ND
Bay 10	H3 (extract)	6/14/80	10	ND
Bay 11	H1 (extract)	6/14/80	1	ND
Bay 11	H1 (extract)	6/14/80	5	ND
Bay 11	H1 (extract)	6/14/80	10	ND
Bay 9	H5 (whole water)	6/22/80	1	ND
Bay 9	H5 (whole water)	6/22/80	5	ND
Bay 9	H5 (whole water)	6/22/80	10	ND
Blank (solvent and field extraction)				9-50

[a]As equivalents of No. 2 fuel oil measured at 312 nm; corrected for blank.

[b]Not detected.

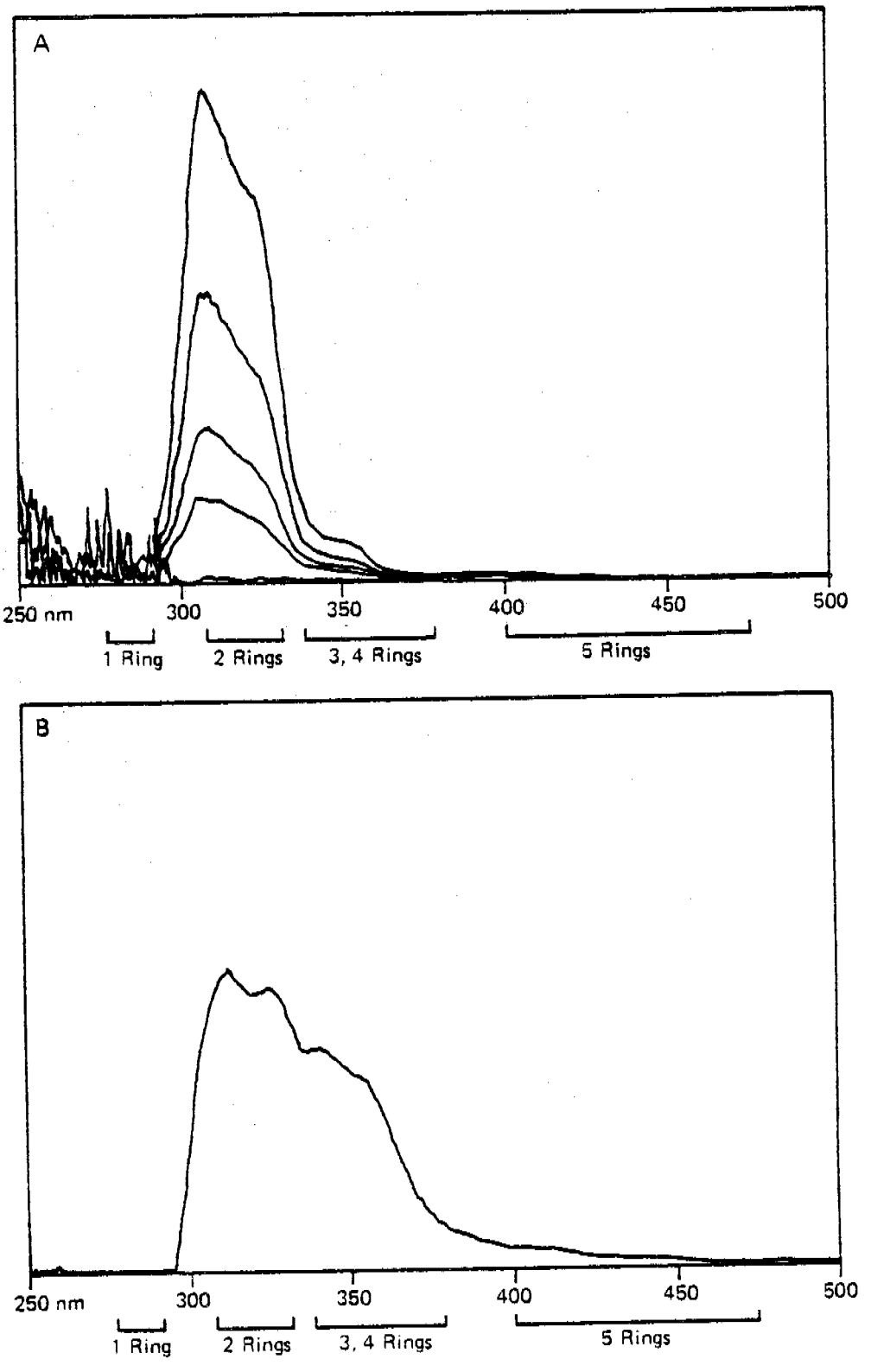


Figure 3.10. UV/F Spectra of A—Number 2 Fuel Oil and B—Water Sample with Detectable Petroleum Hydrocarbons.

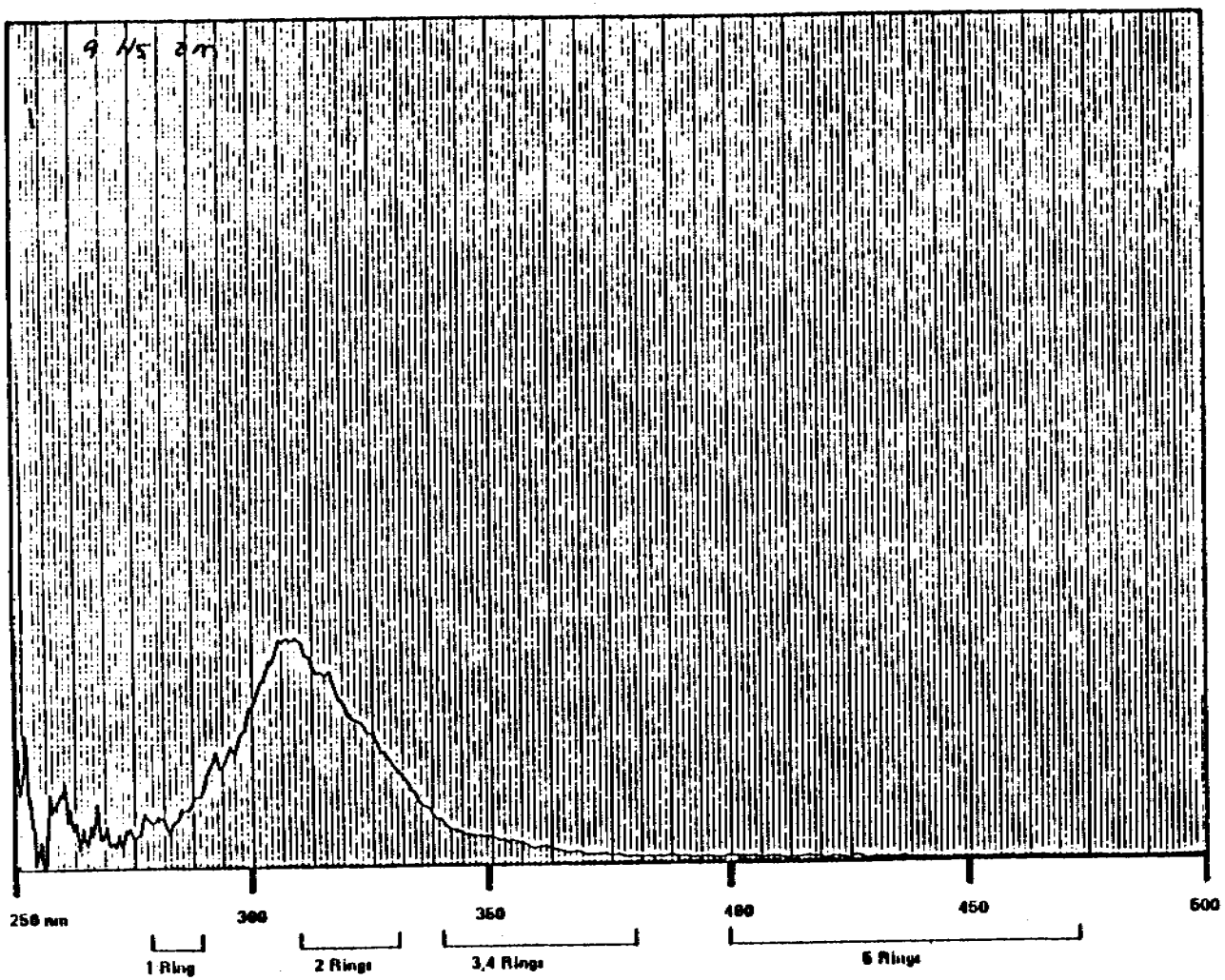


Figure 3.11. UV/Fluorescence (Synchronous Scan) of Bios Water—Baseline.

TABLE 3-8

UV/FLUORESCENCE RESULTS OF AUGUST/SEPTEMBER SAMPLES

SAMPLE	COLLECTION DATE	SAMPLING DEPTH (m)	CONCENTRATION[a,b] (µg/l)
Bay 9	8/26/80	1	ND[c]
Bay 9	8/26/80	5	ND
Bay 9	8/26/80	10	ND
Bay 9	9/20/80	1	ND
Bay 9	9/20/80	5	ND
Bay 10	8/26/80	1	ND
Bay 10	8/26/80	10	ND
Bay 10	8/26/80	10	86
Bay 10	9/19/80	1	ND
Bay 10	9/19/80	5	ND
Bay 10	9/19/80	10	ND
Bay 11	8/26/80	1	ND
Bay 11	8/26/80	5	ND
Bay 11	9/19/80	1	ND
Bay 11	9/19/80	5	ND
Bay 11	9/18/80	10	ND
Bay 102 Prespill	8/18/80	1	3
Bay 102 Prespill	8/18/80	4	ND
Z Lagoon Bay 103 Prespill NBS	8/17/80	1	ND
Z Lagoon Bay 103 Prespill NBS	8/17/80	10	13
Bay 103 Prespill	8/18/80	1	ND
Bay 103 NBS Prespill	8/18/80	7	67
Z Lagoon Prespill	8/20/80	1	ND
Z Lagoon Prespill	8/20/80	10	ND
Bay 103 Afterspill	8/21/80	1	ND
Bay 103 Afterspill	8/21/80	10	ND
Bay 103	9/20/80	1	ND
Bay 103	9/20/80		ND

TABLE 3-8 (Cont.)

SAMPLE	COLLECTION DATE	SAMPLING DEPTH (m)	CONCENTRATION[a,b] (µg/l)
Blank #1	9/19/80	-	ND[c]
Blank #1 (leaked; probably contaminated)	9/20/80	-	ND
Blank #2 (120 ml Freon)	9/17/80	-	ND
Blank #1	9/17/80	-	ND
Blank #2 UV/F	9/19/80	-	3
Blank #3	9/19/80	-	ND
Blank #3	9/20/80	-	ND
Blank (leaked; probably contaminated)	9/18/80	-	ND

[a]Concentrations expressed as micrograms of API No. 2 fuel oil equivalents/liter of seawater extracted.

[b]Detection limit is 3 µg/l.

[c]Not detected.

samples, three taken in Z Lagoon prior to the shoreline experiment, and one taken in Bay 10. We believe these determinations to be the result of sporadic sample contamination or of inclusion of significant amounts of sediment in the sample prior to extraction.

3.2.2 Seawater Samples (GC^2 GC^2/MS)

Three 4-liter seawater samples from Bay 9 (1, 5, and 10 m) were obtained and analyzed by UV/F (see previous section) and by GC^2 (Table 3-9). Whole, unfractionated extracts were analyzed by GC^2 as were fractionated (f_1 and f_2) extracts. No detectable hydrocarbons were observed in any of these 4-liter samples although the unfractionated (total lipid) extracts did reveal several non-hydrocarbon components (Figure 3-12).

The results from the September sampling were more definitive due to the larger volume of water sampled. The NBS-sampler-obtained set contained samples laden with total lipid material (primarily non-hydrocarbon) (Table 3-10). A typical GC^2 trace of the unfractionated extract (e.g., Figure 3-12) revealed a complex set of major (methyl esters, wax esters) and minor (unidentified) compounds, all having their origin in planktonic residues captured in the water samplers. Fractionation of these extracts into f_1 and f_2 hydrocarbon fractions removed most of this polar material and revealed very low levels (<1 ppb) of hydrocarbon material, most often of a biogenic origin (i.e., olefinic material in the f_2 fraction). In two samples (Table 3-10), a small amount of petrogenic material was detected in the f_1 fraction. This material resembled tar residues (tar specks), being

TABLE 3-9

SEAWATER SAMPLES OBTAINED FOR GC^2 AND GC^2/MS ANALYSES

LOCATION	DATE	DEPTH (m)	VOLUME (liter)	TYPE
Bay 10	9/19/80	1	20.5	NBS[a]
Bay 10	9/19/80	5	19.5	NBS
Bay 10	9/19/80	5	19.5	NBS
Bay 10 (Filtered Seawater)	9/7/80	1	210	Risebrough LVWS[b]
Bay 10 (Particulates	9/7/80	1	210	Risebrough LVWS
Bay 11	9/1/80	1	16	NBS
Bay 11	9/17/80	5	20	NBS
Bay 11 (Filtered Seawater)	9/11/80	8	130	Risebrough LVWS
Bay 11 (Particulates	9/11/80	8	130	Risebrough LVWS
Z Lagoon	9/20/80	1	16.2	NBS
Z Lagoon	9/20/80	5	15.5	NBS
Blank Polyplug #1				
Blank Filter #1				
Blank Polyplug #2				
Blank Filter #2				

Key:
NBS = 4-liter drop sampler
LVWS = large-volume water sampler

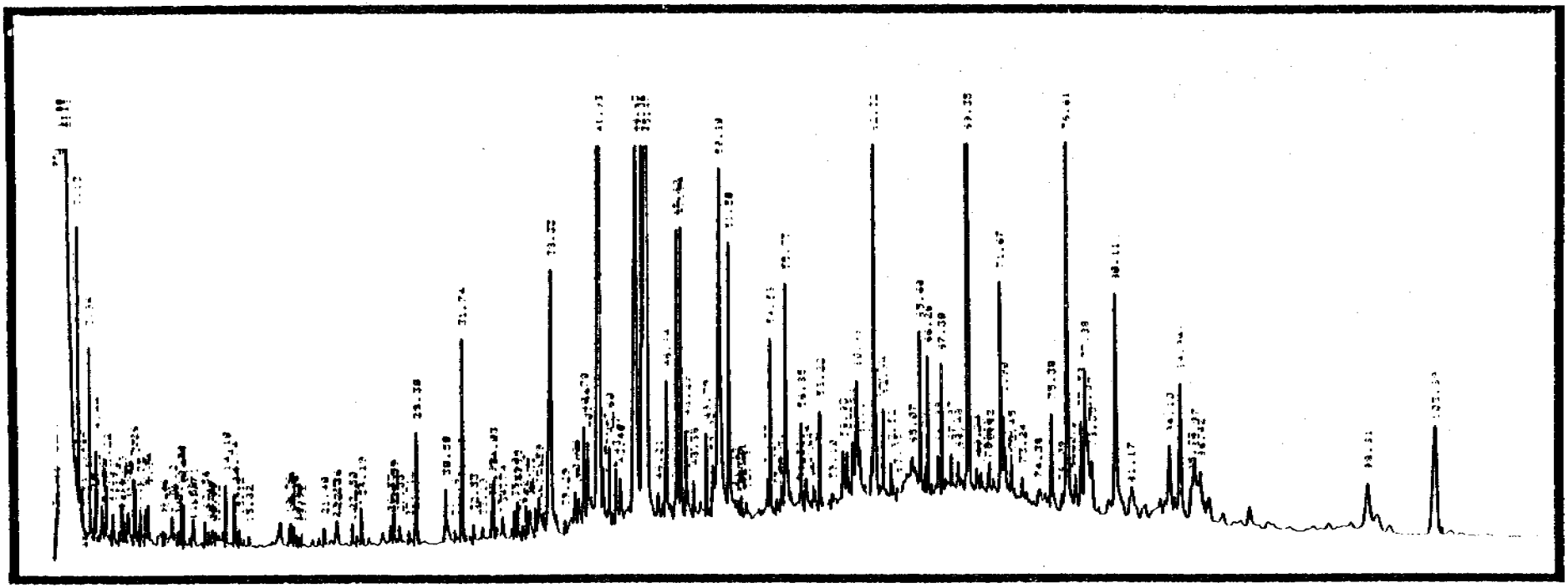


Figure 3.12. GC^2 of Unfractionated Seawater Extract Showing Polar Lipid Material.

TABLE 3-10

ANALYTICAL RESULTS - SEAWATER SAMPLES

SAMPLE	LIPID WEIGHT (UNFRACTIONATED) (µg/l)	HYDROCARBONS (FRACTIONATED) (µg/l)	GC-TYPE (FRACTIONATED
June			
Bay 9 (1 m)	ND	ND[a]	3
Bay 9 (5 m)	ND	ND[a]	3
Bay 9 (10 m)	ND	ND[a]	3
September			
Bay 10 (1 m) - NBS	2.8	ND[b]	3/1
Bay 10 (5 m) - NBS	1.2	ND[b]	3
Bay 10 (5 m) - NBS	0.5	ND[b]	3
Bay 11 (1 m) - NBS	2.7	ND[b]	3/1
Bay 11 (5 m) - NBS	3.4	ND[b]	3
Z Lagoon (1 m) - NBS	3.2	ND[b]	3
Z Lagoon (5 m) - NBS	2.7	ND[b]	3
	f1 (ng/l)[c]	f2 (ng/l)[c]	
Bay 10 (1 m) LVWS - filtered seawater	1.4	2.0	4
Bay 10 (1 m) LVWS - particulates	0.6	0.7	1
Bay 11 (8 m) LVWS - filtered seawater	0.9	1.1	4/1
Bay 11 (8 m) LVWS - filtered seawater	0.7	0.6	1

[a] ND = <5 µg/l.

[b] ND = <0.3 µg/l.

[c] Gravimetric weight.

GC-type:
1. Petrogenic - tarry material
2. Terrigenous biogenics (sediments)
3. Marine biogenics (plankton)
4. Aromatic hydrocarbon residues

highly paraffinic in nature. However, the absolute levels of this tarry material were less than half of the "hydrocarbon" values presented in Table 3-10.

The large-volume water samplers yielded information on particulate and filterable ("dissolved") hydrocarbons from Bays 10 and 11. Once again, the unfractionated extract contained substantial quantities (10-30 µg/l) of lipid material mainly of a planktonic origin. GC^2 and GC^2/MS analyses were performed on these samples and yielded the quantitative data shown in Table 3-10. The very low levels of petroleum-like hydrocarbons presented are real. The sampling technique allows very low levels of hydrocarbons to be detected (0.7-2.0 ng/l = parts per trillion).

The particulate/dissolved couples proved extremely interesting. The Bay 10 filtered seawater contained extremely low levels of hydrocarbons in the saturated fraction (f_1) with no GC^2 detectable components. However, the aromatic (f_2) fraction contained detectable alkylated naphthalene phenanthrene and dibenzothiophene by GC^2 (Figure 3-13) and quantifiable GC^2/MS (Table 3-11). Similarly, the Bay 11 water had very low, but detectable, levels of the same compounds.

The particulates were quite unlike the dissolved fraction, the composition of the former being dominated by saturated hydrocarbons of combined tar-like and terrigenous biogenic sources (Figure 3-14), and having no detectable aromatics. Thus, the dissolved and particulate fractions are decoupled with respect to source and probably transport paths as well.

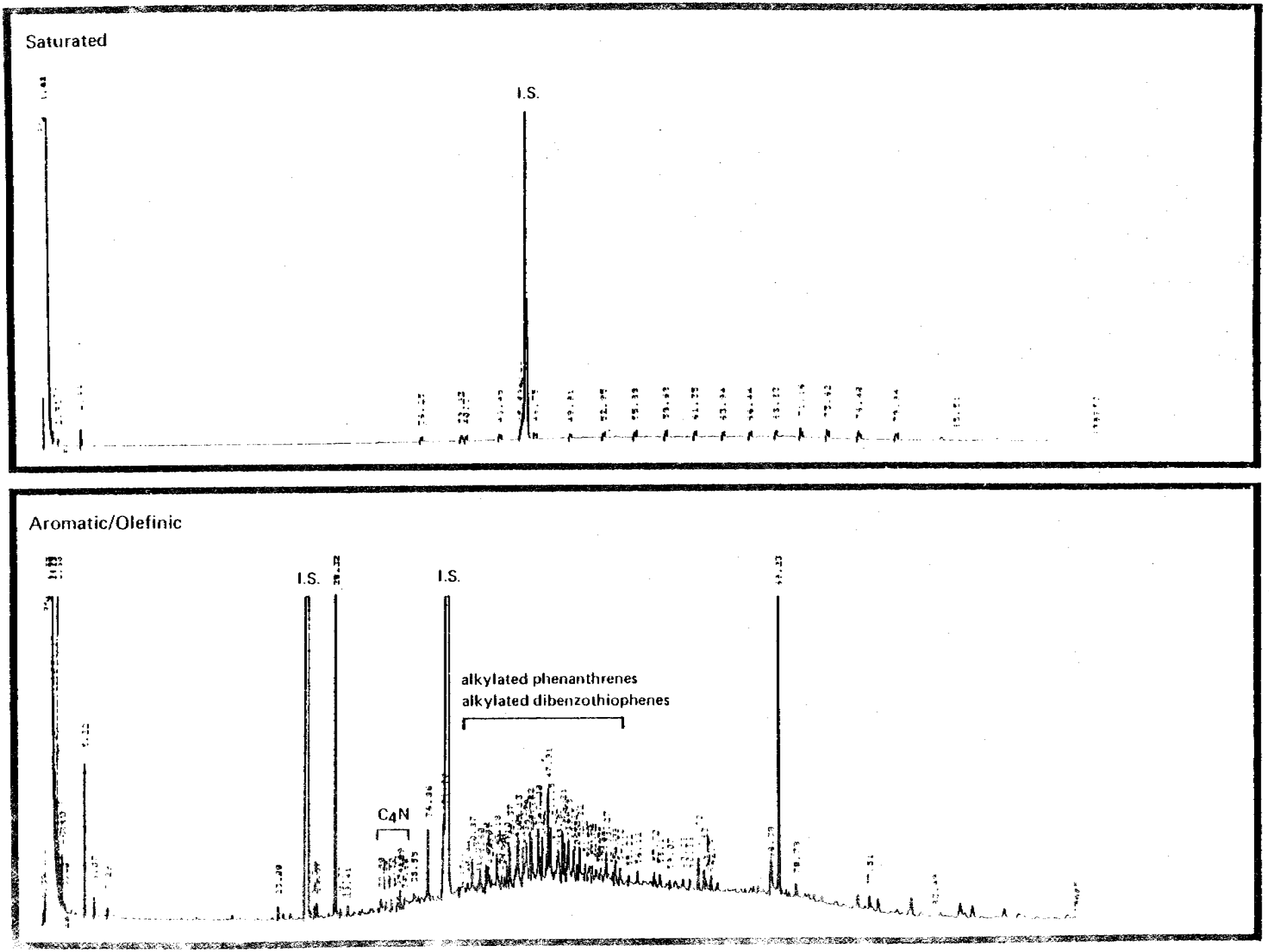


Figure 3.13. GC^2 Traces of Hydrocarbons in Bay 10 Filtered Seawater (LVWS).

TABLE 3-11

AROMATIC HYDROCARBON LEVELS IN LARGE-VOLUME WATER SAMPLES BY GC^2/MS

SAMPLE COMPOUND	BAY 10 FILTERABLE (ng/l)	BAY 10 PARTICULATE (ng/l)	BAY 11 FILTERABLE (ng/l)	BAY 11 PARTICULATE (ng/l)
Tetramethyl naphthalene	0.2	ND	ND	ND
Methyl phenanthrene	0.3	ND	0.05	ND
Dimethyl phenanthrene	0.5	ND	0.1	ND
Trimethyl phenanthrene	0.15	ND	ND	ND
Methyl dibenzothiophene	0.25	ND	0.05	ND
Dimethyl dibenzothiophene	0.4	ND	0.1	ND
Trimethyl dibenzothiophene	0.4	ND	0.1	ND

ND = <0.05 ng/l.

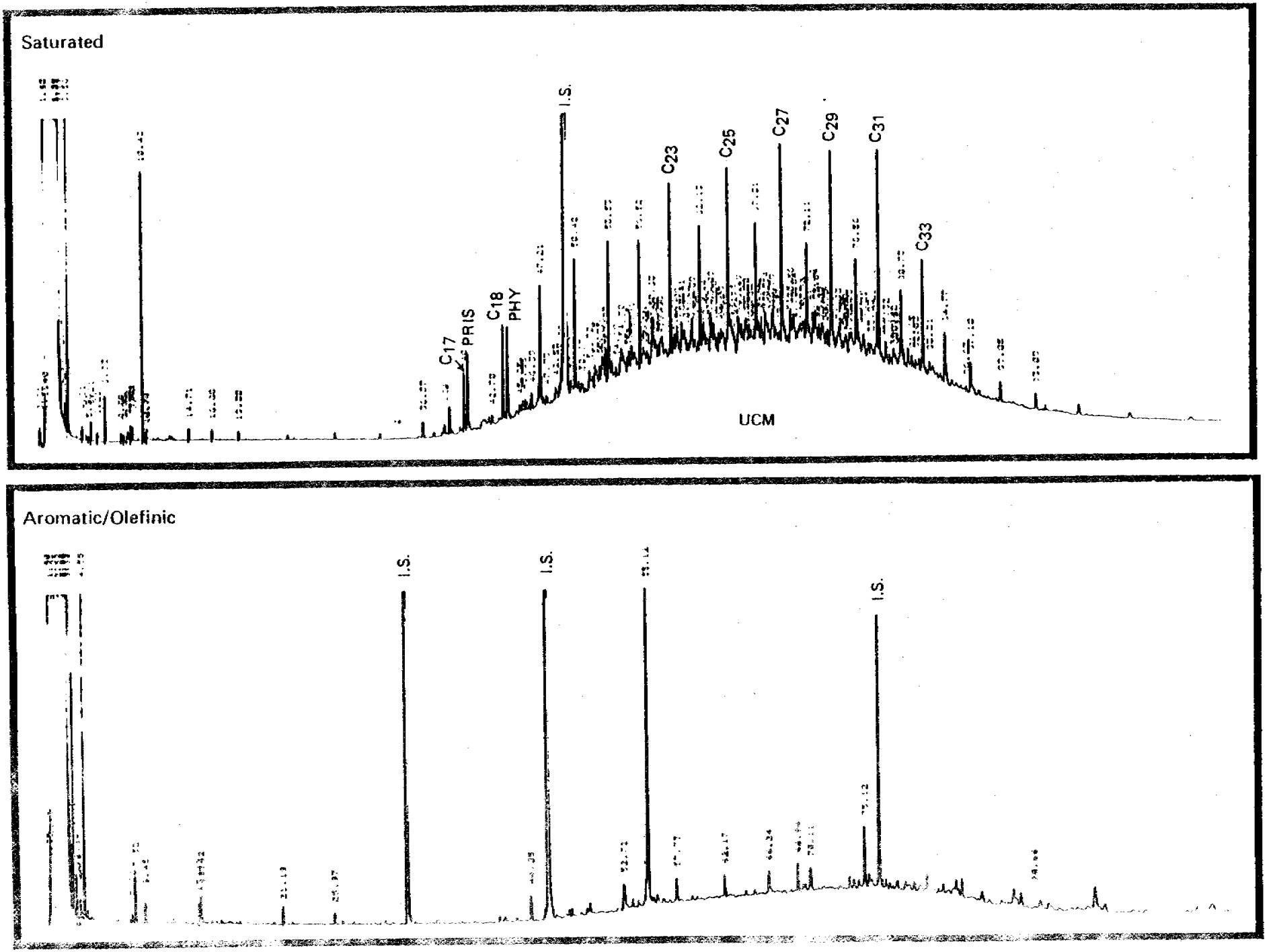


Figure 3.14. GC² Traces of Hydrocarbons in Particulates from Bay 10 (LVWS).

3.2.3 Sediment Samples - UV/F Analyses

The results of the UV/F analyses of sediments are presented in Tables 3-12 and 3-13 for the June offshore and August/September beach samples, respectively. Several replicate analyses (two subsamples) were performed in the June sample batch (#14-2-15-CC-16) and the results indicate that the quantification of hydrocarbons by this method is at least internally consistent.

UV/F measurements of one possible sample contaminant, the core caps, indicated this potential error was not a problem in this study.

Qualitatively, the UV/F spectra (Figure 3-15) reveal the presence of low levels of 3-, 4-, and 5-ring aromatic compounds and the readily identified perylene doublet. The spectra are quite unlike that of the Lagomedio crude (Figure 3-15A), thus pointing to a weakness in the quantification method used (i.e., Lagomedio as a standard) as well as a strength in the method for later distinguishing background from spill-related inputs. The perylene doublet was detected in many offshore sediments as well as several beach sediment samples.

3.2.4 Sediment Samples - GC^2 Analyses

To reveal the details of the hydrocarbons in the baseline sediments, GC^2 analyses were performed. The quantitative results are presented in Tables 3-14 and 3-15. The values presented give both the total saturated (Fraction 1) and aromatic/olefinic (Fraction 2) concentrations, determined gravimetrically and the amount of

TABLE 3-12

UV/FLUORESCENCE-DETERMINED CONCENTRATIONS OF HYDROCARBONS IN SEDIMENT SAMPLES - JUNE 1980

SAMPLE NUMBER	CONCENTRATION[a] (µg/g)
13-A-2-17-CC4	1.0
13-A-3-24-CC5	0.5
10-2-13-CC10	0.8
10-3-22-CC11	ND
14-1-2-CC12	ND
14-2-15-CC16 (0-4 cm)	0.6
14-2-15-CC16 (10-15 cm) #1	2.2
14-2-15-CC16 (10-15 cm) #2	3.0
14-2-15-CC16 (28-33 cm)	1.3
14-3-26-CC17	7.5
105-1-10-CC20 #1	0.9
105-1-10-CC20 #2	0.7
Core caps	ND
Procedural blank	ND

[a]Weathered Lagomedio equivalents @356 nm.

TABLE 3-13

UV/FLUORESCENCE-DETERMINED CONCENTRATIONS OF HYDROCARBONS IN BEACH SEDIMENT SAMPLES - AUGUST 1980

SAMPLE NUMBER	CONCENTRATION[a] (µg/g dry weight)
9-C-H	0.3
9-C-L	0.6
9-N-H	0.2
9-N-L	0.2
9-S-H	0.2
9-S-L	0.1
10-C-H	0.2
10-C-L	0.6
10-N-H	0.3
10-N-L	0.6
10-S-H	0.1
10-S-L	0.2
11-C-H	0.1
11-C-L	0.1
11-N-H	ND
11-N-L	0.1
11-S-H	0.4
11-S-L	0.3
Bay 102 - Beach Sediment Prespill	ND
Bay 103 - Beach Sediment Prespill	0.3
Backshore Beach T-1	0.2
ERCO Blank	0.0

[a]Concentrations expressed as micrograms of Lagomedio crude oil equivalents/gram of dry sediment.

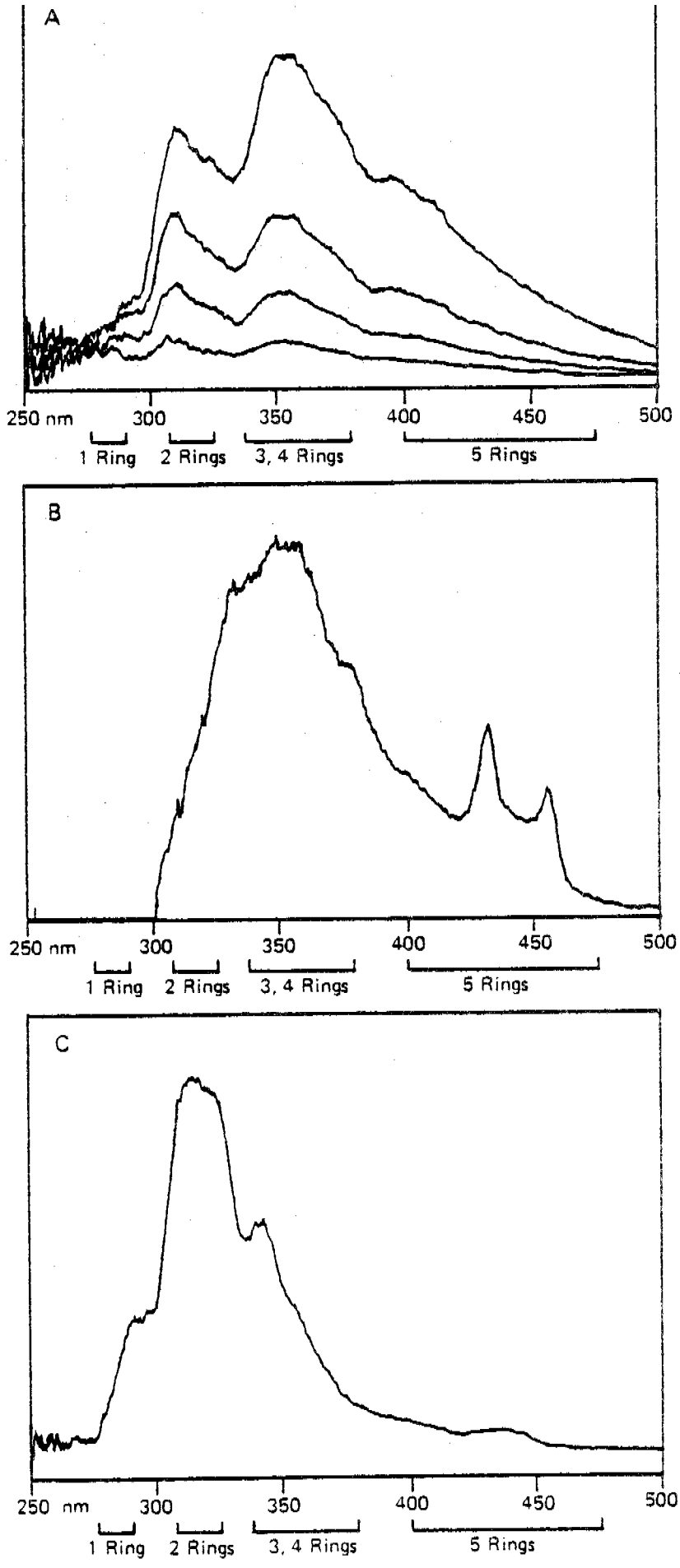


Figure 3.15. UV/F Spectra of A—Several Dilutions of Lagomedio Crude; B—A Beach Sediment; C—An Offshore Sediment.

chromatographically resolvable material (i.e., GC^2 peaks), the latter always being a part of the former. Fraction 1 contains two diagnostic parameters, the relative amounts of the isoprenoids pristane and phytane (PRIS/PHY) and the carbon preference index (CPI). Pristane is a commonly produced biogenic hydrocarbon originating in zooplankton and is also present in all fossil fuels. Phytane on the other hand is found only in fossil fuels. A PRIS/PHY near unity indicates a petroleum-related hydrocarbon source while clean sediments contain much more pristane. The PRIS/PHY ratios range from 3 to 20 in all offshore samples and 13 to 1,500 in the beach samples.

The second diagnostic ratio, CPI, examines the relative amount of n-alkanes containing an odd number of carbon atoms to those containing an even number, in the C_{26} to C_{30} range. Terrigenous plant waxes are abundant in odd carbon n-alkanes (i.e., CPI >>1) while crude oils have ratios near unity due to abiotic synthesis. CPI values in all samples are high, i.e., 3 to 12, indicating a preponderance of terrigenous plant detritus in these samples.

The data on the aromatic/olefinic fraction is fairly nonspecific as many of the compounds readily apparent by GC^2 are unidentified biogenic olefins. The total gravimetric f_2 values are higher than the GC traces would indicate, suggesting that much of the f_2 material does not elute from the GC column. This material is comprised of greenish-pigmented material which in spite of the fact that it elutes in the f_2 of the silicic acid column is either non-hydrocarbon in nature or thermally labile, but in any event of little consequence in this study.

The "total extractables" number indicates the total lipoidal, or solvent extractable organic material, only a small part of which is hydrocarbon (i.e., f_1 or f_2). The f_3 fraction corresponds to material eluting off the silicic acid column in the so-called polar (mainly oxygenated compounds) fraction. The total extractable and Fraction 3 values will be of more use in the post-spill assessment.

There appears to be little variation in the analytical plus small-scale spatial (i.e., two subsamples of the same sediment) variability (Table 3-14). Furthermore there is little variation in the concentration and composition of hydrocarbons within the top 30 cm of the sedimentary record (Core Sample 14-2-15-CC16; Table 3-14).

The concentrations of hydrocarbons in the beach samples range from being much lower to equal to the offshore samples. The source of saturated hydrocarbons to both sets of samples is similar, but both qualitative and quantitative differences occur in the aromatic/olefinic fraction. This is apparent in comparing (1) total resolved and (2) total gravimetric Fraction 2 values and perhaps more importantly, by examining GC^2 traces.

The Fraction 2 GC^2-determined compositions of all offshore samples are similar (Figure 3-16) and different than the beach f_2 assemblage (Figures 3-17 and 3-18). The primary difference is in the amount of olefinic clusters which have strictly a marine origin and are thus deposited offshore. The saturated (f_1) fractions of both sets of samples are similar, illustrating major terrigenous inputs for both sets and a marine biogenic component for the offshore samples. The presence of an unresolved complex mixture in several offshore samples is in this case suggestive of the

TABLE 3-14

JUNE 1980 BIOCHEMISTRY DATA SUMMARY

	LAB CODE	FRACTION 1 RESOLVED (GC) (µg/g)	FRACTION 1 TOTAL (GRAV) (µg/g)	PRISTANE (ng/g)	PHYTANE (ng/g)	PRIS/PHY	CPI	FRACTION 2 RESOLVED (G/C) (µg/g)	FRACTION 2 TOTAL (GRAV) (µg/g)	FRACTION 3 (µg/g)	TOTAL EXTRACTABLES (µg/g)
13A-2-17-CC4	06-910	0.4	1.6	5	1	5	5.1	0.5	32.3	143	329
13A-3-24-CC5	06-912	0.7	6.0	6	1	6	6.1	0.7	11.4	42.5	155
10-2-13-CC10	06-914	0.4	2.0	2	0.3	7	10.8	0.8	175	121	660
10-3-22-CC11	06-916	0.1	7.6	2	0.6	3	6.2	0.1	9.3	13.4	92
14-1-2-CC12	06-918	0.4	0.8	2	<0.1	20	7.2	0.7	29.4	69.6	301
14-2-15-CC16 (0-4 cm)	06-920	0.4	2.0	8	1	8	10.0	0.4	31.2	43.2	228
14-2-15-CC16 #1 (10-15 cm)	06-922	0.7	3.6	7	1	7	6.1	1.1	23.5	73.3	289
14-2-15-CC16 #2 (10-15 cm)	06-924	0.6	2.0	7	0.5	14	9.2	0.8	40.6	116	341
14-2-15-CC16 (28-33 cm)	06-926	0.7	1.6	15	1	15	5.8	0.7	18.8	94	333
14-3-26-CC17	06-928	0.2	1.6	12	1	12	3.3	0.1	11.3	33	89
105-1-10-CC20 #1	06-930	0.6	2.6	10	1	10	5.3	1.1	63.7	107	337
105-1-10-CC0 #2	06-932	0.6	1.9	9	1	9	5.5	1.4	47.4	115	323

CPI = carbon preference index = $\frac{2([n\text{-}C_{27}] + [n\text{-}C_{29}])}{[n\text{-}C_{26}] + 2[n\text{-}C_{28}] + [n\text{-}C_{30}]}$

TABLE 3-15

BEACH SEDIMENT GEOCHEMISTRY DATA SUMMARY - AUGUST/SEPTEMBER 1980

SAMPLE NUMBER	FRACTION 1						FRACTION 2		TOTAL EXTRACTABLES (µg/g)
	RESOLVED (GC^2) (µg/g)	TOTAL (GRAV) (µg/g)	PRISTANE (ng/g)	PHYTANE (ng/g)	PRIS/PHY	CPI	RESOLVED (GC^2) (µg/g)	TOTAL (GRAV) (µg/g)	
9-N-L	0.03	0.17	ND	ND	-	2.5	0.007	0.26	0.7
9-N-H	0.1	0.12	ND	ND	-	4.1	0.06	0.12	6.2
9-C-L	0.03	0.1	ND	ND	-	9.3	ND	0.1	1.0
9-C-H	0.02	1.7	1	ND	-	2.8	0.008	0.1	4.5
9-S-L	0.03	0.2	2	0.2	10	ND	0.013	0.4	2.0
9-S-H	0.04	0.6	ND	ND	-	4.0	0.05	0.2	3.9
10-N-L	0.28	10.6	17	1	17	3.5	0.11	3.0	65.8
10-N-H	0.05	0.2	5	0.2	25	4.2	0.02	0.3	1.3
10-C-L	0.35	2.5	22	1.3	17	4.0	0.15	5.2	81.3
10-C-H	0.04	0.3	1	ND	-	2.7	0.05	0.2	3.5
10-S-L	0.08	3.1	5.0	0.3	17	5.5	0.1	1.2	13.9
10-S-H	0.04	3.0	1	ND	-	2.3	0.02	1.1	14.8
11-N-L	0.12	5.9	17	0.1	170	4.6	0.17	2.4	38.6
11-N-H	0.10	2.7	2	0.2	10	8.8	0.5	2.5	55.7
11-C-L	0.04	2.1	5.8	0.2	29	4.5	0.04	1.0	14.7
11-C-H	0.28	4.8	3.4	0.1	34	4.6	0.39	2.1	54.1
11-S-L	0.30	2.2	6.5	0.5	13	9.5	0.10	1.7	26.0
11-S-H	0.45	2.9	10	0.2	50	10.8	0.37	2.7	70.0
BSB-T1	0.5	0.6	75	0.05	1500	12.4	0.17	0.3	2.2
Bay 102 (pre-spill)	0.01	0.2	ND	ND	-	7.8	0.04	0.3	1.3
Bay 103 (pre-spill	0.07	0.1	ND	ND	-	7.3	0.05	0.1	1.5

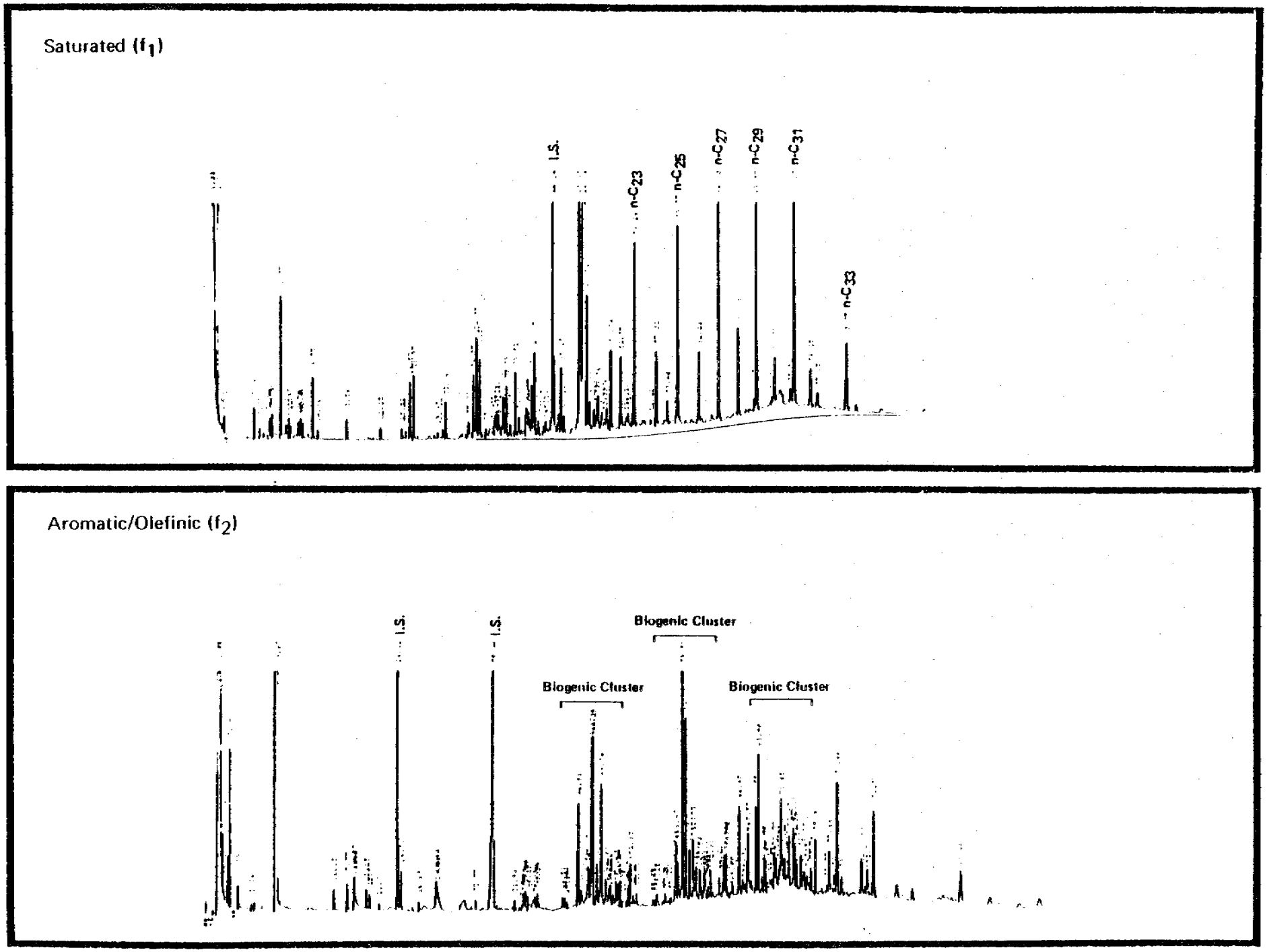


Figure 3.16. Representative GC^2 Traces of Hydrocarbons in Offshore Samples.

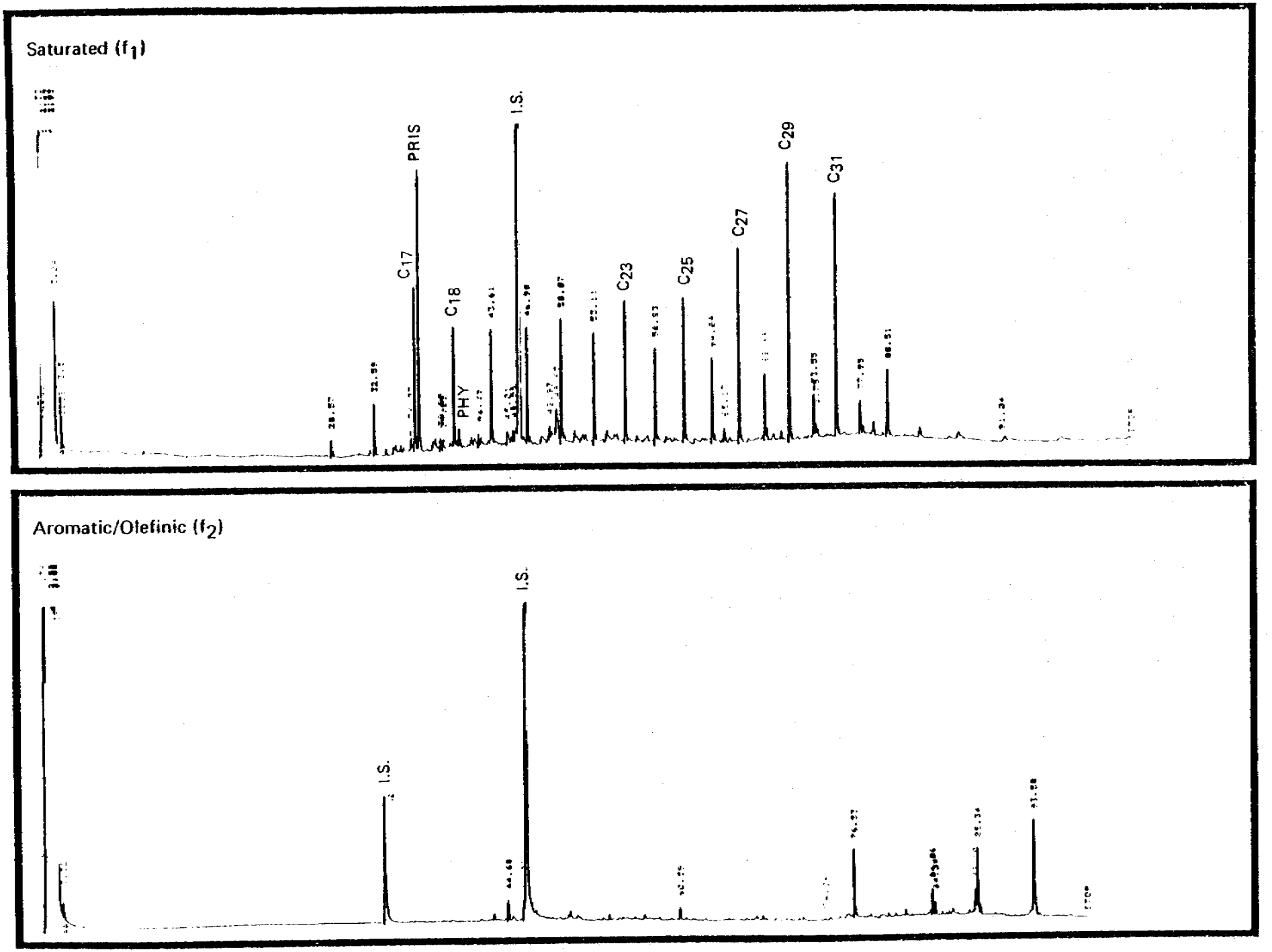


Figure 3.17. Representative GC^2 Traces of Hydrocarbons in Beach Samples (No. 1).

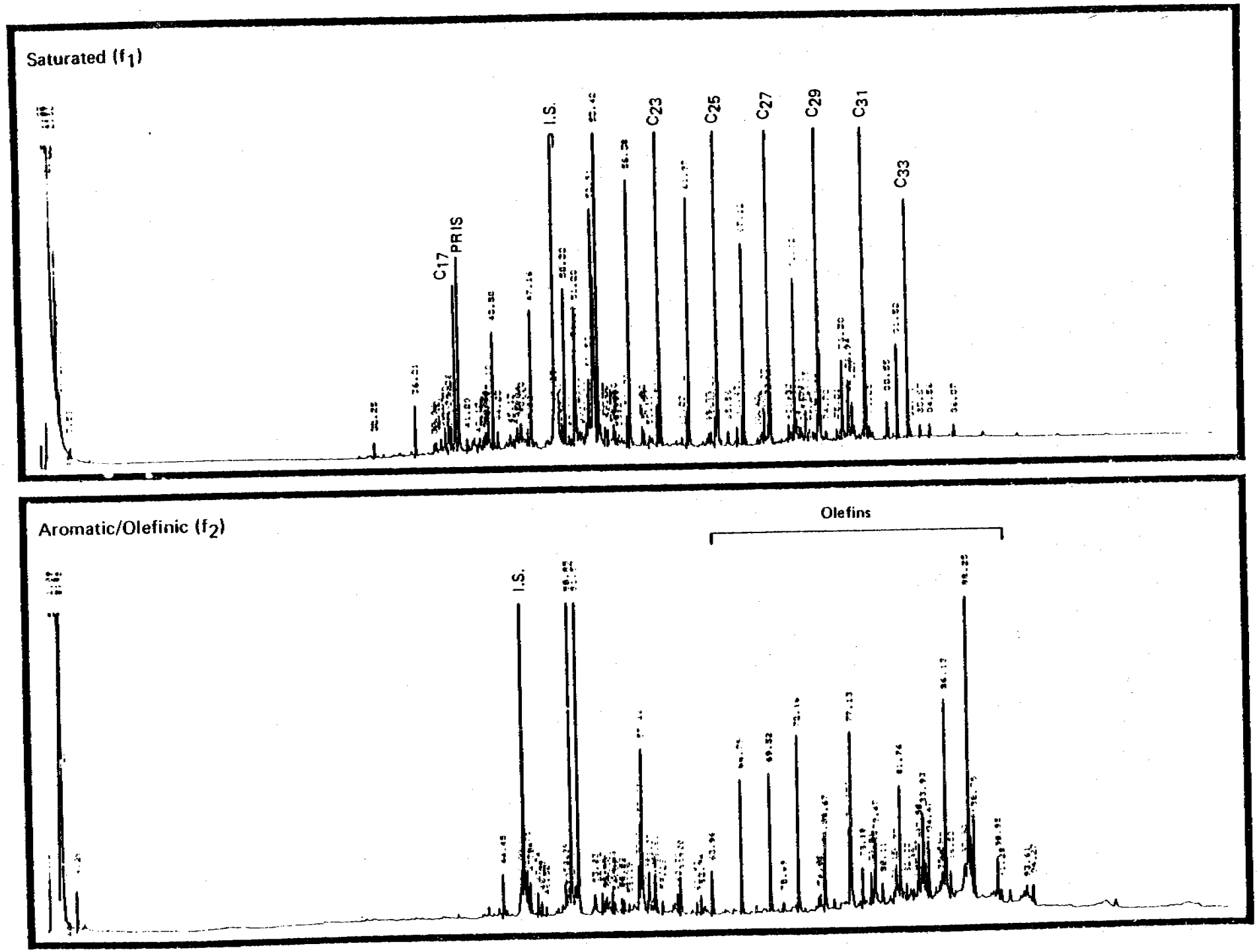


Figure 3.18. Representaive GC^2 Traces of Hydrocarbons in Beach Samples (No. 2).

addition of anthropogenic material, the result of long-range transport of a global nature.

An illustrative set of generic GC^2 traces of hydrocarbons of various marine sediment samples illustrating the variety of possible source material is presented in Figure 3-19 for comparison.

3.2.5 Sediment Samples - GC^2/MS

GC^2/MS analyses were utilized to examine (1) the identities and levels of PAH compounds in f_2 fractions of selected samples, (2) the nature of the background pentacyclic triterpane (hopane) compounds, and (3) the nature of the azaarene composition.

3.2.5(a) PAH Compounds

Very low, but detectable, levels of PAH compounds containing 3 to 5 rings were detected in both the beach samples and the offshore sediment samples. The PAH distributions have a markedly pyrolytic and/or diagenetic source, being comprised mainly of the phenanthrenes, the fluoranthene/pyrene doublet, and perylene. Other minor quantities of benzanthracene, chrysene, and fluorene compounds are present as well (Table 3-16).

The amount of perylene, a diagenetic pentacyclic PAH compound, appears strongly related to the quantity of total extractable organic material and to the level of hydrocarbons in the samples.

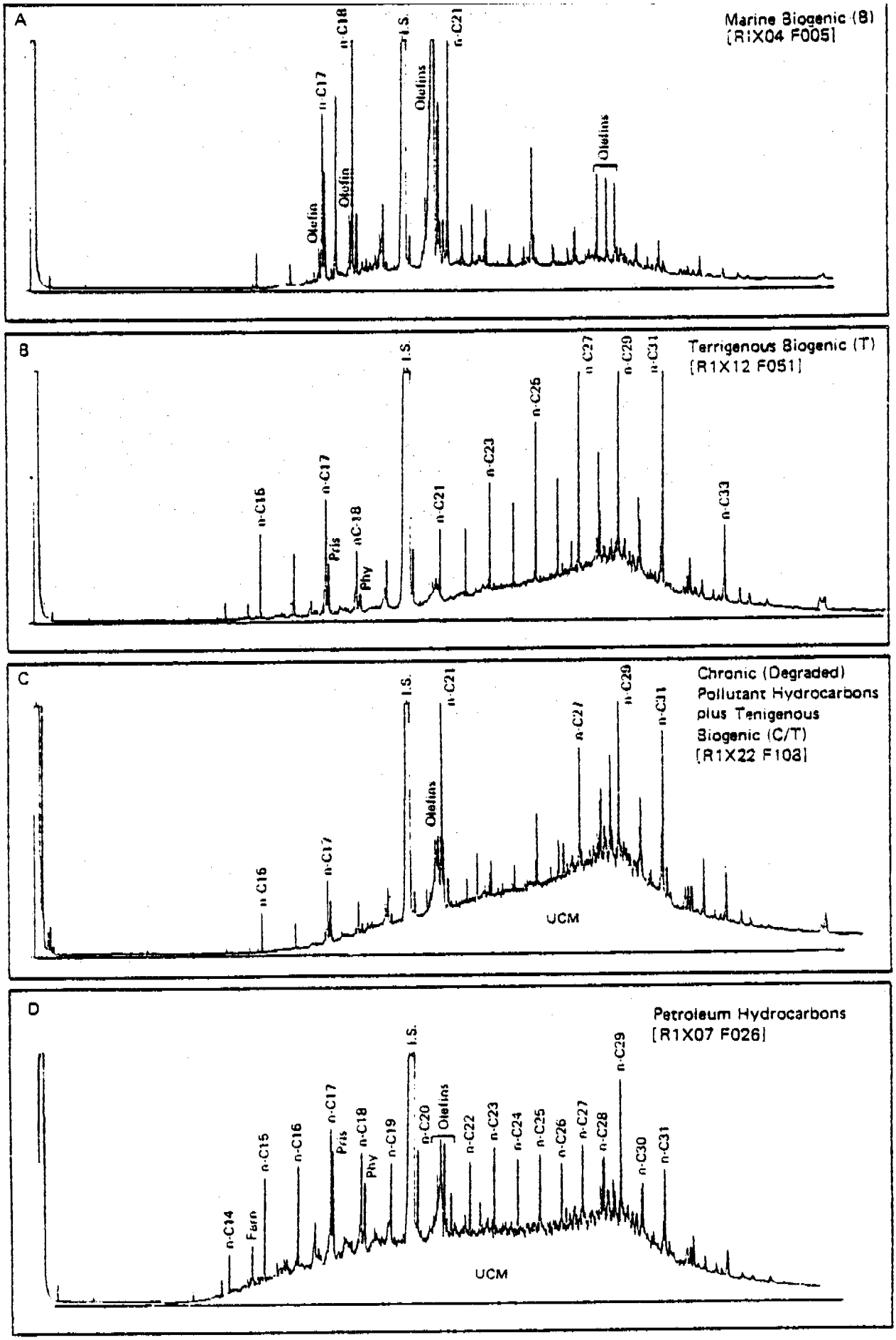


Figure 3.19. Glass capillary gas chromatograms of saturated hydrocarbons in non-oiled & oiled surface sediments.

TABLE 3-16

BASELINE PAH CONCENTRATIONS IN OFFSHORE AND BEACH SEDIMENT BY GC^2/MS

	OFFSHORE			BEACH				
BAY:	13	9	10	9	9	10	10	11
SAMPLE ID	13-3-24-CC5	14-2-15-CC16	10-2-13-CC10	9-C-L	9-C-H	10-N-L	10-N-H	11-C-L
COMPOUND								
Phenanthrene (m/e 178)	1.6	1.6	1.4	0.3	0.3	2.6	0.5	0.3
Methyl phenanthrene (m/e 190)	1.9	2.0	1.2	0.4	ND	5.6	0.9	0.5
Dimethyl phenanthrene (m/e 206)	ND	ND	ND	0.2	ND	3.1	0.5	ND
Trimethyl phenanthrene (m/e 220)	ND	ND	ND	0.1	ND	1.9	0.3	ND
Σ Phenanthrenes	3.5	3.6	2.6	1.0	0.3	13.2	2.2	0.8
Fluorene (m/e 166)	ND	ND	ND	0.03	ND	0.4	0.03	ND
Methyl fluorene (m/e 180)	ND	ND	ND	0.1	ND	1.6	0.2	ND
Dimethyl fluorene (m/e 194)	ND	ND	ND	ND	ND	1.7	0.2	ND
Trimethyl fluorene (m/e 208)	ND	ND	ND	ND	ND	1.0	ND	ND
Methyl dibenzothiophene (m/e 198)	ND	ND	ND	ND	ND	0.8	ND	ND
Benzanthracene (m/e 228)	ND	ND	ND	0.1	ND	1.0	0.1	0.07
Chrysene (m/e 228)	0.6	ND	ND	0.1	ND	1.0	0.1	0.07
Fluoranthene (m/e 202)	0.3	ND	ND	0.4	ND	0.3	0.1	0.05
Pyrene (m/e 202	0.4	0.5	ND	0.3	ND	1.6	0.2	0.15
Benzofluoranthene	ND	ND	ND	0.1	ND	1.6	0.2	0.02
Benzoyrenes	ND	ND	ND	0.1	ND	1.8	0.2	0.04
Perylene	0.9	2.8	10.4	ND	ND	3.3	0.1	0.2
Total extractable organics (µg/g)	155	228	660	1.0	4.5	65.8	1.3	14.7
Total hydrocarbons (µg/g)	17.4	33.2	176.0[a]	0.2	1.8	13.6	0.5	3.1

[a]May contain non-chromatographable pigment material
ND = none detected

One of the beach samples (10-N-L) differed considerably from the others in terms of its PAH content and composition. A petroleum-derived source for the PAH compounds is suggested by the larger quantity (13 ppb) of the phenanthrenes, by the relative abundance of the alkylated phenanthrenes and by the presence of a small quantity of alkylated dibenzothiophenes. However, whatever small quantities of petroleum material may contribute to the PAH, no evidence for petroleum contamination is seen in the saturated hydrocarbon fraction (see Section 3.2.5). Thus, the main contribution to both the total hydrocarbon (13.6 ppm) and total extractable (65.8 ppm) levels are biogenic inputs.

Two curious but unexplained differences exist between the beach samples and the offshore samples. The difference concerns the isomeric composition of the methyl phenanthrenes and the abundance of the higher alkylated phenanthrenes in the samples. The offshore samples examined showed the existence of only 3-methyl phenanthrene and 2-methyl phenanthrene (Figure 3-20) while the beach samples contained these two compounds plus the 9-methyl and 1-methyl isomers (Figure 3-21). Furthermore, the offshore samples do not contain detectable (>0.1 ng/g) levels of the dimethyl and higher alkylated phenanthrene homologs. (Compare Figures 3-20 and 3-21). The phenanthrene mass chromatograms of Lagomedio crude oil are presented in Figure 3-22 for comparison.

3.2.5(b) Pentacyclic Triterpanes (Hopanes)

A set of two offshore and six beach sediment samples was analyzed by GC^2/MS to determine the nature of the pentacyclic triterpane (PT) compounds and their approximate quantities, for use as baseline measurements of this set of

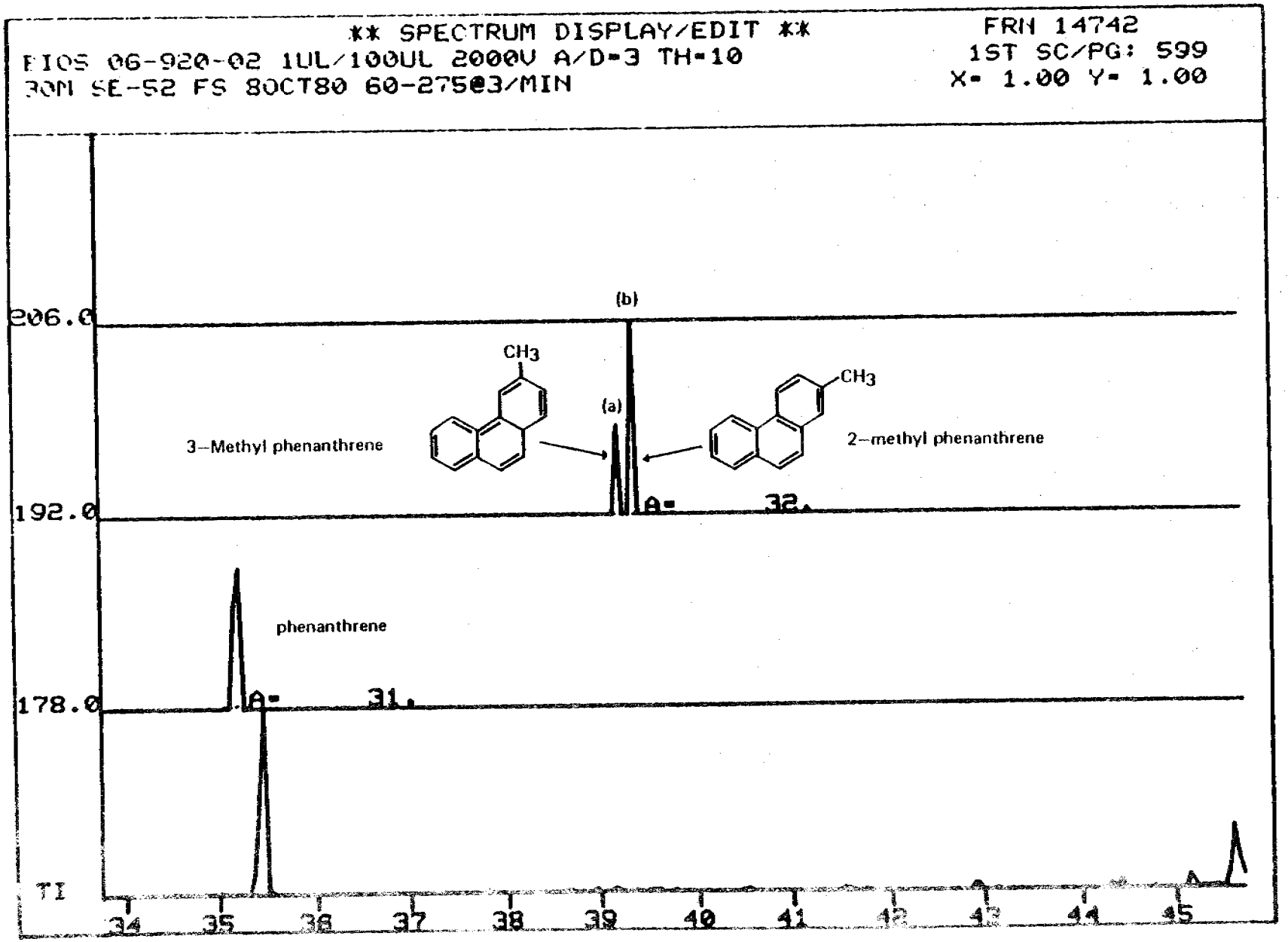


Figure 2.20. Phenanthrene mass chromatograms of offshore sediment sample.

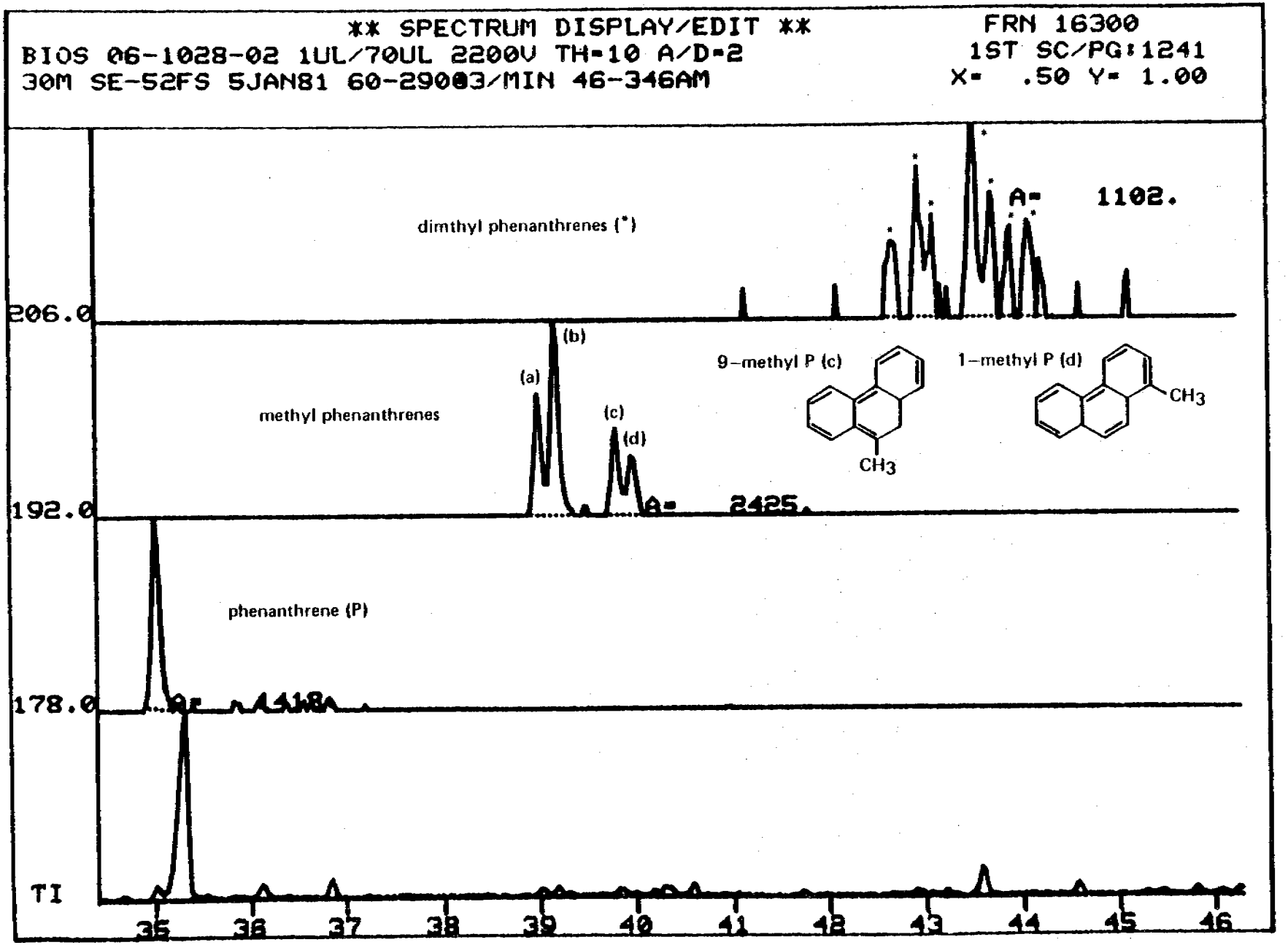


Figure 3.21. Phenanthrene mass chromatograms of beach baseline sample No. 10–N–L.

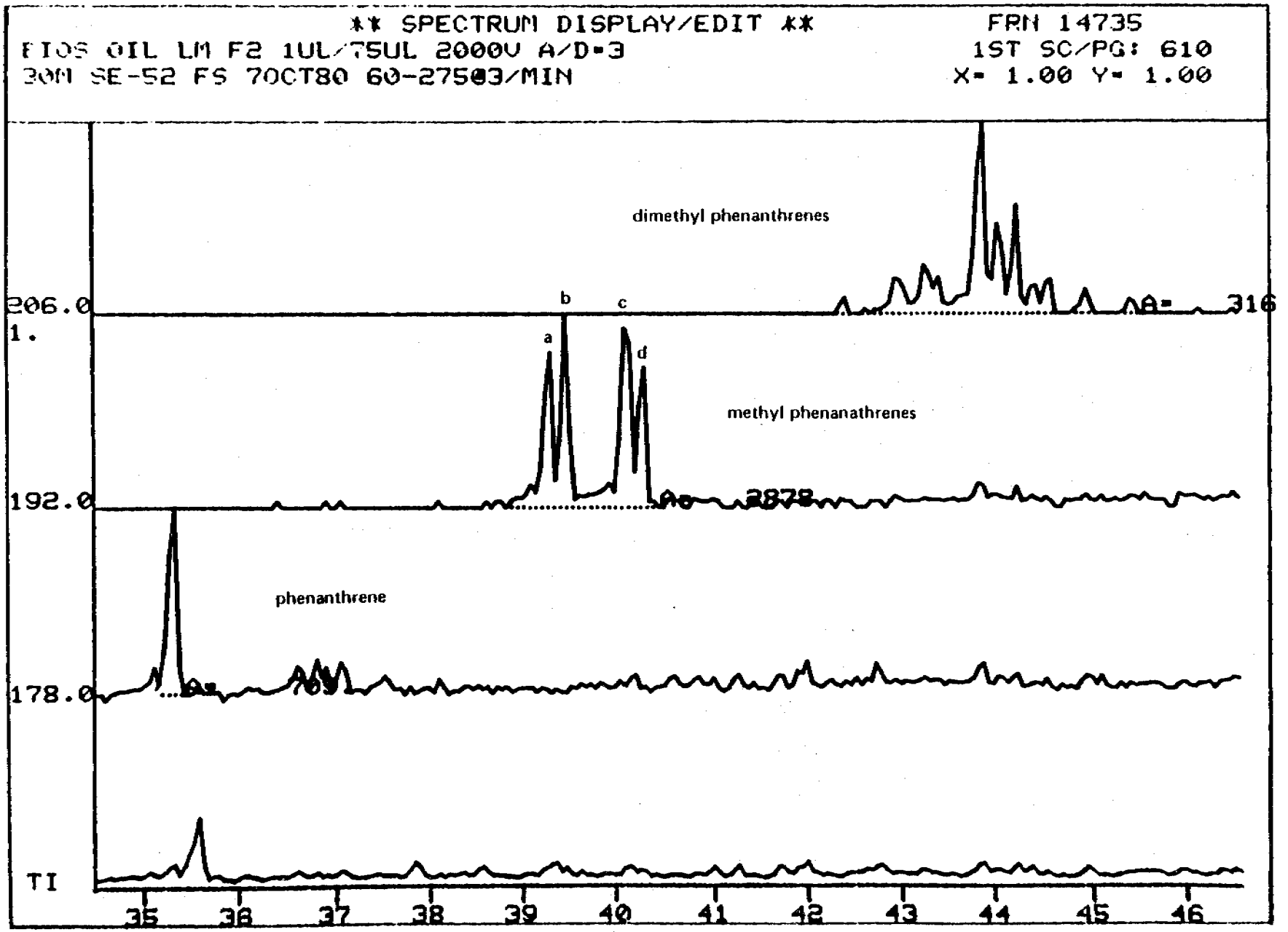


Figure 3.22. Phenanthrene Mass Chromatograms of Lagomedio Crude Oil.

petroleum marker compounds. Eight compounds have been focused on through selected ion searches for the characteristic fragment ion, m/e 191, and through confirmation of identity by molecular ion confirmations.

The distribution of compounds is shown in Figure 3-23.

The saturated fraction of the offshore sample 13-3-24-CC5 contains several prominent PT compounds as determined by m/e = 191 mass spectral searches. A series of eight of these compounds are observed: Compound A = C_{27} hopane ($C_{27}H_{44}$); Compound B = C_{27} trisnorhopane ($C_{27}H_{46}$); Compound C = C_{29} norhopane ($C_{29}H_{50}$); Compound D = C_{30} hopane ($C_{30}H_{52}$); Compounds E, E' = C_{31} homohopanes ($C_{31}H_{54}$); and Compounds F, F' = C_{32} bishomohopanes $C_{32}H_{56}$). All compounds appear to be of the 17α type and the nearly 1:1 ratio of the two C_{31} and C_{32} diastereomers indicates that the hopanes are representative of those associated with "mature" sediments and/or oils (anthropogenic inputs). This fact, coupled with the overall appearance of the GC trace with a prominent UCM (Figure 20), indicates that an anthropogenic input of hydrocarbons characterizes part of the hydrocarbon distribution of this sample.

The results are summarized in Table 3-17. The offshore samples do contain low levels of PT compounds with generally stronger m/e = 191 fragments than the Lagomedio oil itself (see Section 3.1; oil characterizations). The beach samples do not contain any detectable PT compounds. Thus the potential use of PT compounds for molecular markers of oil pollution in this environment may only be useful on the shore where PT compounds are absent.

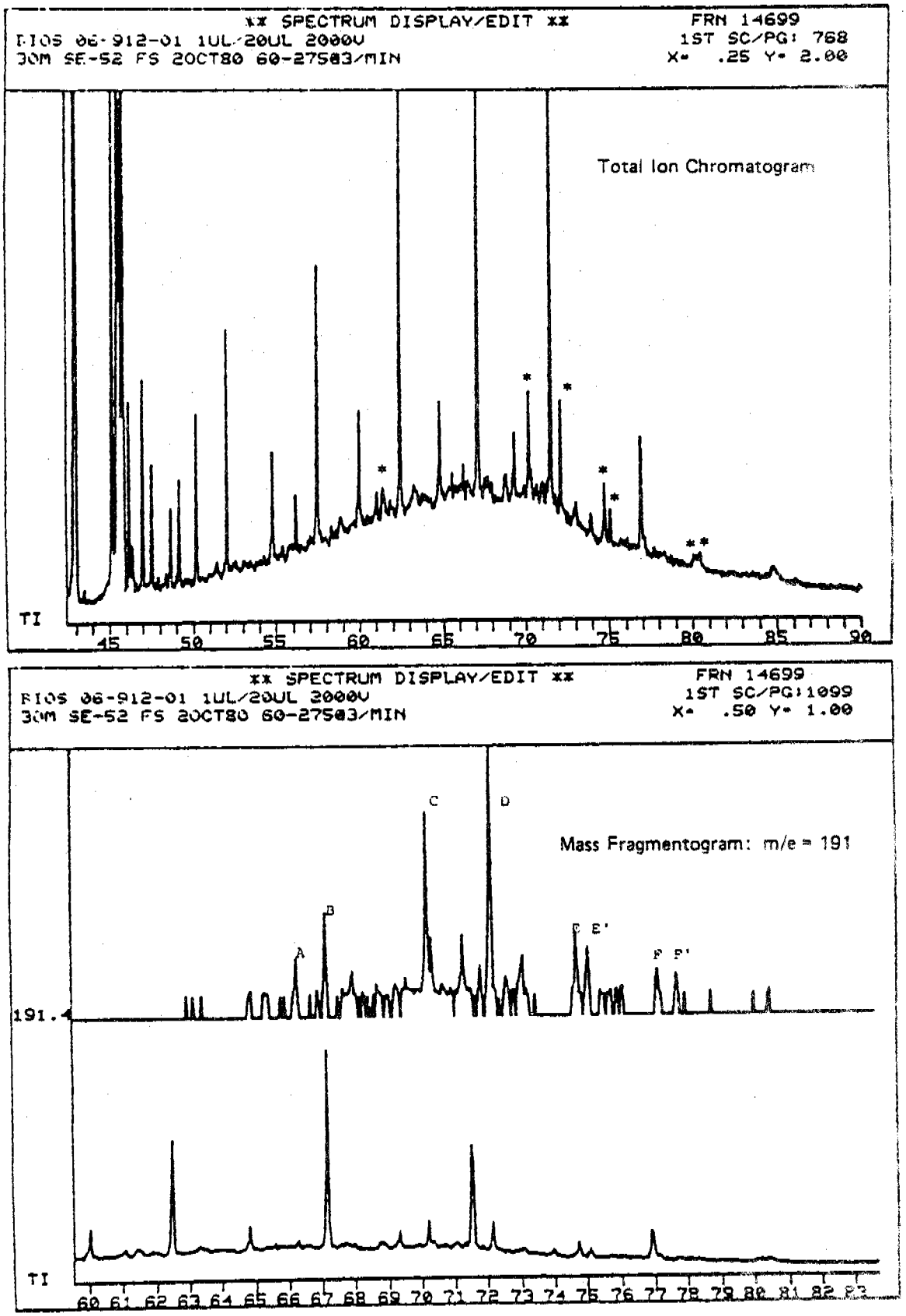


Figure 3.23. Bios Sediment—Hopane Analysis (GC^2/MS).

TABLE 3-17

RESULTS OF MASS SPECTRAL SEARCHES FOR
PENTACYCLIC TRITERPANES IN BASELINE SEDIMENTS

COMPOUND[a]	BEACH		BEACH					
	13-3-24-CC5	10-2-13-CC10	10-C-L	10-N-L	10-N-H	9-C-L	9-C-H	11-C-L
A ($C_{27}H_{44}$)	+ (3)	+ (3)	-	-	-	-	-	-
B ($C_{27}H_{46}$)	+ (5)	+ (4)	-	-	-	-	-	-
C ($C_{29}H_{50}$)	+ (9)	+ (10)	-	-	-	-	-	-
D ($C_{30}H_{52}$)	+ (11)	+ (15)	-	-	-	-	-	-
E ($C_{31}H_{54}$)	+ (4)	+ (6)	+	-	-	-	-	-
E' ($C_{31}H_{54}$)	+ (3)	+ (5)	-	-	-	-	-	-
F ($C_{32}H_{56}$)	+ (3)	-	+	-	-	-	-	-
F' ($C_{32}H_{56}$)	+ (2)	-	+	-	-	-	-	-

[a]Refer to text for compound name.

Key:

( ) Numbers in parentheses refer to approximate concentrations of identified compounds (ng/g = ppb).

+ = positive detection and identification.

- = negative detection.

3.2.5(c) Azaarenes

Two baseline samples (beach samples) were analyzed to determine if azaarenes were found as baseline components of the organic geochemical makeup of the sediment. Trace levels (<1 ppb) of several azaarenes were detected (e.g., Figure 3-24 and 3-25). Table 3-18 shows that one of the samples (10-C-L) appeared to contain compounds in the 3-ring acridine/phenanthridine series (m/e 207,221,235) while only questionable identifications of several alkyl quinolines were noted in sample 11-C-L. Thus the existence of these prominent series of quinolines, acridine, and benzacridine series in the Lagomedio crude (see Section 3.1) suggests that azaarenes may be sensitive long-term chemical markers.

3.2.6 Tissue Hydrocarbons (GC^2)

Seventy-two tissue samples were analyzed for their hydrocarbon content and composition by GC^2. Hydrocarbon concentrations were measured using two techniques:

1. The sum of components as determined by GC^2.

2. The microgravametric weights of the saturated (f_1) and aromatic/olefinic (f_2) fractions.

The results are presented in Tables 3-19 and 3-20. As gravimetrically-determined hydrocarbon values often include non-chromatographable lipoidal material, the weights are often gross overestimates of the GC^2-analyzable material. Thus, the more relevant numbers with repect to "before-and-after-spill" comparisons are the GC^2-determined values in Table 3-18.

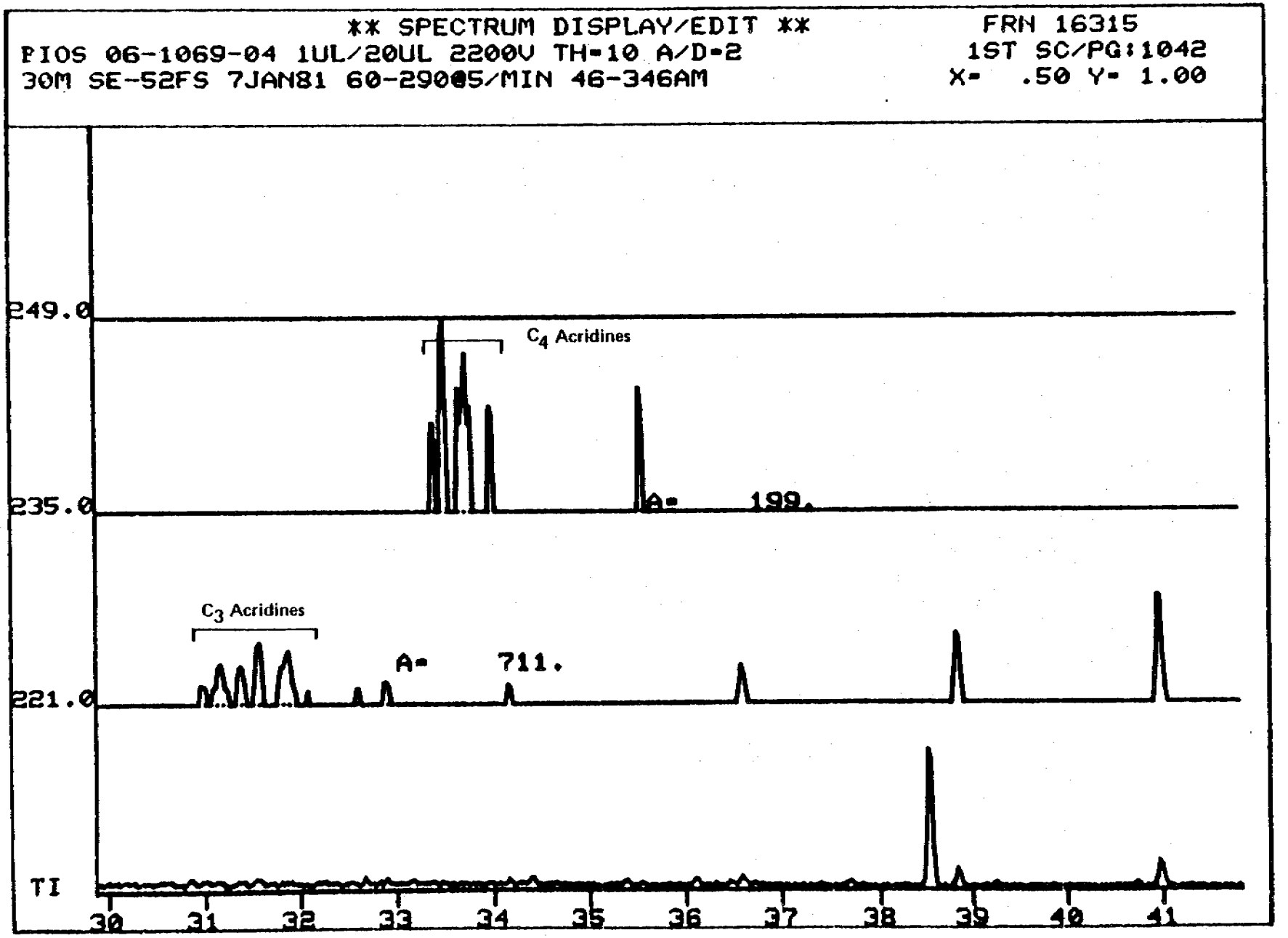


Figure 3.24. Azaarene Mass Spectral Searches—Beach Sediment.

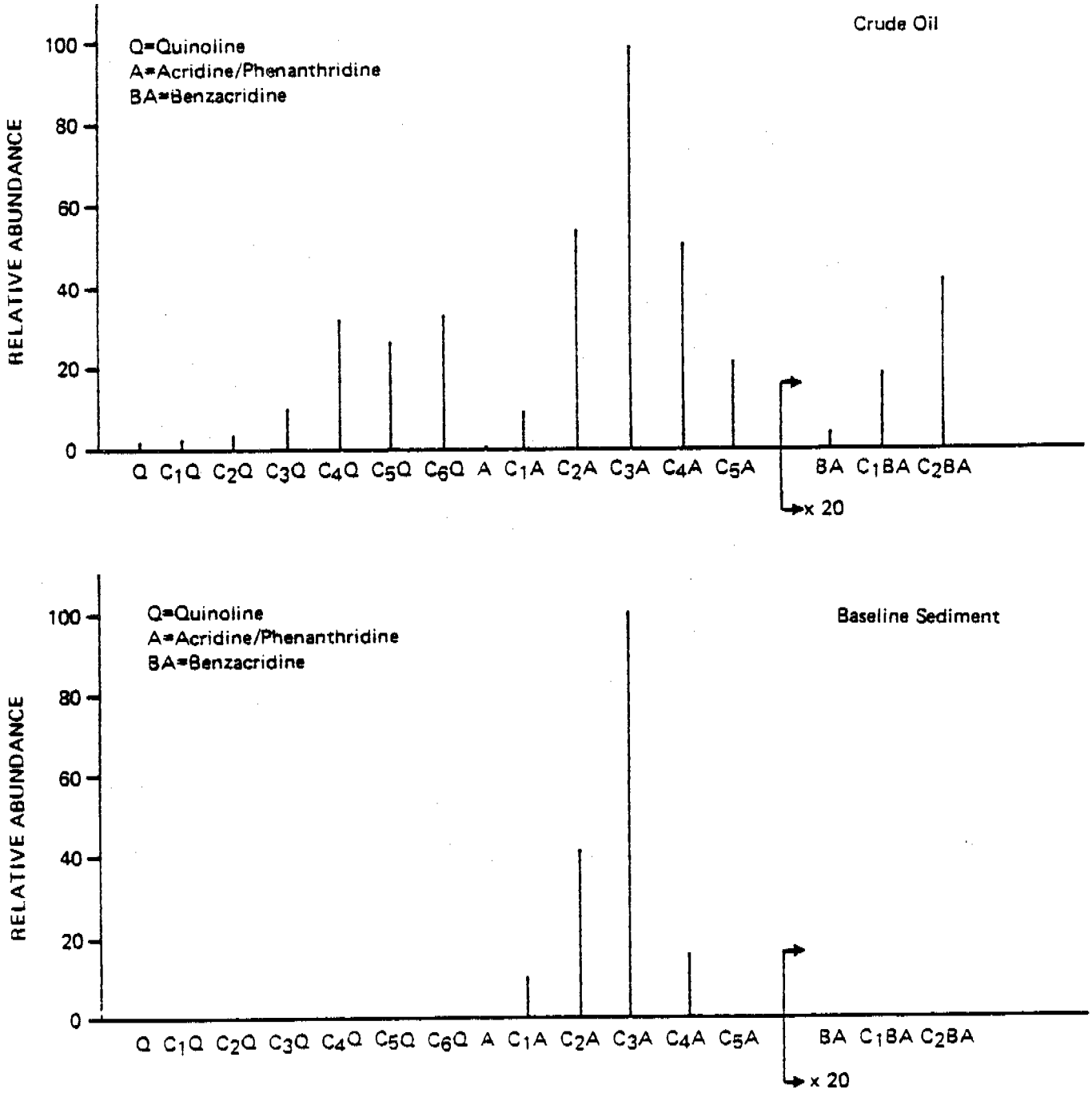


Figure 3.25. Comparison of Azaarene Composition in Baseline Sediment Samples with Lagomedio Crude C

TABLE 3-18

MASS SPECTRAL RESULTS FOR AZAARENE CONTENT OF BASELINE BEACH SAMPLES

	10-C-L	11-C-L
Quinoline (Q)	-	-
C_1Q	-	-
C_2Q	-	-
C_3Q	-	-
C_4Q	-	-
C_5Q	-	-
C_6Q	-	-
Acridine/ Phenanthridine (AP)	+	-
C_1AP	+	-
C_2AP	+	+
C_3AP	+	+
C_4AP	+	-
C_5AP	-	-
Benzacridine (B)	-	-
C_1B	-	-
C_2B	-	-

TABLE 3-19

SUMMARY OF BIOS TISSUE ANALYTICAL RESULTS

BAY	ERCO ID	SPECIES[a]	COLLECTION DATE	DEPTH (m)	HYDROCARBON CONCENTRATIONS (GC)		HYDROCARBON CONCENTRATIONS (GRAVIMETRIC)	
					f^1 (µg/g)	f^2 (µg/g)	f^1 (µg/g)	f^2 (µg/g)
9	728	Fucus	9/13/80	4	1.0	17.1	12.6	21.9
9	729	Leptosterias polaris (L)	9/10/80	9	0.1	3.3	0.3	5.5
9	730	L. polaris (L)	9/10/80	9	1.8	3.4	1.0	7.6
9	731	L. polaris (L)	9/10/80	9	1.9	3.6	0.1	3.1
9	732	L. polaris (M)	9/10/80	9	2.9	13.3	8.4	53.9
9	733	L. polaris (M)	9/10/80	9	1.0	2.8	0.3	6.0
9	734	L. polaris (S)	9/10/80	9	0.3	0.2	0.1	3.2
9	735	Psolus sp.	9/10/80	17	3.9	10.6	12.4	33.1
9	736	Psolus sp.	9/10/80	17	0.6	29.2	1.0	15.3
9	737	Psolus sp.	9/10/80	17	0.8	6.5	2.4	6.4
9	738	Psolus frabricii	9/10/80	17	4.9	43.2	0.1	13.3
9	739	Strongylocentratus droebachiensis (M to L)	9/10/80	9	17.0	35.5	13.7	91.8
9	740	S. droebachiensis (M)	9/10/80	9	1.0	45.0	4.1	20.0

[a](L) = large; (M) = medium; (S) = small.

TABLE 3-19 (Cont.)

BAY	ERCO ID	SPECIES	COLLECTION DATE	DEPTH (m)	HYDROCARBON CONCENTRATIONS (GC) f^1 (µg/g)	HYDROCARBON CONCENTRATIONS (GC) f^2 (µg/g)	HYDROCARBON CONCENTRATIONS (GRAVIMETRIC) f^1 (µg/g)	HYDROCARBON CONCENTRATIONS (GRAVIMETRIC) f^2 (µg/g)
9	741	S. droebachiensis (S)	9/10/80	9	38.5	42.0	13.0	62.0
9	742	S. droebachiensis (S)	9/10/80	9	51.0	10.0	20.0	9.0
9	743	Serripes groenlandica (L)	9/10/80	9	0.3	30.7	3.7	22.3
9	744	S. groenlandica (S)	9/10/80	9	8.7	9.6	3.7	15.5
9	745	Mya truncata (L)	9/10/80	9	0.8	5.9	11.4	35.8
9	746	Mya truncata (L)	9/10/80	9-12	1.3	7.0	6.5	54.2
9	747	Mya truncata (L)	9/10/80	9-10	2.6	13.7	48.4	95.5
9	748	Mya truncata (L)	9/10/80	9-10	0.6	2.5	2.3	11.6
9	749	Mya truncata (M)	9/10/80	9-10	1.7	22.0	1.2	21.3
10	750	Fucus	9/13/80	4	6.9	10.2	5.6	17.6
10	751	Laminaria	9/13/80	5	2.8	11.6	1.1	6.7
10	752	L. polaris (L)	9/13/80	5	2.3	1.7	0.1	1.5
10	753	L. polaris (M)	9/13/80	5	3.6	36.3	2.4	24.3
10	754	L. polaris (S)	9/13/80	5	0.3	10.5	1.0	30.1
10	755	S. droebachiensis (L)	9/13/80	7	6.7	15.4	13.8	34.5
10	756	S. droebachiensis (M)	9/13/80	7	25.1	40.2	22.1	44.1

TABLE 3-19 (Cont.)

BAY	ERCO ID	SPECIES	COLLECTION DATE	DEPTH (m)	HYDROCARBON CONCENTRATIONS (GC) f^1 (µg/g)	HYDROCARBON CONCENTRATIONS (GC) f^2 (µg/g)	HYDROCARBON CONCENTRATIONS (GRAVIMETRIC) f^1 (µg/g)	HYDROCARBON CONCENTRATIONS (GRAVIMETRIC) f^2 (µg/g)
10	757	Mya truncata (L)	9/13/80	7	0.3	1.1	0.9	6.4
10	758	Mya truncata (M)	9/13/80	7	1.7	32.3	6.9	23.9
10	759	Mya truncata (S)	9/13/80	7	1.7	25.1	1.5	21.9
11	777	Agarum	9/8/80	20	3.5	48.7	1.3	4.8
11	778	Fucus	9/13/80	3	5.3	6.7	2.3	7.6
11	779	L. polaris	9/8/80	15-20	5.2	37.4	7.9	61.1
11	780	L. polaris	9/8/80	15-20	1.4	24.1	2.4	37.4
11	781	L. polaris	9/8/80	15-20	1.5	29.3	3.3	32.9
11	782	L. polaris	9/8/80	15-20	8.9	20.6	7.9	27.8
11	783	L. polaris	9/8/80	15-20	3.4	24.5	3.3	34.4
11	784	Psolus frabricii (L)	9/8/80	15-20	2.6	33.9	22.4	90.4
11	785	Psolus frabricii (L)	9/8/80	15-20	2.5	33.3	5.2	74.5
11	786	Psolus frabricii (S)	9/8/80	15-20	4.1	44.8	14.0	158.0
11	787	S. droebachiensis (L)	9/8/80	15-20	30.3	156.0	51.0	321.1
11	788	S. droebachiensis (M)	9/8/80	15-20	16.3	79.8	45.7	123.5
11	789	S. droebachiensis (M)	9/8/80	15-20	17.1	82.4	30.8	167.0

TABLE 3-19 (Cont.)

BAY	ERCO ID	SPECIES	COLLECTION DATE	DEPTH (m)	HYDROCARBON CONCENTRATIONS (GC) f^1 (µg/g)	HYDROCARBON CONCENTRATIONS (GC) f^2 (µg/g)	HYDROCARBON CONCENTRATIONS (GRAVIMETRIC) f^1 (µg/g)	HYDROCARBON CONCENTRATIONS (GRAVIMETRIC) f^2 (µg/g)
11	790	S. droebachiensis (S)	9/8/80	15-20	2.5	24.7	9.9	39.5
11	791	S. droebachiensis (S)	9/8/80	15-20	5.0	25.5	17.4	79.7
11	792	S. droebachiensis (S)	9/8/80	15-20	41.5	11.6	38.8	177.6
11	793	Serripes groenlandica	9/12/80	5	1.5	7.5	1.6	10.7
11	794	Mya truncata (L)	9/8/80	15-20	1.9	30.1	1.5	27.5
11	795	Mya truncata --	9/8/80	15-20	0.8	31.1	0.8	18.6
11	796	Mya truncata (M)	9/8/80	15-20	2.5	7.6	0.5	11.9
11	797	Mya truncata (S)	9/8/80	15-20	0.7	13.3	5.5	16.6
11	798	Mya truncata (S)	9/8/80	15-20	7.3	20.9	2.4	20.2
11	799	Myoxocephalus scorpius	9/8/80	15-20	40.0	51.0	110.0	110.0
Z Lagoon	760	Laminaria	9/14/80	3	23.0	18.4	2.8	18.9
	761	L. polaris	9/16/80	-	0.4	4.1	1.1	10.3
	762	L. polaris	9/16/80	-	2.0	43.4	2.9	60.6
	763	L. polaris	9/16/80	-	0.1	1.0	0.9	6.5
	764	Psolus frabricii	9/16/80	-	3.0	43.6	7.5	88.2
	765	S. droebachiensis (L)	9/16/80	-	46.9	22.1	46.5	34.3

TABLE 3-19 (Cont.)

BAY	ERCO ID	SPECIES	COLLECTION DATE	DEPTH (m)	HYDROCARBON CONCENTRATIONS (GC) f^1 (µg/g)	HYDROCARBON CONCENTRATIONS (GC) f^2 (µg/g)	HYDROCARBON CONCENTRATIONS (GRAVIMETRIC) f^1 (µg/g)	HYDROCARBON CONCENTRATIONS (GRAVIMETRIC) f^2 (µg/g)
Z La-goon	766	S. droebachiensis (L)	9/16/80	-	10.4	59.9	42.3	185.0
	767	S. droebachiensis (M)	9/16/80	-	14.6	125.1	24.4	177.4
	768	S. droebachiensis (S)	9/16/80	-	39.0	45.4	157	392
	769	Serripes	9/16/80	-	16.2	112.0	16.9	129.5
	770	Astarte borealis	9/16/80	-	0.3	1.2	1.0	6.3
	771	Mya truncata (L)	9/16/80	-	7.3	4.8	1.4	1.7
	772	Mya truncata (M)	9/16/80	-	8.0	20.6	1.9	42.0
	773	Mya truncata (M)	9/16/80	-	2.8	6.1	4.7	25.1
	774	Mya truncata (S)	9/16/80	-	0.4	9.0	1.6	34.5
	775	Scallop	9/16/80	-	1.4	27.0	6.3	22.8
	776	Myoxocephalus scorpius	9/16/80	-	2.3	4.5	3.4	4.9

TABLE 3-20

COMPARATIVE ANALYSIS OF CONCENTRATIONS OF HYDROCARBONS IN MARINE TISSUE

SPECIES	BAY 9 RANGE	BAY 9 MEAN	BAY 9 STD. DEVIATION	BAY 9 n	BAY 10 RANGE	BAY 10 MEAN	BAY 10 STD. DEVIATION	BAY 10 n	BAY 11 RANGE	BAY 11 MEAN	BAY 11 STD. DEVIATION	BAY 11 n	Z LAGOON RANGE	Z LAGOON MEAN	Z LAGOON STD. DEVIATION	Z LAGOON n
Mya truncata																
f_1: saturated	0.6-2.6	1.4	0.8	5	0.3-1.7	1.2	0.8	3	0.7-7.3	2.6	2.7	5	0.4-8.0	4.6	3.6	4
f_2: aromatic/ olefinic	2.5-22.0	10.2	7.7	5	1.1-32.3	19.5	16.3	3	7.6-31	20.6	10.3	5	4.8-21	10.1	7.2	4
Strongylocentratus droebachiensis																
f_1: saturated	1.0-51	26.8	22.2	4	6.7-25	15.9	13.0	2	2.5-41	18.8	14.9	6	15-47	27	18	4
f_2: aromatic/ olefinic	10-45	33.1	15.9	4	15-40	27.8	17.5	2	11-156	63.5	49.5	6	22-125	63	44	4
Leptosterias polaris																
f_1: saturated	0.1-2.9	1.3	1.1	6	0.3-3.6	2.0	2.3	3	1.4-8.9	4.1	3.1	5	0.1-2.0	0.8	1.0	3
f_2: aromatic/ olefinic	0.2-13	4.4	4.5	6	1.7-36	16	18	3	20-37	27	6.5	5	1-43.4	16	24	3
Psolus																
f_1: saturated	0.6-4.9	2.6	2.2	4	--	--	--	--	2.5-4.1	3.1	0.9	3	--	3.0	--	1
f_2: aromatic/ olefinic	6.5-43	22	17	4	--	--	--	--	33-45	37	6.5	3	--	46	--	1
Serripes groenlandica																
f_1: saturated	0.3-8.7	4.5	5.9	2	--	--	--	--	--	1.5	--	1	--	16	--	1
f_2: aromatic/ olefinic	9.6-31	20	15	2	--	--	--	--	--	7.5	--	1	--	112	--	1
Fucus																
f_1: saturated	--	1	--	1	--	6.9	--	1	--	5.3	--	1	--	--	--	--
f_2: aromatic/ olefinic	--	17	--	1	--	10	--	1	--	6.7	--	1	--	--	--	--
Laminaria																
f_1: saturated	--	--	--	--	--	2.8	--	1		--	--	--	--	23	--	1
f_2: aromatic/ olefinic	--	--	--	--	--	12	--	1		--	--	--	--	18	--	1
Agarum																
f_1: saturated	--	--	--	--	--	--	--	--	--	3.5	--	1	--	--	--	--
f_2: aromatic/ olefinic	--	--	--	--	--	--	--	--	--	49	--	1	--	--	--	--
Sculpin																
f_1: saturated	--	--	--	--	--	--	--	--	--	40	--	1	--	2.3	--	1
f_2: aromatic/ olefinic	--	--	--	--	--	--	--	--	--	51	--	1	--	4.5	--	1

As presented in Table 3-20, there is a wide range of hydrocarbon concentrations within each species, reflecting variations mainly in biogenic components. For example, concentrations of f_1 and f_2 hydrocarbons in Mya range over an order of magnitude within a given bay. However variations between bays are small. These large variations in the biogenic hydrocarbon makeup of a particular species are common in baseline investigations (e.g., Boehm et al., 1979). Rather than suggesting analytical "chaos," these observed variations fall into definable compositional groups if one views the information given in Table 3-20 for each species over the four-bay region in light of the GC^2-determined compositions.

Perhaps the most important information on these baseline tissue hydrocarbons comes from the GC^2 traces. Representative GC^2 traces reveal that each species groups into one or two main compositional patterns. For example, Mya fall into one of the three related compositional patterns which are similar in their f_1 compositions or combinations thereof (Figures 3-26, 3-27, and 3-28). These compositions are mainly of a biogenic origin although there is some evidence of the presence of small amounts of aromatic hydrocarbon compounds (see Figure 3-25 and next section).

The sea urchins, Strongylocentrotus droebachiensis, contain large amounts of natural lipid material and hence biogenic hydrocarbons. No evidence of petroleum contamination was observed in this species. The hydrocarbon compositions are strikingly similar in all samples of this species examined (e.g., Figure 3-29).

Similarly Psolus samples are free of petroleum inputs and are characterized by biogenic hydrocarbon compositions (Figure 3-30).

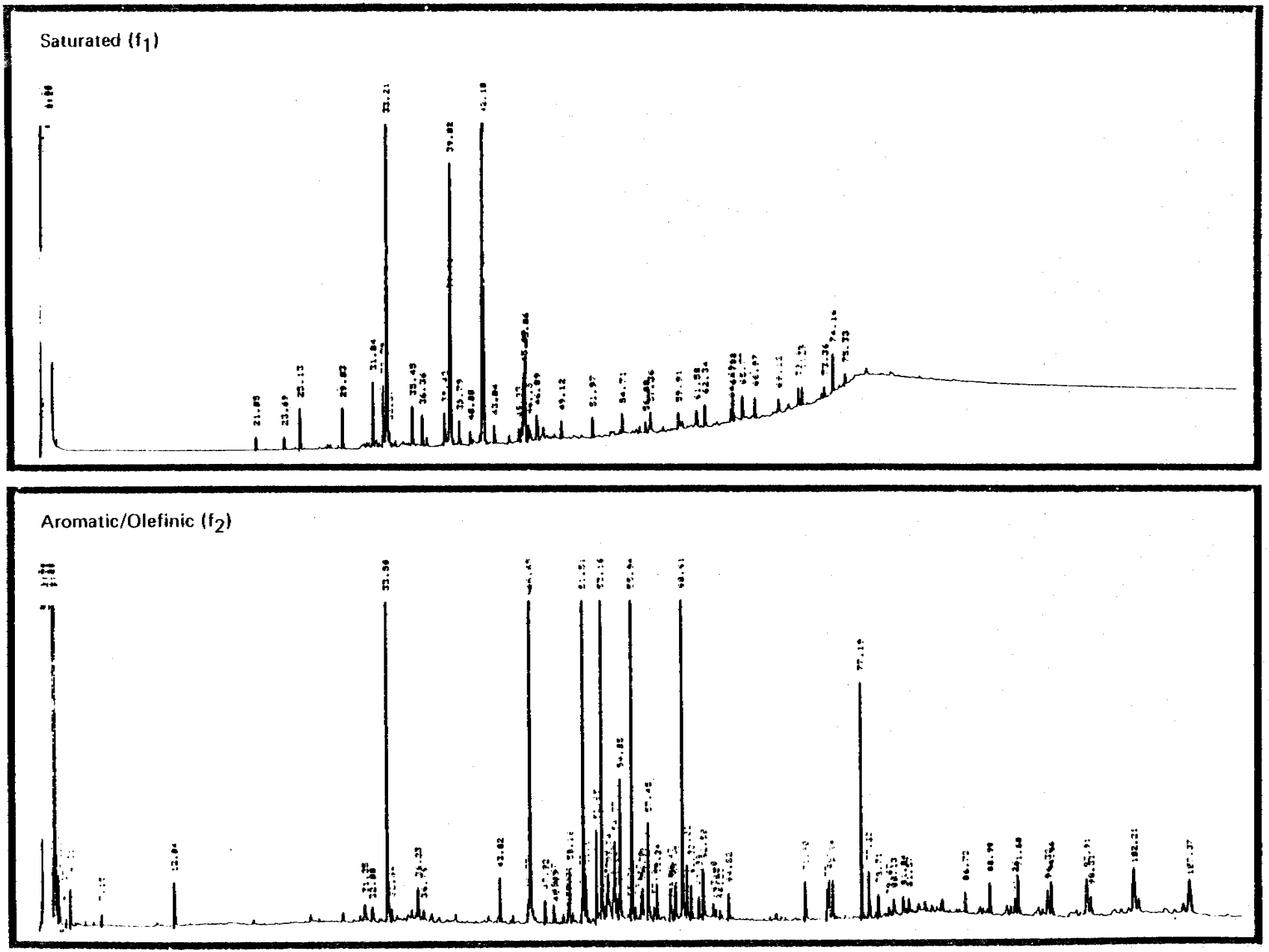
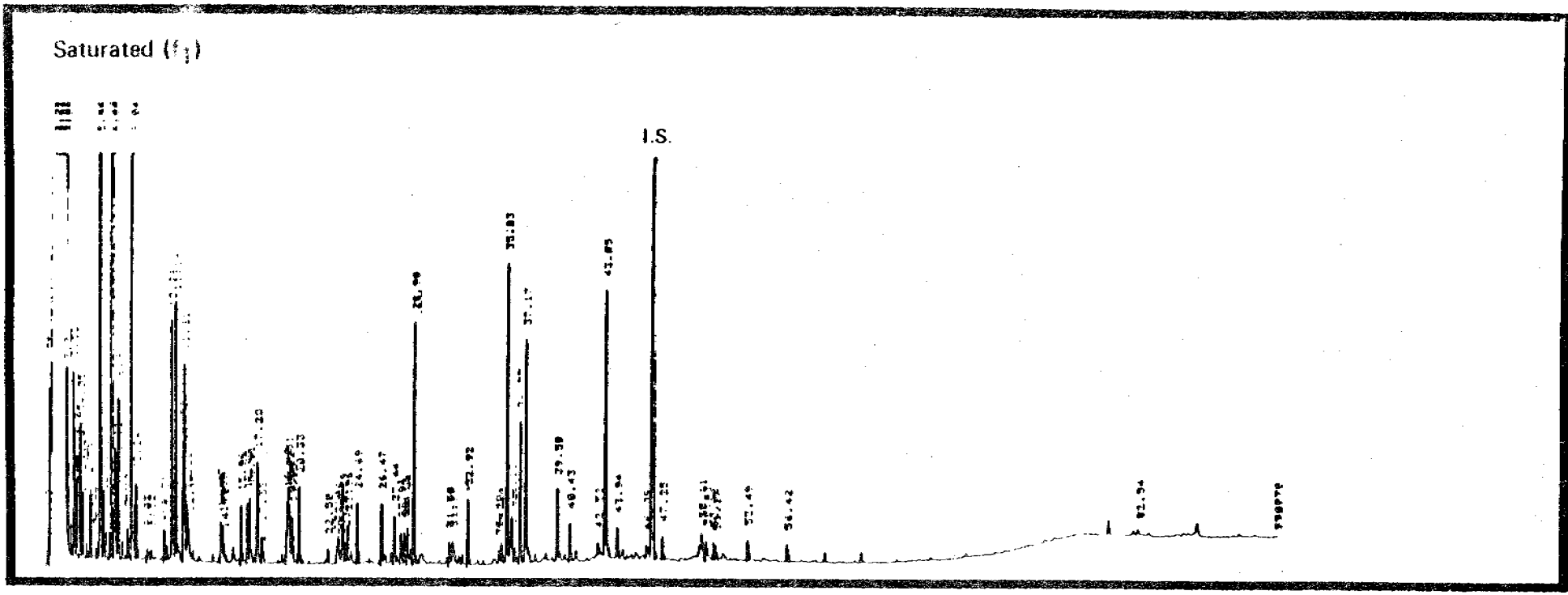
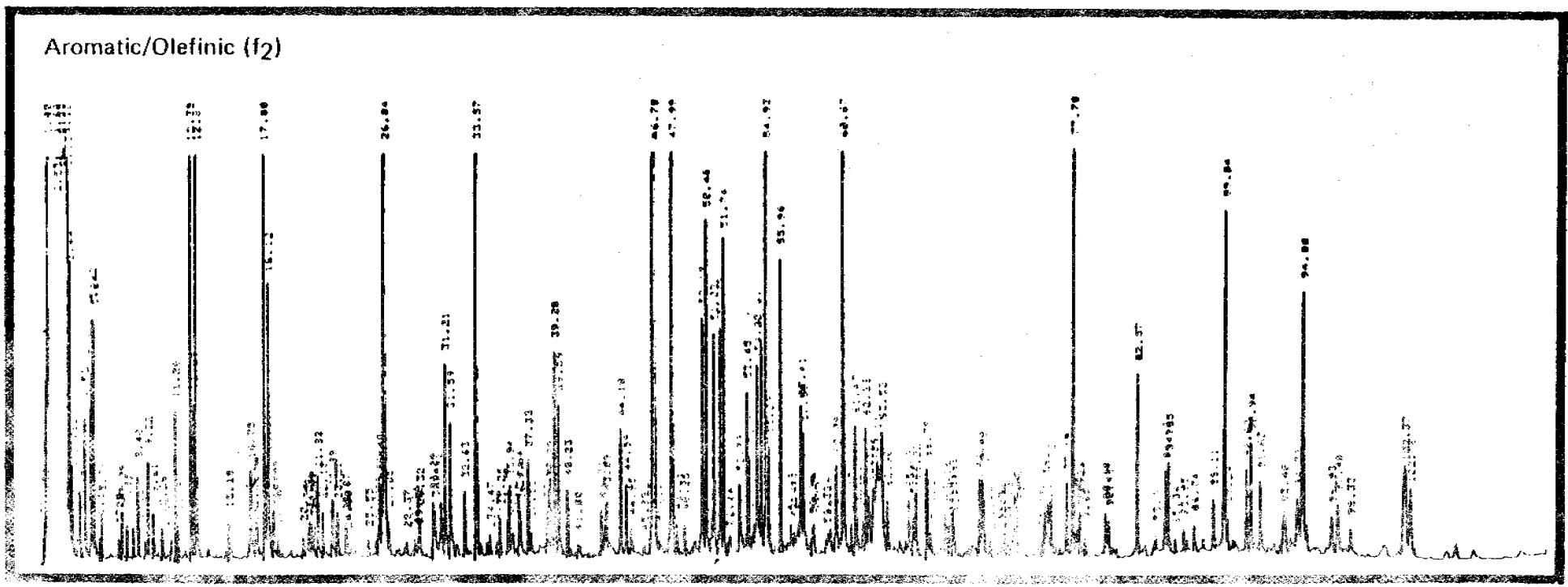


Figure 3.26. GC^2 Traces of *Mya truncata*—Bay 9.


Saturated (f_1)
I.S.


Aromatic/Olefinic (f_2)

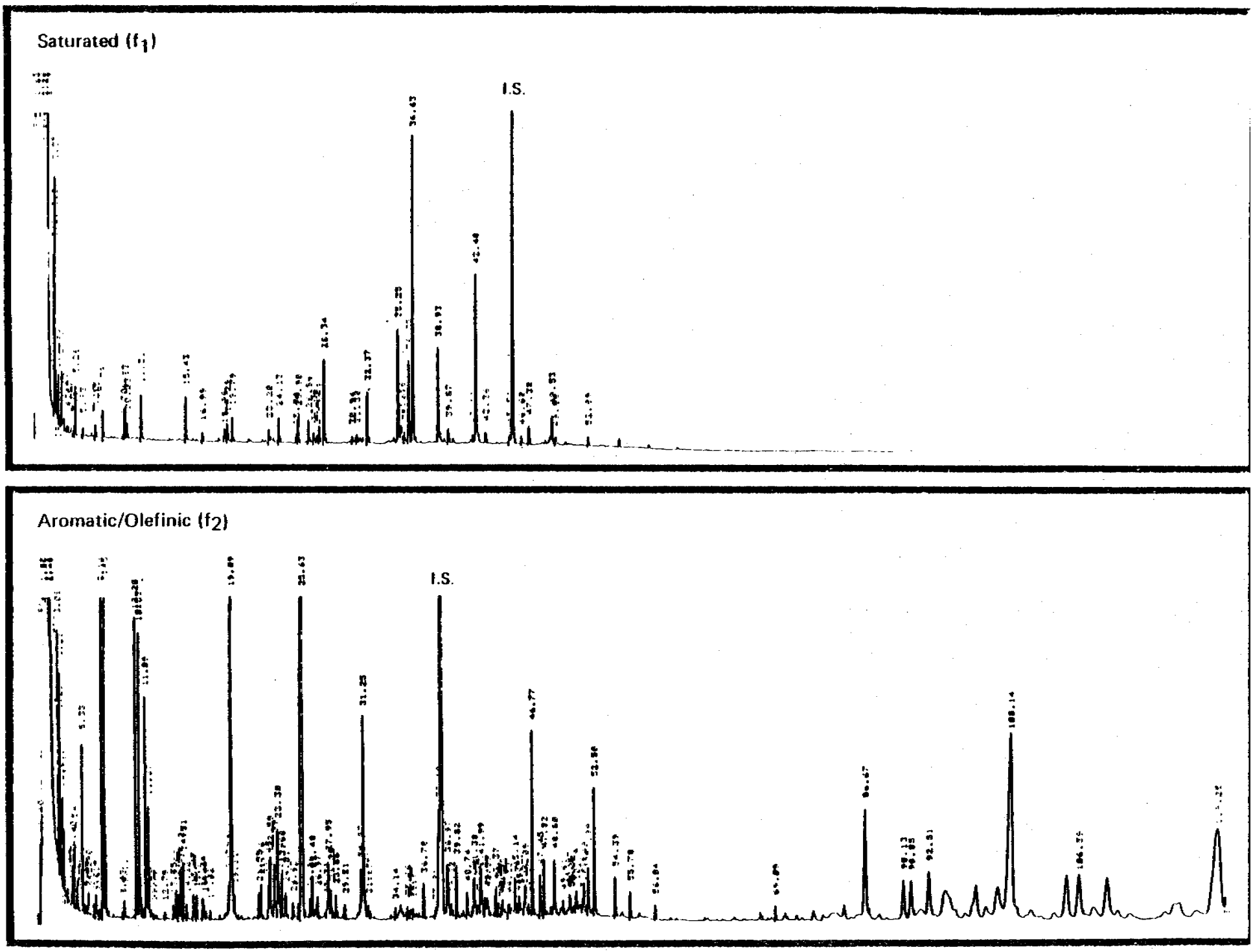
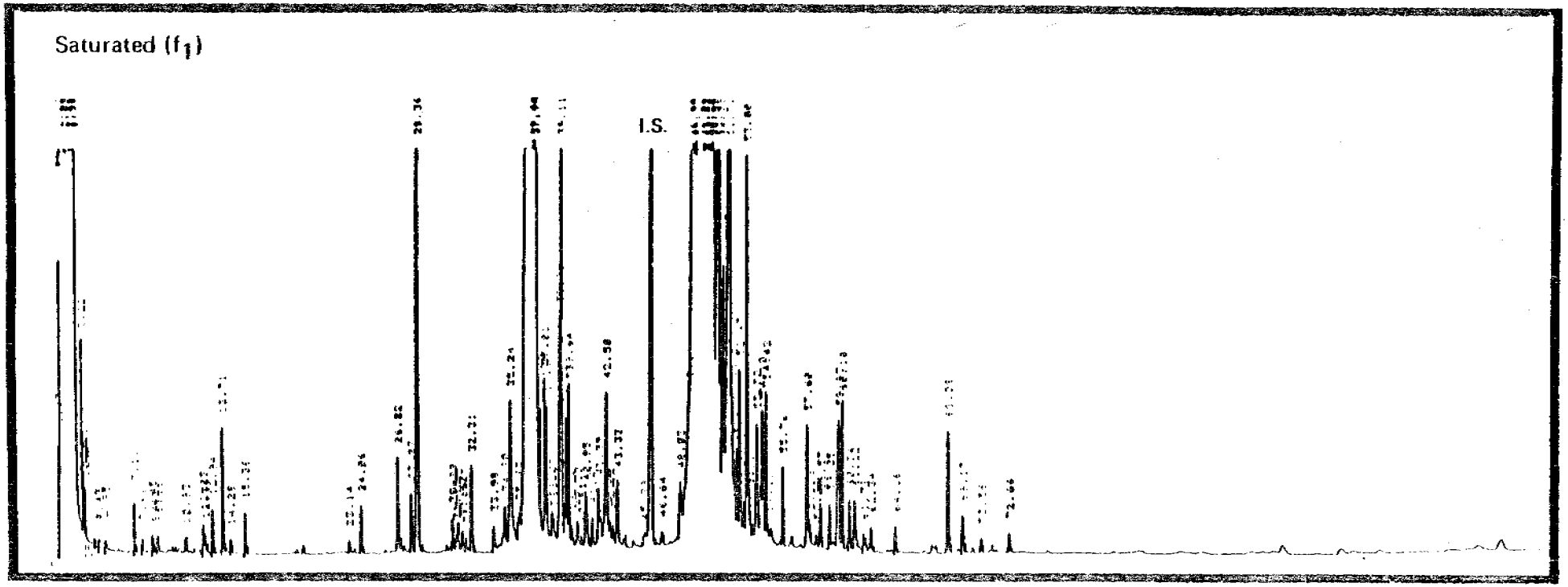
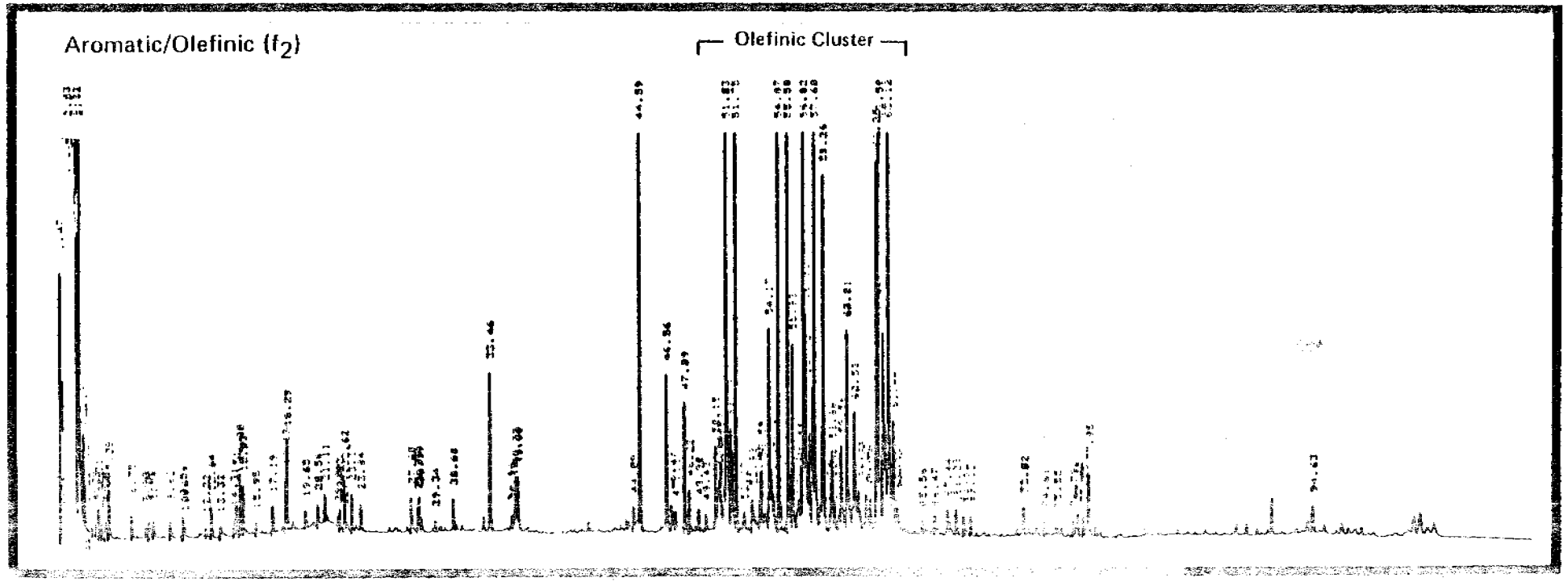
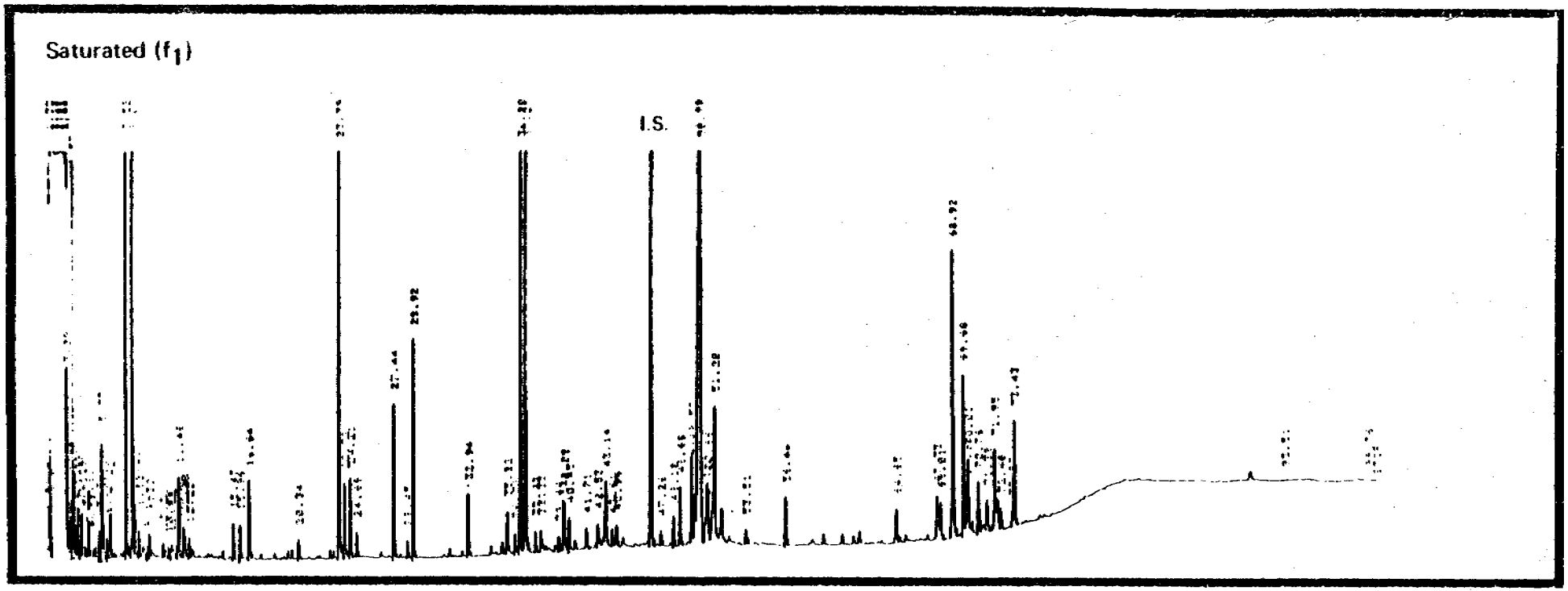


Figure 3.28. GC^2 Traces of *Mya*–Z–Lagoon.


Saturated (f_1)
I.S.


Aromatic/Olefinic (f_2)
Olefinic Cluster



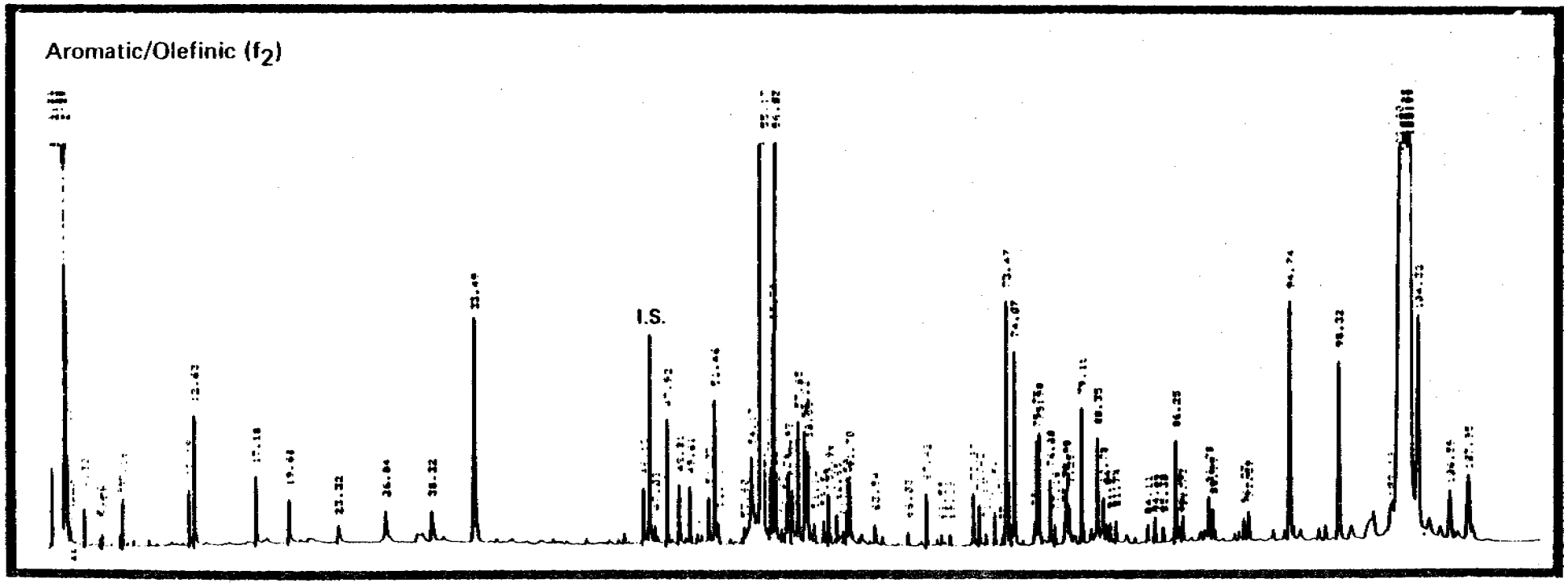


Figure 3.30. GC^2 Traces of *Psolus* Bay 11.

Samples of Leptosterias were comprised of a more complex set of saturated and aromatic/olefinic (f_2) hydrocarbons (Figure 3-31). While no evidence for petroleum hydrocarbon input is seen in the f_1 fraction, GC^2/MS analyses of the f_2 fraction (e.g., Figure 3-29) (see next section) reveal important levels of light aromatic hydrocarbons (alkyl benzenes, naphthalenes) suggestive of low level contaminant input. While most of the samples were comprised of f_1 and f_2 hydrocarbons similar to those shown in Figures 3-31 and 3-32, several of the samples (4 out of a total of 17) appeared to contain obvious petroleum contaminants (2-100 ppm) presumably due to sampling-related contamination (Figure 3-33).

Note that the compositional pattern shown in Figure 3-31 very much resembles the seaweed compositions. All of the remaining sample types contained a variety of biogenic hydrocarbons and no petroleum-related inputs. Several of the seaweeds (Laminaria, Fucus) (Figures 3-34 and 3-35) were comprised of sets of biogenic hydrocarbons very similar in composition to Leptosterias (Figure 3-31) and Strongylocentrotus (Figure 3-31) compositions thus implying a food chain relationship.

3.2.7 Tissue Hydrocarbons (GC/MS)

In order to ferret out any low levels of aromatic hydrocarbons in the biogenic-dominated f_2 distributions, $G2^C$/MS was used. The GC^2/MS/computer system focused on levels of 1- to 5-ring aromatics in 14 samples chosen on the basis of their GC^2 traces and in an effort to get adequate areal and species coverage.

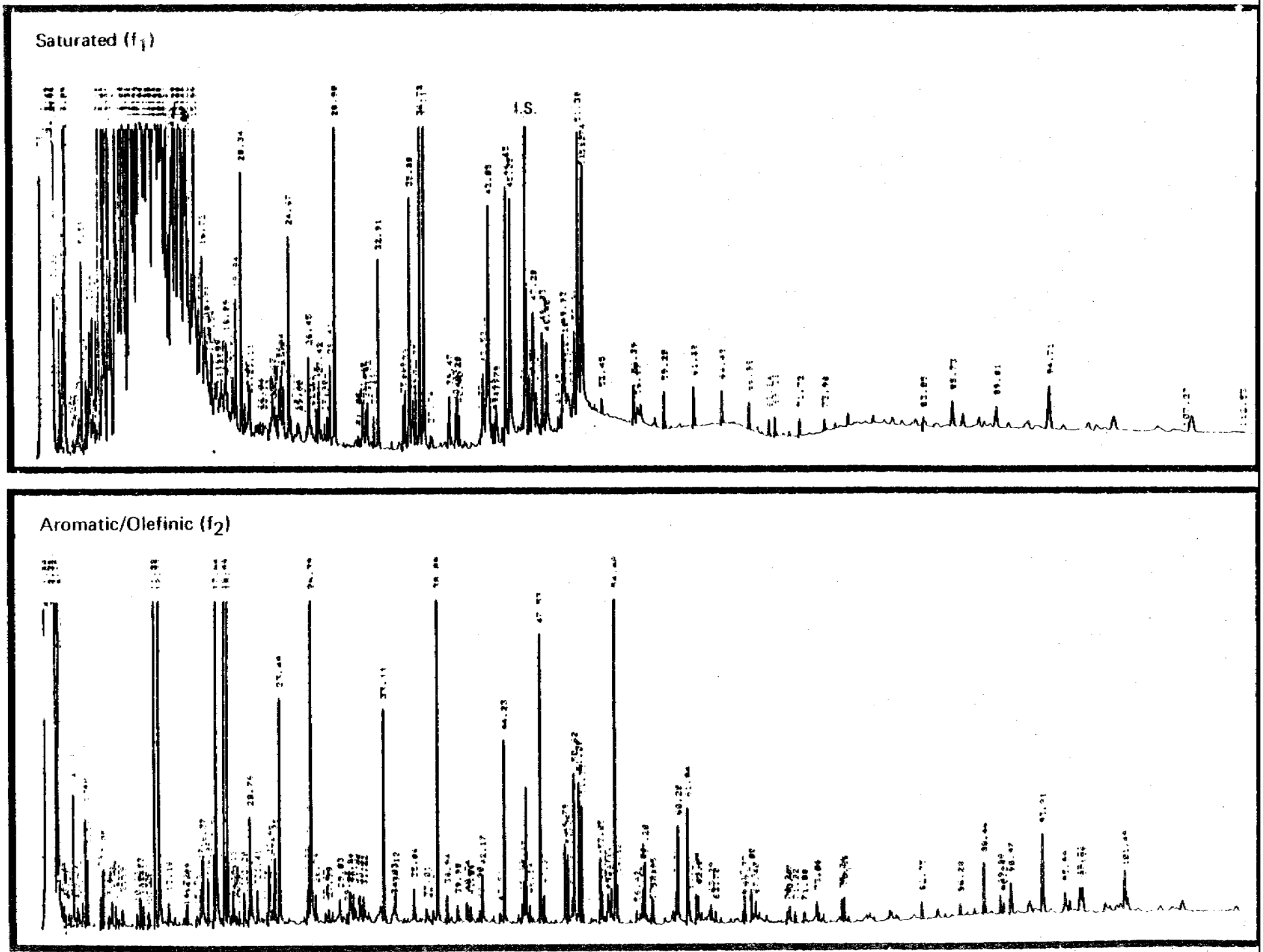
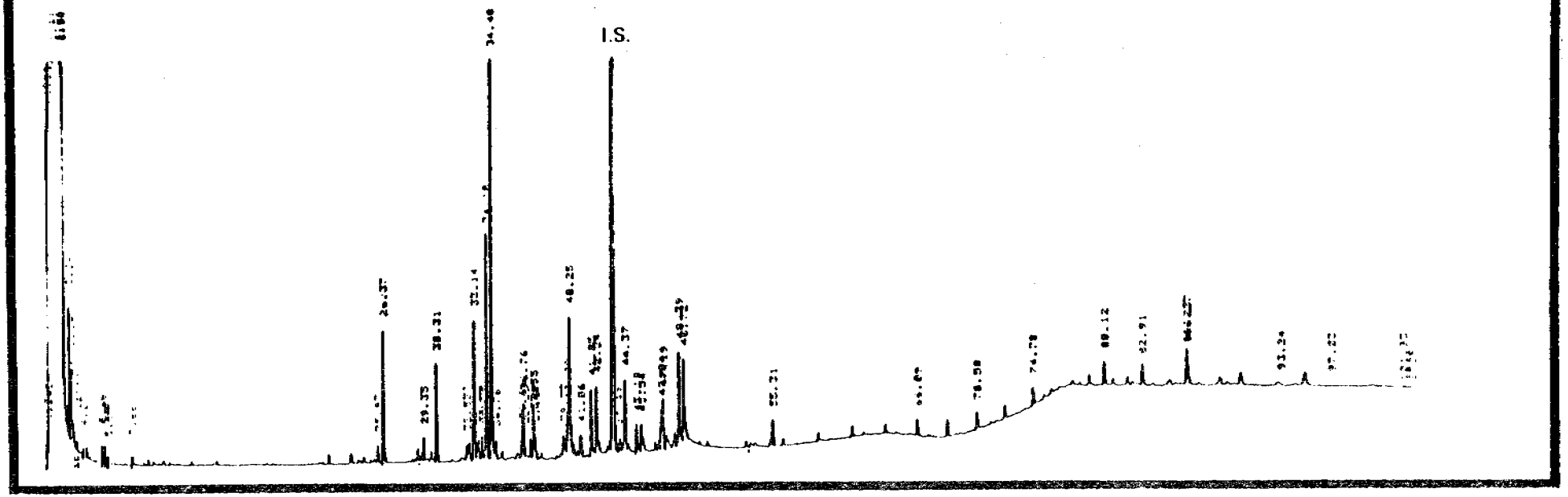
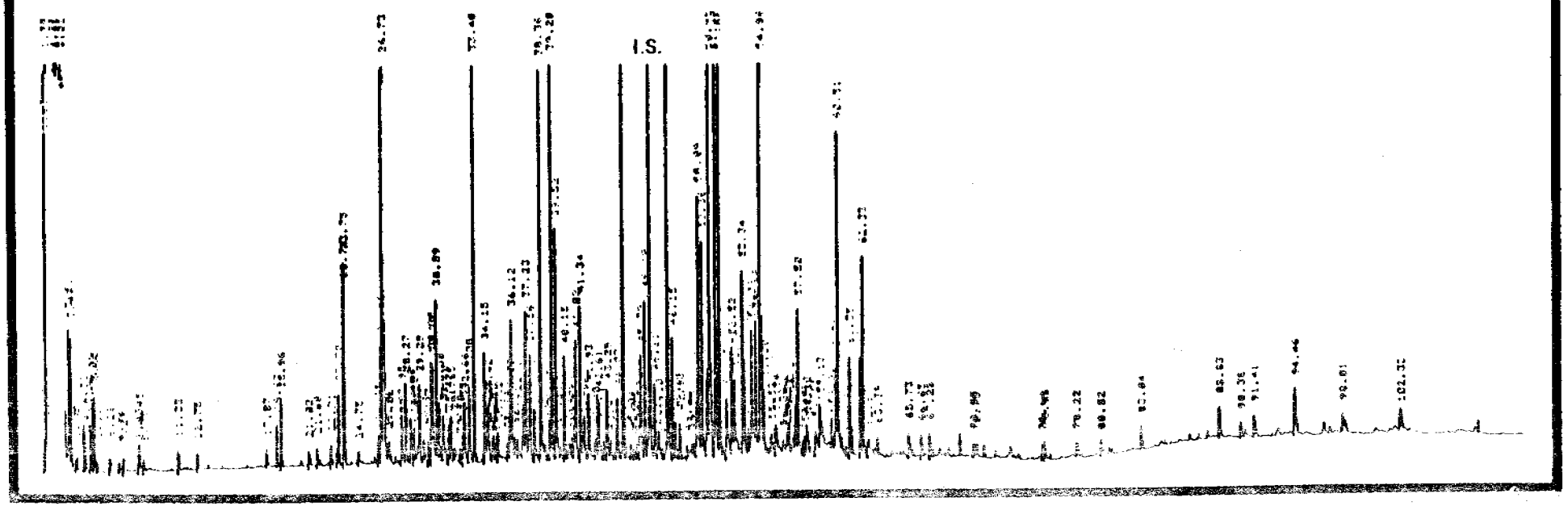


Figure 3.31. GC^2 Traces of *L. polaris*—Bay 10.

Saturated (f_1)



Aromatic/Olefinic (f_2)



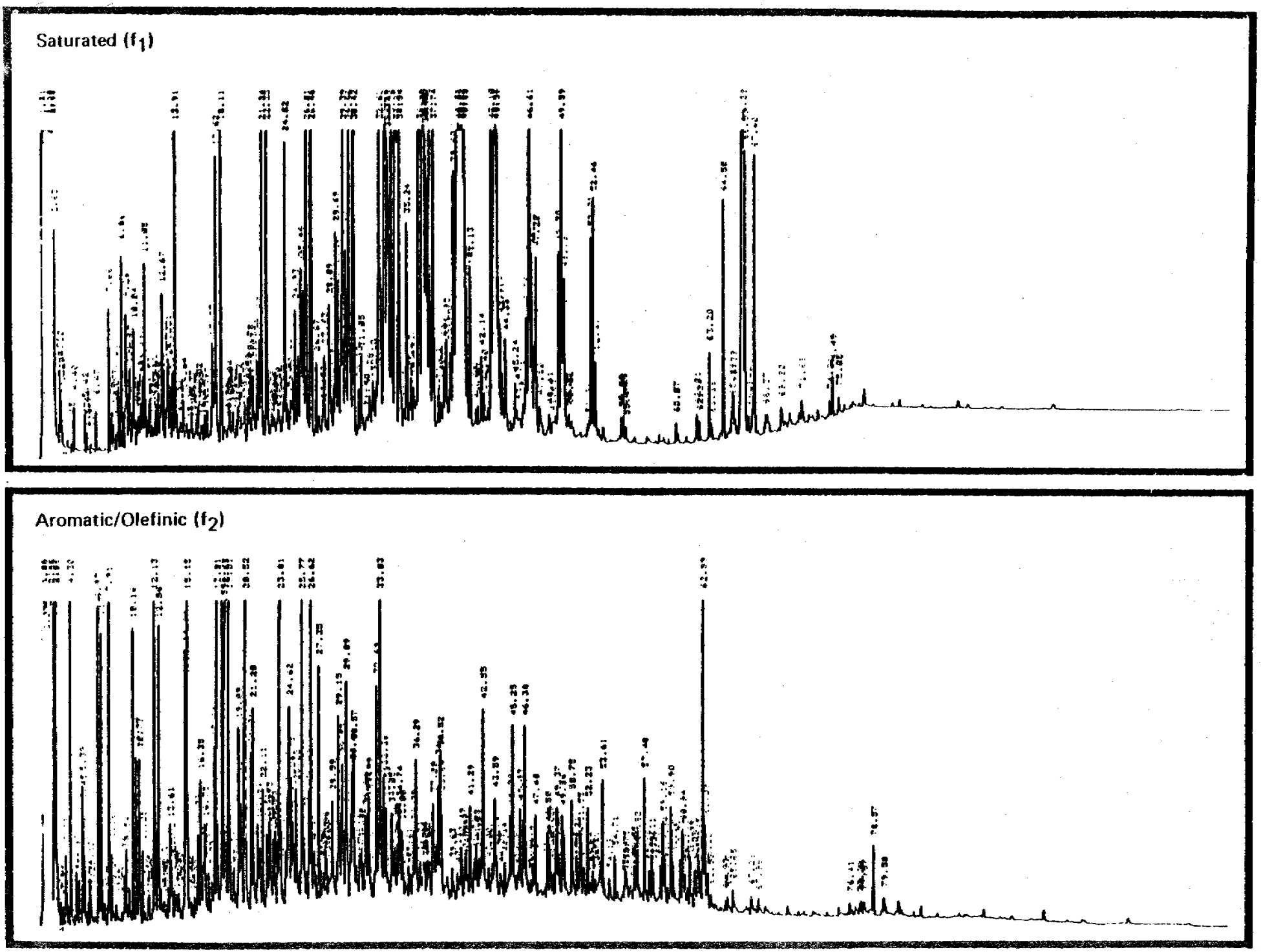


Figure 3.33. GC^2 Traces of *Leptosterias polaris*, Bay 10 Showing Probable Sampling Contamination.

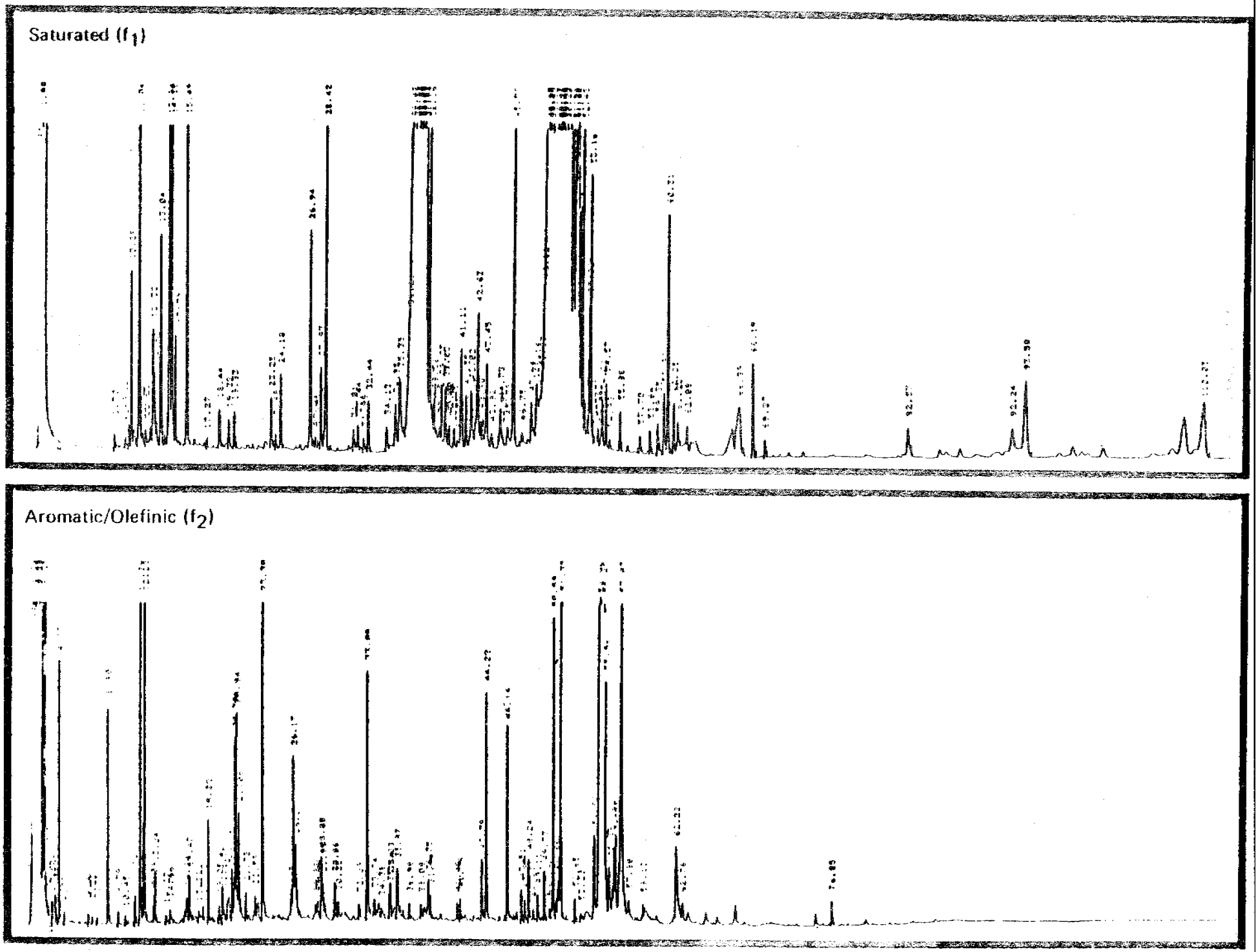
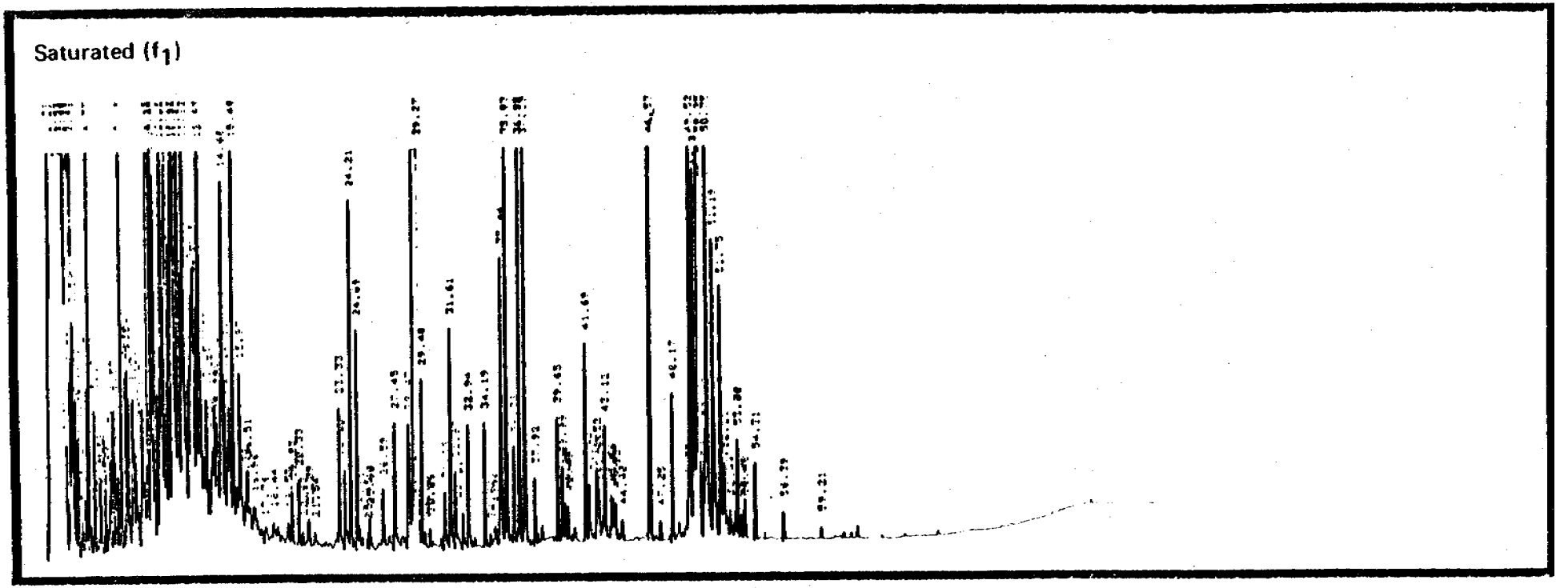


Figure 3.34 GC^2 Traces of *Laminaria*—Z—Lagoon



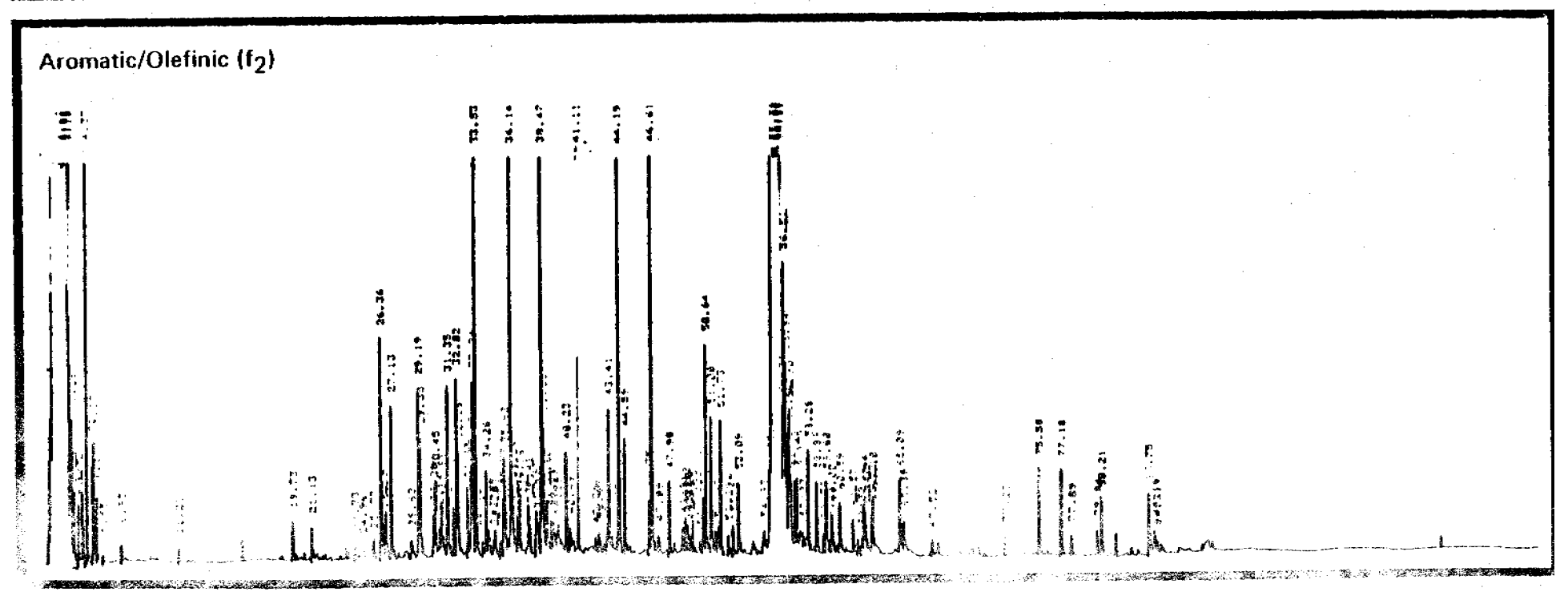


Figure 3.35. GC^2 Traces of *Fucus*—Bay 11.

The resultant data is summarized in Table 3-21. Low levels (2-12 ppb) of naphthalenes, and phenanthrene compounds were found in most of the samples. In the samples with extremely low levels (e.g., all of the Mya samples) the parent (unsubstituted) naphthalene and phenanthrene compounds were detected with none of their alkylated homologues present. In those samples showing moderate to gross petroleum contamination, entire families (C_0 to C_4) of naphthalene, fluorene, and C_3 to C_5 alkylated benzenes were readily detected.

Note that these incidents of contamination affected several Leptosterias samples, as previously mentioned, and may have affected several seaweed samples. The presence of aromatic hydrocarbons in the seaweed was not readily apparent in the GC^2 traces due to the much higher levels of biogenic hydrocarbons present. The results of the Mya analyses indicate that, except for minor inputs of phenanthrene and naphthalene from long-range transport sources (e.g., fallout), this species is quite free of any contamination and is thus quite suitable for use as a sensitive monitor of inputs of low levels of petroleum to the suspended particulate load.

TABLE 3-21

BASELINE STUDY - AROMATIC HYDROCARBON LEVELS IN TISSUES (BY GC/MS) (nanograms/gram dry weight)

	LAB ID													
	749	772	758	797	743	731	732[a]	762	753	729	733[a]	750[a]	777	751
SPECIES:[b]	1	1	1	1	2	3	3	3	3	3	3	4	5	6
BAY:	9	2	10	11	9	9	9	2	10	9	9	10	11	10
Naphthalenes (m/e 128, 142, 156, 170)	7	5	ND	ND	2	28	1270	140	140	8	5100	6700	150	20
Alkyl Benzenes (m/e 120, 134, 148)	ND	ND	ND	ND	ND	11	1300	220	190	3	3500	1700	10	10
Phenanthrenes (m/e 178, 192, 206, 220, 234)	3	6	8	1	10	ND	ND	10	5	ND	40	490	11	8
Fluorenes (m/e 166, 180, 194, 208)	ND	ND	ND	ND	ND	ND	100	ND	ND	ND	130	70	2	1
Biphenyl (m/e 154)	ND	ND	ND	ND	ND	ND	100	8	11	ND	360	200	8	2
Fluoranthene/Pyrene (m/e 202)	ND	ND	ND	ND	4	ND	ND	ND	19	ND	ND	ND	5	ND
Benzopyrenes (m/e 252)	ND	ND	ND	ND	ND	ND	ND	ND	14	ND	ND	ND	ND	ND

[a]GC[2] trace indicates gross contamination.

[b]Species 1 = Mya truncata
2 = Serripes groenlandica
3 = Leptosterioas polaris
4 = Fucus
5 = Laminaria

ND = not detected.

3.3 Shoreline Experiments

Samples from four pairs of oiled test plots were analyzed to determine the detailed hydrocarbon chemical composition in order to discern subtle time-dependent changes owing to weathering processes. The samples, taken at times from 1 to 16 days after the oil applications, consisted of a single composite surface sample (see details in Volume 1).

3.3.1 Hydrocarbon Concentrations

A summary of the analytical data on the gross compositional features (i.e. resolved by GC^2) and total (by microgravimetry) hydrocarbons are presented in Table 3-22 for the 16 test plots. These results indicate large differences in residual concentrations of oil in the test plots from the intertidal zone, dependent mainly on whether the spilled oil was emulsified (site L-2; H-2) or unemulsified (site L-1; H-1). The unemulsified (or aged) oil concentrations remained high throughout the experiments (after day 1 at H-1), but increased at the sites with the emulsified oil, probably due to oil removal followed by redeposition. The oil concentrations at the backshore plots were higher than the intertidal plots throughout the experiments, although some temporal variations were noted at all plots.

3.3.2 Saturated Hydrocarbon Composition (GC^2)

The detailed saturated hydrocarbon compositional information is presented for each test plot in a tabular form and in a graphic form. The tabular information (Tables 3-23 to 3-30) presents concentrations of individual n-alkanes

TABLE 3-22

SHORELINE STUDY - PETROLEUM HYDROCARBON CONCENTRATIONS

SITE	DAY	SAMPLE ID NO.	SATURATED HYDROCARBONS		AROMATIC HYDROCARBONS	
			TOTAL RESOLVED (GC) (µg/g)	TOTAL GRAVI-METRIC (µg/g)	TOTAL RESOLVED (GC) (µg/g)	TOTAL GRAVI-METRIC (µg/g)
L-1	1	GC-11	307	2,650	166	1,970
	2	GC-12	421	2,650	134	1,971
	4	GC-13	301	4,300	201	2,542
	8	GC-14	824	4,210	350	3,890
L-2	2	GC-17	6.0	78	1.8	65
	4	GC-18	57.1	469	30.7	385
	8	GC-19	60.8	278	20.0	188
H-1	1	GC-1	0.7	15.1	ND	0.2
	2	GC-2	84.4	1,100	46.0	905
	4	GC-3	244	1,880	99.3	1,850
	8	GC-4	236	2,790	57.9	1,360
	16	GC-5	98.9	1,290	29.8	963
H-2	1	GC-6	0.14	1.0	0.005	1.8
	2	GC-7	0.4	0.8	0.002	1.6
	4	GC-8	14.8	95.0	9.0	58.1
	8	GC-9	17.5	206	4.0	158
	16	GC-10	250	4,420	77.8	2,840
LT-1	1	GC-21	696	19,900	863	14,700
	2	GC-22	1,350	17,300	632	10,500
	4	GC-23	150	5,170	220	5,240
	8	GC-24	1,550	10,200	758	10,500
LT-2	1	GC-26	2,710	11,300	399	8,970
	2	GC-27	4,380	11,900	835	7,480
	4	GC-28	961	4,400	106	3,720
	8	GC-29	3,920	20,900	487	16,700

TABLE 3-22 (Cont.)

SITE	DAY	SAMPLE ID NO.	SATURATED HYDROCARBONS		AROMATIC HYDROCARBONS	
			TOTAL RESOLVED (GC) (µg/g)	TOTAL GRAVI-METRIC (µg/g)	TOTAL RESOLVED (GC) (µg/g)	TOTAL GRAVI-METRIC (µg/g)
HT-1	1	GC-40	623	3,990	185	2,560
	2	GC-42	490	4,480	155	3,020
	4	GC-44	403	5,030	140	4,030
	8	GC-46	2,270	12,000	872	8,660
HT-2	1	GC-41	3,970	18,300	520	16,500
	2	GC-43	1,100	5,790	361	4,680
	4	GC-45	1,350	13,700	373	6,900
	8	GC-47	1,337	9,120	646	6,920
	16	GC-49	1,260	7,880	304	4,840

TABLE 3-23

SHORELINE STUDY - SATURATED HYDROCARBONS (SITE L-1)

	DAY				
	1	2	4	8	16
Sample ID No.	GC-11	GC-12	GC-13	GC-14	None
Lab ID No.	06-1060	06-1061	06-1104	06-1062	
Constituent (µg/g)					
$n\text{-}C_{10}$	9.2	4.5	3.6	20.1	
$n\text{-}C_{11}$	13.8	13.9	11.6	36.1	
$n\text{-}C_{12}$	15.9	20.1	17.1	43.9	
$n\text{-}C_{13}$	15.6	22.3	20.0	45.0	
$n\text{-}C_{14}$	15.3	23.1	18.2	43.5	
Farnesane	6.1	9.3	8.5	15.3	
$n\text{-}C_{15}$	14.6	22.7	20.5	42.4	
$n\text{-}C_{16}$	12.9	20.0	18.6	37.9	
$n\text{-}C_{17}$	11.2	18.1	16.7	34.8	
Pristane	4.9	7.3	5.7	15.2	
$n\text{-}C_{18}$	10.1	14.9	13.6	2.8	
Phytane	5.5	9.4	8.2	17.7	
$n\text{-}C_{19}$	9.8	15.8	10.0	31.5	
$n\text{-}C_{20}$	7.6	12.5	10.6	25.3	
$n\text{-}C_{21}$	6.3	10.7	8.3	21.2	
$n\text{-}C_{22}$	5.6	9.7	7.5	21.9	
$n\text{-}C_{23}$	4.4	8.2	6.1	15.0	
$n\text{-}C_{24}$	4.8	7.4	5.3	13.5	
$n\text{-}C_{25}$	3.6	6.0	4.2	11.2	
$n\text{-}C_{26}$	2.9	5.1	3.5	10.9	
$n\text{-}C_{27}$	2.7	4.3	2.8	8.0	
$n\text{-}C_{28}$	2.2	3.7	2.2	5.3	
$n\text{-}C_{29}$	2.2	3.0	1.8	6.0	
$n\text{-}C_{30}$	1.5	2.7	1.4	3.5	
$n\text{-}C_{31}$	1.6	2.1	1.3	4.2	
$n\text{-}C_{32}$	1.0	1.5	1.0	ND[a]	

[a]ND = not detected.

TABLE 3-23 (Cont.)

	DAY				
	1	2	4	8	16
Total Alkanes (GC) (µg/g)	175	288	206	483	None
Total Resolved (GC)(µg/g)	307	421	301	829	
Total Saturates (grav. wt.)(µg/g)	2,650	2,650	4,300	4,210	
ALK/ISO	2.36	2.48	2.64	2.55	
SHWR	2.54	1.89	2.33	2.52	

TABLE 3-24

SHORELINE STUDY - SATURATED HYDROCARBONS (SITE L-2)

	DAY				
	1	2	4	8	16
Sample ID No.	None	GC-17	GC-18	GC-19	None
Lab ID No.		06-1064	06-1065	06-1066	
Constituent (µg/g)					
$n\text{-}C_{10}$		ND[a]	0.26	0.30	
$n\text{-}C_{11}$		0.03	1.5	1.2	
$n\text{-}C_{12}$		0.16	2.9	2.3	
$n\text{-}C_{13}$		0.31	3.6	3.0	
$n\text{-}C_{14}$		0.40	3.7	3.5	
Farnesane		0.15	1.2	1.4	
$n\text{-}C_{15}$		0.44	3.5	3.7	
$n\text{-}C_{16}$		0.40	3.2	3.3	
$n\text{-}C_{17}$		0.38	2.9	3.1	
Pristane		0.14	1.1	1.2	
$n\text{-}C_{18}$		0.35	2.7	2.8	
Phytane		0.20	1.6	1.6	
$n\text{-}C_{19}$		0.35	2.6	1.9	
$n\text{-}C_{20}$		0.29	2.2	2.3	
$n\text{-}C_{21}$		0.25	1.9	1.9	
$n\text{-}C_{22}$		0.22	1.7	1.7	
$n\text{-}C_{23}$		0.18	1.4	1.4	
$n\text{-}C_{24}$		0.15	12.0	1.2	
$n\text{-}C_{25}$		0.12	1.0	1.1	
$n\text{-}C_{26}$		0.10	0.82	0.86	
$n\text{-}C_{27}$		0.07	0.72	0.69	
$n\text{-}C_{28}$		0.05	0.60	0.60	
$n\text{-}C_{29}$		0.05	0.61	0.50	
$n\text{-}C_{30}$		0.04	0.58	0.45	
$n\text{-}C_{31}$		0.03	0.47	0.36	
$n\text{-}C_{32}$		0.02	0.37	0.23	

TABLE 3-24 (Cont.)

	DAY				
	1	2	4	8	16
Total Alkanes (GC)(µg/g)	None	5.1	39.7	38.6	None
Total Resolved (GC)(µg/g)		5.98	57.1	60.8	
Total Saturates (grav. wt.)(µg/g)		78.3	469	278	
ALK/ISO		2.70	2.62	2.80	
SHWR		2.09	2.25	2.00	

TABLE 3-25

SHORELINE STUDY - SATURATED HYDROCARBONS (SITE H-1)

	DAY				
	1	2	4	8	16
Sample ID No.	GC-1	GC-2	GC-3	GC-4	GC-5
Lab ID No.	06-1097	06-1073	06-1074	06-1075	06-1076
Constituent (µg/g)					
$n\text{-}C_{10}$	ND[a]	ND	5.	ND	ND
$n\text{-}C_{11}$	ND	0.8	10.4	0.29	ND
$n\text{-}C_{12}$	ND	2.8	13.2	3.0	0.44
$n\text{-}C_{13}$	ND	4.5	13.1	9.7	2.6
$n\text{-}C_{14}$	0.01	5.5	12.7	15.0	5.4
Farnesane	0.002	2.2	4.4	6.4	2.5
$n\text{-}C_{15}$	0.03	5.6	12.3	16.9	6.7
$n\text{-}C_{16}$	0.05	5.1	11.1	15.0	6.6
$n\text{-}C_{17}$	0.05	4.5	10.2	13.9	6.0
Pristane	0.02	2.0	4.2	5.4	2.6
$n\text{-}C_{18}$	0.05	4.0	9.3	12.5	5.1
Phytane	0.03	2.2	5.5	6.8	2.9
$n\text{-}C_{19}$	0.04	2.4	9.2	7.7	3.8
$n\text{-}C_{20}$	0.04	3.1	7.8	9.5	4.5
$n\text{-}C_{21}$	0.04	2.5	6.6	8.0	3.6
$n\text{-}C_{22}$	0.03	2.2	5.7	7.0	3.3
$n\text{-}C_{23}$	0.03	1.9	4.9	5.9	2.8
$n\text{-}C_{24}$	0.03	1.7	4.3	5.3	2.5
$n\text{-}C_{25}$	0.03	1.4	3.7	4.3	2.3
$n\text{-}C_{26}$	0.02	1.2	3.4	3.8	1.8
$n\text{-}C_{27}$	0.03	1.0	2.8	3.3	1.5
$n\text{-}C_{28}$	0.02	0.89	2.3	2.9	1.3
$n\text{-}C_{29}$	0.02	0.79	2.1	2.6	1.0
$n\text{-}C_{30}$	0.02	0.70	1.7	2.4	0.88
$n\text{-}C_{31}$	0.02	0.64	1.6	2.0	0.73
$n\text{-}C_{32}$	0.01	0.44	1.3	1.6	0.43

[a]ND = not detected.

TABLE 3-25 (Cont.)

	DAY				
	1	2	4	8	16
Total Alkanes (GC)(µg/g)	0.55	54.4	155	145	63.6
Total Resolved (GC)(µg/g)	0.74	84.4	244	236	98.9
Total Saturates (grav. wt.)(µg/g)	15.1	1,100	1,880	2,790	1,290
ALK/ISO	2.67	2.54	2.53	2.78	2.58
SHWR	1.27	2.03	2.26	1.81	1.63

TABLE 3-26

SHORELINE STUDY - SATURATED HYDROCARBONS (SITE H-2)

	DAY				
	1	2	4	8	16
Sample ID No.	GC-6	GC-7	GC-8	GC-9	GC-10
Lab ID No.	06-1077	06-1078	06-1079	06-1080	06-1081
Constituent (µg/g)					
n-C_{10}	ND[a]	ND	ND	ND	2.5
n-C_{11}	ND	ND	ND	ND	18.0
n-C_{12}	ND	ND	0.04	0.14	38.3
n-C_{13}	ND	ND	0.25	0.47	52.8
n-C_{14}	ND	ND	0.57	0.82	22.0
Farnesane	ND	ND	0.27	0.40	23.5
n-C_{15}	0.5[b]	ND	0.88	0.95	57.6
n-C_{16}	2.0[b]	0.2[b]	0.96	1.1	52.3
n-C_{17}	3.4[b]	1.5[b]	0.99	1.0	46.5
Pristane	4.5[b]	2.2[b]	0.43	0.45	20.8
n-C_{18}	4.8[b]	3.0[b]	0.97	0.88	40.2
Phytane	2.4[b]	1.4[b]	0.58	0.53	24.2
n-C_{19}	1.6[b]	0.2[b]	0.90	0.67	44.5
n-C_{20}	5.4[b]	3.0[b]	0.76	0.76	35.3
n-C_{21}	4.7[b]	2.8[b]	0.62	0.78	30.6
n-C_{22}	5.3[b]	2.4[b]	0.51	0.58	26.4
n-C_{23}	6.2[b]	2.0[b]	0.44	0.51	22.7
n-C_{24}	8.9[b]	1.6[b]	0.38	0.46	19.4
n-C_{25}	11.5[b]	1.6[b]	0.31	0.39	18.1
n-C_{26}	14.0[b]	1.2[b]	0.27	0.35	14.8
n-C_{27}	10.2[b]	1.2[b]	0.25	0.30	13.0
n-C_{28}	8.0[b]	1.0[b]	0.22	0.25	11.0
n-C_{29}	6.1[b]	1.0[b]	0.21	0.24	9.5
n-C_{30}	4.6[b]	0.6[b]	0.20	0.25	11.2
n-C_{31}	3.4[b]	0.6[b]	0.16	0.18	7.7
n-C_{32}	2.2[b]	0.4[b]	0.12	0.17	6.6

[a]ND = not detected.

[b]ng/g.

TABLE 3-26 (Cont.)

	DAY				
	1	2	4	8	16
Total Alkanes (GC)(µg/g)	64[b]	26[b]	11.0	8.7	591
Total Resolved (GC)(µg/g)	141[b]	444[b]	14.8	17.5	1,000
Total Saturates (grav. wt.)(µg/g)	970[b]	810[b]	95.0	206	4,220
ALK/ISO	3.73	3.06	2.54	2.36	2.06
SHWR	1.04	1.02	1.40	1.18	1.82

[a]ND = not detected.
[b]ng/g.

TABLE 3-27

SHORELINE STUDY - SATURATED HYDROCARBONS (SITE LT-1)

	DAY				
	1	2	4	8	16
Sample ID No.	GC-21	GC-22	GC-23	GC-24	None
Lab ID No.	06-1067	06-1082	06-1068	06-1084	
Constituent (µg/g)					
$n\text{-}C_{10}$	17.1	23.3	4.3	8.9	
$n\text{-}C_{11}$	28.3	60.8	6.7	36.7	
$n\text{-}C_{12}$	33.5	82.3	7.6	58.2	
$n\text{-}C_{13}$	33.8	86.1	7.5	64.6	
$n\text{-}C_{14}$	34.4	84.2	7.4	68.6	
Farnesane	13.6	28.7	3.0	27.5	
$n\text{-}C_{15}$	33.0	81.4	7.2	68.1	
$n\text{-}C_{16}$	29.8	68.2	6.2	59.9	
$n\text{-}C_{17}$	26.8	76.1	5.6	53.7	
Pristane	11.9	24.8	2.5	23.5	
$n\text{-}C_{18}$	22.7	58.8	4.5	43.9	
Phytane	13.0	34.4	2.8	27.8	
$n\text{-}C_{19}$	22.9	41.3	4.7	33.6	
$n\text{-}C_{20}$	19.5	48.9	4.1	37.9	
$n\text{-}C_{21}$	16.3	41.7	3.4	31.1	
$n\text{-}C_{22}$	14.8	36.9	3.7	27.4	
$n\text{-}C_{23}$	12.5	30.6	2.6	23.1	
$n\text{-}C_{24}$	11.0	26.9	2.3	20.3	
$n\text{-}C_{25}$	9.4	21.5	1.9	19.9	
$n\text{-}C_{26}$	8.1	17.8	1.6	16.7	
$n\text{-}C_{27}$	7.5	14.0	1.4	15.6	
$n\text{-}C_{28}$	6.7	11.6	1.2	13.2	
$n\text{-}C_{29}$	6.6	10.9	1.0	10.9	
$n\text{-}C_{30}$	6.5	7.9	0.91	10.5	
$n\text{-}C_{31}$	5.7	5.2	0.74	8.9	
$n\text{-}C_{32}$	4.2	4.2	0.48	6.3	

TABLE 3-27 (Cont.)

	DAY				
	1	2	4	8	16
Total Alkanes (GC)(µg/g)	411	930	87.2	738	None
Total Resolved (GC)(µg/g)	696	1,350	150	1,550	
Total Saturates (grav. wt.)(µg/g)	19,000	17,300	5,170	10,200	
ALK/ISO	2.43	2.57	2.45	2.96	
SHWR	2.35	2.30	2.42	2.25	

TABLE 3-28

SHORELINE STUDY - SATURATED HYDROCARBONS (SITE LT-2)

	DAY				
	1	2	4	8	16
Sample ID No.	GC-26	GC-27	GC-28	GC-29	None
Lab ID No.	06-1085	06-1086	06-1087	06-1088	
Constituent (µg/g)					
$n\text{-}C_{10}$	9.9	13.7	3.6	47.7	
$n\text{-}C_{11}$	55.6	56.9	17.9	127	
$n\text{-}C_{12}$	114	104	36.4	169	
$n\text{-}C_{13}$	141	119	47.8	199	
$n\text{-}C_{14}$	140	127	41.5	204	
Farnesane	60.9	46.4	17.89	65.9	
$n\text{-}C_{15}$	140	119	43.0	200	
$n\text{-}C_{16}$	139	113	56.5	188	
$n\text{-}C_{17}$	124	126	52.9	172	
Pristane	57.3	48.1	21.8	76.9	
$n\text{-}C_{18}$	115	97.2	47.3	165	
Phytane	61.8	153.7	28.0	91.6	
$n\text{-}C_{19}$	119	67.7	48.8	164	
$n\text{-}C_{20}$	95.1	74.5	39.8	135	
$n\text{-}C_{21}$	86.0	61.7	34.4	121	
$n\text{-}C_{22}$	77.0	49.6	29.3	105	
$n\text{-}C_{23}$	76.2	39.8	25.7	90.6	
$n\text{-}C_{24}$	60.4	33.3	22.9	81.8	
$n\text{-}C_{25}$	52.6	33.4	18.4	68.8	
$n\text{-}C_{26}$	48.3	23.7	16.5	70.3	
$n\text{-}C_{27}$	45.1	22.0	13.5	59.9	
$n\text{-}C_{28}$	42.6	18.5	10.1	52.6	
$n\text{-}C_{29}$	35.0	15.0	8.99	48.9	
$n\text{-}C_{30}$	33.7	14.7	8.97	38.2	
$n\text{-}C_{31}$	35.2	10.6	4.97	23.9	
$n\text{-}C_{32}$	25.9	9.21	4.89	19.9	

TABLE 3-28 (Cont.)

	DAY				
	1	2	4	8	16
Total Alkanes (GC)(µg/g)	1,800	1,330	655	1,450	None
Total Resolved (GC)(µg/g)	2,710	4,380	961	3,920	
Total Saturates (grav. wt.)(µg/g)	11,300	11,900	4,400	20,900	
ALK/ISO	2.58	2.45	2.08	2.29	
SHWR	1.93	2.12	1.79	2.03	

TABLE 3-29

SHORELINE STUDY - SATURATED HYDROCARBONS (SITE HT-1)

	DAY			
	1	2	4	8
Sample ID No.	GC-40	GC-42	GC-44	GC-46
Lab ID No.	06-1089	06-1091	06-1093	06-1044
Constituent (µg/g)				
$n\text{-}C_{10}$	4.1	5.0	2.4	26.9
$n\text{-}C_{11}$	18.6	16.7	9.5	78.6
$n\text{-}C_{12}$	30.1	26.5	17.2	122
$n\text{-}C_{13}$	33.0	29.3	20.8	135
$n\text{-}C_{14}$	33.8	29.3	21.4	130
Farnesane	13.4	10.0	7.6	53.1
$n\text{-}C_{15}$	32.8	28.2	21.6	137
$n\text{-}C_{16}$	28.9	24.0	19.4	121
$n\text{-}C_{17}$	25.3	21.5	18.3	112
Pristane	7.1	9.3	8.1	39.9
$n\text{-}C_{18}$	21.3	19.6	16.2	100
Phytane	13.0	10.7	9.5	56.8
$n\text{-}C_{19}$	15.1	19.7	15.7	93.8
$n\text{-}C_{20}$	18.6	15.0	13.4	75.8
$n\text{-}C_{21}$	16.0	13.6	11.9	62.6
$n\text{-}C_{22}$	16.5	11.9	10.4	54.2
$n\text{-}C_{23}$	12.1	9.9	9.0	44.2
$n\text{-}C_{24}$	11.0	8.6	8.0	37.4
$n\text{-}C_{25}$	11.5	7.9	6.3	31.8
$n\text{-}C_{26}$	7.8	6.0	5.3	26.6
$n\text{-}C_{27}$	7.3	4.9	4.1	24.2
$n\text{-}C_{28}$	5.5	3.8	3.6	18.3
$n\text{-}C_{29}$	4.1	3.1	3.2	16.0
$n\text{-}C_{30}$	3.8	2.1	1.9	11.1
$n\text{-}C_{31}$	2.9	2.1	1.9	7.4
$n\text{-}C_{32}$	1.8	1.3	1.1	6.8

TABLE 3-29 (Cont.)

	DAY			
	1	2	4	8
Total Alkanes (GC)(µg/g)	362	310	242	1,460
Total Resolved (GC)(µg/g)	623	490	403	2,270
Total Saturates (grav. wt.)(µg/g)	3,990	4,500	5,030	12,000
ALK/ISO	2.63	2.60	2.52	2.84
SHWR	2.23	2.24	2.18	2.21

TABLE 3-30

SHORELINE STUDY - SATURATED HYDROCARBONS (SITE HT-2)

	DAY				
	1	2	4	8	16
Sample ID No.	GC-41	GC-43	GC-45	GC-47	GC-49
Lab ID No.	06-1090	06-1092	06-1105	06-1095	06-1096
Constituent (µg/g)					
$n\text{-}C_{10}$	46.2	2.65	4.0	8.10	6.96
$n\text{-}C_{11}$	139	18.9	67.2	32.0	29.3
$n\text{-}C_{12}$	215	45.0	77.2	62.9	49.8
$n\text{-}C_{13}$	219	63.0	73.2	69.9	69.5
$n\text{-}C_{14}$	215	69.8	68.8	81.0	73.9
Farnesane	90.0	20.5	28.4	35.9	31.0
$n\text{-}C_{15}$	205	70.0	63.6	89.8	75.2
$n\text{-}C_{16}$	198	62.3	63.2	78.4	68.2
$n\text{-}C_{17}$	180	56.6	57.2	70.4	63.2
Pristane	78.5	25.4	24.8	32.5	28.4
$n\text{-}C_{18}$	165	50.9	51.2	63.3	52.8
Phytane	91.1	30.0	26.4	36.3	31.2
$n\text{-}C_{19}$	166	51.4	52.4	54.0	36.6
$n\text{-}C_{20}$	131	41.5	42.4	49.2	43.8
$n\text{-}C_{21}$	114	36.0	36.8	43.8	37.3
$n\text{-}C_{22}$	100	30.7	31.2	36.6	32.5
$n\text{-}C_{23}$	87.1	26.0	26.0	31.2	26.5
$n\text{-}C_{24}$	78.4	24.4	24.0	25.1	22.8
$n\text{-}C_{25}$	80.7	20.7	22.4	22.3	18.2
$n\text{-}C_{26}$	56.5	18.2	18.4	15.3	15.7
$n\text{-}C_{27}$	52.3	15.2	18.4	11.9	10.5
$n\text{-}C_{28}$	41.1	13.9	14.8	11.4	7.53
$n\text{-}C_{29}$	36.6	13.6	15.6	8.75	6.88
$n\text{-}C_{30}$	30.0	13.0	14.4	6.0	5.65
$n\text{-}C_{31}$	21.0	6.56	12.4	4.0	4.12
$n\text{-}C_{32}$	21.7	7.18	9.2	1.6	2.80

TABLE 3-30 (Cont.)

	DAY				
	1	2	4	8	16
Total Alkanes (GC)(µg/g)	2,600	763	864	877	760
Total Resolved (GC)(µg/g)	3,970	1,110	1,350	1,337	1,260
Total Saturates (grav. wt.)(µg/g)	18,300	5,790	13,700	9,120	7,880
ALK/ISO	2.57	2.80	2.98	2.64	2.38
SHWR	2.12	1.96	2.31	2.07	2.12

(C_{10}-C_{32}) and three key isoprenoids (branched alkanes) farnesane, pristane, and phytane. The alkanes are summed and presented in relation to the entire suite of resolved (GC^2) saturates. The total saturates (= resolved plus unresolved complex mixture plus non-chromatographables) was determined by microgravimetry. Two key ratios, the ALK/ISO (alkanes from n-C_{14} through n-C_{18} ÷ five key isoprenoids in this boiling range including farnesane, pristane phytane and two others), and the SHWR - saturated hydrocarbon weathering ratio:

$$\text{SHWR} = \frac{\text{Sum of alkanes from n-}C_{10}\text{ to n-}C_{25}}{\text{Sum of alkanes from n-}C_{17}\text{ to n-}C_{25}}$$

are calculated. The ALK/ISO is sensitive to biodegradation as alkanes are preferentially biodegraded (Boehm et al., 1981a; Boehm et al., 1981b; Atlas et al., 1981). The SHWR approaches unity as the lighter components are lost due mainly to evaporation and some dissolution (Boehm and Fiest, 1981a).

The ALK/ISO and SHWR values in the "fresh" and "aged" Lagomedio crude oils are:

	ALK/ISO	SHWR
Fresh	2.36	2.87
Aged	2.50	2.28

The graphic results (Figures 3-36 to 3-43) are derived from the tables and present compositional information relative to n-C_{24}, which is asssumed to be unaffected by weathering processes. The compositional information can be compared to the fresh oil in the figures. Perhaps it would be more appropriate to compare the results to the "aged" oil but, as discussed previously (Section One), the "aged" oil's composition is more weathered than most of the residues in the test

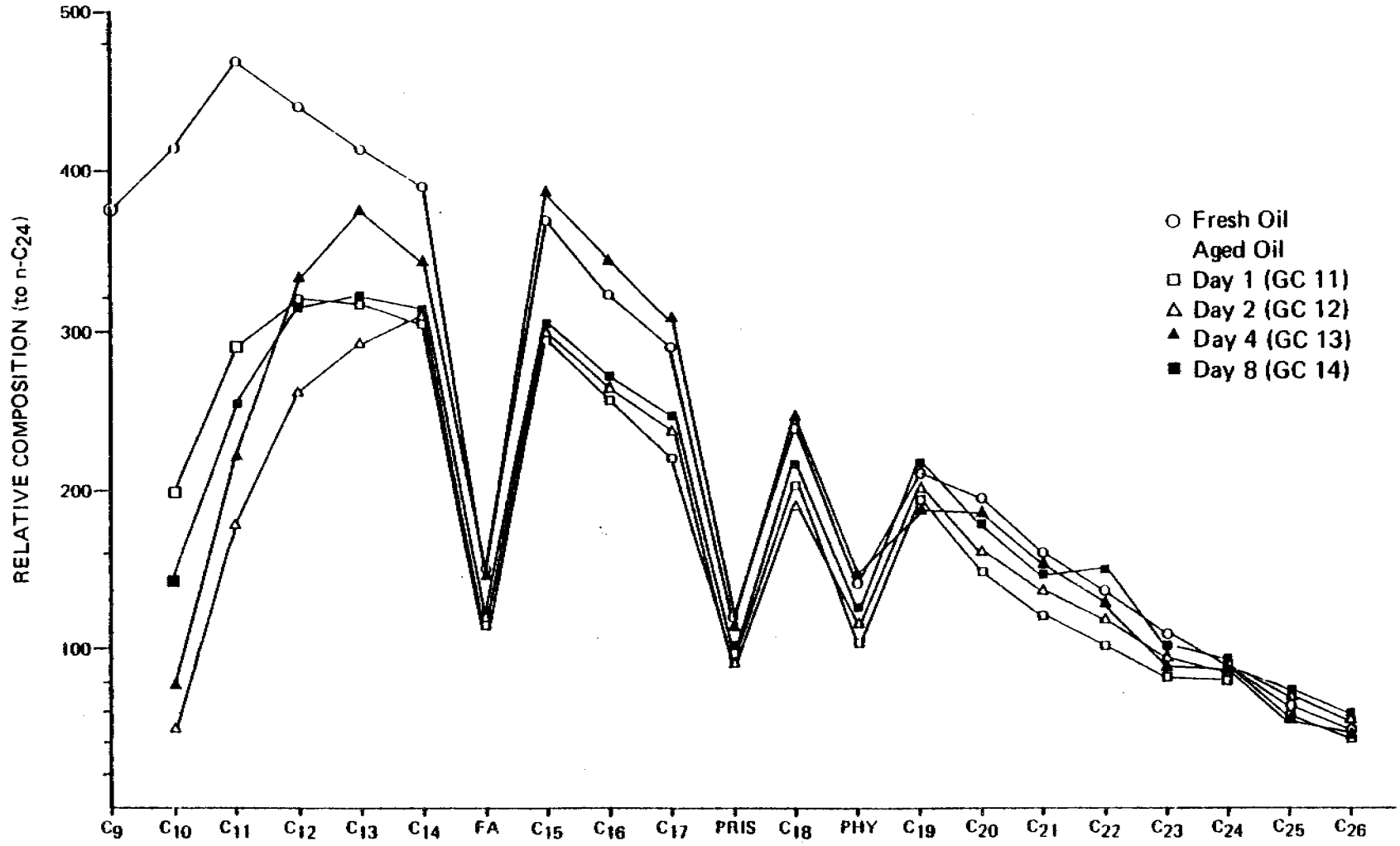


Figure 3.36. Comparative Saturated Hydrocarbon Composition of Lagomedio Crude-Shoreline Experiment, Site L–1.

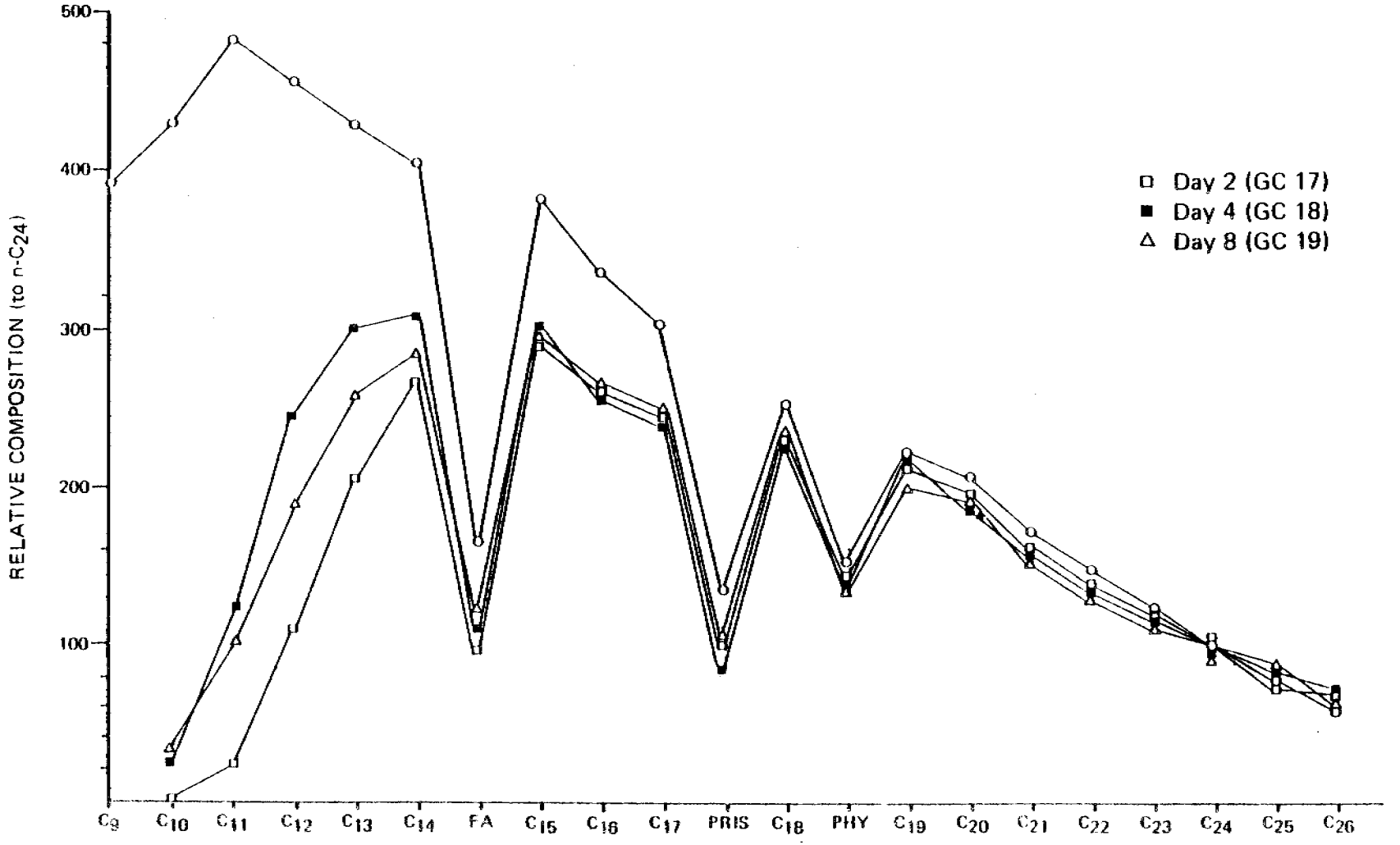


Figure 3.37. Comparative Saturated Hydrocarbon Composition of Lagomedio Crude Shoreline Experiment, Site [illegible].

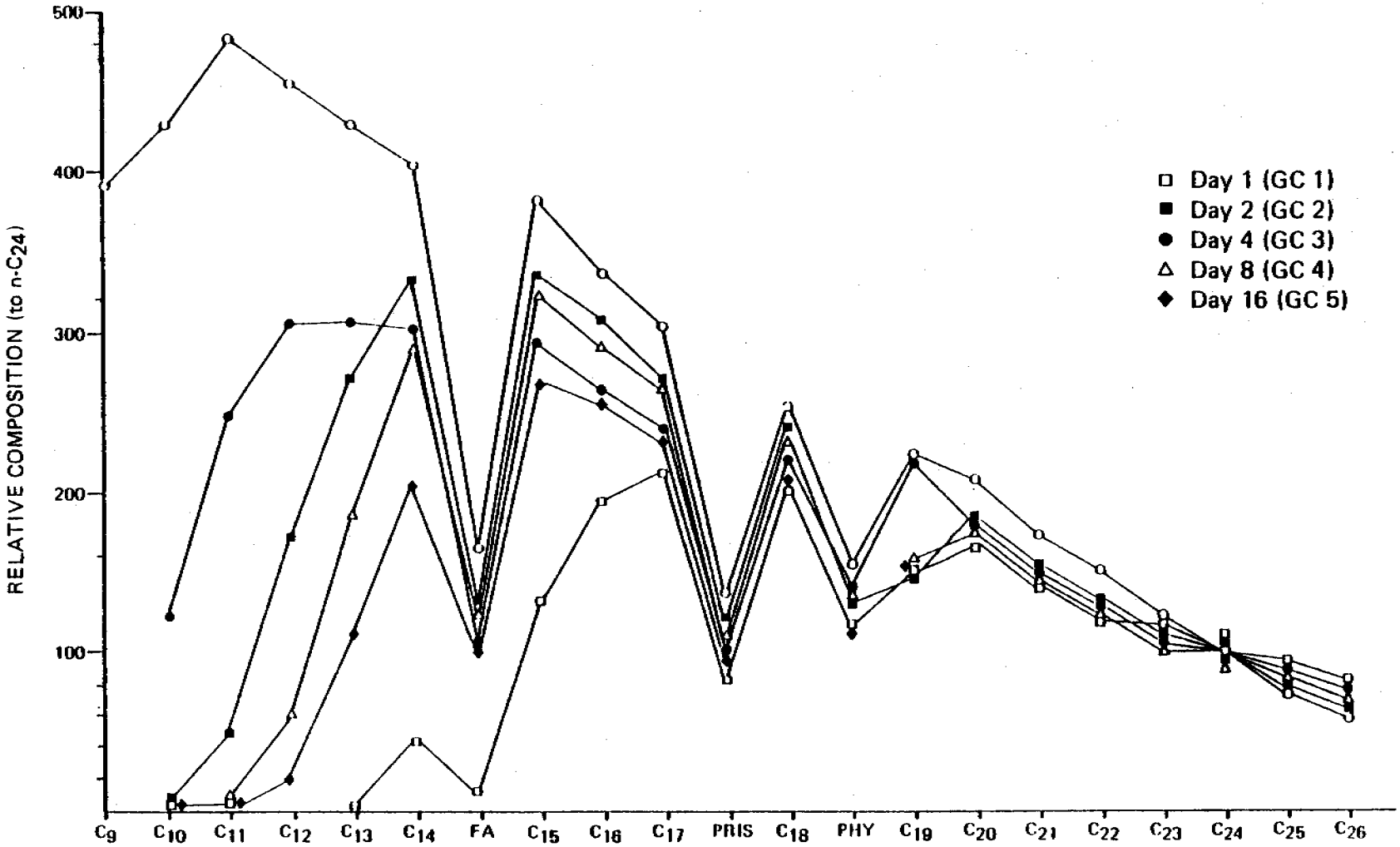


Figure 3.38. Comparative Saturated Hydrocarbon Composition of Lagomedio Crude-Shoreline Experiment, Site H–1.

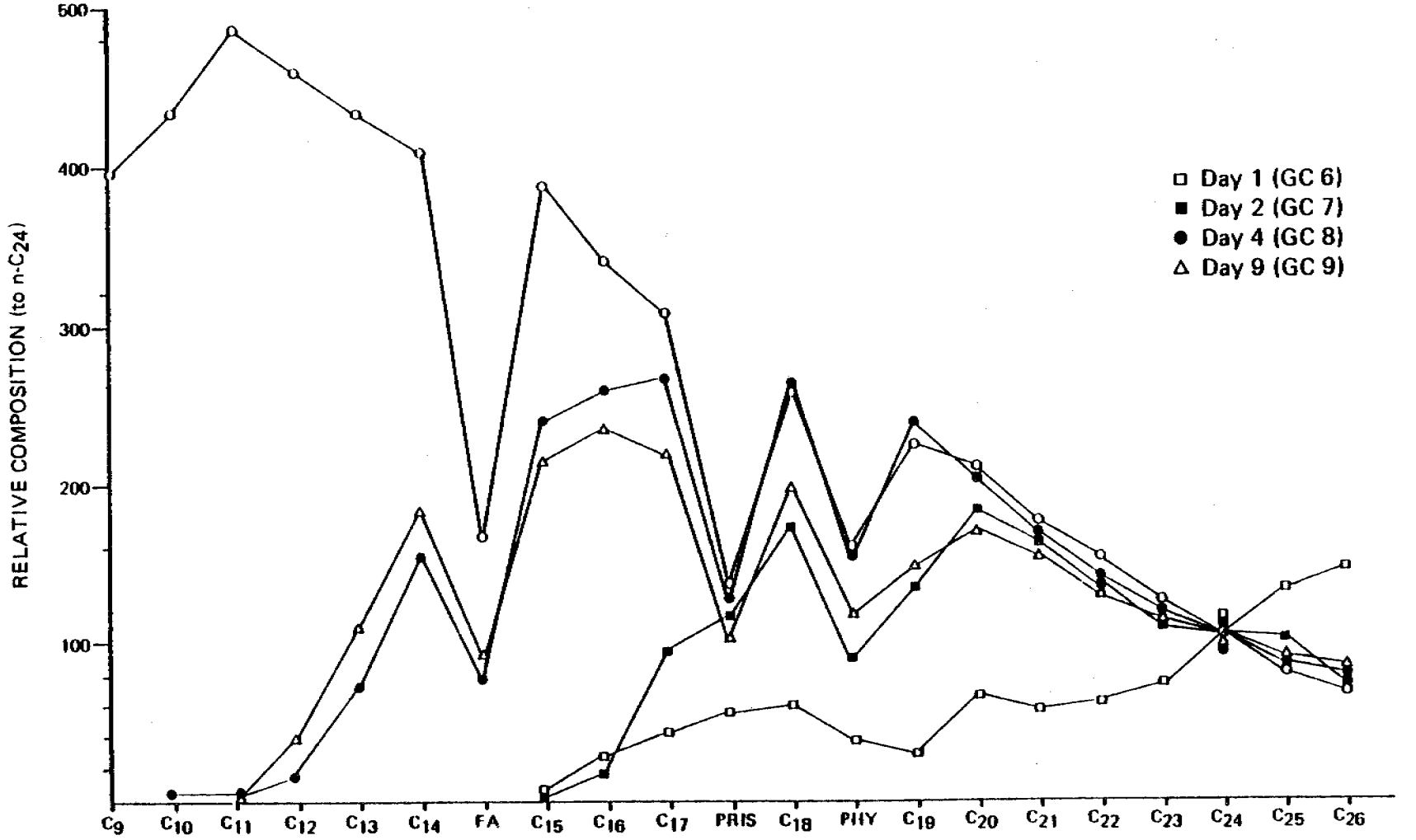


Figure 3.39. Comparative Saturated Hydrocarbon Composition of Lagomedio Crude-Shoreline Experiment, Site H--2.

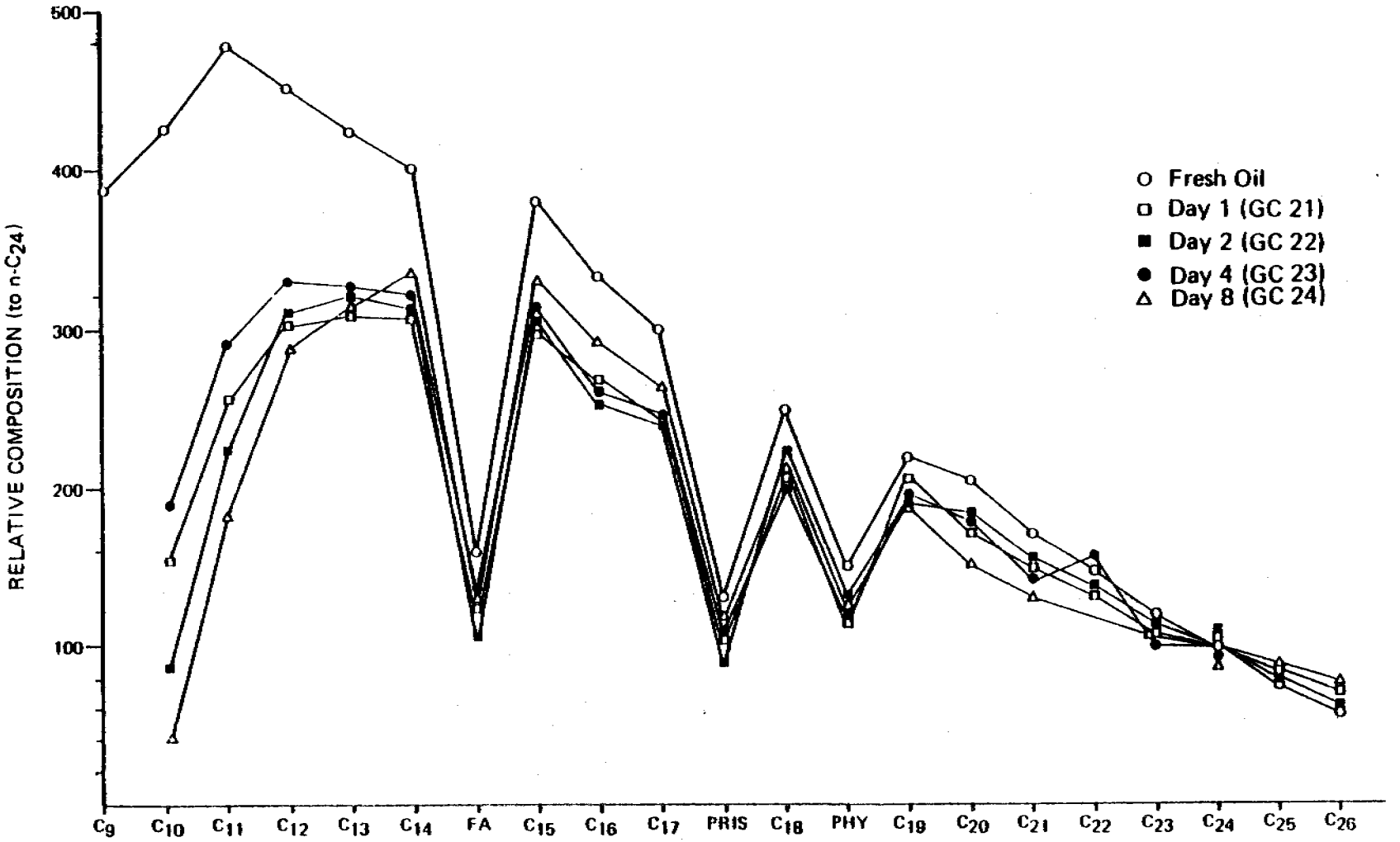


Figure 3.40. Comparative Saturated Hydrocarbon Composition of Lagomedio Crude-Shoreline Experiment, Site LT–1.

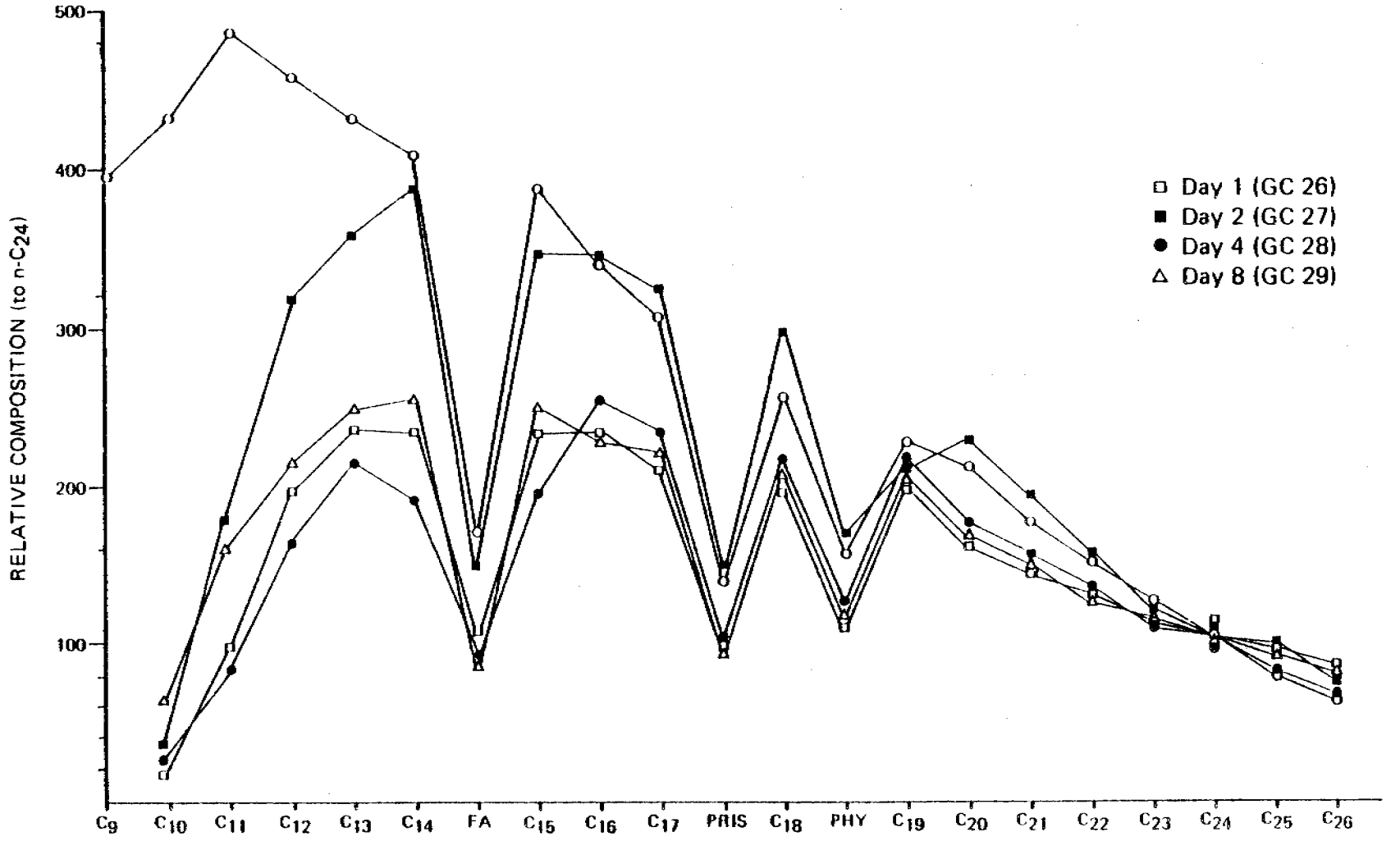


Figure 3.41. Comparative Saturated Hydrocarbon Composition of Lagomedio Crude-Shoreline Experiment, Site LT–2.

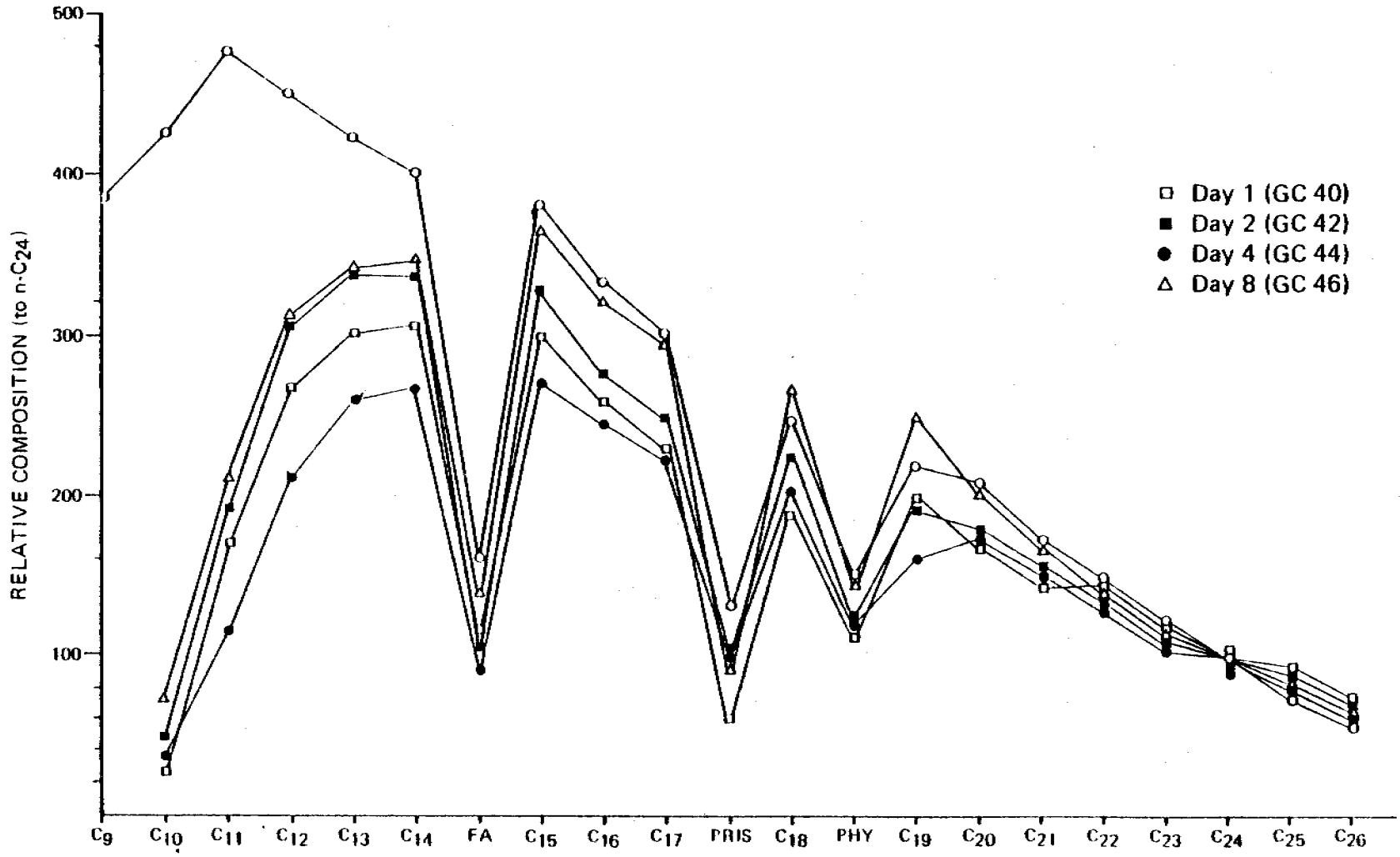


Figure 3.42. Comparative Saturated Hydrocarbon Composition of Lagomedio Crude-Shoreline Experiment, Site HT–1.

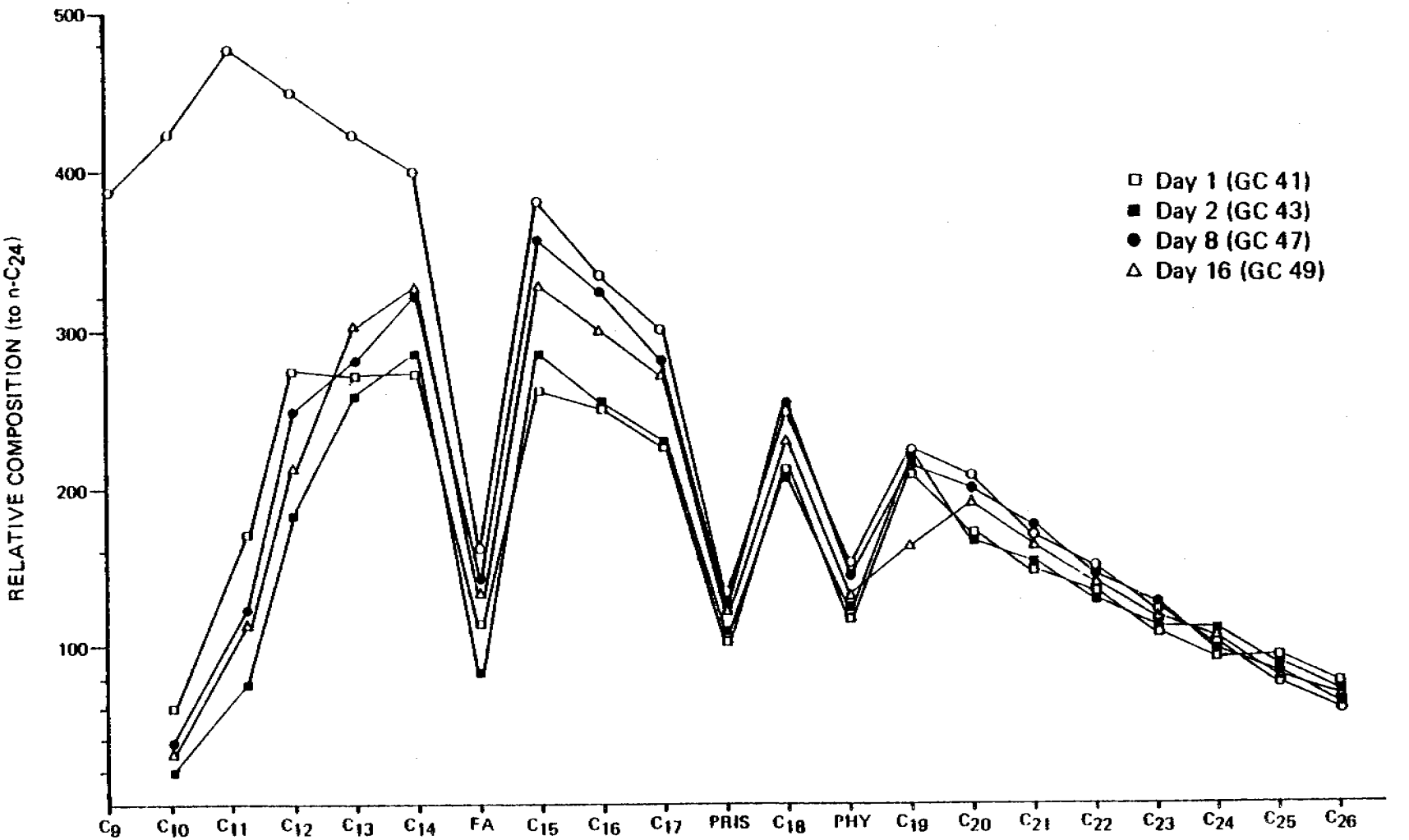


Figure 3.43. Comparative Saturated Hydrocarbon Composition of Lagomedio Crude-Shoreline Experiment, Site HT–2.

plots. Note, however, that we did not analyze oil from the field prior to application. Our "aged" oil was supplied by the project office as sampled from the railroad car (aging chamber) and thus must differ significantly from that used in the field. We suspect that the test oil actually used in the field was intermediate in composition between the fresh and "aged" oil supplied by the project office.

The compositional plots indicate much non-predicted behavior with the "older" residues (4 to 16 days) often "fresher" or less weathered than the early samples. However, it is probable that compositional heterogeneity occurs in the test plots and it is certain that most of the 16 days of weathering occurred within several (1-2) days in all plots other than perhaps Site H-1. Note how the samples containing much lower concentrations (e.g., site H-1 day 1; H-2 days 1 and 2) are more highly weathered. This is probably due to gross removal of oil by waves leaving the remaining low level oil residues more highly leached. Subsequently, fresher oil is redeposited.

The results from one site, H-1, illustrate that evaporative weathering does proceed during the entire 16 days although the compositional situation during days 1 through 4 appears highly variable. Thereafter (days 8 and 16), weathering proceeds steadily (SHWR = 2.26, day 4; 1.81, day 8; 1.63, day 16).

3.3.3 Aromatic Hydrocarbons (GC/MS)

The samples from two test plots, L-1 and LT-1, formed a subset of shoreline plots which were analyzed by GC^2/MS to determine the detailed aromatic hydrocarbon compositions and,

hence, weathering patterns. The analytical results are summarized in Table 3-31. Four families of aromatic hydrocarbon compounds and one organo-sulfur family were focused on: naphthalenes and alkyl naphthalenes; alkyl benzenes; fluorenes; phenanthrenes and alkyl phenanthrenes; dibenzothiophanes and alkyldibenzothiophenes. The total polynuclear aromatics (PAH) represent the sum of the compounds of interest. The AWR or aromatic weathering ratio is similar in concept to the SHWR (Section 3.3.2)

$$AWR = \Sigma\ (\Sigma AB + \Sigma N + \Sigma F + \Sigma P + \Sigma DBT)/\ \Sigma\ (\Sigma P + \Sigma DBT)$$

and approaches unity as the more volatile, soluble compounds (N, AB, F) are weathered. The AWR values in fresh and aged Lagomedio oil are 4.29 and 3.47, respectively.

The values in Table 3-31 parallel the trends previously noted. For the L-1 test plots weathering of the aromatic fraction is complete by the first day after application. This is supportive of the saturated hydrocarbon data (see Section 3.3.2). The LT-1 plot results also parallel the saturated hydrocarbon trends. Figures 3-44 and 3-45 graphically illustrate the comparison of the "aged" oil to the "fresh" oil and to the test samples as well. In these plots the aromatics are normalized to trimethyl (C_3) dibenzothiophene. Most of the compositional change is seen in the lighter compounds (i.e., alkyl benzenes and, to a lesser extent, naphthalenes).

3.3.4 Azaarenes (GC^2/MS)

Detailed analyses of three families of prominent nitrogen heterocyclics (azaarenes) in the Lagomedio Crude

TABLE 3-31

SHORELINE STUDY - GC/MS DATA SUMMARY
OF AROMATIC HYDROCARBON RESULTS

	Site L-1			Site LT-1			
	Day 1 GC-11 (µg/g)	Day 2 GC-12 (µg/g)	Day 8 GC-14 (µg/g)	Day 1 GC-21 (µg/g)	Day 2 GC-22 (µg/g)	Day 4 GC-23 (µg/g)	Day 8 GC-24 (µg/g)
N	1.2	1.2	2.9	13.0	6.8	2.4	6.9
C_1N	7.1	7.6	15.7	66.0	44.2	12.0	43.4
C_2N	17.7	17.7	38.0	139.0	102.2	28.1	106.0
C_3N	14.7	16.3	30.0	114.0	87.2	22.6	90.5
C_4N	7.7	7.9	15.9	694.0	54.0	13.3	48.3
ΣN	48.4	50.7	102.5	426.0	294.4	78.4	295.1
C_3AB	3.7	3.2	4.1	99.6	21.8	7.3	19.0
C_4AB	6.4	5.7	4.2	86.6	35.6	11.4	33.3
C_5AB	5.3	5.1	3.4	61.0	31.8	9.9	32.2
ΣAB	15.4	14.0	11.7	246.2	89.2	28.6	84.5
BP	0.6	0.6	1.2	4.6	3.2	-	3.2
F	0.4	0.4	0.6	3.2	2.4	0.6	2.2
C_1F	1.2	1.3	2.4	8.2	6.2	1.7	6.6
C_2F	1.8	1.9	3.3	16.6	11.2	3.1	12.3
C_3F	2.4	2.5	4.4	21.0	15.4	3.8	13.4
ΣF	5.8	6.1	10.7	49.0	35.2	9.2	34.5
P	0.8	0.9	1.8	7.6	4.8	1.4	5.0
C_1P	4.1	3.8	7.2	32.0	18.9	5.1	22.5
C_2P	5.0	5.2	8.6	40.0	25.4	6.6	28.2
C_3P	3.5	3.1	6.0	37.8	22.0	5.8	21.7
C_4P	2.8	2.4	2.1	22.6	13.2	3.4	18.1
ΣP	16.2	15.4	25.7	140.0	84.3	22.3	95.5

TABLE 3-31 (Cont.)

	Site L-1			Site LT-1			
	Day 1 GC-11 (µg/g)	Day 2 GC-12 (µg/g)	Day 8 GC-14 (µg/g)	Day 1 GC-21 (µg/g)	Day 2 GC-22 (µg/g)	Day 4 GC-23 (µg/g)	Day 8 GC-24 (µg/g)
DBT	1.0	1.1	2.0	8.4	5.6	1.4	5.2
C_1DBT	3.8	3.7	6.9	30.8	20.4	5.2	20.2
C_2DBT	6.9	7.2	13.7	62.2	41.4	9.9	38.9
C_3DBT	5.6	5.6	10.0	50.8	35.2	7.6	37.5
ΣDBT	17.3	17.6	32.6	152.2	102.6	24.1	101.8
Total PAH (µg/g)	103.7	104.4	184.4	1,018	608.9	162.6	614.6
Total Aromatics (Grav.) (µg/g)	1,970	1,971	3,890	19,900	17,300	5,170	10,200
AWR	3.08	3.15	3.14	3.46	3.24	3.50	3.10

N = naphthalenes
AB = alkyl benzenes
BP = biphenyl
F = Fluorenes
P = phenanthrenes
DBT = dibenzothiophenes
C_1,C_2,C_3,C_4,C_5 = mono-, di-, tri-, tetra- and penta-methyl homologues.

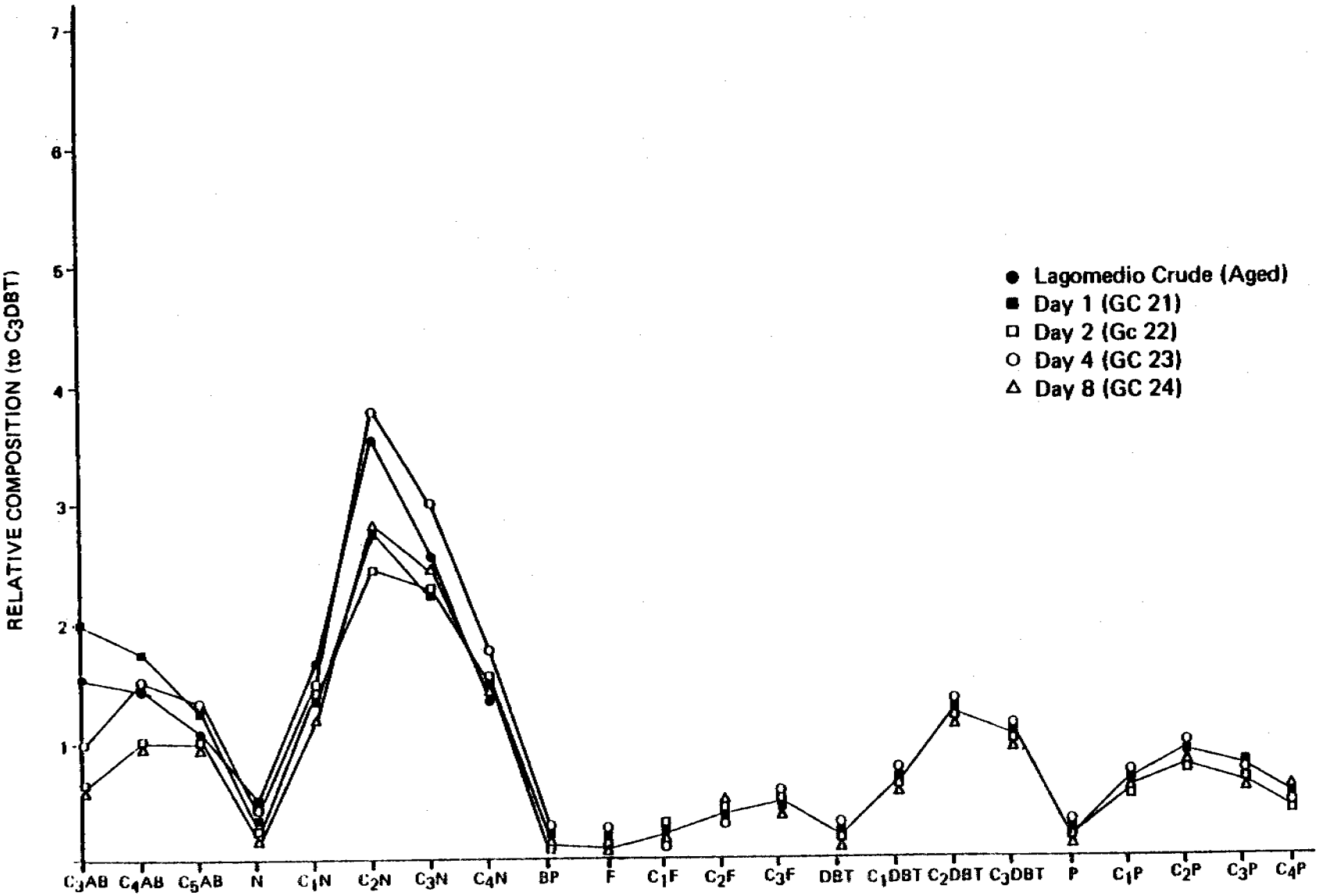


Figure 3.44. Comparative Aromatic Hydrocarbon Compositions, Site L–1: Aged Oil.

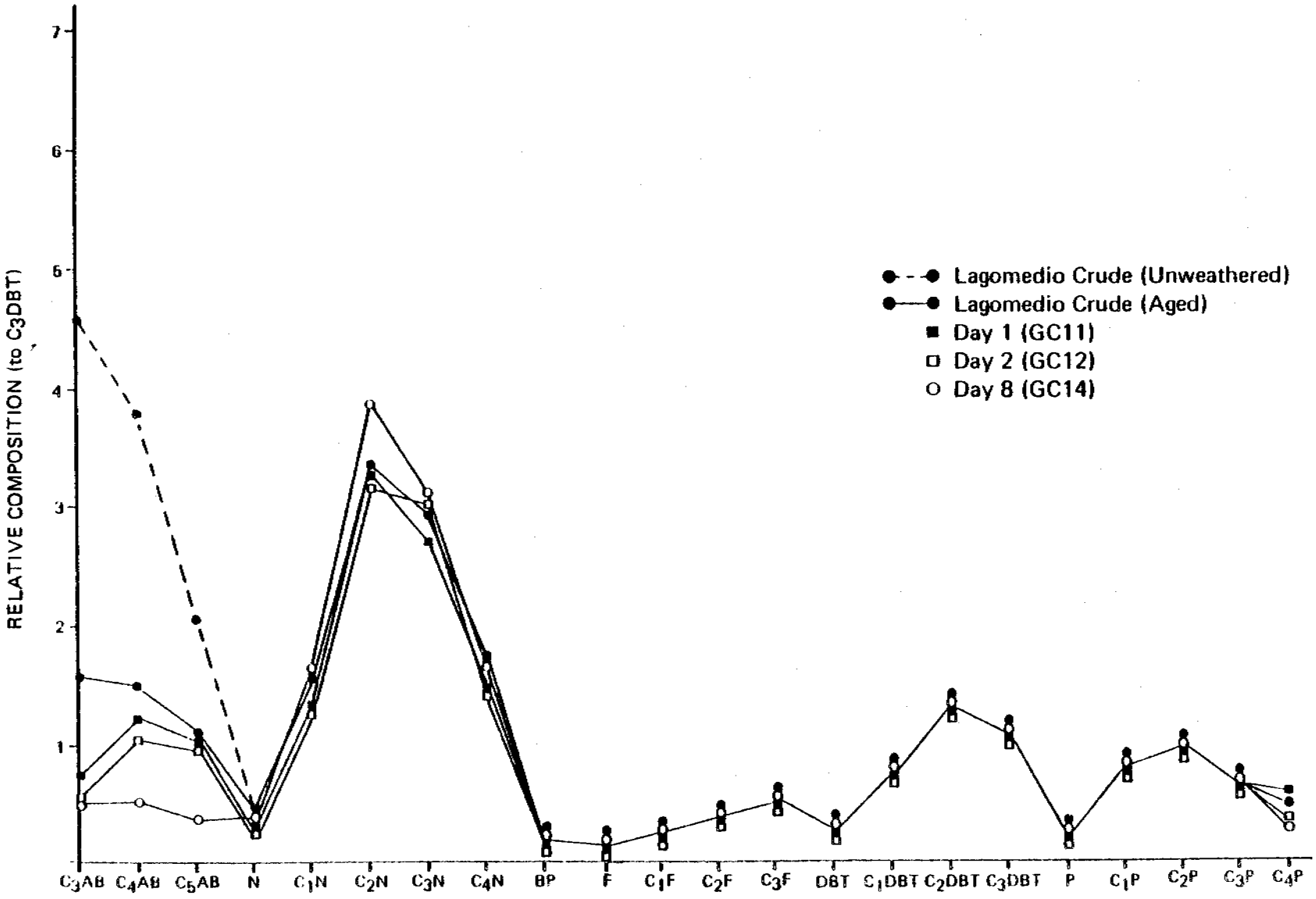


Figure 3.45. Comparative Aromatic Hydrocarbon Compositions, Site LT-1: Aged Oil

were performed on a set of samples (Table 3-32) from the oiled test plots. The compositions of the quinoline, acridine/phenanthridene families remained invariant throughout the 8 days of sampling. A substantial, unique data base on the range (however narrow) of azaarene compositions has been amassed which will be extremely important for the use of these compounds as markers for this oil.

Figure 3-46 compares the azaarene composition in the test oils with that in the samples confirming the narrow range of variation of these compounds. Differences between fresh and aged Lagomedio are also small. Significant variations do occur in the benzacridine samples. The low relative amounts of these compounds (note scale expansion) probably account for the noticeable variability.

3.3.5 Pentacyclic Triterpanes (GC^2)

Pentacyclic triterpane hydrocarbons (PT) were the subject of a GC^2/MS analytical program involving nine heavily oiled shoreline samples. The objective was to examine post-spill PT compositional changes to document weathering-induced changes, if any. As shown in Table 3-33, the PT compounds identified in the oil itself are present in the oiled samples throughout the time period studied in nearly the same ratios to each other. However, the compounds are present in very low levels and are often barely detectable above instrumental noise. The PT fingerprint is certainly less clear than that revealed by other oils in spill situations (e.g., Amoco Cadiz; Atlas et al., 1981) (Figure 3-47) and hence, although the weak Lagomedio PT fingerprint persists, it is doubtful whether it will be useful as a long-term marker, especially in view of the background PT fingerprint (see Section 3.2.5).

TABLE 3-32

AZAARENES IN OIL RESIDUES FROM SHORELINE TEST PLOTS - RELATIVE CONCENTRATIONS[a]

	SITE L-1		
	DAY 1 GC-11	DAY 2 GC-12	DAY 8 GC-14
M/e			
129 Q	0.8	0.7	--
143 C_1Q	2.2	1.6	1
157 C_2Q	1.9	2.9	1.4
171 C_3Q	7.8	10	15
185 C_4Q	25	31	41
199 C_5Q	21	25	32
213 C_6Q	29	30	30
179A	0.3	0.2	0.5
193 C_1A	8	11	11
207 C_2A	55	51	71
221 C_3A	100	100	100
235 C_4A	54	63	39
249 C_5A	18	22	10
229 BA	0.2	0.3	0.1
243 C_1BA	3	3	1
257 C_2Ba	4	4	2

[a] = Concentrations normalized to C_3A

A = Acridines/phenanthridines
Q = Quinolines
BA = Benzacridines
C_n = Alkyl homologues with n-Carbon atoms

TABLE 3-32 (Cont.)

	SITE LT-1			
	DAY 1 GC-21	DAY 2 GC-22	DAY 4 GC-23	DAY 8 GC-24
M/e				
129 Q	4	3	.3	0.9
143 C_1Q	6	5	3	1.7
157 C_2Q	5	4	1	2.5
171 C_3Q	15	11	8	11
185 C_4Q	43	33	26	32
199 C_5Q	31	27	23	28
213 C_6Q	30	29	25	28
179A	0.4	0.2	0.3	0.3
193 C_1A	14	10	8	11
207 C_2A	69	58	49	52
221 C_3A	100	100	100	100
235 C_4A	41	53	51	59
249 C_5A	12	19	20	21
229 BA	0.2	0.2	0.3	0.2
243 C_1BA	1	2.4	3	2.5
257 C_2Ba	1	3.0	5	3.4

[a] = Concentrations normalized to C_3A

A = Acridines/phenanthridines
Q = Quinolines
BA = Benzacridines
C_n = Alkyl homologues with n-Carbon atoms

TABLE 3-32 (Cont.)

	Site H-1				
	Day 1 GC-1	Day 2 GC-2	Day 4 GC-3	Day 8 GC-4	Day 16 GC-5
M/e					
129 Q	0.5	0.3	0.3	0.6	1.0
143 C_1Q	1.1	0.7	1.4	2.3	2.6
157 C_2Q	1.8	2	1	1.5	1.5
171 C_3Q	8	7	8	10	9.5
185 C_4Q	25	22	27	32	30
199 C_5Q	19	16	24	22	23
213 C_6Q	28	22	27	28	27
179A	0.3	0.2	0.2	0.3	0.3
193 C_1A	10	9	9	10	9
207 C_2A	49	47	57	55	47
221 C_3A	100	100	100	100	100
235 C_4A	51	47	56	55	51
249 C_5A	18	15	20	20	0.9
229 BA	0.2	0.2	0.3	0.3	0.1
243 C_1BA	2.1	2	1.5	3.2	3.0
257 C_2Ba	4.3	5	3	5.1	4.6

[a] = Concentrations normalized to C_3A

A = Acridines/phenanthridines
Q = Quinolines
BA = Benzacridines
C_n = Alkyl homologues with n-Carbon atoms

TABLE 3-32 (Cont.)

	Site HT-1		
	Day 1 GC-40	Day 2 GC-42	Day 4 GC-44
M/e			
129 Q	0.5	0.3	0.2
143 C_1Q	1.7	0.7	0.7
157 C_2Q	1.7	1.6	1.1
171 C_3Q	9	9	6.5
185 C_4Q	27	25	20
199 C_5Q	24	22	20
213 C_6Q	29	29	29
179A	0.3	0.2	0.1
193 C_1A	9	10	9
207 C_2A	52	53	50
221 C_3A	100	100	100
235 C_4A	58	58	58
249 C_5A	24	24	24
229 BA	0.2	0.3	0.2
243 C_1BA	3.4	3.1	2.9
257 C_2Ba	5.6	5.4	4.6

[a] = Concentrations normalized to C_3A

A = Acridines/phenanthridines
Q = Quinolines
BA = Benzacridines
C_n = Alkyl homologues with n-Carbon atoms

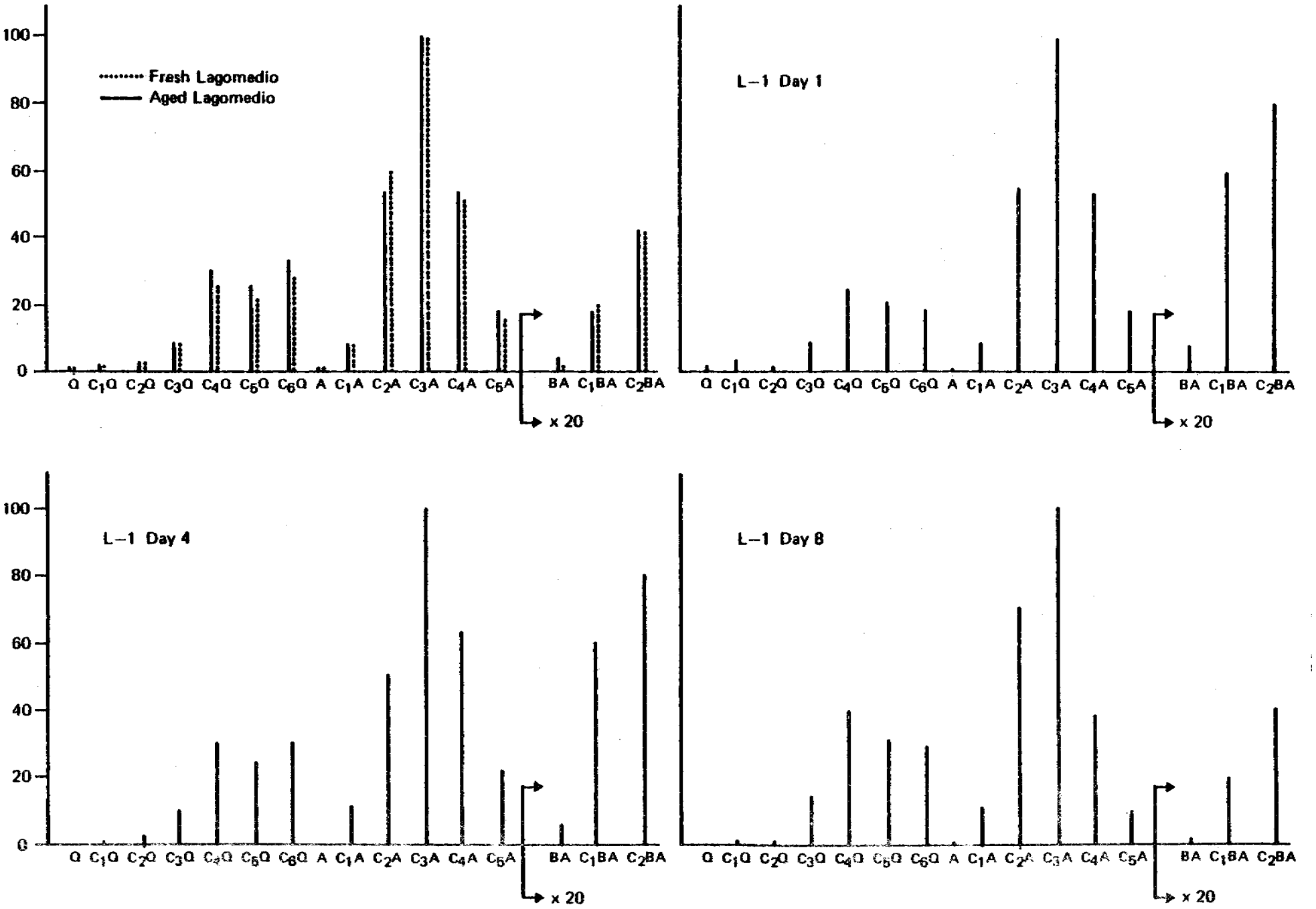


Figure 3.46. Comparative compositional plots of Azaarenes in aged Lagomedio oil and oil residues from Site L–1.

TABLE 3-33

QUALITATIVE GC/MS ANALYSES OF OILED SHORELINE SAMPLES FOR PENTACYCLIC TRITERPANE COMPOUNDS

SITE:	-	L-1	L-1	L-1	LT-1	LT-1	LT-1	LT-1	HT-1	HT-1
DAY:	-	1	2	8	1	2	4	8	1	4
SAMPLE:	AGED OIL	GC-11	GC-12	GC-14	GC-21	GC-22	GC-23	GC-24	GC-40	GC-44
COMPOUND[a]										
A	+	+	+	+	+	+	+	+	+	+
B	+	+	+	+	+	+	+	+	+	+
C	+	+	+	+	+	+	+	+	+	+
D	+	+	+	+	+	+	+	+	+	+
E	+	+	+	+	+	+	+	+	+	+
E'	+	+	+	+	+	+	+	+	+	+
F	-	-	-	-	-	-	-	-	-	-
F'	-	-	-	-	-	-	-	-	-	-

[a]See Section 3.2.5(b) for explanation of compound identification.

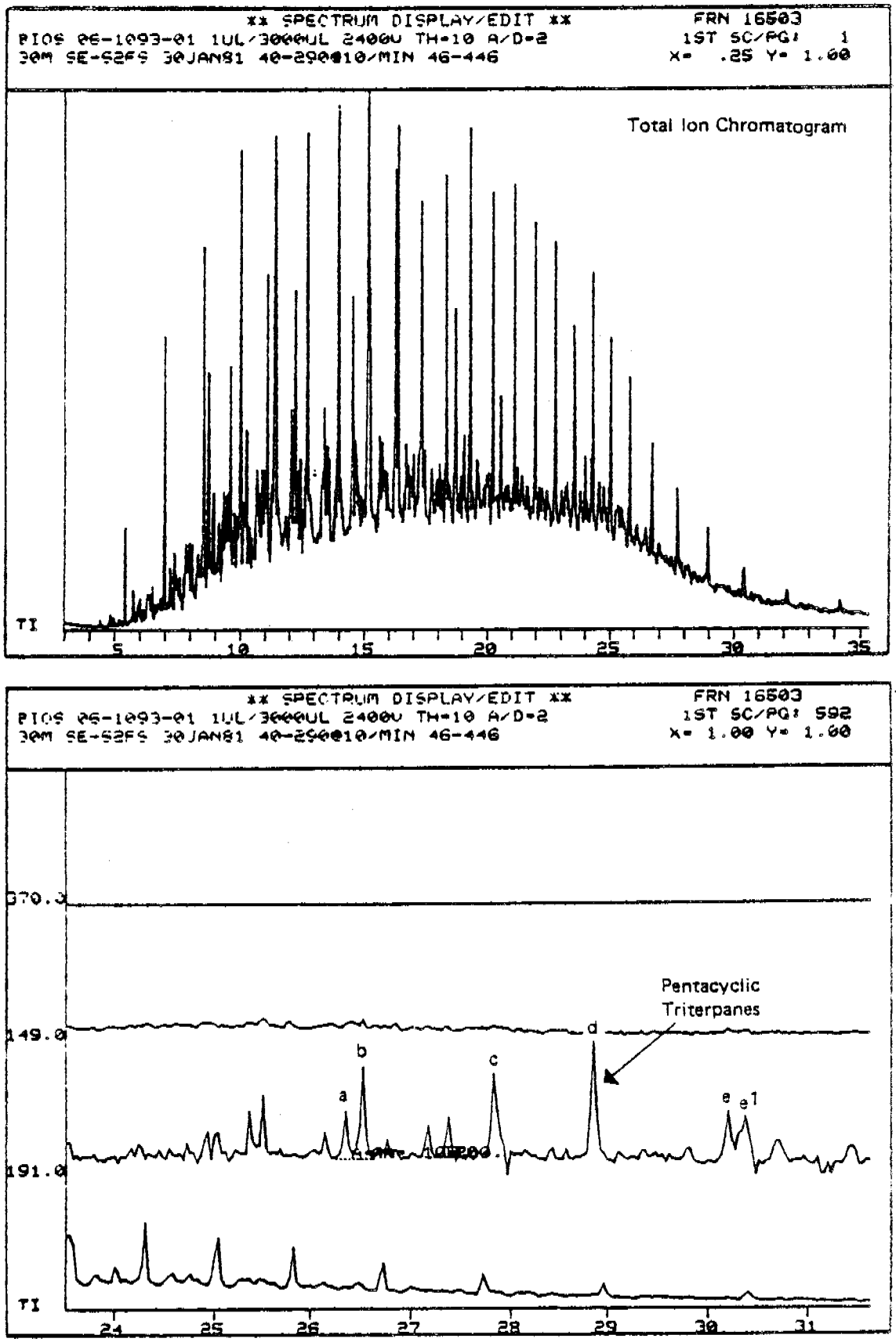


Figure 3.47. GC/MS of Pentacyclic Triterpanes in Oil Sample from shoreline sample.

SECTION FOUR

DISCUSSION

The marine environment of the Cape Hatt, N.W.T., area is comparable to other Arctic environments studied recently (e.g., Wong et al., 1976; Johansen et al., 1977; Shaw et al., 1978) with respect to its pristine nature. This similarity is reflected in the low petroleum hydrocarbon concentrations observed in the seawater, sediment, and tissue samples examined. As analytical methods have improved in recent years, the ability to measure minute levels of pollutant compounds has increased. Consequently, we find low levels (<1 ppb) of polynuclear aromatic hydrocarbons (PAH) and polycyclic aromatic nitrogen compounds (PAN = azaarenes), in both offshore and beach sediment. These levels of PAH and PAN compounds can be ascribed to the global atmospheric transport of a high-temperature combustion (mainly anthropogenic) origin (Lee et al., 1977). The other source for some of these aromatic compounds (e.g., perylene) is through early diagenesis of organic matter deposited in the sediments and preserved in a reducing environment.

Along with PAH and PAN compounds, an array of polycyclic saturated hydrocarbons (PSH = diterpane and triterpane) of an anthropogenic source are also detected in the sediment.

Tissue samples would be expected to be influenced by the deposition of these minute levels of PAH, PAN, and PSH compounds. However, for the most part, the tissue hydrocarbon components are of a biogenic origin. A complex array of biogenic compounds characterizes the hydrocarbon distribution of the species examined. Intraspecies compositional

uniformity was revealed through compositional similarities in GC^2 traces. Low levels of naphthalene and phenanthrene compounds (1-5 ppb) were identified in a set of Mya truncata samples. Thus the uniform levels and the composition of the very low, but detectable levels of petroleum-related aromatics, as opposed to the widely varying absolute levels of biogenic hydrocarbons, create a solid baseline for future oil-spill impact studies.

Seawater samples revealed little indication of petroleum inputs until the large volume water samples (LVWS) (150 to 200 liters) were analyzed. Subpart-per-trillion levels of petrogenic saturates were observed in the particulate hydrocarbons while smaller levels of petroleum-related alkylated naphthalene, phenanthrene and dibenzothiophene were observed by GC^2/MS in the filterable or dissolved fraction. These findings point to four important facets of the study: (1) the LVWS are essential for the scrutiny of background and low-level post-spill water column investigations, (2) the Cape Hatt waters do contain minute levels of weathered petroleum-related material, (3) it is necessary to fractionate the water column into "dissolved" and particulate fractions to reveal the true physical-chemical nature of the hydrocarbon distribution (strongly related to their bioavailability), and (4) the "dissolved" and particulate fractions are decoupled with respect to chemical nature and probable transport mechanisms, thus confirming previous such baseline hydrocarbon measurements (Boehm, 1980).

The results of the study confirm the appropriateness of blending types of analyses to balance informational needs and cost aspects of the study. The low background levels of fluorescing material (i.e., aromatic hydrocarbons) make the UV/F technique extremely useful for screening both post-spill

seawater and sediment samples for the existence of petroleum contamination prior to sample selection for more detailed methods. During the spills UV/F can be used effectively, with appropriate standardization, in a continuous mode to monitor levels in the water column.

In order to examine (1) weathering of oil in shoreline and nearshore spillages, (2) the existence and the chemical nature of petroleum components in the dissolved and particulate forms in the water column, (3) the exposure levels and chemical fractionation of oil in biological samples, and (4) the detailed chemical fate of oil in sediments, GC^2 analysis must parallel or follow UV/F analysis.

Specified chemical marker compounds (PAH, PAN, PSH) must be analyzed by GC^2/MS to accurately identify and quantify components. Analysis for those marker compounds in baseline and post-spill shoreline sediment samples indicates that the most promising markers are the PAH (organo-sulfur and three-ringed alkylated aromatics) and PAN compounds. The pentacyclic triterpanes seem to be both too abundant in offshore sediments and too "unimportant" as components of the oil to be used effectively as post-spill biogeochemical marker compounds. In addition, the PAH and PAN are the most biologically active components with the potential to cause long-term biological effects.

The character of the oil was revealed in great detail in this study and consists of a surprisingly strong suite of azaarenes and an equally surprisingly weak suite of pentacyclic triterpanes. Analysis of the composition of the artifically aged oil compared to the freshest residues (1 day) obtained in the shoreline experiments indicates that

much heterogeneity exists in what is being termed as the test oil. This fact added to the very important determination of non-Newtonian behavior through precipitation of wax from the oil and the 10:1 oil/dispersant mix at 0°C, suggests that close scrutiny of both field oil storage and the application of the oil should occur in order to avoid and/or monitor wax precipitation. Also, samples of oil for chemical analysis from the field should never be replaced by other surrogate sources.

Finally, the first year of oil weathering studies from the Z Lagoon and Eclipse Bay shorelines indicates that a small degree of weathering due to evaporation and dissolution occurs shortly (1 day) after the application but only minimal (but measurable) weathering proceeds further during the first 16 days. No indication for biodegradation was noted, probably due to the very high oil levels versus available nutrients.

Thus the BIOS project is in an excellent position to proceed, having obtained a comprehensive knowledge of (1) the baseline organic chemical aspects of the Cape Hatt marine environment, (2) the chemical and physical nature of the test oil, (3) the applicability of long-term marker compound analysis, (4) the expected early shoreline weathering-induced chemical changes in the oil's composition, and (5) the appropriate blend of analytical techniques to be used in the real-time spill monitoring and post-spill assessment.

SECTION FIVE

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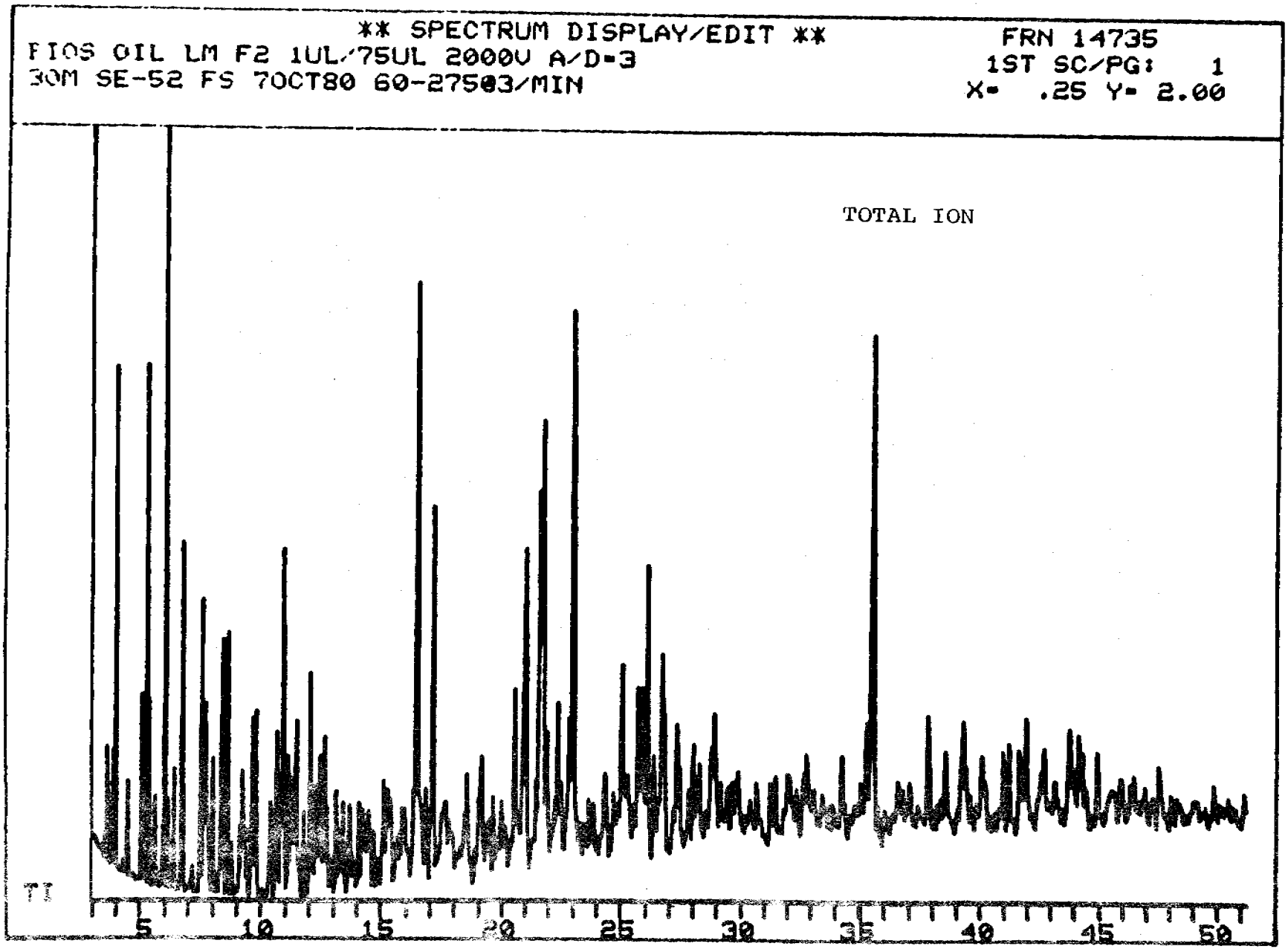
APPENDIX A

GC^2/MS ANALYSIS OF AROMATIC FRACTION OF LAGOMEDIO CRUDE OIL

Key to mass spectral searches:

m/e	Compound
120	C_3 alkyl benzenes
134	C_4 alkyl benzenes
148	C_5 alkyl benzenes
128	Naphthalene (N)
142	C_1 N
156	C_2 N
170	C_3 N
184	C_4 N
188	Deuterated anthracene (internal standard)
184	Dibenzothiophene (DBT)
198	C_1 DBT
212	C_2 DBT
226	C_3 DBT
154	Biphenyl
166	Fluorene (F)
180	C_1 F
194	C_2 F
208	C_3 F
202	Fluoranthene/pyrene
178	Phenanthrene (P)
192	C_1 P
206	C_2 P
220	C_3 P
234	C_4 P
228	Benzanthracene/chrysene
252	Benzopyrenes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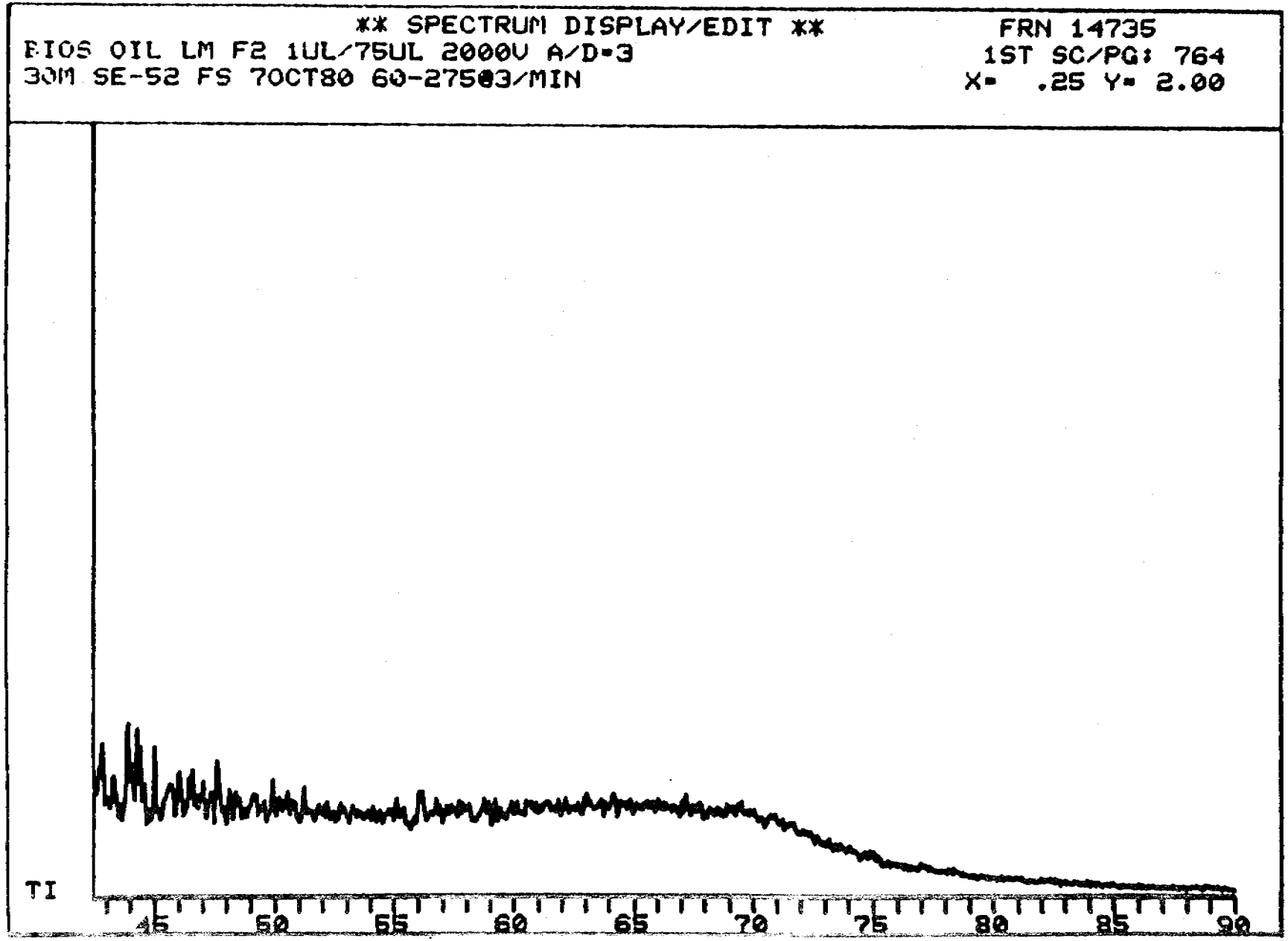
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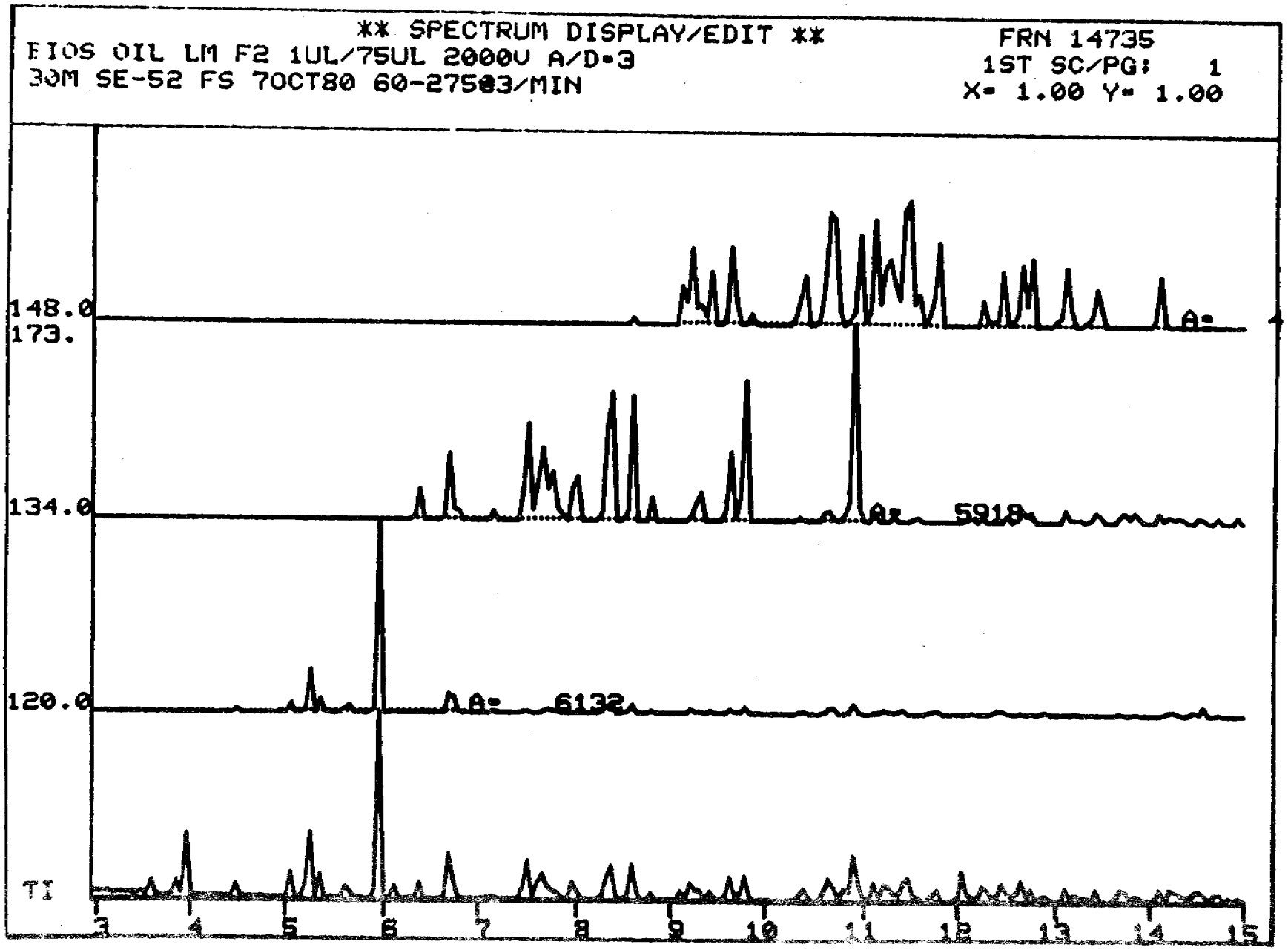
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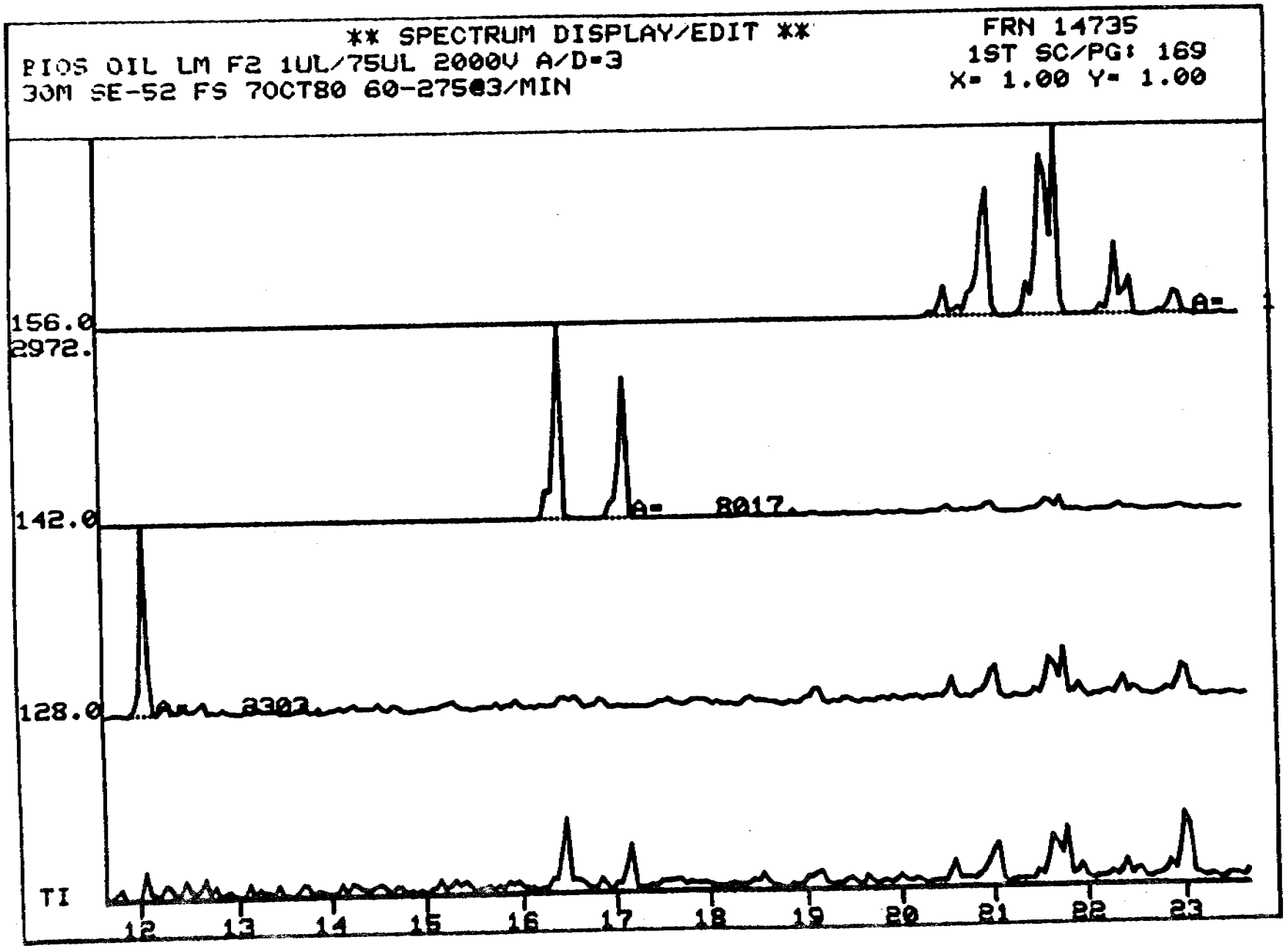
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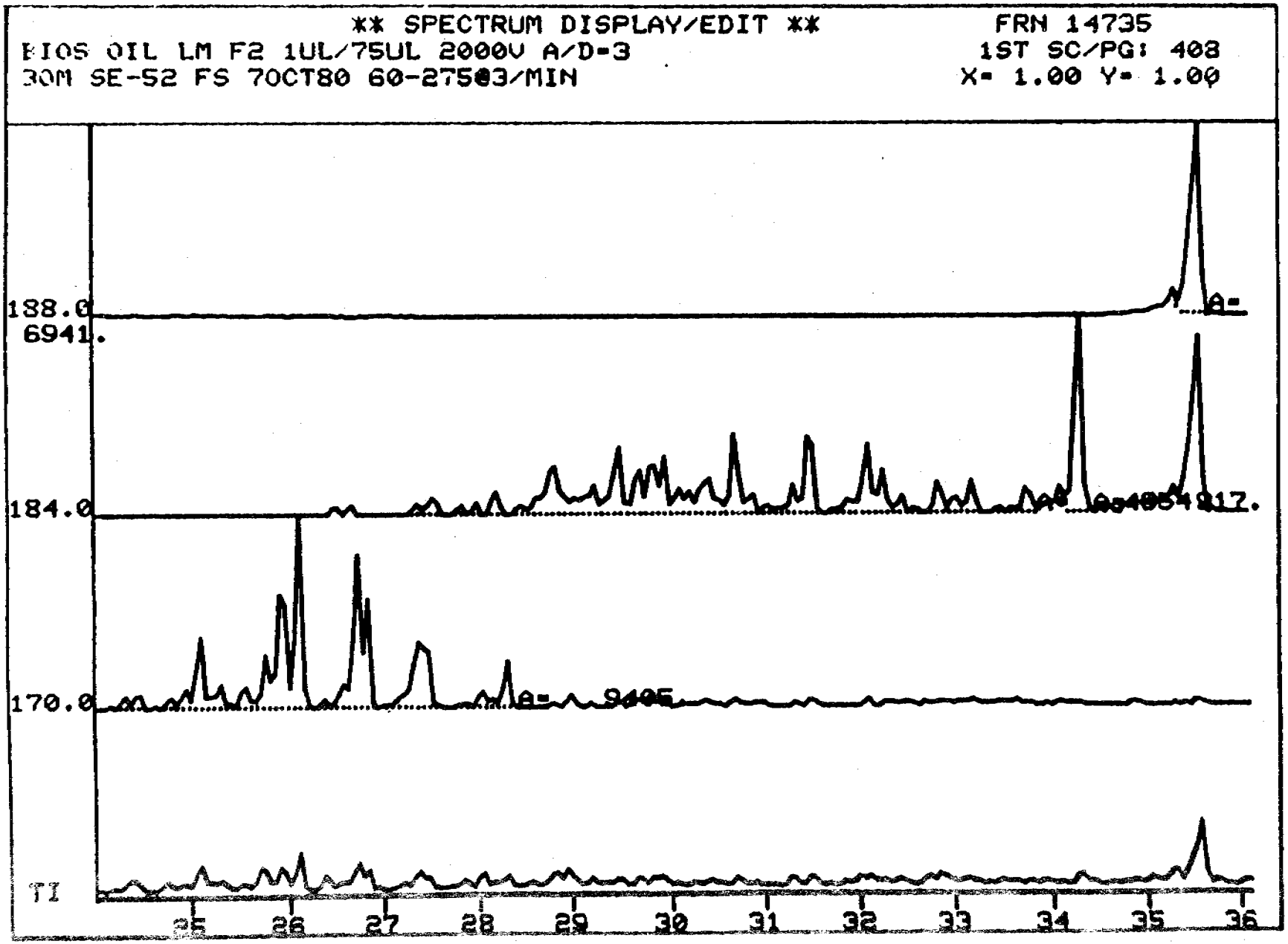
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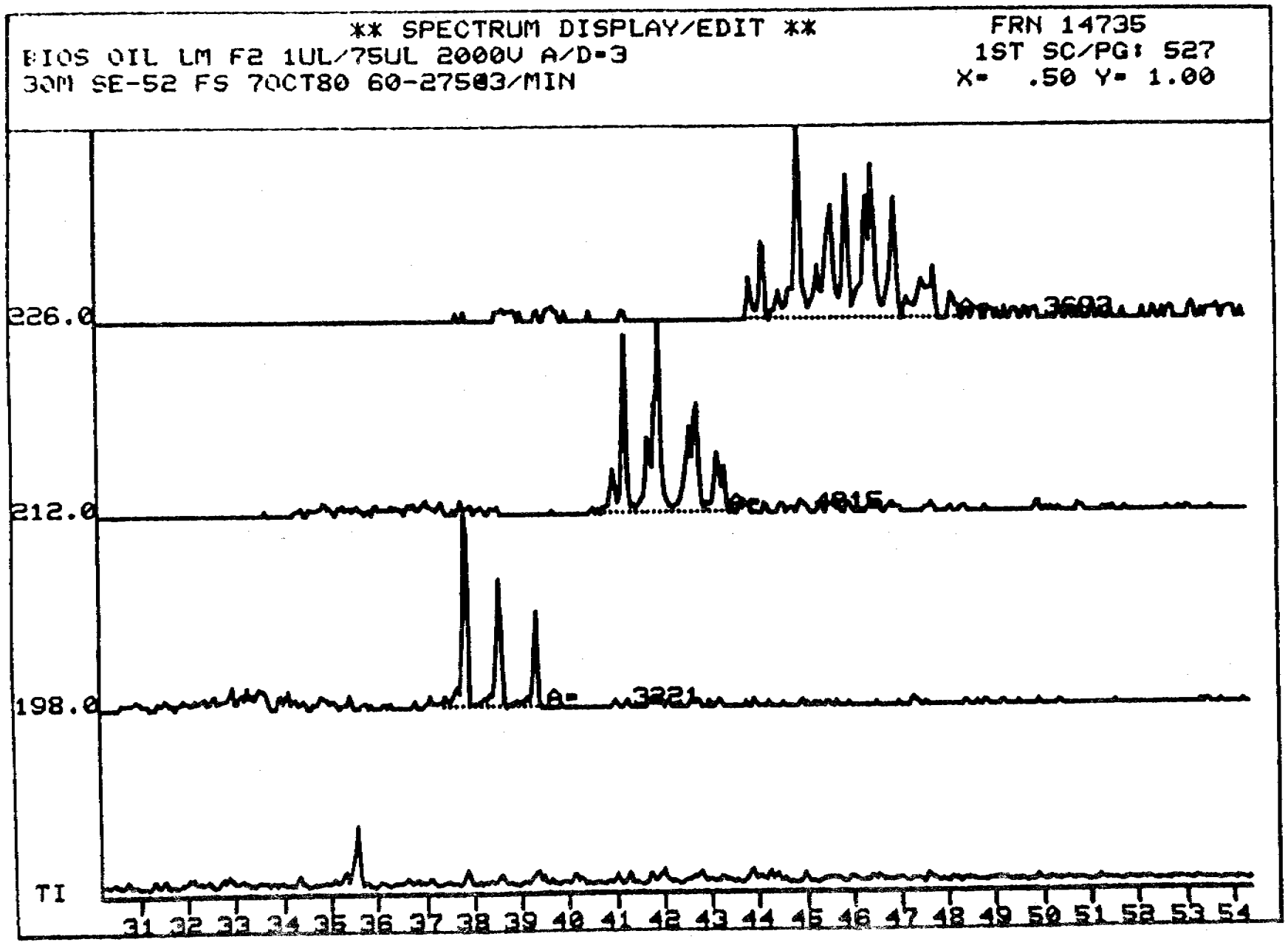
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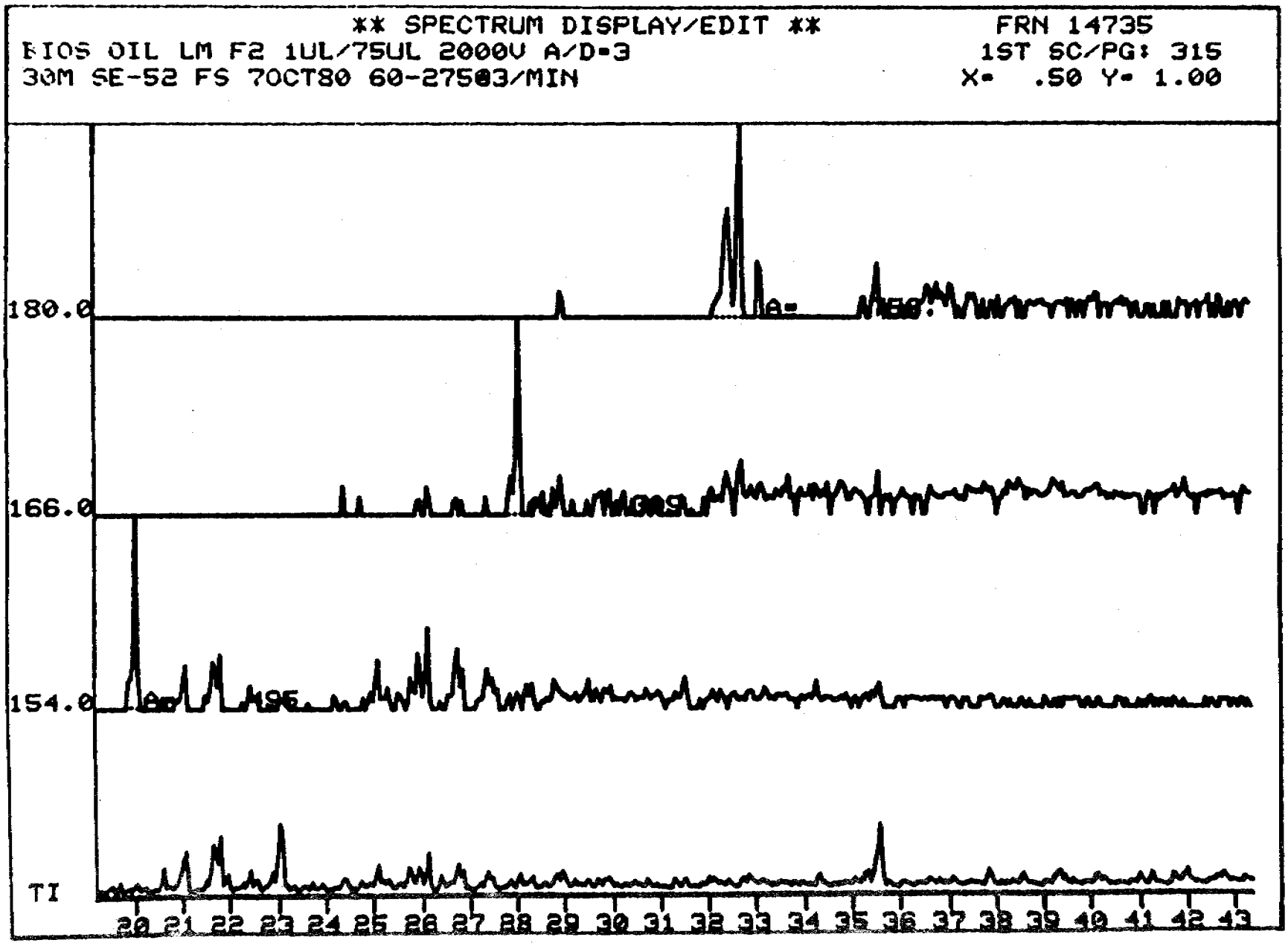
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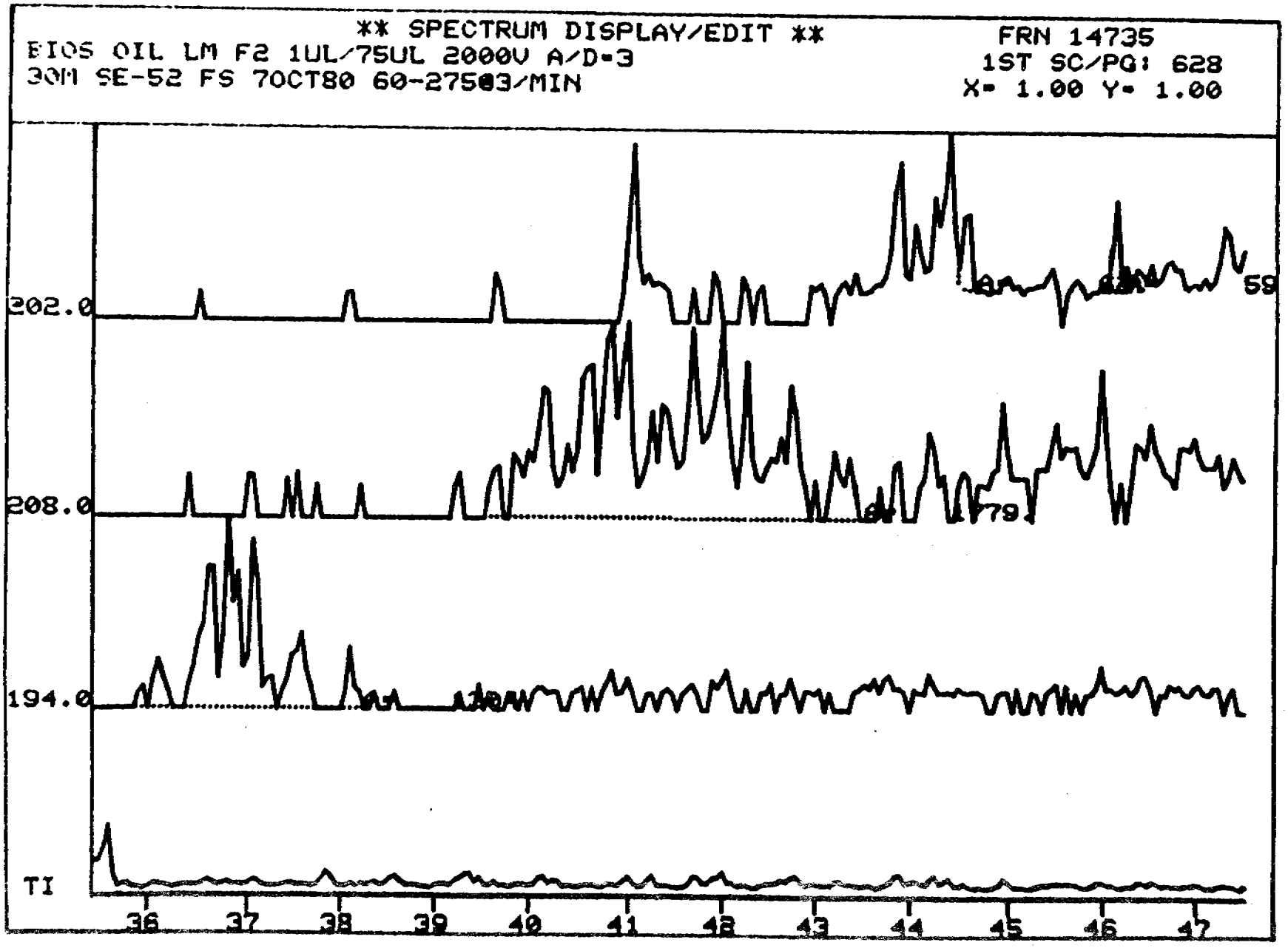
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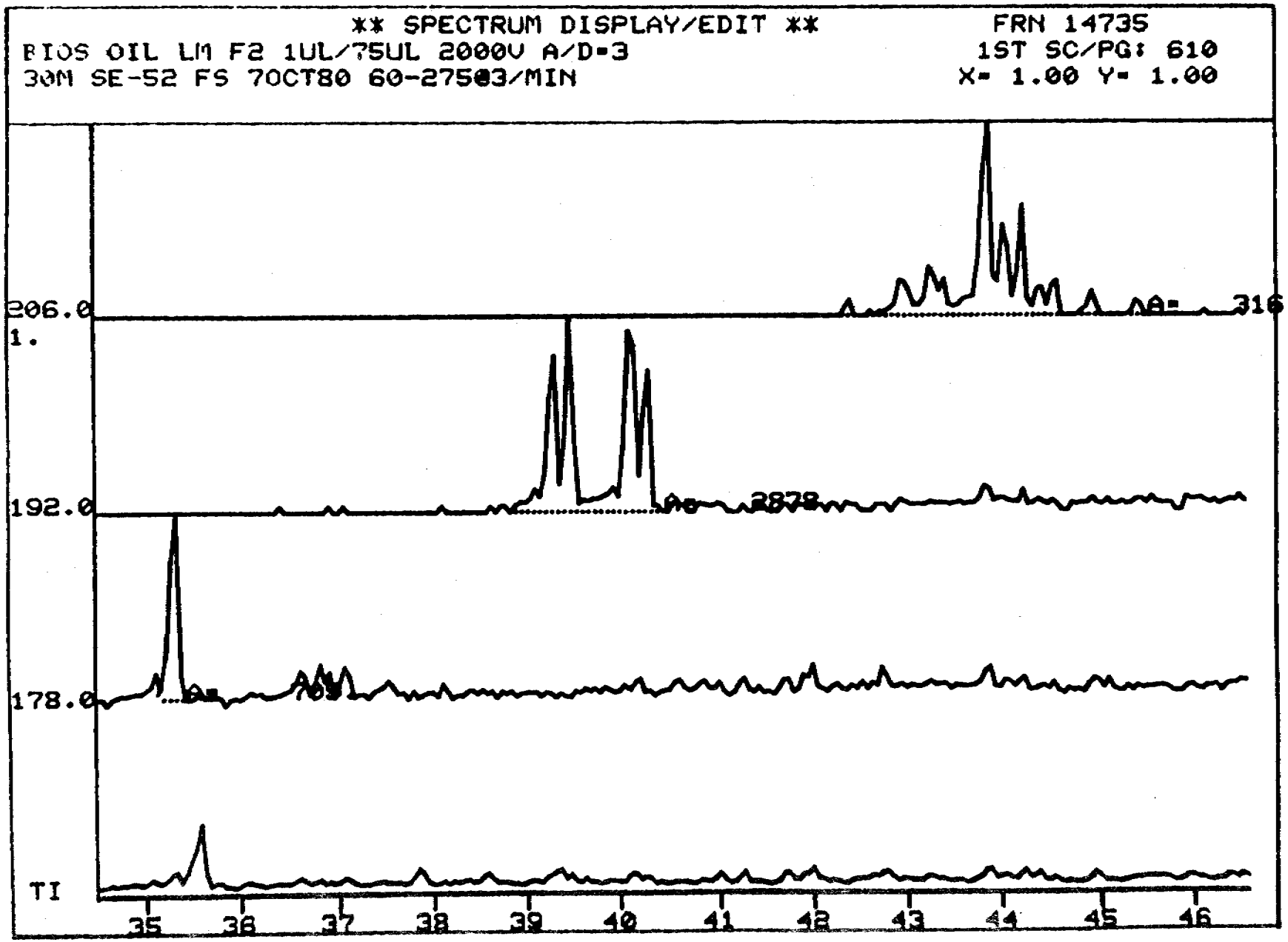
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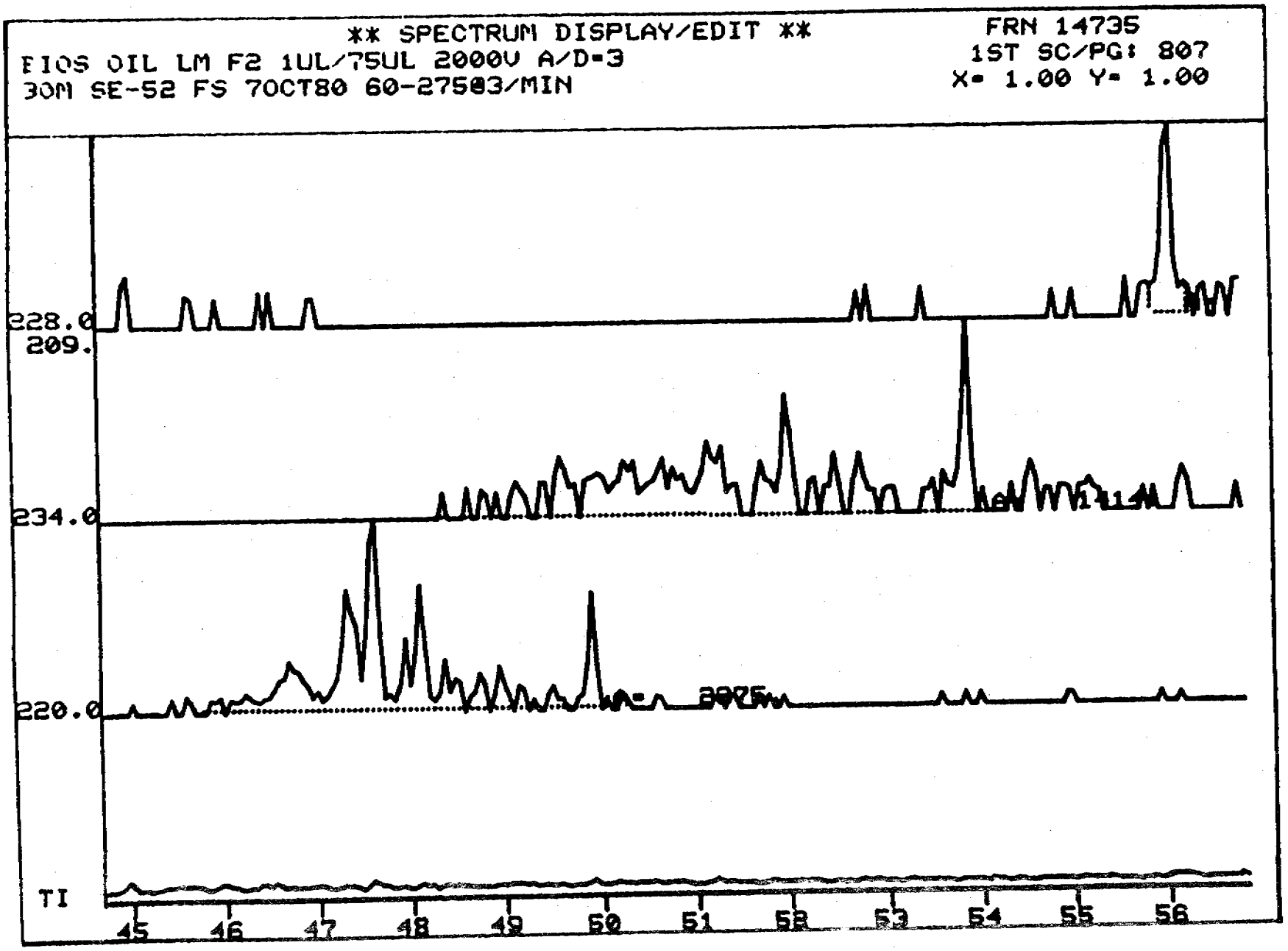
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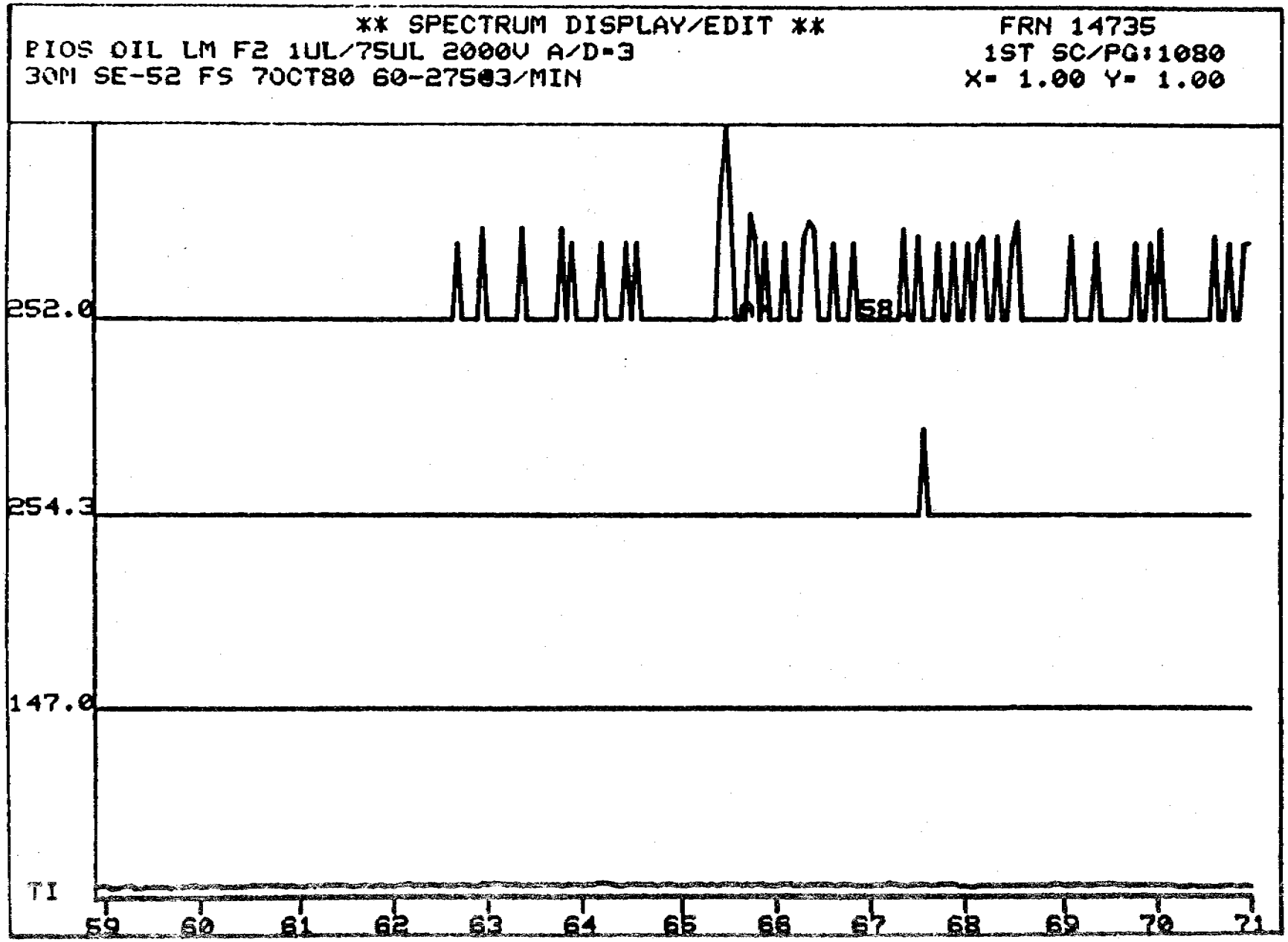
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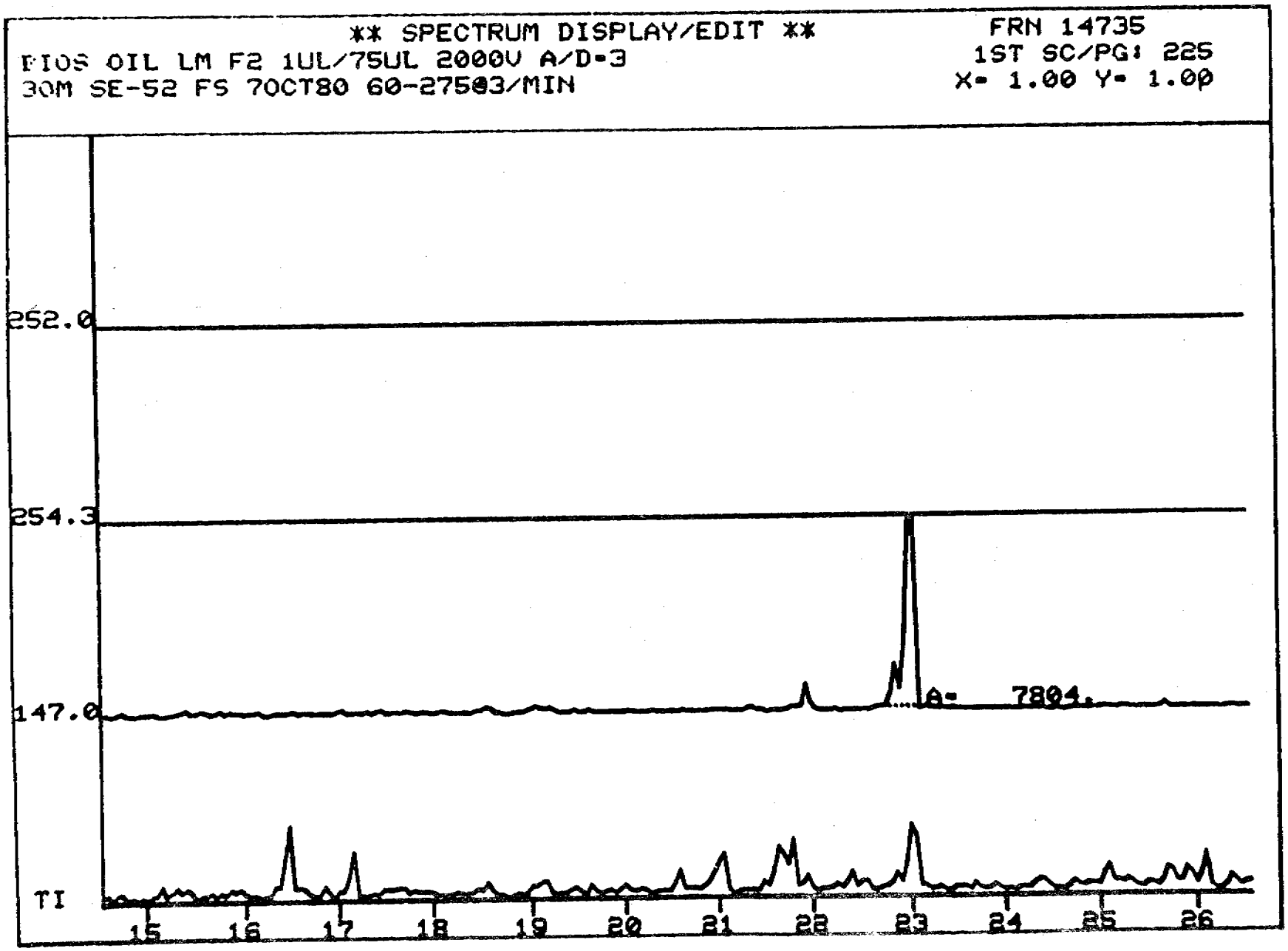
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252.0
254.3
147.0
A= 7804.
TI
15
16
17
18
19
20
21
22
23
24
25
26

FILE NUMBER 14735

ENTRY	TIME	MASS	AREA	%
1	35.6	188.0	7078.	100.00
2	30.7	184.0	4780.	67.54
3	34.3	184.0	879.	12.42
4	26.2	170.0	9415.	133.02
5	12.0	128.0	2303.	32.54
6	16.5	142.0	8017.	113.27
7	21.8	156.0	12972.	183.27
8	20.0	154.0	517.	7.30
9	28.1	166.0	309.	4.37
10	32.7	180.0	730.	10.32
11	36.8	194.0	1304.	18.42
12	41.1	208.0	1779.	25.13
13	44.6	202.0	62.	.88
14	46.2	202.0	59.	.83
15	35.3	178.0	709.	10.02
16	39.5	192.0	2878.	40.66
17	43.9	206.0	3161.	44.66
18	23.1	147.0	8075.	114.10

CAL % ON ENTRY?

FILE NUMBER 14735

ENTRY	TIME	MASS	AREA	%
1	66.4	252.0	35.	.49
2	65.8	252.0	34.	.48
3	65.5	252.0	58.	.82
4	23.1	147.0	7985.	112.82
5	47.6	220.0	2375.	33.56
6	53.9	234.0	1414.	19.97
7	56.1	228.0	209.	2.95
8	37.9	198.0	3221.	45.50
9	42.0	212.0	4815.	68.03
10	45.0	226.0	3693.	52.17
11	6.0	120.0	6132.	86.64
12	10.9	134.0	5918.	83.62
13	11.5	148.0	4173.	58.95
14	35.6	188.0	7078.	100.00

CAL % ON ENTRY?

FILE NUMBER 14735

ENTRY	TIME	MASS	AREA	%
1	35.6	188.0	7078.	87.65
2	30.7	184.0	4780.	59.20
3	34.3	184.0	879.	10.88
4	26.2	170.0	9415.	116.59
5	12.0	128.0	2303.	28.52
6	16.5	142.0	8017.	99.28
7	21.8	156.0	12972.	160.63
8	20.0	154.0	517.	6.40
9	28.1	166.0	309.	3.83
10	32.7	180.0	730.	9.04
11	36.8	194.0	1304.	16.15
12	41.1	208.0	1779.	22.03
13	44.6	202.0	62.	.77
14	46.2	202.0	59.	.73
15	35.3	178.0	709.	8.78
16	39.5	192.0	2878.	35.64
17	43.9	206.0	3161.	39.14
18	23.1	147.0	8075.	100.00

CAL % ON ENTRY?

FILE NUMBER 14735

ENTRY	TIME	MASS	AREA	%
1	66.4	252.0	35.	.44
2	65.8	252.0	34.	.43
3	65.5	252.0	58.	.72
4	23.1	147.0	7985.	100.00
5	47.6	220.0	2375.	29.75
6	53.9	234.0	1414.	17.70
7	56.1	228.0	209.	2.61
8	37.9	198.0	3221.	40.33
9	42.0	212.0	4815.	60.31
10	45.0	226.0	3693.	46.25
11	6.0	120.0	6132.	76.79
12	10.9	134.0	5918.	74.12
13	11.5	148.0	4173.	52.26
14	35.6	188.0	7078.	88.64

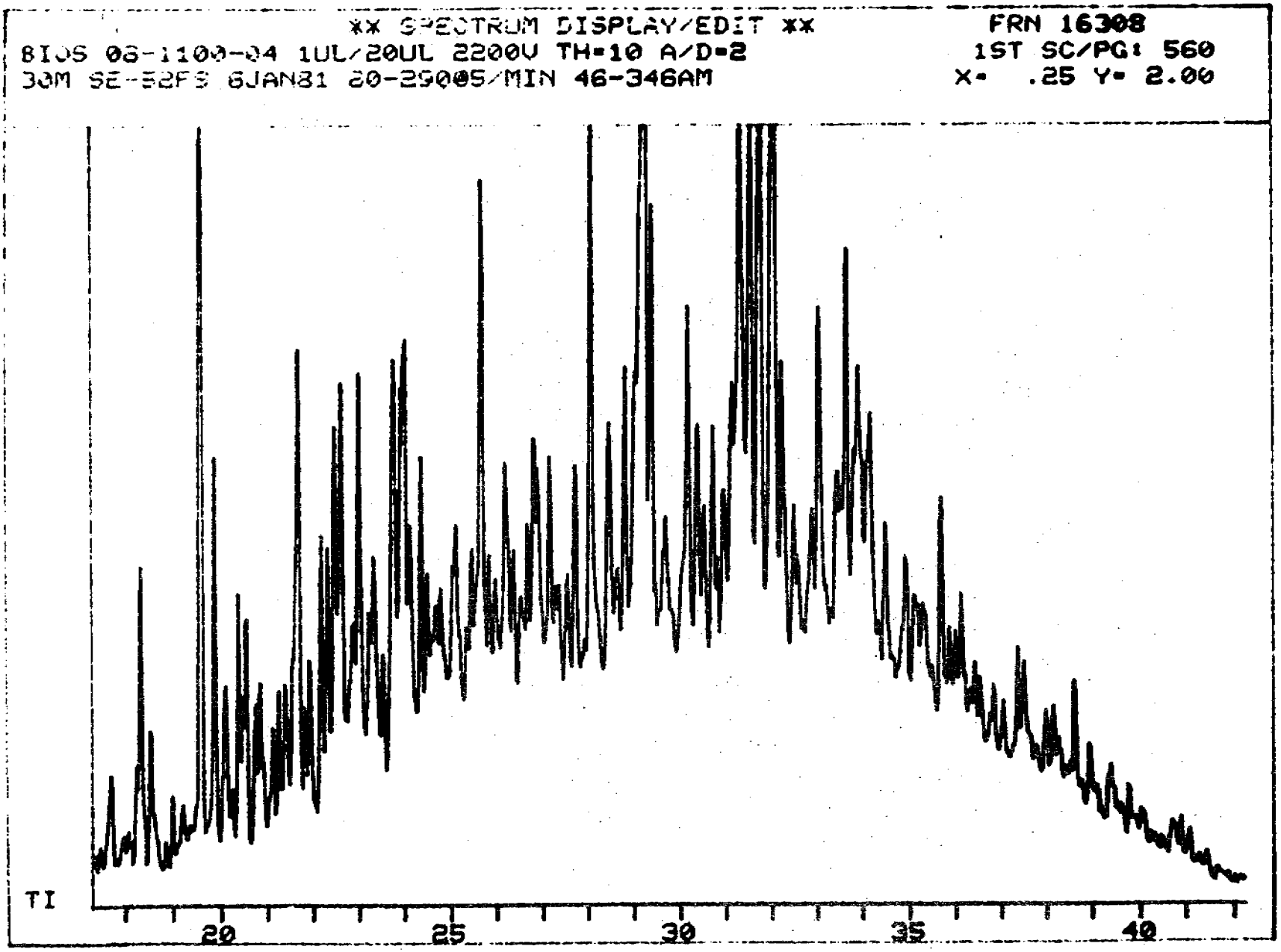
CAL % ON ENTRY?

APPENDIX B

GC^2/MS ANALYSIS OF AZAARENE FRACTION OF LAGOMEDIO CRUDE OIL

Key to mass spectral searches:

m/e	Compound
129	Quinoline, isoquinoline (Q)
143	C_1 Q
157	C_2 Q
171	C_3 Q
185	C_4 Q
199	C_5 Q
213	C_6 Q
179	Acridine/phenanthridine (AP)
193	C_1 AP
207	C_2 AP
221	C_3 AP
235	C_4 AP
249	C_5 AP
167	Carbazole (C)
181	C_1 C
195	C_2 C
209	C_3 C
223	C_4 C
229	Benzacridine (BA)
243	C_1 BA
257	C_2 BA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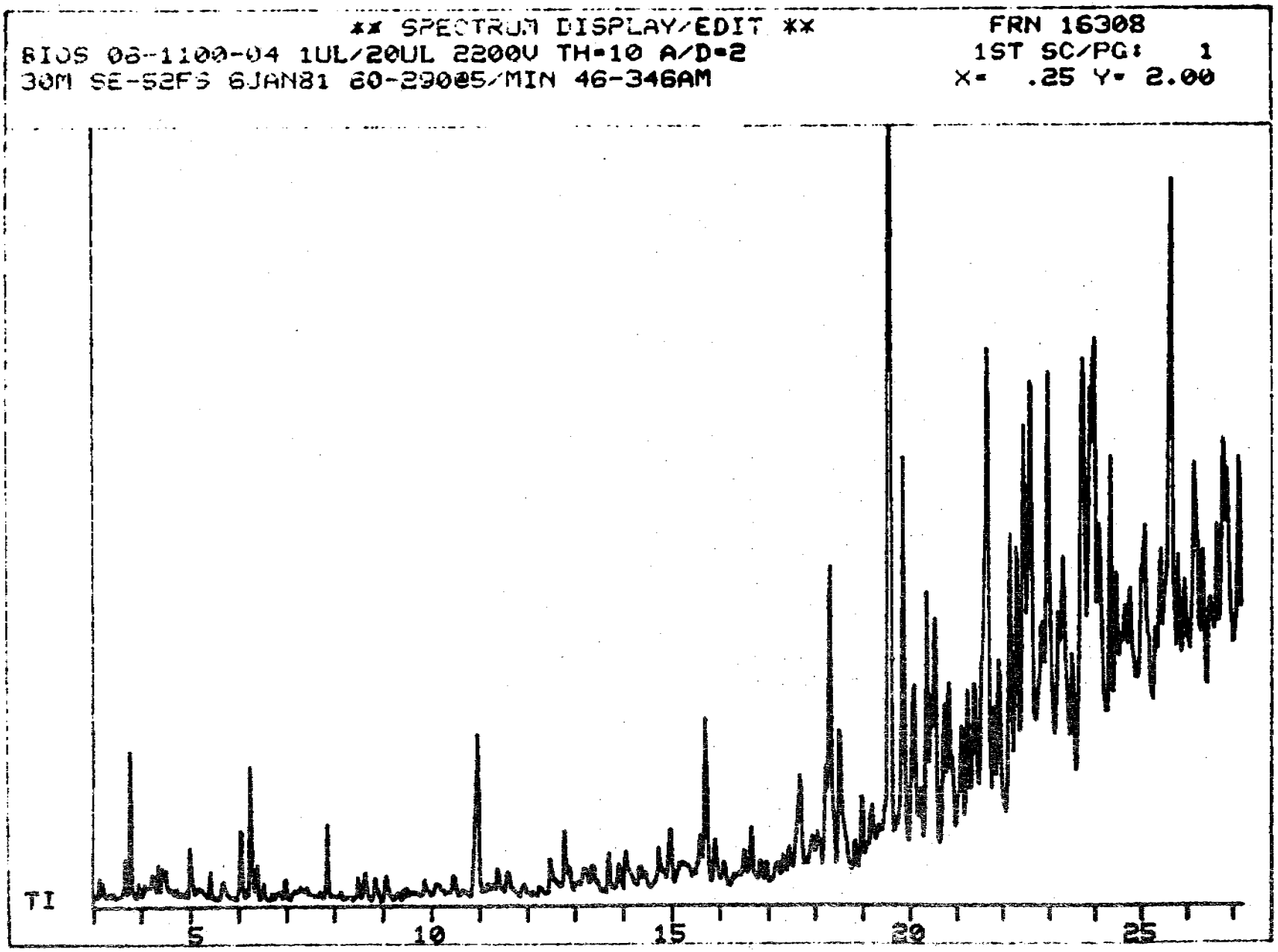
TI
20
25
30
35
40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1ST SC/PG: 1
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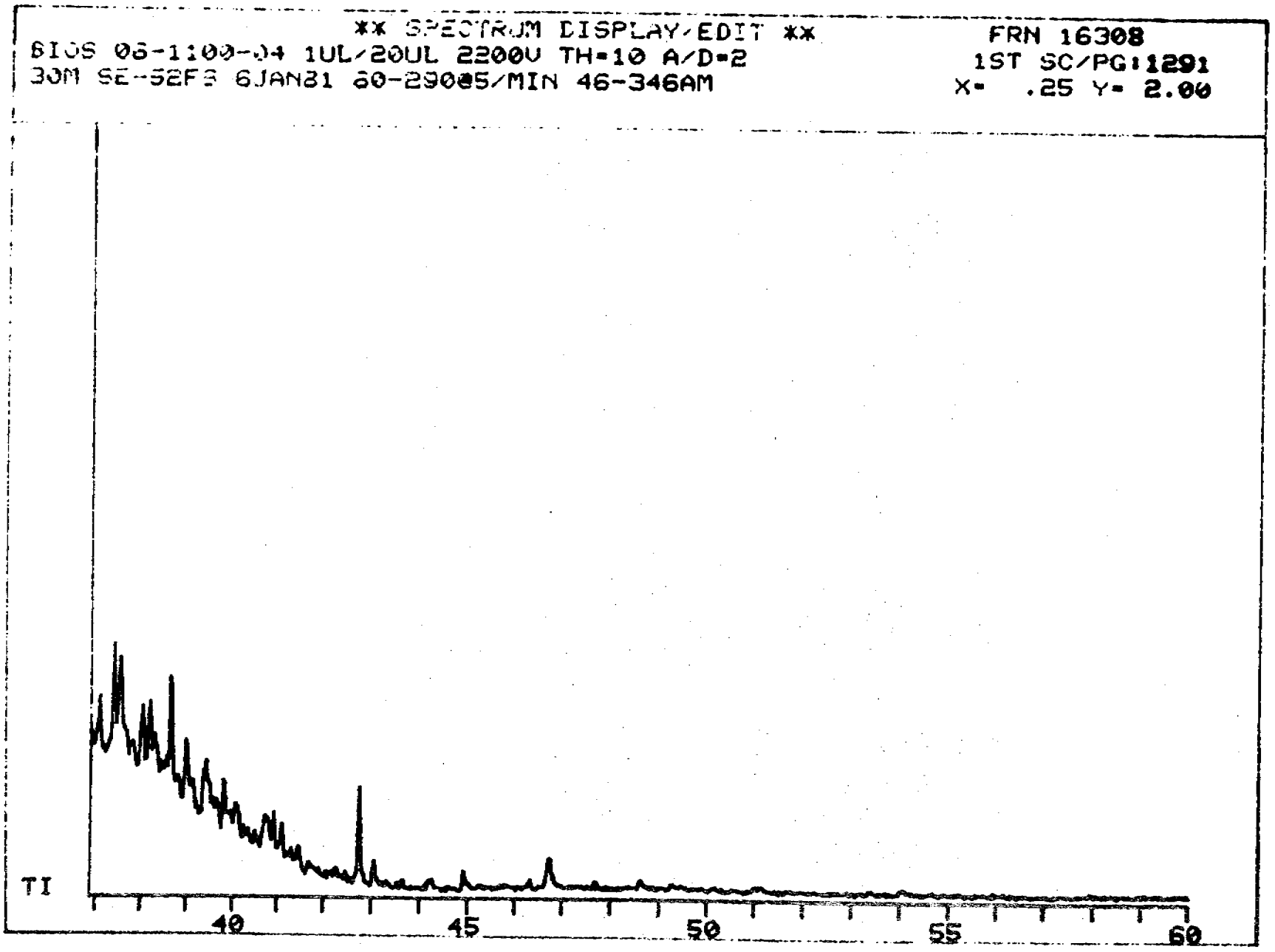
X= .25 Y= 2.00
TI
5
10
15
20
25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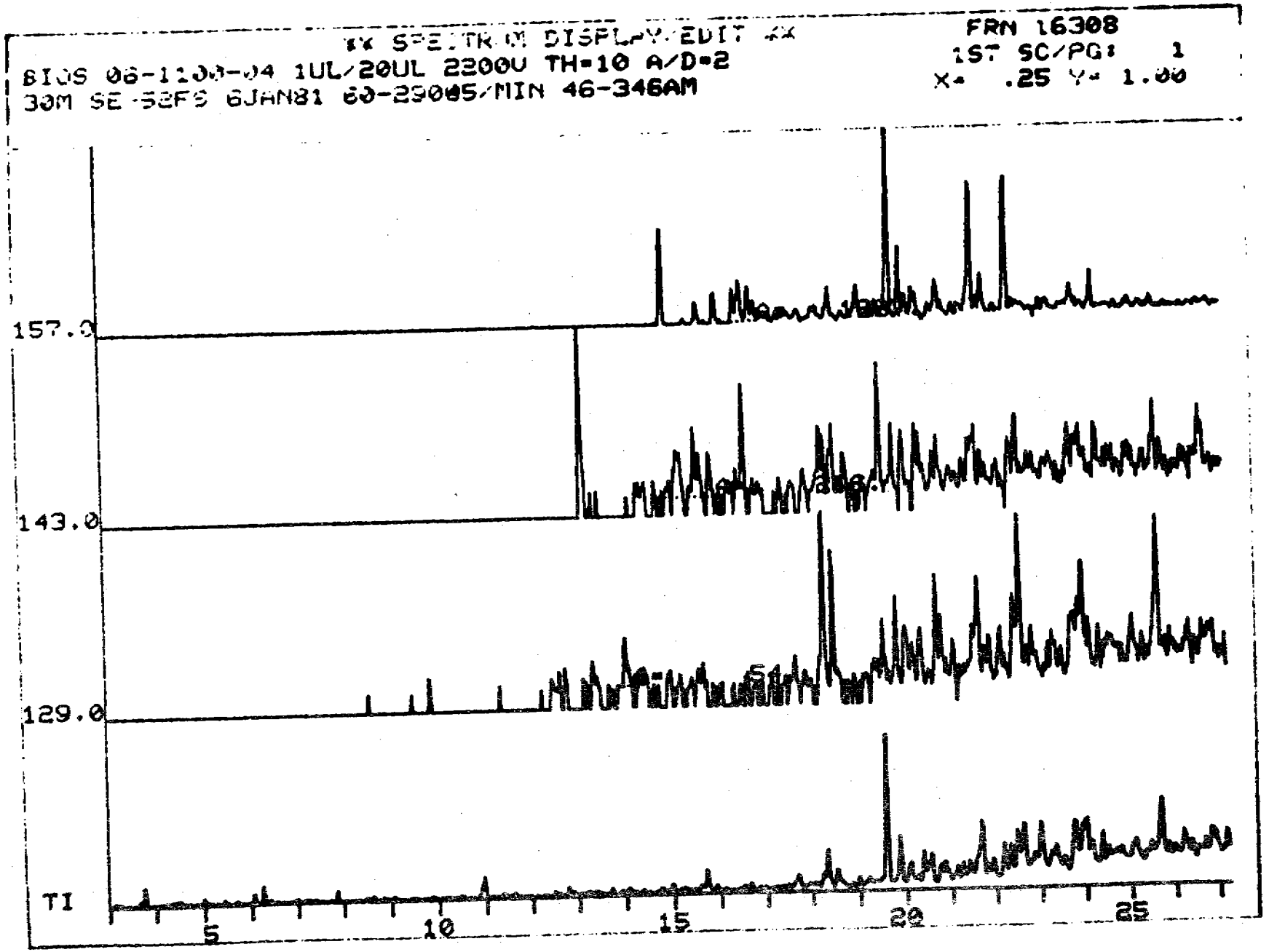
TI
40
45
50
55
60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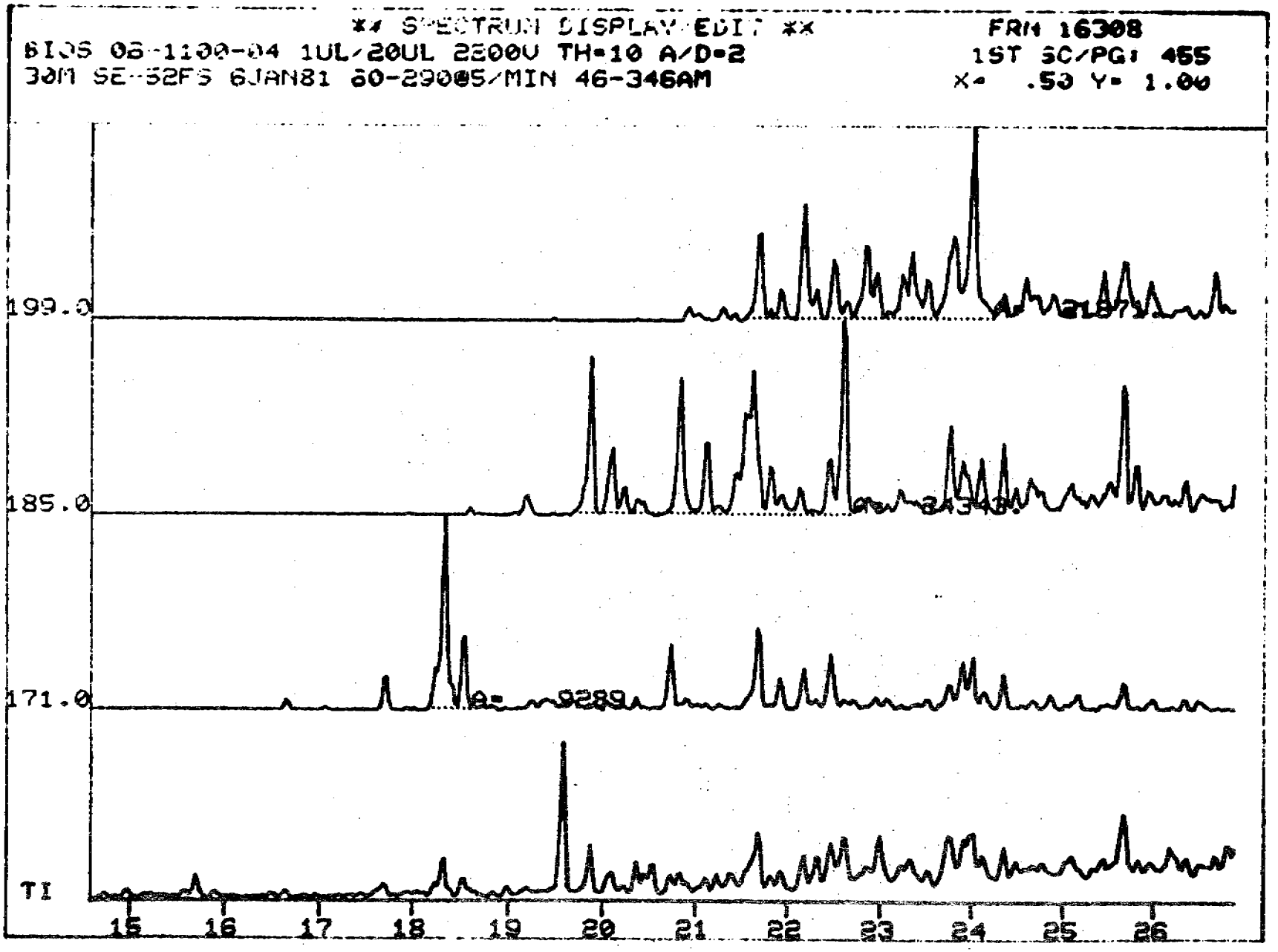
143.0
129.0
TI
5
10
15
20
25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185.0
171.0
TI
15
16
17
18
19
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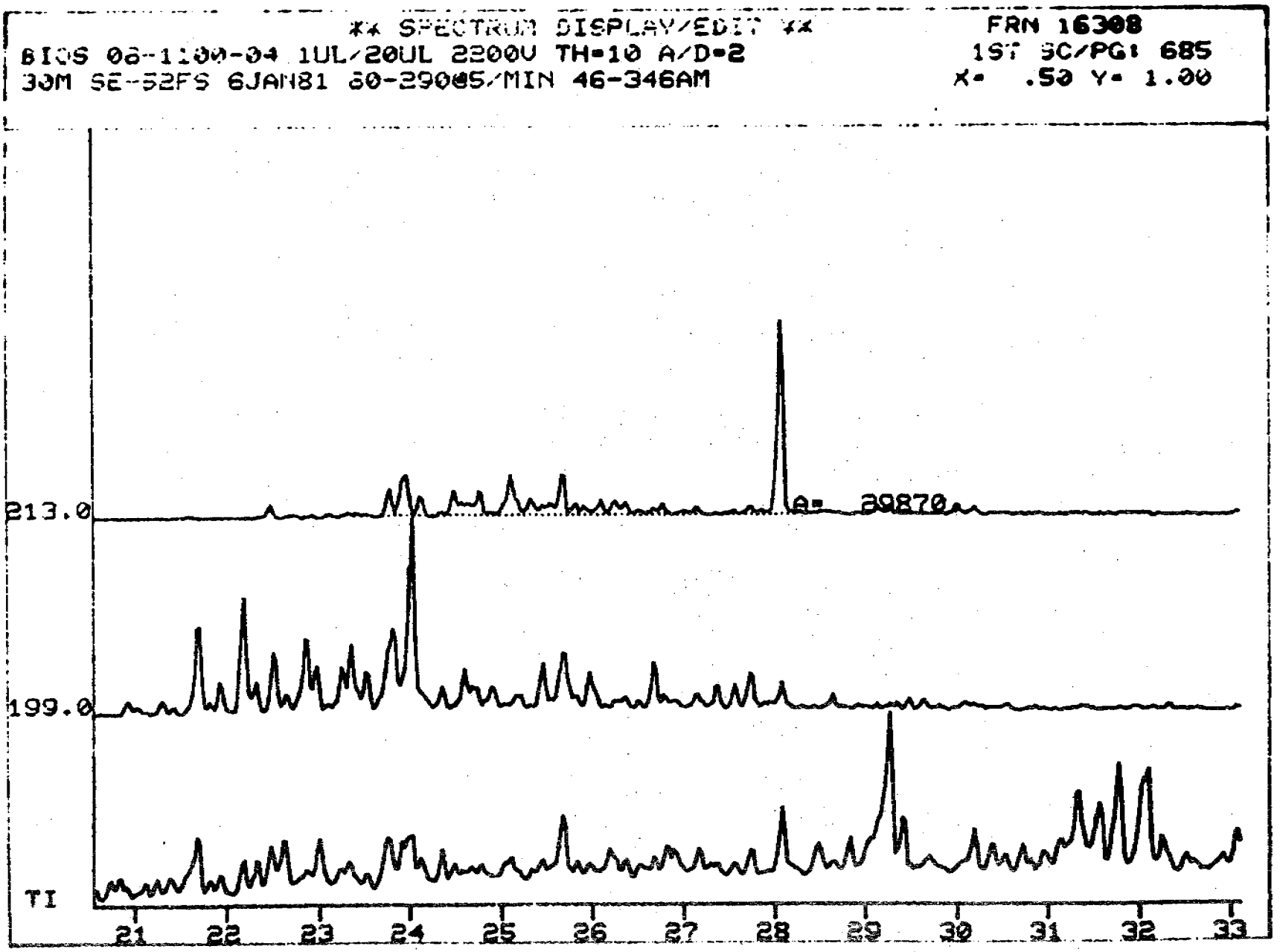
22
23
24
25
26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1ST SC/PG: 685
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199.0
TI
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23
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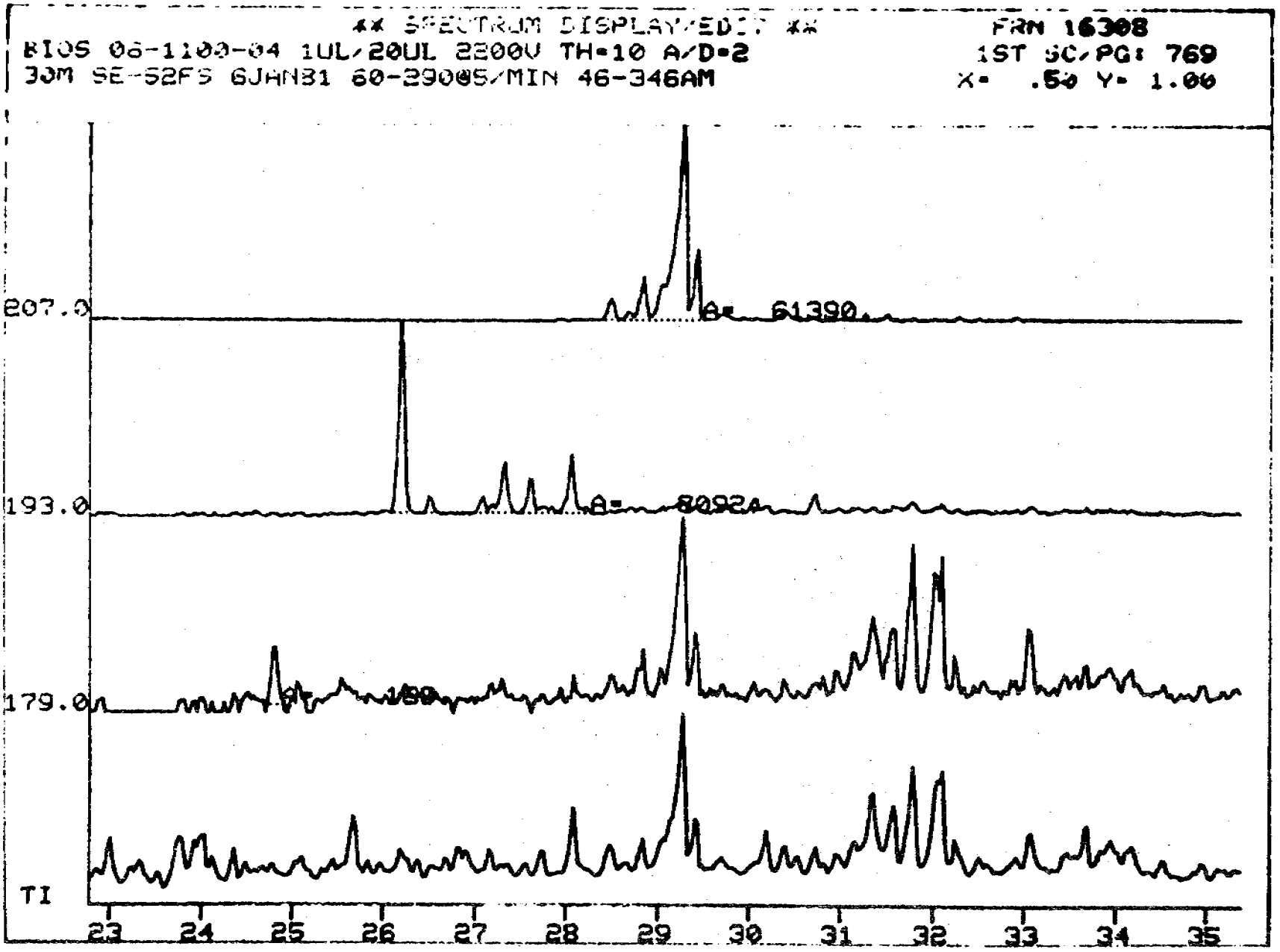
28
29
30
31
32
33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3OM SE-52FS 6JAN81 60-29005/MIN 46-346AM
X= .50 Y= 1.00
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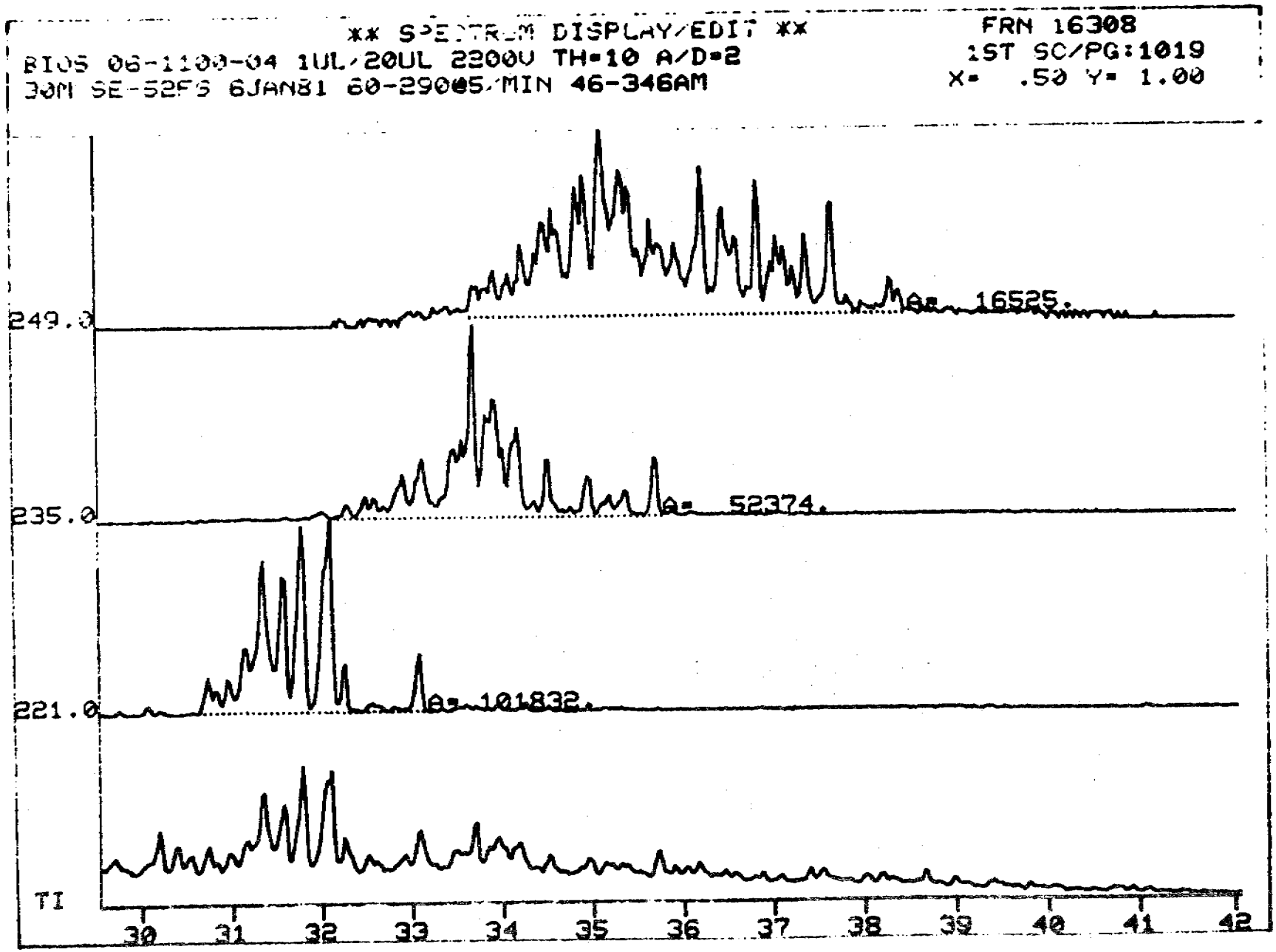
193.0
179.0
TI
23
24
25
26
27
28
29
30
31
32
33
34
35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B= 16525.
235.0
B= 52374.
221.0
B= 101832.
TI
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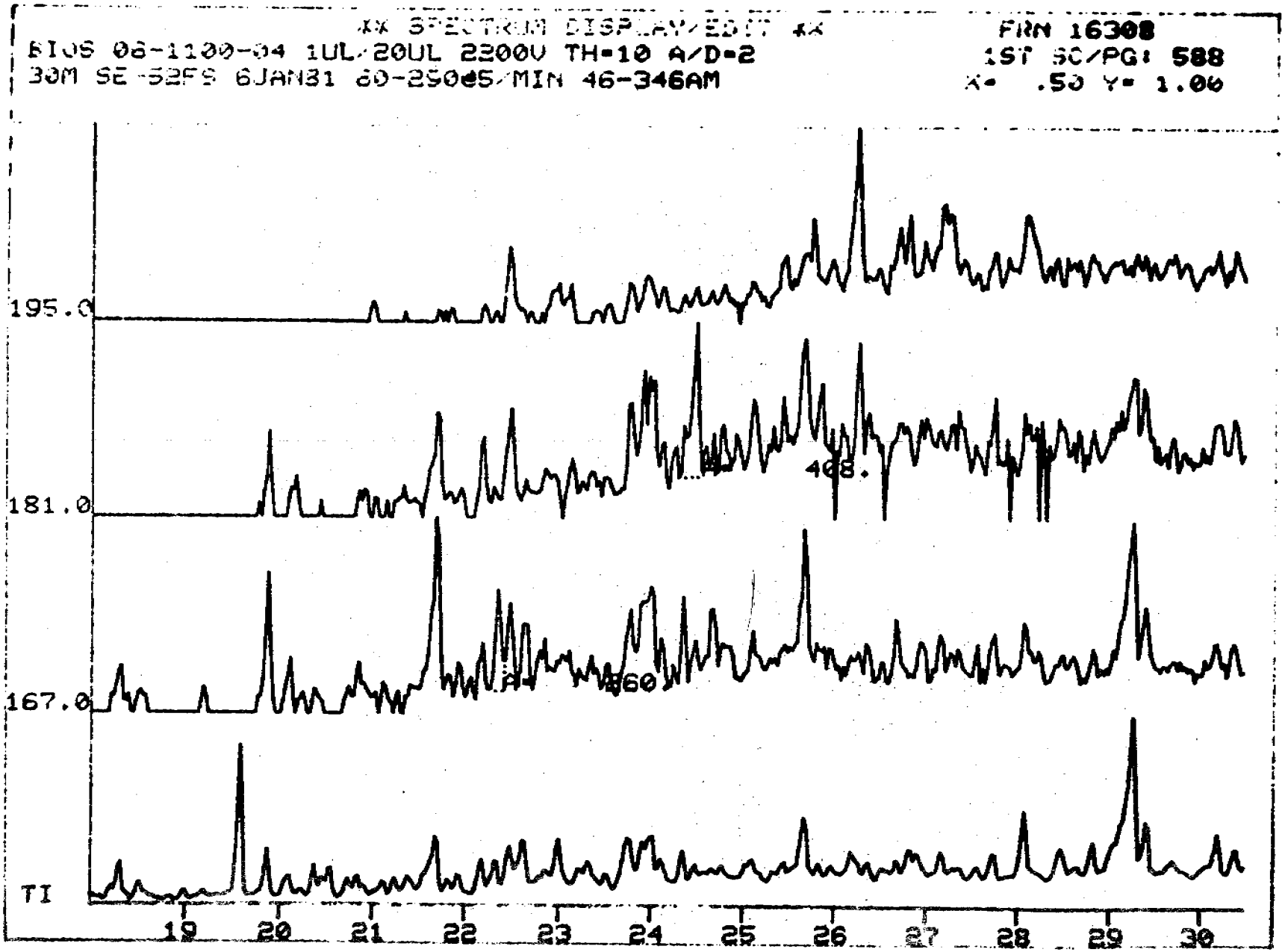
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X= .50 Y= 1.00
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181.0
167.0
TI
19
20
21
22
23
24
25
26
27
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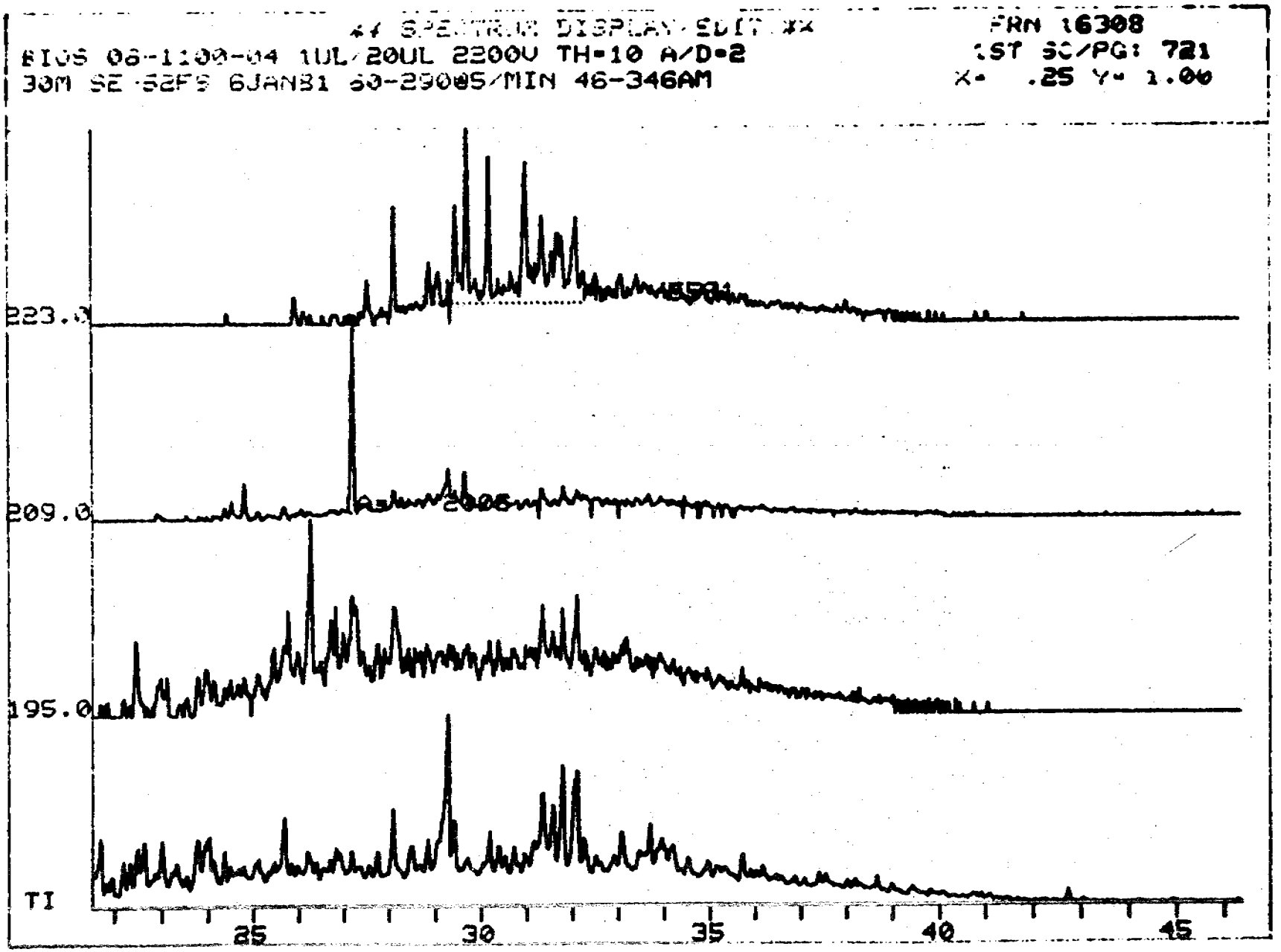
29
30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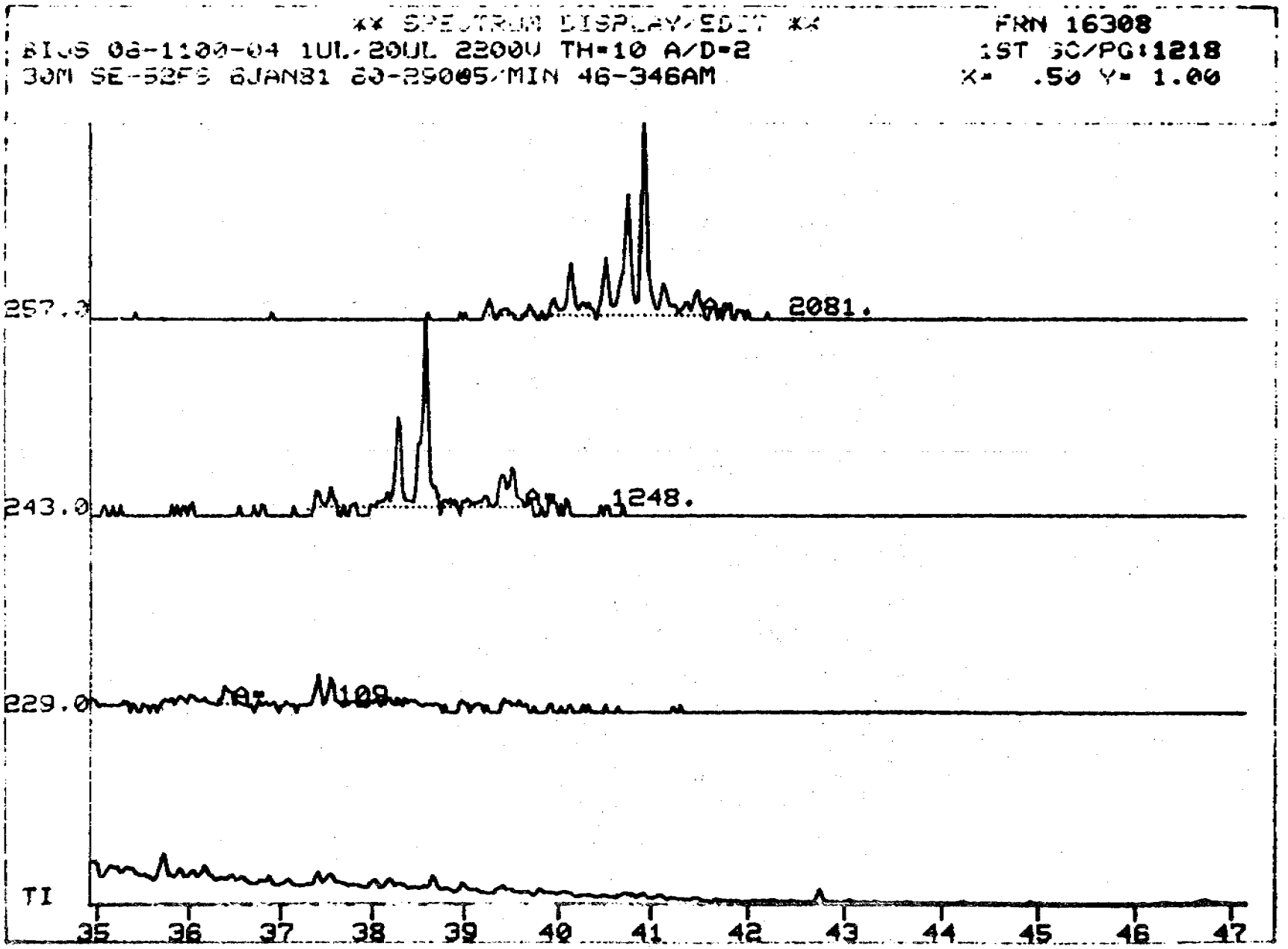
223.0
209.0
195.0
TI
25
30
35
40
45

460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2081.
243.0
1248.
229.0
M108
TI
35 36 37 38 39 40 41 42 43 44 45 46 47

FILE NUMBER 16308

ENTRY	TIME	MASS	AREA	%
1	14.0	129.0	54.	.05
2	15.6	143.0	296.	.29
3	16.7	157.0	1287.	1.26
4	18.3	171.0	9289.	9.12
5	22.6	185.0	24343.	23.90
6	24.0	199.0	21871.	21.48
7	23.1	213.0	29870.	29.33
8	24.8	179.0	169.	.17
9	26.2	193.0	8092.	7.95
10	29.3	207.0	61390.	60.29
11	32.1	221.0	101832.	100.00
12	33.7	235.0	52374.	51.43
13	35.2	249.0	16525.	16.23

CAL % ON ENTRY?

FILE NUMBER 16308

ENTRY	TIME	MASS	AREA	%
1	27.2	209.0	2006.	1.94
2	39.7	223.0	5521.	5.34
3	26.2	195.0	1093.	1.06
4	22.3	167.0	260.	.25
5	24.5	181.0	408.	.39
6	36.4	229.0	109.	.11
7	38.6	243.0	1248.	1.21
8	40.9	257.0	2081.	2.01
9	32.1	221.0	103353.	100.00
10	32.1	221.0	100268.	97.01

CAL % ON ENTRY?

EFFECTS OF OIL AND DISPERSED OIL ON NEARSHORE MACROBENTHOS AT CAPE HATT, NORTHERN BAFFIN ISLAND. I. RESULTS OF 1980 PRE-SPILL STUDIES

by

William E. Cross

and

Denis H. Thomson

for

Baffin Island Oil Spill Project

Environmental Protection Service

Environment Canada

Edmonton, Alberta

March 1981

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the study. The advice and encouragement of Biological Technical Committee members Ron Foreman, Roger Green, Gary Sergy, Norm Snow and John Vandermeulen are gratefully acknowledged.

INTRODUCTION

The pace of exploration and development of hydrocarbon resources in arctic and subarctic marine areas is likely to increase in the future. Already, exploratory drilling is occurring in the Canadian Beaufort Sea, Sverdrup Basin, Davis Strait and the Labrador Sea, and plans call for offshore drilling in the Alaskan Beaufort Sea, Lancaster Sound and Baffin Bay in the near future. Plans for major offshore oil production are being developed for the Canadian Beaufort Sea by industry, and the Federal Environmental Assessment Review Office is preparing to evaluate the plans. Year-round transport of oil through the Northwest Passage, Baffin Bay and Davis Strait is now a distinct possibility.

Clearly, as the amount of activity increases, the possibility of an accidental release of oil also increases. If oil is released there will be substantial pressure to use chemical dispersants to try to keep the oil from accumulating on the surface of the water or on shorelines where extremely small amounts can have dramatic effects on birds.

With or without the use of chemical dispersants, released oil will enter the water column and, especially in nearshore locations, impinge upon the bottom. The initial biological effects will occur among planktonic and benthic invertebrates, although effects at higher levels of the food web may result from the mortality of or accumulation of oil in important food species. The use of chemical dispersants may increase biological effects because of dispersant toxicity, increased dissolution of toxic oil fractions, or increased opportunity for the accumulation of oil in sediments. Also, the primary use of dispersants will likely be as a countermeasure to prevent the

impingement of oil on shorelines; hence they will be used primarily in shallow, productive nearshore waters, many of which are important feeding areas for birds and marine mammals.

Recently, considerable attention has been given to the effects of oil and dispersants on individual species of arctic marine flora and fauna under experimental conditions (Percy and Mullin 1975, 1977; Percy 1976, 1977, 1978; Busdosh and Atlas 1977; Malins 1977; Atlas et al. 1978; Foy 1978, 1979; Hsiao et al. 1978), but to date the potential effects on whole communities are unknown. During the recent TSESIS oil spill investigations, a comparison of approaches towards detecting biological effects supported the 'ecosystem approach' advocated by Mann and Clark (1978): data on reproductive abnormalities in a sensitive species only confirmed effects that were already obvious at the community level (Elmgren et al. 1980). In temperate waters community studies have been carried out for up to 10 years after a spill (e.g. Sanders et al. 1980), but most of these studies have been after the fact; hence they lack adequate 'control' data on pre-spill conditions, on naturally occurring changes that would have occurred in the absence of the spill, or on both (National Academy of Sciences 1975; cf. Bowman 1978). Another shortcoming of many spill studies has been the lack of supporting data on oceanographic and atmospheric conditions, and on hydrocarbon concentrations in the impacted environment (National Academy of Sciences 1975).

To date, no major oil spill has occurred in Canadian arctic waters. In 1978-1979 the Arctic Marine Oilspill Program (AMOP) examined the need for research associated with experimental oilspills in cold Canadian waters, and

thereafter instigated the Baffin Island Oilspill (BIOS) project to study a controlled introduction of oil and dispersed oil onto shorelines and into nearshore arctic waters. The objectives of this project were to assess the environmental impact of chemical dispersants and the relative effectiveness and impact of other shoreline protection and clean-up techniques. The BIOS project is an internationally funded, multidisciplinary study being carried out by engineers, meteorologists, physical oceanographers, geologists, chemists and biologists from various government departments, industry and research organizations. The nearshore component of the BIOS project includes studies of microbiology and benthic macrobiology, atmospheric and oceanographic conditions, and chemical properties of the water column and surface sediments, with special emphasis on concentrations of petroleum hydrocarbons.

The objectives of the macrobiological component of the BIOS project are to assess the effects of oil and dispersed oil on the macrophytic algae, the relatively immobile benthic infauna (e.g. bivalves, polychaetes) and the motile epibenthos (e.g. amphipods, urchins) in shallow arctic waters. Variables to be examined include total abundance/biomass and community structure, as well as the abundance, biomass, population age structure and length-weight relationships of dominant species in these communities. The statistical design of the study is 'optimal' for impact assessment (in the sense of Green 1979) in that it includes both temporal (pre-spill) and spatial (unoiled bay) controls. The present report provides baseline data from the first of two pre-spill sampling periods (September 1980, August 1981). These data, together with post-spill data from an uncontaminated (control) bay, will be used as a basis of comparison with post-spill data from the experimental bays.

METHODS

Field Collection Procedures

Field work was carried out during 7-13 August and 29 August-17 September 1980 from the BIOS project camp located at Cape Hatt, Baffin Island (Fig. 1). All sampling was carried out by divers working from inflatable boats (Zodiacs) moored in Bay 11 (August) or Bay 12 (September). Processing and preservation of samples were performed in tents erected on the beach at the same locations (Fig. 1). During the preliminary survey in early August, Bays 9, 10, 11 and 13 were examined with the primary objective of selecting three suitable bays and three suitable transects within each bay; the aim was to select sites with similar substrates and faunal assemblages. During September, systematic sampling was carried out in Bays 9, 10, and 11, and additional collections were made in Z lagoon to provide specimens for tissue analysis (Fig. 1). The following sections apply to the systematic work in September.

Sampling Locations

Three contiguous 50 m transects were set parallel to the shoreline at each of two depths in each of Bays 9, 10 and 11 (Fig. 2). A depth of 7 m was selected as the primary sampling depth, and transect locations at that depth were chosen in each bay using as criteria (1) similarity in substrate characteristics and infaunal community composition among transects and bays (as determined during the preliminary survey), and (2) facility of sampling (soft substrate with as little surface rock as possible). The second

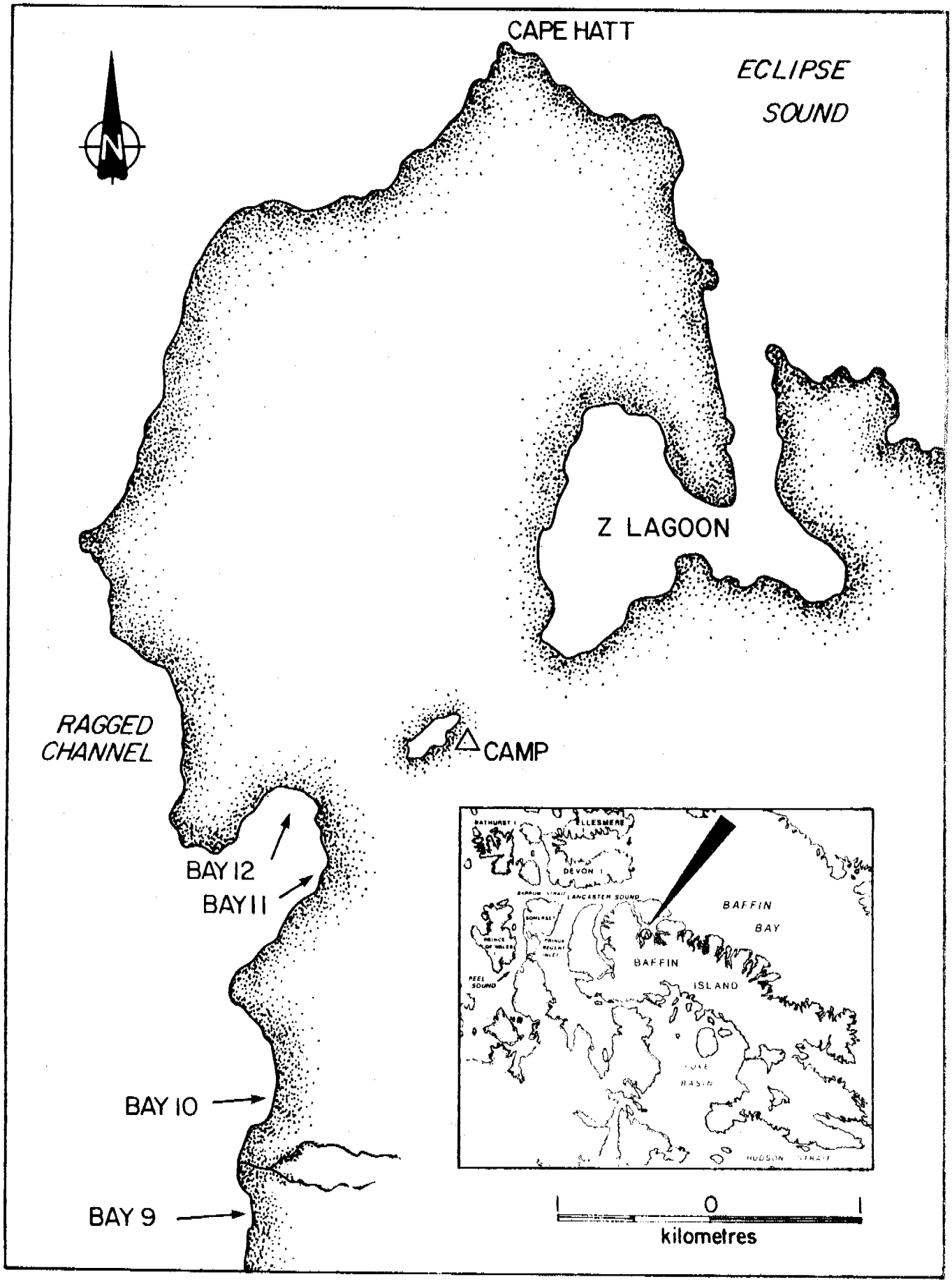


FIG. 1. Locations of study bays at Cape Hatt, northern Baffin Island.

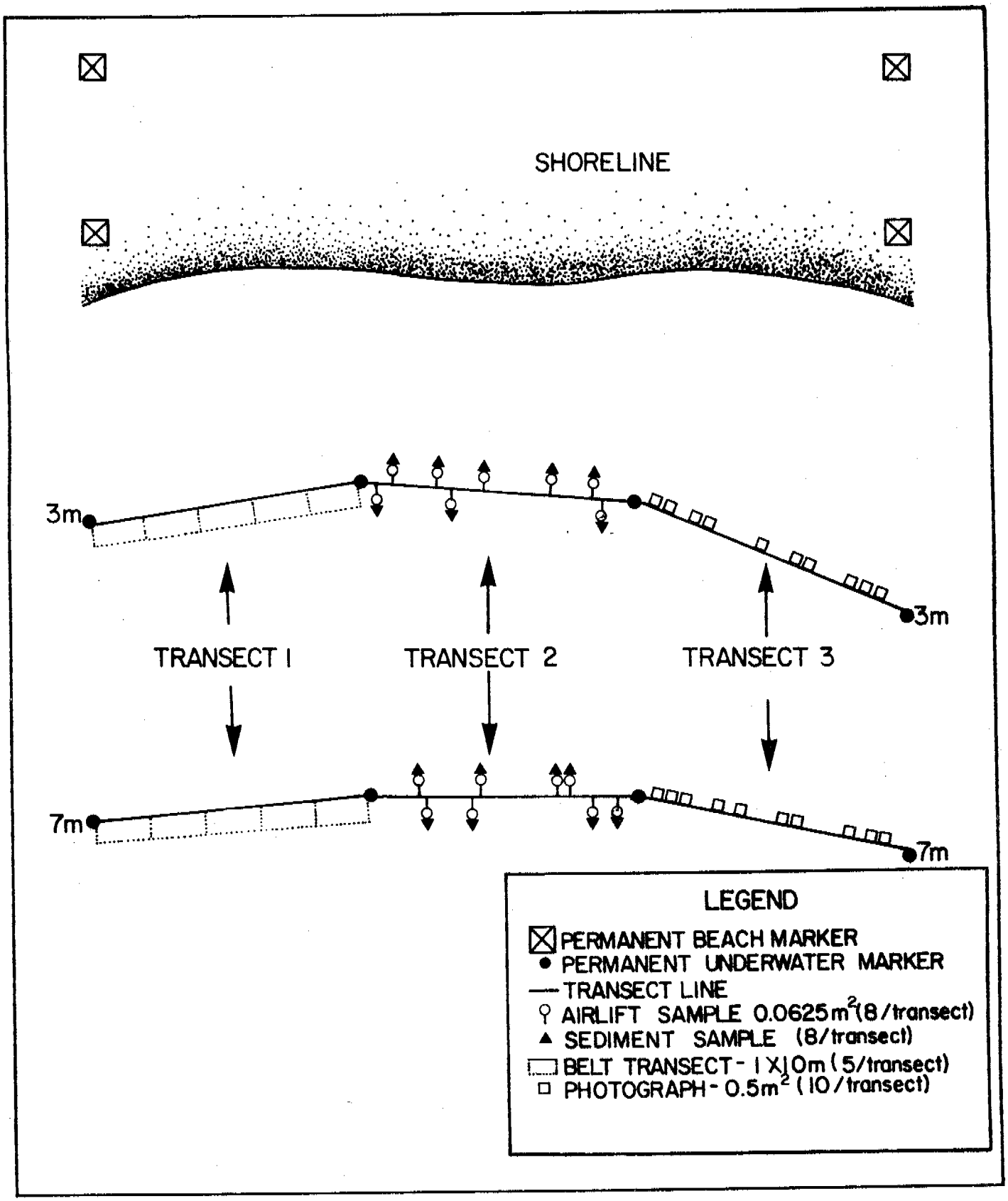


FIG. 2. Schematic representation of sampling design for BIOS benthic study. Each of the four sample types shown in the legend was collected on each of three transects at each of two depths in each of three bays. For clarity, only one or two sample types are shown per transect in this figure.

transect in each bay was located immediately inshore of the 7 m transect at a depth of 3 m, where a relatively even cover of algae occurred in each bay.

Transect locations at 3 and 7 m depths were marked by driving steel rods approximately 1 m into the substrate at 50 m intervals along a 150 m line. In each bay, sighting lines toward the ends of the lines of transects were established on the shore by placing pairs of markers on the beach.

A 150 m transect rope marked at 1 m intervals was set between the permanent stakes before (and removed after) sampling at each bay/depth combination. Numbered plastic tags attached to the line indicated randomly selected 1 m^2 airlift sampling locations immediately seaward or shoreward of the line; the exact location of the sample within each of these 1 m^2 areas was that with the least amount of surface rock. Photograph locations along each transect were also randomly selected, and were indicated on a list attached to the camera. Sample locations for airlifts and photographs were re-randomized for each transect. In situ counts and supporting collections were made within 1 x 10 m belts along each transect line.

Airlift Sampling

Infauna were sampled by means of a self-contained diver-operated airlift. Eight replicate samples were obtained on each of 3 transects at each of 2 depths in each of 3 bays (total of 144 samples).

The airlift consisted of a weighted 2 m length of pipe 8 cm in diameter fitted at the top with a 1 mm mesh net which retained the sample and could be

removed quickly and capped. Air was supplied from a 3000 psi (20 MPa) air cylinder fitted with the first stage of a diving regulator which reduced air pressure to approximately 125 psi (860 kPa) above ambient. Areas to be sampled were demarcated by a ring containing an area of 0.0625 m^2.

The ring was placed on the bottom and pushed as far as possible into the substrate to contain shallow infauna. The airlift was inserted into the ring, the air was turned on, and the mouth of the airlift was moved around to thoroughly cover the area within the ring. The air was turned off when all visible organisms had been collected, and the net on the airlift was then removed, capped and replaced. The depth of penetration of the airlift into the substrate (range 2-25 cm; mean ± SD = 13.2 ± 4.4) was then measured to the nearest cm, and a sample of surface sediment was taken immediately beside the excavated area. Large rocks remaining within the ring were removed and either weighed underwater using a plastic bucket and a brass fish scale and then discarded, or placed in a numbered sample bag cross-referenced with the airlift sample. After 3 or 4 samples had been taken they were raised to the boat and rinsed in the collecting bags from the side of the boat in order to remove fine sediments. Immediately after each dive all samples were returned to the field laboratory.

Quantitative Photography

A photographic record of each transect was obtained on colour slide film using a Nikonos camera with a 15 mm lens, paired Vivitar electronic flashes and a fixed focus framer covering a bottom area of approximately 0.5 m^2. Ten photographs were taken at randomly located intervals along each transect

line. In addition to providing a permanent visual record of each study area, photographs were used to estimate densities of visible surface fauna that were too sparsely distributed to be represented adequately in airlift samples.

In Situ Counts

Macrophytes and those invertebrates too large and sparsely distributed to be representatively sampled by airlift or camera were counted *in situ*. On each transect, counts of urchins, starfish and individual kelp plants, as well as estimates of percent bottom cover by algae, were made within five 1 x 10 m strips parallel to and immediately adjacent to the transect line. Collections of representative plants and animals were also made for species identification.

Laboratory Analysis Procedures

All samples were processed in the field within 12 h of collection. Samples were emptied into large plastic trays, and nets were carefully rinsed and picked clean. Large conspicuous animals and algae were separated from the sample, labeled and preserved in 10% formalin in separate jars. Large rocks and gravel were picked from the sample and weighed on an Ohaus triple-beam balance. The balance of the sample was labeled and preserved in 10% formalin. Macrophytic algae from systematic transect collections were pressed on herbarium paper and dried at room temperature.

Detailed laboratory processing and analysis of samples was carried out within 1 to 3.5 months of collection. Samples were initially rinsed to remove formalin and sediment, and then separated into 5 fractions. All material passing through a 1 mm mesh screen and retained on a 0.45 mm mesh screen was preserved in alcohol for future reference. A 'floating' fraction, separated by rinsing, contained algae, detritus and most soft bodied animals. This fraction was examined under a binocular microscope and animals $\geq$1 mm in length were sorted into major taxonomic groups; the remaining algae and detritus was blotted dry and weighed on a Mettler PT 200 balance. In 7 of the 144 samples (when large volumes of algae were present), large and conspicuous organisms were picked from the entire sample but only a subsample of known weight was examined microscopically. Different size fractions (1-2.8 mm; 2.8-5.6 mm; $\geq$5.6 mm) of the balance of the sample, separated in nested seives, contained sand, gravel, bivalves and some soft bodied animals. Each fraction was sorted separately in glass trays into major taxonomic groups. Shell fragments from the largest size fraction and entire bivalve shells from each fraction were separated, labeled and stored for future reference. Gravel from the largest size fraction was blotted dry and weighed.

All animals were identified to species level whenever possible; unidentified or tentatively identified species were sent to appropriate authorities for identification or verification (see Acknowledgements). For each taxon identified, individuals were counted, gently blotted dry and weighed together to the nearest milligram on a Mettler PT 200 balance. Unless otherwise specified (see below), all weights presented in this report are preserved (10% formalin) wet weights, including mollusc shells but

excluding polychaete tubes. Lengths of individuals of all bivalve species, and diameters of the calcareous oral rings of the holothurian Myriotrochus rinkii, were measured to the nearest millimetre. After laboratory examination, all taxa were stored in 75% alcohol; a solution of 3% propylene glycol in 75% alcohol was used for crustaceans.

For each of three common bivalve species (Mya truncata, Astarte borealis and Macoma calcarea), the relationships between length, wet weight and dry weight were derived as follows: For each bay, approximately fifty undamaged individuals of each species were selected from airlift samples taken along the middle transect at 7 m depth. If necessary to obtain a sample size of 50 per bay, animals from the inner ends of the outer two transects were also used. For each individual the length, wet weight including shell, wet meat weight, and dry (constant) meat weight were determined. Constant dry weight was obtained by drying at 60°C in a Fisher Isotemp Oven Model 301 and weighing at daily intervals until constant weight was found.

Species identifications of macrophytic algae were carried out on all herbarium specimens. Dominant macrophytes in one randomly selected airlift sample from the 3 m depth in each bay were identified, and a species list was made for subsamples of two of these airlift samples.

Data Processing and Analysis

All data collected in the field and all results from laboratory analyses were coded for computer processing. Computer programs developed by LGL were

used to generate the sample by sample, transect by transect, and bay by bay tabulations that were used to select species and taxa for further analyses. Other LGL programs were used to organize the data into a format acceptable to packaged statistical programs. Prior to analyses a logarithmic transformation (log [x+1]) was applied to density and biomass data in order to reduce the skewness inherent in such data.

Two-factor (depths and bays) fixed-effects analyses of variance with transects nested within depths and bays were used to examine and test the variability in the benthic community. Many of these analyses showed significant bay by depth interactions, so separate single factor (bays) analyses were run on data from each depth; again, transects were nested within bay. Analyses of variance were performed by the SAS computer programs (Helwig and Council 1979).

Factor analysis with varimax rotation (BMDP4M, Dixon and Brown 1977) was used to identify recurring groups of species. Factor scores generated by this analysis were used as dependent variables in a multivariate analysis of variance (SAS general linear models program, Helwig and Council 1979) that was used to test for differences in community composition among bays and between depths.

The appropriate transformation used to determine length-weight relationships of dominant bivalve species was selected after analysis of (1) scatter plots of the original data, and (2) plots of residuals generated by regression analyses. Analyses of covariance with length as the covariate were used to test for differences in dry meat weight among bays. All of these analyses utilized the BMDP computer programs (Dixon and Brown 1977).

The mean lengths of selected bivalve species were calculated for each sample and analyses of variance were used to test whether mean lengths of these species differed among bays or between depths.

Because of the large number of analyses carried out in this study and the even larger number that will be employed throughout the project, some type I errors in statistical inference would be expected if the conventional $\alpha = 0.05$ criterion of statistical significance were applied. Hence, a criterion of $\alpha = 0.01$ was used to distinguish significant ($P \leq 0.01$) from non-significant ($P > 0.01$) results in all univariate analyses. The multivariate analysis of variance is an extremely powerful test and hence we used an $\alpha = 0.05$ criterion in this analysis.

SITE DESCRIPTIONS

The study area for the nearshore component of the 1980 Baffin Island Oil Spill Project consisted of three shallow embayments in Ragged Channel, some 5-8 km SSE of Cape Hatt, Eclipse Sound (72°27'N, 79°51'W). Bays 9 and 10 are shallow indentations in the coastline, each about 500 m in length, separated by the delta of a small stream and a distance of somewhat less than 500 m. Bay 11 has been designated as the lower half (and Bay 12 as the upper half) of a deeper embayment some 1 km x 1 km in dimensions, located approximately 1 km north of Bay 10 (Fig. 1).

Information on the nearshore geology of the study area may be found in Barrie et al. (1981); the following data on grain size distribution are abstracted from that work. Sediments in the nearshore areas of the study

bays were generally coarse to fine sand at the shallowest depths, and increasingly fine with increasing depth (Fig. 3). Sediments at the 3 m sampling depth were fine sand in all 3 bays. At the 7 m depth sediments were very fine sand in Bays 9 and 10 and coarse silt in Bay 11 (Table 1). On a volumetric basis, rock formed between 13.6 and 18.9% of the volume sampled by the airlift sampler at the 3 m depth, and from 7.2 to 16.8% at the 7 m depth. At both depths, airlift samples from Bay 11 contained the smallest amount of rock.

The three study bays (9, 10 and 11) were generally similar in substrate and in floral and faunal characteristics. The beaches and intertidal zones were composed of a gravel/cobble pavement overlying sand with scattered rocks and boulders. At depths of 1-2 m a relatively flat, predominantly sand bottom occurred, and the rockweed Fucus sp., together with a relatively sparse cover of smaller algae and patches of tunicates (including Rhizomolgula globularis) were the only conspicuous biota. In bays 9 and 10 a steep, rocky slope with a relatively dense cover of Fucus sp. occurred between 2 and 3 m depths. At the bottom of this slope, and at an equivalent depth in Bay 11, a zone with a relatively even and nearly complete cover of algae stretched seaward for distances from 1 to 10 m on a substrate that included silt, sand, gravel and larger rocks. In bays 10 and 11 there followed a similarly narrow zone of kelp (predominantly Laminaria spp.) that extended to depths of 4-5 m.

In all bays a relatively fresh surface layer of water was observed during both August and September. On many occasions a distinct boundary was observed at 3-4 m depths, whereas at other times the mixing of fresh and salt

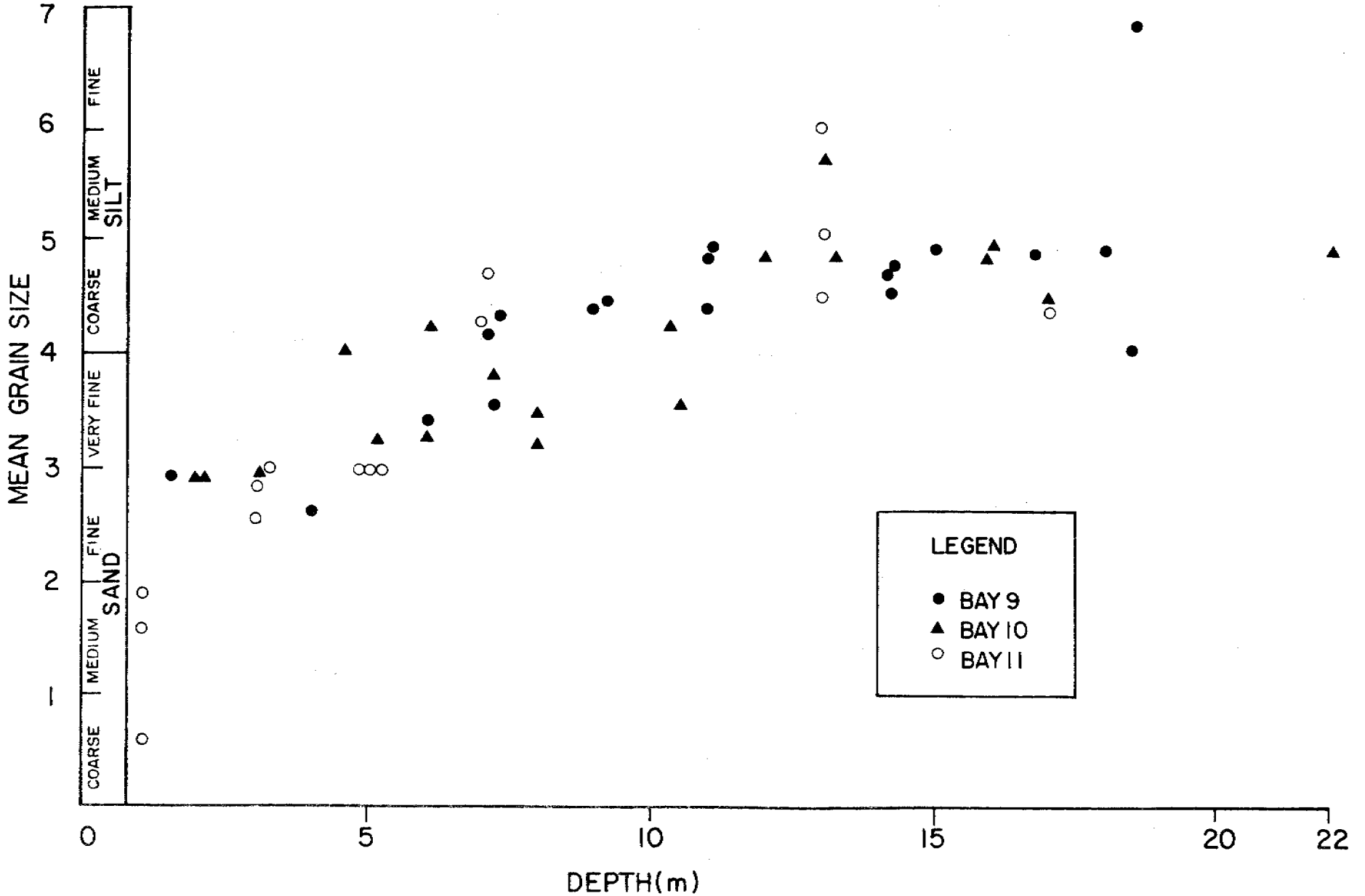


FIG. 3. Depth distribution of mean grain size in sediments from the three study bays at Cape Hatt, northern Baffin Island. Data were supplied by Barrie et al. 1981.

Table 1. Sediment characteristics[1] and volume of sediment and rock sampled by airlift sampler at two depths in three bays at Cape Hatt, northern Baffin Island. For sediment characteristics (lines 1-3), n = 6 (column 2), 4 (col. 1), 3 (col. 5, 6) or 2 (col. 3, 4); for airlift samples (lines 4-6), n = 24 (all columns). Data are expressed as mean ± SD.

	7 m Depth			3 m Depth		
	Bay 9	Bay 10	Bay 11	Bay 9	Bay 10	Bay 11
Mean grain size	3.89 ± 0.43	3.54 ± 0.42	4.50 ± 0.31	2.77 ± 0.22	2.95 ± 0.12	2.77 ± 0.14
Sorting coefficent	2.71 ± 0.45	2.41 ± 0.24	3.52 ± 0.04	2.27 ± 0.15	1.57 ± 0.08	2.97 ± 0.09
Skewness	1.48 ± 0.62	1.47 ± 0.40	0.68 ± 0.11	1.64 ± 0.70	2.96 ± 0.28	1.29 ± 0.08
Airlift penetration depth (cm)	16.4 ± 2.7	15.8 ± 3.7	16.9 ± 3.0	10.7 ± 2.7	11.2 ± 2.7	8.0 ± 2.8
Sediment volume[2] sampled (L)	8.5 ± 1.7	8.7 ± 2.0	9.8 ± 1.8	5.7 ± 1.8	5.6 ± 1.7	4.3 ± 1.7
Rock content of sample (kg)	4.3 ± 1.2	3.1 ± 1.3	1.9 ± 1.0	2.4 ± 0.9	3.3 ± 1.2	1.7 ± 0.9
Rock volume in total volume[3] (%)	16.8	12.6	7.2	14.4	18.9	13.6

[1] From Barrie et al. 1981.
[2] Not including rock.
[3] Including rock.

water was apparent as deep as 7 or 8 m. Recent kills of bivalves, brittle stars, urchins and gastropods, probably resulting from this influx of fresh water, were observed at depths of 3-5 m during both August and September.

In the deeper (5-10 m) portion of the sublittoral zone in each bay, the substrate consisted of an unconsolidated silt veneer overlying a mixture of silt, sand, gravel and considerable amounts of cobble and rock. Sparsely distributed boulders and large rocks colonized by the kelps Laminaria sp. and Agarum cribrosum were common in bays 10 and 11, and less common in Bay 9. In all bays the conspicuous infauna were the bivalve Mya truncata and the fan worm Chone infundibuliformis, and fauna commonly occurring on the substrate surface included the urchin Strongylocentrotus droebachiensis, the sea star Leptasterias groenlandicus and several species of brittle stars. The relative densities of these and other organisms, less conspicuous due to size or habit, are presented in the following section.

RESULTS AND DISCUSSION

The benthos in the study bays at Cape Hatt is comprised of a wide variety of animals which, for the purposes of the present study, have been classified into two groups according to their relative mobility. The term infauna will be used to refer to those animals that are either incapable of motion or are only able to move slowly in the sediment or on the sediment surface. This group includes bivalves, polychaetes, gastropods, priapulids, nemerteans and some echinoderms. The term epibenthos will be used for those animals capable of relatively rapid motion, including amphipods, cumaceans and ostracods, and large echinoderms capable of moving relatively

large distances on the sediment surface (urchins and starfish). Both of these groups are included in the infauna as defined by Thorson (1957).

The infauna and epibenthos (as defined above) will be treated separately in the present study. Most analysis and discussion will concern infauna, primarily because their relative immobility will expose them to the full impact of oil or dispersed oil and facilitate the interpretation of results. With mobile epibenthos, it is often impossible to distinguish between mortality and emigration as the cause of disappearance following an oilspill (e.g. Elmgren et al. 1980). Infauna are also of interest because of their dominance of total benthic biomass (99.4% in the study bays at Cape Hatt), and because of their long life spans in the Arctic (Curtis 1977; Petersen 1978). The latter further facilitates interpretation of results because it is indicative of reduced seasonal and annual variability.

Infauna

Sampling Efficiency

Preliminary sampling in August 1980 indicated that all of the species and most of the individuals found in the Cape Hatt benthic community could be sampled adequately with a sampler penetration depth of no more than 8 to 10 cm. However, a large proportion of the benthic biomass was contributed by large individuals of the bivalve Mya truncata, which occurred to depths of 15 cm in the sediment. Mean depth of penetration of the airlift sampler used in the present study was at least 15 cm at the 7 m depth in each of the bays (Table 1). Visual and tactile inspection of sampling plots by divers during

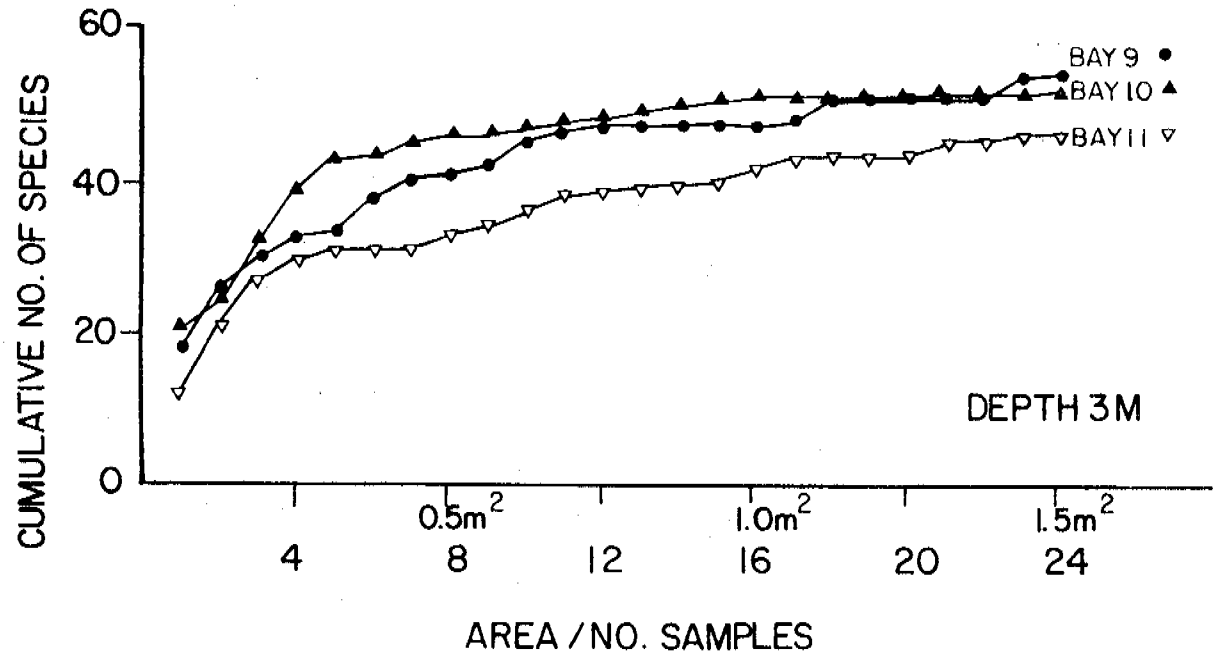
and after sampling insured that all large individuals of Mya truncata were collected by the sampler. Sampler penetration was shallower at the 3 m depth in all bays (Table 1) due to the presence of a consolidated inpenetrable sediment layer and/or rock. Inspection of the sampling plots insured that all large individuals of Mya truncata were collected.

Although the area and depth of sediment sampled by the airlift could be accurately controlled by the divers, the amount of rock in the sampling plots was variable (Table 1) and hence the volume of sediment sampled was also variable. The effect of a variable volume of sediment on the abundance of infauna under a fixed area was assessed by regression analysis (BMDP1R, Dixon and Brown 1977). The biomasses and densities of polychaetes, bivalves and total infauna were regressed against volume of sediment removed by the airlift sampler minus the volume of rock. The results (Table 2) show that only the biomass of polychaetes in Bay 11 at 3 m and the biomasses of bivalves and total infauna in Bay 9 at 7 m were significantly related to variable sediment volume. Volume of rock in the sediment does not appear to have been a major factor influencing the quantity of animals collected in the samples.

Species-area curves are useful in determining the area that must be sampled in order to yield a representative estimate of the number of species present. The curves shown on Fig. 4 show the cumulative number of species that have been collected after an increasing number of samples has been considered at each depth in each bay. At both depths the curves flatten after 0.5 to 1.0 m^2 has been sampled. Depending on depth and bay, the number of species found in 1.0 m^2 (16 samples) represented 87 to 98% of the total number of species collected in all 24 samples.

Table 2. Significance levels for regression of quantity of polychaetes, bivalves and total infauna vs. volume of sediment sampled. Sedement volume adjusted for volume of rock; NS = $P>0.01$, ** = $P\leq 0.01$.

	Depth ->	7 m				3 m			
	Bay ->	9	10	11	All	9	10	11	All
Taxon	Sample size ->	24	24	24	72	24	24	24	72
Polychaeta	(no./m^2)	NS	NS	NS	NS	NS	NS	NS	NS
	(g/m^2)	NS	NS	NS	NS	NS	NS	**	NS
Bivalvia	(no./m^2)	NS	NS	NS	NS	NS	NS	NS	NS
	(g/m^2)	**	NS	NS	NS	NS	NS	NS	NS
Total infauna	(no./m^2)	NS	NS	NS	NS	NS	NS	NS	NS
	(g/m^2)	**	NS	NS	NS	NS	NS	NS	NS



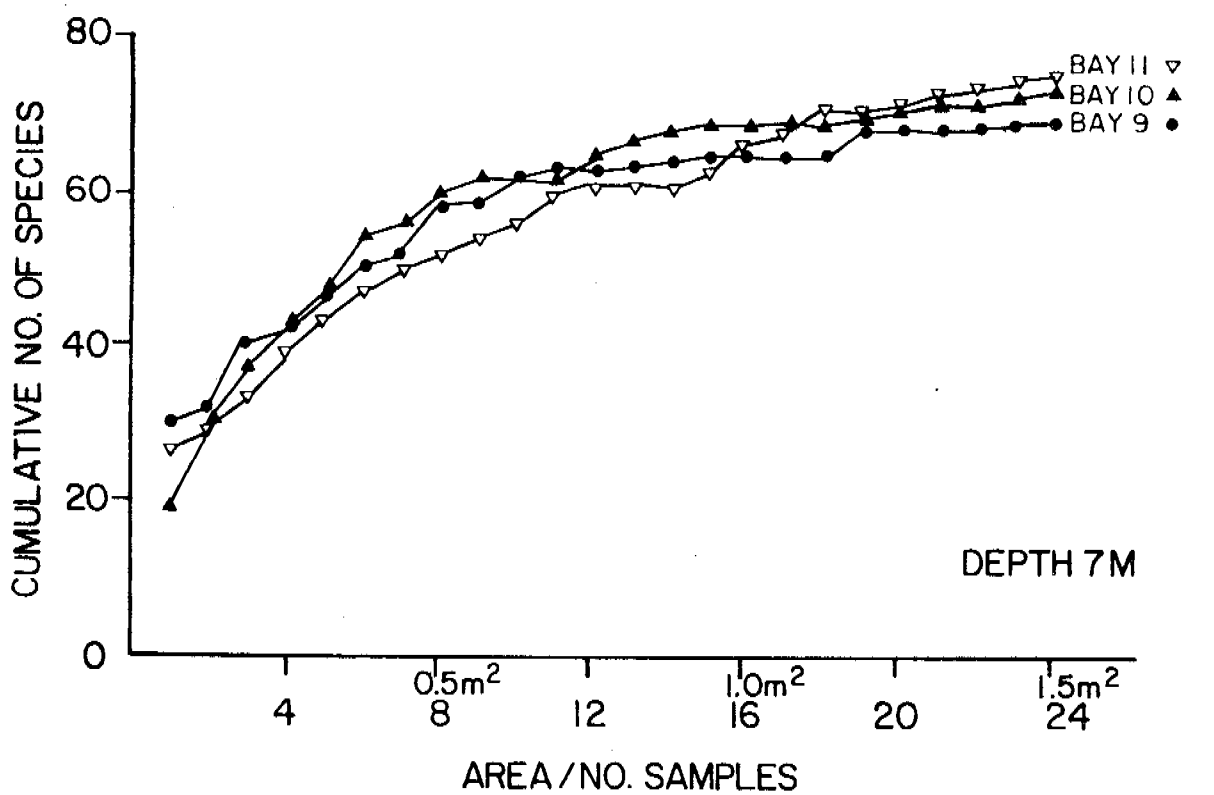


FIG. 4. Species-area curves for each of the three experimental bays at Cape Hatt, northern Baffin Island. Only the infauna is considered.

The penetration depth and the total area sampled by the airlift in each bay appear to be sufficient to yield samples that adequately represent the types and quantities of animals present.

Group and Species Composition

Group composition of the infauna collected in the study area at Cape Hatt (all bays and depths considered) is shown in Table 3. Bivalves accounted for most of the biomass (93.4%) and bivalves and polychaetes, in approximately equal proportions, accounted for most of the numbers of animals collected (85.6%).

The most common animals taken from samples at Cape Hatt are shown in Table 4. Twelve species accounted for 93.2% of the infaunal biomass and a partially overlapping list of 12 taxa accounted for 65.5% of the numbers of animals collected. Only four species were dominant (i.e. among the top 12 species) in terms of both biomass and density: Mya truncata, Astarte borealis, Astarte montagui and Macoma calcarea (Table 4).

In general, the benthos of the study area at Cape Hatt appears to be typical of that in nearshore high arctic areas. Several of the dominant infaunal species, including several of those contributing most to biomass (Mya truncata, Macoma calcarea, M. moesta, Astarte borealis, A. montagui, Serripes groenlandicus, and Cistenides granulata), belong to the arctic Macoma community (Thorson 1957; Ockelmann 1958; Ellis 1960; Thomson MS). This community is a widespread and common feature of nearshore high arctic areas and is displaced only under local circumstances (e.g. under estuarine

Table 3. Group composition of infauna collected in the study bays at Cape Hatt, northern Baffin Island, during September 1980. Based on 144 samples, each covering 0.0625 m^2, from 3 and 7 m depths.

Taxon	% of total biomass	% of total density
Bivalvia	93.40	45.36
Polychaeta	3.75	40.27
Gastropoda	1.14	8.27
Echinoidea	0.98	0.04
Holothuroidea	0.36	4.02
Ophiuroidea	0.15	0.06
Asteroidea	0.05	0.77
Ascidiacea	0.03	0.08
Other	0.14	1.11
Total infauna	1170.8 g/m^2	2904.9 $ind./m^2$

Table 4. Percent contribution to total infauna by dominant species in the study bays at Cape Hatt, northern Baffin Island. Based on 144 samples, each covering 0.625 m^2, from 3 and 7 m depths.

Biomass		Density	
Dominant species	% of total infauna	Dominant species	% of total infauna
Mya truncata (B)	50.9	*Phloe minuta* (P)	11.8
Astarte borealis (B)	18.4	*Thyasiridae* sp. (B)	8.3
Serripes groenlandicus (B)	8.4	*Astarte borealis* (B)	8.1
Astarte montagui (B)	4.4	*Nereimyra punctata* (P)	6.9
Hiatella arctica (B)	2.9	*Mya truncata* (B)	6.1
Macoma calcarea (B)	2.9	*Astarte montagui* (B)	5.1
Cistenides granulata (P)	1.1	*Astarte* sp. juveniles (B)	4.2
Strongylocentrotus droebachiensis (E)	1.0	*Myriotrochus rinkii* (H)	4.0
Musculus niger (B)	0.9	*Euchone analis* (P)	3.8
Musculus discors (B)	0.9	*Chaetozone setosa* (P)	2.8
Macoma moesta (B)	0.8	*Cingula castanea* (G)	2.2
Trichotropis borealis (G)	0.6	*Macoma calcarea* (B)	2.2
Total % contribution	93.2	Total % contribution	65.5
Biomass of all infauna (g/m^2)	1170.8	Density of all infauna (no./m^2)	2904.9

B = bivalve, P = polychaete, G = gastropod, H = holothurian, E = echinoid.

influences). A quantitative analysis of community structure in the study bays is presented in a following section.

Biomass

Average infaunal biomass in the study area at Cape Hatt (all bays and depths considered) was 1171 g/m^2 (Table 4); at 3 m and 7 m depths, respectively, mean biomass was 328 g/m^2 and 2013 g/m^2. These values are considerably higher than mean depth-integrated biomass (5 to 50 m) in other arctic areas (Table 5).

Table 5. Mean depth-integrated biomass (g/m^2) of benthic infaunal animals from arctic areas. Only the depth range from 5 to 50 m is considered.

Location*	Sample size	Mean biomass (g/m^2)	Source
Alaskan Beaufort Sea	131	41	Carey (1977)
Bridport Inlet, Melville Is.	78	94	Buchanan et al. (1977)
Brentford Bay, Boothia Pen.	21	188	Thomson et al. (1978)
Lancaster Sound	110	319	Thomson and Cross (1980)
Northern Baffin Is.	51	200-438	Ellis (1960)

* Relatively high biomass (up to 1482 g/m^2) has also been reported at locations in West Greenland (Vibe 1939).

The apparently high infaunal biomass at Cape Hatt relative to that in other arctic locations is likely due largely to the effectiveness of our sampler. About half of the biomass found at the 7 m depth at Cape Hatt represented Mya truncata. Preliminary sampling indicated that this deeply burrowing species was only sampled effectively if the sediment was excavated to a depth of 15 cm. Buchanan et al. (1977) compared results of quantitative underwater photographs with those of shallow penetrating samples and found that their shallow samples underestimated infaunal biomass by as much as 960

g/m^2. Many of the other low values reported in Table 5 may also be due to inadequate sampling.

In most high arctic areas a barren zone extends from the shoreline to depths of 2 to 10 m. This zone is devoid of infauna except for the tunicate Rhizomolgula globularis, and is populated almost exclusively by motile amphipods. At Cape Hatt, however, a relatively high infaunal biomass consisting mainly of bivalves was found at the 3 m depth. Here and elsewhere in Eclipse Sound the barren zone occurs only at shallower depths, likely due to the relatively protected location (Thomson and Cross 1980).

Distribution--Analysis of Variance

At the end of the experiment, the overall test for an effect of oil and/or oil and dispersant will be a test for any change in benthic community composition. This test will be based on a multivariate analysis of variance (see below). This analysis will compare temporal changes (if any) in the experimental bays with temporal changes in the control bay. This overall test will be supplemented with similar analyses of the density and biomass of major infaunal groups and selected species. The analyses outlined in the present report describe the nature of variablility of these groups and species under pristine conditions. These data on pre-spill conditions, along with similar data to be collected just before the spill in 1981, will be the basis against which post-spill data will be compared.

Mean density (no./m^2) and biomass (g/m^2) of bivalves, polychaetes, total infauna and species that are dominant either in terms of density or biomass

are given in Tables 6 and 7. Considerable spatial variability is evident for most groups and species at all scales, viz. among samples within transects, among transects within depths and bays, between depths, and among bays. This section describes the results of statistical procedures used to examine the relative magnitudes and significances of these sources of variation. The smallest-scale variability, that among replicate samples within transects, is used as a basis of comparison for variation among transects. Among-transect variation, in turn, is used to determine the significance of variation among bays and between depths.

The smallest-scale variability in infaunal distribution that can be examined in the present study is that occurring among the eight samples (each covering 1/16 m^2) along each of the 50 m transects. The amount of within-transect variability is indicated by the coefficients of variation (CV = SD/Mean, expressed as a percent); a low CV is indicative of an even distribution; a high CV (e.g., where CV>100%) is indicative of a patchy distribution. For groups of species (bivalves, polychaetes and total infauna), the CV always was less than 100% at the 7 m depth, and usually was <100% at the 3 m depth (23 of 27 cases for biomass, 26 of 27 for density). The distributions of the individual species listed in Tables 6 and 7, however, were less even, especially at the 3 m depth. At 3 m, the CV for biomass was <100% in only 6 of 45 cases (13%), and that for density was <100% in only 27 of 50 cases (54%). At a depth of 7 m, the coefficient of variation for biomass was >100% in 35 of 54 species/transect combinations (65%), and that for density was >100% in 35 of 49 combinations (71%). A more detailed analysis of small-scale distribution will not be presented here, but inspection of the data in Tables 6 and 7 shows that distributions range from

Table 6. Mean density (no./m^2) of major taxa and dominant species of infauna on transects at two depths in three bays at Cape Hatt, northern Baffin Island, during September 1980. Data are expressed as mean ± standard deviation and are based on 8 replicate 0.0625 m^2 airlift samples at each depth and transect.

Taxa	Transect	3 m Depth			7 m Depth		
		Bay 9	Bay 10	Bay 11	Bay 9	Bay 10	Bay 11
Total infauna[1]	1	3226.0 ± 462.7	3494.0 ± 1548.0	1470.0 ± 1218.5	3866.0 ± 1082.5	2974.0 ± 1214.0	2790.0 ± 483.9
	2	3370.0 ± 950.4	2894.0 ± 653.3	1804.0 ± 764.3	3954.0 ± 1475.4	2772.0 ± 601.3	2562.0 ± 663.1
	3	4700.0 ± 832.0	2652.0 ± 448.0	1184.0 ± 379.0	2918.0 ± 1250.9	2774.0 ± 722.9	2804.0 ± 756.7
	All	3765.3 ± 1005.1	3013.3 ± 1025.2	1486.0 ± 860.4	3759.3 ± 1313.3	2840.0 ± 852.6	2743.3 ± 631.2
Polychaeta	1	2016.0 ± 407.7	1604.0 ± 529.4	840.0 ± 613.0	880.0 ± 235.0	884.0 ± 383.9	828.0 ± 252.7
	2	1536.0 ± 280.8	1760.0 ± 638.7	1174.0 ± 468.7	910.0 ± 363.2	948.0 ± 301.5	738.0 ± 217.8
	3	2086.0 ± 782.6	1432.0 ± 462.9	618.0 ± 229.0	850.0 ± 409.7	988.0 ± 325.4	962.0 ± 319.7
	All	1879.3 ± 568.7	1598.7 ± 541.7	877.3 ± 501.6	880.0 ± 329.7	940.0 ± 326.6	842.7 ± 271.7
Bivalvia	1	646.0 ± 245.3	1258.0 ± 789.1	228.0 ± 402.1	2656.0 ± 783.0	1850.0 ± 965.2	1718.0 ± 515.0
	2	992.0 ± 615.9	598.0 ± 327.0	418.0 ± 246.6	2388.0 ± 725.4	1676.0 ± 434.2	1526.0 ± 521.3
	3	1672.0 ± 371.5	866.0 ± 286.4	264.0 ± 139.2	1808.0 ± 760.6	1654.0 ± 635.4	1502.0 ± 498.6
	All	1103.3 ± 604.4	907.3 ± 568.9	303.3 ± 284.1	2284.0 ± 808.4	1726.7 ± 686.9	1582.0 ± 498.8
Mya truncata	1	278.0 ± 170.4	310.0 ± 337.7	60.0 ± 81.0	238.0 ± 61.3	208.0 ± 110.2	158.0 ± 65.4
	2	244.0 ± 205.6	180.0 ± 123.9	66.0 ± 55.7	170.0 ± 75.5	150.0 ± 90.9	162.0 ± 87.8
	3	326.0 ± 89.7	186.0 ± 76.9	46.0 ± 32.5	120.0 ± 73.1	100.0 ± 62.7	208.0 ± 107.5
	All	282.7 ± 159.1	225.3 ± 211.9	57.3 ± 57.8	176.0 ± 83.3	152.7 ± 97.2	176.0 ± 87.8
Astarte borealis	1	68.0 ± 124.5	80.0 ± 89.7	20.0 ± 40.8	372.0 ± 253.4	320.0 ± 295.8	454.0 ± 183.2
	2	196.0 ± 129.6	68.0 ± 127.4	18.0 ± 23.3	610.0 ± 257.2	354.0 ± 163.3	320.0 ± 131.4
	3	316.0 ± 102.2	24.0 ± 29.6	22.0 ± 28.3	290.0 ± 200.3	386.0 ± 216.9	334.0 ± 256.2
	All	193.3 ± 154.0	57.3 ± 90.9	20.0 ± 30.3	424.0 ± 266.6	353.3 ± 223.2	369.3 ± 198.0
Astarte montagui	1	8.0 ± 17.1	16.0 ± 39.2	2.0 ± 5.7	240.0 ± 228.8	142.0 ± 103.5	554.0 ± 245.3
	2	114.0 ± 118.6	0	4.0 ± 11.3	182.0 ± 105.1	126.0 ± 119.6	418.0 ± 286.1
	3	288.0 ± 198.0	0	0	114.0 ± 69.7	154.0 ± 91.3	306.0 ± 215.6
	All	136.7 ± 173.8	5.3 ± 23.0	2.0 ± 7.2	178.7 ± 153.4	140.7 ± 101.4	426.0 ± 261.0
Thyasiridae sp.	1	62.0 ± 77.2	178.0 ± 113.0	12.0 ± 28.0	740.0 ± 232.3	400.0 ± 265.7	88.0 ± 115.1
	2	230.0 ± 183.2	108.0 ± 75.4	0	518.0 ± 233.9	552.0 ± 210.7	62.0 ± 33.6
	3	370.0 ± 311.6	88.0 ± 96.0	12.0 ± 22.2	512.0 ± 186.2	348.0 ± 216.0	58.0 ± 75.0
	All	220.7 ± 241.1	124.7 ± 99.9	8.0 ± 20.6	590.0 ± 235.3	433.3 ± 238.8	69.3 ± 79.2
Euchone analis	1	236.0 ± 154.1	108.0 ± 127.9	160.0 ± 123.6	10.0 ± 22.5	2.0 ± 5.7	18.0 ± 32.5
	2	288.0 ± 155.8	258.0 ± 130.9	38.0 ± 43.6	2.0 ± 5.7	14.0 ± 23.3	0
	3	760.0 ± 744.1	52.0 ± 35.0	40.0 ± 41.9	0	2.0 ± 5.7	6.0 ± 17.0
	All	428.0 ± 491.0	139.3 ± 135.9	79.3 ± 95.7	4.0 ± 13.6	6.0 ± 14.8	8.0 ± 21.6
Myriotrochus rinkii	1	290.0 ± 201.9	160.0 ± 235.5	114.0 ± 124.9	104.0 ± 98.6	0	2.0 ± 5.7
	2	332.0 ± 180.4	148.0 ± 60.9	80.0 ± 49.9	92.0 ± 102.9	0	0
	3	428.0 ± 128.5	118.0 ± 60.4	82.0 ± 42.3	76.0 ± 111.4	2.0 ± 5.7	76.0 ± 99.6
	All	350.0 ± 175.5	142.0 ± 139.4	92.0 ± 79.4	90.7 ± 100.5	0.7 ± 3.3	26.0 ± 65.9

[1] All taxa but ostracods, cumaceans and amphipods.

Table 7. Mean biomass (g/m^2) of major taxa and dominant species of infauna on transects at two depths in three bays at Cape Hatt, northern Baffin Island, during September 1980. Data are expressed as mean ± standard deviation and are based on 10% formalin wet weight in 8 replicate 0.0625 m^2 airlift samples at each depth and transect.

Taxa	Transect	3 m Depth			7 m Depth		
		Bay 9	Bay 10	Bay 11	Bay 9	Bay 10	Bay 11
Total infauna[1]	1	254.8 ± 163.7	401.1 ± 297.1	113.4 ± 179.0	3632.7 ± 1115.9	1983.6 ± 1033.4	1388.6 ± 617.9
	2	596.5 ± 420.8	246.4 ± 184.0	93.3 ± 61.0	2959.2 ± 651.2	1383.9 ± 733.1	1728.1 ± 890.8
	3	936.5 ± 306.9	265.2 ± 154.7	43.2 ± 29.0	1934.5 ± 847.8	1423.7 ± 277.5	1689.3 ± 1073.2
	All	595.9 ± 414.2	304.2 ± 222.3	83.3 ± 109.8	2842.1 ± 111.5	1597.1 ± 768.3	1602.0 ± 855.7
Bivalvia	1	207.4 ± 160.0	341.2 ± 273.1	75.6 ± 141.8	3523.9 ± 1142.3	1879.9 ± 1036.7	1239.2 ± 595.6
	2	528.7 ± 423.1	209.9 ± 185.0	62.9 ± 57.0	2874.3 ± 672.3	1302.9 ± 750.4	1622.4 ± 886.5
	3	870.5 ± 296.2	233.2 ± 152.5	24.9 ± 25.4	1818.9 ± 842.9	1338.7 ± 269.0	1528.5 ± 1019.8
	All	535.5 ± 406.8	261.4 ± 208.8	54.5 ± 88.3	2739.0 ± 1125.2	1507.2 ± 770.2	1463.4 ± 831.5
Polychaeta	1	33.2 ± 13.0	47.3 ± 30.0	18.7 ± 19.0	48.8 ± 39.8	61.9 ± 46.8	45.1 ± 19.2
	2	44.8 ± 13.1	29.1 ± 12.6	25.2 ± 12.3	66.0 ± 39.2	75.0 ± 51.4	42.9 ± 22.2
	3	40.6 ± 16.2	23.0 ± 9.2	11.6 ± 4.8	49.7 ± 33.6	54.4 ± 29.2	72.4 ± 35.0
	All	39.5 ± 14.4	33.1 ± 21.4	18.5 ± 14.0	54.8 ± 36.9	63.8 ± 42.5	53.5 ± 28.7
Mya truncata	1	150.3 ± 159.6	176.7 ± 235.2	38.2 ± 82.2	2314.5 ± 1048.6	1018.6 ± 792.3	517.0 ± 492.6
	2	250.9 ± 180.7	156.2 ± 169.4	9.6 ± 8.4	1528.8 ± 750.1	728.0 ± 583.2	1066.8 ± 679.7
	3	334.5 ± 165.1	97.4 ± 101.4	4.8 ± 7.6	1149.4 ± 492.4	430.8 ± 287.2	756.6 ± 680.3
	All	245.2 ± 178.6	143.4 ± 172.8	17.6 ± 48.2	1664.2 ± 908.5	725.8 ± 616.2	780.1 ± 638.9
Astarte borealis	1	13.5 ± 22.2	54.6 ± 104.9	4.4 ± 8.6	293.5 ± 275.1	366.6 ± 464.9	404.0 ± 240.7
	2	164.9 ± 182.9	15.3 ± 27.0	19.9 ± 44.2	475.2 ± 375.6	287.9 ± 218.6	256.8 ± 225.9
	3	339.4 ± 121.2	28.8 ± 46.0	10.3 ± 17.6	189.5 ± 120.2	426.6 ± 222.9	528.8 ± 734.6
	All	172.6 ± 182.5	32.9 ± 67.0	11.6 ± 27.4	319.4 ± 291.4	360.4 ± 314.4	396.5 ± 458.6
Astarte montagui	1	1.5 ± 2.9	9.3 ± 19.3	0.3 ± 0.7	78.1 ± 84.3	43.3 ± 26.8	177.7 ± 78.8
	2	49.9 ± 56.6	0	0.1 ± 0.2	50.7 ± 42.3	45.9 ± 44.8	154.6 ± 119.9
	3	98.3 ± 75.1	0	0	45.6 ± 45.0	64.9 ± 56.0	109.7 ± 77.4
	All	49.9 ± 65.7	3.1 ± 11.5	0.1 ± 0.4	58.2 ± 59.5	51.4 ± 43.4	147.3 ± 94.5
Serripes groenlandicus	1	13.2 ± 21.8	0	6.6 ± 18.7	429.0 ± 267.6	189.2 ± 182.6	15.4 ± 31.1
	2	21.5 ± 37.7	3.5 ± 9.8	0	462.5 ± 558.2	110.1 ± 193.5	40.0 ± 79.9
	3	6.1 ± 15.3	0	0	190.1 ± 98.2	260.4 ± 238.4	29.8 ± 67.7
	All	13.6 ± 26.3	1.2 ± 5.7	2.2 ± 10.8	360.5 ± 367.3	186.6 ± 206.8	28.4 ± 61.1
Hiatella arctica	1	26.4 ± 35.2	53.9 ± 97.3	1.4 ± 3.3	185.5 ± 207.3	4.0 ± 5.7	13.0 ± 36.7
	2	18.2 ± 31.4	15.5 ± 41.3	8.5 ± 20.9	109.2 ± 127.8	0.3 ± 0.9	0.1 ± 0.1
	3	57.5 ± 74.1	0.9 ± 1.8	0.1 ± 0.2	85.4 ± 241.6	14.9 ± 27.8	10.4 ± 24.1
	All	34.0 ± 51.5	23.4 ± 62.6	3.3 ± 12.3	126.7 ± 194.2	6 .4 ± 16.9	7.8 ± 24.9
Macoma calcarea	1	0.2 ± 0.7	6.3 ± 8.8	7.2 ± 10.1	100.5 ± 40.1	60.2 ± 38.5	54.3 ± 28.6
	2	11.2 ± 21.7	6.2 ± 7.6	0	68.0 ± 44.3	70.0 ± 48.3	33.9 ± 36.0
	3	15.7 ± 15.0	1.2 ± 1.8	0	50.7 ± 22.9	70.8 ± 41.6	47.9 ± 48.1
	All	9.0 ± 16.0	4.6 ± 6.9	2.4 ± 6.6	73.1 ± 41.1	67.0 ± 41.4	45.4 ± 37.7

[1] All taxa but ostracods, cumaceans and amphipods.

relatively even to relatively patchy, depending on species. The number of species for which data are presented is admittedly small, and even greater extremes in variability are expected among the large number of less common species that occur in the study area.

Two types of nested analysis of variance were carried out: two-factor (bays, depths) analyses in which all data were included, and one-factor (bays) analyses in which data from the two depths were analyzed separately. In each case transects were nested within bays and (if considered together) depths. An additional source of variation in the two-factor analysis is the interaction between bay and depth effects. Where this term is significant, the pattern of among-bay variation differs from depth to depth; in this circumstance the interpretation of main effects (bay and depth) is confounded, and separate one-factor analyses must be carried out. In the following sections the results of both one- and two-factor ANOVAs are presented.

Transect Effects

Of the three groups of organisms whose densities and biomasses were examined by ANOVA (bivalves, polychaetes and total infauna), variation among transects was significant only in the case of bivalve density (Table 8). However, among the individual species whose densities were examined, among-transect variation was significant for all but *Mya truncata*. For each of these cases where two-factor ANOVA indicated significant among-transect variation, one-factor ANOVA's showed the variation to be significant at only one of the two depths. Densities of total bivalves, *Astarte montagui*,

Table 8. One- and two-factor[1] analyses of variance (ANOVA) for the biomasses and densities of major taxa and selected infaunal species in the study bays at Cape Hatt. Transects are nested within depths and bays; bay, depth and bay-depth interaction effects are tested over the transect MS, and transect effects over the residual MS. F-values are shown with associated significance levels (ns = P>0.01; ** P≤0.012, *** P≤0.001).

		Two-factor analyses (3 and 7 m depth, n = 144)				One-factor analyses[3]			
						3 m depth (n = 72)		7 m depth (n = 72)	
Source of variation →		Bay	Depth	Bay x Depth	Transect	Bay	Transect	Bay	Transect
df →		2,12	1,12	2,12	12,126	2,6	6,63	2,6	6,63
Biomass (g/m^2)	Total infauna	10.07[2]	36.77[2]	8.18 **	1.84 ns	17.21 **	2.05 ns	8.28 ns	1.27 ns
	Polychaeta	5.91 ns	31.32 ***	6.04 ns	1.46 ns	8.81 ns	1.66 ns	0.69 ns	1.21 ns
	Bivalvia	22.49[2]	143.93[2]	9.16 **	1.83 ns	16.95 **	2.01 ns	8.74 ns	1.17 ns
Density ($no./m^2$)	Total infauna	28.1[2]	8.46[2]	14.59 ***	1.31 ns	28.79 ***	1.44 ns	3.52 ns	1.06 ns
	Polychaeta	13.27[2]	28.43[2]	10.65 **	1.43 ns	14.17 **	2.17 ns	0.79 ns	0.52 ns
	Bivalvia	10.18 **	45.90 ***	5.47 ns	2.98 **	8.00 ns	3.43 **	5.29 ns	1.04 ns
	Mya truncata	16.88[2]	8.17[2]	19.19 ***	0.80 ns	33.13 ***	0.49 ns	0.37 ns	2.94 ns
	Astarte borealis	4.24 ns	52.46 ***	3.62 ns	2.95 **	4.23 ns	3.10 ns	0.23 ns	1.87 ns
	Astarte montagiu	4.80 ns	62.48 ***	6.16 ns	5.95 ***	5.08 ns	10.27 ***	10.82 ns	0.90 ns
	Thyasiridae spp.	27.80 ***	36.08 ***	1.33 ns	3.16 **	11.16 **	3.58 **	53.03 ***	1.36 ns
	Euchone analis	2.16 ns	104.46 ***	3.67 ns	3.01 **	4.15 ns	3.92 **	0.20 ns	2.00 ns
	Myriotrochus rinkii	12.76 **	53.17 ***	1.28 ns	2.90 **	5.41 ns	2.11 ns	7.81 ns	3.56 **

[1] One-factor ANOVA (bays) for each depth; two-factor ANOVA for bays and depths.
[2] Ambiguous because of significance of bay-depth interaction term.

Thyasiridae spp. and Euchone analis were variable only at the 3 m depth, and the density of Myriotrochus rinkii varied significantly only at the 7 m depth. Among-transect variation in the density of Astarte borealis was not significant at either depth when separate one-factor ANOVA's were considered.

Depth Effects

The significance of the bay-depth interaction effect in many groups and species tested precluded unambiguous interpretation of depth effects for these taxa, but the interaction itself can be considered to be indicative of a depth effect. For all other groups and species where no significant interaction occurred (biomass of polychaetes; densities of bivalves, Astarte borealis, Astarte montagui, Thyasiridae spp., Euchone analis and Myriotrochus rinkii), variation between depths was highly significant ($P<0.001$). Inspection of density and biomass data (Tables 6 and 7) shows that all of these bivalves (including total bivalves) were more abundant, and the biomass of polychaetes was higher, at the 7 m depth. On the other hand, the polychaete E. analis and the holothurian M. rinkii were more abundant at the 3 m depth. Table 7 also indicates that there were higher bivalve biomasses on the deeper transects for Serripes groenlandicus and Macoma calcarea (not tested) and for Mya truncata and total bivalves (significant interaction effects). In the last two cases biomass was higher at 7 m than at 3 m in all bays, but disproportionately so in one bay (>25 x higher at 7 m) than in the other two bays (5.1-6.8 x higher).

Bay Effects

For all groups and species where the bay-depth interaction term was significant (biomass of bivalves and total infauna; density of polychaetes, total infauna and Mya truncata), variation among bays was significant at the 3 m depth but not at the 7 m depth (Table 8). Among the remaining groups and species (those with no significant bay-depth interaction), no significant variation among bays was evident in the biomass of polychaetes or in the density of Astarte borealis, A. montagui and Euchone analis. On the other hand, the densities of bivalves, Thyasiridae spp. and Myriotrochus rinkii did vary significantly among bays. Results of one- and two-factor ANOVAs were consistent for all cases except the densities of bivalves and M. rinkii, where the among-bay variation evident in two-factor ANOVAs was significant at neither depth based on one-factor ANOVAs.

Capitella capitata

The polychaete worm Capitella capitata is an opportunistic species that is often used as an indicator of pollution (Grassle and Grassle 1977; Pearson and Rosenberg 1978). After an oil spill in Buzzards Bay, Massachussets, C. capitata 'monopolized the biologically denuded substrata at the heavily oiled stations for the first eleven months after the spill' (Sanders et al. 1980). At Cape Hatt, the mean density of Capitella capitata in all samples was 27.7 ± SD 71.5 indiv./m^2 (n = 144). It appeared to be most abundant in shallow water, especially in Bay 9 (Table 9).

Table 9. Mean density ± SD (indiv./m^2) of Capitella capitata on transects at two depths in three bays at Cape Hatt, northern Baffin Island. n = 8 for each transect.

	3 m Depth			7 m Depth		
Transect	Bay 9	Bay 10	Bay 11	Bay 9	Bay 10	Bay 11
1	52.0 ± 82.4	52.0 ± 49.7	42.0 ± 27.0	24.0 ± 30.8	8.0 ± 8.6	14.0 ± 15.9
2	106.0 ± 261.9	52.0 ± 28.0	8.0 ± 17.1	18.0 ± 21.7	26.0 ± 41.8	8.0 ± 12.1
3	4.0 ± 7.4	14.0 ± 15.9	10.0 ± 22.5	0.0 ± 0.0	48.0 ± 79.8	12.0 ± 16.6
All	54.0 ± 157.4	39.3 ± 37.4	20.0 ± 26.8	14.0 ± 23.3	27.3 ± 52.6	11.3 ± 14.5

Two factor, nested ANOVA showed that the density of this species differed significantly among transects ($F = 0.005$, $df = 12,126$; $P = 0.005$) indicating a patchy distribution. Variations among bays and between depths, however, were not significant (for bays $F = 2.29$, $df = 2,12$; $P = 0.144$; and for depths $F = 0.94$, $df = 1,12$; $P = 0.351$). The occurrence of this species in the experimental bays at Cape Hatt is fortuitous. Changes in the density of this species as the experiment progresses will be closely monitored.

Size-Frequency Distribution

Exposure to oil may cause size-selective mortality of benthic animals in a variety of ways. Not all life stages of marine animals are equally susceptible to the effects of oil (Rice et al. 1975; Linden 1978). Larval stages are generally more susceptible than are adults (Wells and Sprague 1976). Dow (1978) has demonstrated, on the other hand, an instance of selective mortality of large individuals of a bivalve. The juveniles inhabited clean surface sediments, but as they grew they tended to burrow deeper into the substrate and died when they reached an oil-contaminated layer.

Mean lengths of four bivalve species and oral ring diameters of a holothurian are shown in Table 10. Mean lengths (log transformed) of individuals in each sample were compared among bays and depths, using one- and two-factor nested ANOVAs (Tables 11 to 15). Insufficient data from some bays and depths precluded all-inclusive analyses for three of the species. For species in which mean lengths differed between depths (Mya truncata,

Table 10. Mean lengths (mm) of five species of infaunal benthic animals from three bays at Cape Hatt, northern Baffin Island.

Species		7 m			3 m			7 m	3 m
		Bay 9	Bay 10	Bay 11	Bay 9	Bay 10	Bay 11	All	All
Mya truncata	$\bar{x}$	28.0	19.3	17.6	12.6	9.9	8.9	21.8	11.1
	SD	13.9	13.6	13.0	7.5	7.8	5.4	14.3	7.6
	n	222	178	224	369	296	79	624	744
Macoma calcarea	$\bar{x}$	13.0	15.2	15.2	11.9	10.7	19.4	14.1	11.9
	SD	5.5	6.8	6.9	5.1	4.7	3.8	6.3	5.3
	n	253	146	88	42	29	5	487	76
Astarte borealis	$\bar{x}$	12.7	13.2	13.6	12.8	8.1	10.9	13.1	11.7
	SD	7.0	8.5	8.6	7.5	8.7	7.5	8.0	8.0
	n	633	527	551	290	86	29	1711	405
Astarte montagui	$\bar{x}$	9.8	9.9	10.0	11.1	12.6	5.7	9.9	11.1
	SD	3.5	4.0	3.8	2.7	2.7	3.1	3.8	2.8
	n	268	209	638	205	8	3	1115	216
Myriotrochus rinkii[1]	$\bar{x}$	3.15	4.00	3.56	2.29	2.74	2.70	3.25	2.46
	SD	1.03	-	0.79	1.06	1.06	1.00	0.99	1.07
	n	136	1	39	517	210	137	176	864.00

[1] Diameter of calcareous oral ring.

Table 11. Results of analyses of variance on mean lengths in each sample of Mya truncata and Astarte borealis from three bays at Cape Hatt, northern Baffin Island.

Source	Mya truncata				Astarte borealis			
	df	MS	F	P	df	MS	F	P
Among bays	2,12	0.5387	14.82	0.001	2,12	0.0212	0.79	0.476
Between depths	1,12	2.5558	70.32	0.000	1,12	0.2884	10.75	0.007
Bay x depth interaction	2,12	0.0980	2.70	0.108	2,12	0.0801	2.99	0.088
Among transects within bays	12,124	0.0363	1.29	0.279	12,101	0.0268	0.74	0.709
Error	124	0.0282			101	0.0360		

Table 12. Results of analyses of variance on mean lengths in each sample of Macoma calcarea and Astarte montagui from three bays at Cape Hatt, northern Baffin Island. Only samples from 7 m depth are considered.

Source	Macoma calcarea				Astarte montagui			
	df	MS	F	P	df	MS	F	P
Among bays	2,6	0.0212	0.98	0.428	2,6	0.0014	0.26	0.779
Among transects within bays	6,63	0.0217	1.39	0.233	6,61	0.0054	0.70	0.651
Error	63	0.0157			61	0.0078		

Table 13. Results of analysis of variance on the mean oral ring diameter in each sample of the holothurian Myriotrochus rinkii from three bays at Cape Hatt, northern Baffin Island. Only samples from 3 m depth are considered.

Source	df	MS	F	P
Among bays	2,6	0.0344	7.01	0.027
Among transects within bays	6,57	0.0049	0.60	0.729
Error	57	0.0082		

Table 14. Results of analyses of variance on mean lengths in each sample of *Astarte montagui* and *Myriotrochus rinkii* from Bay 9 at Cape Hatt, northern Baffin Island.

Source	*Astarte montugui*				*Myriotrochus rinkii*			
	df	MS	F	P	df	MS	F	P
Between depths	1,35	0.0148	2.69	0.110	1,36	0.2443	32.53	0.000
Among transects	2,35	0.0004	0.07	0.933	2,36	0.0150	2.00	0.150
Transect x depth interaction	2,35	0.0064	1.15	0.328	2,36	0.0248	3.30	0.048
Error	35	0.0055			36	0.0075		

Table 15. Results of analysis of variance on mean lengths in each sample of the bivalve *Macoma calcarea* from Bays 9 and 10 at Cape Hatt, northern Baffin Island.

Source	df	MS	F	P
Among bays	1,8	0.0183	0.80	0.397
Between depths	1,8	0.1969	8.57	0.019
Bay x depth interaction	1,8	0.0247	1.08	0.329
Among transects within bays	8,60	0.0230	1.78	0.099
Error	60	0.0129		

Astarte borealis, Myriotrochus rinkii), the larger animals were found at the deeper depth. Mean length differed among bays only in the case of Mya truncata. The largest individuals were found in Bay 9. Patchiness (variability among transects within bays and depths) was not significant for any species on the scale tested (50 m), apparently due to rather high within-transects variability. In none of the three instances where tests involving more than one bay were possible was there a significant bay by depth interaction. Thus the main factor related to size was water depth.

In Lancaster Sound and Eclipse Sound, Thomson and Cross (1980) also found some bivalve species to be smaller in shallow water, and attributed this to periodic mortality of shallow water animals. One cause of mortality in the Cape Hatt area could be an effect of the rather pronounced freshwater influence in the bays. Mortality of shallow water animals was in fact observed during the present study (see 'Site Descriptions').

Length-Weight Relationships of Bivalves

Exposure to crude oil may cause physiological changes in marine invertebrates. In bivalves these changes may be reflected in the length-dry weight relationship (Stekoll et al. 1980). The length-dry weight relationship of three bivalve species will be used as an indicator of sublethal effects of oil in the experimental bays at Cape Hatt.

For three species of bivalves, approximately 50 individuals from each of the three bays were measured and weighed. These animals were taken from the middle transect at 7 m depth in each bay:

Species	Sample size	Length (mm)		Dry meat weight (g)	
		Mean	Range	Mean	Range
Mya truncata	144	18.5	3-49	0.119	0.001-5.021
Macoma calcarea	132	12.7	3-28	0.022	0.001-0.230
Astarte borealis	152	12.9	3-38	0.018	0.001-0.467

Analysis of scatter plots of the original data and of residuals produced by regresson analyses indicated that the length-weight relationship of these animals was best expressed by a power curve ($y = ax^b$) rather than by exponential ($y = ae^x$), linear ($y = a + bx$) or logarithmic ($y = a \log x$) functions. Length-weight relationships are shown in Fig. 5.

The resultant regression equations (Table 16) explained, on the basis of the variable length, 86 to 98% of the variance of dry meat weight. Apparent among-bay differences in the slopes and intercepts of the regression lines were assessed with analysis of covariance. Significant ($P<0.01$) among-bay differences were evident in the slopes of the regression lines for Mya truncata and Astarte borealis (Table 16). Individuals of Mya truncata from Bay 10 and Astarte borealis from Bay 9 do not gain as much weight with increasing length as do individuals of the same species from other bays. These differences preclude the second step of the analysis of covariance--the test for differences in mean weight of individuals among bays after compensating for the effect of length on weight. For Macoma calcarea, the among-bay differences in the slope of the length-dry weight regression were not significant (Table 16). After adjusting for length-weight relationships, there was no significant among-bay difference in the dry meat weight of Macoma calcarea.

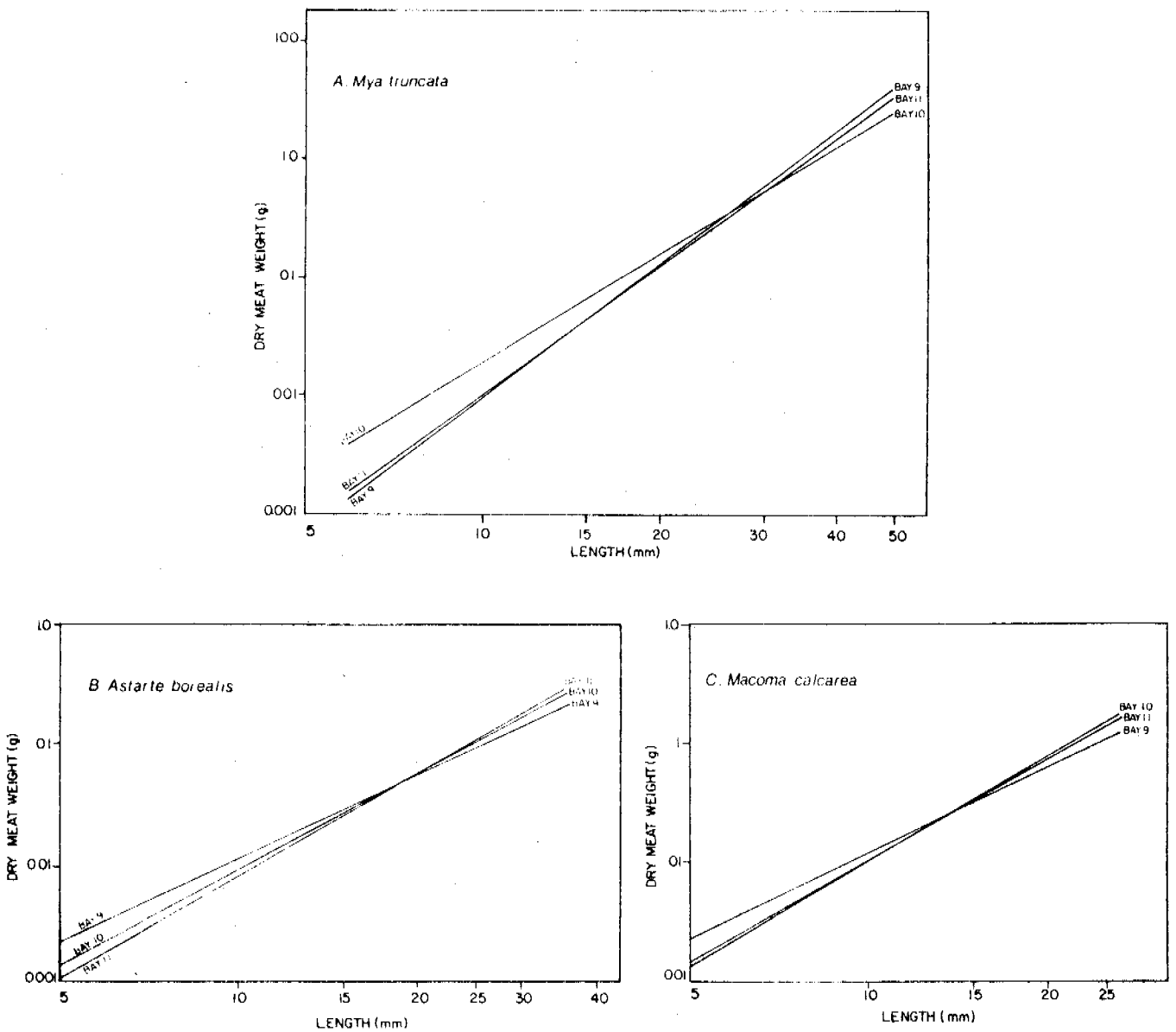


FIG. 5. Least squares regression lines of dry meat weight vs. length for (A) Mya truncata, (B) Astarte borealis and (C) Macoma calcarea from three bays at Cape Hatt, northern Baffin Island.

Table 16. Regressions and analyses of covariance for the length–dry meat weight relationships of three species of bivalve molluses from three bays at Cape Hatt, northern Baffin Island.

	Regression			Covariance			
	Equation[1]	Correlation Coefficent[2] r	Sample size	Adjusted group means (Σ Log wt./n)[3]	Linearized group means (g)[4]	Test of equality of group means	Test of equality of slopes
Mya truncata							
Bay 9	$y = 2.28 \times 10^{-6} x^{3.696}$	0.989	47	-0.93571	0.116		
Bay 10	$y = 14.31 \times 10^{-6} x^{3.112}$	0.929	47	-0.85566	0.139	F = 2.24[5] (df = 2,140)	F = 8.35 (df = 2,138) p = 0.000
Bay 11	$y = 1.78 \times 10^{-6} x^{3.761}$	0.991	50	-0.97601	0.106		
Macoma calcarea							
Bay 9	$y = 3.88 \times 10^{-5} x^{2.493}$	0.957	50	-1.65140	0.022		
Bay 10	$y = 1.46 \times 10^{-5} x^{2.873}$	0.980	45	-1.65293	0.022	F = 0.03 (df = 2,128) p = 0.975	F = 4.42 (df = 2,126) p = 0.014
Bay 11	$y = 1.66 \times 10^{-5} x^{2.830}$	0.980	37	-1.65815	0.022		
Astarte borealis							
Bay 9	$y = 5.35 \times 10^{-5} x^{2.300}$	0.968	50	-1.70059	0.020		
Bay 10	$y = 2.10 \times 10^{-5} x^{2.626}$	0.986	50	-1.75567	0.018	F = 4.37[5] (df = 2,148)	F = 11.36 (df = 2,146) p = 0.000
Bay 11	$y = 1.25 \times 10^{-5} x^{2.792}$	0.984	52	-1.79281	0.016		

[1] y = dry meat weight (g); x = length (mm).
[2] Determined using a log transformation of both variables. The percent of variance explained is 100_r^2.
[3] Mean transformed weight adjusted for the slope of the regression.
[4] Back transformed weight adjusted for the slope of the regression.
[5] Statistical significance not directly testable because of among-bay difference in slopes.

Community Structure

Perturbation of the benthic marine environment often results in large scale changes in the infaunal community structure (Pearson and Rosenberg 1978). Faunal changes resulting from the introduction of oil may be drastic and the degree of change is related to the intensity and duration of oiling (Sanders et al. 1980). One of the best approaches to detecting oil effects appears to be the community, or ecosystem approach (Mann and Clark 1978; Elmgren et al. 1980).

The relative change in species composition resulting from the experimental introduction of crude oil and crude oil plus a dispersant into two bays at Cape Hatt will be assessed with a multivariate analysis of variance (MANOVA). The following analyses were performed to describe the benthic community structure and its spatial variability under pristine conditions. Factor analysis was used to identify recurring groups of species and to reduce the dimensionality of the large number of possibly intercorrelated variables presented to the MANOVA.

The species considered in the analysis were those that accounted for at least 1% of the total number of infauna collected in any bay at either depth. In this way, 35 species were selected; together, these comprised 89.3% of the total number of infauna collected. Either density or biomass data would be adequate for the detection of large scale change, but subtle faunal changes would be more readily detected in density data. The biomass data are dominated by the presence and abundance of older individuals and would be relatively insensitive to numerical changes in younger individuals. Hence analyses were performed on density data.

The correlation matrix of transformed species abundances was calculated; principal components were then extracted from this matrix, and finally factors were generated by Varimax rotation. Eight factors were extracted (8 principle components had eigenvalues >1); these eight factors accounted for 65.2% of the variance represented by the 35 species variables. Each of these factors can be considered as representing a group of species that tend to occur together and whose densities vary more or less proportionately.

The results of the factor analysis are summarized in Table 17. This table lists the species whose densities were strongly correlated with each of the eight factors. Species that were strongly and positively correlated with any one factor tended to occur together. A measure of the abundance of each such group in a particular sample can be obtained by calculating the corresponding 'factor score'. A factor score is a linear, additive function of the original variables (log-transformed species densities), with each variable weighted proportionately to its correlation with the factor. A high factor score indicates that the group of species represented by the factor is common in the sample in question; a low or negative factor score indicates that those species are rare or absent. The mean factor scores for samples from each transect, bay and depth are shown in Fig. 6.

The first factor, representing mostly the bivalves, was the dominant assemblage in the samples; it accounted for 28% of variance represented in the 35 species variables. It was prominent on almost all transects at the 7 m depth (Fig. 6). This assemblage is very similar to the high arctic Macoma community described by Thorson (1957) and reported from other Canadian high arctic areas (Ellis 1960; Sekerak et al. 1976; Thomson MS).

Table 17. Results of factor analysis of the 35 most abundant benthic animals collected at Cape Hatt, northern Baffin Island, during September 1980. The values shown are the correlations between the log transformed densities of various species (the original variables) and each of the 8 factors determined in the analysis. Species whose densities were weakly correlated with a factor ($-0.4 < r < 0.4$) are not shown.

1.	
Astarte montagui	0.827
Macoma calcarea	0.806
Astarte borealis	0.790
Nuculana minuta	0.749
Macoma juveniles	0.720
Cistenides granulata	0.698
Trichotropis borealis	0.692
Astarte juveniles	0.673
Thyasiridae sp.	0.634
Macoma moesta	0.593
Aricidea sp.	0.553
Maldane sarsi	0.540
Phloe minuta	0.473
Serripes groenlandicus	0.415
Mya truncata	0.453
Scoloplos armiger	0.449
Proxillella proetermissa	0.498
Nereimyra punctata	-0.599
Euchone analis	-0.464

2.	
Myriotrochus rinkii	0.752
Retusa obtusa	0.724
Phloe minuta	0.594
Chaetozone setosa	0.543
Euchone analis	0.538
Cingula castanea	0.484

3.	
Musculus niger	0.670
Serripes groenlandicus	0.669
Owenia fusiformis	0.661
Nereimyra punctata	-0.427
Etone longa	-0.452

4.	
Terebellides stroemi	0.832
Ampharetidae (unidentified)	0.655
Mya truncata	0.559
Cingula castanea	0.419

5.	
Musculus juveniles	0.872
Musculus discors	0.765

6.	
Capitella capitata	0.759
Ophelia limacina	0.454
Hiatella arctica	0.406

7.	
Harmothoe imbricata	0.720
Gastropod species G	-0.678

8.	
Scoloplos armiger	0.614
Thyasiridae sp.	0.499

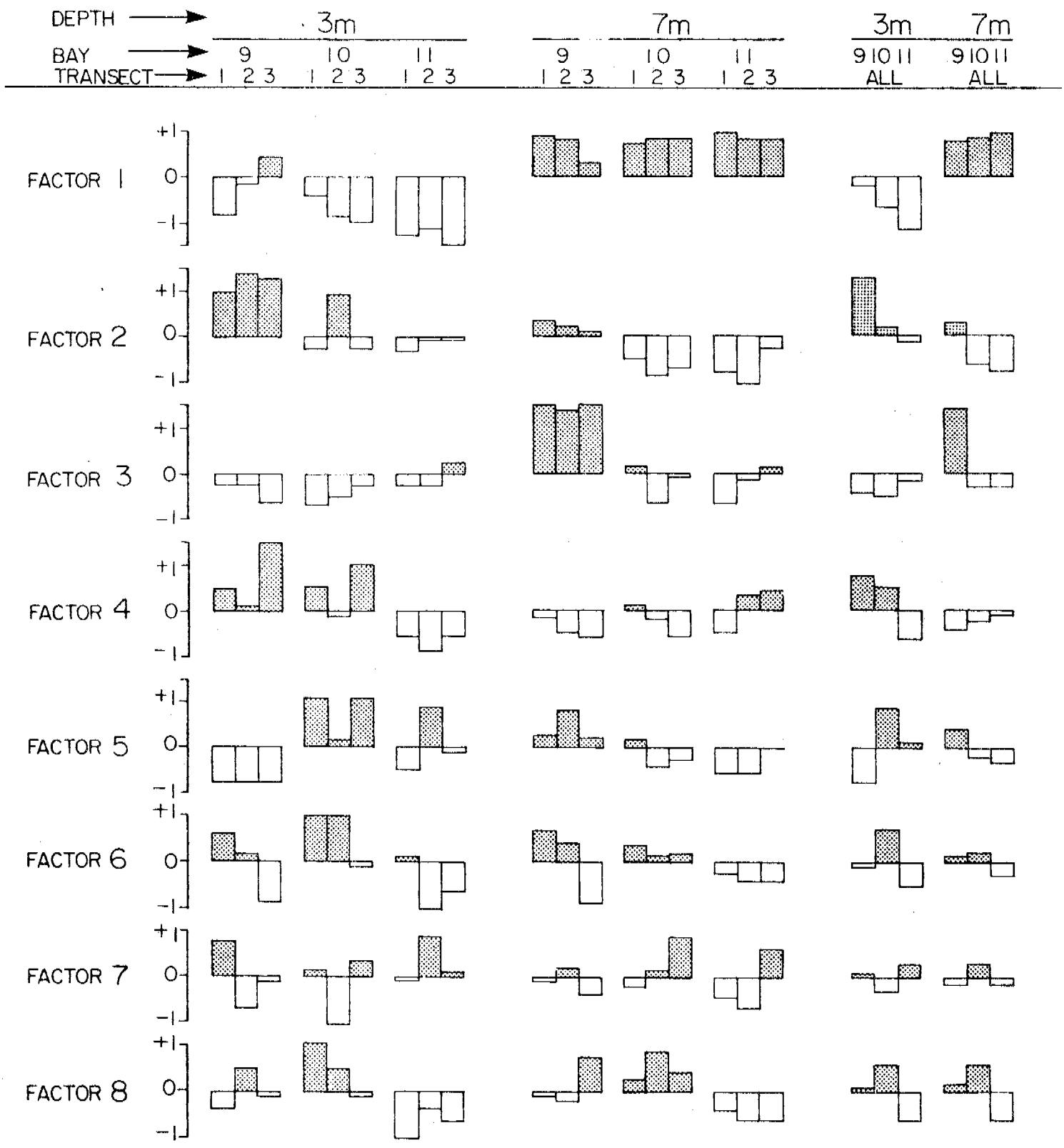


FIG. 6. Mean factor scores for each transect and bay at two depths in Cape Cape Hatt, northern Baffin Island.

The second factor, which represented primarily the synaptid holothurian Myriotrochus rinkii, two gastropods and three polychaetes, assumed high values only in shallow water on all transects in Bay 9 and on the centre transect in Bay 10.

The three species whose densities were strongly positively correlated with the third factor are all filter feeders. This assemblage was conspicuous only in the deep samples from Bay 9, and this may be indicative of high current velocities in this bay. A strong current generally enhances food supply for filter feeders (Ölscher and Fedra 1977).

Factors 4 and 5 appeared to represent primarily shallow water assemblages, while the remaining three assemblages did not appear to be characteristic of or restricted to any one depth or bay.

Differences in community composition among bays and depths were assessed with a multivariate analysis of variance (MANOVA) using, as dependent variables, the factor scores for each of the eight factors derived in the previous analysis. The design was a fixed-effects 2-way factorial MANOVA with bays and depths as the factors and with transects nested within depths and bays. This analysis tests for differences in community composition among bays and depths by simultaneously considering the scores for all eight factors. Since these eight factors, in turn, represent 35 species, the MANOVA tests for overall differences in community composition.

The 2-way MANOVA results (Table 18) show significant among-transect variability, indicating that, overall, the distribution of infaunal

Table 18. One- and two-factor[1] multivariate and univariate analyses of variance for factor scores determined in factor analysis of infaunal density in the study bays at Cape Hatt. Transects are nested within depths and bays; bay, depth and bay-depth interaction effects are tested over the transect MS, and transect effects over the residual MS. F-values are shown with associated significance levels (ns = P>0.01; ** P≤0.01, *** P≤0.001) for univariate ANOVAS and actual probabilities for multivariate ANOVAS.

	Two-factor analyses (3 and 7 m depth, n = 144)				One-factor analyses[3]			
					3 m depth (n = 72)		7 m depth (n = 72)	
Source of variation ->	Bay	Depth	Bay x Depth	Transect	Bay	Transect	Bay	Transect
df ->	2,12	1,12	2,12	12,126	2,6	6,63	2,6	6,63
MANOVA[3]								
Pillaistrace	5.32[2]	50.97[2]	3.13	2.73	3.18	2.68	1.10	1.67
df ->	(16,8)	(8,5)	(16,12)	(96,1008)	(10,6)	(30,315)	(10,6)	(30,315)
P ->			0.026	0.000	0.085	0.000	0.475	0.017
ANOVAS								
Factor 1	4.35[2]	112.62[2]	8.54 **	3.09 ***	7.89 ns	4.35 ***	0.85 ns	1.46 ns
Factor 2	21.19 ***	27.77 ***	0.76 ns	1.62 ns	10.55 ns	1.87 ns	12.01 **	1.23 ns
Factor 3	16.72[2]	21.80[2]	19.44 ***	1.32 ns	1.73 ns	1.51 ns	29.30 ***	1.22 ns
Factor 4	16.63[2]	2.93[2]	7.06 **	2.73 **	5.67 ns	2.67 ns	1.56 ns	2.88 ns
Factor 5	2.51[2]	0.26[2]	12.06 **	1.89 ns	7.54 ns	2.36 ns	6.51 ns	1.18 ns
Factor 6	3.56 ns	0.14 ns	0.63 ns	4.06 ***	2.73 ns	4.43 ***	1.03 ns	3.57 **
Factor 7	0.05 ns	0.02 ns	0.86 ns	4.11 ***	0.42 ns	5.06 ***	0.51 ns	3.13 **
Factor 8	14.25 ***	0.22 ns	0.02 ns	1.65 ns	5.74 ns	2.14 ns	9.66 ns	1.16 ns

[1] In the one-factor ANOVA, for each depth, bays were compared. In the two-factor ANOVA, bays and depths were both compared.
[2] Ambiguous because of significance of bay-depth interaction term.
[3] Only factors 1 to 5 were considered in one-factor MANOVA (see text).

assemblages was patchy on the 50 m scale. After accounting for this among-transects variability, it was found that differences between depths within bays were inconsistent among bays (i.e. the bays by depths interaction term was significant). This interaction precluded interpretation of the among-bays or between-depths terms of the 2-way analysis.

Separate MANOVA's were therefore performed on samples from each depth.* These results--like the 2-way MANOVA--showed significant among-transects variability, especially at 3 m depth. However, no significant among-bays differences in community composition were evident at either the 3 m or 7 m depth (Table 18). ANOVA results for individual factors also showed no significant among-bays differences for any of the factors at 3 m, and showed significant among-bays variation for only two of the factors at 7 m.

Figure 7 is a visual portrayal of the relative similarities and differences of the animals present in each bay and at each depth. This 'ordination' of bay-depth combinations (locations) was generated by multiple discriminant analysis (BMDP7M, Dixon and Brown 1977) of the six locations using as variables the eight factors described above. Discriminant analysis derives canonical variables, which are specific linear additive functions of the variables on which the analysis is based (in this case, the eight species assemblages or factors). The particular functions chosen by the analysis are those which 'maximize the separation' of the locations. The analysis was structured such that only two canonical variables were derived, and such that the first of these emphasized factors differing among depths while the second

*In each of these analyses we were able to consider only the first five factors because of the small number of degrees of freedom of the transect term in the test of the bays effect. Inspection of the ANOVA results for individual factors showed that values of the three factors excluded from the 1-way MANOVA did not differ significantly among bays at either the 3 m or the 7 m depth (Table 18).

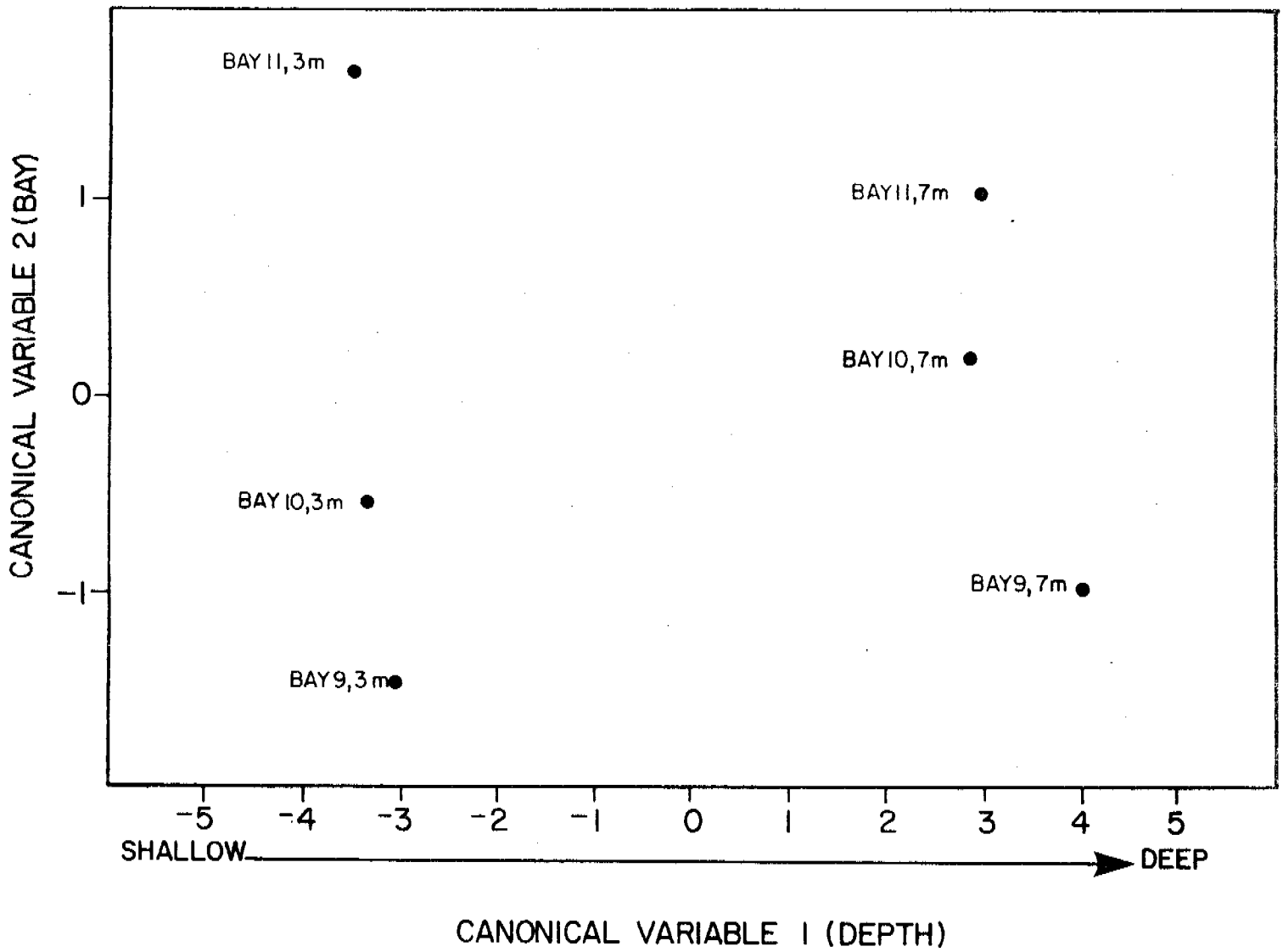


FIG. 7. Similarity of animals found in six depth/bay combinations. The centroid of each combination is plotted against two canonical variables (discriminant functions). The variables were derived by stepwise multiple discriminant analysis of the depth-area combinations, using as predictors the eight factors derived from factor analysis of the 35 dominant infaunal animals. The analysis was structured such that the first canonical variable emphasized depth and the second emphasized location.

emphasized factors differing among bays. Both canonical variables afforded significant ($P<0.005$) discrimination of locations on the basis of the animals present. The visual portrayal represents the mean value of each canonical variable for each location (Fig. 7). This approach, extended to include the time dimension, will be especially useful in comparing pre- and post-spill community composition in the various bays.

Figure 7 shows, on the basis of the types and numbers of animals present, a clear separation of deep and shallow samples, and also shows a consistent pattern of differences among bays. In general, differences between depths appear to be larger than differences among bays within depths. This can be confirmed numerically: The mean Euclidean distance (Walker et al. 1979) between all possible pairs of centroids of similar depths is 1.88 ± SD 0.90 (n = 6), while the deep and shallow centroids for particular bays differ by 6.54 ± SD 0.48 (n = 3).

Trophic Relationships

The infaunal animals (excluding gastropods) collected by the airlift sampler were classified into feeding guilds based on data available in the literature (Table 19). The feeding modes follow those described by Fauchald and Jumars (1979).

Filter feeders extract particulate material from the water. Sabellid polychaetes do this externally with a brachial 'fan' while bivalves pump water through their body and filter out particulate material with their gills. *Mya truncata* lies buried deep in the sediment and extends a siphon to

Table 19. Feeding mode of benthic infaunal animals (excluding gastropods) from Cape Hatt, northern Baffin Island. Species tentatively assigned to feeding modes indicated by ?; numbers in parentheses are number of species in that family or group.

	Polychaetes	Bivalves	Others
Filter feeders	Sabellidae (2+) Owenia fusiformis? Chaeopterids (1)	Mya truncata Thyasiridae Astarte spp. (2) Musculus spp. (2) Hiatella arctica Serripes groenlandicus	Rhizomolgula globularis
Carnivores	Polynoidae (4) Phyllodocidae (4) Phloe minuta Lumbrineris sp. (1)? Glycera capitata Nephtys ciliata?		
Deposit feeders	Capitella capitata Cistenides spp. (2) Opheliidae (3) Scoloplos armiger Scalibregma Maldanidae (4) Scalibregma inflatum		Myriotrochus rinkii?
Surface deposit feeders	Chaetozone setosa Terebellidae (3) Ampharetidae (2+) Spionidae (5) Trichobranchus glacialis Diplocirrus sp. (1) Aricidea sp. (1)	Macoma calcarea Macoma moesta? Nuculans minuta	Strongylocentrotus droebachiensis

References for feeding type: Ockelmann 1958; Reid and Reid 1969; Himmelman and Steele 1971; Ansell and Parulekar 1978; Mohlenberg and Riisgård 1978; Fauchald and Jumars 1979.

the surface. Mussels (Musculus sp.) are usually attached to rocks or algae and water enters through a gape in their shell. It should be pointed out that many benthic filter feeders ingest material of benthic rather than pelagic origin (Marshall 1970).

Some deposit feeders burrow through the mud (Capitella capitata) or live in tubes (maldanid polychaetes) and ingest the substate (Fauchald and Jumars 1979). These animals derive their nutrition from bacteria associated with the organic matter and detritus found in the sediments. The deposit feeders listed in Table 19 burrow or live in tubes and generally feed at some depth below the surface of the sediment. The activity of these animals is especially important in reworking the surface layers of the sediment (e.g. Cadée 1979).

Surface deposit feeders feed at the sediment-water interface. Their food includes benthic microalgae and bacteria. Most of the polychaetes included in this group (Table 19) feed by means of tentacles (Fauchald and Jumars 1979). Nuculana minuta extends the palp proboscides over the surface of the sediment (Ansell and Parulekar 1978).

The carnivores are all motile predators.

An animal's mode of feeding may determine its degree of exposure to oil. A short exposure to dispersed oil may not affect filter feeders, which may stop feeding temporarily, but the resultant oil-containing flocs that accumulate on the surface of the sediment may seriously affect the surface deposit feeders. In active benthic environments, wave action and sediment

transport may incorporate undispersed oil into the sediment and seriously affect burrowing deposit feeders.

Filter feeding was the dominant feeding mode in the three experimental bays at Cape Hatt (Table 20). Biomass of filter feeders was highest in Bay 9, again indicating that current velocities may be higher in this bay than in the others (see also 'Community Structure'). Surface deposit feeding was the second most common mode of feeding at the 7 m depth. Surface deposit feeders were much less abundant at the 3 m depth, perhaps owing to instability of the sediment surface due to wave action. Considering both depths together, carnivores and burrowing and tubiculous deposit feeders showed the least variability among bays.

Epibenthos

For the purposes of the present study, the term 'epibenthos' refers to motile members of the benthic community, including those animals capable of rapid movement through the water column (e.g. crustaceans, fish) and those moving relatively slowly on the sediment surface, but capable of covering relatively large distances due to their size (e.g. urchins, starfish). As previously mentioned, the purpose of this distinction is largely to facilitate the interpretation of any changes in faunal densities in the study bays after oiling. The relative roles of mortality and emigration in determining any changes in the densities of the above animals will not be distinguishable with certainty. Hence relatively little effort is directed to the analysis of their distributions. A further justification for the inclusion of urchins and starfish in this section are the different (and less

Table 20. Mean biomass of animals comprising the four major feeding modes of infaunal animals (excluding gastropods) found at Cape Hatt, northern Baffin Island.

		3 m depth			7 m depth		
		Bay 9	Bay 10	Bay 11	Bay 9	Bay 10	Bay 11
Carnivores	g/m^2	10.56	9.72	7.49	12.48	6.31	4.95
	%	1.82	3.28	9.70	0.45	0.42	0.32
Filter feeders	g/m^2	522.95	256.98	52.52	2579.50	1365.31	1386.23
	%	90.33	86.66	68.04	93.08	91.34	89.99
Deposit feeders	g/m^2	25.79	19.83	14.19	33.46	32.98	33.14
	%	4.45	6.69	18.38	1.21	2.21	2.15
Surface deposit feeders	g/m^2	19.63	9.99	3.00	145.76	90.09	116.18
	%	3.39	3.37	3.89	5.26	6.03	7.54
Total	g/m^2	578.93	296.52	77.20	2771.20	1494.69	1540.50

intensive) sampling methods employed due to the large size and sparse distribution of these animals.

The available data on highly motile epibenthic crustaceans at Cape Hatt are from the same airlift samples upon which infaunal results are based. Estimates for epibenthic crustaceans likely are not as accurate as those for infauna, however, due both to escape of organisms from the area sampled and to inclusion of those inadvertently drawn into the airlift from outside the 0.0625 m^2 sampling area. A modification to the sampler, developed for EAMES studies to overcome this shortcoming (see Thomson and Cross 1980), was not practical in the present study due to difficulties in operating the airlift in the mixed sediment-rock substrate. No quantitative estimates are available for the extent to which epibenthic crustaceans were over- or under-estimated in the present study.

The densities of large surface-dwelling epibenthos (urchins and starfish) were estimated by two methods: counts from 8 to 12 photographs (each covering 0.5 m^2) along each transect, and counts performed *in situ* within five 1 x 10 m strips beside each transect. No urchins or starfish were present at 3 m; mean density estimates at 7 m for each bay and method, and the total areas on which the estimates are based, are as follows:

	In situ counts			Counts in photographs		
	Urchins (no./m^2)	Starfish (no./m^2)	Area (m^2)	Urchins (no./m^2)	Starfish (no./m^2)	Area (m^2)
Bay 9	4.42	0.10	150	1.46	0.08	13.0
Bay 10	1.56	0.19	150	0.91	0.12	16.5
Bay 11	0.99	0.07	150	0.55	0.00	14.5

Urchin density estimates from photographs were lower than those based on in situ counts on every transect (9 of 9 cases). The observed patchy distribution of urchins along transects may account for this discrepancy. Patches were included in the large areas surveyed, but not in the relatively small area covered by photographs. Differences between techniques in the density estimates for the large starfish Leptasterias polaris did not differ as greatly between techniques (on a per bay basis), and the differences were not in a consistent direction on all transects. All transects contained at least one starfish (based on in situ counts); on 7 of 9 transects no L. polaris were present in photographs, and on the two transects where starfish did occur in photographs the resulting density estimates were higher than those based on in situ counts. This indicates that this starfish was too widely distributed for its density to be accurately estimated by the photographic technique. The photographic method employed in the present study, therefore, appears to be inadequate for estimating the densities of urchins or starfish due to large-scale patchiness and sparse distribution, respectively. Hence only data based on in situ counts are discussed below.

Epibenthic crustaceans collected in airlift samples consisted entirely of ostracods (56.3%), amphipods (34.0%) and cumaceans (9.6%). Ostracods, six species of amphipods, and two species of cumaceans accounted for 86.0% and 82.7% of total numbers and biomass, respectively (Table 21). All of these species are common in nearshore high arctic waters (Sekerak et al. 1976; Buchanan et al. 1977; Thomson and Cross 1980).

Mean densities of major epibenthic taxa and dominant species are shown by transect, bay and depth in Table 22. Densities of ostracods, all

Table 21. Percent contribution to total epibenthic biomass and density by 10 dominant crustaceans in the study bays at Cape Hatt, northern Baffin Island, during September 1980. Based on 144 samples, each covering 0.625 m^2, from 3 and 7 m depths.

Taxon	% of total biomass	% of total density
Ostacoda (Myodocopa)	54.85	27.77
Anonyx nugax (A)	7.42	47.27
Guernea sp. (A)	6.99	0.71
Lamprops fuscata (C)	6.65	0.87
Paroediceros lynceus (A)	2.49	3.06
Monoculodes borealis (A)	2.04	0.92
Pontoporeia femorata (A)	1.50	1.55
Ostracoda (Podocopa)	1.44	0.11
Brachydiastylis resima (C)	1.31	0.29
Stenothoidae sp. a (A)	1.29	0.17
Total	85.99	82.71
Total epibenthos	1152.1 ind./m^2	7.17 g/m^2

(A) = amphipod (C) cumacean.

Table 22. Mean density (no./m^2) of major epibenthic taxa and dominant species of epibenthic animals on transects at two depths in three bays at Cape Hatt, northern Baffin Island, during September 1980. Data are expressed as mean ± standard deviation and are based on 8 replicate 0.0625 m^2 airlift samples at each depth and transect.

Taxa	Transect	3 m Depth			7 m Depth		
		Bay 9	Bay 10	Bay 11	Bay 9	Bay 10	Bay 11
Ostacoda	1	40.0 ± 46.8	34.0 ± 77.2	10.0 ± 14.7	714.0 ± 293.4	1264.0 ± 679.6	1458.0 ± 549.6
	2	58.0 ± 58.6	72.0 ± 159.8	8.0 ± 12.1	564.0 ± 427.5	1902.0 ± 691.1	938.0 ± 383.2
	3	30.0 ± 30.2	28.0 ± 33.9	10.0 ± 17.0	1620.0 ± 872.1	2020.0 ± 1024.3	944.0 ± 1033.3
	All	42.7 ± 46.1	44.7 ± 101.6	9.3 ± 14.1	966.0 ± 735.5	1728.7 ± 848.7	1113.3 ± 723.6
Amphipoda	1	188.0 ± 81.0	332.0 ± 244.0	432.0 ± 215.0	394.0 ± 368.7	746.0 ± 484.9	614.0 ± 376.6
	2	322.0 ± 194.7	436.0 ± 235.1	226.0 ± 96.2	178.0 ± 81.8	552.0 ± 262.0	542.0 ± 273.2
	3	158.0 ± 62.5	264.0 ± 324.3	200.0 ± 122.2	236.0 ± 159.7	628.0 ± 341.2	594.0 ± 264.5
	All	222.7 ± 141.5	344.0 ± 268.6	286.0 ± 180.7	269.3 ± 244.7	642.0 ± 366.8	583.3 ± 296.9
Anonyx nugax	1	2.0 ± 5.7	10.0 ± 17.0	64.0 ± 142.9	192.0 ± 372.2	442.0 ± 464.8	124.0 ± 102.2
	2	6.0 ± 11.9	26.0 ± 67.3	2.0 ± 5.7	34.0 ± 42.3	184.0 ± 64.6	46.0 ± 45.6
	3	4.0 ± 7.4	2.0 ± 5.7	6.0 ± 11.9	32.0 ± 37.3	262.0 ± 231.1	100.0 ± 74.4
	All	4.0 ± 8.5	12.7 ± 39.7	24.0 ± 84.2	86.0 ± 221.3	296.0 ± 309.0	90.0 ± 81.3
Guernea sp.	1	52.0 ± 29.3	64.0 ± 95.2	18.0 ± 21.7	108.0 ± 41.7	100.0 ± 68.2	192.0 ± 89.7
	2	112.0 ± 80.2	46.0 ± 47.9	20.0 ± 38.0	58.0 ± 41.8	130.0 ± 70.7	132.0 ± 81.0
	3	52.0 ± 57.8	14.0 ± 18.0	4.0 ± 7.4	108.0 ± 91.2	102.0 ± 101.1	132.0 ± 116.6
	All	72.0 ± 63.8	41.3 ± 63.3	14.0 ± 25.5	91.3 ± 64.6	112.7 ± 78.9	152. 0 ± 97.0
Paroediceros lynceus	1	6.0 ± 8.2	18.0 ± 44.8	22.0 ± 14.7	0.0 ± 0.0	40.0 ± 25.7	52.0 ± 74.4
	2	8.0 ± 17.1	76.0 ± 108.8	8.0 ± 12.1	14.0 ± 13.4	44.0 ± 63.9	62.0 ± 90.3
	3	0.0 ± 0.0	8.0 ± 17.1	4.0 ± 7.4	0.0 ± 0.0	40.0 ± 50.6	114.0 ± 108.0
	All	4.7 ± 11.0	34.0 ± 72.4	11.3 ± 13.7	4.7 ± 10.0	41.3 ± 47.2	76.0 ± 92.1
Cumacea	1	30.0 ± 46.4	48.0 ± 105.8	30.0 ± 43.1	152.0 ± 48.4	190.0 ± 93.1	328.0 ± 184.8
	2	42.0 ± 38.2	60.0 ± 100.4	2.0 ± 5.7	144.0 ± 143.4	126.0 ± 104.5	286.0 ± 155.2
	3	8.0 ± 8.6	2.0 ± 5.7	0.0 ± 0.0	130.0 ± 128.7	92.0 ± 65.6	312.0 ± 166.3
	All	26.7 ± 36.4	36.7 ± 84.5	10.7 ± 27.8	142.0 ± 110.0	136.0 ± 94.8	308.6 ± 162.7
Lamprops fuscata	1	26.0 ± 44.4	48.0 ± 105.8	30.0 ± 43.1	112.0 ± 51.3	162.0 ± 83.5	146.0 ± 135.1
	2	40.0 ± 40.1	18.0 ± 18.0	2.0 ± 5.7	114.0 ± 131.8	92.0 ± 92.4	166.0 ± 140.0
	3	6.0 ± 8.3	2.0 ± 5.7	0.0 ± 0.0	100.0 ± 108.1	76.0 ± 53.9	240.0 ± 136.6
	All	24.0 ± 36.2	22.7 ± 62.4	10.7 ± 27.8	108.7 ± 98.4	110.0 ± 84.0	184.0 ± 137.5

cumaceans and the cumacean Lamprops fuscata were considerably higher at the 7 m depth than at 3 m. Amphipods were more evenly distributed at the two depths, although a tendency toward higher numbers at the 7 m depth was evident, both for total amphipods and for the individual species included in Table 22. Differences among bays were also apparent for all taxa considered. For reasons outlined above, no statistical treatment of the distribution of epibenthic crustaceans is presented.

The densities of the urchin Strongylocentrotus droebachiensis and the starfish Leptasterias polaris at 7 m depth in the study bays are shown in Table 23. No urchins or starfish were present on transects at the 3 m depth. At a depth of 7 m L. polaris occurred at a relatively low and even density in the three bays. Strongylocentrotus droebachiensis was most abundant in Bay 9 and least abundant in Bay 11 (Table 23). Based on one-factor nested ANOVA of urchin density, variation among transects within bays was non-significant ($F = 0.833$, $df = 6,36$; $P = 0.552$), whereas variation among bays was highly significant ($F = 63.271$, $df = 2,6$; $P<0.001$).

Strongylocentrotus droebachiensis is widely distributed and often relatively abundant (up to 14 individuals/m^2) in the Lancaster Sound area, whereas the distribution of Leptasterias polaris is more restricted (Thomson and Cross 1980). Both species are of interest due to their trophic positions. Strongylocentrotus droebachiensis is a herbivore whose impact on benthic algal populations has been found to be considerable on both the east and west coasts of Canada (Miller and Mann 1973; Foreman 1977). L. polaris is a top predator feeding primarily on large bivalves, and hence may be indirectly affected by oil through changes in bivalve populations. Thus,

Table 23. Density (no/m^2) of urchins and starfish in the study bays at Cape Hatt, northern Baffin Island, during September 1980. Based on *in situ* counts within five 1 x 10 m areas along each of three transects in each of three bays at a depth of 7 m.

Species	Transect	7 m Depth		
		Bay 9	Bay 10	Bay 11
Strongylocentrotus droebachiensis	1	3.26 ± 1.47	1.66 ± 0.66	0.92 ± 0.31
	2	5.04 ± 1.10	1.62 ± 0.86	1.00 ± 0.42
	3	4.96 ± 1.33	1.40 ± 0.64	1.06 ± 0.59
	All	4.42 ± 1.48	1.56 ± 0.69	0.99 ± 0.42
Leptasterias polaris	1	0.04 ± 0.09	0.24 ± 0.15	0.08 ± 0.08
	2	0.14 ± 0.11	0.12 ± 0.08	0.08 ± 0.18
	3	0.12 ± 0.11	0.22 ± 0.13	0.06 ± 0.05
	All	0.10 ± 0.11	0.19 ± 0.13	0.07 ± 0.11

in spite of the above-mentioned interpretational difficulties caused by the mobility of these animals, the densities of urchins and starfish should be carefully monitored throughout the course of this study. Observations on behaviour and mortality in these species may also provide information on oil effects.

Fish were not a conspicuous feature of the marine fauna in the study bays at Cape Hatt. Pelagic fish were not observed, and benthic fish were rarely encountered. A total of 10 fish belonging to four species were collected in airlift samples (Table 24). Gymnocanthus tricuspis and juvenile Gymnocanthus sp. were most common, and only one individual was collected of each of Gymnelis viridis, Eumicrothemus derjugini and Icelus sp. Most of the fish collected were from the 3 m transects in bays 9 and 11; the absence of fish on 3 m transects in Bay 10, however, may be of little significance considering the small total number collected. All of the species collected are previously known from arctic Canada (Leim and Scott 1966).

Table 24. Species of fish collected in airlift samples in the three study bays at Cape Hatt, northern Baffin Island, during September 1980.

Depth	Bay	Transect	Species	Biomass (g)	Total Length (mm)
2 m	9	1	Gymnocanthus tricuspis	5.45	82
		2	Gymnocanthus tricuspis	4.14	76
		3	Gymnelis viridis	0.55	61
	11	1	Gymnocanthus sp. (juvenile)	0.02	22
		2	Gymnocanthus tricuspis	23.85	119
			Gymnocanthus sp. (juvenile)	0.28	35
		3	Gymnocantus sp. (juvenile)	0.03	17
			Gymnocantus sp. (juvenile)	0.02	19
7 m	10	2	Eumicrothemus derjugini (juvenile)	0.01	11
	11	1	Icelus sp. (juvenile)	0.14	22

Macrophytic Algae

The benthic marine algae of the North American Arctic have been studied intermittently since the early nineteenth century, but early reports consisted of little more than species lists (Kent 1972). Recently, floristic and ecological studies have been performed in Labrador and Ungava Bay (Wilce 1959), West Greenland (Wilce 1964), Prince Patrick Island (Lee 1966), Pangnirtung Fiord (Kent 1972), and in several areas in the northern and southwestern Canadian Arctic (Lee 1980). These studies have shown that macrophytic algae are a common feature of arctic and subarctic nearshore waters, both on exposed rocky coasts and on soft bottoms. In the latter case they are either loose-lying and still viable or are attached to mud, small rocks, shells and polychaete tubes (Lee 1966; Lee 1973, 1980). These floristic studies have provided much valuable information on species composition, zonation and reproduction of littoral and sublittoral macrophytes in high latitudes. Quantitative studies of kelps and conspicuous understory algae have also been carried out at several locations in the Lancaster Sound area (Thomson and Cross 1980), but to date combined floristic/biomass studies of benthic macroalgae have not been reported for the Canadian Arctic.

The overall effects of oil on macroalgal communities have not been studied in the Arctic, but Hsiao et al. (1978) determined that *in situ* primary production in two macroalgal species in the Beaufort Sea was significantly inhibited by all types and concentrations of oil tested. In other latitudes, studies of the effects of oil spills with and without the use of chemical dispersants have often demonstrated changes in the abundance

of littoral and sublittoral macrophytic algae (see Natural Academy of Sciences 1975, Table 4-1). In some cases widespread mortality has been observed (e.g. Bellamy et al. 1967; Thomas 1973), whereas in other cases no mortality was apparent immediately following the spill (e.g. Nelson-Smith 1968). Subsequent changes following spills have included a proliferation of macroalgal growth, which has been attributed to the oil-related absence of herbivores including sea urchins (North et al. 1965) and limpets (Nelson-Smith 1968).

Species Composition

A total of 29 species of macroalgae were collected in the study bays at Cape Hatt (Table 25). This is a relatively small number when compared with the 126 species known in the arctic sublittoral (Wilce 1973) or the 183 species and varieties (littoral and sublittoral) recorded by Lee (1980). This difference undoubtedly is largely attributable to the small area studied at Cape Hatt relative to the wide coverage in the above investigations. To a smaller extent, the difference probably also reflects the focus of the present study on dominant species and the lack of particular effort to collect small or rare (and hence easily overlooked) species. Species determinations were based on (1) herbarium specimens collected by hand on each transect, plus (2) single airlift samples from each of Bays 10 and 11 (2 m depth), which were quickly scanned for species present. Two of the 29 species collected in the study area were found only in these airlift samples (Table 25).

Table 25. Species list and distribution of macrophytic algae collected in three bays at Cape Hatt, northern Baffin Island, during August and September 1980. Depth distribution is shown for species present in systematic hand collections along transects at 3 m and 7 m depths.

Species and Authority	Bay 9		Bay 10			Bay 11		
	3m	7m	3m		7m	3m		7m
Chlorophyceae								
Ulothrix flacca (Dilwyn) Thuret in LeJolis							A	
Chlorochytrium schmitzii Rosenvinge	P		P			P		
Spongomorpha sonderi Kuetzing		P		*			A	P
Chaetomorpha linum (O.F. Mueller) Kuetzing			P		P	P	A	P
Chaetomorpha melagonium (Weber et Mohr.) Kuetzing			P			P	A	
Phaeophyceae								
Pilayella littoralis (L.) Kjellman	P	P	P	A	P	P	A	P
Symphyocarpus strangulans Rosenvinge				A				
Phaeostroma pustulosum Kuckuck					P			
Elachistea lubrica Ruprecht						P		
Stictyosiphon tortilis (Ruprecht) Reinke	P	P	P	A	P	P	A	P
Platysiphon verticillatus Wilce	P	P			P			P
Dictyosiphon foeniculaceus (Hudson) Greville	P	P	P	A	P		A	P
Desmarestia aculeata (L.) Lamouroux	*			*				P
Desmarestia viridis (O.F. Mueller) Lamouroux	P		P		P			
Chorda filum Linnaeus	P			A		P		
Chorda tomentosa Lyngbye	P		P	A	P	P		P
Agarum cribrosum (Mertens) Bory	*				P			P
Laminaria saccharina (L.) Lamouroux			P			P		
Laminaria solidungula J. Agardh	*		P					
Laminaria sp.		P			P			P
Haplospora globosa Kjellman	*							
Sphacelaria plumosa Lyngbye	P		P	A				
Sphacelaria arctica Harvey	*		P	A	P		A	
Fucus distichus L. subsp. *distichus*	P	P	P	A		P		
Rhodophyceae								
Ahnfeltia plicata (Hudson) Fries	*							
Neodilsea integra (Kjellman) A. Zinova	P		P	A		P		
Halosaccion ramentaceum (L.) J. Agardh						P		
Palmaria palmata (L.) O. Kuntze			P					
Polysiphonia arctica J. Agardh	*			*				
Rhodomela confervoides (Hudson) Silva f. *flagellaris* Kjellman	*						A	

P = Present in systematic hand collections.
* = Present only in off-transect collections, 2-12 m depths.
A = Present in airlift collections at 3 m depth (see text).

The distributions of algal species collected by hand and by airlift are shown for each bay and depth in Table 25. Comparison of the two types of data for locations with both hand and airlift samples further points out the inadequacy of the hand collection technique for small or rare species; in addition to the two species found only in airlifts, airlifts provided five new 'location records' for species that were present in hand collections from other depths and bays. Hence, floristic comparisons among transects, depths and bays must await more detailed analyses of algae from 1980 airlift samples, to be carried out in 1981.

A brief description of the 3 m algal community is warranted, however, based on in situ observations, and on the general appearance of algae from airlift samples. The bulk of the algae in most samples was a tangled mat of filamentous and fine dendritic forms; Pilayella littoralis, Dictyosiphon foeniculaceous and Stictyosiphon tortilis were apparently the dominant species in each of three samples (one from each bay) that were examined microscopically. Likely because of their abundance, these species were also present in hand collections from all or most bays and depths (Table 25). Extending above this tangled mat, and conspicuous both due to size and abundance, were long, unbranched Chorda spp. and short foliose species including Fucus distichus, Neodilsea integra, Palmaria palmata and small Laminaria spp. These 'canopy' species were apparently more unevenly distributed than was the lower algal stratum, both within and among bays.

Biomass

Mean biomasses of algae collected in airlift samples along transects at 3 m and 7 m depths in each of the study bays are shown in Table 26. Overall

Table 26. Mean biomass of macrophytic algae at 3 m and 7 m depths in three bays at Cape Hatt, northern Baffin Island, during September 1980. Biomass expressed as 10% formalin-preserved wet weight (g/m^2); n = 8 airlift samples per transect; each sample covered 0.0625 m^2.

Depth	Transect	Bay 9	Bay 10	Bay 11
		mean ± SD	mean ± SD	mean ± SD
3 m	1	611 ± 298	1351 ± 812	320 ± 337
	2	295 ± 134	689 ± 528	566 ± 421
	3	514 ± 159	1294 ± 835	1005 ± 826
	All	473 ± 241	1112 ± 769	631 ± 616
7 m	1	369 ± 159	554 ± 491	206 ± 220
	2	179 ± 89	442 ± 226	138 ± 65
	3	175 ± 44	334 ± 166	310 ± 306
	All	241 ± 139	444 ± 235	218 ± 223

average biomass of macroalgae was 739 and 301 g/m^2 at 3 and 7 m depths, respectively. The maximum transect mean was 1351 g/m^2 and the maximum single-sample estimate was 3020 g/m^2. These values (based on formalin-preserved wet weight) probably underestimate fresh weight; Thomson and Cross (1980) reported a considerable (>30%) formalin-induced reduction in the weight of understory algae from Cape Fanshawe, Bylot Island. Algal biomass at Cape Hatt (Table 26) was higher than the biomass of macroalgae other than kelp at most of the 5 and 10 m stations studied by Thomson and Cross (1980). However, kelp biomass in the Lancaster Sound area was considerably higher (0.5-12.7 kg/m^2 fresh wet weight). No estimates of kelp biomass were made at Cape Hatt, either on transects or in the narrow Laminaria zone at 4-5 m depth.

Algal biomass varied considerably among replicate samples within transects (Table 26). Two-factor, nested ANOVA on log-transformed data showed significant ($P<0.005$) variation both among transects and between depths. Variation among bays was not significant ($P>0.01$), however, when compared with variation among transects within depths and bays. No interaction between the depth and bay factors was evident:

Source	df	MS	F	P
bays	2,12	1.7018	6.67	0.0113
depths	1,12	4.4865	17.59	0.0012
Bay x depth	2,12	0.0324	0.13	0.8819
transects within bays and depths	12,126	0.2551	3.13	0.0006
Error	126	0.0814		

Substrate Cover

Mean percent of the substrate covered by macrophytic algae on each transect and in each bay, based on *in situ* estimates within 10 m^2 areas, are shown in Table 27. Separate estimates were made for larger foliose algae (*Fucus distichus*, *Neodilsea integra* and *Palmaria palmata*), and for the lower stratum of mixed filamentous and dendritic forms. Because both types occurred together in some areas, the sum of the two estimates does not necessarily equal total bottom cover. Percent cover by the mixed lower stratum was usually relatively high (68 to 90%) on 3 m transects and relatively low (2 to 12%) on 7 m transects. Intermediate values (19 to 33%) were estimated for some transects at both depths, however (Table 27). Larger foliose algae provided little bottom cover at the 3 m depth in Bay 9, but contributed substantially (10 to 35%) to bottom cover on the shallow transects in the other two bays. On the 7 m transects, smaller foliose algae were replaced by large and generally solitary individuals of *Laminaria* spp. and *Agarum cribrosum*. Kelp was present at 7 m depth in Bay 9 (counts were not made), and was widely distributed in Bays 10 and 11, averaging a little more than one plant per 10 m^2 (Table 27).

No correlation between estimated percent cover of 'understory' algae and biomass, on a transect by transect basis, was evident at either depth ($r = -0.20$ and -0.02 for 3 m and 7 m, respectively). This is likely due both to variation among transects in the thickness of the algal mat and to variation among bays in species composition, particularly with respect to the larger foliose algae. For example, at the 3 m depth the highest cover estimate and the lowest biomass estimate are both from Bay 9 (Tables 26 and

Table 27. Estimates of macrophytic algal density based on *in situ* counts at 3 m and 7 m depths in the study bays at Cape Hatt, northern Baffin Island, during September 1980. Data are expressed as mean ± SD; n = five 1 x 10 m areas on each transect.

		3 m Depth		7 m Depth	
Bay	Transect	Understory[1] (%)	Canopy[2] (%)	Understory[1] (%)	Kelp[3] (no./10 m^2)
9	1	68 ± 13	2 ± 3	10 ± 5	-
	2	87 ± 10	<1	3 ± 2	-
	3	90 ± 5	<1	2 ± 2	-
	All	82 ± 14	1 ± 2	5 ± 5	-
10	1	72 ± 8	11 ± 2	11 ± 4	1.6 ± 0.9
	2	33 ± 6	13 ± 5	7 ± 5	1.2 ± 1.1
	3	19 ± 10	35 ± 25	6 ± 4	1.4 ± 1.1
	All	41 ± 24	20 ± 18	8 ± 5	1.4 ± 1.0
11	1	28 ± 17	10 ± 7	19 ± 18	2.0 ± 1.0
	2	70 ± 34	18 ± 18	12 ± 16	1.2 ± 1.1
	3	79 ± 24	22 ± 11	26 ± 31	0.4 ± 0.9
	All	59 ± 33	17 ± 13	19 ± 22	1.2 ± 1.1

[1] Primarily *Dictyosiphon foeniculaceus*, *Stictyosiphon tortilis* and *Pilayella littoralis* (see text).
[2] Includes *Fucus distichus*, *Neodilsea integra* and *Palmaria palmata*.
[3] Includes *Laminaria* sp. and *Agarum cribrosum*.
- Data not collected.

27) where foliose algae were scarce. This relationship should be examined further when biomass data for dominant species become available.

The present report is the first to present quantitative results concerning arctic macrophytic algae occurring on the mixed sediment-rock bottom type such as that found at Cape Hatt. The qualitative appearance of algal communities in transect photographs, the percent cover estimates from *in situ* counts, and the data on biomass and species composition from airlift samples constitute pre-spill information on a variety of variables. These data will be used to detect and assess any post-spill changes in the macrophytic algae of the study bays.

SUMMARY

On the basis of preliminary surveys at Cape Hatt, northern Baffin Island, during August 1980, three bays, two depths and three contiguous 50 m transects at each depth in each bay were selected using as selection criteria (1) similarity of substrate, flora and fauna, and (2) facility of sampling. During September 1980 the first pre-spill suite of systematic sampling was carried out on each transect. The work on each transect included (1) collection of eight samples, each covering 0.0625 m^2, using a diver-operated airlift sampler, (2) collection of 8-12 photographs, each covering 0.5 m^2, and (3) *in situ* counts of large organisms within five areas, each 1 x 10 m in dimensions. All fauna $\geq$1 mm in length were sorted from airlift samples, identified to species where possible, counted and weighed. All bivalves and holothurians were measured, and wet and dry weights were obtained for a subsample of three dominant bivalve species from each bay. Photographs and

in situ counts were used to provide a permanent visual record of the study area and to enumerate large and widely distributed organisms.

Summary--Infauna

The shallow water infaunal benthic community found at Cape Hatt was typical of that found in nearshore regions of Eclipse Sound, Lancaster Sound and channels to the west. Benthic biomass estimates from the present study were higher than those recorded in most other arctic areas, probably because our sampler penetrated farther into the sediment and provided a more complete collection of the animals present. Grabs and other samplers used in previous studies probably did not penetrate to a sufficient depth to collect all of the large, deeply burrowing individuals of Mya truncata. This and other bivalve species accounted for most of the biomass of infauna collected at Cape Hatt, and polychaetes and bivalves in approximately equal proportions accounted for most of the numbers.

We compared the infauna in the various sampling areas (3 bays, 2 depths, 3 transects per bay and depth) using analysis of variance (ANOVA). The density and biomass of bivalves, polychaetes and total infauna, and the density of seven selected infaunal species (13 variables altogether), were examined using a fixed-effects, two-factor (bays and depths) ANOVA design in which transects were nested within bays and depths. For many variables, bay x depth interaction effects were significant, indicating that the patterns of among-bay variation at 3 m and 7 m depths were not consistent between depths. This confounded the interpretation of main effects (variation among bays and between depths) and necessitated analysis by separate one-factor

(bays) ANOVAs for data from each depth. The latter analyses showed that six of the 13 variables differed significantly ($P<0.01$) among bays at the 3 m depth, but only one did so at the 7 m depth. Variation between depths was highly significant for seven of the eight variables whose bay x depth interactions were not significant. The comparative similarity of the pre-spill infauna at 7 m depth in the three bays will facilitate the analysis of oil spill effects.

Factor analysis of the densities of the 35 most common species (89.3% of total infauna) identified eight 'assemblages' of animal species that tended to occur together. The first of these assemblages bore a very close resemblance to the ubiquitous arctic Macoma community. We used one- and two-factor nested (see above) multivariate analyses of variance (MANOVA), with the eight sets of factor scores as dependent variables, to compare the infaunal communities in relation to bays, depths and transects. The two-factor MANOVA showed a significant bay by depth interaction. One-factor MANOVA showed no significance among-bay difference in community composition at either the 3 m or the 7m depth.

Mean lengths of two bivalve species (Mya truncata and Astarte borealis) and the mean oral ring diameter of the holothurian Myriotrochus rinkii were significantly larger at 7 m depth than at 3 m. Of the five species tested, only Mya truncata showed significant among-bay differences in length. The length-weight relationships of the three bivalve species that were studied were best expressed by power curves ($y = ax^b$). Analysis of covariance showed that the exponent in this relationship differed significantly among bays for Mya truncata and Astarte borealis. For Macoma calcarea, neither the exponent nor the dry meat weight after adjusting for length differed among bays.

Infauna were classified into feeding guilds based on data available in the literature. In the study bays at Cape Hatt, filter feeding was the dominant feeding mode, and surface deposit feeding the second most common mode. Carnivores and burrowing and tubiculous deposit feeders showed the least among-bays variability in biomass.

Summary--Epibenthos

For the purposes of the present study, the 'epibenthos' was defined as those animals capable of motion. For these animals, any temporal changes in density might be a result of either mortality or emigration, or both, and the contributions of these two sources of variation would not be readily distinguishable. This group was, therefore, treated in less detail than the relatively immobile infauna.

Epibenthic crustaceans collected in airlift samples consisted of ostracods, amphipods and cumaceans. Relatively few species, all of which are common in nearshore high arctic waters, comprised the majority of the numbers and biomass collected. Ostracods, cumaceans and, to a lesser extent, amphipods were more abundant at the 7 m depth than at 3 m, and among-bay differences in densities were also apparent.

A total of 10 fish belonging to four species were collected in airlift samples. Most were Gymnocanthus tricuspis and juvenile Gymnocanthus sp. collected on the 3 m transects.

Density estimates of the urchin Strongylocentrotus droebachiensis and the starfish Leptasterias polaris were more accurate when based on in situ

counts than when based on counts from photographs. This was attributable to the patchy (urchins) and sparse (starfish) distributions of these animals and the relatively small area covered by photographs. No urchins or starfish were observed on the 3 m transects; at 7 m, densities of L. polaris were low and relatively even in the three bays, whereas S. droebachiensis was more abundant and significantly variable among bays.

Summary--Macroalgae

The macrophytic algal community in the study bays at Cape Hatt was dominated by a basal stratum of filamentous and dendritic forms consisting primarily of Pilayella littoralis, Stictyosiphon tortilis and Dictyosiphon foeniculaceus. A 'canopy' of foliose algae including Fucus distichus, Neodilsea integra and Laminaria spp. was unevenly distributed at the 3 m depth both within and among bays. At 7 m, sparsely distributed kelps (Laminaria spp. and Agarum cribrosum) were the only conspicuous canopy macroalgae.

Based on two-factor nested ANOVA (see above), the biomass of macroalgae varied significantly ($P \leq 0.01$) among transects within bays and depths, and was significantly higher at the 3 m depth than at 7 m. Variation among bays, however, was not significant when compared with variation among transects within bays and depths. Percent bottom cover (based on in situ estimates) by the lower algal stratum was usually high at 3 m at low at 7 m, but intermediate values were estimated for some transects at both depths. No correlation was evident between these estimates and biomass estimates on a transect by transect basis, likely due to variation in thickness of the lower stratum and in the distribution of foliose 'canopy' species.

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APPENDIX 1. Dates and locations (depth, bay, transect, and number of metres from N to S along the transect line) of each airlift sample collected at Cape Hatt in 1980.

Depth	Bay	Transect	Replicate								Date(s)
			1	2	3	4	5	6	7	8	
7 m	9	1	2	8*	20*	23*	31	35	36	43	1 Sept
		2	6	24*	25	32*	37	43	43*	48	31 Aug, 1 Sept
		3	2	8*	20*	23*	31	35*	36*	43	31 Aug
	10	1	1	2*	7*	13*	14	16	29*	31	3 Sept
		2	5*	8	11	16*	20*	24	33*	41*	3 Sept
		3	5	9*	16*	20	24*	25	38*	44	3 Sept
	11	1	1	5*	12*	23*	24	33	39*	40*	4, 5 Sept
		2	6	12*	14*	16*	27*	29	40*	41	5, 6 Sept
		3	4*	20	25*	27	28	40	43*	45*	6 Sept
3 m	9	1	2*	5	10	11*	14	20	33	39*	10 Sept
		2	1*	9	10	17	24*	30	36	44	10 Sept
		3	6*	16*	21	27	30*	33	38	45	10 Sept
	10	1	4*	11	30	33*	37*	43	45	46*	7 Sept
		2	3*	6*	11	13	17*	32*	44	46	7 Sept
		3	2*	5*	8	13*	14	30	31	37*	8 Sept
	11	1	0*	6*	12	16*	21	41	44	45*	9 Sept
		2	1*	12*	19*	26	27	30	39*	47	9 Sept
		3	0	1*	6	7	14*	16*	18	42*	9 Sept

* Indicates sample taken seaward of transect line.

Abundance and Activity of Heterotrophic Marine Bacteria in Selected Bays at Cape Hatt, N.W.T. 1980

First report to the Baffin Island
Oil Spill (BIOS) Project

by

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ABSTRACT

On the basis of bacterial abundance and activity, three bays (9, 10, 11) at Cape Hatt, N.W.T. have been judged to be similar and therefore suitable for comparative microbiological studies during and after experimental petroleum spills in 1981. Analyses of variance of counts of total viable heterotrophs (TVH), and concentrations of particulate and dissolved organic carbon in water columns of the three bays did not reveal any differences at the 1.0% level of significance. Maximum velocities (V_{max}) of glutamic acid uptake and total counts (TC) of bacterial cells did not show significant differences between bays 9 and 10 or 10 and 11, but a significant difference was found between bays 9 and 11. This has been attributed to the residence time of water in bay 11. Maximal velocities at the beginning of the open water season averaged 5.35 $\mu g\ L^{-1}d^{-1}$, similar to other areas in Baffin Bay, Lancaster Sound and Frobisher Bay.

Variations in values between the sediments of stations and bays for V_{max}, total count and total viable heterotrophs were attributed to changes in the areas of sediment sampling and methods of collection. Mean total counts of bacteria appeared to reach a peak early in the open water season and then slowly decline, whereas the means of V_{max} increased slowly across the sampling period to mid-September.

Mineralization of n-(1-^{14}C)hexadecane in the three bays was uniformly low, or not detectable by our analytical procedure. Few oleoclastic or petroleum-degrading cells were determined by a similar analysis.

The measurement of glutamic acid uptake in benthic in situ incubation systems (BISIS) corresponded closely to laboratory results. Glutamic acid uptake at the sediment-water interface was found to be different from uptake in the water immediately above the sediment. Experimental work with BISIS units is planned for 1981.

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1.0 INTRODUCTION

The Baffin Island Oil Spill (BIOS) project at Cape Hatt, N.W.T. is a four year study designed to evaluate the effects of chemical dispersants during and after experimental oil spills in 1981. Integrated physical, chemical and biological studies during 1980 yielded baseline data. The "core" biological studies include benthic and microbiological studies supported by environmental chemistry. Aspects of oil degradation by microorganisms are being studied during this project by a group of Norwegian microbiologists. Our laboratory is concerned with the seasonal abundance and activities of heterotrophic bacteria in water columns and sediments and the effect of petroleum and petroleum-dispersant mixtures on these activities, both experimentally and as a consequence of the spills. Limited studies on the bacterial degradation of a hydrocarbon fraction were also undertaken.

On the basis of gross biological and physical similarities, two bays were selected in June and a third bay in August to serve as sites for the experimental spilling of crude petroleum and crude petroleum mixed with dispersant. The third bay will serve as a control. Sampling was conducted through the ice in June and in open water in August and September.

The objectives for the first year of study (1980) were:

1. characterization of numbers and heterotrophic activities of bacteria in the water and sediment of selected bays at Cape Hatt;
2. assessment of the above measurements during all seasons in relation to other biological and chemical cycles.

Microorganisms, including bacteria, yeasts, other fungi and phytoplankton are vital components of the world's oceans. Carbon is fixed and utilized by phytoplankton, providing foragable biomass and dissolved organic nutrients for the marine food chain. As part of the carbon cycle, heterotrophic bacteria, through a process of oxidation, decompose complex organic molecules to smaller monomolecular units for incorporation into their protoplasm, thereby utilizing the greater part of the dissolved organic nutrients formed by phytoplankton. In so

doing, particulate bacterial biomass is made available for grazing by organisms at other trophic levels, and essential nutrients such as ammonia and phosphate are released by mineralization for re-entry into nutrient cycles. Bacterial floras have been estimated to comprise upwards of 50% of the biomass of the world's oceans (Morita, 1977) and therefore are important marine constituents in terms of both abundance and function.

When introduced into the marine ecosystem petroleum forms a highly complex part of the dissolved and particulate organic material in the water. Heterotrophic activity by bacteria and fungi comprises the sole route for degradation and mineralization of petroleum as part of the carbon cycle. This is an oxidative process which requires a source of nitrogen, phosphorus, oxygen and trace elements and is very dependent on temperature. The degradation and mineralization of petroleum hydrocarbons releases carbon and energy for the maintenance, growth and multiplication of some heterotrophic marine bacteria, defined here as oleoclasts.

The heterotrophic activity of bacteria in the marine environment is regulated by the availability of inorganic nutrients and organic substrates required for growth and multiplication. As previously indicated, the availability of substrates depends on the activities of other biological components of the system, especially the primary producers. Heterotrophic activity is, therefore, linked to the cycle of primary production. Oleoclastic bacteria, capable of deriving carbon and energy from hydrocarbon fractions in addition to naturally-occurring organic substrates, appear to form a proportion of the bacterial floras of most marine environments even when petroleum is not present. Oleoclasts demonstrate a cycle of abundance and activity in concert with other bacterial activities in northern ocean areas which are ice-covered much of the year. With declining biological activity during the period of ice formation and the paucity of organic substrates in the water, heterotrophic floras including oleoclasts decline.

Little is known about the effects of petroleum on bacteria other than effects on their capacity for biodegradation of petroleum fractions. The controlled influx of petroleum and dispersants into a measured environment provides an opportunity to observe possible changes in the cycles of bacterial abundance and activities. These changes may be a result of alterations in other biological cycles, or a consequence of direct effects on bacterial populations. In the first year of the BIOS project, various parameters have been quantitated and compared in three bays. A description of these baseline data forms the substance of this report.

2.0 STUDY AREA

As part of the BIOS project, located at Cape Hatt on northern Baffin Island (Fig. 1), eight stations were sampled for microbiological activity in 1980 (Fig. 2). The stations sampled during June in bays 9, 10 and 13, were on transects surveyed on the ice by Dr. J. M. Semples of Petro-Canada. These were located at water depths of 12 to 14 m along the two outer of three transect lines. The stations were moved slightly in August after ice break-up to coincide with the shore markers placed by Gary Sergy of the Environmental Protection Service (EPS), Department of the Environment. Temporary floats were used to mark the location of the stations and permanent markers will be deployed in 1981. From 19 August onwards, bay 11 was sampled in preference to bay 13. Stations in bay 11 were located at depths of 11 to 12 m.

In each of the bays chosen, two stations were sampled for water and sediments. The field season was divided into seven sampling cycles, two from 6 to 16 June under winter and early spring conditions and five cycles from 11 August to 16 September under summer and fall conditions. Cycles were defined as the time taken to sample all three bays once (Table 1).

3.0 METHODS AND MATERIALS

3.1 Sampling Procedures

3.1.1 Water Column

Water samples from depths of 1, 5, and 10 m were collected at all microbiology stations using a 5.0 L Niskin bottle and hand-line. The samples were transferred to 4.0 L bottles and transported in a sample box to the Cape Hatt laboratory where they were processed within several hours of collection.

3.1.2 Sediments

During the first three sampling cycles sediments were collected at all stations using a Petersen-type grab or a Phleger corer. Station 1A on 6 June was not sampled since the bottom was found to be rocky. Beginning with the fourth cycle of sampling that started on 19 August, divers from LGL Ltd. made collections at a depth of 7 m, slightly inshore from the station markers, by scraping a 2.0 cm layer of sediment into a wide mouth jar. From the original sample, two modified 50 mL disposable syringes were filled with surface sediment. One syringe was frozen and later transported to Ste-Anne-de-Bellevue for organic carbon and dry weight determinations. The other was refrigerated for microbiological processing the following day. From the refrigerated syringe, two 1.0 mL wet subsamples were measured with a modified 3 mL disposable syringe and were suspended in 2.0 L of filter-sterilized 10 m water collected from the same station. The 0.1% by volume sediment-water mixture was then manually agitated and the suspension maintained in a crushed ice bath on a magnetic stirrer during processing.

3.2 Chemical and Physical Oceanography

The values for chlorophyll _a_, reactive phosphate and nitrate used in this report originated from Green (1981). Collection of the material for these analyses was made at the same time as microbiological sampling.

3.3 Total Viable Count of Heterotrophs (TVH)

Petri dishes containing a modified formulation of the Lib X agar

medium of Griffiths, Hanus and Morita (1974) were prepared in Ste-Anne-de-Bellevue, shipped to Cape Hatt, and stored at 2.0°C until used. The medium consisted of 2.3 g trypticase (Baltimore Biological Laboratories), 1.2 g Bacto-yeast extract (Difco), 7.69 g tris buffer (Trizma-7.2; Sigma Chemical Co.), 0.3 g sodium citrate, 0.3 g L-glutamic acid, 0.05 g sodium nitrate, 0.001 g ferric chloride, and 12.0 g Bacto-agar (Difco). The ingredients were dissolved in 1.0 L of 34°/$_{oo}$ Instant Ocean (Aquarium Systems, Inc.). The medium was autoclaved, cooled and dispensed in 100 mm petri dishes. Final pH at 5.0°C was 7.8. Plate count agar was prepared in a similar fashion using Plate Count Broth (Difco) dissolved in distilled water and supplemented with Bacto-agar. A spin-plate technique was employed to dispense a 0.1 mL aliquot of the water sample on the surface of triplicate cold Lib X agar plates with a 0.2 mL sterile pipette. From the original 0.1% by volume (10^{-3}) sediment-suspension sample, two sequential 10 fold dilutions were prepared such that 10^{-4} and 10^{-5} dilutions of sediment in seawater were obtained. Triplicate petri dishes of Lib X and plate count agar were spun for each of the sediment dilutions. After incubation at 2.0°C for three weeks, the plates were examined and those with an uneven distribution of colonies were discarded. The colonies of a replicate set were enumerated and the mean value was expressed as the total number of viable heterotrophs (TVH) per litre of water sample or litre of 0.1% by volume sediment-water suspension.

3.4 Most Probable Number (MPN) of Oleoclasts

The abundance of oleoclastic or petroleum-degrading heterotrophs was determined by the most probable number (MPN) procedure (American Public Health Association, 1971). Ten mL samples of seawater or sediment-suspension, and 10 fold serial dilutions up to 10^{-9} in 9.0 mL of sterile Instant Ocean (34°/$_{oo}$) were prepared in triplicate using screw-cap test tubes. Each tube was supplemented with 0.2 mL of nitrate-phosphate nutrient solution, 10.0 µL of sterile 8.0% weathered Lago Medio crude petroleum, and 15.0 µL of n-(1-^{14}C)hexadecane (Amersham

Corp.). The nitrate-phosphate concentrate yielded a final concentration of 1.0 g NH_4NO_3 and 0.1 g K_2HPO_4 per litre of sample water. Final activity of hexadecane was 3.0 μCi L^{-1} of sample water. The tubes were incubated at 5.0°C for a 60 day period after which time they were acidified by addition of 0.2 mL of 5.0 N H_2SO_4 through the septum of the cap.

In the laboratory at Ste-Anne-de-Bellevue, the tubes were purged of CO_2 in a gas train. The CO_2 was collected in NaOH and precipitated as $BaCO_3$. The precipitates were filtered through two 24.0 mm Whatman GFC filters and the filters added to scintillation vials containing 7.0 mL of Aquasol (New England Nuclear Corp.). The vials were shaken on a vortex mixer to disperse the precipitate, and 3.0 mL of distilled water were added to gel the Aquasol and ensure that a uniform distribution of the precipitate was maintained in the vial. Vials were counted in a Nuclear-Chicago Isocapp 300 scintillation counter and corrected for quenching by the channel ratios method. Sample counts of more than double the background value were considered positive. Vials were scored for the highest dilutions yielding positive results; these were used to obtain the MPN of oleoclasts by reference to a standard MPN statistical table.

3.5 Total Counts

Total counts of bacteria in seawater were made using a procedure slightly modified from that described by Watson, Nowitsky, Quinby and Valois (1977). Polycarbonate membrane filters (Nucleopore Corp.) of pore size 0.2 μm and 25.0 mm diameter were prestained in a 0.2% solution of irgalan black in 2.0% by volume acetic acid. The filter was then rinsed in cell-free distilled water and placed on a 25.0 mm glass filter holder (Millipore Corp.). A seawater or sediment-suspension sample of 2.0 to 15.0 mL, fixed at the time of collection with 0.2% gluteraldehyde, was added to the filter funnel after shaking on a vortex mixer and sufficient acridine orange (80% dye content), at a concentration of 0.1% in 0.02 mol tris (pH 7.2), was added to the sample to yield a final stain

concentration of 0.02%. After two minutes, the sample was filtered and rinsed with 5.0 mL of cell free water. The membrane was then placed on a glass slide, wetted with a drop of Cargille Type A immersion oil, and covered with a cover glass. A Zeiss model WL microscope equipped with an epifluorescent condenser, a 50 watt mercury lamp, a BG 12 excitation filter, a No. 50 barrier filter, and a No. 500 beam splitter was used to view the fluorescing cells. For counting, a reticule with a 10 mm grid was used. A sufficient amount of seawater or sediment-suspension sample was filtered to yield about 100 cells per grid field. Ten randomly selected grid fields of each sample membrane were counted and the mean value expressed as the number of cells per litre of seawater or sediment-suspension sample.

3.6 Bacterial Heterotrophic Potential

An extensive modification of the procedure described by Harrison, Wright and Morita (1971) was employed throughout this study. Water samples and sediments were collected, as previously described, and processed immediately. To measure substrate assimilated and retained by bacteria, 10.0 mL of water sample or sediment-suspension were added to 14 chilled and sterile 55 mL screw-cap bottles containing varying amounts of glutamic acid substrate. Seven varying ratios of labelled to unlabelled glutamic acid were used in these supplements, yielding duplicate vessels containing total glutamic acid concentrations of 1.0 to 60.0 $\mu g\ L^{-1}$ of sample water, and activity of 2.0 or 20.0 $\mu Ci\ L^{-1}$. The specific activity of the L-[$^{14}C(U)$] glutamic acid (New England Nuclear Corp.) was 292.0 $mCi\ mmol^{-1}$. A fifteenth vessel containing 1.0 $\mu g\ L^{-1}$ of glutamic acid with 2.0 $\mu Ci\ L^{-1}$ of radioactivity served as a background control for the seven concentrations. Upon addition of the seawater or sediment-suspension aliquot, the reaction volume of the control vessel was immediately filtered through a 25.0 mm membrane filter (Millipore Corp.) with a pore size of 0.45 μm, and rinsed twice with 15.0 mL portions of cold, filtered seawater. The fourteen vessels were incubated at 2.0°C for 9.0 or 18.0 hours.

Suspended sediment samples were incubated at 2.0°C for 5.0 or 9.0 hours. Incubation times were varied in view of the expected bacterial activity at the time of sampling. Incubation was stopped by simultaneous filtration of the 14 vessels followed by cold rinsing. Rinsed membranes were transferred to scintillation vials containing 8.0 mL of Aquafluor (New England Nuclear Corp.), a dioxane-based fluor, as suggested by Thompson and Hamilton (1974).

To measure substrate respired by bacteria, fifteen 50 mL serum bottles were prepared with substrate and sample water or sediment-suspension as above. Upon addition of the sample to the control vessel, 0.2 mL of 5.0 N H_2SO_4 was immediately added to reduce the pH of the sample to below 2.0. Bottles were stoppered with serum caps fitted with plastic reaction wells (Kontes Glass Co.). The wells, suspended above the reaction volume, contained a fluted wick of two glass filters (Whatman GFA-24 mm). After incubation as above, the reaction in the serum vessels was stopped by the addition of 5.0 N H_2SO_4 through the rubber serum caps by means of a syringe. At the same time, 0.2 mL of β-phenethylamine (New England Nuclear Corp.) was added through the cap into the plastic well where it was completely absorbed by the wick. The bottles were then further incubated for 12 h at 40.0°C, during which time $^{14}CO_2$ was evolved from the seawater and absorbed by the phenethylamine-soaked wicks. The bottles were then opened and wicks were transferred to scintillation vials containing 8.0 mL of Aquasol. Scintillation vials were transported to Ste-Anne-de-Bellevue for counting. Results of membrane and wick counts were combined to yield total uptake of the glutamic acid substrate. Uptake kinetics were generated using computer programs (Section 3.10).

3.6.1 Theory

Kinetic parameters from the uptake of the glutamic acid substrate were calculated from a modified Michaelis-Menten equation (Dowd and Riggs, 1965):

$$\frac{D_\mu t}{d} = \frac{(K+S)}{V_{max}} + \frac{A}{V_{max}}$$

where D_μ = radioactivity added, d = radioactivity taken up, t = incubation

time in hours, K = an uptake constant, S = concentration of the natural substrate, V_{max} = the maximum velocity of uptake and A = concentration of the substrate added. Plotting $\frac{(D\mu t)}{d}$ against A yields a straight line where the reciprocal of the slope = V_{max}, Y intercept = turnover time in hours (T), and X intercept = (K+S).

The maximum velocity (V_{max}), or potential of heterotrophic activity, is the velocity of uptake at which the substrate saturates the uptake system such that the velocity can no longer increase. V_{max} indicates the physiological state of the bacterial flora by demonstrating the potential ability of the flora to use a particular substrate; i.e. its degree of adaptedness to that substrate.

3.7 Determination of Mineralization of Hexadecane

To assess the amount of mineralization of n-(1-^{14}C)hexadecane in water samples from a depth of 5 m or sediment-suspension samples, 30.0 mL aliquots of the samples were added to fifteen 50.0 mL serum bottles and supplemented with 0.6 mL of a sterile tris buffer solution, 30 µL of sterile 8.0% weathered Lago Medio crude and 30 µL of hexadecane containing n-(1-^{14}C)hexadecane to yield a final concentration of 2.0 µCi L^{-1} of sample. To account for the possibility of nutrient limitation, a second set of bottles was also prepared and supplemented with 0.6 mL of a sterile nitrate-phosphate concentrated solution with a final concentration of 1.0 g NH_4NO_3 and 0.1 g K_2HPO_4 per litre of sample. To measure the effect of dispersant on hexadecane mineralization, two additional sets of bottles were prepared as above and supplemented with 30 µL of 10% by volume Corexit 9527 (Exxon Chemical Co.) in water solution. The final ratio of Corexit to oil was 1:10. In each of the four sets, the 15th bottle was immediately acidified to pH 2.0 with 0.6 mL of 5.0 N H_2SO_4 and served as a background control. All vessels were incubated at 5.0°C and, at five or ten day intervals up to fifty days, two bottles from each set were acidified. Evolved $^{14}CO_2$ was quantitated as in the MPN procedure. Maximum DPM was generally obtained after the 50 day incubation.

3.8 Organic Carbon Determinations

Water samples were analysed for carbon by a procedure which modified and combined those of Menzel and Vaccaro (1964) and Stainton (1973). Freshly collected 100.0 mL water samples were filtered through previously ashed Whatman GFC-24 mm glass filters. Filters and filtrates were immediately frozen for subsequent analysis.

Dissolved organic carbon (DOC) was determined from the thawed filtrate by wet oxidation with potassium persulphate in a sealed ampule after removal of inorganic carbonate. Particulate organic carbon (POC) was determined from the thawed glass filters in a similar fashion. In both cases, evolved CO_2 was reduced to methane on a nickel catalyst in a continuous stream of hydrogen. Production of methane was determined by flame ionization using a Hewlett-Packard 5700A gas chromatograph and recorded and quantitated by a Hewlett-Packard 3380 recorder-integrator. Procedures for organic carbon determinations in the sediments will be reported at a later date.

3.9 In Situ Sediment Incubation

The presence of divers at the BIOS project site permitted the development and use of a benthic in situ incubation system (BISIS) (Fig. 3). The BISIS was designed to study and compare the in situ uptake of radio-labelled substrates by bacteria at the water-sediment interface and the water immediately above the sediment.

As illustrated in Figure 3, the BISIS consists of a cylinder divided in two compartments by a centre plate. The open bottom of the BISIS is forced into, and closed off by, the sediment. Rubber springs between the top end plate, centre support collar, centre plate, and bottom support collar maintain openings A, B, and C, thereby allowing a free flow of water through the compartments until the unit is closed. When closed by tightening the clamp handle, the two compartments each contain approximately 1 L of water. The bottom support collar acts as a flange to control the depth to which the BISIS unit can be placed into the sediment, thereby defining the water volume of the lower compartment. Incubation proceeds with the injection of radio-labelled

substrate into each compartment from the previously-loaded substrated syringes. After incubation, a 60.0 mL volume of sample water is withdrawn from the bottom compartment *in situ* using the attached 50 mL sampling syringe. Upon retrieval of the BISIS from the sediment, 60.0 mL of water from the top compartment is sampled at the surface by attaching another 50 mL sampling syringe to the top compartment sampling port.

BISIS units were deployed by divers on three occasions at Cape Hatt:

13 August, Station 4
6 September, Station 2
15 September, Station 2

Sufficient L-[^{14}C(U)] glutamic acid was injected into top and bottom compartments to yield a final concentration of 10.0 μg L^{-1} with 20.0 μCi L^{-1} activity. After incubations of approximately 9 h, water samples were withdrawn from top and bottom compartments. In two instances, surface sediment was collected from the area under the units. In each instance, water was collected outside and immediately adjacent to the units for comparative *in vitro* studies. All samples were maintained on crushed ice and, when returned to the laboratory, immediately processed.

To measure the amount of substrate retained by bacteria, incubation was stopped in the laboratory by simultaneous filtration of three 10.0 mL aliquots of sample water through a 25.0 mm membrane filter with a pore size of 0.45 μm and two subsequent rinsings with 15.0 mL portions of cold, filtered seawater. Rinsed filters were transferred to scintillation vials containing 8.0 mL of Aquafluor. To measure the amount of substrate respired, three 10.0 mL aliquots of sample water were transferred to 50 mL serum bottles and stoppered with serum caps fitted with a plastic reaction well. Samples were then treated as described in section 3.6.

Assuming a linear relationship between uptake and incubation time for each BISIS, a correction factor was introduced to standardize results to a 9 h incubation period. A linear correction was also

introduced when sample volumes were less than 10.0 mL due to collection problems with some sampling syringes.

The surface sediment sample collected by the diver from under the BISIS, was subsampled in the laboratory with a modified 3 mL syringe. A 1.0 mL aliquot of this wet sediment was diluted to 10^{-3} with sterile filtered seawater. Three 10.0 mL aliquots of this suspension were filtered in the same fashion as the water samples to quantitate the retention of radio-labelled substrate by bacteria in the sediment.

Finally a kinetic uptake experiment, as described in section 3.6, was made using water from outside the BISIS unit.

3.10 Statistics

Kinetic parameters of glutamic acid uptake and their correlation coefficients were generated using computer programs developed by D. Burrage (Department of Computer Sciences, McGill University) and J. N. Bunch. Multi-way analysis of variance (ANOVA) was determined at the 1.0% level of significance. This statistical procedure is part of the Statistical Analysis System (SAS Institute Inc., Cary, N.C., USA) available through the McGill University Computing Centre.

4.0 RESULTS

4.1 Water Columns

Statistical analysis of the results indicated that no significant differences existed between depths at the same station, or stations in the same bay. For the purposes of this report, values from the three depths sampled at both stations in a bay have been averaged and are presented in Figures 4 to 8. Data for all stations and depths are presented in Tables 7 and 8. Since bay 13 was found to be unsuitable for these studies, data obtained at stations 1A and 2A have been reported (Tables 7 and 8) but are not included in figures and other tables, nor in discussions and comparisons.

4.1.1 Total Count of Bacterial Cells

The total count of bacterial cells found in the June sampling period (cycles 1 and 2) ranged from 5.2×10^7 to 8.3×10^7 cells per litre (Fig. 4). When collections resumed in August (cycle 3), values had increased to 3.4×10^8 and 3.3×10^8 cells per litre for bays 10 and 9 respectively. The number of cells continued to increase in all bays, with the greatest population size occurring in late August. The largest population, 9.6×10^8 L^{-1} was found in bay 11 on 28 August. Water samples taken during the last cycle indicated a decline in total cell numbers in all bays, with population size in bays 9 and 10 returning to that found during cycle three.

4.1.2 Total Viable Heterotrophs

In all bays, the total number of viable heterotrophs, as determined by the number of colony-forming units, was less than 4.0×10^5 L^{-1} on plates inoculated with water samples collected up to 21 August (Fig. 5). Most results are missing for 13 and 15 August because plated samples froze in a faulty incubator. Heterotrophic numbers increased rapidly after 21 August, the maximum heterotrophic population size occurring in Cape Hatt waters in late August and early September. There was only a slight decline in numbers from early September until the last collection on 16 September.

4.1.3 Dissolved and Particulate Organic Carbon

Dissolved organic carbon (DOC) remained stable in June at an average of 1.5 mg L^{-1}. Early August results were up slightly to 1.9 mg L^{-1} and 2.0 mg L^{-1} for bays 10 and 9 respectively and increased to a maximum between 28 August and 1 September (Fig. 6). The highest concentration (3.4 mg L^{-1}) was found in bay 10 on 30 August. During the last sampling cycles, values in all bays had decreased and were similar to June values.

Particulate organic carbon (POC) concentrations were low in June samples, ranging from 35 µg L^{-1} to 60 µg L^{-1}. As illustrated in Figure 7, August and September values were similar in all bays, and roughly three times greater than June values, ranging from a low of 130 µg L^{-1} (bay 11 on 28 August) to a high of 188 µg L^{-1} (bay 9 on 15 August). A trend towards lower values appeared to be forming as the field season ended.

4.1.4 ^{14}C-Glutamic Acid Uptake

The maximum velocity (V_{max}) at which bacteria can potentially utilize glutamic acid is one of the kinetic parameters used to indicate the physiological state of bacterial floras.

During the June sampling period, activity was minimal and increased slightly from 0.23 µg $L^{-1}d^{-1}$ to 0.57 µg $L^{-1}d^{-1}$. When sampling was resumed in August, V_{max} in bay 10 had already reached an observed maximum of 5.49 µg $L^{-1}d^{-1}$. In bays 9 and 11, similar maximum values were obtained in the fourth cycle. As indicated in Figure 8, mid-August was the period of greatest potential activity after which time values decreased steadily for the balance of the field season. Values obtained in mid-September were roughly half those in mid-August.

4.1.5 Most Probable Number of Oleoclastic Cells

The numbers of oleoclastic cells in water samples from 5 m determined by the most probable number procedure (MPN) using 8% weathered Lago Medio crude as a carrier for n-(1-^{14}C)hexadecane are presented in Table 2. Counts of between 40 and 90 L^{-1} were observed

on some occasions, but generally the numbers of oleoclastic cells appeared to be too low to be detected.

4.1.6 Hexadecane Mineralization

Hexadecane mineralization in a 30.0 mL water sample, using 8% weathered Lago Medio crude as a carrier for ^{14}C-hexadecane are presented in Table 2. A long lag period was observed and activity was shown only after incubation for 40 or 50 days. Although overall response was low, the most active samples were those supplemented with nutrients. Hexadecane mineralization was negligible in the presence of Corexit 9527 and was not enhanced by nutrient supplementation. A comparison of the use of 8% weathered Lago Medio crude and well-weathered Norman Wells crude as carriers for n-(1-^{14}C)hexadecane did not show any significant difference in hexadecane mineralization even with nutrient supplementation.

The two highest results obtained after 50 days of incubation were at station 6 on 16 September and station 1 on 5 September, with values of 19 825 disintegrations per minute (dpm) and 14 902 dpm respectively.

4.2 Sediments

One sediment sample was collected from each station during each of the seven collection cycles for determining the V_{max} of glutamic acid uptake, total counts of bacteria and total numbers of viable heterotrophs. The data are reported in Table 9. The lack of homogeneity in the few sediment samples, together with the use of several techniques for sediment collection, yielded varying results. Figure 9 and Table 3 show the means of six sediment collections from three bays during sampling cycles. Sediment studies were performed with 0.1% by volume sediment-water suspensions and the results for total count, total viable heterotrophs and V_{max} are expressed per litre of suspension.

4.2.1 Total Counts of Bacterial Cells

The means of total counts for the June sampling cycles were 2.9×10^8 and 1.9×10^8 L^{-1} of sediment suspension (Table 3, Fig. 9). A mean count

of 5.8 X 10^8 L^{-1} was obtained from the bays during 13-15 August and a maximum mean count of 7.5 X 10^8 L^{-1} was obtained in the following cycle of collection (19-23 August). Counts declined slowly in the sediments of the three bays in subsequent collections to the end of the sampling season.

4.2.2 Total Viable Heterotrophs

The means of counts of total viable heterotrophs (TVH) from sediments (Table 3, Fig. 9) refer to counts of colonies which developed on a marine plating medium (Lib X) at 2.0°C. Some data in August (Table 9) are missing because plates froze in a faulty incubator and were therefore discarded. Mean counts from sediment samples taken during the June sampling cycles were 5.7 X 10^5 and 3.3 X 10^5 L^{-1}. The fluctuation in mean counts during the first two cycles in August can be regarded as spurious since only one sediment sample was counted for the sampling cycle of 13-15 August. Mean counts of 11.9 X 10^5 to 15.5 X 10^5 L^{-1} were observed for the balance of the season, values that were approximately three times higher than during June.

No colony formation was observed on plate count agar, a terrestrial plating medium prepared with fresh water, after inoculation with sediment suspensions.

4.2.3 ^{14}C-Glutamic Acid Uptake Kinetics

The maximum velocity (V_{max}) of glutamic acid by bacterial floras in sediments showed large fluctuations, probably as a result of variation in sediment samples and methods of collection. In addition, the uptake activity in the sediments was higher than any observations previously made in other areas by our laboratory (unpublished data). The bacterial flora was rapidly saturated by all but the lowest concentrations of glutamic acid and the kinetic parameters were difficult to estimate. The means of V_{max} determined from the two cycles of collection in June were 10.8 and 13.4 μg $L^{-1}d^{-1}$ (Table 3, Fig. 9). A similar mean was determined from the first sampling cycle in open water (13-15 August). The means of uptake activity were observed to increase approximately

2.5 fold across the balance of the sampling season to a mean of 34.0 $\mu g\ L^{-1}d^{-1}$ in the sediments of the three bays during 12-16 September.

4.2.4 Most Probable Number of Oleoclasts

With two exceptions, the mineralization of n-(1-^{14}C)hexadecane by sediment samples could not be detected by our procedure. Most probable numbers, therefore, could not be calculated. The two exceptions were cell counts of 1.9 X 10^2 L^{-1} from sediment at station 6 and 2.9 X 10^3 L^{-1} from sediment at station 4 on 10 June and 13 August respectively.

4.2.5 Amounts of Hexadecane Mineralization

With one exception, no determinations of amounts of hexadecane mineralization were made for the reason given above (4.2.4). Had incubation intervals extended beyond fifty days, a possible lag might have been overcome and positive results obtained. The only exception was a single result of 1432 dpm obtained from evolved $^{14}CO_2$ after 50 days incubation of sediment collected at station 4 on 15 September.

4.3 In Situ Sediment Incubations

On 13 August, three benthic in situ incubation systems (BISIS) were installed at station 4. For comparative purposes, all results were equalized in terms of a 9.0 hour incubation time and 10.0 mL of water or sediment-suspension sample.

The three upper compartments yielded reproducible results both in uptake (filter membranes) and respiration (wicks) (Table 4). These results also related very closely to those obtained by means of the in vitro incubation carried on simultaneously in the laboratory (Table 4). A respiration percentage of 43 was obtained in the top chamber in the BISIS whereas 44% respiration was recorded by the in vitro uptake experiment using 10 m water from station 4.

Greater fluctuation was recorded in the results of the lower compartments, both for uptake and respiration. The lower compartment of one unit was not sampled because of a valve malfunction. The average

uptake value in lower compartments was five times lower than in the outside water, while the average percent respiration was twice as high as in the surrounding water. No sediment samples were collected from under the compartment following incubation.

On 6 September, three BISIS units were placed at station 2. The results obtained from the upper compartments were similar in uptake and respiration (Table 4). They also compared favourably with the results obtained *in vitro*. Uptake values increased from approximately 33 000 dpm on 13 August to nearly 40 000 dpm on 6 September, whereas respiration values and percent respiration decreased. The respiration values obtained from all three bottom compartments showed little variation and were similar to the respiration values observed in the surrounding water. However, uptake results, with a mean value almost 20% higher than the control, showed considerable fluctuation. An uptake value of about 105 000 dpm was obtained for a 10 mL sediment-suspension sample.

In situ incubations on 16 September at station 2 yielded reproducible results in top compartments both for uptake and respiration (Table 4). Respiration was similar to the *in vitro* control value but uptake showed a 1.5 fold increase with respect to the surrounding water at 10 m. When these results were compared to those obtained on 13 August, a decrease of 20% for uptake and of more than 50% for respiration was noted. The lower compartments showed moderate fluctuations in both uptake and respiration, the average uptake values being slightly lower and the average respiration value being more than three times higher than the *in vitro* control. A sediment uptake value of 216 623 dpm showed a two fold increase over 6 September.

5.0 DISCUSSION

5.1 Water Columns

Analysis of variance (Section 3.10) of the data for V_{max}, total count, DOC and POC did not demonstrate any significant difference between the three depths of a station or between the two stations within a bay during August and September. For this reason, the data are presented as six replicate samples of a given bay on a particular sampling date. The uniformly low values for the same parameters in the June period did not lend themselves to inclusion in the statistical analyses, nor was it deemed necessary. The data from bay 13 were not included in statistical analyses. Although the three bays (9, 10, and 11) were occupied across a four day sampling period, (Table 1) simultaneous sampling was assumed in the summary of data presented in Figure 10 and Table 5, where the results of three bays are given as a cycle mean.

No significant difference between the three bays was evident in the concentrations of POC or DOC during the August-September period. Concentrations of DOC in the three bays did show a significant difference with time. The average concentration of DOC increased two fold across the sampling periods and returned to a concentration of 1.49 mg L^{-1} by mid-September (Fig. 10A). This is probably the concentration which persists in the water during the winter months. The seasonal increase can be attributed largely to extruded or degraded products of phytoplankton blooms and the decline corresponded to the increase in bacterial numbers. Bada and Lee (1977) suggested that bacterial floras utilize the readily oxidizable products of primary production. The remainder, which in part forms a baseline level of DOC, is refractory to bacterial utilization or is only slowly oxidized and incorporated into bacterial biomass.

The concentrations of POC in water columns of the three bays did not show significant differences with time although the mean cycle value had decreased from 185 to 149 μg L^{-1} from early August to mid-September (Fig. 10C). High levels of POC are a consequence of phytoplankton blooms and the biomass of phytoplankton forms a part of measured POC. It is probable that much of an earlier bloom of phytoplankton settled

out of the near-shore environment at Cape Hatt. Sediment traps placed at a depth of 10 m in Frobisher Bay, N.W.T. during 1980 demonstrated that a large amount of detrital material settled out in a two day period within a few weeks of maximum bloom conditions (J. W. Wacasey, personal communication). Had POC measurements been made closer to the time of ice breakup at Cape Hatt, (27 July) values higher than those of 11-13 August would probably have been obtained. POC concentrations in excess of 1000 $\mu g\ L^{-1}$ have been measured at Frobisher Bay after a week of the maximum bloom of phytoplankton (unpublished data). Data from Cape Hatt in September were similar to those from Frobisher Bay during the same period and a slow decline across the winter to June levels or lower seems evident.

The abundance of bacterial cells in the water columns of the bays increased across the open water season. The number of viable heterotrophic cells (TVH) determined by colony formation on marine plating medium increased approximately 10 fold to $1.4 \times 10^6\ L^{-1}$ (Fig. 10B) and remained at that level to the end of the sampling season. This increase was in response to primary production and paralleled the increase in DOC. Seasonal 10 fold increases in TVH occur yearly at Frobisher Bay, and the bays at Cape Hatt appear to be following previously observed trends in arctic marine waters. There was no significant difference between the three bays in TVH determinations.

A similar increase was observed in the total counts of bacterial cells as determined by fluorescent microscopy (Fig. 10B). The maximum number of cells, expressed as a cycle average, occurred during the 28 August to 1 September cycle when counts averaging $6.8 \times 10^8\ L^{-1}$ were obtained. Predictably, this value was two orders of magnitude higher than that of TVH, and is a more realistic estimate of bacterial numbers. As previously noted, total counts of bacterial cells paralleled the increase and subsequent decrease in the concentrations of DOC. There was a significantly higher count of bacteria in bay 11 than in bay 9. This may be attributable to the longer residence time of water in bay 11.

The highest values for maximal velocity (V_{max}) of glutamic acid

uptake occurred at the beginning of the summer sampling period in the bays and these rates persisted into the next cycle of sampling. The cycle means during this period were approximately 5.5 μg $L^{-1}d^{-1}$ (Fig. 10A). Similar rates have been obtained at Frobisher Bay (unpublished data) approximately two weeks following ice breakup, and larger rates are seldom observed in this area. At Cape Hatt, sampling began during the time when V_{max} was probably greatest in the bays. The subsequent decrease in V_{max} in the bays to the end of the sampling season corresponded to decreases in DOC and total counts of bacteria, and appears to reflect declining activities and numbers of bacteria under conditions of diminishing organic substrate.

A significant difference was found between bays 9 and 11 with respect to V_{max} of glutamate uptake (Fig. 8). This may be attributable to the four day time difference between the sampling of bay 11 and bay 9 (Table 1). Values of V_{max} tend to peak rapidly and this rapidity may be reflected in the differences observed. Alternatively, the larger V_{max} values in bay 11 can be construed to be a result of the activity of the larger number of bacterial cells observed in bay 11 over those in bay 9 (Fig. 4).

Analyses of sample waters for reactive nitrate and phosphate and chlorophyll *a* were performed by Seakem Oceanography Ltd. and reported reported by Green (1981). The data summarized in Figure 10 and Table 5 are from that source.

Reactive nitrate and phosphate levels were high in water columns during the June sampling period and uniformly low throughout the open water sampling period. Phosphate values were not significantly different between bays but were significantly different across the open water season, increasing slightly during the last sampling cycle in September. This has been attributed to a storm surge which occurred at that time. Low levels of phosphate and nitrate from early August on can be attributed to deplation of these nutrients by an earlier bloom of phytoplankton.

The bloom of phytoplankton which occurred prior to the commencement of sample collection in August was not reflected in the concentrations of

chlorophyll a obtained during the sampling cycle of 13-15 August. Chlorophyll a levels had presumably decreased rapidly in the near-shore waters with nutrient depletion and the cessation of the bloom. The slight increase in chlorophyll a levels in September was attributed to the storm surge replenishing nutrients, which could enhance phytoplankton production (i.e. increased chlorophyll a). Alternatively, higher levels of chlorophyll were present in deep offshore water which may have been brought near-shore by the storm surge. As an adjunct to the chlorophyll measurements, water samples were collected for enumeration of phytoplankton cells. These data will be reported at a later date.

Determinations of V_{max} of glutamic acid uptake from other regions near Cape Hatt and at Frobisher Bay are presented in Table 6 as a comparison to determinations from bay 11 at Cape Hatt. Measured concentrations of chlorophyll a, reactive nitrate and particulate organic carbon are also included. These stations (unpublished data) were chosen for comparison because of their spatial and temporal proximity to Cape Hatt. In the case of Frobisher Bay, the given rates of V_{max} were observed at an interval after ice breakup similar to the time after breakup at Cape Hatt. Station 4A was occupied near the mouth of Navy Board Inlet and station 16A near the mouth of Pond Inlet. Data from station 4A suggest that the station was occupied during the commencement of a bloom of phytoplankton. The V_{max} in the top 5 m of the water column indicated a high level of bacterial activity in response to primary production. A V_{max} of 1.69 $\mu g\ L^{-1}d^{-1}$ at 20 m, together with a chlorophyll a value of 0.7 $\mu g\ L^{-1}$ and POC of 40.0 $\mu g\ L^{-1}$ indicated that phytoplankton and bloom products had not yet descended into the water column. Reactive nitrate was not yet depleted at this depth.

In water samples taken from 1 m and 5 m at station 16A, levels of bacterial activity, together with concentrations of POC and reactive nitrate, suggested post-bloom conditions whereas at 20 m, a high V_{max}, and high levels of chlorophyll a and POC verified that the water column was in an advanced state of bloom. At Frobisher Bay, intensive site-specific work across the seasons in 1979 allowed us to follow the course of phytoplankton and bacterial activity very closely. The largest

values of chlorophyll a occurred approximately 2.5 weeks after ice breakup and during this time, reactive nitrate was exhausted in the water column and POC in excess of 600 μg L^{-1} was noted. V_{max} values were uniformly high in the 20 m water column.

When these data are compared to those obtained in bay 11 at Cape Hatt 19 August, it would appear that the sampled waters were in a post-bloom condition, and the V_{max} of glutamate uptake remained high. Reactive nitrate was essentially depleted and concentrations of chlorophyll a and POC were probably decreasing. To ensure that the development of the bloom is observed in 1981, sample collections should commence prior to ice breakup.

5.2 Sediments

The fluctuations observed in sediment data are possibly due to the small one mL wet samples used. When values from six stations in each sampling cycle (Fig. 9) are averaged, the mean V_{max} slowly increased during August and September, giving an almost straight line response. This gradual increase in heterotrophic potential suggests that seasonal changes in the sediment occur much more slowly than in the water which had already peaked between June and August, and was showing a gradual decrease in values during the same period when sediment activity was increasing. Since sampling was terminated before a maximum V_{max} could be observed, it would be difficult to estimate when or at what value this peak might occur.

Averaging of total counts or total viable heterotrophs indicated no significant change during the August and September sampling cycles. The total count values for the June sampling cycles were slightly lower than in August-September. The large seasonal fluctuations found in the water column were not apparent in the sediment. This will be investigated more thoroughly in the coming season with changes in sediment sampling procedures, which should reduce the fluctuation between individual samples.

5.3 In Situ Sediment Incubations

Since one of the three in situ experiments was carried out in a different bay, only comparisons between the two chambers of a sampler and between each sampler and the control water was attempted. The possible influence of bivalves filtering out radio-labelled substrate and bacteria in the bottom chambers rendered the interpretation of the activity displayed by each group of organisms difficult.

Throughout the experiments, water from the top chamber yielded uptake and respiration results similar to those obtained from water surrounding the BISIS. The uptake and respiration values observed in the bottom chambers were different from those in the surrounding water or in the top chambers. Generally lower uptake values in water from bottom chambers can be explained by the removal of bacteria with incorporated radio-labelled substrate by filter-feeding organisms. In addition, sediment bacteria would utilize a fraction of the labelled substrate and consequently reduce the quantity of available radio-labelled glutamic acid for bacteria in the overlying water. Carbon dioxide produced by the sediment bacteria would escape to the overlying water and add to the $^{14}CO_2$ generated by water bacteria, thus increasing respiration values to the high levels observed in the bottom chamber. Utilization of radio-labelled bacteria and substrate by filter-feeders would also contribute to evolved $^{14}CO_2$ in the lower compartments.

The implication of sediment bacteria in the production of $^{14}CO_2$ was reflected by sediment samples taken from under the BISIS units after incubation. Those sediments yielding very high uptake results indicated that bacteria within the surface sediment layer were assimilating and presumably respiring ^{14}C-glutamic acid present in the overlying water. No attempt was made to evaluate respiration in the sediment on the assumption that most of the $^{14}CO_2$ had been liberated into the water.

First experiments with the BISIS units have provided in situ data of bacterial activity at the sediment-water interface. In addition, in situ data have compared favourably with laboratory results. Some

modifications are to be made to the units for the 1981 season. Experiments will be performed to assess the influence of filter-feeding invertebrates in the bottom compartment, enumerate bacteria inside the compartments before and after incubations, and measure the effects of petroleum and dispersants on glutamate uptake. Mineralization of hexadecane at the sediment-water interface will also be evaluated.

5.4 Hexadecane Mineralization

Mineralization of n-(1-^{14}C)hexadecane was used to estimate the numbers of oleoclastic bacteria in water and sediment samples and their rates of activity. With maximum recorded observations of 90 oleoclastic bacteria per litre of water at Cape Hatt (Table 2), the three 10.0 mL undiluted sample tubes used in the most probable number determinations contained a maximum of 2.7 oleoclastic bacteria. One or two or even three of the tubes would therefore probably have no oleoclastic bacteria. This was the limit of detection using a 10.0 mL sample. The carrier for labelled hexadecane was 8% weathered Lago Medio crude (D. Mackay, personal communication) and was a subsample of the large volume of weathered Lago Medio crude presently on-site at Cape Hatt. The results could have been confounded by the small sample volume or the use of slightly weathered Lago Medio crude. If various indigenous populations of oleoclasts were present and preferred a fraction or component of the Lago Medio crude other than hexadecane, this would not be obvious by measuring n-(1-^{14}C)hexadecane mineralization. This possibility, however, has been ruled out since visual inspection of MPN tubes for turbidity (i.e. growth and multiplication at the expense of the crude) yielded the same results as the measurement of hexadecane mineralization. Alternatively, the presence of the Lago Medio crude inhibited growth and hexadecane mineralization due to toxic fractions remaining in the crude after 8% weathering. This possibility was also ruled out in view of the limited results obtained in three trials at Cape Hatt with highly weathered Norman Wells crude, a sample of which has been used routinely for several years by our laboratory with favourable results in several geographic locations in the Arctic and on the Grand Banks of Newfoundland.

Where determinations of rates of n-(1-^{14}C)hexadecane mineralization were attempted, a 30.0 mL subsample of seawater was incubated in a serum bottle as opposed to the 10.0 mL subsample contained in screw-cap test tubes for the MPN procedure. Evidence of hexadecane mineralization was obtained three times as often with the 30.0 mL sample volume as with the 10.0 mL volume. This again suggests that the indigenous population capable of mineralizing hexadecane was sparse and not always available in 10.0 mL aliquots. This possibility will be tested in the coming year with the use of larger sample volumes.

In attempting to measure rates of hexadecane mineralization, replicates were removed at intervals up to 50 days. In most cases, the lag before detectable activity extended beyond the 40 day interval and some positive results were obtained only at the 50 day and final interval. A lag period of more than 50 days, therefore, would not yield a positive result. Supplementation of nitrate and phosphate in the incubation vessels reduced the lag sufficiently to almost double the number of positive observations at 50 days.

The addition of Corexit 9527, with and without nutrient supplementation, increased the lag to the extent that there was no activity recorded after 50 days of incubation. The Corexit either suppressed hexadecane mineralization or provided a substrate preferred by oleoclasts over the hexadecane; evidence from experiments at Frobisher Bay suggest the latter. In the presence of nitrate and phosphate, hexadecane mineralization proceeded at a rapid rate after a long lag, during which time it seems probable that one or more fractions of Corexit were utilized by oleoclasts. In view of the marginal oleoclastic activity detected at Cape Hatt, the maximum incubation times for hexadecane mineralization experiments may be increased to 60 days for the 1981 season. With increased sample volume as well, the sensitivity of this procedure should increase significantly.

Only meagre evidence of hexadecane mineralization was observed in the sediments, either from our rate experiments or the most probable number determinations. It can only be concluded that oleoclastic bacteria, capable of hexadecane mineralization, were inactive or not present in the sediment in detectable numbers. In future studies, the

sample size will be increased by using smaller sediment dilutions for hexadecane mineralization studies.

Oleoclastic activity in the water was not observed until the last three sampling cycles. It is reasonable to assume that since seasonal trends occur more slowly in the sediment, oleoclastic activity in the sediment might have been observed later in September had sampling continued for a longer period.

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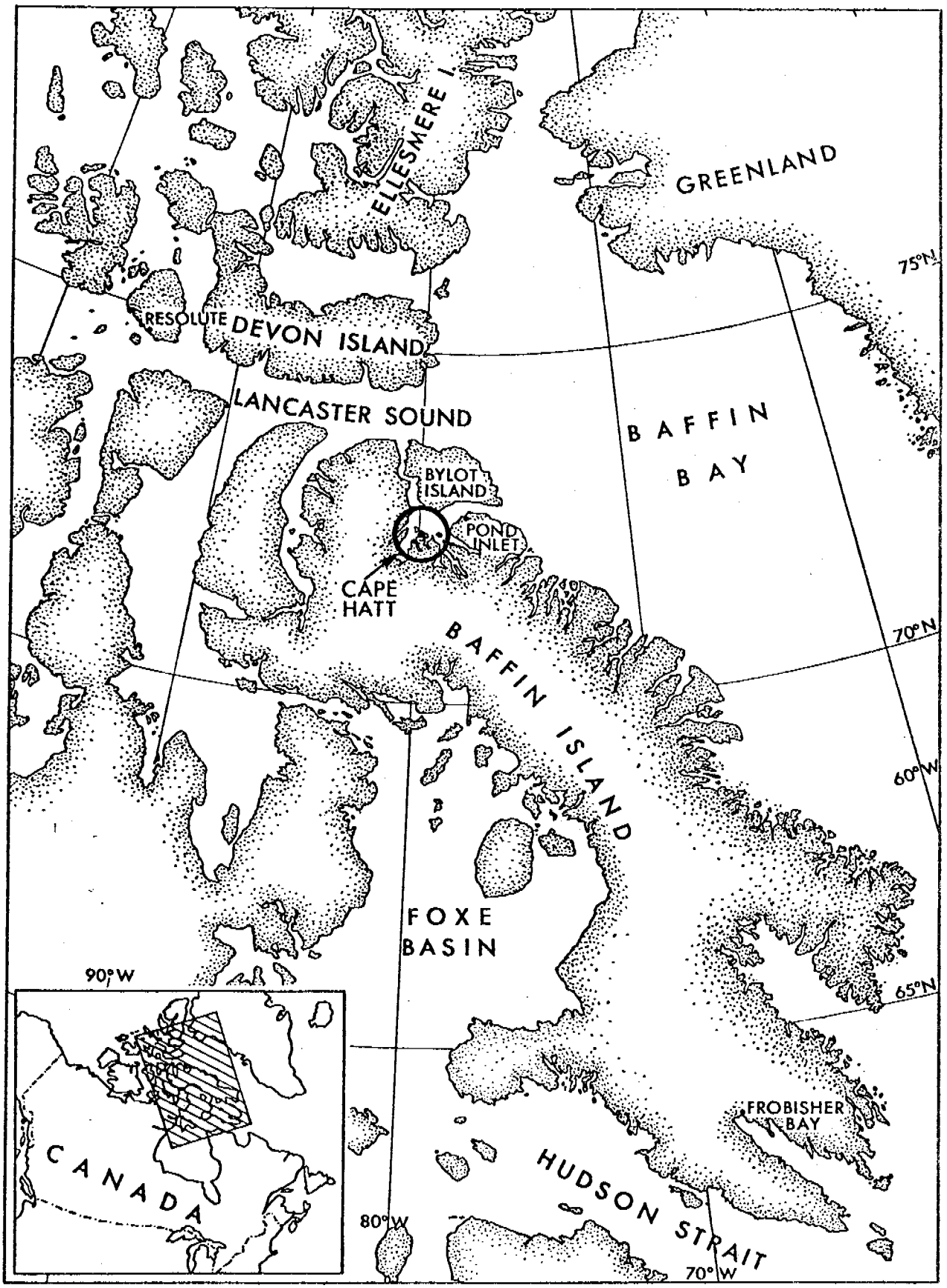


Figure 1. Location of the BIOS project, Cape Hatt, N.W.T.

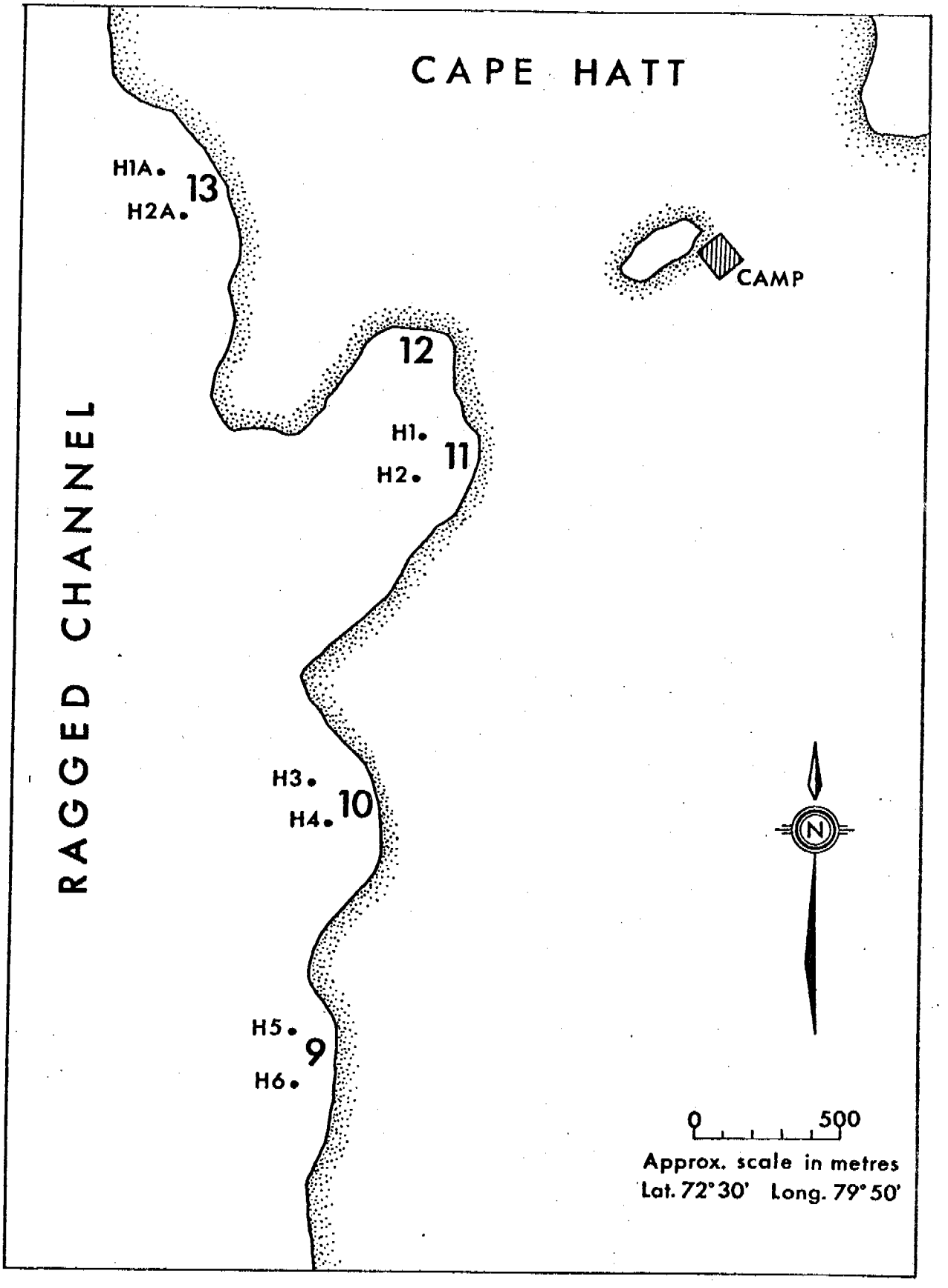
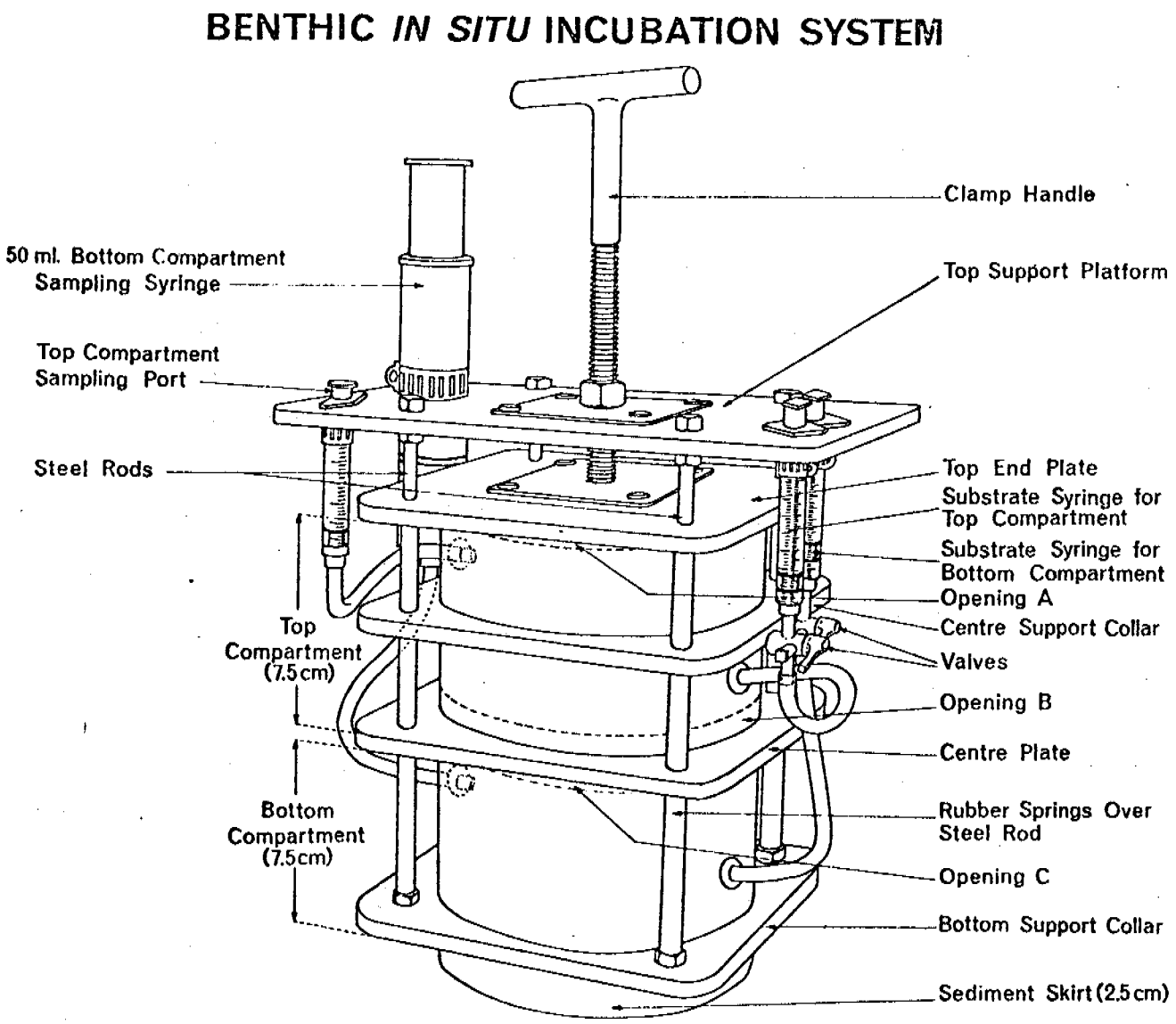


Figure 2. Microbiological stations occupied at Cape Hatt during 1980.

Figure 3


BENTHIC *IN SITU* INCUBATION SYSTEM
Clamp Handle
50 ml. Bottom Compartment Sampling Syringe
Top Support Platform
Top Compartment Sampling Port
Steel Rods
Top End Plate
Substrate Syringe for Top Compartment
Substrate Syringe for Bottom Compartment
Opening A
Centre Support Collar
Valves
Top Compartment (7.5cm)
Opening B
Centre Plate
Bottom Compartment (7.5cm)
Rubber Springs Over Steel Rod
Opening C
Bottom Support Collar
Sediment Skirt (2.5cm)

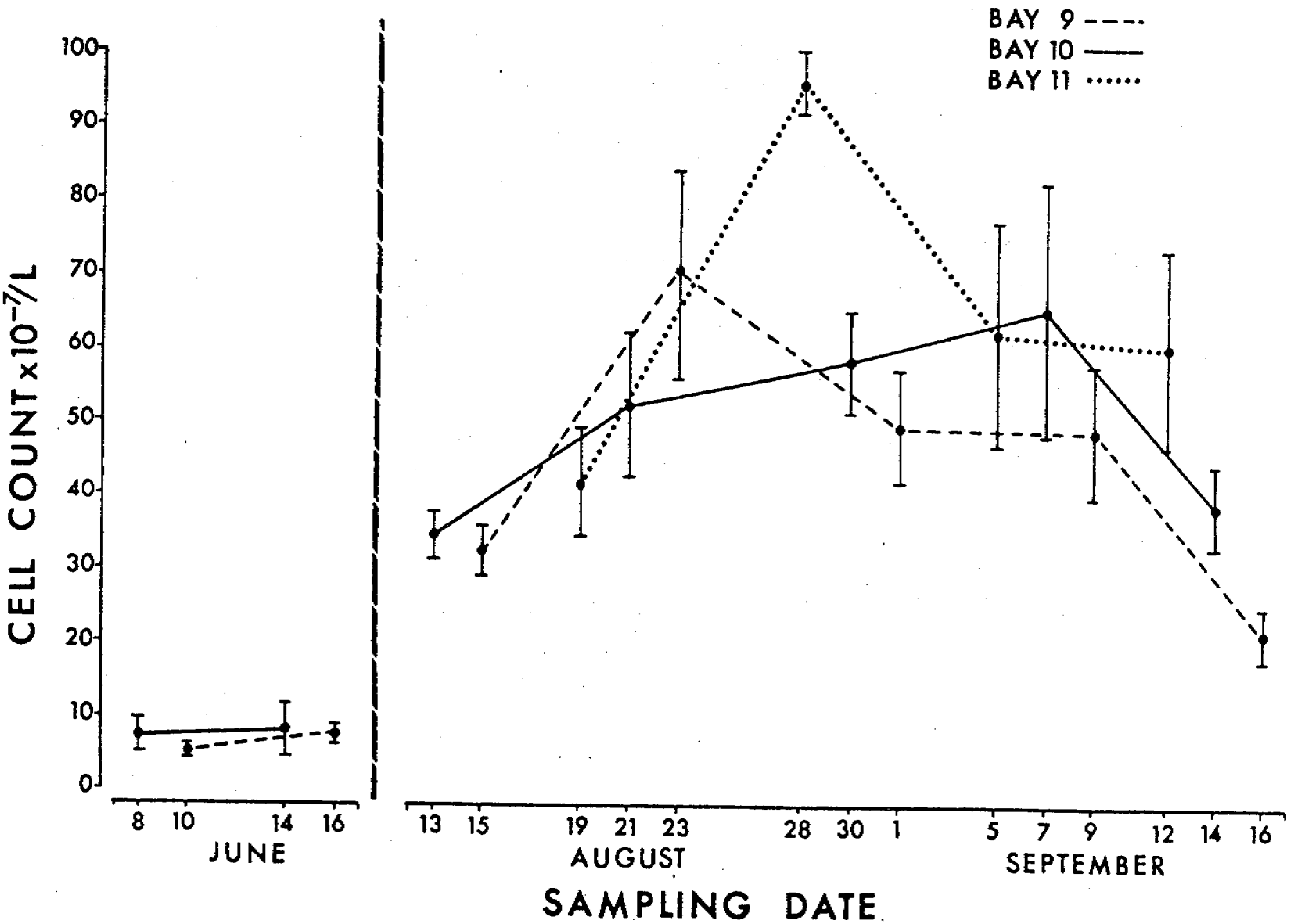


Figure 4. Total count (TC) of bacterial cells in water samples collected at Cape Hatt, 1980. Results are presented as means and standard deviations of six values from three depths at two stations in each bay.

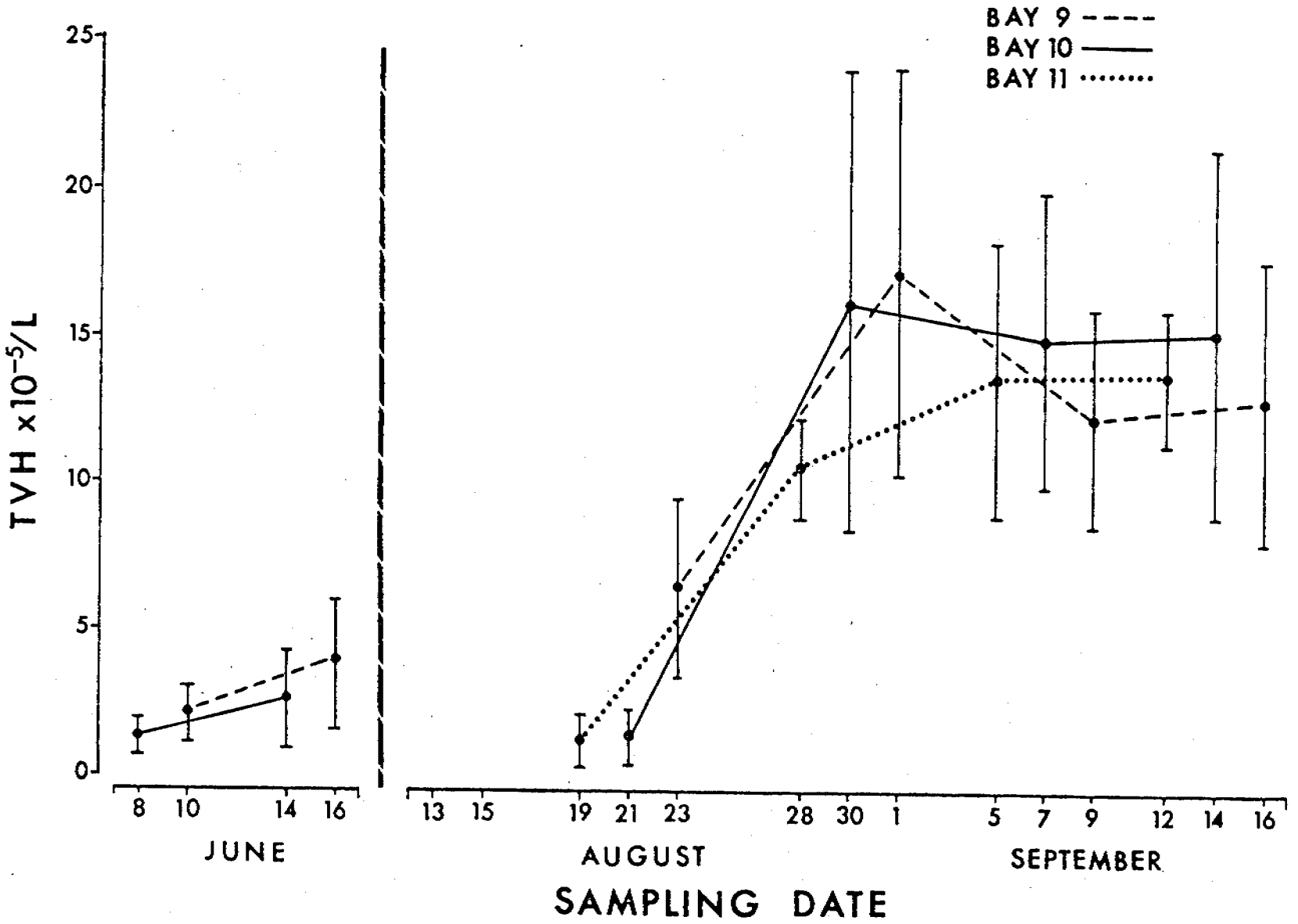


Figure 5. Total viable heterotrophs (TVH) determined by counts of colonies developed on a marine medium inoculated with water samples collected at Cape Hatt, 1980. Results are presented as means and standard deviations of six values from three depths at two stations in each bay.

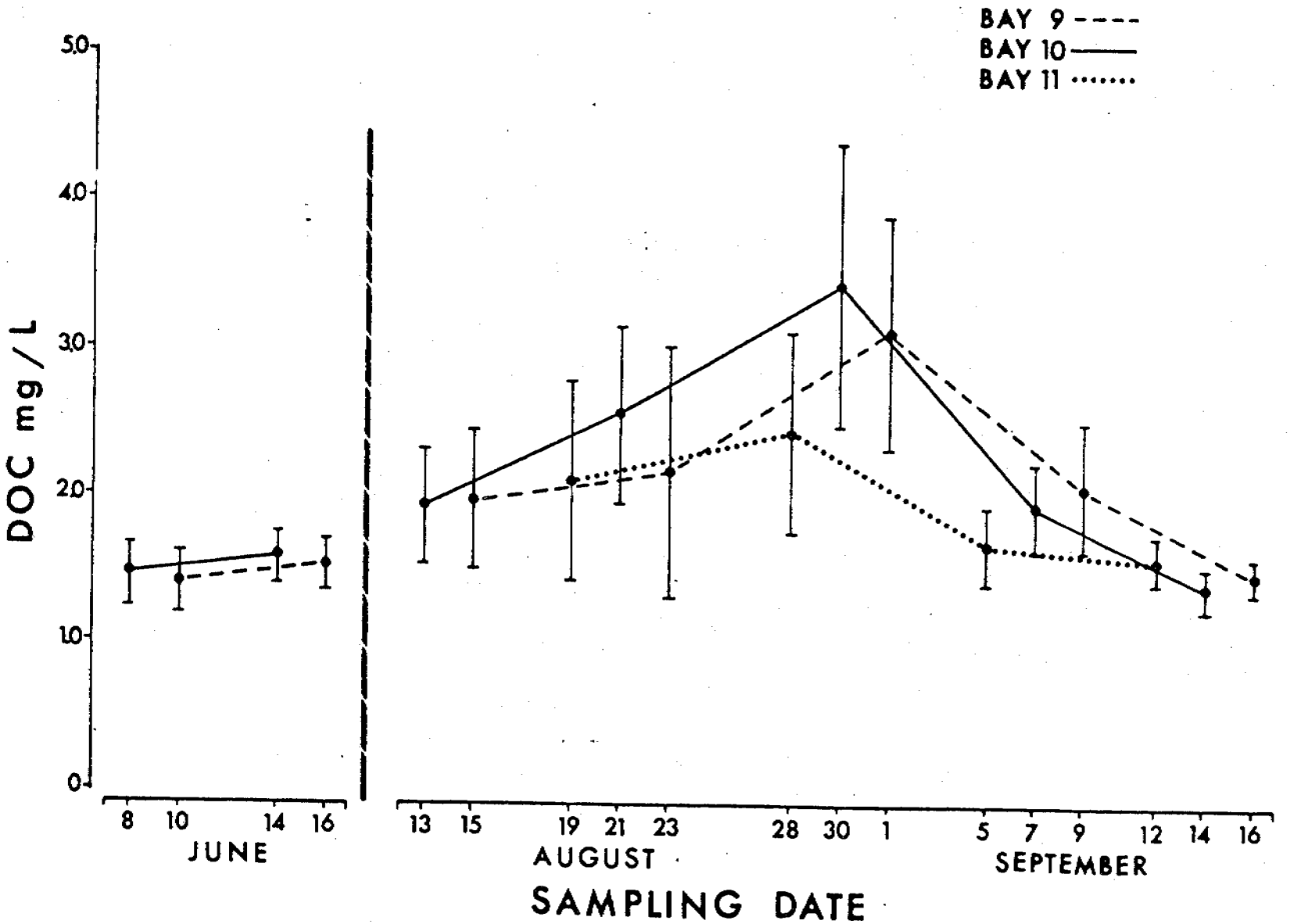


Figure 6. Concentrations of dissolved organic carbon (DOC) in water samples collected at Cape Hatt, 1980. Results are presented as means and standard deviations of six values from three depths at two stations in each bay.

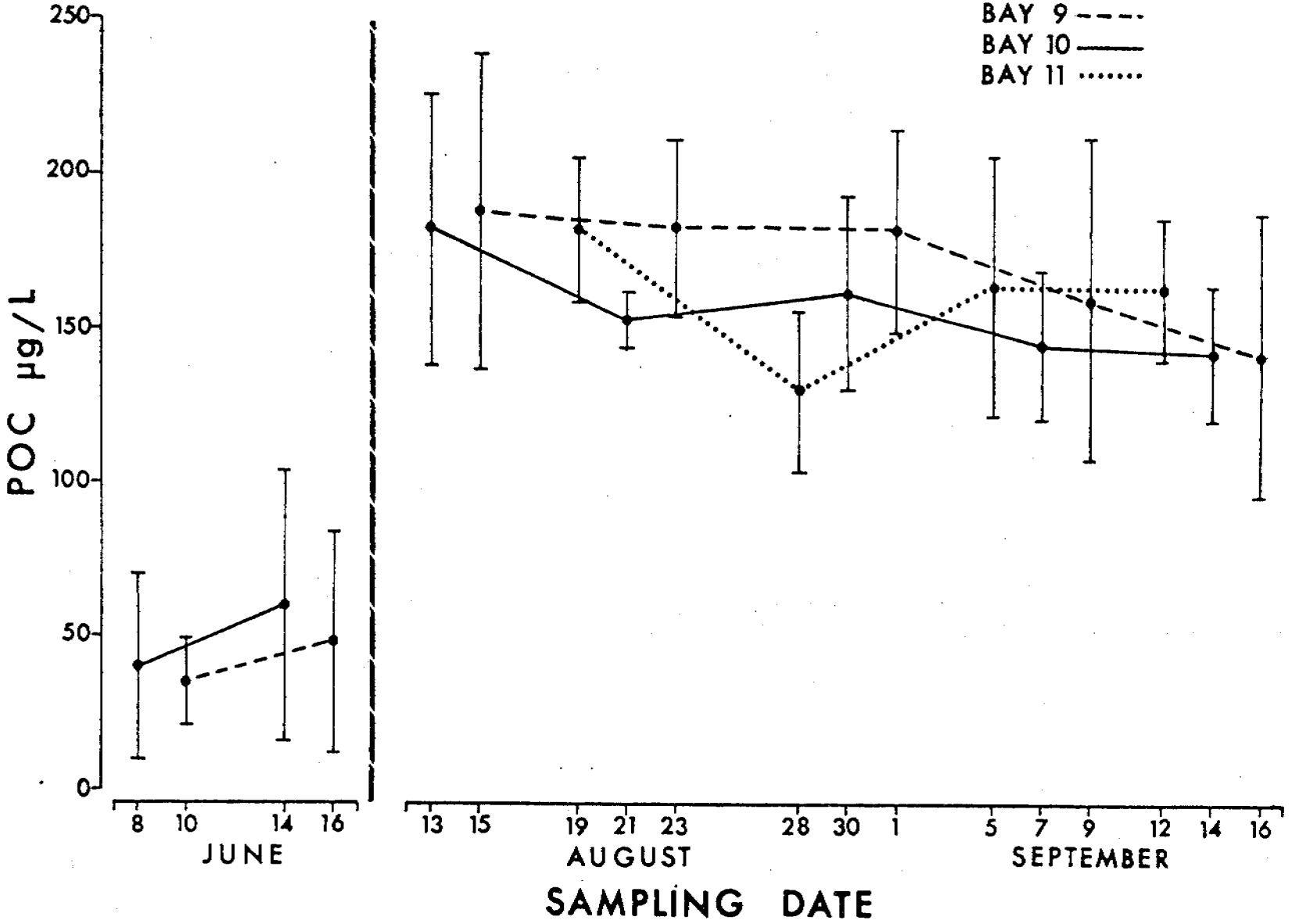


Figure 7. Concentrations of particulate organic carbon (POC) in water samples collected at Cape Hatt, 1980. Results are presented as means and standard deviations of six values from three depths at two stations in each bay.

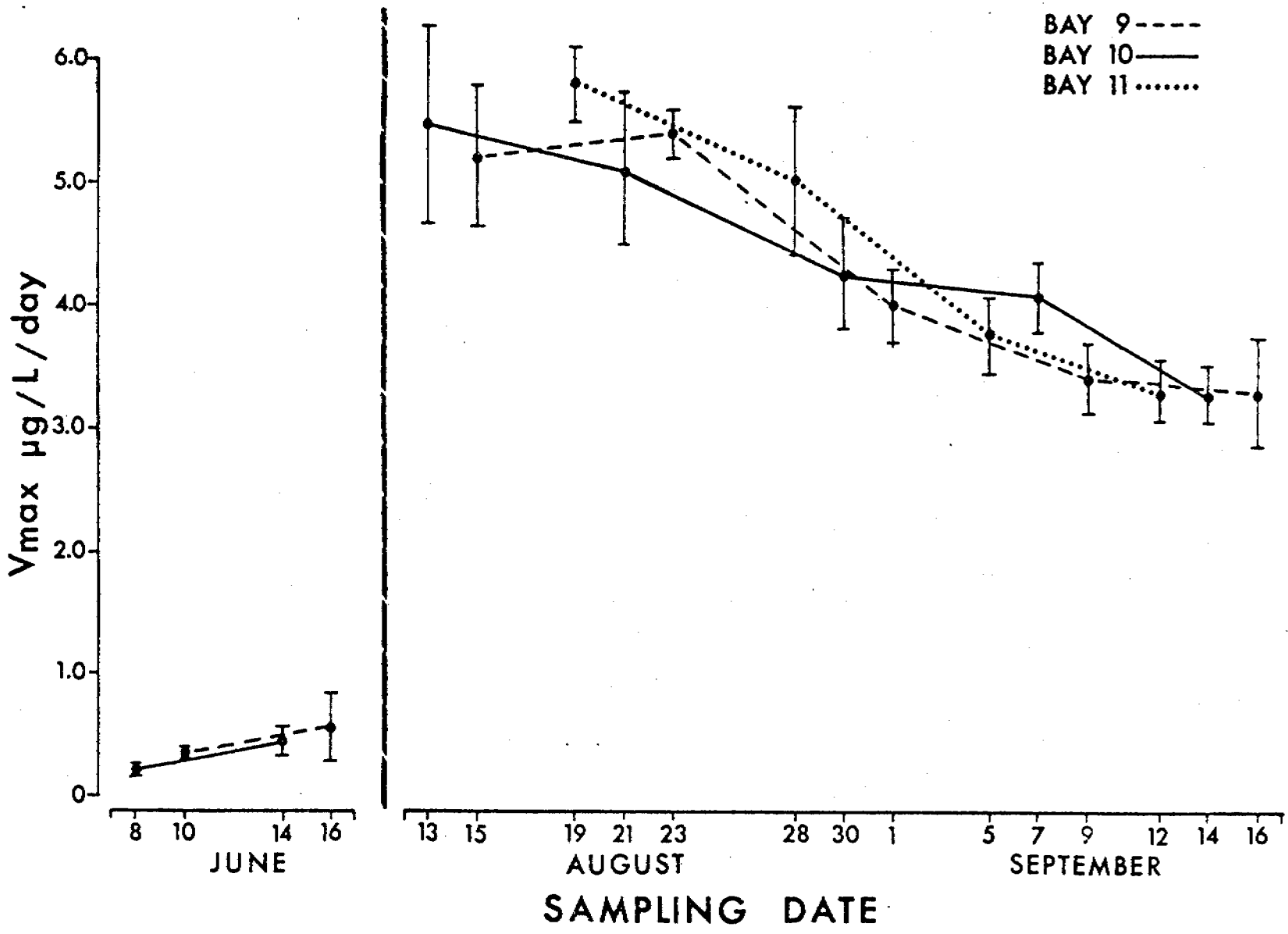


Figure 8. Maximum velocity (V_{max}) of glutamic acid uptake determined in water samples collected at Cape Hatt, 1980. Results are presented as means and standard deviations of six values from three depths at two stations in each bay.

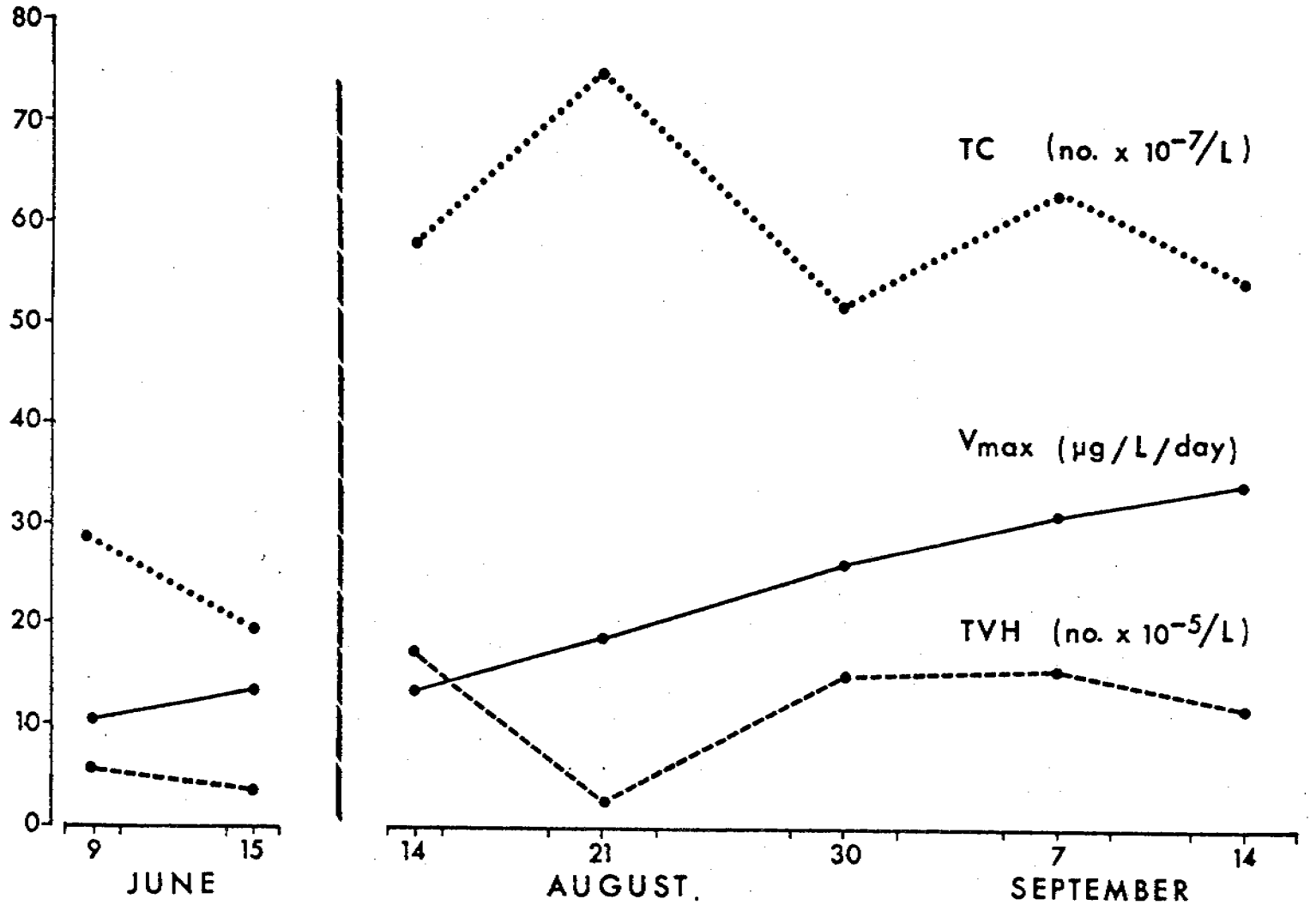


Figure 9. Means of values determined for total count (TC) of bacteria, maximum velocity (V_{max}) of glutamic acid uptake and total viable heterotrophs (TVH) in sediment samples. These means are of two stations for each of two or three bays that represented one sampling cycle (see Table 3). Units of volume (L) refer to a 0.1% by volume sediment-water suspension.

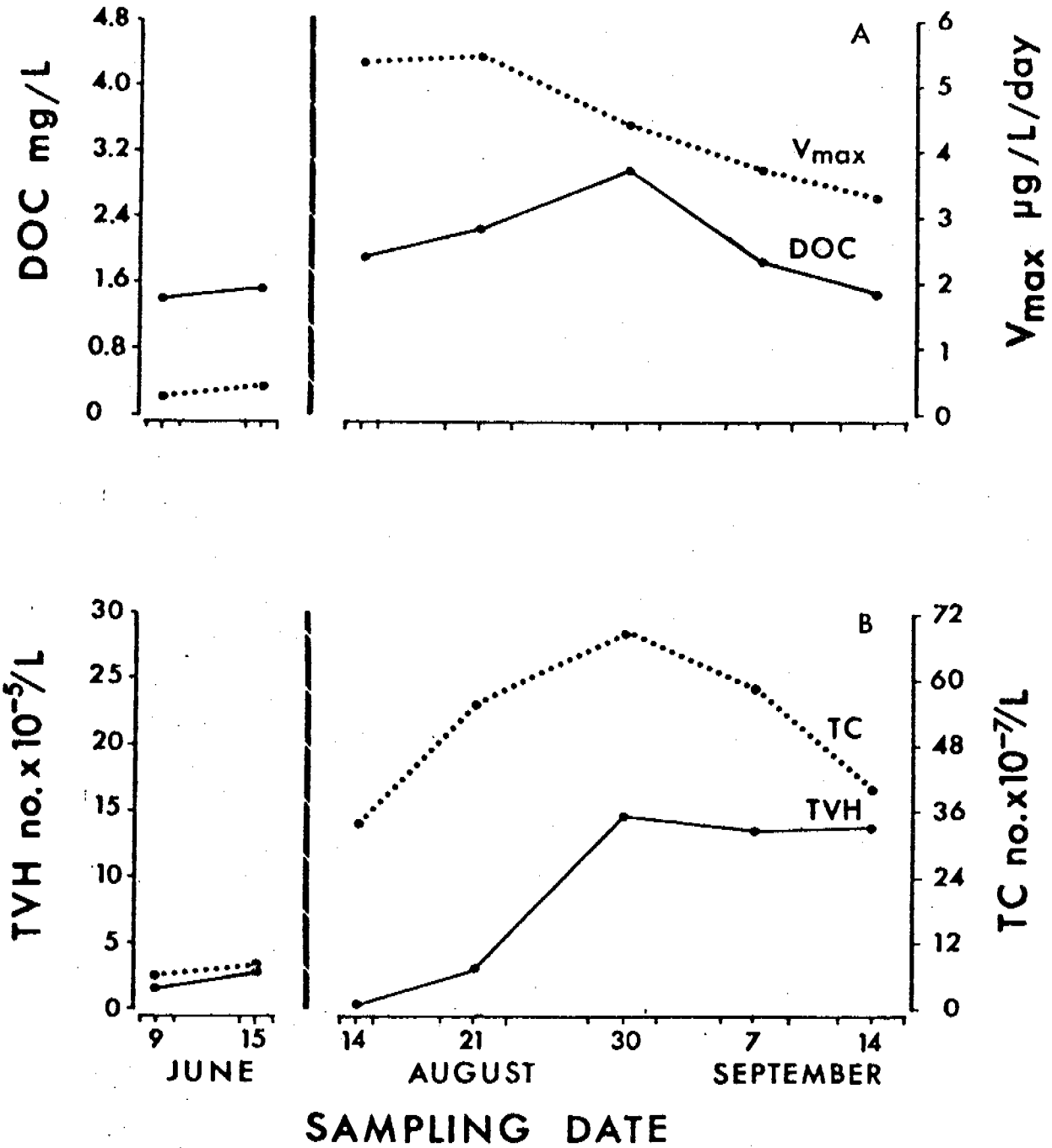


Figure 10 (A-D). Summary graphs of data obtained from three bays (9, 10 and 11) during eachsampling cycle. Data are expressed as the mean of three depths from each of two stations in the three bays for a total of eighteen samples (see Table 5). Data for chlorophyll a, reactive nitrate and phosphate were obtained from Green (1981).

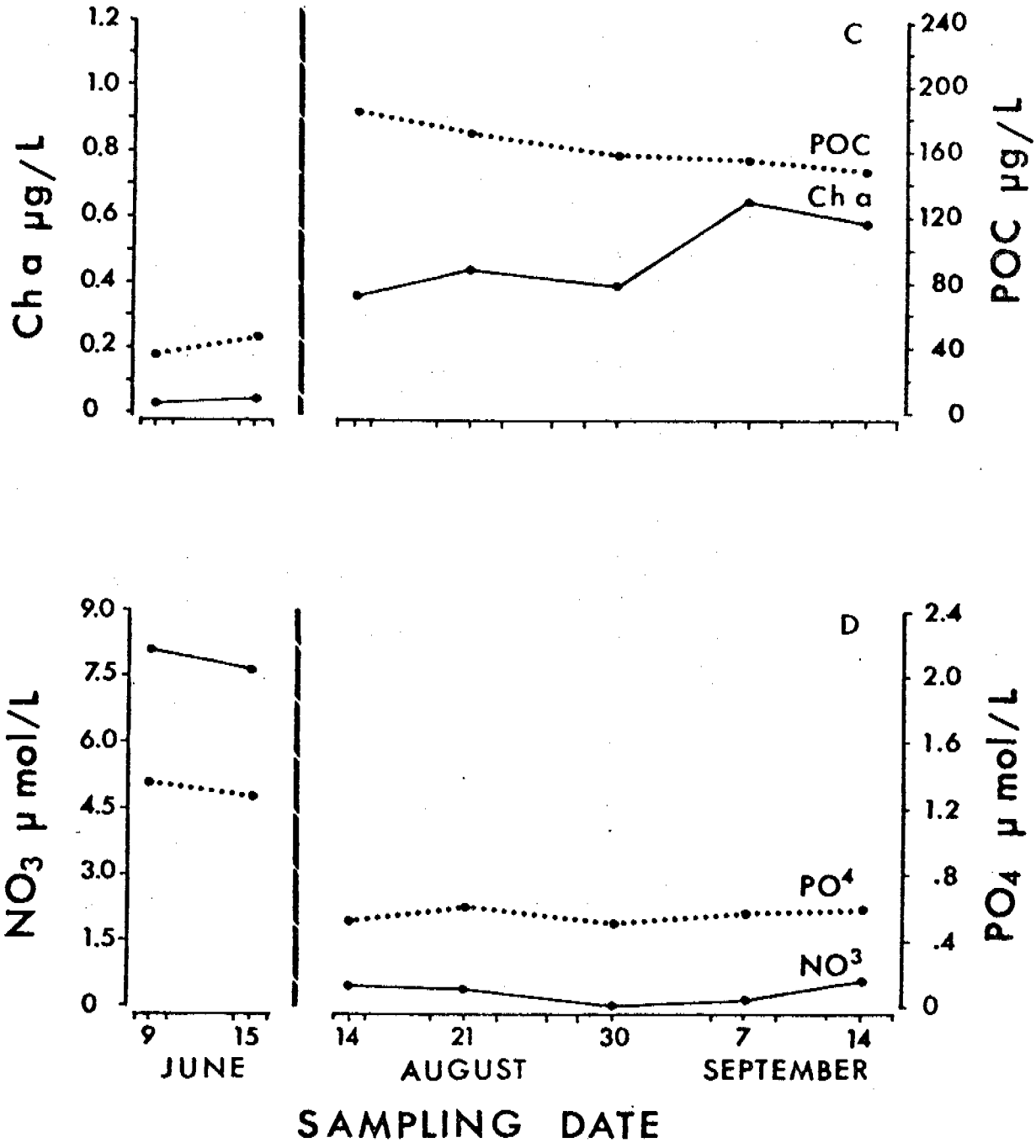


Figure 10 (Cont.)

Table 1. Station occupations at Cape Hatt 1980.

Date	Cycle	Bay	Stations	
6 June		13	1A	2A
8 June	1	10	3	4
10 June		9	5	6
12 June		13	1A	2A
14 June	2	10	3	4
16 June		9	5	6
11 Aug		13	1A	2A
13 Aug	3	10	3	4
15 Aug		9	5	6
19 Aug		11	1	2
21 Aug	4	10	3	4
23 Aug		9	5	6
28 Aug		11	1	2
30 Aug	5	10	3	4
1 Sept		9	5	6
5 Sept		11	1	2
7 Sept	6	10	3	4
9 Sept		9	5	6
12 Sept		11	1	2
14 Sept	7	10	3	4
16 Sept		9	5	6

Note: in other BIOS reports these station numbers may be prefixed by H (Hatt).

Table 2. Most probable number (MPN), determinations of oleoclastic cells and maximum disintegrations per minute (dpm) obtained from samples collected from 5 m at Cape Hatt during 1980 and incubated for 50 days with radiolabelled hexadecane and Lago Medio (L.M.) crude. **

	Date	Stn. no.	L.M. crude hexadecane	L.M. crude hexadecane nutrients	N.W. crude hexadecane nutrients	Oleoclasts
			dpm	dpm	dpm	no.L^{-1}
Bay 9	10-6	5	0	0	--	0
		6	0	0	--	40
	16-6	5	0	0	--	0
		6	0	0	--	0
	15-8	5	0	0	--	0
		6	0	0	--	0
	23-8	5	0	0	--	0
		6	0	0	--	0
	1-9	5	320	6 349*	6 291	0
		6	301	0	--	0
	9-9	5	0	0	0	0
		6	0	401	--	0
	16-9	5	0	512	0	0
		6	7 152	19 825	--	0
Bay 10	8-6	3	0	0	--	0
		4	0	0	--	0
	14-6	3	0	0	--	0
		4	0	0	--	0
	13-8	3	0	163*	--	90
		4	0	0	--	40
	21-8	3	0	0	--	0
		4	0	0	--	0
	30-8	3	0	5 700	0	0
		4	0	1 108	--	0
	7-9	3	0	0	0	0
		4	0	0	--	0

Table 2. (cont'd)

	Date	Stn. no.	L.M. crude hexadecane	L.M. crude hexadecane nutrients	N.W. crude hexadecane nutrients	Oleoclasts
			dpm	dpm	dpm	no.L^{-1}
Bay 10	14-9	3	1 416	276	698	40
		4	1 809	1 394	--	0
Bay 11	19-8	1	0	0	--	0
		2	0	0	--	0
	28-8	1	0	0	12 590	0
		2	0	0	--	0
	5-9	1	0	14 902	5 602	0
		2	3 572	6 721	--	0
	12-9	1	0	0	0	40
		2	0	0	--	0

* Forty day incubation.

** Identical samples were incubated with or without nutrient supplementation. Some samples were also incubated with Norman Wells (N.W.) crude.

Table 3. Means of values determined for total count (TC) of bacteria, total viable heterotrophs (TVH), and maximum velocity (V_{max}) of glutamic acid uptake in sediment samples collected at Cape Hatt during 1980. **

Cycle	Date	TC	(n)*	TVH	(n)*	V_{max}	(n)*
		no. L^{-1} (10^{-7})		no. L^{-1} (10^{-5})		$\mu g L^{-1} d^{-1}$	
1	8-10 June	28.88±10.14	4	5.70± 2.17	4	10.79± 7.50	4
2	14-16 June	19.48±13.32	4	3.28± 2.72	4	13.44± 0.49	2
3	13-15 Aug	57.99±16.38	4	17.10± 0.0	1	13.35± 7.02	4
4	19-23 Aug	74.79±24.90	6	2.60± 2.91	5	18.70±15.11	6
5	28 Aug-1 Sept	52.06±11.75	6	15.18±10.12	6	26.85±16.17	6
6	5- 9 Sept	63.28±14.83	6	15.50± 9.54	6	30.80±10.82	6
7	12-16 Sept	54.78±18.94	6	11.90± 3.09	6	34.08± 9.77	6

* number of samples

** Data are expressed as mean ± standard deviation of three depths from each of two stations in the three bays for a total of eighteen samples unless otherwise stated. Units of volume (L) refer to a 0.1% by volume sediment-water suspension

Table 4. Disintegrations per minute (dpm) obtained from wicks and filter membranes (FM) after *in situ* incubations in top and bottom compartments of BISIS units at Cape Hatt during 1980. **

In situ uptake		13 August		6 September		16 September	
Compartment		Top	Bottom	Top	Bottom	Top	Bottom
Wick	(DPM)	25 362±1 289	35 721±3 476	18 921± 265	26 120± 3 899	9 759±371	27 212±2 767
FM	(DPM)	33 028± 447	5 516±3 051	39 976±1 604	46 111±27 088	25 657±192	14 167±4 801
W+FM	(DPM)	58 390±1 718	41 236±5 526	58 896±1 622	72 231±30 934	35 416±443	41 379±4 822
Respiration (%)*		43	87	32	36	28	66

In vitro uptake		13 August	6 September	16 September
Wick	(DPM)	24 491± 53	21 714± 774	8 262± 592
FM	(DPM)	31 394±201	36 776± 247	18 072±1 365
W+FM	(DPM)	55 885±254	58 490±1 021	26 334±1 956
Respiration (%)*		44	37	31

Sediment collection from under bottom compartment		13 August	6 September	16 September
FM	(DPM)	--	10 5010±4 942	216 623±137 623

* % respiration = $\frac{\text{Wick}}{\text{W+FM}} \times 100$

** Water collected at the same depth and treated in a similar fashion *in vitro* provided results for comparison only with top compartments. Sediment was collected from under the bottom compartments after incubation *in situ* to determine glutamic acid uptake by sediment bacteria (FM) values. Results are expressed as means ± standard deviations of determinations from three units.

Table 5. Summary table of data obtained in water samples from three bays (9, 10 and 11) during each sampling cycle. **

Cycle	Date	TVH	TC	V_{max}	NO_3^{-1} *	PO_4^{-3} *	DOC	POC	Ch *a* *
		no. L^{-1} (10^5)	no. L^{-1} (10^7)	$\mu g L^{-1} d^{-1}$	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$mg\ L^{-1}$	$\mu g\ L^{-1}$	$\mu g\ L^{-1}$
1 †	8-10 June	1.73±0.92	6.32± 2.07	0.30±0.08	8.13±0.42	1.36±0.12	1.43±0.20	37.50±23.50	0.03±0.02
2 †	14-16 June	3.21±2.02	8.00± 2.64	0.50±0.22	7.65±0.44	1.28±0.05	1.55±0.17	54.17±40.71	0.05±0.03
3 †	13-15 Aug	0.43±0.19	33.48± 3.32	5.35±0.71	0.47±0.30	0.51±0.15	1.94±0.43	185.00±47.70	0.36±0.12
4	19-23 Aug	3.01±3.08	55.00±15.80	5.45±0.50	0.43±1.06	0.59±0.13	2.27±0.75	172.78±25.78	0.44±0.17
5	28 Aug-1 Sept	14.64±6.72	68.18±21.13	4.43±0.63	0.08±0.13	0.50±0.12	2.99±0.92	157.78±36.90	0.39±0.13
6	5- 9 Sept	13.57±4.63	59.05±15.87	3.76±0.40	0.36±0.68	0.59±0.15	1.89±0.37	155.88±41.59	0.65±0.57
7	12-16 Sept	13.88±4.85	40.37±18.05	3.30±0.32	0.59±0.68	0.61±0.03	1.49±0.16	148.89±33.65	0.58±0.09

† Bays 9 and 10 (12 samples)

* Data obtained from Green (1981)

** Data are expressed as the mean ± standard deviation of three depths from each of two stations in the three bays for a total of eighteen samples.

Table 6. Comparison of measurements of V_{max} of glutamic acid uptake, chlorophyll *a*, reactive nitrate and particulate organic carbon (POC) from several arctic regions.

Location	Station	Date	Depth	V_{max}	Ch *a*	POC	Nitrate
			m	$\mu g\ L^{-1}d^{-1}$	$\mu g\ L^{-1}$	$\mu g\ L^{-1}$	$\mu mol\ L^{-1}$
Cape Hatt 72°30'N 79°50'W	Average of Stations 1 and 2 (Bay 11)	80.8.19	1	5.96	0.59*	160.0	0.50*
			5	5.81	0.73*	200.0	0.0 *
			10	5.66	0.42*	185.0	0.15*
			20	--	--	--	--
Lancaster Sound 73°49'N 80°11'W	4A	79.8.25	1	6.05	1.44	210.0	1.10
			5	5.24	1.60	250.0	1.10
			10	--	--	--	--
			20	1.69	0.70	40.0	5.80
Baffin Bay 72°37'N 74°56'W	16A	79.8.27	1	3.51	0.87	120.0	1.10
			5	4.56	1.38	260.0	0.20
			10	--	--	--	--
			20	10.45	4.73	320.0	0.30
Frobisher Bay 63°43'N 68°31'W	1	79.7.10	1	6.42	0.79	240.0	0.30
			5	5.16	6.37	640.0	0.0
			10	5.53	10.20	620.0	0.0
			20	4.59	10.77	330.0	1.73

* Data obtained from Green (1981)

Table 7. Determinations of total viable heterothrophs (TVH), total count (TC) of bacteria, maximum velocity V_{max} of glutamic acid uptake, dissolved organic carbon (DOC) and particualate organic carbon (POC) from water samples collected at Cape Hatt during June 1980.

Date	Bay	Station	Depth	TVH	TC	V_{max}	DOC	POC
			m	no. L^{-1} (10^{-5})	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	mg L^{-1}	µg L^{-1}
06.06	13	1A	1	9.80	8.08	0.13	1.10	20.0
		1A	5	2.00	5.64	0.23	1.30	20.0
		1A	10	1.20	6.70	0.21	1.50	10.0
06.06	13	2A	1	0.90	7.83	0.23	2.20	10.0
		2A	5	1.00	6.01	0.21	1.60	10.0
		2A	10	0.90	9.82	0.22	1.60	20.0
06.08	10	3	1	2.40	4.99	0.19	1.80	80.0
		3	5	1.00	10.84	0.22	1.40	40.0
		3	10	1.00	10.38	0.18	1.50	80.0
06.08	10	4	1	0.70	6.29	0.27	1.50	20.0
		4	5	0.90	6.35	0.24	1.40	10.0
		4	10	2.00	5.63	0.27	1.10	10.0

Table 7 (Continued)

Date	Bay	Station	Depth	TVH	TC	V_{max}	DOC	POC
				no. L^{-1} (10^{-5})	no. L^{-1} (10^{-7})	µg L^{-1} d^{1}	mg L^{-1}	µg L^{-1}
06.10	9	5	1	1.70	3.61	0.39	1.70	50.0
		5	5	1.30	5.28	0.32	1.30	10.0
		5	10	2.30	5.46	0.35	1.60	50.0
06.10	9	6	1	1.80	4.58	0.42	1.30	30.0
		6	5	1.40	6.28	0.37	1.10	40.0
		6	10	4.20	6.15	0.33	1.40	30.0
06.12	13	1A	1	11.70	8.62	0.29	1.70	60.0
		1A	5	0.80	6.50	0.28	1.80	30.0
		1A	10	1.50	9.95	0.27	1.50	30.0
06.12	13	2A	1	2.00	7.07	0.28	1.40	10.0
		2A	5	1.40	6.68	0.25	1.50	30.0
		2A	10	4.40	6.74	0.30	1.30	10.0
06.14	10	3	1	4.40	11.96	0.53	1.80	130.0
		3	5	1.10	9.73	0.37	1.50	50.0
		3	10	5.30	10.51	0.35	1.60	30.0
06.14	10	4	1	2.10	1.06	0.66	1.80	110.0
		4	5	1.00	8.26	0.36	1.40	20.0
		4	10	1.80	7.99	0.35	1.40	20.0

Table 7 (Continued)

Date	Bay	Station	Depth	TVH	TC	V_{max}	DOC	POC
				no. L^{-1} (10^{-5})	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	mg L^{-1}	µg L^{-1}
06.16	9	5	1	7.60	9.42	0.86	1.60	100.0
		5	5	1.20	7.23	0.42	1.30	10.0
		5	10	4.60	8.47	0.39	1.60	20.0
06.16	9	6	1	2.90	8.87	1.04	1.80	70.0
		6	5	1.60	6.93	0.37	1.40	10.0
		6	10	4.90	5.61	0.35	1.40	80.0

Table 8. Determinations of total viable heterotrophs (TVH), total count (TC) of bacteria, maximum velocity (V_{max}) of glutamic acid uptake, dissolved organic carbon (DOC) and particulate organic carbon (POC) from water samples collected at Cape Hatt during August-September 1980.

Date	Bay	Station	Depth	TVH	TC	V_{max}	DOC	POC
			m	no. L^{-1} (10^{-5})	no. L^{-1} (10^{-7})	$\mu g\ L^{-1}\ d^{-1}$	mg L^{-1}	$\mu g\ L^{-1}$
08.11	13	1A	1	--	35.83	4.63	2.70	150.0
		1A	5	--	35.33	2.61	2.10	110.0
		1A	10	--	47.29	3.33	3.50	210.0
08.11	13	2A	1	9.00	44.17	5.17	2.90	100.0
		2A	5	3.00	25.78	3.68	1.80	130.0
		2A	10	5.40	31.61	5.20	1.90	240.0
08.13	10	3	1	--	34.22	6.16	2.00	140.0
		3	5	--	37.24	5.63	2.60	150.0
		3	10	--	39.04	4.62	1.90	260.0
08.13	10	4	1	--	30.40	6.75	1.70	190.0
		4	5	0.70	34.02	5.22	1.30	140.0
		4	10	--	31.91	4.53	2.00	210.0

Table 8 (Continued)

Date	Bay	Station	Depth	TVH	TC	V_{max}	DOC	POC
			m	no. L^{-1} (10^{-5})	no. L^{-1} (10^{-7})	$\mu g\ L^{-1}\ d^{-1}$	mg L^{-1}	$\mu g\ L^{-1}$
08.15	9	5	1	--	30.30	4.63	1.70	130.0
		5	5	0.30	32.31	5.44	1.90	190.0
		5	10	0.30	33.01	5.28	2.50	210.0
08.15	9	6	1	--	27.39	5.58	1.50	280.0
		6	5	--	33.42	6.01	1.50	130.0
		6	10	--	38.54	4.33	2.70	190.0
08.19	11	1	1	--	42.76	5.68	1.80	150.0
		1	5	1.00	44.37	5.51	1.70	190.0
		1	10	2.70	35.33	5.94	3.60	160.0
08.19	11	2	1	0.90	45.48	6.24	1.70	170.0
		2	5	0.30	30.70	6.10	2.00	210.0
		2	10	--	53.62	5.37	1.80	210.0
08.21	10	3	1	0.30	33.01	5.83	2.50	150.0
		3	5	0.90	63.37	5.48	1.60	160.0
		3	10	2.80	57.64	4.70	3.40	170.0
08.21	10	4	1	2.10	48.39	5.75	2.10	150.0
		4	5	0.30	54.72	4.85	2.60	150.0
		4	10	1.80	58.54	4.11	3.10	140.0

Table 8 (Continued)

Date	Bay	Station	Depth	TVH	TC	V_{max}	DOC	POC
			m	no. L^{-1} (10^{-5})	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	mg L^{-1}	µg L^{-1}
08.23	9	5	1	2.80	54.42	5.70	1.70	170.0
		5	5	11.20	55.43	5.61	1.60	160.0
		5	10	4.20	81.76	5.48	1.90	150.0
08.23	9	6	1	8.60	72.71	5.13	4.00	180.0
		6	5	5.30	65.27	5.20	1.50	230.0
		6	10	--	92.51	5.33	2.30	210.0
08.28	11	1	1	12.70	88.19	4.56	3.30	140.0
		1	5	9.90	95.02	4.62	1.80	110.0
		1	10	10.70	96.88	4.48	2.30	100.0
08.28	11	2	1	8.60	96.78	6.18	2.40	130.0
		2	5	8.50	97.69	5.41	1.50	180.0
		2	10	12.80	102.71	4.90	3.30	120.0
08.30	10	3	1	30.20	65.22	3.62	4.50	190.0
		3	5	16.60	59.19	4.15	3.80	210.0
		3	10	13.90	52.66	4.37	2.20	170.0
08.30	10	4	1	4.90	66.83	5.08	3.40	140.0
		4	5	19.70	59.90	4.29	2.20	140.0
		4	10	12.00	47.64	4.08	4.50	120.0

Table 8 (Continued)

Date	Bay	Station	Depth	TVH	TC	V_{max}	DOC	POC
			m	no. L^{-1} (10^{-5})	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	mg L^{-1}	µg L^{-1}
09.01	9	5	1	31.00	48.34	3.93	3.20	160.0
		5	5	16.10	42.01	4.29	2.40	240.0
		5	10	17.90	43.72	3.57	2.90	160.0
09.01	9	6	1	8.80	65.83	4.30	4.20	140.0
		6	5	13.20	49.25	4.27	4.00	190.0
		6	10	16.10	49.45	3.66	2.00	200.0
09.05	11	1	1	20.30	62.01	3.81	1.70	150.0
		1	5	12.40	50.75	3.48	1.40	170.0
		1	10	11.10	48.74	3.72	1.50	170.0
09.05	11	2	1	13.30	46.83	4.32	2.00	--
		2	5	18.10	82.91	3.90	2.00	100.0
		2	10	6.10	82.41	3.36	1.40	230.0
09.07	10	3	1	15.20	82.21	3.78	1.80	140.0
		3	5	20.10	84.92	3.88	1.60	120.0
		3	10	22.50	80.50	4.13	2.40	160.0
09.07	10	4	1	9.70	51.66	3.83	2.10	120.0
		4	5	8.70	50.65	4.25	1.70	140.0
		4	10	13.10	44.52	4.60	2.10	190.0

Table 8 (Continued)

Date	Bay	Station	Depth	TVH	TC	V_{max}	DOC	POC
			m	no. L^{-1} (10^{-5})	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	mg L^{-1}	µg L^{-1}
09.09	9	5	1	12.30	47.94	3.53	1.80	120.0
		5	5	14.90	38.69	3.60	1.50	140.0
		5	10	9.60	39.14	3.78	2.50	210.0
09.09	9	6	1	16.80	60.15	2.95	1.90	130.0
		6	5	14.40	61.66	3.14	2.80	110.0
		6	10	5.70	47.18	3.54	1.90	250.0
09.12	11	1	1	13.80	56.53	3.58	1.50	150.0
		1	5	9.40	43.67	2.97	1.60	170.0
		1	10	14.90	66.78	3.34	1.60	200.0
09.12	11	2	1	12.10	83.26	3.61	1.90	130.0
		2	5	16.30	65.68	3.03	1.40	180.0
		2	10	15.30	46.18	3.36	1.50	150.0
09.14	10	3	1	23.80	41.56	3.55	1.20	120.0
		3	5	13.80	41.76	2.94	1.60	130.0
		3	10	19.90	42.36	3.24	1.30	140.0
09.14	10	4	1	5.60	42.56	3.20	1.40	160.0
		4	5	9.40	26.28	3.63	1.40	120.0
		4	10	18.60	39.85	3.20	1.50	180.0

Table 8 (Continued)

Date	Bay	Station	Depth	TVH	TC	V_{max}	DOC	POC
			m	no. L^{-1} (10^{-5})	no. L^{-1} (10^{-7})	µg L^{-1} d^{-1}	mg L^{-1}	µg L^{-1}
09.16	9	5	1	13.40	26.68	3.30	1.50	120.0
		5	5	14.40	19.04	2.82	1.70	80.0
		5	10	19.70	22.16	2.81	1.50	160.0
09.16	9	6	1	4.40	16.26	4.12	1.50	100.0
		6	5	9.50	25.80	3.30	1.30	210.0
		6	10	15.60	20.28	3.42	1.40	180.0

Table 9. Determinations of maximum velocity (V_{max}) of glutamic acid uptake, total count (TC) of bacteria, and total viable heterotrophs (TVH) from sediment samples collected at Cape Hatt during 1980.

Date	Station	V_{max}	TC	TVH
		$\mu g\ L^{-1}d^{-1}$	no. L^{-1} (10^{-7})	no. L^{-1} (10^{-5})
8-6	3	3.57	24.49	2.10
8-6	4	23.21	46.36	5.90
10-6	5	6.62	21.80	7.60
10-6	6	9.74	22.88	7.20
14-6	3	--	4.87	0.10
14-6	4	13.93	31.37	5.50
16-6	5	12.95	34.09	6.40
16-6	6	--	7.60	1.10
13-8	3	22.24	60.20	--
13-8	4	14.08	58.49	--
15-8	5	14.51	79.70	17.10
15-8	6	2.58	33.57	--
19-8	1	18.32	47.74	1.00
19-8	2	16.82	66.13	--
21-8	3	20.75	74.87	1.50
21-8	4	48.91	125.73	8.40
23-8	5	4.10	77.18	1.30
23-8	6	3.32	57.08	0.80
28-8	1	11.24	70.05	9.50
28-8	2	17.31	48.34	5.70
30-8	3	55.75	63.62	29.30
30-8	4	37.08	42.61	8.80
1-9	5	9.97	35.68	8.30
1-9	6	29.76	52.06	29.50

Table 9. (cont'd)

Date	Station	V_{max}	TC	TVH
		$\mu g\ L^{-1}d^{-1}$	no. L^{-1} (10^{-7})	no. $L^{-1}(10^{-5})$
5-9	1	35.76	74.37	9.40
5-9	2	52.13	51.76	2.10
7-9	3	18.87	57.69	23.40
7-9	4	24.58	42.01	7.30
9-9	5	28.90	87.13	26.70
9-9	6	24.58	66.73	24.10
12-9	1	24.43	67.03	9.60
12-9	2	26.47	63.37	7.30
14-9	3	33.72	65.58	15.80
14-9	4	44.09	75.53	15.50
16-9	5	25.78	26.54	12.70
16-9	6	49.97	30.60	10.50

BAFFIN ISLAND OIL SPILL PROJECT

MICROBIAL DEGRADATION OF OIL

MEASUREMENTS IN RAGGED CHANNEL, Z-LAGOON AND ECLIPSE SOUND, CAPE HATT 1980, A BASELINE ASSESSMENT.

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ABSTRACT

During the period 26 August to 9 September 1980 analyses of baseline parameters related to microbial degradation of oil were determined in samples from the nearshore waters and sediments of Ragged Channel (Bay 9, 10 and 11) and in shoreline sand and sediments inside and outside the Z-lagoon of Cape Hatt.

Most probable number techniques were used to assess counts of oildegrading microorganisms (bacteria), generally heterotrophic bacteria and antibiotica resistant oildegrading microorganisms (fungi). An experimental laboratory technique was used to determine rates of mineralization of generally tritiated Lago Medio crude oil and ^{14}C-labelled *n*-hexadecane, naphtalene and benz(a)pyrene. As a measure of general biological activity in shoreline sand and sediments a field method for determining CO_2-production.

In the coastal water an average content of $4 \cdot 10^4 L^{-1}$ oildegrading bacteria was detected, constituting about 1% of the assessed viable count for generally heterotrophic bacteria. Only 5 bottom sediments were analyzed. They contained oildegrading bacteria in the range $2{,}5 \cdot 10^3$ to $2{,}5 \cdot 10^4$ ml^{-1} sediment.

Experiments with tritiated Lago Medio demonstrated mineralization of oil by the microorganisms in the water and maximal rates (V_{max}) from 11 to 30 $\mu g/m^3$ water, d. were measured. Tentative results indicated a somewhat lower activity for mineralization of *n*-hexadecane. Definite mineralization of naphtalene and benz(a)pyrene needs confirmation.

The shoreline sand and sediment contained low but detectable numbers of oil degrading microorganisms. After spraying with weathered Lago Medio crude oil or the same oil as 50% water-oil emulsion an increase in oildegrading bacteria from $\sim 10^2$ to 10^5 respectively 10^7 cells per ml sand/oil mixture could be registered over a period of 15 days.

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1. INTRODUCTION

The participation of the Norwegian microbiology group in the BIOS-project has two major general objectives which can be phrased through these questions:

1. How will the microbial community of the cold waters of the Arctic coast react to a massive exposure to oil? How fast and to what extent will the microbial potential for oil degradation change? And, in this context, will the physical state of the oil as slick or as a dispersion have any significant influence on the microbial response?

2. Under the harsh condition of the Arctic shoreline is it still possible within the limits of practicality to enhance the normal potential of the shoreline for microbial degradation of stranded oil? We are thinking along two lines of approach: a) increased levels of nutrients, b) improved conditions for aeration.

Our work in 1980 was aimed at

- testing our methods of analysis and experimentation in the field and to gain experiences for work in the Arctic.

- to assess the baseline levels of oildegrading microorganisms and generally heterotrophic microorganisms in the nearshore waters and sediments of Ragged Channel and in the sand and tidal sediments of the shorelines inside and outside the Z-lagoon.

- to assess the biochemical activity for mineralization of radiolabelled oil and hydrocarbon in water and bottom sediments.

- to start and monitor the initial phase of the first experiment related to the fate of oil stranded and washed up on the shoreline.

This report summarizes our activities and results from the fieldwork at Cape Hatt 23 August to 15 September 1980.

2. MATERIALS AND METHODS

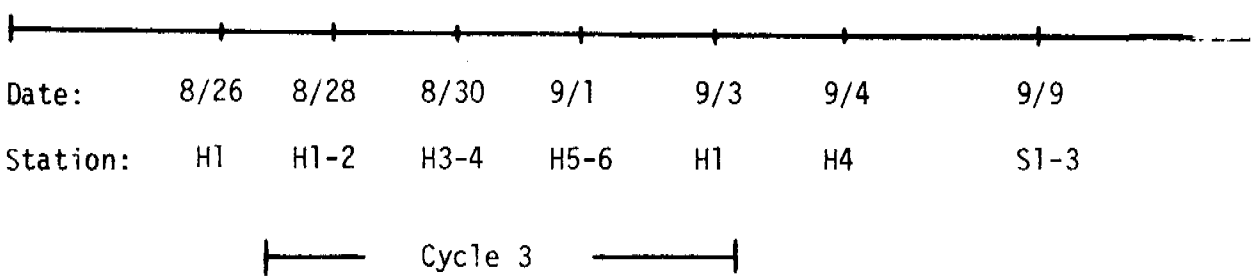
2.1.1. Water samples.

Water samples were taken from Zodiac by using a 5 l Niskin bottle. The bottle was rinsed by surface water at each station prior to the first sample. The sampled water was immediately dispersed into sterile flasks or sterile plastic bags (for large water samples), brought to the lab and stored at $+2^{o}C$.

At each station samples were normally taken at 1m, 5m and 10m depth. In Bay 9, 10 and 11 stations H1 to H6 (see map on Fig. 1) were sampled according to the schedule given below and the main sampling period coincided with the cycle 3 of Canadian microbiology and the analysis program for environmental chemistry. Station number, date and depth specifically identify the samples concomittently analyzed by all three partners.

For comparative purposes on one occasion water samples were taken at the west shore of the Ragged Channel; these stations are designated S1 - S3 (see Fig. 1).

Water sampling period:



2.1.2. Sediment and beach sand samples.

One sample from subtidal Channel area (H2 - 8/29) was taken with a Peterson gravity sampler from Zodiac. The other sediment samples analyzed by us were recovered by divers from 7 m depth, by scooping sediments from the top 2 cm layer at convenient locations into a plastic container. The samples were stored at $+2^{o}C$ and analyzed within 2-3 hours. In one case (H1 - 9/2) the sample was frozen and thawed the next day immediately prior to the analysis.

For sampling sand from beaches (oiled or not oiled) and sediment from tidal areas the tip of a sterile disposable syringe was cut off leaving a syringe barrel with full opening. By inserting the syringe into the sand a measured volume of sand, usually 5 ml, could be collected. In some cases several 5 ml samples from the same general area were mixed in a sterile plastic beaker, and a 5 ml collective subsample was taken in the same manner described above. In most cases a collective 5 ml sample was prepared directly by filling the initial syringe with sand from 4-6 sampling locations. Both

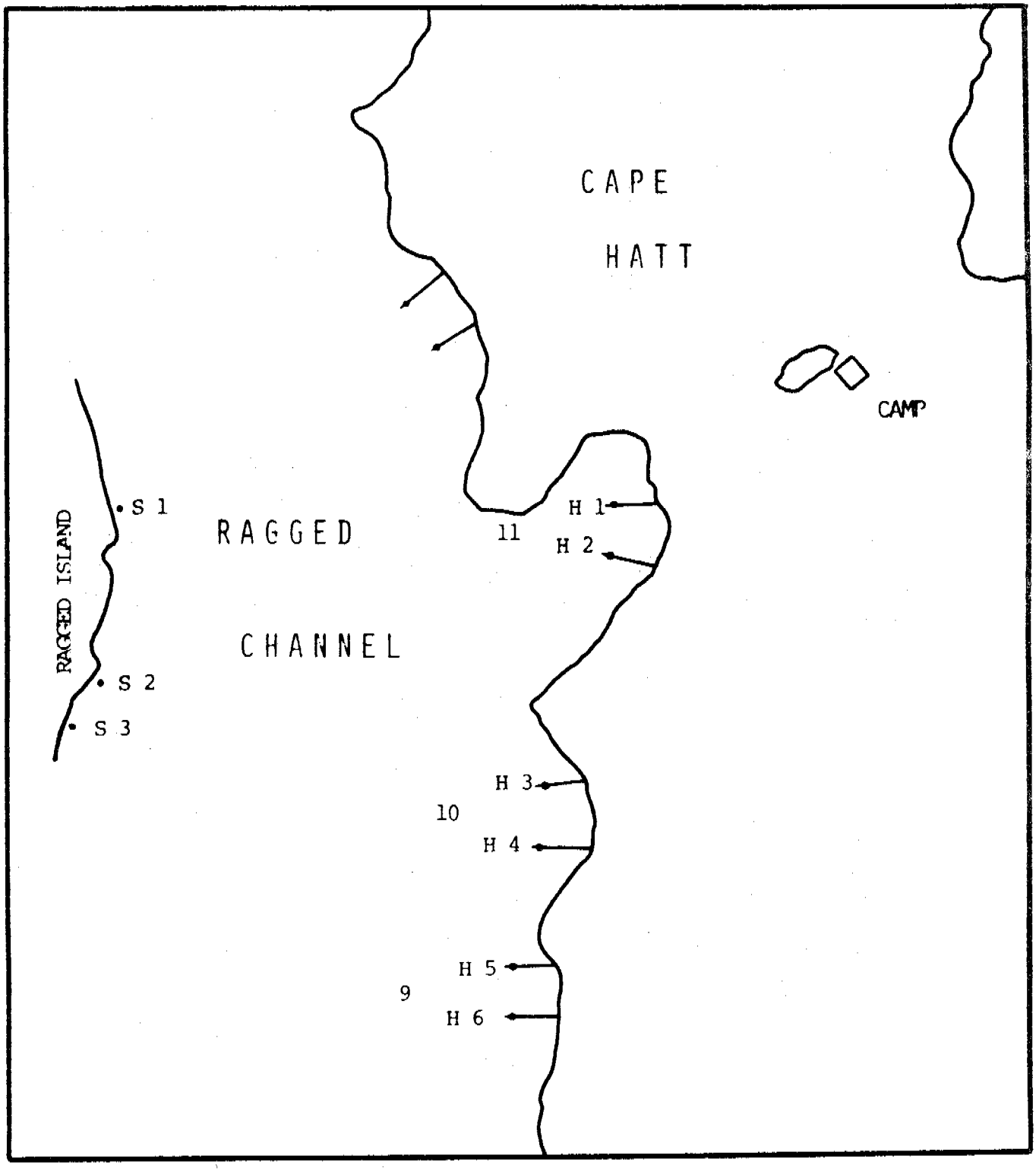


Fig. 1. Location of sampling stations H 1 - H 6 and S 1 - S 3 in Ragged Channel, Baffin Island; August/September 1980.

types of collective samples were brought to field lab in a sterile 50 ml plastic container for analysis.

2.2. EXPERIMENTAL AND ANALYTICAL METHODS

2.2.1. Radioactive chemicals.

Generally tritiated Lago Medio crude oil (3H-Lago Medio) was prepared by The Radiochemical Centre, Amersham, England, by exchange reaction with $^{3}H_2O$. Components of the crude oil with boiling points below 200°C had been removed prior to the tritiation and the final product corresponded to a medium weathered oil and had a specific activity of approx. 1 m C_i/mg. Applied to a column of activated silica the ^{3}H-Lago Medio could be separated into three fractions in the usual way by successive elutions by heptane, benzene and methanol. Fig. 2 gives the graphic profile for the distribution of radioactivity in the various fractions eluted from the column. The specific activity by weight in the alkane fraction (eluted by heptane) was substantial, but as expected somewhat (about 40%) lower than in the more polar aromatic fractions.

1-^{14}C-*n*-hexadecane (sp.activ 235 μC_i/mg), [1(4,5,8)-^{14}C] - naphthalene (sp.activ. 40 μC_i/mg) and [7,10-^{14}C] - benz(a)pyrene (sp.act. 86 μC_i/mg) were purchased from The Radiochemical Centre, Amersham, England. Solutions of appropriate concentrations and specific activity were prepared by dissolving "hot" and when required cold chemical in cyclohexane (^{3}H-Lago Medio), hexane (^{14}C-*n*-hexadecane), and toluene (^{14}C-naphthalene and benz(a)pyrene). All four radioactive substrates were at times used at two levels of specific activity, 100% and 1%. Except for the *n*-hexadecane which was diluted to 100 μC_i/mg the 100% corresponded to the specific activity of the commercial products.

2.2.2. Hydrocarbon mineralization experiments.

2.2.2.1. Preparations of water and sediment samples.

The water for assessment of the biochemical activity for mineralization of hydrocarbons was taken from 5 m depth at all stations. 500 ml (in some cases 1500 ml) of water was filtered through sterile 50 mm polycarbonate membrane filters (Uni-Pore, pore size 0,4 µm), the filter rolled up and immediately deposited into a 120 mm sterile screwcapped tube containing 10 ml of the same sample water. The screwcap had an inner liner of aluminum. As the filter folded out and lined the wall of the tube, the surface containing the particles including the bacteria retained from the filtered water sample faced the inside of the tube.

For analyses of sediments the following technique was used. 5 to 20 ml sediment sample was suspended to 50 ml with freshly sampled water from the 10 m depth of the same station and 1 ml samples of the resulting slurry

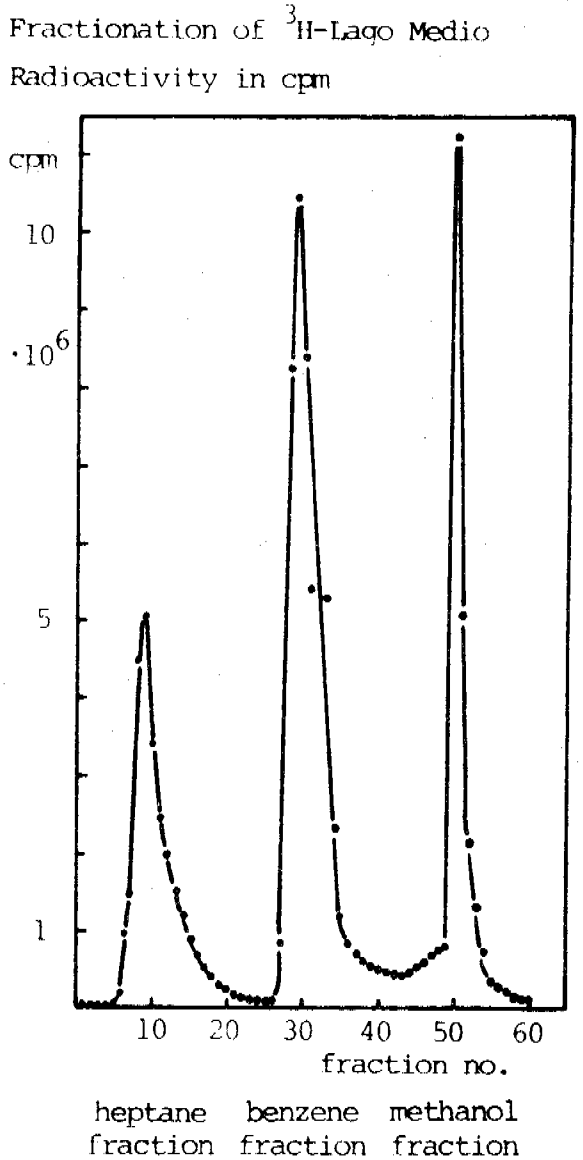


Fig. 2. Chromatographic analyses of generally tritiated Lago Medio crude oil.

Profile of radioactivity in fractions of hydrocarbon eluted from a column of activated silica.

(0.1 to 0.4 ml original sediment volume) were dispensed into 120 mm tubes containing 9 ml of water (10 m, same station).

When the necessary number of identical tubes had been prepared from the same sample, sterile 3 mm cellulose-pads containing known amounts of radioactive oil or hydrocarbon were added, the tubes securely closed and placed radially on a rotating cylinder inside a specially designed water bath. The rotor revolved at approximately 30 rmp.

Due to a failure of the cooling thermostat brought to Cape Hatt a constant temperature of $0^{o}C$ had to be selected as an incubation temperature. Constant temperature was maintained by a mixture of water and crushed ice.

2.2.2.2. Analyses of mineralization products.

At 4-6 intervals over 10-16 days 0.5 or 1.0 ml aliquots of the water phase of each tube were drawn for the extraction and determination of the radioactive mineralization products.

$^{14}CO_2$ were adsorbed by 1 N NaOH applied to a glassfiber filter using standard extracting procedure. After complete absorption the filter was baked at $100^{o}C$ for 6-10 hours to strip off co-adsorbed ^{14}C-hydrocarbons and placed in vials for later counting.

Aliquots from experiments with ^{3}H-Lago Medio were applied to a column of successive layers of Silicone SE-80 (Methyl) and Chromosorb B, BIO-Rad A6 50W x 8 (kation exchange resin), BIO-Rad A6 1 x 8 (anion exchange resin) and activated charcoal mixed with Porapak Type Q (1:1). The column was designed to retain essentially quantitatively all organic radioactive compounds from the tritiated oil or organic products derived from them, leaving $^{3}H_2O$ the only radioactive species of significance. The latter was recovered by elution with 4 one ml portions of distilled water, each collected separately in counting vials; 0.1 ml toluene added and vials kept frozen (when possible) until they were counted.

After return to Trondheim 10 ml Scint Hei 3 was added to each vial and radioactivity of ^{14}C or ^{3}H measured in a Packard model 3375 Liquid Scintillation Spectrometer.

2.2.2.3. Calculation of V_{max}.

For each water or sediment sample to be assessed a set of experiment consisted of 5 tubes identical except for the added hydrocarbon substrate which were present in increasing amounts from tube one to five. The amounts added varied from 5 to 200 µg/tube for substrates with 1% specific activity and from 2 to 50 or 0,5 to 20 µg/tube for substrates with the highest activity.

In successful experiments the accumulation of products directly related to the mineralization of the added hydrocarbon substrate proceeds in a linear fashion, often after a characteristic lag period. In some cases this

linear rate is used to express the biochemical activity for hydrocarbon mineralization.

The observed linear rates will increase with increasing amounts of added substrate, when varied within a certain range of concentration. The set of 5 rates for each experiment was used to calculate a maximal rate for mineralization, V_{max}, according to Michaelis-Menten kinetics using the technique described by Wright and Hobbie (1965). This value represents the rate of mineralization at conditions when substrate concentration no longer limits the reaction rate. V_{max} therefore can be considered a term expressing the potential of the microorganisms in the sample for the utilization and mineralization of hydrocarbons and suitable for comparative purposes.

2.2.3. Microbiological analyses.

Water samples were processed immediately after returning to camp. Serial tenfolds dilutions were made in sterile seawater.

Sediment samples from Bay 9 to 11 were used as brought to camp and not homogenized in any way. Subsamples were taken by inserting and filling the barrel of a 5 ml sterile disposable syringe, the tip of which had been cut off to leave full barrel opening. 5 ml sample was mixed with ice-cold sterile 0.1% Tween 80 in seawater and the suspension shaken by hand for 2 minutes. Tenfold serial dilutions of the resulting slurry were made in sterile seawater.

Samples of beach sand and tidalzone sediments were treated with 0.1% Tween 80 in the same manner as for bottom sediments. Sand containing oil needed more than 2 minutes shaking to produce a homogeneous slurry. Seawater - distilled water (1:1) was used for preparing Tween 80 solution and the tenfold dilution series.

2.2.3.1. MPN, generally heterotrophic microorganisms (bacteria).

For assessing the total viable count of generally heterotrophic bacteria a liquid most probable number (MPN) technique was used. 3 times 0.1 ml aliquots from each selected dilution were mixed with 0.1 ml medium in 3 wells of presterilized tissue culture plates 3596 (Costar, Cambridge, Mass.). Each plate had 96 wells permitting 4 analyses (each 3x8 microcultures of 0.2 ml). The final growth medium contained per liter: 5 g peptone, 1 g yeast extract, 15 g NaCl, 0.1 g K_2HPO_4, 30 mg phenol red, pH 7.8, in seawater - distilled water (1:1).

Positive cultures were scored by turbidity after incubation for 15 days at 10°C.

2.2.3.2. MPN-oildegrading microorganisms (bacteria).

For assessing a number for oildegrading microorganisms a liquid most probable number (MPN) technique was used, with a mixture of *n*-hexadecane and weathered Lago Medio crude being the selective source for energy and carbon.

Three 1 ml aliquots from each selected dilution were added to three one dram flasks (approx. 5 ml) containing a mineral-hydrocarbon medium which after inoculation had the following composition per l: 0.1 g NH_4NO_3, 0.1 g K_2HPO_4, 0.05 g phenol red and 25 g of a mixture of weathered Lago Medio crude (weathered to "boiling point" above 200°C) and *n*-hexadecane (1:1) in sea-water, pH adjusted to 7.8-8.0.

10 and 100 ml water samples were filtered through sterile 25 mm polycarbonate membrane filters (Uni-Pore 0.2 µm) and the filters, as inoculum, pushed into the MPN-flasks by a sterile toothpick.

Normally 6 dilutions were used as inoculum for the 3 flask MPN series; for water samples 100, 10, 1, 10^{-1}- 10^{-3} ml served as inoculum and for sediment and sand samples 10^{-1}-10^{-6} dilutions were made with reference to 1 ml of the sample volume. The flasks were incubated at 10°C and scored after 15-20 and 25-30 days. A positive oildegrading culture was assessed by a clear change in the indicator color from red/purple to yellow. Blanks without hydrocarbon substrate will under such circumstances remain unaltered.

This MPN-method for enumeration of oil degrading microorganisms has been used for sample materials giving numbers for oil degrading bacteria ranging from 0.001 to 100% of the viable count for heterotrophic bacteria in the same sample.

The selection of 10°C for incubation temperature is deemed a reasonable compromise between the wish for a procedure with a high degree of analytical expediency and our experience with the requirements of the most sensitive marine psychrophilic microorganisms.

2.2.3.3. MPN-antibiotica resistant oildegrading microorganisms (fungi).

The method was a slight modification of the described most probable number (MPN) technique for oildegrading microorganisms and aimed at assessing the content of oildegrading fungi in beach sand and tidal zone sediments. The presence of a mixture of three antibiotica were intended to subdue any growth of procaryotic bacteria and thus favor growth of oildegrading fungi. But in addition to creating a more selective medium, a lower analytical sensitivity was expected due to the drastic reduction in the possibilities for secondary growth on intermediate products of the initial hydrocarbon degradation. All cultures that eventually were scored positive with this technique did contain fungi, but bacteria were also almost invariably present. Therefore we prefer the given designation of the method.

Three 1 ml aliquots from each selected dilution were added to three 2 ml tubes containing 0.1 ml medium base and approx. 25 mg of the mixture of weathered Lago Medio crude oil and *n*-hexadecane (1:1). The tubes were closed by sterile plastic caps. After inoculation the medium had the following composition per l: 0.1 g NH_4NO_3, 0.01 g K_2HPO_4, 50 mg each of phenol red, streptomycin, chloramphenicol and tetracycline, in seawater - distilled water (1:1) and pH adjusted to 7.8-8.0.

The MPN-tubes were assessed after 15-20 and 25-30 days at 10°C.

2.2.4. Measurement of CO_2-production in beach sand and tidal sediment.

The open end of a plexiglass cylinder serving as a CO_2 measurement cell (see Fig. 3) were pressed firmly into sand or intertidal sediment. The panshaped CO_2-absorption chamber was loaded with 3 ml 1 N CO_2-free NaOH-solution by injection through a diafragm on the top airtight end of the cylinder. A thermometer mounted into the same end permitted the temperature inside the cell to be recorded. To minimize the heating effect of radial energy the cylinders were coated with a reflecting paint.

Over a period of 6-10 (tidal zone) to 20-25 hours (beach sand) samples of the NaOH-solution were drawn by a syringe piercing the needle through the diafragm. Before the actual samples were drawn the content of the absorption chamber was thoroughly mixed by pumping the solution in and out of the syringe. The samples were sealed in glass-ampules until analyzed for CO_2 in an Envirotech DC50 Total carbon analyzer.

2.2.5. Detritovore invertebrate assessment.

Pit-fall traps containing 3% formaldehyde were mounted in the beach sand with the edge of the trap at level with the surface.

2.2.6. Experimental plots for observation of natural decay of oil.

An area of apparent uniform character in the supralitoral zone of Bay 102 was divided into three plots, each 2x10 m. One area (102B) was sprayed with weathered (~8%) Lago Medio crude oil to give 10 $l \cdot m^{-2}$ and one (102C) with the same oil emulsified with equal volume of water to give 20 $l \cdot m^{-2}$. The technique of spraying is described in the Report of Baffin Island Oil Spill Project - Shoreline component. Interim Report. Woodward-Clyde Consultants. Nov. 28, 1980.

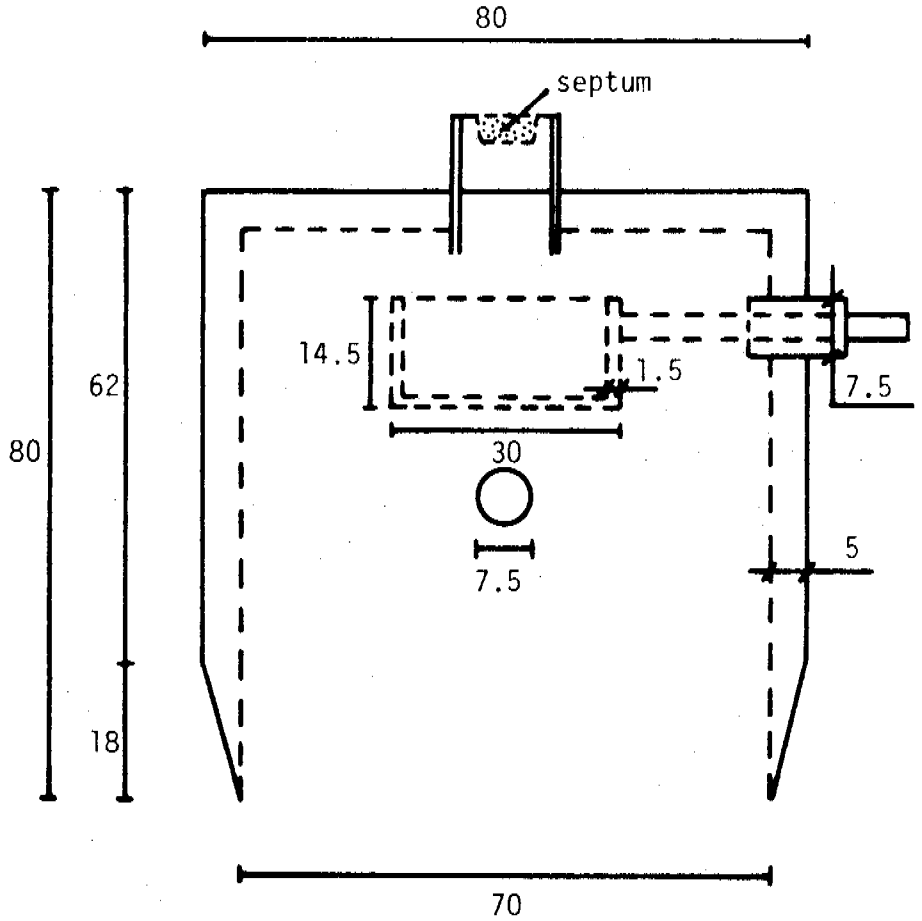


Fig. 3. Absorption chamber for analyses of carbon dioxide in sand and tidal sediments.

All measures in mm.

3. RESULTS AND COMMENTS

Within the framework of the objectives for the BIOS Project the results of our work at Cape Hatt in 1980 constitutes a baseline for the effect studies to follow in 1981 and 82-83. Except for some comments directly relevant to the analytical values obtained, there is therefore no grounds for any extensive discussions in this report.

3.1. NEAR SHORE PROJECT

3.1.1. Analyses of the microbial population in the watercolumn.

Table 1 and Fig. 4 summarize the results of the microbiological analyzes for viable count of generally heterotrophic microorganisms (essentially bacteria) and oildegrading microorganisms (essentially bacteria) in 1m, 5m and 10m water samples from the fixed sampling stations in Bay 9, 10 and 11 over the short time period from 28 August to 4 September. The measurements have to be considered an assessment of the level of bacteria present in these waters during the period of highest water temperature (see Appendix with quotations of the results given by Seakem for the environmental physical and chemical parameters) and the fluctuations mainly caused by the short-term physical processes and do not represent any attempt to assess seasonal fluctuations.

A liquid most probable number technique has been used to obtain values for the viable count of generally heterotrophic bacteria. In our hands this technique gives higher values than other techniques used to obtain the same parameter.

The analytical figures for general heterotrophs varied between $1.5 \cdot 10^5$ and $9.5 \cdot 10^6$ L^{-1}; in one instance a high $4.5 \cdot 10^7$ L^{-1} was recorded. The data for total bacteria (direct count using an epifluorescence method) in the same samples were not at our disposal when writing this report, so detailed comparisons cannot be made.

All methods for enumerating socalled oildegrading microorganisms are based on the selectivity of an oil/hydrocarbon substrate and an observable response directly or indirectly connected to the biochemical attack on one or an unknown number of the hydrocarbons in the substrate. The use of MPN methods for this purpose essentially determine the smallest aliquot of the sample that will give a positive response. This minimum requirement for a response is defined as equivalent to one cell and the result of the analysis expressed as a number of oildegrading cells present per unit volume or weight of the sample. The latter definition is most like not true.

For a variety of other reasons the various methods designed will vary considerably in their sensitivity and concequently yield different numerical values when analyzing the same sample. Nevertheless, each method may be fully useful for making conclusions when comparing two or more situations. All methods, however, share the characteristic common to all methods for viable counts of microorganisms, the analytical figures are lower than the actual, true figure.

TABLE 1. Bacterial counts in the nearshore water column of Bays 9, 10 and 11, Ragged Channel, Cape Hatt, Baffin Island; August-September 1980.

Station (cycle)		Date	Bacteria liter^{-1}	
			Oil degraders	Total heterotrophs
H1	1	8/26/80	$2.5 \cdot 10^4$	$2.5 \cdot 10^6$
	5	"	$9.5 \cdot 10^3$	$4.5 \cdot 10^7$
	10	"	$2.5 \cdot 10^4$	$7.5 \cdot 10^6$
H1(3)	1	8/28/80	$2.5 \cdot 10^5$	$4.5 \cdot 10^6$
	5	"	$2.0 \cdot 10^3$	$9.5 \cdot 10^6$
	10	"	$4.5 \cdot 10^3$	$9.5 \cdot 10^5$
H2(3)	1	"	$4.5 \cdot 10^3$	$2.5 \cdot 10^6$
	5	"	$2.5 \cdot 10^3$	$7.5 \cdot 10^6$
	10	"	$2.5 \cdot 10^3$	$1.1 \cdot 10^6$
H3(3)	1	8/30/80	$2.5 \cdot 10^3$	$7.5 \cdot 10^5$
	5	"	$4.5 \cdot 10^3$	$2.5 \cdot 10^6$
	10	"	$4.5 \cdot 10^2$	$2.5 \cdot 10^6$
H4(3)	1	"	$2.5 \cdot 10^4$	$4.5 \cdot 10^6$
	5	"	$9.5 \cdot 10^3$	$4.5 \cdot 10^6$
	10	"	$1.5 \cdot 10^3$	$2.5 \cdot 10^6$
H5(3)	1	9/1/80	$2.5 \cdot 10^5$	$2.0 \cdot 10^6$
	5	"	$2.5 \cdot 10^5$	$9.5 \cdot 10^5$
	10	"	$2.5 \cdot 10^4$	$4.5 \cdot 10^5$
H6(3)	1	"	$4.5 \cdot 10^4$	$7.5 \cdot 10^5$
	5	"	$2.5 \cdot 10^4$	$7.5 \cdot 10^6$
	10	"	$4.5 \cdot 10^4$	$9.5 \cdot 10^5$
H1	1	9/3/80	$2.5 \cdot 10^5$	$1.5 \cdot 10^7$
	5	"	$1.5 \cdot 10^5$	$1.5 \cdot 10^6$
	10	"	$4.5 \cdot 10^4$	$4.5 \cdot 10^6$
H4	1	9/4/80	$1.1 \cdot 10^6$	$*2.5 \cdot 10^6$
	5	"	$4.5 \cdot 10^4$	$*1.5 \cdot 10^6$
	10	"	$2.5 \cdot 10^4$	$*2.5 \cdot 10^5$

* Assessed after 10 days of incubation.

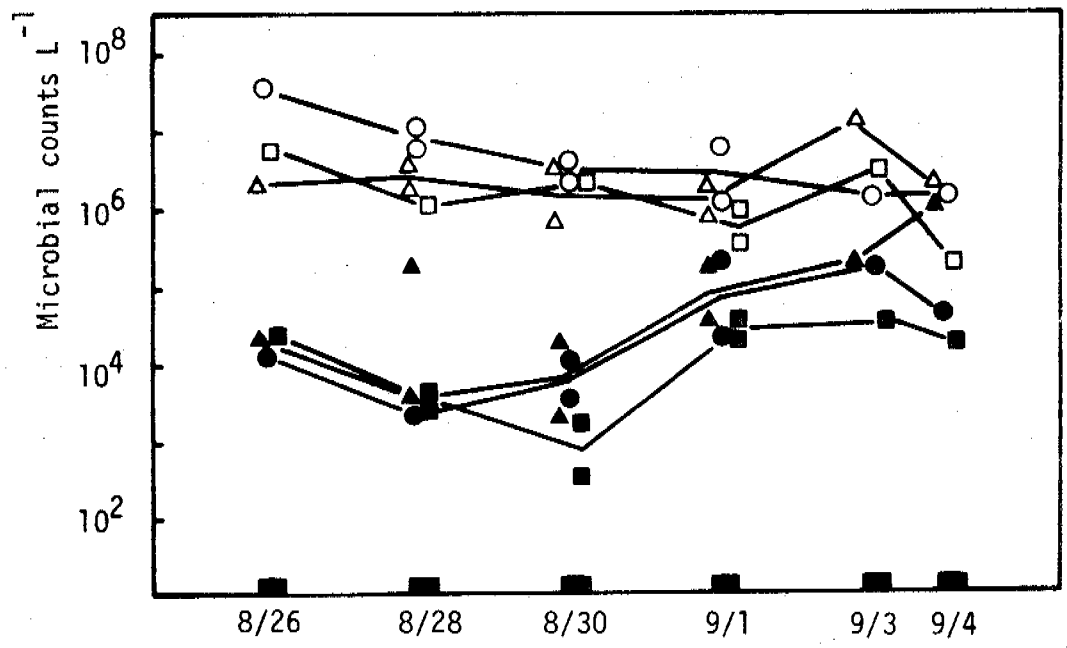


Fig. 4. Analyses of the microbial population in the nearshore waters of Bay 9, 10 and 11 of Ragged Channel, Baffin Island; August/September 1980.

Viable counts for oildegrading and generally heterotrophic bacteria at 1, 5 and 10 m.

The number of oildegrading bacteria in the water samples of Bay 9 to 11 found by using our MPN method varied normally between $2.5 \cdot 10^3$ and $2.5 \cdot 10^5$ L^{-1}, with an occational low value of 450 and a high value of $1.1 \cdot 10^6$ L^{-1}. This corresponds to concentrations found by us in the Barents Sea using the same method. Bunch (1979) reported the high range values for oleoclastic bacteria in the Davis Strait to the 10^3 to 10^4 L^{-1}, with occasional stations with concentrations above 10^4 L^{-1} (August 1978). In Prudhoe Bay Horowitz and Atlas (1977) recorded high values of 10^3-10^5 oildegrading bacteria per ml during the summer season. The method of analysis were different in all three cases.

For the samples taken during the cycle 3 period an average concentration of $4 \cdot 10^4$ L^{-1} oildegrading bacteria can be calculated. This means that an average about 1% of the population of the generally heterotrophic bacteria (based on viable count) in the coastal water of Ragged Channel possess the physiological ability to attack hydrocarbons of mineral oil. This does not necessarily implicate that these waters are recipients of mineral oil components. Naturally occurring hydrocarbons different in nature to the hydrocarbons of mineral oil may be responsible.

Most of the highest values for oildegraders (except H1-1m-8/28) occurred during a period of great stratification of the surface water (see figures for salinity in the Appendix). A connection between high surface counts and land drainage cannot be ruled out.

It has to be admitted that the concentration of oildegraders found in the waterphase was somewhat surprising based on otherwise unsubstantiated notions. For this reason some watersamples were taken in the nearshore waters on the west side of the Ragged Channel, in areas assumed to be entirely free of any accidental pollution due to logistic activity. The results of these analyses are given in Table 2. The level of oildegrading bacteria in these samples were the same as found for Bay 9 to 11 and giving no support to any suspicion of contamination of the latter bays.

3.1.2. Analyses of the microbial population of the bottom sediments.

5 sediment samples, at least one from each bay, were analyzed. This was somewhat less than planned. The results are presented in Table 3. Per ml sediment $2.5 \cdot 10^3$ to $2.5 \cdot 10^4$ oildegrading units could be detected and the viable count of general heterotrophs varied from $2.5 \cdot 10^5$ to 10^7 ml^{-1}. Relative to the concentrations in the watercolumn the total bacterial population of the sediments are not high, but there is a considerable variation in the relative proportions between the two groups of bacteria. This needs clarification next year.

3.1.3. Mineralization of Lago Medio crude oil and defined hydrocarbons.

The method developed to measure quantitatively the biochemical activity for mineralization of oil and hydrocarbons (i.e. oxidation to the products of carbondioxide and water) is particularly designed to assess the low activities associated with the water of open ocean. For this reason we thought the method might be useful for assessment of the oildegradation potential of the cold environment of the Arctic Sea.

TABLE 2. Bacterial counts in nearshore water column at the west side of Ragged Channel (stations S1-S3), Ragged Island, Baffin Island; September 1980.

Station		Date	Bacteria liter^{-1}	
			Oil degraders	Total heterotrophs
S1	1	9/9/80	$2.5 \cdot 10^5$	$*1.1 \cdot 10^6$
	5	"	$2.5 \cdot 10^5$	$*9.5 \cdot 10^4$
S2	1	"	$1.1 \cdot 10^6$	$*9.5 \cdot 10^4$
	5	"	$4.5 \cdot 10^4$	$*1.5 \cdot 10^5$
S3	5	"	$2.5 \cdot 10^4$	$*9.0 \cdot 10^3$

* Assessed after 5 days of incubation.

TABLE 3. Bacterial counts in nearshore bottom sediments* of Bay 9, 10 and 11 of Cape Hatt, Ragged Channel, Baffin Island; August/September 1980.

Sample H2 collected as indicated, the other samples were taken at 7 m depth close to station indication.

Station	Date	ml sediment^{-1}	
		Oil degraders	Total heterotrophs
H2(3)	8/29/80	$2.5 \cdot 10^3$	$9.5 \cdot 10^6$
H4(3)	8/31/80	$7.5 \cdot 10^3$	$9.5 \cdot 10^6$
H5(3)	9/2/80	$9.5 \cdot 10^4$	$2.0 \cdot 10^5$
H6(3)	--	$9.5 \cdot 10^3$	$9.5 \cdot 10^5$
H1 **I	9/2/80	$2.5 \cdot 10^4$	*** $2.5 \cdot 10^4$
H1 **II	"	$2.5 \cdot 10^4$	*** $1.5 \cdot 10^5$
H1 **III	"	$2.5 \cdot 10^3$	*** $9.5 \cdot 10^4$

* Uncorrected for water content in the sediment.

** I-III successive 1 ml samples drawn from same sediment sample container.

*** Results assessed after only 11 days.

TABLE 4. Mineralization of Venezuelan Lago Medio crude oil at 0°C by microorganisms in water from Bay 9, 10 and 11, Ragged Channel, Cape Hatt, Baffin Island; August/September 1980.

Calculated maximal rates of mineralization (V_{max}) based on laboratory experiments with radioactive tritiated weathered Lago Medio crude.

Station	Date	V_{max}, $\mu g/m^3$,d ^{3}H-Lago	Oil degrading bacteria L^{-1}	Generally heterotrophic bacteria L^{-1}
H2	8/28/80	32	$4.5 \cdot 10^3$	$7.5 \cdot 10^6$
H4	8/30/80	29	$9.5 \cdot 10^3$	$4.5 \cdot 10^6$
H5	9/01/80	26	$2.5 \cdot 10^5$	$9.5 \cdot 10^5$
H1	9/03/80	11	$1.5 \cdot 10^5$	$1.0 \cdot 10^6$
H4	9/04/80	13	$4.5 \cdot 10^4$	$1.0 \cdot 10^6$

The method has certain limitations as to the magnitude of activity of the sample and since the latter was entirely unknown we had to design the experimental program to cover a considerable range to enhance the chance for at least some successful measurements. For this reason concentration of substrate, spesific activity of substrate and to some extent sample size were varied in a systematic manner.

Due to the failure of the low temperature thermostat brought to Cape Hatt to control the temperature of the incubator 0°C had to be selected as temperature of incubation in stead of the *in situ* temperature.

In total 22 experiments were carried out in attempt to assess the mineralization activity of water samples and 13 experiments for assessment of bottom sediments. Generally tritiated Lago Medio crude was used as substrate in 14 experiments and ^{14}C-*n*-hexadecane, naphthalene and benz(a)-pyrene in 21.

Only 5 experiments using ^{3}H-Lago Medio crude oil with the highest specific activity were successful in the sense of giving results permitting calcukaton of V_{max} (rate of mineralization at saturating concentration of oil). The results are given in Table 4.

For convenience the rates of mineralization determined experimentally have been recalculated to µg of oil mineralized per day by the microorganisms in one cubic meter of the sample water. The values for V_{max} determined in this way varied between 11 and 30 µg/m^3, d.

The experimentally determined rates bear little apparent relationship to the content of oildegrading bacteria in the same samples. The recorded V_{max} values corrolate better to the viable count for general heterotroph. Although the degradation of oil obligatory depends on oildegrading bacteria the general heterotrophs may also contribute substantially to the total mineralization.

This apparent lack of corrolation between the specific microbiological and biochemical analyses for oil degradation has been observed by Bunch (1979) for a much larger sample material from Davis Strait in 1978 and to a lesser extent for the North Sea. Several possible explanations for this situation can be offered. Bunch favors the idea that the mineralization of *n*-hexadecane in the August water of Davis Strait was primarily limited by nutrients. The water of Ragged Channel was extremely low in nitrate in August-September. For the Barents Sea and the North Sea we have no experimental grounds for supporting any hypothesis.

Although we have very few measurements of this type to make good comparisons, our measurements at Cape Hatt, Barents Sea and the North Sea have been done with identical methods. Below the results of these analyses are summarized in terms of giving the ranges found for the rate of mineralization of oil (based on the use of tritiated oil) and the content of oildegrading bacteria in the same water sample. The rate of mineralization were determined at somewhat different temperatures in the various investigations and for the Barents Sea samples the rate (V) were calculated from single concentration experiments. The corresponding V_{max} will be somewhat higher.

	Temperature for mineralization rate measurement	Mineralization $\mu g/m^3,d$	Oildegrading bacteria L^{-1}
Barents Sea	-0.5 - 4.2°C	V: 7 - 21.8	$2.5 \cdot 10^3$ - $5.5 \cdot 10^5$
North Sea	8 - 9°C	V_{max}: 26 - 114	$9.5 \cdot 10^3$ - $4.5 \cdot 10^5$
Ragged Channel	0°C	V_{max}: 11 - 30	$4.5 \cdot 10^3$ - $2.5 \cdot 10^5$

In spite of these differences it can be seen that values for V_{max} found for the Ragged Channel water in a general way fit into expected range based on the content of oildegrading bacteria.

The other experiments with Lago Medio crude oil may also have yielded useful data in spite of a much lower specific activity of the substrate. But contrary to previous experience with titriated Ekofisk crude oil the tritiated Lago Medio preparation gave unduly high background counts and thus lowered the sensitivity of the analysis. We have reason to believe that this problem will be solved prior to the 81 season.

In experiments with the defined ^{14}C-hydrocarbons only the substrates with the highest spesific activity yielded radioactive CO_2 in measureable amounts, but unfortunately the results could not be used for calculation of V_{max} in the usual manner.

The best indications for mineralization were obtained with ^{14}C-*n*-hexadecane and some rates of mineralization calculated from single concentration experiments are reported in Table 5. Although no strict comparison between V_{max} for one substrate and V for a single concentration for another substrate can be made, the results indicate that the mineralization rate for *n*-hexadecane is slower than for crude oil in water (H4, Table 4). The apparent higher rate observed for the sediment sample is in this case consistant with a higher concentration of oildegrading bacteria in the sediment relative to the water (Table 1 and 3).

In some experiments we have indications for mineralization of naphthalene and benz(a)pyrene, but the results are not consistant and need to be confirmed next year before any conclusions can be made.

TABLE 5. Mineralization of ^{14}C-*n*-hexadecane in water (Bay 10) and sediment (Bay 9) of Ragged Channel, Baffin Island; August/September 1980. Linear rates of mineralization (V) based on single concentration experiments.

Added *n*-hexadecane µg/tube	Water (H4-8/31) µg/m^3,d	Sediment (H6-9/2) µg/L,d
0.5	0.6	2.5
5	2.6	7.5
10	-	15.8

3.2. ON SHORE PROJECT

In 1981 a series of experiments will be started to test the effect of fertilizers (nitrogen and phosphate) and mechanical treatment of the sand-oil mixture (increased aeration) on the natural microbial wheathering and mineralization of oil stranded on the shoreline. The plots for these experiments were selected this year and baseline analyses of microbial population and biological CO_2 production were carried out.

It is assumed that the natural processes for oil decay may take a long time in these cold regions and to gain a year of observation, one set of testplots were started in 1980.

3.2.1. Baseline analyses of microorganisms and CO_2-production in shoreline sand and sediments.

The test area in Bay 103 of the Z-lagoon (low energy beach) was monitored for microbial population and CO_2-production over the period from 28 August to 9 September and the results are given in Table 7 and Table 9. The results for the control area (Table 8) may also be included.

The total number of analyses are admittedly low. In the supralitoral area low but detectable numbers of oildegrading bacteria were observed. The count of antibiotica resistant oildegraders (fungi) were even lower. It is, however, expected that this method of analysis may have a low sensitivity, so a direct comparison may not be entirely justified. The analyses for generally heterotrophic bacteria vary considerably, $4.5 \cdot 10^3$ - $2 \cdot 10^7$ ml. This may reflect the method of sampling. The great abundance of snow-geese droppings in this area may also contribute to the quantitative variability. In the tidal zone (Table 7) the content of oildegrading bacteria were consistantly higher and increased quite markedly over the period of sampling. A similar increase in general heterotrophs was not noticeable, but analyses for the last samplingday are lacking. Already before the first sampling sheens of oil were observed in the tidal zone later to be sampled. The oil most likely had drifted to the innermost beaches of 103 from testsites closer to the opening of the bay. These sites were sprayed with Lago Medio crude and emulsified crude during the days of 20-21 August. In spite of the boom some oil may have escaped. Later on (9/20) chemical analyses revealed substantial concentrations of oil in Bay 103 (Seakem, see Appendix). This might offer an explanation for the changes taken place in the microbial flora of the tidal sediments. The rather short responsetime is interesting.

As an overall parameter for the biological activity of the shoreline sand and sediments we have attempted to measure the total CO_2 produced from the biota restricted to a certain volume of sand in communication with a bell-shaped absorption chamber (Fig. 3). Table 9 reports characteristic values obtained for CO_2-production rates in selected areas of apparently uncontaminated beach sand and tidal sediments. A clearcut difference was found between activity in the sand of high and low energy beaches. Analyses of chemical and physical parameter in the sand (Table 6) offer no explanation for the observed differences in biological activity.

During the last measurements on the high energy beach (Bay 102) the temperature in the top layer of the sand dropped to -0.4°C. This temperature decrease did not seem to reduce the rate of CO_2 production noticeably (see Bay 102 D-H, Table 9).

3.2.2. Natural decay of oil on beach sand - Initial observations.

In the supralitoral zone of Bay 102 plots (2x10 m) were sprayed to give a 1 cm layer of weathered Lago Medio crude or a 2 cm layer of 50% emulsified Lago Medio crude oil. A nearby plot was selected as a control. The plots were oiled 24 August and left undesturbed for observation of the natural decay of oil and for comparison of the natural decay of emulsified and not emulsified oil. At the same time these plots will serve as respective controls for series of experiments on enhancement of natural decay of oil to be started in 1981. The former experiments were started this year to leave us the longest possible period of observation.

The results of the microbiological analyses for the initial 2 weeks after oildeposition are given in Table 8. In the same period the rate of CO_2-production was measured 3 times and the results are presented in Table 10. Analyses of the sand in plot B and C prior to the deposition of the oil are lacking. Two days after the deposition of the oil the levels of general heterotrophic and oildegrading bacteria were still rather low and comparable to the level in the control plot. The oil itself thus did not seem to contribute significantly to the microbial population.

7 and 15 days after the spill decisive increases in the population of oildegrading as well as generally heterotrophic bacteria seemed to have taken place. There was a particularly pronounced development of oildegraders in the sand containing the emulsified oil and the same may have been the case for the general heterotrophs. For logistic reasons the incubation period for the analyses of the latter was too short and the counts may certainly be on the low side. The counts for antibiotica resistant oildegrading microorganisms remained low, with the exception of a possible small increase in the emulsified oil plot.

Admittedly the number of analyses are few and the observed difference in the microbial development in the two types of oil needs confirmation. The weather during the period of observation was extremely good with hardly any percipitation, and maximal temperatures of 17-18°C were measured in the top surface in both oiled plots. Under these conditions it is reasonable to assume that the high watercontent of the oil emulsion may have created a more congenial environment for bacterial growth. The effect of water activity in the oil on the microbial growth will be looked into more carefully next year.

The observed rates of CO_2-production dropped markedly when the sand was covered by oil (Table 10). This is commonly observed when massive amounts of oil penetrated down into sand or soil and it is generally assumed to be

caused by the toxic effect of oil on the biota. The plot oiled by weathered Lago Medio crude appeared to recover after a few days, but the CO_2-production in the plot with emulsified oil remained low during the whole period. This was in apparent contrast to the microbial development in the same plots.

A direct comparison of the plots entirely based on the observed rate of CO_2 production may not be warranted. The field method used to determine CO_2 is obviously dependent on the total volume of sand/sediment with biota that contribute CO_2 to the absorption chamber. A massive addition of oil to the sand will reduce this volume more or less as well as affecting its biota. A visual inspection of the oiled plots clearly indicated a pronouned difference between the two plots in this respect. The sand sprayed with weathered Lago Medio crude oil appeared to retain its characteristic airy and rather loose structure whereas the emulsified oil to a great extent remained on the top of the sand forming a continuous liquid surface. The latter most likely formed a boundary that will reduce the volume in diffusion contact with the CO_2-trap. The observed rates of CO_2-production in the two oiled plots are therefore not necessarily comparable, and not representative for the respective microbial activity.

3.2.3. Conclusions.

The beach sand contained low, but detectable levels of oildegrading microorganisms. A week after deposition of oil an increased level of these bacteria were noticeable, in spite of low levels of nitrogen and phosphor. Under the dry conditions of the arctic summer the emulsified oil seemed to offer the best substratum for bacterial development.

TABLE 6. Chemical and physical properties of beach sand, Cape Hatt, Baffin Island; August/September 1980.

Bay	Water %	Organic matter mg/g	Total nitrogen mg/g	Total phosphor mg/g	Nitrate µg/g	Phosphate µg/g	pH
102	0.8	4	1.1	0.13	1	<0.5	8.9
103	17	8	0.8	0.14	0.6	<0.5	7.9

TABLE 7. Baseline levels of microbial activity in sand and sediment of the shoreline of Z-lagoon, Cape Hatt, Baffin Island (Bay 103, low energy beach); August/September 1980.

Counts of generally heterotrophic bacteria, oildegrading bacteria and antibiotica resistant oildegrading microorganisms.

	Cells per ml sample					
	Supralitoral zone		Midtidal zone		Low tidal zone	
Sampling date	Oil degraders	Hetero-trophs	Oil degraders	Hetero-trophs	Oil degraders	Hetero-trophs
8/29/80	95(25*)	$2 \cdot 10^7$	450(25)	$2.5 \cdot 10^5$	450 (25)	$9.5 \cdot 10^5$
9/02/80	950(25)	$4.5 \cdot 10^4$	$3.5 \cdot 10^3$(25)	$9.5 \cdot 10^4$	$2 \cdot 10^3$(250)	$2 \cdot 10^4$
9/09/80	300(25)	-	$1.1 \cdot 10^6$(25)	-	$9.5 \cdot 10^4$(25)	

* Number in parethesis - antibiotica resistant oildegrading microorganisms.

Numbers not corrected for water content.

TABLE 8. Microbial activity in beach sand after spraying with oil. Bay 102, Cape Hatt, Baffin Island; August/September 1980.

Plot B sprayed with 1 cm layer of weathered Venezuelan Lago Medio crude and plot C with 2 cm layer of 50% emulsion of the same oil; spraying date 8/24/80.

	Cells per ml sand					
	Control Area		Plot B		Plot C	
Date	Oil degraders	Hetero-trophs	Oil degraders	Hetero-trophs	Oil degraders	Hetero-trophs
8/26	0(0)	$4.5 \cdot 10^3$	$2 \cdot 10^2$(0)	$7.5 \cdot 10^3$	$1.5 \cdot 10^2$(0)	$1.5 \cdot 10^4$
8/31	95(25*)	$1.5 \cdot 10^4$	$2.5 \cdot 10^4$(25)	$2.5 \cdot 10^5$	$7.5 \cdot 10^5$(250)	$9.5 \cdot 10^6$
9/08	9(25)	**$2.5 \cdot 10^2$	$9.5 \cdot 10^4$(25)	**$4.5 \cdot 10^5$	$1.5 \cdot 10^7$(400)	**$4.5 \cdot 10^7$

* Numbers in parenthesis - antibiotica resistant oildegrading microorganisms.

** Assessed after only 6 days of incubation at 10°C.

TABLE 9. CO_2-production in beach sand and in tidal sediments at Cape Hatt, Baffin Island; August/September 1980.

The average rate of CO_2-production measured over minimum periods of 24 hours (supralitoral areas) or 6-10 hours (tidal areas).

Bay	Temperature (average mean) °C	$mgC \cdot m^{-2} \cdot h^{-1}$*	Beach type
103A	8.8	11 ± 4.6(4)	supralitoral
103B	11.4	6 ± 3.6(3)	midtidal
103C	10.6	8 ± 2.2(3)	lowtidal
103A	4.8	9.8 ± 3.7(5)	supralitoral
102A	5.8	28 ± 6 (4)	supralitoral
102D-H	0.4	47 ± 19(10)	supralitoral

* Number in parenthesis gives number of measurements.

TABLE 10. CO_2-production in oiled beach sand of Bay 102, Cape Hatt, Baffin Island; August/September 1980.

Plots oiled 8/24 with 1 cm layer of weathered Venezuelan Lago Medio crude (Plot B) and 2 cm layer of 50% emulsion (Plot C) of the same oil.

Days after oiling	Temperature max. variation (mean temp.)	$mgC \cdot m^{-2} \cdot h^{-1}$ * Control plot	Plot B	Plot C
2	3.5-7 (5.6-6.3)	28 ± 6(4)	17 ± 4(3)	13 ± 5(3)
7	3.5-18.5 (10-10.6)	34 ± 2(2)	35 ± 6.5(4)	13 ± 13(4)
12	3.0-16 (7.9-8.9)	12 ± 2(2)	21 ± 8(4)	18 ± 12(4)

* Number in parenthesis indicates parallel measurements.

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APPENDIX

Environmental chemistry and hydrocarbon analyses of direct interest to the present report.

Data from:

Baffin Island Oil Spill Project

Chemistry Component: Baseline year

Seakem Oceanography Ltd., 12 January 1981.

ENVIRONMENTAL CHEMISTRY DATA FOR STATIONS OF BAY 9, 10 AND 11 DURING THE PERIOD OF CYCLE 3 AND 4.

Station	depth	Date	Temp. °C	Salinity o/oo	Nitrate µg at.l^{-1}	Phosphate µg at.l^{-1}	DOC mg l^{-1}	*Particulate org. carbon, µg l^{-1}
	1		3.8	25.5	0	0.53	3.03	140
H1	5	8/28	3.3	26.3	0	0.59	2.13	110
	10		2.6	29.4	0.5	0.81	2.11	100
	1		3.8	24.7	0.1	0.54	2.61	130
H2	5	"	3.0	27.4	0.1	0.48	2.63	180
	10		2.5	29.6	-	-	4.09	120
	1		4.1	20.4	0.1	0.38	2.39	190
H3	5	8/30	3.8	24.2	0	-	2.98	210
	10		3.4	26.7	0.2	0.58	2.72	170
	1		4.2	20.3	0	0.31	~5	140
H4	5	"	3.9	23.7	0	0.44	2.75	140
	10		3.4	26.7	0.1	0.48	2.25	120
	1		4.6	22.8	0	0.37	2.37	160
H5	5	9/01	4.2	23.1	0	0.49	2.42	240
	10		3.3	27.0	0	0.58	2.11	160
	1		4.6	19.6	0	0.56	2.97	140
H6	5	"	4.0	24.0	0.2	0.50	3.56	190
	10		3.0	27.4	0	0.32	2.21	200
	1		4.5	20.2	0	0.36	2.08	150
H1	5	9/05	3.2	27.1	0	0.62	2.50	170
	10		2.0	30.0	0.3	0.76	1.97	170
	1		4.5	18.8	0.1	0.33	2.07	120
H4	5	9/07	3.3	27.2	0.2	0.60	3.03	140
	10		2.0	29.2	0.1	0.71	2.84	200

* Analyzed by Arctic Biological Station, DFO

HYDROCARBONS IN THE WATER OF BAYS OF CAPE HATT, 1980. (Seakem, 1981 p. 66-67)

Date	Bay	Depth	Total hydrocarbons µg L^{-1} *	Comments
8/26	9	1 m	D.L.**	August sampling
		5 m	"	
		10 m	"	
"	10	1 m	26	
		5 m	D.L.	
		10 m	"	
"	11	1 m	D.L.	
		5 m	"	
9/20	9	1 m	D.L.	September sampling
		5 m	"	
9/19	10	1 m	D.L.	
		5 m	"	
		10 m	80	
9/18	11	1 m	D.L.	
		5 m	72	
		10 m	1138	
8/18	103	1 m	D.L.	Prespill
		7 m	"	
8/21	103	1 m	D.L.	Prespill
		7 m	"	
9/20	103	1 m	150	Postspill
		5 m	D.L.	

* µg L^{-1} Lago Medio crude equivalents.

** D.L. = below detection limit of 13 µg L^{-1}.

ANALYSIS OF SEDIMENTS OF BAY 9, 10 AND 11.

Analysis of nutrients
(calculated from data p.61, Seakem 1981)

Station	Date	Total organic carbon % d.w.	Interstitial nitrate µg at. l^{-1}	Interstitial phosphate µg at. l^{-1}
H2	8/29	N.A.	N.A.	N.A.
H4	8/31	N.A.	3.1	12.0
H1	9/02	0.42	N.A.	N.A.
H5	"	0.30	2.5	9.0
H6	"	0.35	1.1	20.1

Hydrocarbons
(calculated from data p.68-69, Seakem 1981)

Date	Bay	Total hydrocarbons µg g^{-1} min - max	Total hydrocarbons µg g^{-1} average	Non-polar hydrocarbons µg g^{-1} min - max	Non-polar hydrocarbons µg g^{-1} average
9/12/80	9	7.1-35.9	20.1±10.4	0-2.0	0.8±0.8
9/13/80	10	7.2-24.5	12.7± 6.6	0-2.6	0.8±0.9
9/11/80	11	10.9-26.7	16.5± 6.4	0-1.5	0.9±0.7

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BIODEGRADATION OF AROMATIC COMPOUNDS BY HIGH-LATITUDE PHYTOPLANKTERS

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ANNUAL REPORT

I. Summary.

Microalgae, primarily diatoms, from the Kachecmak Bay area and ice edge in the Bering Sea north of the Pribolof Islands have been collected and isolated into pure culture. The isolates from Kachecmak Bay have the capacity to oxidize napthalene. The principal product was 1-naphthol. The results suggest that cold water adapted diatoms can oxidize naphthalene and they reinforce the view that the capacity for oxidation of aromatic compounds is a general feature in the microalgae. Algal aromatic oxidation may be an important pathway for the degradation of spilled oil, especially in the upper water column (where bacterial activity is less than in sediments) and at the ice-edge where intense phytoplankton blooms can occur). Secondly, the consequences of this oxidation may be serious if mammalian-type reactive oxidation products are formed and persist for a sufficient time to impact higher trophic levels.

II. Results.

The background information, methods and materials, and data are detailed in the enclosed manuscript which will shortly be submitted for publication.

III. Current Work.

Pure cultures of diatoms are being isolated from the enrichment cultures developed from samples collected at the ice edge in the Bering Sea. Several of these cultures will be examined for their capacity to oxidize naphthalene to 1-naphthol and if possible, for rate of $^{14}CO_2$ release from labeled naphthalene. The nature of the primary attack on the naphthalene ring is of major importance, and the "NIH" shift and $^{18}O_2$ incorporation into naphthalene oxidation products are also under investigation in several microalgae.

IV. Summary of Shipboard Collecting, January 1 to March 31, 1981.

Water samples were collected by C. Van Baalen from the ice edge in the Bering Sea north of the Pribolof Islands during cruise of the NOAA Ship SURVEYOR S132, 22 February through March 14, 1981. Phytoplankton were sampled from the SURVEYOR salt water intake system and from ice cores taken by S. Martin from 22 February to 14 March. The purpose of the sampling program was to obtain pure cultures for physiological studies of representative species ednemic to the marginal ice zone. The table below describes the samples taken. Enrichment cultures of the water samples were made using sterile technique. The volume of the samples varied from 0.2 to 2 ml depending on the evident phytoplankton concentrations at 100x. These samples were added to filtered, enriched local seawater. The enrichment medium provided inter alia, nitrate, phosphate, iron EDTA, and vitamins B_{12}, B_1 and biotin. Culture tubes were incubated in two ways; 1) in an icebox (5-10°C) at 12 to 15 cm above a 40w tungsten blub and; 2) in the ship-board flowing sea water sink (0 to -2°C) with illumination from overhead fluorescent lamps. In both methods the illumination was continuous. Representative subsamples were also preserved with neutral formalin for later comparison with the results of the enrichment cultures.

Water and Ice Samples Collected, Leg II.

GMT	Date	Latitude	Longitude	Type
1800	Feb. 23	55°44.4'	157°24.1'	Shipboard pumped sea water filtered through 10 μm Nitex nylon net
1900	Feb. 24	55°4.3'	162°11.1'	Pumped sea water and 10 μm net
1830	Feb. 25	55°57.5'	168°45.3'	Pumped sea water and 10 μm net
1730	Feb. 26	58°7.5'	173°17.4'	Two ice cones, courtesy of Seelye Martin, bottom 3-4 cm thawed and filtered through 0.2 μm sterile filter
1830	Feb. 26	58°13.4'	173°8.6'	Pumped sea water and 10 μm net
1745	Feb. 27	58°26.6'	172°51.2'	Piece of "brown ice" thawed in 10 μm net
1930	Feb. 27	58°29.0'	172°37.1'	Pumped sea water and 10 μm net
1900	Feb. 28	58°07.6'	173°17.8'	Pumped sea water and 10 μm net
1830	Mar. 1	58°39.9'	172°19.6'	Pumped sea water and 10 μm net
1800	Mar. 3	59°15.5'	171°12.0'	"Brown ice" thawed in 10 μm net
1800	Mar. 4	59°18.2'	171°29.2'	Pumped sea water and 10 μm net
0400	Mar. 5	59°17.0'	171°41.8'	Surface tow, 10 μm net in clearings around ice
1900	Mar. 5	59°08.8'	171°55.0'	Pumped sea water and 10 μm net
2300	Mar. 5	59°07.7'	171°50.4'	Pumped sea water 10 μm net
1830	Mar. 7	58°43.0'	172°15.4'	Pumped sea water 10 μm net
1930	Mar. 8	58°46.3'	172°51.7'	Pumped sea water 10 μm net
1900	Mar. 9	58°32.9'	173°29.4'	Pumped sea water 10 μm net

AROMATIC HYDROCARBON OXIDATION BY DIATOMS ISOLATED FROM THE KACHEMAK BAY REGION OF ALASKA

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Three pure cultures of diatoms, a Navicula sp., a Nitzschia sp. and a Synedra sp. grown in the presence of naphthalene at $6^{o}C$ or $12^{o}C$ oxidized naphthalene to ethyl-acetate soluble and water-soluble metabolites. The major ethyl acetate soluble metabolite was identified as 1-naphthol by gas chromatographic and mass spectral analysis. Experiments with [^{14}C] naphthalene indicated that the extent of naphthalene metabolism ranged from 0.7 to 1.2%.

INTRODUCTION

Aromatic hydrocarbons have been found to be widely distributed in open ocean waters (Brown and Huffman, 1976). Many of these compounds and/or their metabolites have toxic properties which include the initiation of tumor formation and cancer (Miller and Miller, 1976). In studies of the fate of hydrocarbons in aquatic ecosystems, a considerable amount of information is available on the bacterial and fungal degradation of these compounds and their derivatives (Atlas, 1981; Cerniglia, 1981). In view of the fact that cyanobacteria and microalgae are widely distributed in many aquatic environments and may be important in the catabolism of hydrocarbons, we initiated a research program on the algal oxidation of aromatic hydrocarbons (Cerniglia et al. 1979, 1980 a,b,c,).

Most of the studies on the microbial oxidation of hydrocarbons have been conducted at temperatures between 20°C to 30°C. Since there has been increased activities of oil exploration and transport of petroleum in Alaskan waters, there has been recent interest in the microbia degradation of crude oil at low water temperatures (Atlas, 1981).

In this investigation, we report on three diatoms isolated from the Kachemak Bay region of Alaska which have the ability to metabolize the aromatic hydrocarbon, nephthalene at low temperatures.

METHODS

Organisms and Growth Conditions. The diatoms K1A (Navicula sp.), K8A (Nitzschia sp.) and 4D (Synedra sp.) were isolated via enrichment culture at 6 to 10°C from oblique net (20 µm Nitex nylon) tows made during August 1979 and April, 1980 in the Kachemak Bay Region, south of Homer, Alaska. The enrichment medium was local sea water plus 5, 20, or 50% ASP-2 medium (Van Baalen, 1962). Pure cultures were obtained by repeated streaking or by several minutes treatment with ultraviolet radiation (254 nm, 15W germicidal lamp) and subsequent pour plates. Organism N-1 (Cylindrotheca sp.) was isolated from a water sample taken from the Pass adjacent to the Port Aransas Marine Laboratory (Estep et al., 1978). The organisms were grown on ASP-2 medium containing 125 mg l^{-1} $Na_2 SiO_3.9H_2O$, 4 µg l^{-1} vitamin B_{12} and 250 µg l^{-1} thiamine in 22 x 175 mm Pyrex test tubes at 12°C. The growth tubes were illuminated with fluorescent lamps F2oT12-WWX two on each side of the water bath, 8 cm from the front edge of the lamp to the tube center. The cultures were continuously aerated with 1 ± 0.1% CO_2 enriched air. The generation times under these conditions for the four organisms were about 24 hours.

Naphthalene Biotransformation Experiments. [1-^{14}C]-Naphthalene experiments were conducted in order to determine the amount of naphthalene oxidized by each organism. Cells (0.5 to 0.8 mg) were pooled from several growth tubes and placed in 22 x 175 mm screw cap tubes, final volume 10 ml. [^{14}C]-Naphthalene (1 uCi in 20 ul ethanol, 6.9 mg/liter) was added just before closing the tube with a plastic top lined with a chromatography septum, aluminum foil and 1 ml Teflon film. Carbon dioxide was added through a

small hole in the plastic top with a gas tight syringe to an initial concentration of 1%. The screw cap tubes were clamped to a glass rod and rotated slowly in the same illuminated water bath used for growing the cultures. The tubes were incubated at either 6°C or 12°C.

After 22 hr incubation, cells were removed by centrifugation and each supernatant extracted with five thirty ml volumes of ethyl acetate. The organic extracts were dried over anhydrous sodium sulfate and the solvent was removed in vacuo at 42°C. Each residue was redissolved in methanol and analyzed by high pressure liquid chromatography. The ratio of ethyl acetate soluble metabolites to water soluble metabolites was determined by taking each organic soluble extract and redissolving in 50 ul of acetone and 10 ul aliquots was added to vials containing 10.0 ml of scintillation fluid. The radioactivity present was determined in a liquid scintillation counter. Corrections were made for machine efficiency and quenching.

An experiment with unlabeled naphthalene was conducted with organism K8A in order to obtain sufficient material for the isolation and structure elucidation of the naphthalene biotransformation products. Four 10 ml samples of organism K8A were incubated in screw cap tubes as described above with 6.9 mg/liter naphthalene at 12°C. After 22 hr the cells were centrifuged and the supernatant was extracted and concentrated as described above. The residue was redissolved in methanol and analyzed by gas-chromatography and mass spectrometry.

Analysis of Metabolic Products. High pressure liquid chromatography (hplc) was used for the separation of metabolies. All hplc analysis were performed on a Beckman Model 332 hplc and Model 155-10 variable wavelength absorbance

detector (Beckman Instruments, Inc., Berkeley, CA, USA) operated at 254 nm. An Altex Ultrasphere-ODS Column (25 cm x 4.6 mm id) [Altex Scientific, Inc., Berkely, CA, USA] was used for the separation of naphthalene metabolites, which was achieved with a programmed methanol/water gradient (50 to 95%, v/v, 30 min.) with a flow rate of 1 ml/min. In experiments with [^{14}C]-naphthalene, 0.5 ml fractions were collected at 0.5 min. intervals in scintillation vials and 5.0 ml of Aquasol-2 (New England Nuclear Corp., Boston, MA, USA) was added to each vial. The radioactivity present in each fraction was determined in a Beckman LS-250 liquid scintillation counter.

Gas chromatographic and mass spectral analysis (GC-MS) of naphthalene metabolites was performed on a Finnigan Model 3100 mass spectrometer coupled to a gas chromatograph equipped with a glass column (2 m x 1.5 mm id) packed with 3% OV-1 on Chromosorb Q. The injection temperature was 50^{o}C with a temperature program of 100-250^{o}C at 8^{o}C/min. The carrier gas was helium, with a flow rate of 30 ml/min. The following conditions were used for mass spectrometry: molecular separator temperature 350^{o}C; ion source temperature 100^{o}C ionization beam 70 eV; and ionization current 200 uA.

Chemicals: Naphthalene (99.9%) was from Aldrich Chemical Co., Milwaukee, Wis., USA. [1(4,5,8)-^{14}C]-Naphthalene [5 mCi/mmo l] was from Amersham Searle, Arlington Heights, Il., USA. All naphthalene derivatives were purified as described previously (Cerniglia and Gibson, 1977). Solvents for hplc were purchased from Burdick and Jackson Laboratories, Muskegon, Mich., USA.

RESULTS AND DISCUSSION

Three pure cultures of diatoms isolated from Alaskan waters (strains K8A, 4D and K1A) were incubated with [^{14}C]-naphthalene at either 6^{o}C or 12^{o}C. The hplc elution profile of the ethyl-acetate soluble naphthalene metabolites formed by each diatom is shown in Fig. 1. For comparative purposes the chromatographic properties of synthetic naphthalene derivatives is shown in Fig. 1A. All of the organisms oxidized naphthalene to a compound which co-chromatographed with 1-naphthol. These results are similar to our earlier studies on the oxidation of naphthalene by cyanobacteria and microalgae (Cerniglai *et al.*, 1980b).

In order to confirm that 1-naphthol was the major metabolite in the oxidation of naphthalene, cells of *Nitzschia sp.* strain K8A were incubated for 22 hr. in the presence of naphthalene and the ethyl acetate soluble extract analyzed by GC-MS. The GC-MS analysis of the ethyl acetate extract of the metabolism of naphthalene by *Nitzschia sp.* strain K8a showed a compound that had a similar retention time (9.5 min.) and mass spectrum (m/e 144) to that of authentic 1-naphthol.

Table 1 shows that these diatoms oxidized naphthalene to both organic soluble and water soluble derivatives. The amount of naphthalene oxidized ranged from 0.7 to 1.2%. It is also interesting to note that *Cylindrotheca sp.* strain N-1 when grown at 12^{o}C, wherein it had a similar rate as organism 4D gave less total naphthalene oxidation (Table 1). This data suggests that cold-water adapted microalgae may prove to be more metabolically active than is implied by their slow growth rates.

In an earlier study we showed that the cyanobacterium *Oscillatoria sp.* strain JCM oxidized 4.8% of the added naphthalene. The ratio of ethyl

acetate soluble metabolites to water-soluble metabolites was 41:59. Table 1 shows that all of the diatoms formed water-soluble products. The identification of these products remains to be determined but these results suggest that diatoms may have the ability to oxidize naphthalene to ring cleavage or conjugated products.

The results herein extend the original observations on the oxidation of naphthalene by temperate forms (Cerniglia et al., 1980b) to cold-adapted diatoms and reinforce the view that the capacity for oxidation of aromatic compounds is a general metabolic feature in the microalgae. Algal rates of aromatic oxidation versus rates for the aerobic heterotrophic microbial populations in the photic zone are unknown. However, the photic zone in the sea may prove to be a major sink for transformations of aromatic compounds in nature. Whether this will increase or decrease their toxicity for zooplankton and higher trophic levels is unknown.

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FIGURES

Figure 1. Hplc elution profile of metabolites formed from [^{14}C]-naphthalene by different diatoms. A, resolution of a mixture of synthetic naphthalene derivatives. B, *Nitzschia sp.* strain K8A; C, *Synedra sp.* strain 4D; D, *Navicula sp.* strain K1A.
Hplc conditions were as described in Methods.

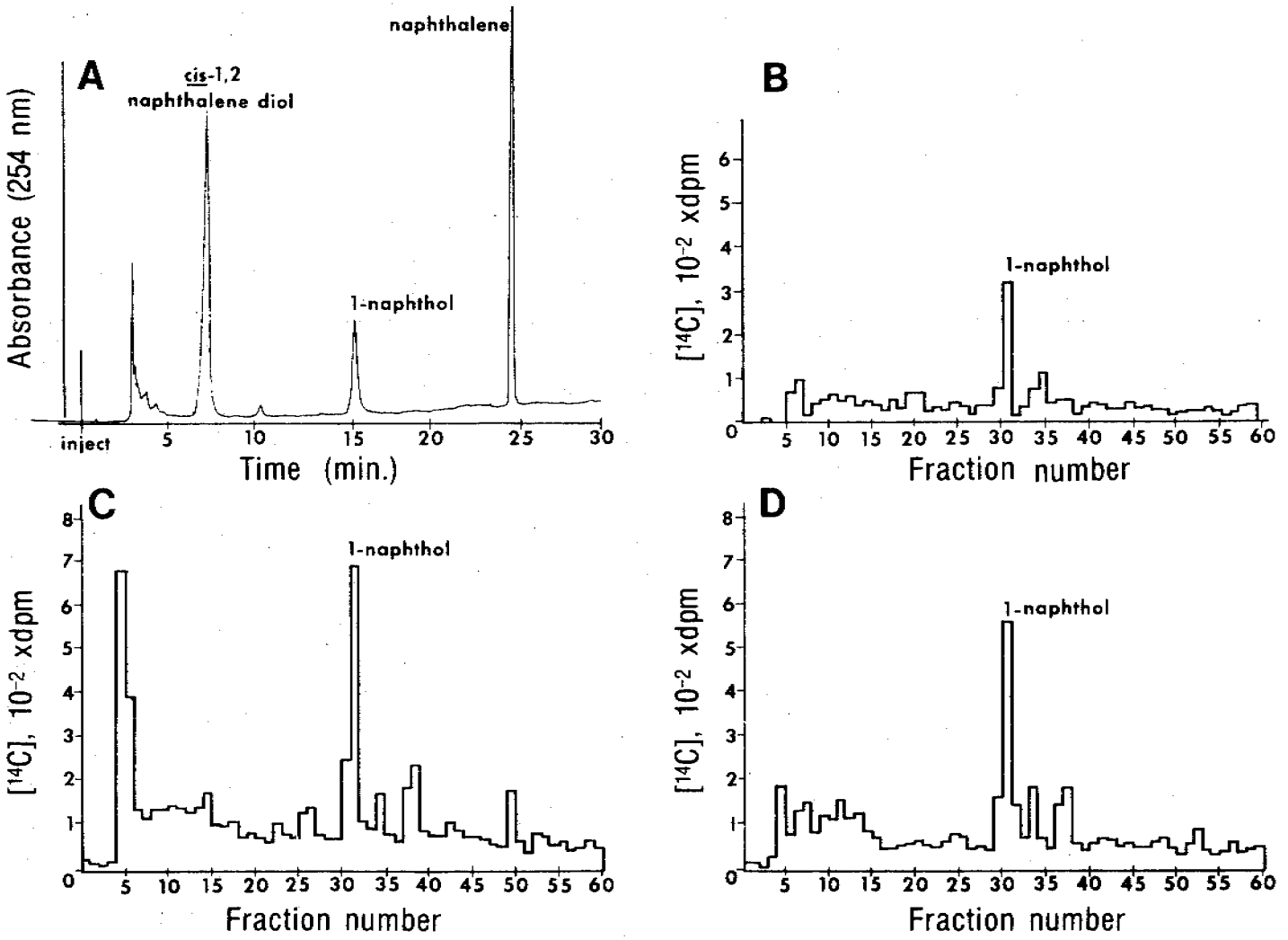
A
cis-1,2
naphthalene diol
naphthalene
1-naphthol
Absorbance (254 nm)
inject
5
10
15
20
25
30
Time (min.)
B
1-naphthol
[14C], 10-2 xdpm
Fraction number
C
1-naphthol
[14C], 10-2 xdpm
Fraction number
D
1-naphthol
[14C], 10-2 xdpm
Fraction number

Table 1. Distribution of Radioactivity in the Ethyl Acetate Soluble and Water-Soluble Metabolites Formed from [^{14}C]-Naphthalene by Diatoms.

Organism	d.p.m. mg dry wt^{-1}			Percentage Metabolism of Naphthalene
	Organic-Soluble	Water-Soluble	Total Radioactivity	
Nitzschia sp. strain K8A	8,965 (49)*	9,311 (51)	18,276	0.7
Synedra sp. strain 4D	18,044 (58)	13,332 (42)	31,376	1.2
Navicula sp. strain K1A	9,658 (55)	7,987 (45)	17,645	0.7
Cylindrotheca sp. strain N-1	6,550 (43)	8,784 (57)	15,334	0.6

* percent of total metabolites

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