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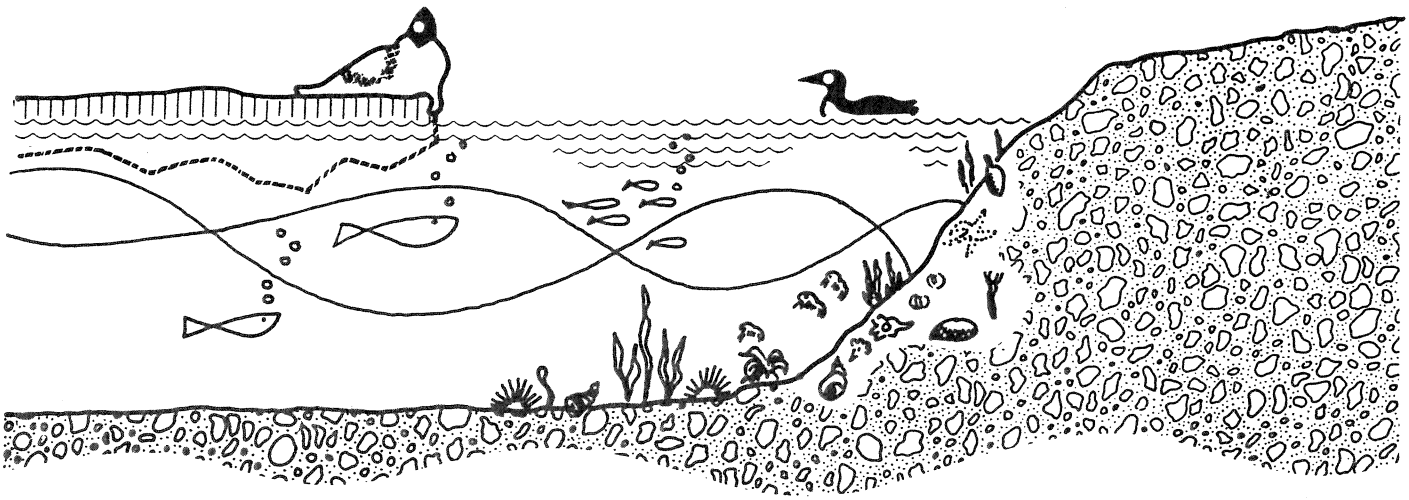
MICROBIOLOGY

2. Biodegradation of Oil



Baffin Island Oil Spill Project

WORKING REPORT SERIES

BIOS CAPE HATT

1981 STUDY RESULTS

The Baffin Island Oil Spill Project

OBJECTIVES

The Baffin Island Oil Spill (BIOS) Project is a program of research into arctic marine oil spill countermeasures. It consists of two main experiments or studies. The first of these, referred to as the Nearshore Study, was designed to determine if the use of dispersants in the nearshore environment would decrease or increase the impact of spilled oil. The second of the two experiments in the BIOS Project is referred to as the Shoreline Study. It was designed to determine the relative effectiveness of shoreline cleanup countermeasures on arctic beaches.

The project was designed to be four years in length and commenced in 1980.

FUNDING

The BIOS Project is funded and supported by the Canadian Government (Environment Canada; Canadian Coast Guard; Indian and Northern Affairs; Energy, Mines & Resources; and Fisheries & Oceans), by the U.S. Government (Outer Continental Shelf Environmental Assessment Program and U.S. Coast Guard), by the Norwegian Government and by the Petroleum Industry (Canadian Offshore Oil Spill Research Association; BP International [London] and Petro-Canada).

WORKING REPORT SERIES

This report is the result of work performed under the Baffin Island Oil Spill Project. It is undergoing a limited distribution prior to Project completion in order to transfer the information to people working in related research. The report has not undergone rigorous technical review by the BIOS management or technical committees and does not necessarily reflect the views or policies of these groups.

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BAFFIN ISLAND OIL SPILL PROJECT

MICROBIAL DEGRADATION OF OIL

MEASUREMENTS IN RAGGED CHANNEL TEST BAYS AND BAY 102, CAPE HATT 1981.

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SUMMARY

Baseline assessment of water and sediments in the BIOS Project test bays of Ragged Channel, Baffin Island, during August 3-17 1981 indicated same level of total viable heterotrophic bacteria (TVH), somewhat lower levels of oil degrading bacteria (ODB) compared to 1980 study. Bay 7 (new control bay) and Bay 11 had significantly higher levels of ODB in the surface water than Bay 9 and 10.

During the 3 to 4 weeks of post spill sampling no drastic changes in the population of TVH and ODB could be recorded. The thin surface sheen in Bay 11 seemed to affect TVH in the surface water (one order of magnitude reduction), normal level was reestablished 2 weeks after the spill. In the same period significant increases of ODB were registered in surface water (about 5x) and 5 m water (about 3x) over and above the levels in corresponding samples from the control bay.

Apart from a possible small enhancement in the count of ODB at 5 m in Bay 9 no effect of dispersed oil on the microbial populations in the water column could be detected.

The bacterial populations in the sediment samples were very similar to last year, and no effects of the oil spill could be found.

Based on experiments using tritiated Lago Medio weathered crude oil the rate of oil mineralization was assessed in samples of sediments and in water from 5 m depth. In early August the average mineralizing activity in the water of the bays rapidly reached a plateau level of about V_{10} = 35 $\mu g \cdot m^{-3} \cdot d^{-1}$ (V_{10} = rate determined in water at equilibrium with 1 ppm of oil), which unexpectedly dropped to half this value just prior to the first oil spill (surface spill). During the first two weeks of September the activity again rose to 18-25 $\mu g \cdot m^{-3} \cdot d^{-1}$ in all bays except Bay 11. No definite explanation for the latter can be given.

The mineralizing activity in the sediment ranged from 15-39 $\mu g \cdot L^{-1} \cdot d^{-1}$.

All water and sediment samples analyzed could mineralize ^{3}H-Lago Medio oil as well as *n*-(1-^{14}C)-hexadecane. The average V_{10}-rates for *n*-hexadecane mineralization for the various bays ranged from 9.5-43.8 $\mu g \cdot m^{-3} \cdot d^{-1}$ for water (highest rates in early August), from 4 to 114 $\mu g \cdot L^{-1} \cdot d^{-1}$ for the sediment samples. Only a minor percentage of the samples exhibited ability to mineralize ^{14}C-naphthalene (28%) and benz(a)pyrene (10%).

The supralittoral test plots oiled in 1980 still had enhanced levels of TVH, ODB and respiratory carbon dioxide production compared to unoiled plots. The crude oil plot had less remaining oil than the oil-emulsion plot.

6 new test plots with oil emulsion were prepared in 1981. Compared to last year the application of oil to the sand caused no stagnation in the respiratory carbon dioxide production which rose to 30 ±7 $mgC \cdot m^{-2} \cdot h^{-1}$ and populations of 7-13$\cdot 10^{6}$ TVH ml^{-1} and 0.7-28$\cdot 10^{4}$ ODB ml^{-1} sand were recorded. After treatment of the oiled sand with BP solidifying agent about 50% of the oil could be removed as solid sand-oil-polymer lumps, and enhanced respiratory activity ($81^{\pm 8}$ $mgC \cdot m^{-2} \cdot h^{-1}$) indicated no negative influence on the microbial

activity. The addition of a composite fertilizer (nitrate, phosphate and minerals) at low level (0.04 kg·fertilizer·m^{-2}) supported a rapid increase in bacteria (TVH about $3 \cdot 10^9$ ml^{-1}, ODB $0.7\text{-}13 \cdot 10^6$ ml^{-1}) and carbon dioxide production (69±8 mgC·m^{-2}·h^{-1}). At a higher level of fertilizer (0.4 kg·m^{-2}) carbon dioxide production increased to 156±18 mgC·m^{-2}·h^{-1} and the oil-sand housed a ODB population of $7 \cdot 10^7$ ml^{-1} after 2½ weeks. A mechanical mixing of the oil and fertilizer (high level) into the sand caused no additional effect apart from giving a dryer surface.

Unforseen high tides in late August covered the test plots with 15-30 cm sand and gravel and further sampling had to be postponed until 1982.

ACKNOWLEDGEMENTS

We thank our Canadian microbiology colleagues J. Bunch, T. Cartier, F. Dugré, R. Harland, J. Laliberté and L. Mînard for their friendly cooperation at Cape Hatt and for permission to use their carefully prepared sediment samples. The untiring smile and skill of William Werner was always very much appreciated. The personal and logistical support of the BIOS Project Office is gratefully acknowledged. We also wish to thank the Royal Ministry of Environment, Norway, for their financial support through their research programs FOH and PFO.

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1. INTRODUCTION

The Baffin Island Oil Spill Project (BIOS Project) is a study designed to evaluate the short-term and the long-term effects of spilled oil on arctic shorelines and shallow waters. The study is devided into two projects, a near shore project for comparison of effect and fate of two different types of oil spills to extract the essential consequence of using a chemical dispersant in an arctic environment, and an on shore project aimed at assessing the effect of natural physical forces on standard oil and to test practical cleaning techniques.

The Norwegian groups of microbiologists has been involved in both projects. In the near shore project we have been concerned with the microbial activity for degradation of oil in the water column and in sediments before and after the oil spills. A baseline investigation in 1980 and a prespill period in 1981 should indicate the microbial population potentially able to handle oil hydrocarbons. The main objective for the post spill study is to monitor the effect of petrogenic hydrocarbon on this population.

In the on shore study we are interested in the possibility to influence positively the biodegradation of oil which is stranded above the storm-line.

This report summarizes the results of our work in 1981. The further relation of the results from the nearshore study to environmental chemistry and oil chemistry parameters has to wait until the latter data become available.

2. MATERIALS AND METHODS

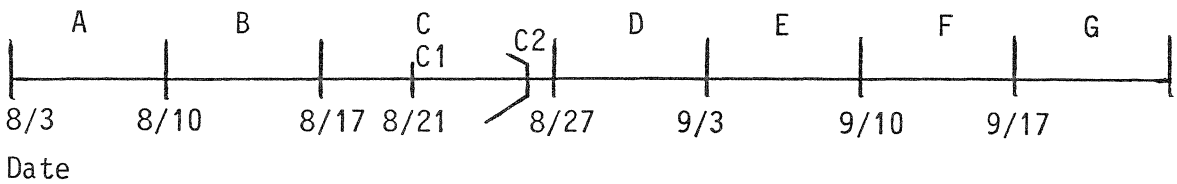
2.1. SAMPLING PROCEDURES

2.1.1 Water samples.

Water samples at 5 and 10 m were taken and handled as described previously (1). The 1 m sample used in 1980 was replaced by a surface sample (designated 0.1 m) drawn directly into 500 ml sterile screw-capped bottles.

Sampling cycle coinsided with Canadian microbiology group for cycle intervals A-G with starting dates as indicated below. In cycle interval C, Bay 11 was sampled more frequently by us; these samples are called C_1 and C_2. All 4 bays, including the new control Bay 7, were sampled throughout the cycle, from August 3 to September 17.

Cycle interval.



2.1.2 Sediment and beach sand samples.

Samples for microbiological analyses of oiled and unoiled beach sands were taken as described previously (1). In all cases the 5 ml samples were composed of at least 5 subsamples collected arbitrarily within the sampling area.

Sediment samples from the test bays were taken by divers at locations close to the markers for the microbiological stations, at approx. 12 m depth. Each sample consists of 7-8 subsamples drawn by 50 ml disposable syringes (with cut-off front part), mixed thoroughly and appropriate subsample-volumes were taken for the various analyses. The quantitative data for the sediments are calculated on a wet-volume basis.

2.2. EXPERIMENTAL AND ANALYTICAL METHODS

2.2.1 Radioactive materials.

The generally tritiated Lago Medio weathered crude oil (^{3}H-Lago Medio), same preparation as described in (1), had a specific activity of approx. 1 m Ci/mg and was used undiluted.

1-^{14}C-*n* hexadecane (sp. activ. 235 µCi/mg), [1(4,5,8)-^{14}C] -naphthalene (sp. activ. 40 µC/mg) and [7,10-^{14}C]-benz(a)pyrene (sp. activ. 86 µCi/mg) were purchased from the Radiochemical Centre, Amersham, England. The radiochemicals were used undiluted in mineralization experiments. The ampule of naphthalene was sterilized by flaming and crushed in sterile 3% sodium chloride solution to make a solution containing 10 mg/L naphthalene. ^{14}C-*n*-hexadecane and benz(a)pyrene solution were used as delivered by supplyer.

2.2.2 Hydrocarbon mineralization experiments.

Water from 5 m depth or sediments were used for assessment of rate of mineralization of hydrocarbons by the methods described in (1). Following modifications were introduced for work in 1981:

For measurement of mineralization of ^{3}H-Lago Medio the caps of the incubation tubes had a teflon instead of an aluminum liner. The experiments with ^{14}C-hydrocarbons were carried out in 120 mm Hungate tubes with a butane rubber septum in the screwcaps.

For single concentration rate measurements 10 µg of hydrocarbon substrate was added to each tube, naphthalene as an appropriate volume of the ^{14}C-naphthalene solution in water. ^{14}C-*n*-hexadecane, ^{14}C-benz(a)pyrene and ^{3}H-Lago Medio as 5 mm cellulose pads containing the radiochemicals.

For determination of V_{max} identical samples prepared from water or sediments were exposed to 1, 2, 5, 10 and 20 µg ^{3}H-Lago Medio in separate tubes.

Blanks for analytical background controls and assessment of the spontaneous release of $^{3}H_2O$ from ^{3}H-Lago Medio contained 10 ml sterile seawater with 50 ppm Hg^{++}, sterile poly-carbonate filter and 10 µg of the ^{14}C-hydrocarbons and 1, 2, 5, 10 or 20 µg ^{3}H-Lago Medio.

The tubes were incubated in the special water bath (1) maintained at 3°C. After 3, 6, 10 and 15 days for tubes with ^{3}H-Lago Medio, *n*-hexadecane and naphthalene, 3, 10 and 20 days for benz(a)pyrene, samples were taken for determination of mineralization products, as described in (1). From tubes containing ^{14}C-substrate 1.0 ml samples were drawn through the membrane seal using a 1 ml sterile disposable syringe. The tubes containing ^{3}H-Lago Medio were opened aseptically to draw samples in the normal way.

The linear rate of accumulation of radioactive mineralization products obtained in experiments with 10 µg substrate per tube was used to calculate a rate of mineralization, called V_{10} = µg/m^3, d for water and V_{10} = µg/L, d for sediments. The kinetic maximal rate of mineralization, V_{max}, were calculated as previously described (1) and expressed in the same units.

For the given specific activity of the radioactive hydrocarbon substrates the limit of detection corresponds to V_{10} = 0.5 µg/m^3, d for benz(a)pyrene and V_{10} = 1.0 µg/m^3, d for ^{3}H-Lago Medio, ^{14}C-*n*-hexadecane and naphthalene for water samples and the same numerical value per liter for the sediments.

2.2.3 Microbiological analyses.

Water, sediments and beach-sand samples were analyzed for total viable heterotrophic bacteria (TVH) and oil degrading bacteria (ODB) by the most probable number techniques (MPN) described in (1).

2.2.4 Analyses connected to onshore experimental plots.

Respiratory carbon dioxide measurement and assessment of detritovore invertebrate population were performed as described in (1).

2.2.4.1 Sampling of oiled beach sand.

Two types of samples were taken at 0-5 cm and 5-10 cm. Each sample of approx. 1L were composed of 25 subsamples arbitrarily collected over the total plot-area. After thorough mixing subsamples were taken for analyses.

2.2.4.2 Oil analysis.

Total oil in beach sand was determined gravimetrically by CCl_4 extraction. Final given value is based on analyses of 5 parallel subsamples from the same mixed sample.

2.2.4.3 Total nitrogen analysis.

Total nitrogen (including nitrate) in beach sand sample was analyzed according to the Kjeldahl Jodlaubers method (2).

2.2.4.4 Experimental plots, preparations.

Plot 102A-C were established in Bay 102 August 23 1980, two of which were sprayed with crude Lago Medio oil (102B), respectively 50:50 emulsion of crude Lago Medio oil and seawater (102A).

August 1 1981 5 new plots, 102D-H, each 4x5 m were prepared according to the table below, all sprayed with 50:50 Lago Medio crude-seawater emulsion. One plot, 102D, was further divided into 3 unequal parts, $102D_0$, D_1 and D_2. The method of oil deposit is described in Woodward-Clyde Consultants' BIOS-report for 1981 (3). The fertilizer, Norsk Hydro, Fullgjødsel C, was evenly spread over the fertilized plots by a lawn fertilizer sprayer. The product declaration reads: 16% N, 6.6% P, 11.9% K, 1.2% Mg, 1.6% S, 3.0% Ca and 0.02% B. The solidifying agent used in plot $102D_1$ and $102D_2$ was applied to the beach sand by an expert from British Petroleum, England (4). Zygol, a commercial pinebark product, was used as oil-sorbent in $102D_0$ in the oil-sorbent volume ratio of 1:1. After addition of fertilizer to plot 102E the oil was mixed into the top 15-20 cm of beach-sand by running a garden tiller twice of the plot area.

The experimental plots were prepared in this way:

Exp. plot	Type and amount of oil	Treatment	Starting date
102A	$20L \cdot m^{-2}$ emulsion	none	Aug. 23 1980
102B	$10L \cdot m^{-2}$ crude	none	"
102C	none, control	none	"
$102D_0$	$20L \cdot m^{-2}$ emulsion	oil sorbent	Aug. 1 1981
$102D_1$	"	BP solidifying agent added Aug. 4	"
$102D_2$	"	BP solidifying agent added Aug. 4; lumps manually removed after 4 days	"
102E	"	$0.4kg \cdot m^{-2}$ fertilizer and oil-fertilizer mixed by tilling	"
102F	"	none, control 1981	"
102G	"	$0.04kg \cdot m^{-2}$ fertilizer	"
102H	"	$0.4kg \cdot m^{-2}$ fertilizer	"

2.2.4.5 Special information.

The designation of plots A and C have been reversed since 1980, to obtain a sequence corresponding to the field situation.

3. RESULTS AND COMMENTS

3.1. NEAR SHORE PROJECTS

Our intensions for the near shore shork in 1981 included a prespill baseline study for at least 2 cycle intervals starting August 3 (cycle intervals A and B), continued observations until the two oil spills were carried out (cycle interval C), and an extension of the post spill observations as far as possible into September. Due to time needed for preparations for the dispersed oil spill the cycle interval C was somewhat lengthened.
This period was to some extent utilized for a closer look at possible effects of the surface oil spill taken place August 19 in Bay 11. The dispersed oil spill was very successfully performed August 27 at Bay 9. This initiated our sampling cycle at interval D. Thanks to maintaining the Cape Hatt camp open until September 23, we were able to complete 4 post spill sampling cycle intervals, with the last samples taken September 17.

The second control bay (Bay 7) was selected early in August and sampled throughout the cycle A-G.

3.1.1 Microbiological investigations.

3.1.1.1 Water column.

The data for total viable heterotrophic bacteria and oil degrading bacteria in the water column for 2 stations, 2 in each bay, and at 3 depths, 0.1m, 5 m and 10 m are given in Table 1. These data are summarized in two ways. Fig. 1, 3, 5 and 7 present the average values of the cell-counts for the 6 water samples (2 stations and 3 depths) collected in each bay during each cycle interval and indicate the general development with time of the two bacterial groups. In Fig. 2, 4, 6 and 8 the development at each depth in each bay are given as the average of the values for the two sampling stations.

Fig. 11 summarizes all the microbiological data for all 4 bays, comprising 19-24 single analytical figures for each interval. It is quite apparent that no drastic changes in the population of general heterotrophs or oil degrading bacteria in the water bodies after the oil spills can be detected. Such an effect was probably not expected.

In general the population of total viable heterotrophic bacteria was increasing over the first couple of weeks in August to reach a maximum over the last two weeks of August. A marked decline was observed in September. This pattern was fairly uniform for all bays, except the increase in population seemed to have occurred earlier in Bay 10 and 11 (Fig. 5 and 7) than in the other two bays. The maximum population variation in the bays ranged, evaluated by the average values, from $2\text{-}5 \cdot 10^6$ cells L^{-1} and in late September the population was lowered to about one tenth of that value.

Variations in general heterotrophs with depth were not very pronounced in Bay 7 (Fig. 2) and in Bay 10 (Fig. 6). The surface water of Bay 10 in the cycle interval C had the highest count recorded during the season, $7.2 \cdot 10^7$ cells L^{-1}, but could only be found in the water from one station, and may be an error. Except for fairly low counts for general heterotrophs at 5 and 10 m in cycle interval C Bay 9 (Fig. 4) also had fairly uniform populations

at all depths. The situation in Bay 11 seemed to be more fluctual (Fig. 8) with greater than "normal" changes within each depth. In the surface water a rather sudden drop in the total viable heterotrophs was observed 2 days after the surface oil spill and the expected population level was only regained a week or more later. The same low figure was recorded at both stations. During the first days after the spill the surface water of Bay 11 had a sheen from oil continuously being drained from the test area in the tidal zone. A direct connection between these two phenomena cannot be excluded.

At the same time an increase in the population of oil degrading bacteria in the same surface water was observed (Fig. 8), but only in the water samples from one of the stations (H_2), a fact which considerably lowers the significance of the finding. The population of oil degrading bacteria in the surface water of Bay 11 as well as at 5 m did, however, steadily increase over the next 2-3 weeks after the surface oil spill and which may be a direct implication of the continuous release of oil from the beach.

The microbial situation in the surface water of Bay 11 is more closely compared to the control bay in Fig. 9. Both bays exhibited rather similar sizes and developments for both populations of bacteria, except for the "dip" in total viable heterotrophs and the gradual increase in the oil degrading bacteria in the post spill period in Bay 11. This lends support to the contension that both effects may be directly caused by the oil spill.

The surface water of Bay 7 and Bay 11 (Fig. 2 and 8) had the highest populations of oil degrading bacteria of all bays and significantly higher than at the lower depths in the same bays. In bay 9 and 10 the surface population were equal or lower than the population at 5 m (Fig. 4 and 6). There may be physical oceanographic reasons for this. The 10 m population of oil degrading bacteria were in general the lowest.

In Bay 9 (Fig. 4) the population of oil degrading bacteria appeared to increase both at 5 m and in the surface water in the period after the dispersed oil spill, the largest population found in the 5 m samples. The high figure for surface water in cycle interval G is very doubtful due to exceptionally great difference between the values from the two stations. A similar but smaller increase in oil degrading bacteria was observed in the surface water of Bay 10 (Fig. 6). These three observations are the only ones that may possibly be linked to any positive effects of dispersed oil on the bacterial populations investigated.

As found last year the population of oil degrading bacteria in general tended to increase or maintain its level towards the end of the summer. Since the total viable heterotrophs continuously level off in September the ratio between the two groups of bacteria changes quite markedly. In this connection one shall bear in mind that the analytical figure named oil degrading bacteria is derived from a method which is based on a physiological potential for degradation of oil. The induction of this potential may be different from petrogenic hydrocarbons.

3.1.1.2 Sediments.

For cycle intervals A, B, C and D only one sediment sample from each bay was analyzed. In the sampling interval E and F samples from all stations were analyzed. The mixed homogeneous sediment sample was prepared by the Canadian microbiology group of dr. J. Bunch and made accessable to us, for which we are very grateful.

The data for all samples are given in Table 2. The number of samples were few for each bay. The average values of total viable heterotrophs and oil degrading bacteria for all samples collected in each bay are presented in Table 4, showing average populations of general heterotrophs varying between $3.4 \cdot 10^6$ and $9.5 \cdot 10^6$ cells$\cdot$ml^{-1} with fairly constant population sizes of oil degrading bacteria of $1\text{-}2 \cdot 10^5$ cells$\cdot$ml^{-1}. This compares well to the figures found in 1980 (1). 1.7 to 1.5% of the total viable heterotrophs analyzed as oil degraders, with an average of 1.6%. Judged by the average values for the samples analyzed from each sampling cycle interval there appears to be a slight increase in both groups of bacteria over the season, but the data do not indicate any changes induced by the oil spill.

3.1.2 Mineralization of Lago Medio weathered crude oil and defined hydrocarbons.

3.1.2.1 Mineralization of tritiated Lago Medio weathered crude oil.

As in 1980 the water from 5 m depth was used for the biochemical analyses to determine the rate of mineralization of oil and hydrocarbons. Mineralization of oil was assessed by formation of ^3H_2O from tritiated Lago Medio weathered crude oil. With corrections for spontaneous formation of ^3H_2O and the use of preparation with high specific activity rates of mineralization as low as 1 µg/m^3,d may be assessed. In some cases the measurements were carried out to permit calculation of the kinetically defined maximal rate of mineralization V_{max}. In most cases, however, the rate assessments were, for logistical reasons, made from experiments with one and the same substrate concentration. The calculated rate is named V_{10} to indicate that the measurement was carried out in a system containing 10 µg of oil or hydrocarbon substrate. Both V_{max} and V_{10} are given the unit µg$\cdot$m$^{-3}\cdot$d^{-1} for water and µg$\cdot$L$^{-1}\cdot$d^{-1} for sediment.

All data for watersamples and sediment samples are given in Table 3.

The V_{max}-values for water varied from 108 to 11 µg$\cdot$m$^{-3}\cdot$d^{-1}, the highest values were generally found early in August or late in September, the lower values in the intermediate period. The data are too limited and somewhat inconsistent for any trend assessment.

The V_{10}-values for all samples analyzed from the 4 bays are summarized according to sampling time in Fig. 12. From an average rate of about 17 µg$\cdot$m$^{-3}\cdot$d^{-1} in early August the rate rapidly doubled to give a plateau activity of about 35 µg$\cdot$m$^{-3}\cdot$d^{-1} lasting from August 10 to August 18. The capacity to mineralize the tritiated oil then fell rapidly to a level varying between 15 and 20 µg$\cdot$m$^{-3}\cdot$d^{-1}. Towards the end of the sampling cycle, E to F, the rate again rose.

The first phase of development were evident in all four bays (Fig. 13), and the drop in biological activity clearly had nothing to do with the oil spills. We cannot offer any explanation. Perhaps this will be possible when the results of the environmental chemistry parameters become available. The enhancement in activity early August may be associated with the increase in the total population of bacteria.

In the period after the oil spills a clear difference in the development was evident. In Bay 7, 9 and 10 the mineralizing activity increased in September, whereas in Bay 11 the activity decreased drastically. The latter may possibly be negatively linked to contamination of the water by oil; substancial amounts of oil would affect the specific activity of the tritiated substrate used in the analysis and reduce sensitivity. No visual evidence for any such contamination was observed. For Bay 9 the population of oil degrading bacteria at 5 m depth increased in the same cycle period, but for the other bays no correlation is apparent between the observed rates of mineralization and the quantitative microbiological data.

3.1.2.2 Mineralization of ^{14}C-hydrocarbons.

A substantial number of the water and the sediment samples were used in experiments to assess the activity to metabolize ^{14}C-*n*-hexadecane, naphthalene and benz(a)pyrene. The data are given in Table 3 and summarized in Table 6 (*n*-hexadecane) and Table 7 (naphthalene and benz(a)pyrene).

All water samples and sediment samples tested did produce $^{14}CO_2$ from ^{14}C-*n*-hexadecane. For water the average V_{10} rate calculated varied from 11.8 to 43.8 $g \cdot m^{-3} \cdot d^{-1}$ over the cycle period A to F. For the samples within each cycle interval a considerable spread was observed in the results which make evaluation of trends very difficult. The average rates compared favourably with the V_{10}-values for mineralization of tritiated Lago Medio, but some of the individual values are substantially higher than any observed for the latter substrate.

For the sediments the average results are more uniform (except for cycle period C which gave high values and also had the largest range spreading) with V_{10} varying from 8.9 to 22.2 $g \cdot L^{-1} \cdot d^{-1}$.

Metabolism of ^{14}C-naphthalene was recorded in 11 out of 55 water samples tested and 14 of 33 sediment samples gave positive results. The water samples had maximal values about 10 times the detection limit with occasional higher values for the sediment. The percent higher incidences of positive results for sediments compared to water may be significant.

Conversion of ^{14}C-benz(a)pyrene to $^{14}CO_2$ was verifyed in only 4 out of 30 sample tests, two from each sample group. In all cases the results were only slightly above detection limit.

3.1.3 Conclusions.

The overall level of bacteria in the bays of Ragged Channel in 1981 compares fairly well with the results found in 1980; the counts of total viable heterotrophs were very much the same, whereas the counts of oil degraders were somewhat lower than the previous year. The prespill sampling in 1981 covered the first part of August immediately after the disappearance of the land fast ice. The 1980 baseline analyses were done late in summer over one week in August-September. The surface level of oil degrading bacteria was somewhat higher in Bay 7 and Bay 11 than in the other two bays, Bay 10 having on the average the lowest level of oildegraders.

After the oil spills no drastic changes in the bacterial populations could be registered, but some effects are discernible. The oil on the surface water of Bay 11 seemed to have affected negatively the level of general heterotrophic bacteria in the same water layer; after a sudden drop the normal level was only reestablished a couple of weeks later. In the next 2-3 weeks after the surface spill the level of oil degrading bacteria definitely increased in the surface water as well as at 5 m, to levels 3-5 times of those recorded for the control bay.

Apart from a possible small increase in the level of oil degrading bacteria at 5 m water of Bay 9, no direct effect of dispersed oil could be significantly detected. We have, however, no way of distinguishing between dispersed oil or surface oil as the possible cause of the enhancement of oil degrading bacteria in Bay 11.

Over the sampling period from early August to medio September the content of oil degrading bacteria and general heterotrophic bacteria in the sediment appeared to increase slightly, but no effect of spilled oil could be detected.

The microbial activity for mineralizing Lago Medio weathered crude oil in the 5 m water of Ragged Channel reached a maximum during the period August 10 to 15. The sudden drop to half this activity clearly had nothing to do with the two oil spills. At the moment we cannot offer any explanation for this decline. During the two first weeks of September the mineralizing activity again increased in Bay 7, 9 and 10. For Bay 9 this may be associated with a slightly enhanced population of oil degrading bacteria, but such a correlation is not apparent for the other two bays. The contrasting steady decrease in mineralizing activity during this period in Bay 11 is surprising and may be an artifact due to faulty analytical conditions.

All samples of water and sediments tested mineralized Lago Medio weathered crude oil as well as *n*-hexadecane, but the use of the latter substrate seems to have yielded the most consistent results.

3.2. ON SHORE PROJECTS

Two test plots were sprayed with crude Lago Medio oil and a 50:50 seawater emulsion of the same oil in 1980 in the supralittoral zone of Bay 102, a high energy beach. These plots were in good condition in 1981.

In our original plan given to BIOS-project for the Norwegian component of the on shore project we had suggested the experiments on enhanced biodegradation of oil to be carried out[1)] in the supralittoral zone of a high energy as well as a low energy beach[2)], to include two dose-levels for each of the nutrient elements nitrogen and phosphate[3)], the latter in combination with mechanical mixing and the use of sorbents. For economical reasons plans had to be restricted to 5 testplots in one test area. The high energy beach in Bay 102 was chosen. To the best knowledge of the experts of Woodward-Clyde Consultants (we gratefully acknowledge their help) the site was selected to combine a realistic scenario of oil deposited in the supralittoral zone by wave action and relative protection from flooding. The 5 testplots were overlayered by a 50:50 oil-seawater emulsion and treated according to the description given in "Materials and Methods". Two plots were used to test the effect of two dose levels of a combined nitrogen-phosphate-tracemetal fertilizer. In a third plot the fertilizer (highest level) and oil was mechanically mixed into the sand by a motorized garden tiller. One plot was kept untreated as a control. The remaining plot was devided into two parts, one covered immediately with Zygol, a natural product sorbent made of pine-bark and the other treated a few days later with the "BP solidifying agent". The latter product is intended to mix with oil and sand, polymerize and retain the oil in a solid/semisolid sand-polymer mixture.

The initial conditions of the testplots in terms of surface and subsurface oil content and content of total nitrogen are given in Table 8. All plots except 102E had an average oil content of 35 to 50 $g \cdot kg^{-1}$ sand in the upper 5 cm, and less than 10 g $oil \cdot kg^{-1}$ in the 5-10 cm layer. Plot 102E had been tilled and the results from this plot reveal a more uniform distribution of the oil in the top 10 cm of the sand.

Results from unfertilized plots $102D_0$, $102D_1$, $102D_2$ and 102F indicate relatively high levels of nitrogen, particularly in the surface layer of the sand. This may be associated with the rows of seaweed unevenly deposited in the beach area and which were removed immediately prior to the oil deposition. Increased levels of nitrogen were noticeable in the fertilized plots 102E (0.4 $kg \cdot m^{-2}$ fertilizer) and 102G (0.04 $kg \cdot m^{-2}$ fertilizer), but the nitrogen content in 102H was much lower than expected. Except for faults in the sampling we can offer no explanation for this result. Nitrate analysis was not performed.

August 29-31 a sequence of exceptionally high tides flooded the test plots and covered the oil with 10-30 cm sand and gravel. Further sampling was postponed until 1982.

3.2.1 Microbial activity in test plots.

The development of microbial populations in the different test plots are shown in Table 9 and 10.

The enhanced level of oil degrading bacteria and generally heterotrophic bacteria observed in 1980 in oiled plots 102A and 102B were maintained in 1981, and both populations increased considerably (10 to 100 times) during the period from August 1 to 17 1981.

Prior to the use of the site oil degrading bacteria could be detected in the new test area in Bay 102, but at very low levels. The rather uneven distribution of generally heterotrophic bacteria in the various plots may reflect the previous presence of seaweed. 5 days after laying the oil both groups of bacteria increased significantly in number, in relative sence oil degrading bacteria the most. The relative difference between the control (102F) and the treated plots are best judged by evaluation of the results from the samples taken August 17. The plots with increasing populations of oil degrading bacteria can be ranged in the sequence from 102F - 102D - 102G - 102E - 102H, with maximal enhancements of order of 100 times. The difference between the control plot 102F and 102D may not be significant, but the highest level of added fertilizer (0.4 $kg \cdot m^{-2}$) did sustain rather quickly substantial populations of bacteria (102H), and a significant increase may be obtained by 1/10 of this amount of fertilizer (102G). Mechanical mixing did not seem to enhance microbial growth any further (102E), but did give a none-sticky surface. For generally heterotrohps essentially the same consequence is seen, with increases 100 to 300 times over and above the control. For this group the lowest level of fertilizer seems to give an optimal result.

Unfortunately this development could not be followed any further in 1981.

3.2.2 Invertebrate fauna.

The *Collembola* are the dominating invertebrate group in this type of shoreline sediments. Even this systematic taxon is very impoverished. Only two species are common, *Archisotoma besselsi* (Pacard) and *Hydrogastrura* aff. *matura* (Mac Nam). Population assessments, based on pitfall traps, show these two species to inhabit the shoreline with fairly stable populations (Table 11).

When oil was added both aged crude oil and oil emulsion killed the populations totally. After one year (102A and 102B) the populations in these areas were not reestablished.

The only treatment that prevented total elimination of these invertebrates was the one given to plot 102E, where the oil was tilled into the sand. In this environment the population of *A. besselsi* was approximately 10% and of *H. matura* approximately 4% of the normal level.

3.2.3 Total heterotrophic respiratory carbon dioxide production.

As an index of the overall heterotrohpic respiratory activity the rate of carbon dioxide production in the various plots was measured and the results are given in Table 12. In the old oiled plots (102A and 102B) the respiratory carbon dioxide production was significantly raised compared to the rate found in the unoiled beach sand (102C), approximately a doubling was recorded. The difference observed in 1980 between the oiled plots no longer existed in July 1981; this was in line with the assessed microbial populations. The carbon dioxide evolution seemed to stay essentially unaltered over the following 4 weeks.

In the new plots the addition of oil caused no lowering of the indigenous activity as seen last year (1980 results in Table 12). This may be due to a marked difference in oil sand interaction. In contrast to last year the water-oil emulsion drained into the sand very quickly, leaving a relatively porous sand texture. In the untreated plot (102F) the activity seemed to increase slightly over the next 3 weeks. For unexplainable reasons the activity in the plot covered with Zygol decreased with time.

Low amounts of fertilizer (plot 102G) more than doubled the carbon dioxide production compared to the control, and in the plots having received the highest level of fertilizer the production was 4 to 5 times the control. The mechanical tilling did not give any additional effect on the total respiration, in line with the microbiological results.

In general the enhanced carbon dioxide production seem to correlate better to the size of the population of oil degrading bacteria than to the population of total heterotrohpic bacteria, and the results substantiated the findings of the microbial analyses. Direct evidence for the degradation of oil hydrocarbons has not been produced, but one has to assume that most likely the enhanced carbon dioxide production originates from hydrocarbons of the oil deposited in the beach sand.

The measured rates of respiratory carbon dioxide production should not be taken as exact figures for the *in situ* production of carbon dioxide in the beach sand, oiled or unoiled. The results serve primarily the purpose to compare the heterotrophic activity between the various test plots. It is obvious that the temperature inside the absorption chamber, see (1), is somewhat higher than outside the chamber. An example of the temperature-time profiles inside and outside the absorption chamber for one particular day is shown in Fig. 14A, and the average temperature profile for the surface and 3 cm depth subsurface of the beach sand in Bay 102 is shown in Fig. 14B.

The daytime temperature range in 1981 during the period when carbon dioxide production rates were recorded, varied between 5.7°C and 10°C (August 4-5) and 6.3°C and 10.7°C (August 23-24). The variation in respiration rates caused by such relatively small changes in temperature could not be singled out from other environmental factors affecting the heterotrophic respiration at the different sites. The data are, however, comparable to the rates of *in situ* respiration reported by Sparrow *et al* (4) for oiled and unoiled plots in Alaska.

The treatment of oiled beach sand with the BP solidifying agent created very quickly a non-sticky sand surface. The rate of carbon dioxide production in this plot was August 8 found to be 86±9 mg C $m^{-2}h^{-1}$. 12 days later the rate had dropped to 63±8 mg C $m^{-2}h^{-1}$. Both rates are considerably higher than in the control plot (Table 12). This treatment made it possible to manually pick up roughly 50% of the oil as oil-sand-polymer lumps. After the manual cleaning the rate of carbon dioxide production increased to 81±8 mg C $m^{-2}h^{-1}$.

3.2.4 Total oil content.

The analyses of total oil in the samples taken from the plots 102A-H are shown in Table 13 and 14.

Over a time span of little more than a year the disappearance of oil seemed to be greater in the plot with aged crude oil (102B) than in the plot oiled with the emulsion. A deeper penetration and therefore a more extensive physical dilution of the oil in the first case cannot be excluded.

Apart from showing a stable distribution of oil between surface and subsurface sand during the 25 days period, this period was too short to expect any significant change in the total oil content of the various new plots, 102D-H. The manual "cleaning" of plot $102D_2$ explains the significant reduction in oil content in surface sample of August 25 for this plot.

3.2.5 Concluding remarks.

These studies show that the heterotrophic respiration of oil slicks in the supralittoral zone of arctic beaches can be enhanced up to about five times the normal rates of beach sediments. It is also shown that the microbiological community responds to heavy loads of fertilizers. It is only the fertilizer that is actually mixed into the oil film which affect oil biodegradation. Fertilizing combined with land tilling does create a dry soil surface, but rates of heterotrophic respiration are approximately the same as without this treatment. The use of sorbents without the addition of fertilizers did not enhance rates of respiration in this study.

The total decomposition rate of oil during the second summer season is approximately 25 g oil kg $soil^{-1}$ and 10 g oil kg $soil^{-1}$ for aged and emulsified oil, respectively. These rates are comparable to other studies in the temperate zone, where rates between 9-165 g oil kg $soil^{-1}$ are reported when oil content varied between 5% and 10% in the soil /5/.

The use of a solidifying agent created a non-sticky surface where the heterotrophic activity was greater than in the control plot. This treatment made it possible manually to pick up the oil from soil surface. In this process, approximately 50% of oil from the surface was removed. After removal of the solidified oil, total heterotrophic activity increased.

These studies show that, by simple means, it is possible to enhance the oil biodegradation and restrict the negative environmental effects of oil, even in the cold climate of arctic shorelines.

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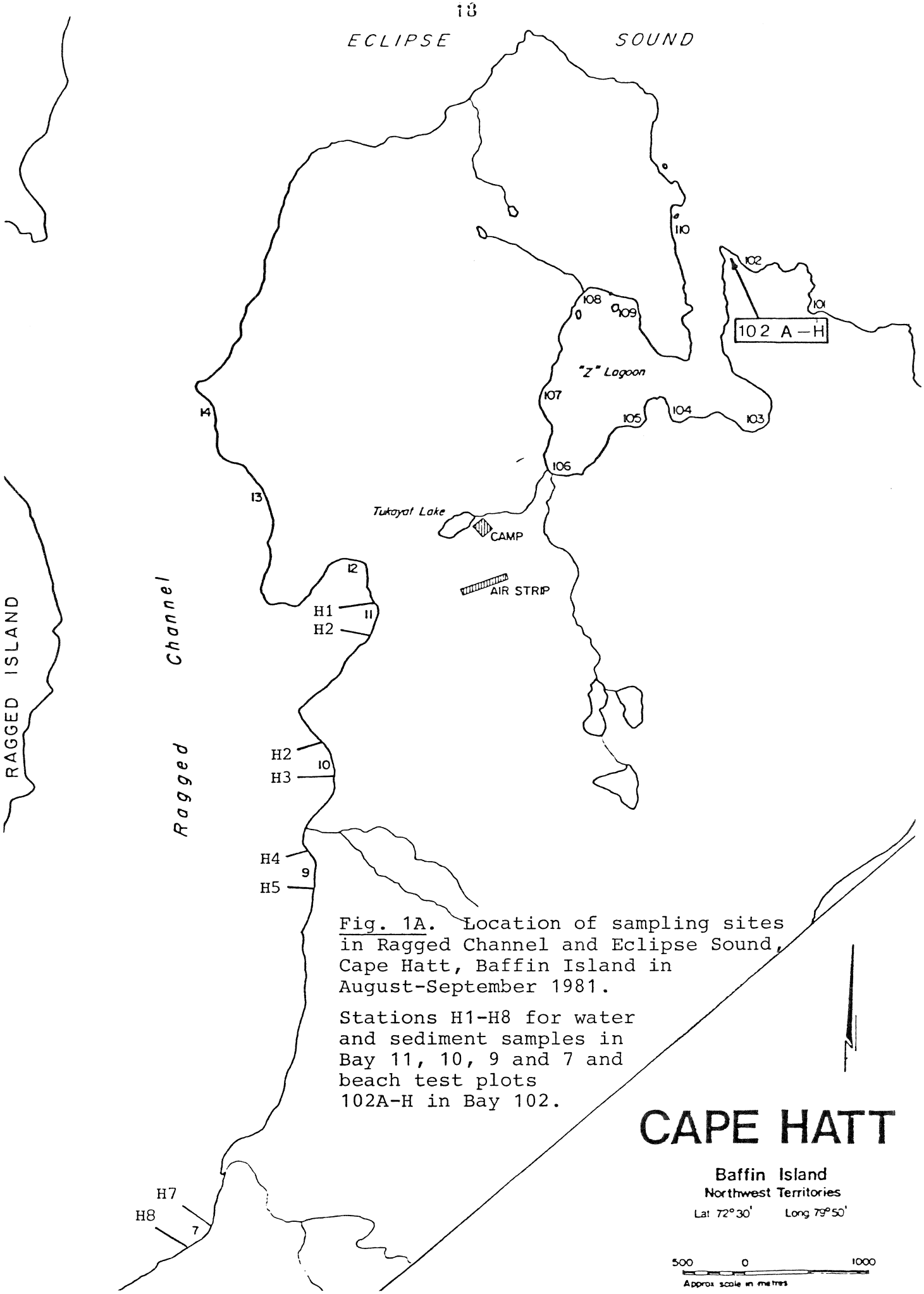

Fig. 1A. Location of sampling sites in Ragged Channel and Eclipse Sound, Cape Hatt, Baffin Island in August-September 1981.

Stations H1-H8 for water and sediment samples in Bay 11, 10, 9 and 7 and beach test plots 102A-H in Bay 102.

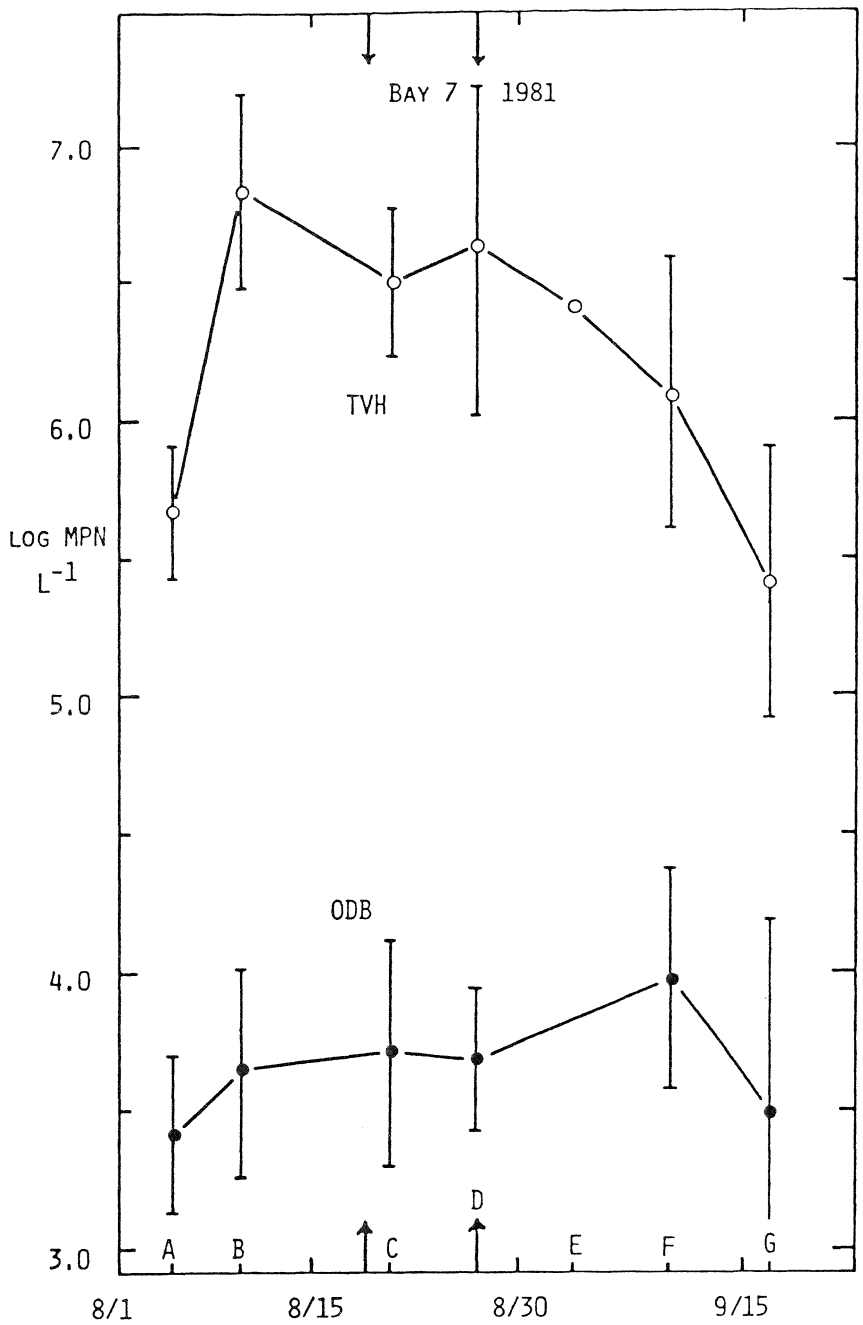

Fig. 1. Total viable heterotrophic bacteria (TVH) and oil degrading bacteria (ODB) in water of Bay 7 in Ragged Channel in August-September of 1981.

Each point represents the mean of logarithmic values for cell-count (MPN-methods) at 0.1, 5 and 10 m depth at 2 stations (H7 and H8), a total of 6 values. The vertical bars indicate the calculated standard deviation of the logarithmic values.

Bay 7 is the new control bay selected in 1981. The arrows at the X-axis mark the days of the oilspills. The capital letters signify the cycle of coordinated microbiological studies.

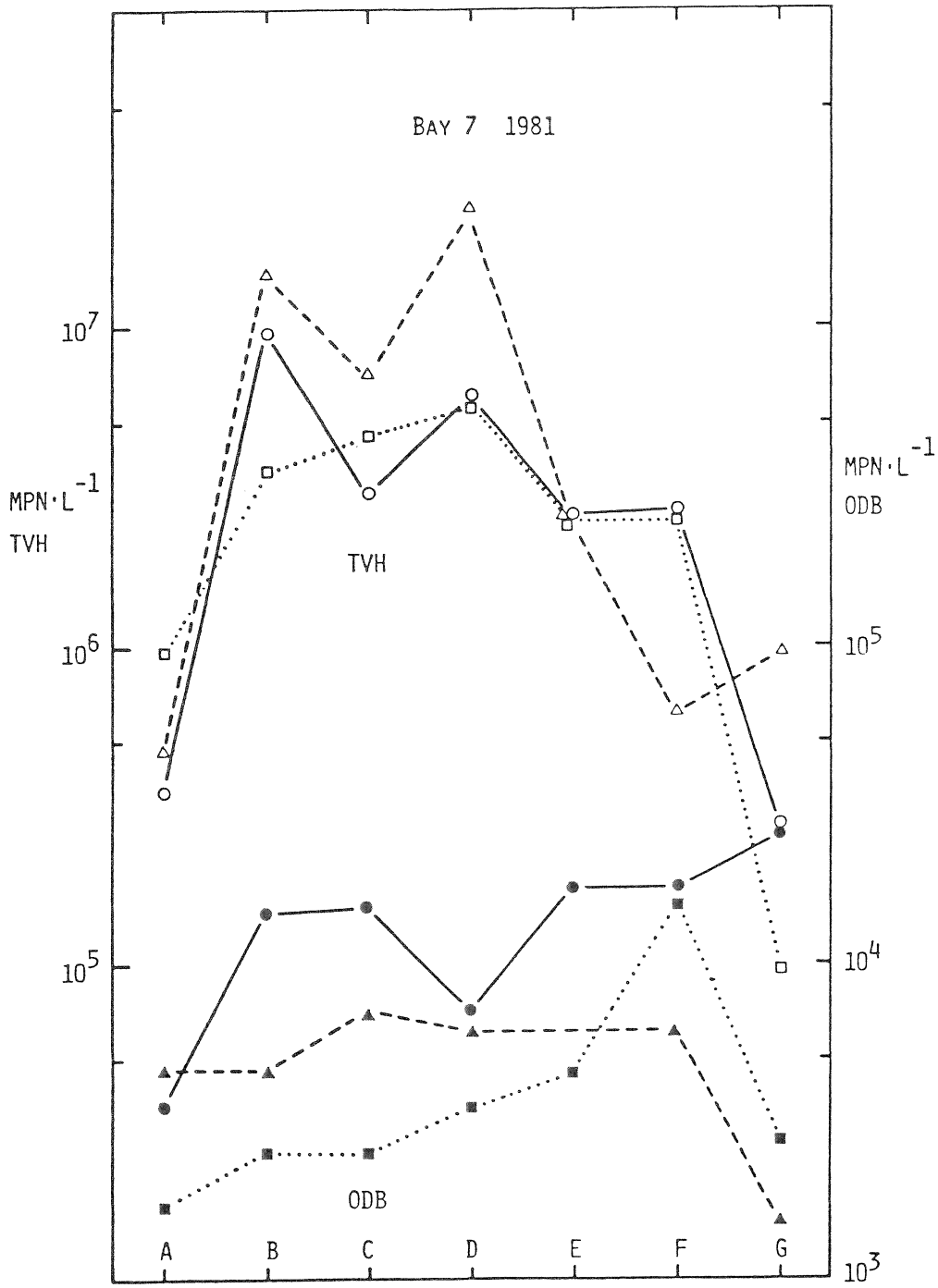

Fig. 2. Total viable heterotrophic bacteria (TVH and open symbols) and oil degrading bacteria (ODB and closed symbols) in the water at 0.1 m (——), 5 m (---) and 10 m (·····) depth in Bay 7 of Ragged Channel in August-September of 1981.

Each point is the mean of the cell-counts determined in samples taken at 2 stations (H7 and H8), at each cycle interval indicated by the capital letters.

This bay was selected in 1981 as the new control bay.

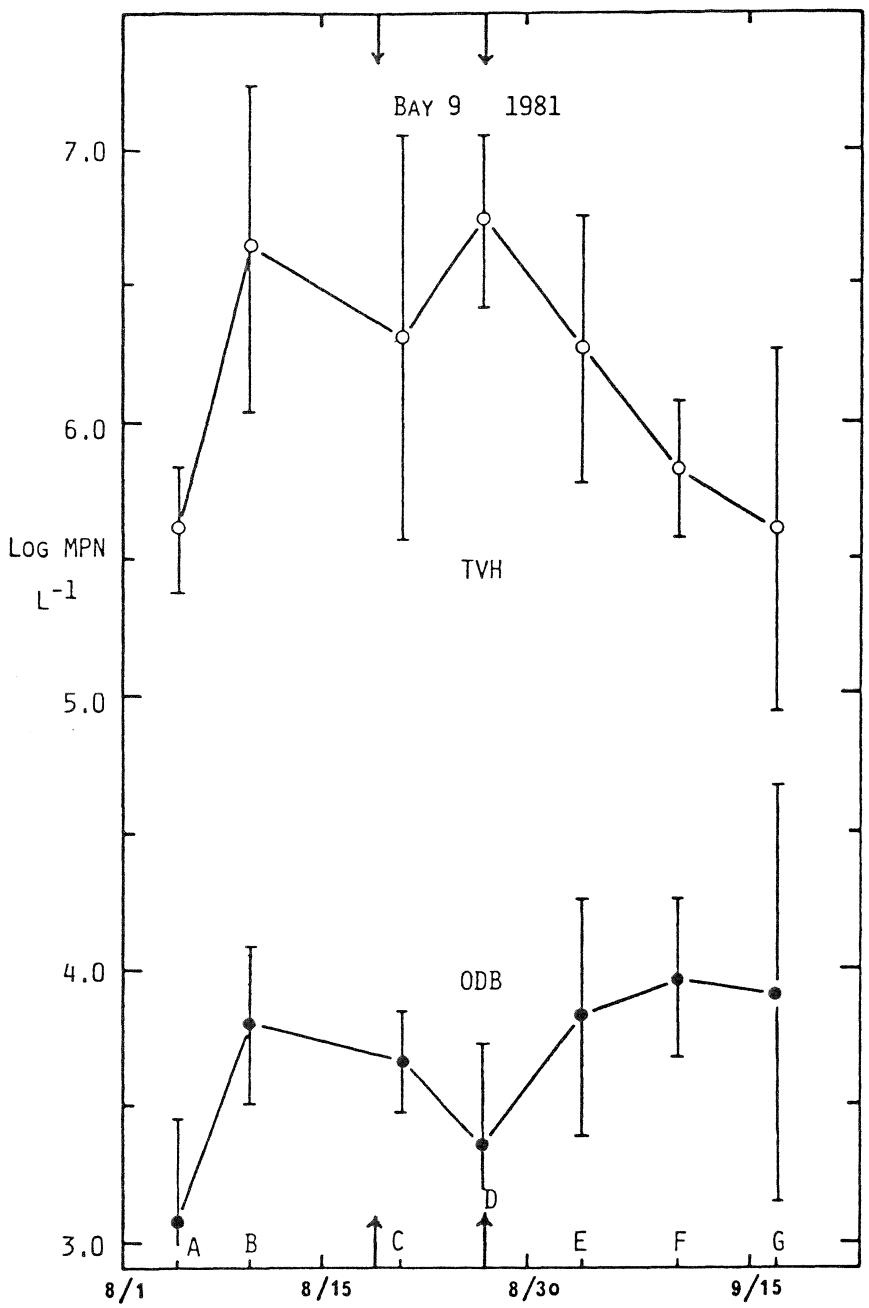

Fig. 3. Total viable heterotrophic bacteria (TVH) and oil degrading bacteria (ODB) in the water of Bay 9 in Ragged Channel in August-September of 1981.

Each point represents the mean of the logarithmic values of the cell-count (MPN-methods) at 0.1, 5 and 10 m depth at 2 stations (H5 and H6), a total of 6 analytical figures. The vertical bars indicate the calculated standard deviation of the logarithmic values.

The dispersed oil-spill was carried out in this bay August 27, at the beginning of cycle D (right arrow). The other arrow indicates the surface oil spill. The capital letters signify the cycle of coordinated microbiological studies.

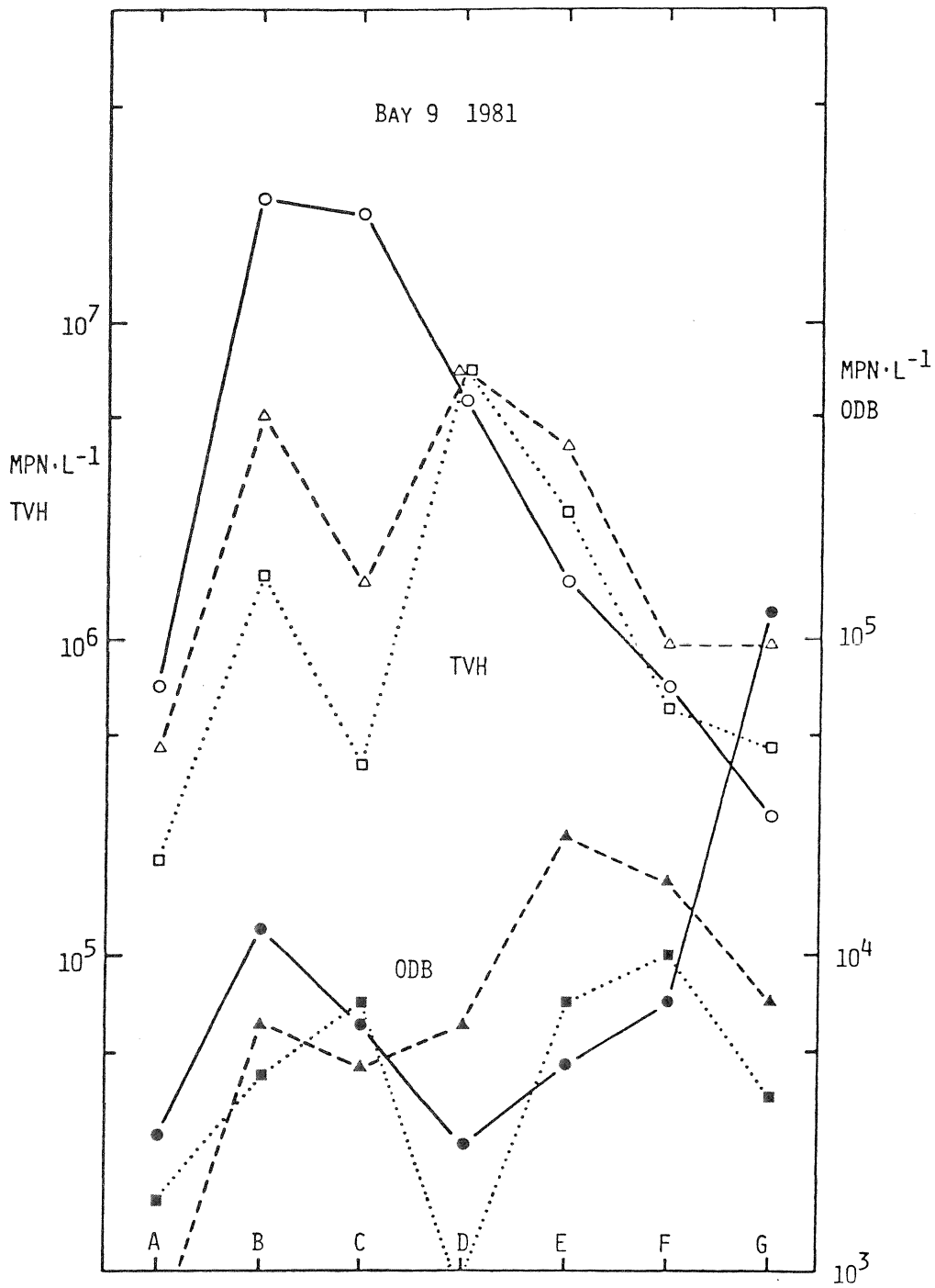

Fig. 4. Total viable heterotrophic bacteria (TVH and open symbols) and oil degrading bacteria (ODB and closed symbols) in water at 0.1 m (——), 5 m (---) and 10 m (·····) depth in Bay 9 of Ragged Channel in August-September of 1981.

Each point is the mean of cell-counts (MPN-methods) determined in samples taken at 2 stations (H5 and H6) collected once in each cycle interval indicated by the capital letters.

The dispersed oil spill was carried out in this bay at the initiation of cycle D.

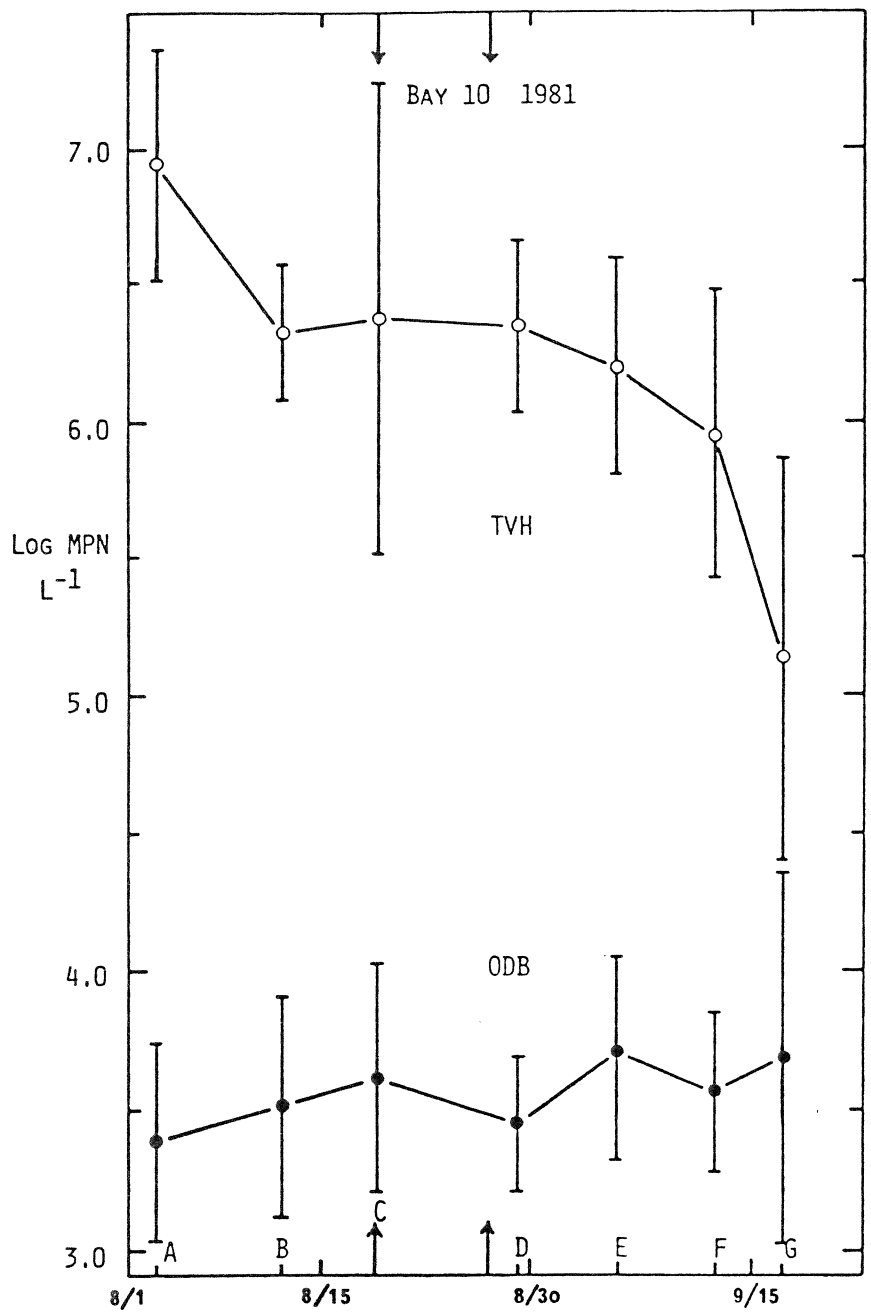

Fig. 5. Total viable heterotrophic bacteria (TVH) and oil degrading bacteria (ODB) in the water of Bay 10 in Ragged Channel in August-September of 1981.

Each point represents the mean of the logarithmic values of the cell-count (MPN-methods) at 0.1, 5 and 10 m depth at 2 stations (H3 and H4), a total of 6 analytical figures. The vertical bars indicate the calculated standard deviation of the logarithmic values. Bay 10 is the "old" control bay. The arrows at the X-axis mark the days of the oil spills. The capital letters signify the cycle of coordinated microbiological studies.

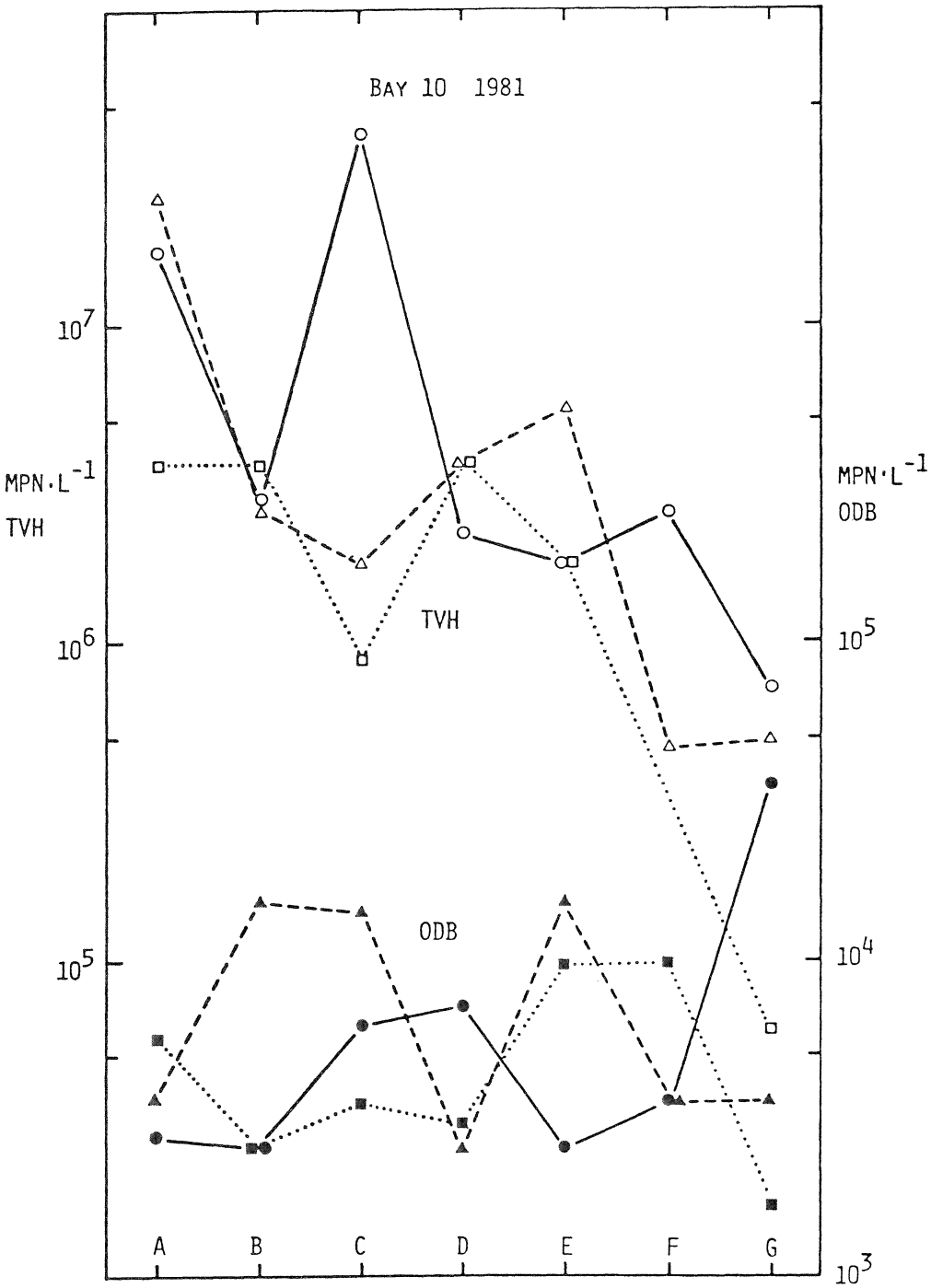

Fig. 6. Total viable heterotrophic bacteria (TVH and open symbols) and oil degrading bacteria (ODB and closed symbols) in water at 0.1 m (——), 5 m (---) and 10 m (·····) depth in Bay 10 of Ragged Channel in August-September of 1981.

Each point is the mean of cell-counts (MPN-methods) determined in samples taken at 2 stations (H3 and H4), collected once in each cycle interval indicated by the capital letters.

Bay 10 is the "old" control bay.

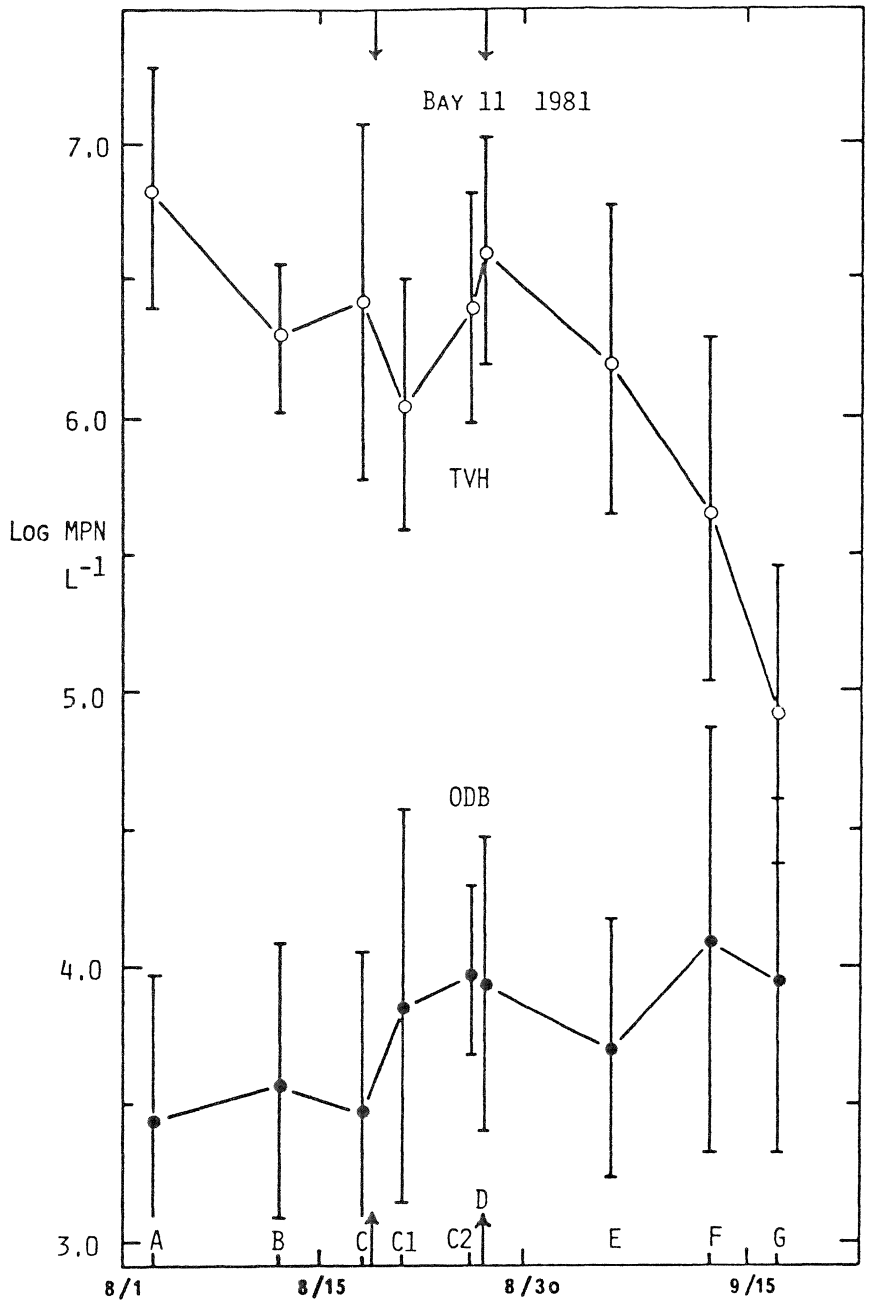

Fig. 7. Total viable heterotrophic bacteria (TVH) and oil degrading bacteria (ODB) in the water of Bay 11 in Ragged Channel in August-September of 1981.

Each point represents the mean of the logarithmic values of the cell-count (MPN-methods) at 0.1, 5 and 10 m depth at 2 stations (H1 and H2), a total of 6 analytical figures. The vertical bars indicate the calculated standard deviation of the logarithmic values. The capical letters signify the cycle of coordinated microbiological studies.

The surface oil spill was carried out in Bay 11 August 19 (the left arrow) during cycle C. Sampling period C1 and C2 are special for this project. The right arrow indicates time for dispersed oil spill.

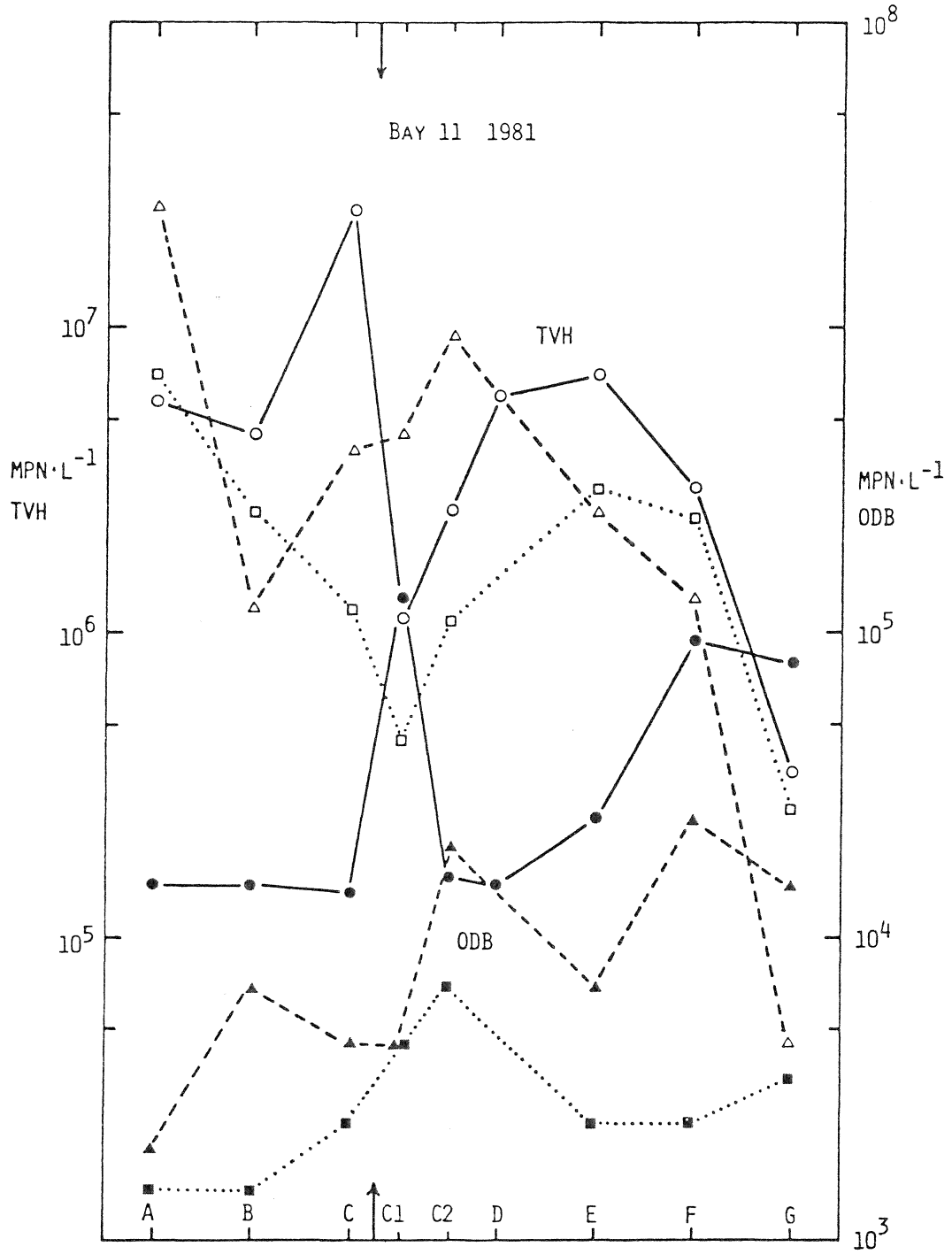

Fig. 8. Total viable heterotrophic bacteria (TVH and open symbols) and oil degrading bacteria (ODB and closed symbols) in water at 0.1 m (——), 5 m (- - -) and 10 m (·····) depth in Bay 11 of Ragged Channel in August-September of 1981.

Each point is the mean of cell-counts (MPN-methods) determined in samples taken at 2 stations (H1 and H2), normally collected once in each cycle interval indicated by the capital letters. C1 and C2 represent special samplings for this project. The surface oil spill was carried out in this bay August 19, indicated by the arrow.

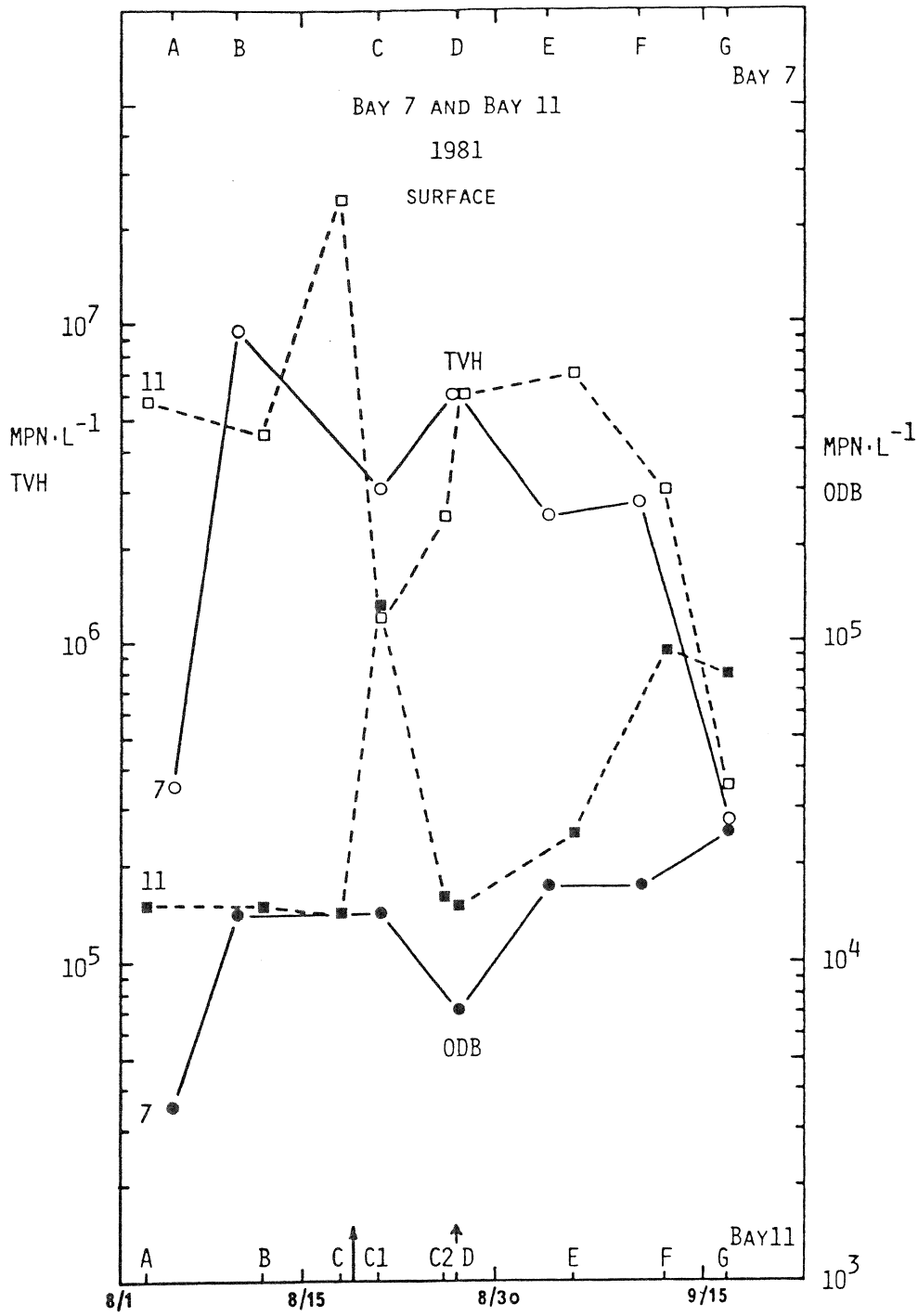

Fig. 9. Comparison of the microbiological development in the surface water of Bay 7 (——) and Bay 11 (---) during August-September of 1981.

The points are mean of the cell-counts for total heterotrophic bacteria (TVH and open symbols) and oil degrading bacteria (ODB and closed symbols) determined in 2 samples from 2 stations in each bay. In Bay 11 a surface oil spill was carried out August 19 (left arrow). The right arrow indicates day of dispersed oil spill in Bay 9. The capital letters signify the cycle of the coordinated microbiological studies. C1 and C2 cycle intervals are special for this project.

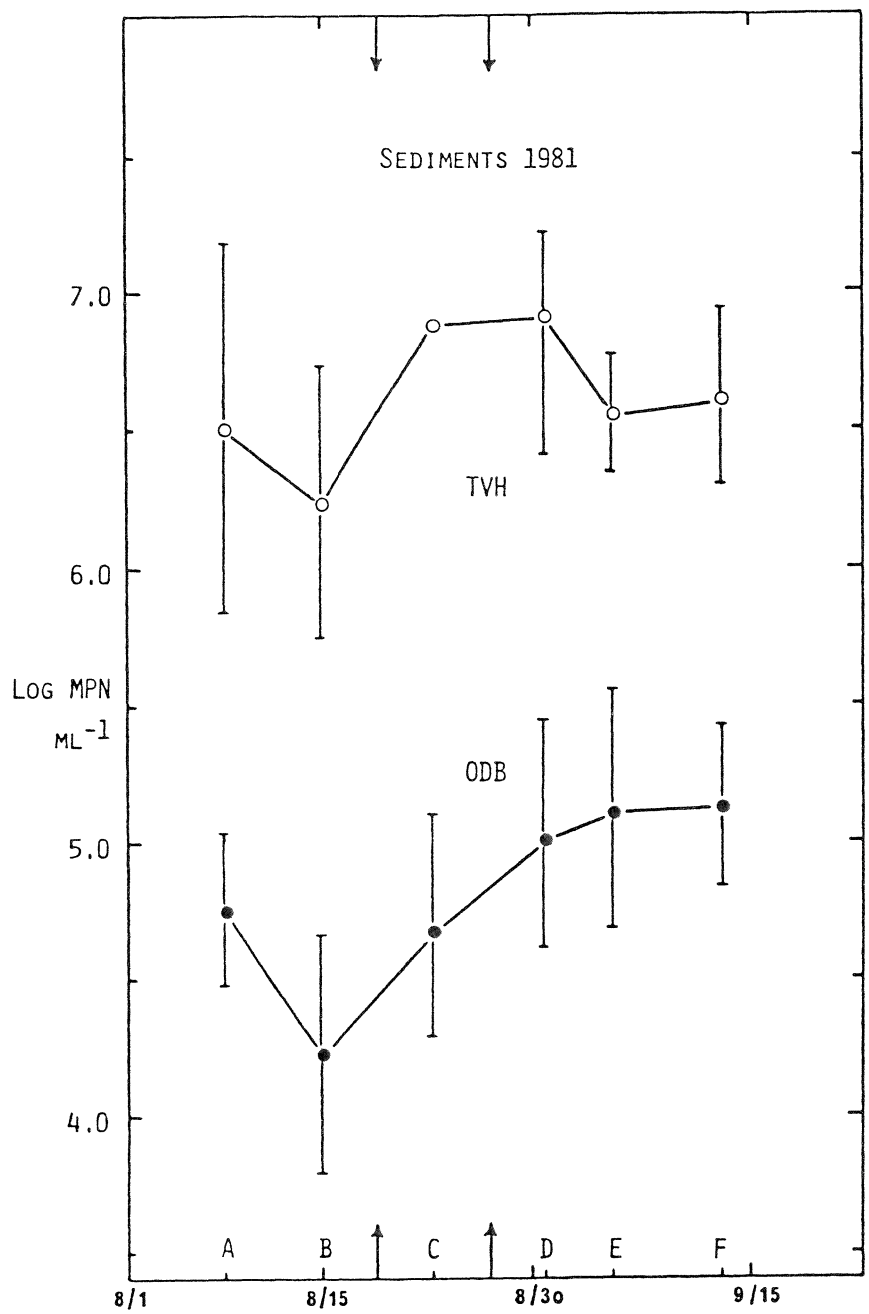

Fig. 10. Total viable heterotrophic bacteria (TVH) and oil degrading bacteria (ODB) in sediments in Bay 7, 9, 10 and 11 of Ragged Channel in August-September of 1981.

Each point represents the mean of the logarithmic values of the cell-count (MPN-methods) in all sediment samples collected during each cycle interval, A (4), B (3), C (2), D (5), E (8) F (8), sample number given in parenthesis. The vertical bars indicate the calculated standard deviation of the logarithmic values. Each sample analyzed was an homogeneous mixture of 8 sediment samples taken individually by a diver using 50 ml sterile disposable syringes. The same sampling areas close to the hydrographic stations marked in each bay were used throughout the period.

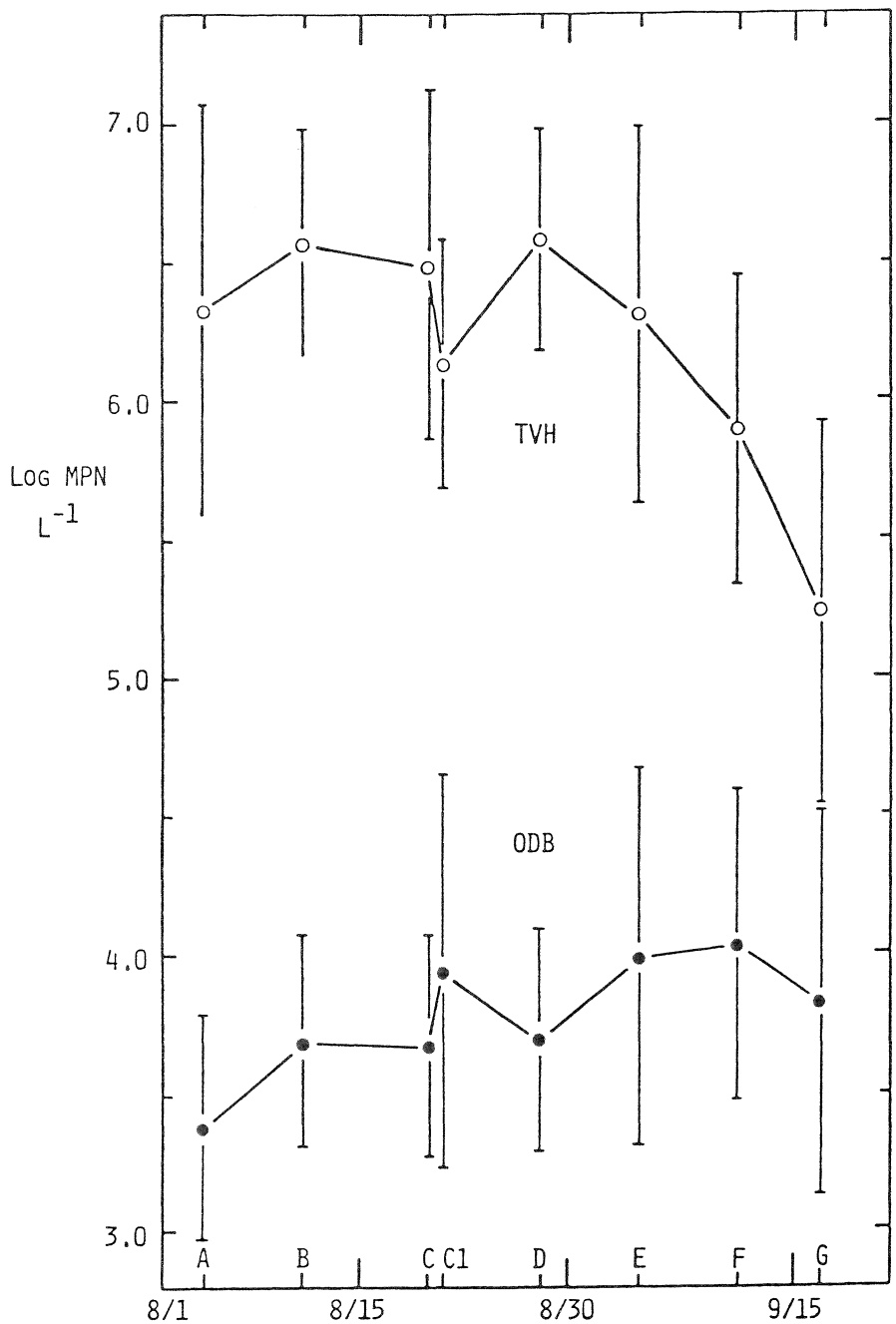

Fig. 11. Summaray graphs for total viable heterotrophic bacteria (TVH) and oil degrading bacteria (ODB) in the water of Ragged Channel in August-September of 1981.

The data are expressed as means of the logarithmic values for cell-counts (MPN-methods) of 19-24 water samples for each cycle interval, 3 depths at 2 stations in Bay 7, 9, 10 and 11. The special cycle interval C1 contains 6 samples (Bay 11). The vertical bars indicate the calculated standard deviation of the logarithmic values.

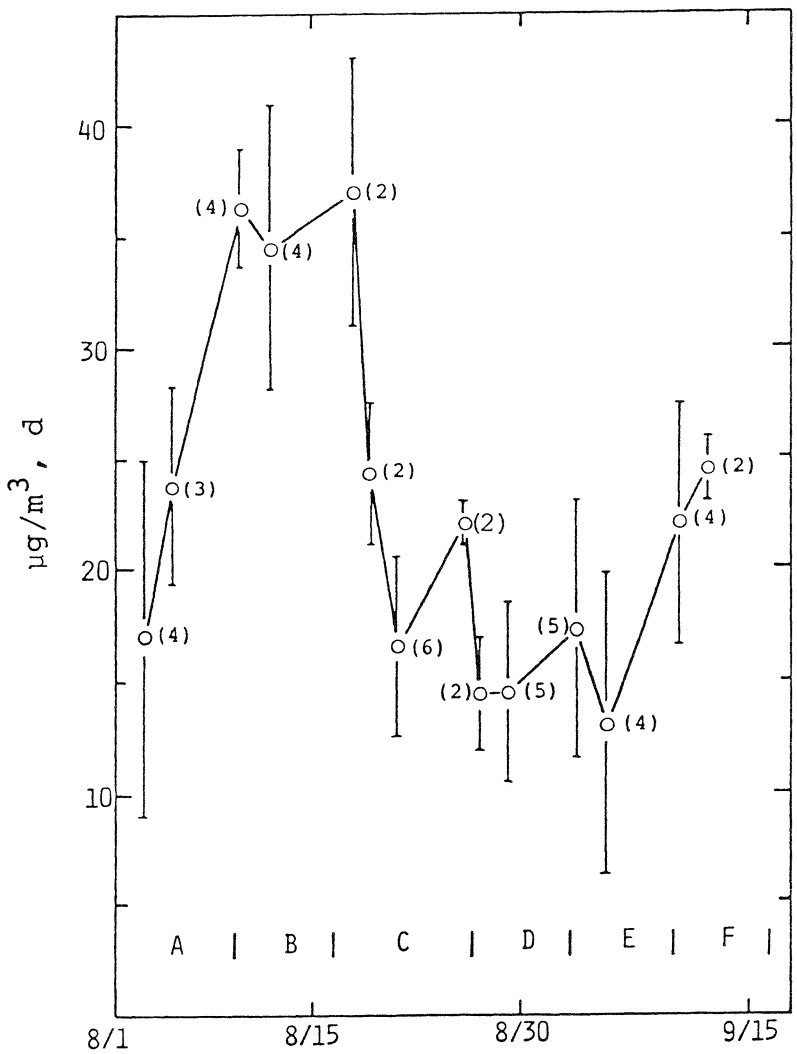

Fig. 12. Summary graph of V_{10}-values for mineralization of ^{3}H-Lago Medio weathered crude oil in water samples of Ragged Channel in August-September of 1981.

Data are means of rate measurements of all water samples collected at 5 m on a particular day. Number of samples analyzed is given in parenthesis.

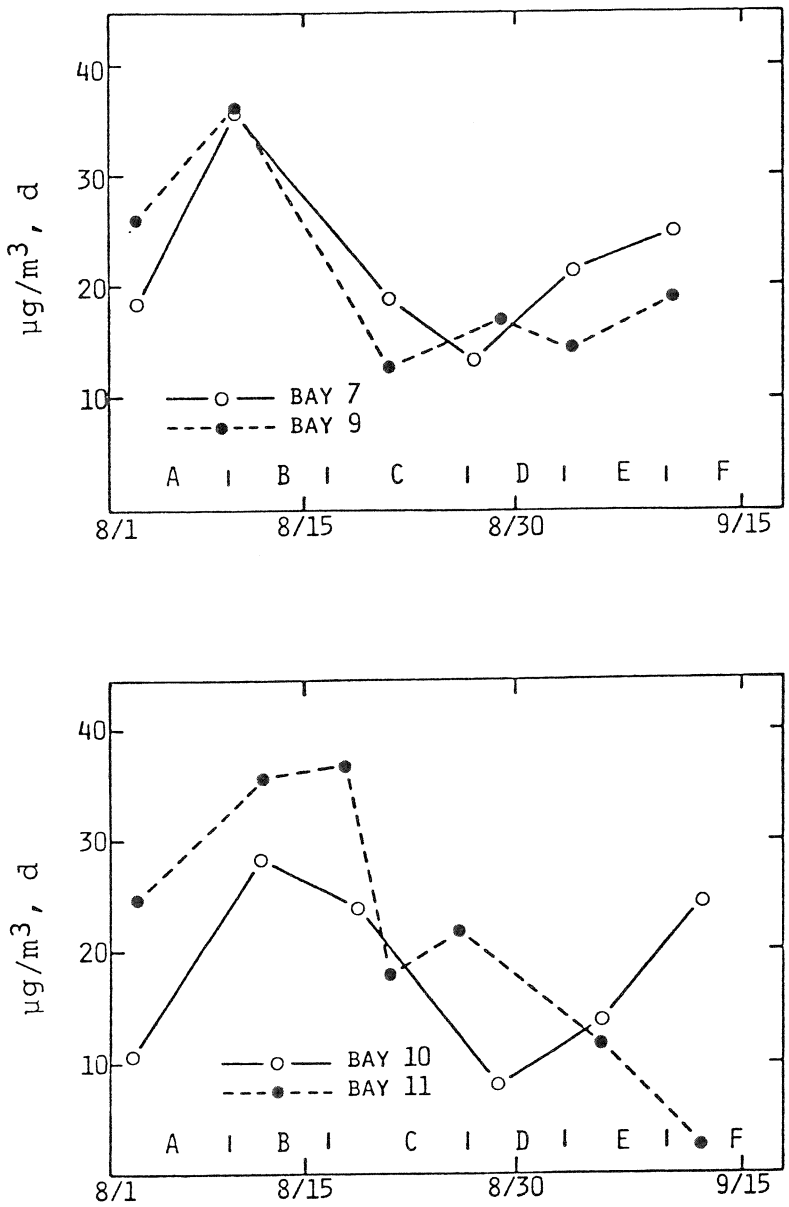

Fig. 13. The rate of mineralization of ^{3}H-Lago Medio weathered oil in water samples from each of Bay 7, 9, 10 and 11 in Ragged Channel during August-September of 1981.

Data given are means of measurement of V_{10} (see Materials and Methods) in 5 m water samples from 2 stations at each bay during each cycle interval (indicated by capital letters).

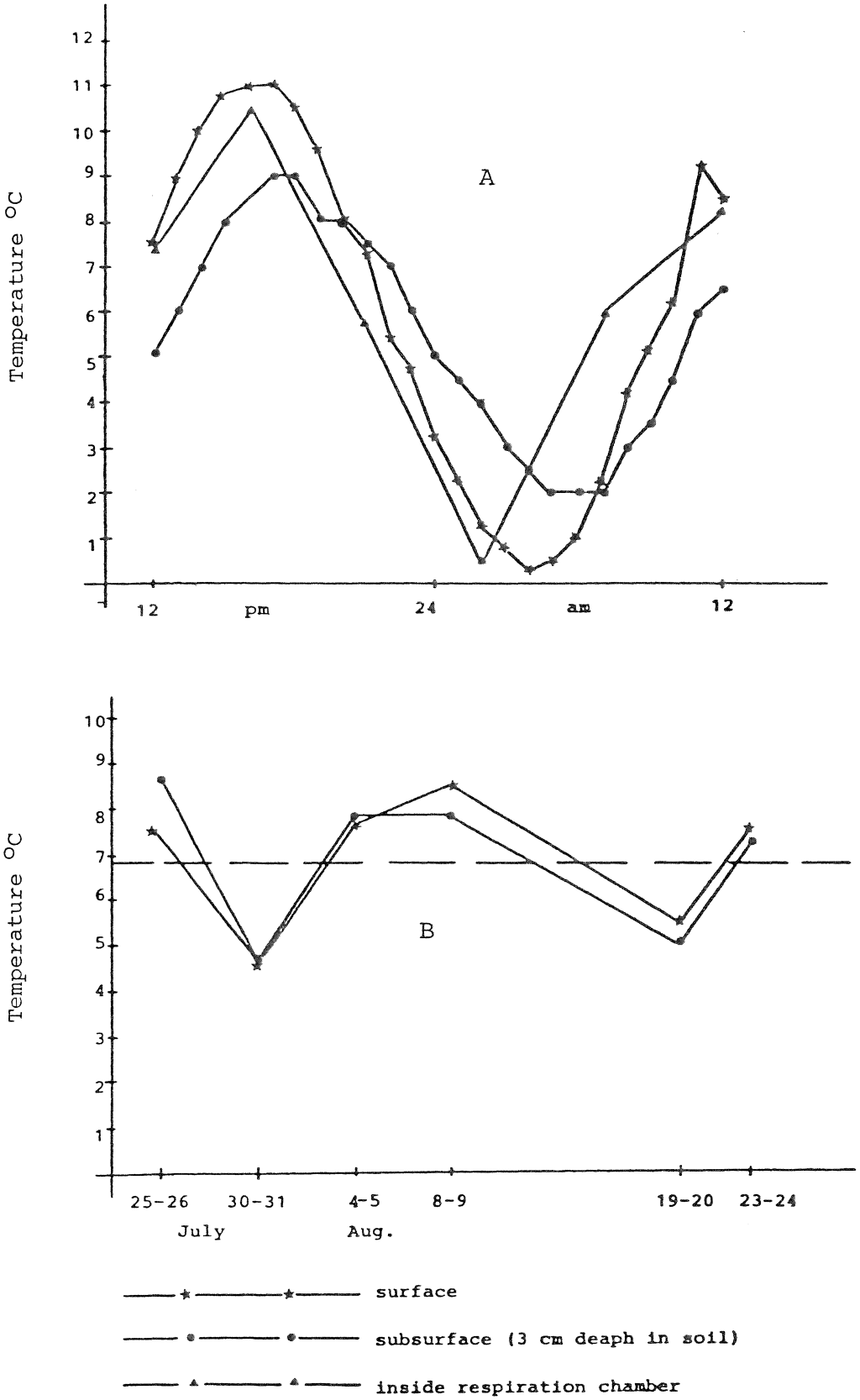

Fig. 14. Temperature profile inside and outside respiration chamber at plot 102F August 14-15 1981 (A) and mean temperature in beach sand of Bay 102 July-August 1981 (B).

Table 1.

Enumeration of total viable heterotrophs (TVH) and oil-degrading bacteria (ODB) in watersamples collected in Ragged Channel, Cape Hatt, August-September 1981.

Bay 7

Date	Station	Depth m	TVH no. $L^{-1} \cdot 10^{-5}$	ODB no $L^{-1} \cdot 10^{-3}$
08.05	7	0.1	4.5	2.5
(cycle A)	7	5	4.5	4.5
	7	10	9.5	0.9
08.05	8	0.1	2.5	4.5
(cycle A)	8	5	-	-
	8	10	9.5	2.5
08.10	7	0.1	95	25
(cycle B)	7	5	250	4.5
	7	10	25	2.5
08.10	8	0.1	95	2.5
(cycle B)	8	5	45	4.5
	8	10	45	2.5
08.21	7	0.1	25	25
(cycle C)	7	5	9.5	9.5
	7	10	45	2.5
08.21	8	0.1	40	2.5
(cycle C)	8	5	45	4.5
	8	10	45	2.5
08.27	7	0.1	9.5	4.5
(cycle D)	7	5	20	2.5
	7	10	65	2.5

Bay 7 (cont.)

Date	Station	Depth m	TVH no. $L^{-1} \cdot 10^{-5}$	ODB no. $L^{-1} \cdot 10^{-3}$
08.27	8	0.1	25	9.5
(cycle D)	8	5	450	9.5
	8	10	45	4.5
09.03	7	0.1	25	25
(cycle E)	7	5	25	950
	7	10	25	4.5
09.03	8	0.1	25	9.5
(cycle E)	8	5	25	450
	8	10	25	4.5
09.10	7	0.1	9.5	25
(cycle F)	7	5	2.5	9.5
	7	10	25	25
09.10	8	0.1	45	9.5
(cycle F)	8	5	9.5	2.5
	8	10	-	4.5
09.17	7	0.1	4.5	45
(cycle G)	7	5	9.5	0.4
	7	10	0.95	4.5
09.17	8	0.1	0.95	4.5
(cycle G)	8	5	-	2.5
	8	10	-	0.9

Bay 9

Date	Station	Depth m	TVH no. $L^{-1} \cdot 10^{-5}$	ODB no. $L^{-1} \cdot 10^{-3}$
08.05	5	0.1	9.5	4.5
(cycle A)	5	5	4.5	0.9
	5	10	2.0	0.9
08.05	6	0.1	4.5	0.9
(cycle A)	6	5	4.5	0.4
	6	10	2.5	2.5
08.10	5	0.1	45	15
(cycle B)	5	5	25	9.5
	5	10	7.2	4.5
08.10	6	0.1	450	9.5
(cycle B)	6	5	72	2.5
	6	10	25	4.0
08.21	5	0.1	400	2.5
(cycle C)	5	5	15	4.5
	5	10	4	4.5
08.21	6	0.1	45	9.5
(cycle C)	6	5	15	4.5
	6	10	4.5	9.5
08.29	5	0.1	15	2.5
(cycle D)	5	5	95	9.5
	5	10	45	0.9
08.29	6	0.1	95	2.5
(cycle D)	6	5	45	2.5
	6	10	95	0.9
09.03	5	0.1	4.5	4.5
(cycle E)	5	5	40	2.5
	5	10	4.5	9.5

Bay 9 (cont.)

Date	Station	Depth m	TVH no. $L^{-1} \cdot 10^{-5}$	ODB no. $L^{-1} \cdot 10^{-3}$
09.03	6	0.1	25	4.5
(cycle E)	6	5	45	45
	6	10	45	4.5
09.10	5	0.1	4.5	4.5
(cycle F)	5	5	9.5	25
	5	10	2.5	4.5
09.10	6	0.1	9.5	9.5
(cycle F)	6	5	9.5	9.5
	6	10	9.5	15
09.17	5	0.1	4.5	250
(cycle G)	5	5	25	9.5
	5	10	9.5	4.5
09.17	6	0.1	0.95	2.5
(cycle G)	6	5	9.5	4.5
	6	10	4.5	2.5

Bay 10

08.03	3	0.1	250	0.9
(cycle A)	3	5	230	2.5
	3	10	25	9.5
08.03	4	0.1	95	4.5
(cycle A)	4	5	250	4.5
	4	10	45	2.0
08.12	3	0.1	9.5	2.5
(cycle B)	3	5	25	25
	3	10	45	2.5

Bay 10 (cont.)

Date	Station	Depth m	TVH no. $L^{-1} \cdot 10^{-5}$	ODB no. $L^{-1} \cdot 10^{-3}$
08.12	4	0.1	45	2.5
(cycle B)	4	5	25	4.5
	4	10	25	2.5
08.19	3	0.1	720	9.5
(cycle C)	3	5	20	25
	3	10	15	4.5
08.19	4	0.1	95	2.5
(cycle C)	4	5	15	2.5
	4	10	2	2.5
08.29	3	0.1	36	4.5
(cycle D)	3	5	36	2.5
	3	10	29	2.5
08.29	4	0.1	6.4	9.5
(cycle D)	4	5	36	2.5
	4	10	45	3.5
09.05	3	0.1	25	2.5
(cycle E)	3	5	9.5	4.5
	3	10	9.5	9.5
09.05	4	0.1	9.5	2.5
(cycle E)	4	5	95	25
	4	10	25	9.5
09.12	3	0.1	-	4.5
(cycle F)	3	5	-	4.5
	3	10	-	15
09.12	4	0.1	25	2.5
(cycle F)	4	5	4.5	2.5
	4	10	-	4.5

Bay 10 (cont.)

Date	Station	Depth m	TVH no. $L^{-1} \cdot 10^{-5}$	CDB no. $L^{-1} \cdot 10^{-3}$
09.17	3	0.1	4.5	45
(cycle G)	3	5	0.25	2.5
	3	10	0.95	0.9
09.17	4	0.1	9.5	45
(cycle G)	4	5	9.5	4.5
	4	10	0.2	2.5

Bay 11

08.03	1	0.1	95	15
(cycle A)	1	5	450	1.5
	1	10	95	2.0
08.03	2	0.1	20	15
(cycle A)	2	5	45	2.5
	2	10	45	0.9
08.12	1	0.1	45	4.5
(cycle B)	1	5	15	4.5
	1	10	25	2.5
08.12	2	0.1	45	25
(cycle B)	2	5	9.5	9.5
	2	10	25	0.9
08.18	1	0.1	450	2.5
(cycle C)	1	5	72	4.5
	1	10	9.5	4.5

Bay 11 (cont.)

Date	Station	Depth m	TVH no. $L^{-1} \cdot 10^{-5}$	ODB no. $L^{-1} \cdot 10^{-3}$
08.18	2	0.1	25	25
(cycle C)	2	5	9.5	4.5
	2	10	15	0.4
08.21	1	0.1	9.5	4.5
(cycle C1)	1	5	45	4.5
	1	10	4.5	4.5
08.21	2	0.1	15	250
(cycle C1)	2	5	45	4.5
	2	10	4.5	4.5
08.26	1	0.1	25	25
(cycle C2)	1	5	95	15
	1	10	15	9.5
08.26	2	0.1	25	7.2
(cycle C2)	2	5	95	25
	2	10	9.5	4.5
08.27	1	0.1	95	25
(cycle D)	2	0.1	25	4.5
09.05	1	0.1	95	45
(cycle E)	1	5	45	9.5
	1	10	4.5	2.5
09.05	2	0.1	45	4.5
(cycle E)	2	5	4.5	4.5
	2	10	15	2.5
09.12	1	0.1	1.5	95
(cycle F)	1	5	1.5	45
	1	10	2.5	2.5

Bay 11 (cont.)

Date	Station	Depth m	TVH no. $L^{-1} \cdot 10^{-5}$	ODB no. $L^{-1} \cdot 10^{-3}$
09.12	2	0.1	4.5	95
(cycle F)	2	5	25	4.5
	2	10	45	2.5
09.17	1	0.1	4.5	150
(cycle G)	1	5	0.45	25
	1	10	0.95	2.5
09.17	2	0.1	0.25	9.5
(cycle G)	2	5	0.45	4.5
	2	10	4.5	4.5

Table 2.

Enumeration of total viable heterotrophs (TVH) and oil-degrading bacteria (ODB) in sediments collected in Ragged Channel, Cape Hatt, in August-September 1981.

Each sample represents a composit homogeneous mixture of 8 sediment samples taken individually by a diver using 50 ml sterile disposable syringes.

Date	Station	TVH no. $ml^{-1} \cdot 10^{-5}$	ODB no. $ml^{-1} \cdot 10^{-3}$
08.07 (cycle A)	8	250	95
08.16 (cycle B)	7	9.5	15
09.01 (cycle D)	8	250	25
09.06 (cycle E)	7	25	45
	8	45	75
09.13 (cycle F)	7	45	250
	8	95	95
08.09 (cycle A)	5	45	45
08.18 (cycle C)	6	95	4.5
08.31 (cycle D)	5	45	250
09.06 (cycle E)	5	45	250
	6	45	45
09.13 (cycle F)	5	25	95
	6	45	-
08.07 (cycle A)	3	9.5	25
08.14 (cycle B)	3	9.5	45
08.31 (cycle D)	4	45	250
09.06 (cycle E)	3	25	250
	4	95	250
09.13 (cycle F)	3	45	-
	4	9.5	250
08.09 (cycle A)	2	9.5	95
08.14 (cycle B)	1	9.5	25
08.23 (cycle C)	1	75	25
	2	75	95
08.31 (cycle D)	1	95	75
	2	75	95
09.06 (cycle E)	1	25	45
	2	20	4.5
09.13 (cycle F)	1	45	200
	2	95	45

Table 3.

Rates of mineralization of tritiated (^3H) weathered Lago Medio oil and ^{14}C-hexadecane, naphthalene and benz(a)pyrene in water and sediment samples from Ragged Channel, Cape Hatt, August-September 1981.

V_{10} designates the rate assessed in presence of 10 µg hydrocarbonsubstrate in the test-system (which corresponds to 1 ppm if all substrate is dissolved in the waterphase).

Watersamples		µg/m³, d				
		3H-Lago	Medio	hexadecane	naphthalene	benz(a)pyrene
Date	Station	V_{10}	V_{max}	V_{10}	V_{10}	V_{10}
08.05.	7	18,5	-	4,3	1,2	0,8/0,9
(cycle A)	8	-	-	-	-	-
08.10.	7	38,0	65,9	12,0	<1,0	-
(cycle B)	8	33,7	66,5	7,9	<1,0	-
08.21.	7	22,5	-	163,3	3,1	-
(cycle C)	8	14,4	-	44,2	5,2	-
08.27.	7	10,9	-	9,5	<1,0	-
(cycle D)	8	16,0	73,4	0,7	<1,0/<1,0	-
09.03.	7	23,1	88,6	111,3	<1,0	<0,5
(cycle E)	8	19,7	-	7,1	<1,0	<0,5
09.10.	7	24,1	-	23,4	<1,0	-
(cycle F)	8	25,7	42,6	19,0	<1,0	-
08.05.	5	27,7	84,6	34,1	1,6/1,2	<0,5
(cycle A)	6	24,8	88,2	10,1	1,5	<0,5

Table 3 (cont.)

		µg/m³, d				
		^{3}H-Lago	Medio	hexadecane	naphthalene	benz(a)pyrene
Date	Station	V_{10}	V_{max}	V_{10}	V_{10}	V_{10}
08.10.	5	39,2	51,8	16,1	<1,0	-
(cycle B)	6	34,1	-	11,1	<1,0	-
08.21.	5	15,1	-	26,9	2,8	-
(cycle C)	6	10,4	-	76,3	3,0	-
08.29.	5	17,5/19,0	-	15,8	<1,0	-
(cycle D)	6	14,8	-	11,2/4,9	<1,0	-
09.03.	5	8,3	11,7	11,2	<1,0/<1,0	<0,5
(cycle E)	6	19,6/15,3	-	29,1	<1,0	<0,5
09.10.	5	24,3	-	10,4/5,7	<1,0	-
(cycle F)	6	13,6	97,9	5,1	<1,0/<1,0	-
08.03.	3	10,4	36,4	-	-	-
(cycle A)	4	11,2	39,9	105,9	<1,0	<0,5
08.12.	3	27,3	84,1	-	<1,0	-
(cycle B)	4	29,5	-	-	<1,0	-
08.19.	3	26,6	-	4,3	2,0	-
(cycle C)	4	21,7	-	5,0	<1,0	-
08.29.	3	7,8	-	28,8	<1,0/<1,0	-
(cycle D)	4	8,8	-	8,6	<1,0	-
09.05.	3	6,5	12,2	12,6	<1,0	<0,5
(cycle E)	4	21,4	-	5,1/5,8	<1,0	<0,5
09.12.	3	23,4	-	3,5	<1,0	-
(cycle F)	4	25,6	46,3	9,5	<1,0/<1,0	-

Table 3 (cont.)

		µg/m³, d				
		^{3}H-Lago	Medio	hexadecane	naphthalene	benz(a)pyrene
Date	Station	V_{10}	V_{max}	V_{10}	V_{10}	V_{10}
08.03.	1	24,4	-	34,8	<1,0	<0,5
(cycle A)	2	25,7	108,9	-	-	-
08.12.	1	41,5	102	-	<1,0	-
(cycle B)	2	29,9	-	-	<1,0	-
08.18.	1	41,4	-	46,8	5,5	-
(cycle C)	2	32,4	-	24,9	2,0	-
08.21.	1	18,1	-	33,9	<1,0	<0,5
(cycle C)	2	17,8	-	12,6	<1,0	<0,5
08.26.	1	20,9	-	57,0	<1,0	-
(cycle D)	2	22,9	60,6	41,6	<1,0	-
09.05.	1	15,7	29,4	29,3	<1,0/<1,0	<0,5
(cycle E)	2	8,2	-	15,8	<1,0	<0,5
09.12.	1	1,7	-	5,5	<1,0/<1,0	-
(cycle F)	2	3,9	-	8,9/4,2	<1,0	-

Table 3 (cont.)

Sediment samples

Date	Station	^{3}H-Lago V_{10}	Medio V_{max}	hexadecane V_{10}	naphthalene V_{10}	benz(a)pyrene V_{10}
				µg/L^{-1}, d		
08.07. (cycle A)	7	44,3	96,2	-	1,8	<0,5
08.16. (cycle B)	7	-	-	23,1/28,8	2,5/1,8	<0,5/<0,5
09.01. (cycle D)	8	48,4	198,7	-	5,3	<0,5
09.06. (cycle E)	7	37,8	-	14,9	1,8	<0,5
09.13. (cycle F)	7	22,2	-	7,8	<1,0	-
	8	24,7	-	8,6	<1,0	-
08.09. (cycle A)	5	33,3	119,4	14,0/15,2	-	<0,5
08.18. (cycle B)	6	-	-	114,0/89,7	5,0/16,8	<0,5/<0,5
08.31. (cycle D)	5	34,5	-	40,3/14,0	-	<0,5
09.06. (cycle E)	5	34,9	220,3	8,4	2,4	<0,5
	6	37,2	-	7,4	11,8	<0,5
09.13. (cycle F)	5	19,1/31,3	-	15,0/6,7	<1,0	-
	6	10,4	-	15,9	<1,0	-

Table 3 (cont.)

		µg/L^{-1}, d				
		^{3}H-Lago	Medio	hexadecane	naphthalene	benz(a)pyrene
Date	Station	V_{10}	V_{max}	V_{10}	V_{10}	V_{10}
08.07.	3	39,8	122,3	13,7	1,4	<0,5
(cycle A)						
08.14.	3	-	-	-	<1,0	1,2
(cycle B)						1,0
08.31.	4	36,6	-	22,1	<1,0	<0,5
(cycle D)						
09.06.	3	27,3	52,2	13,3	<1,0	<0,5
(cycle E)	4	-	-	-	-	-
09.13.	3	3,5	-	5,8	<1,0	-
(cycle F)	4	16,5	-	7,6/7,6	<1,0	-
08.09.	2	-	-	-	16,5	<0,5/<0,5
(cycle A)						
08.14.	1	-	-	-	<1,0	<0,5/<0,5
(cycle B)						
08.23.	1	19,3	-	25,2	7,6	<0,5
(cycle C)	2	10,9	.	77,7	12,5	<0,5
08.31.	1	20,0	-	19,3	<1,0	<0,5
(cycle D)	2	37,8	-	15,7	7,7	<0,5
09.06.	1	37,4	-	18,1/9,6	<1,0	-
(cycle E)	2	-	-	-	-	-
09.13.	1	12,5/23,7	-	6,6/8,6	<1,0	-
(cycle F)	2	17,5	-	7,9/9,2	<1,0	-

Table 4.

Total viable heterotrophic bacteria (TVH) and oil degrading bacteria (ODB) in sediments of Bay 7, 9, 10 and 11 of Ragged Channel in August - September 1981.

Data are expressed as means of the cell-count analyses of all sediment samples from each bay.

Bay	7	9	10	11	all
TVH $ml^{-1} x 10^{-5}$	95	48	34	52	57
ODB $ml^{-1} x 10^{-5}$	0,8	1,1	1,8	1,1	1,5
Mean % of TVH	1,7	2,8	5,1	4,6	(2,6)
Samples analyzed	7	7	7	10	

Table 5.

Rates of mineralization of ^{3}H-Lago Medio weathered crude oil in sediments of Bay 7, 9, 10 and 11 in August - September 1981.

Rates given are means of V_{10}-values (see Materials and Methods) for all sediments for each cycle interval.

Cycle intervals	A	B	C	D	E	F
V_{10} $\mu g/L^{-1}$, d	39,1	-	15,1	32,3	37,2	18,1
Standard deviation	±5,5		±5,9	±8,0	±6,7	±8,0
Sediment samples analyzed	3		2	4	6	10

Table 6.

Rates of mineralization of 1-^{14}C-hexadecane in water and sediments of Ragged Channel, August - September 1981.

Summary of data expressed as mean of V_{10}-values (see Material and Methods) for all samples analyzed in each cycle interval.

Cycle intervals	A	B	C	D	E	F
WATER						
V_{10} µg/m^3,d	33,7	11,8	43,8	11,4	22,9	9,5
Range of V_{10}	4,3-105,9	7,9-16,2	4,3-163,3	0,7-28,8	1,5-111,3	4,2-23,4
n	6	4	10	7	10	10
SEDIMENTS						
V_{10} µg/L^{-1},d	14,3	15,2	76,7	22,2	11,9	8,9
Range of V_{10}	13,7-15,2	4,0-28,8	25,2-114,1	14,0-40,3	7,4-18,1	5,8-15,9
n	3	4	4	5	6	12

Table 7.

Mineralization of [1(4,5,8)-^{14}C]-naphthalene and [(7,10)-^{14}C]-benz(a)pyrene in water and sediments of Ragged Channel August - September 1981.

Table indicates numbers of positive verifications of mineralization of the hydrocarbons relative to the total number of analyses. The range of mineralization rates for positive samples are given in parenthesis. Limits of detection: for water samples 1,0 µg/m^3,d naphthalene and 0,5 µg/m^3,d benz(a)pyrene, for sediment samples 1,0 µg/L^{-1},d naphthalene and 0,5 µg/L^{-1},d benz(a)pyrene.

	Positive measurements		Total measurements	
	Water (range V_{10}=µg/m^3,d)	Sediments (range V_{10}=µg/L^{-1},d)	Water	Sediments
Naphthalene	11 (1,2-5,5)	14 (1,4-16,8)	55	33
Benz(a)pyrene	2 (0,8-0,9)	2 (1,0-1,2)	16	24

Table 8.

On shore biodegradation experiment in the supralittoral zone of Bay 102, Cape Hatt 1980 and 1981.

The initial oil and total nitrogen content in the beach sand immediately after completion of all treatments. The BP solidifying agent was applied to plot 102 D1/D2 a few days later.

Plot	Surface 0-5 cm		Subsurface 5-10 cm	
	g oil/kg sand	mg N/kg sand	g oil/kg sand	mg N/kg sand
102A	51*	129-151	1,7*	92-93
102B	46,2*	130-131	29,4*	72-82
102Do	48,5±1,6	125-177	4,3±0,2	69-87
102D1/D2	37,3±0,9	163-194	9,6±0,4	48-61
102E	16,2±0,2	310-343	11,4±0,1	245-245
102F	35,2±0,4	106-109	5,9±0,2	6-8
102G	39,2±0,3	237-244	6,9±0,2	70-74
102H	49,1±0,5	178-190	9,6±0,3	76-81

*) Analyzed by Woodward-Clyde Consultants (3).

Table 9.

On shore biodegradation experiment in Bay 102, Cape Hatt. Microbial development in the surface layer (0-2 cm) of untreated oil test plots over 2 successive summer seasons. The oiled plots were laid August 23 1980.

TVH = total viable heterotrophic bacteria - $ml^{-1} \cdot 10^{-4}$

ODB = oil degrading bacteria - $ml^{-1} \cdot 10^{-4}$

	102A emulsion		102B crude oil		102C control	
Date	TVH	ODB	TVH	ODB	TVH	ODB
8/26 1980	1,5	0,015	0,75	0,02	0,45	0
8/31 1980	950	75	2,5	2,5	1,5	<0,01
9/9 1980*	4500	1500	45	9,5	0,025	<0,01
8/1 1981	730	0,073	280	0,28	1,3	<0,01
8/17 1981	4400	13	2800	7,3	130	<0,01

*) Results assessed after only 6 days of incubation.

Table 10.

On shore biodegradation experiment in the supralittoral zone of Bay 102, Cape Hatt. Microbial development in the various test plots started August 1, 1981.

TVH = total viable heterotrophic bacteria - $ml^{-1} \cdot 10^{-4}$

ODB = oil degrading bacteria - $ml^{-1} \cdot 10^{-4}$

	102D sorbent		102E fertilized and mixed		102F, control		102G low level fertilizer		102H high level fertilizer	
	TVH	ODB	TVH	ODB	TVH	ODB	TVH	ODB	TVH	ODB
8/1 1982										
0-2 cm*	7,3	0,01	280	<0,01	7,3	<0,01	13	<0,01	4,4	<0,01
5-10 cm	-	-	-	-	-	-	-	-	-	-
8/5 1981										
0-2 cm	1300	0,73	73	0,28	73	28	730	2800	73	2,8
5-10 cm	2,8	1,3	4,4	0,073	130	28	73	7,3	280	7,3
8/17 1981										
0-2 cm	7300	28	730	≥730	730	0,73	280000	73	73000	≥7300
5-10 cm	2800	13	1300	≥730	1300	28	130000	1300	73000	7300

*) Samples taken prior to oil deposition.

Table 11.

Collembola at Bay 102, Cape Hatt, in unoiled supralittoral sand. Numbers trapped per 24 hours.

Species	1980-08-25	1980-09-05	1981-08-05	1981-08-30
Archisotoma besselsi (Pacard)	108±20	145±35	190±45	150±40
Hypogastrura aff. *matura* (Mac Nam)	0	0	40±20	50±25

Table 12.

On shore biodegradation experiment in the supralittoral zone of Bay 102, Cape Hatt. The rates of respiratory carbon dioxide production in unoiled plots and in oiled plots treated differently for tests (see 2.2.4.4) of enhanced biodegradation. The plots were oiled August 23 1980 and August 1 1981.

Rates given as mg carbon x $m^{-2} \cdot h^{-1}$.
Number in parenthesis indicates number of rate determinations.

	102A	102B	102C	102D	102E	102F	102G	102H
8/25 1980	13±5(3)	17±4(3)	28±6(4)	-	-	-	-	-
8/30 "	13±13(4)	35±6(3)	34±2(2)	-	-	-	-	-
9/6 "	18±12(4)	21±8(4)	12±2(2)	-	-	-	-	-
7/25 1981	36±4(38)	34±5(27)	18±2(31)	-	-	-	-	-
8/4 "	-	-	-	21±2(42)	41±5(32)	20±2(27)	21±3(26)	49±8(24)
8/20 "	24±4(27)	40±7(16)	-	-	-	-	-	-
8/24 "	-	-	-	13±3(21)	133±17(20)	30±7(14)	69±8(16)	156±18(16)

Table 13.

On shore biodegradation experiment in the supralittoral zone of Bay 102, Cape Hatt. Total oil content in the surface and subsurface beach sand of plot 102A (oil-emulsion) and 102B (crude oil) 1980 and 1981.

Plots were oiled August 23 1980.
Oil content in g x kg^{-1} sand; number in parenthesis indicates number of parallel analyses of mixed composit sample.

	1980	1981	
	8/23	7/24	8/25
102A			
0-5 cm	51,0* (1)	51,8±0,8 (5)	44,3±1,9 (5)
5-10 cm	1,7* (1)	23,2±0,6 (5)	12,9±0,2 (5)
102B			
0-5 cm	46,2** (1)	42.8±0,3 (5)	19,1±0,3 (5)
5-10 cm	29,4* (1)	18,2±0,4 (5)	19,1±0,1 (5)

*) Analyzed by Woodward-Clyde Consultants (3).

Table 14.

On shore biodegradation experiment in the supralittoral zone of Bay 102, Cape Hatt. Total oil content in surface and subsurface sand in the various test plots oiled August 1 1981.

Oil content in g x kg^{-1} sand as mean of 5 determinations.

Plot	depth, cm	treatment	8/1	8/25
$102D_0$	0-5	sorbent	48,5±1,6	51,6±0,9
	5-10		4,3±0,2	2,3±0,1
$102D_1$	0-5	solidifying	37,3±0,9	35,1±0,8
	5-10	agent	9,6±0,4	6,2±0,6
$102D_2$	0-5	solidifying agent, solid	37,3±0,9	19,2±0,9
	5-10	lumps removed approx. 8/15	9,6±0,4	6,4±0,2
102E	0-5	high fertilizer	16,2±0,2	15,4±0,4
	5-10	+ tilling	11,4±0,1	13,9±0,3
102F	0-5	none	35,2±0,4	39,7±0,4
	5-10		5,9±0,2	9,6±0,3
102G	0-5	low level	39,3±0,3	36,9±1,2
	5-10	fertilizer	6,9±0,2	4,8±0,2
102H	0-5	high level	49,1±0,5	50,2±0,4
	5-10	fertilizer	9,6±0,3	6,0±0,1