ECOLOGICAL STUDY OF THE AMOCO CADIZ OIL SPILL

Report of the NOAA-CNEXO Joint Scientific Commission

U. S DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration

CENTRE NATIONAL POUR l'EXPLOITATION DES OCEANS

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October 1982

U. S. DEPARTMENT OF COMMERCE
Malcolm Baldrige, Secretary
National Oceanic and Atmospheric Administration
John V. Byrne, Administrator

CENTRE NATIONAL POUR l'EXPLOITATION DES OCEANS

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PREFACE

At approximately 11:30 p.m. on Thursday, March 16, 1978, the supertanker Amoco Cadiz went aground on a rock outcropping 1.5 km offshore of Portsall on the northwest coast of France. The vessel contained a cargo of 216,000 tons of crude oil and 4,000 tons of bunker fuel. At 6:00 a.m. on Friday, March 17, the vessel broke just forward of the wheelhouse and thus started the largest oil spill in maritime history. During the course of the next 15 days, the bunker fuel and contents of all 13 loaded cargo tanks, which contained two varieties of light mideastern crude oil, were released into the ocean. The oil quickly became a water-in-oil emulsion (mousse) of at least 50% water, and heavily impacted nearly 140km of the Brittany coast from Portsall to Ile de Brehat. At one time or another, oil contamination was observed along 393 km of coastline and at least 60 km offshore. Impacted areas included recreational beaches, mariculture impoundments, and a substantial marine fishery industry.

On March 18, Dr. Wilmot N. Hess, Director of the Environmental Research Laboratories (ERL) of the National Oceanic and Atmospheric Administration (NOAA), contacted Dr. Lucien Laubier, Director of the Centre Oceanologique de Bretagne (COB) of the Centre National pour l'Exploitation des Oceans (CNEXO), the French national oceanographic organization. Dr. Hess and Dr. Laubier arranged for participation by United States scientists in a joint Franco-American investigation of physical and chemical manifestations of the spill. On March 24, the agreement was expanded to include cooperative biological investigations through contacts initiated by Dr. Eric Schneider, Director of the Environmental Protection Agency's Environmental Research Laboratory in Narragansett, Rhode Island.

NOAA personnel arrived on March 19 to join the investigation initiated on March 17 by several French scientific teams. Initial photographic over-flights and active beach sampling began on Tuesday, March 21, followed by initial chemical sampling by vessel on Friday, March 24. The team was supplemented with EPA biological observers on Sunday, March 26. Sampling has continued by some segments of this original team until the present time.

Throughout the period of investigation, active interaction and coordination with the French scientific community have taken place under the auspices of COB/CNEXO. All sampling has been coordinated with the general ecological impact study designed by the French Ministry of Environment, organized by CNEXO, and operated by several scientific institutions in France[1/], making possible a more thorough evaluation of the effects of the incident than would otherwise have been possible.

[1/] National Museum of Natural History, National Geographic Institute, French Institute of Petroleum, Scientific and Technical Institute of Marine Fisheries, University of Western Brittany, University P. and M. Curie, Paris VI, and the National Center for the Exploitation of the Oceans.

About three months after the oil spill the U.S. team prepared a "Preliminary Scientific Report on the Amoco Cadiz Oil Spill" covering data up to May 15, 1978. This document covered only the period of acute effects. A one-day symposium on the Amoco Cadiz spill was held in Brest on June 7, 1978, and published soon after. It was obvious from these initial observations that a period of years would be required to understand what had happened to these portions of the coast where the oil had settled in and not been cleansed promptly.

During this early period of study of the spill Mr. Russ Mallatt of the Amoco Trading Company had several discussions with Drs. Hess, Laubier and Schneider. Mr. Mallatt was the General Manager for Environmental Conservation and Toxicology of Amoco. Discussion with Mr. Mallatt during the first two months after the spill identified Amoco's interests in carrying out long-term studies of the effects of the oil spill. These early contacts were followed up by substantial discussions between Mr. John Linsner of Amoco and Mr. Eldon Greenberg, General Counsel of NOAA. These discussions culminated with an agreement being signed by Amoco and NOAA to carry out long-term studies of the effects of the spill. The study would cover three years and would be a joint French-U.S. activity. A Joint NOAA/CNEXO Scientific Commission was established through another agreement between the two agencies signed June 2, 1978. Amoco would transfer money to NOAA and the Joint Commission, chaired by Drs. Hess and Laubier, would determine the research program to be carried out, the investigators to do the research, and the funding levels. The Joint Commission would also monitor the progress of the studies and be responsible for making the final report. One of its major goals was to make U.S. and French scientific teams work together in a common effort to better understand the consequences of the wreckage.

The Joint Commission first met in Brest at the CNEXO Laboratory on July 18, 1978. Taking into account the French program to assess the long-term ecological impact of the oil spill funded by the Ministry of Environment, it determined that the most important areas for research were:

1. Heavily impacted subtidal areas like the Abers and the Bays of Morlaix and Lannion.

2. Heavily impacted intertidal areas such as St. Efflam and the salt marsh at Ile Grande.

3. The detailed chemical evolution of the petroleum hydrocarbons.

4. Biodegradation of petroleum.

The second meeting of the Joint Commission, held in Washington, D.C., on October 12, 1978, reviewed the work carried out during the first months of the first year and planned the research program for the second year's study.

In November 1979, an international conference was held in Brest sponsored by CNEXO. Investigators sponsored by the Joint NOAA/CNEXO Scientific Commission, as well as a number of other scientists, gave papers at this conference. The proceedings of this conference entitled "Amoco Cadiz: Fates and Effects of the Oil Spill" make a very good summary of the first one and one-half year study after the spill.

Following the second meeting of the Joint Commission, Dr. Hess left NOAA and was replaced as co-chairman by Dr. Joseph W. Angelovic from the Office of Ocean Programs in NOAA.

The third meeting of the Joint Commission was held in Paris, France, October 28, 1980, in conjunction with the meeting of the U.S.-French Cooperative Program in Oceanography. The previous work was reviewed and the final year of the research program was planned.

Now the three-year study is over and attempts are being made to bring together the findings of the investigators. A workshop was held in Charleston, South Carolina, on September 17-18, 1981, to report on the physical and chemical studies. A second workshop was held in Brest, France, on October 28-30, 1981, to report on the biological effects studies. This document is the report of those workshops and forms the body of the final report to Amoco from the Joint NOAA/CNEXO Scientific Commission.

Speaking for all who worked on the spill, we would like to thank the Amoco Transport Company for sponsoring this three-year study of the effects of the spill. Without Amoco's help, we would be nowhere near our present state of knowledge of what the effects of the spill were or how the recovery back to normal conditions has proceeded. Other studies have been carried out, sponsored by the French Government and other sources, but an important part of the work has been sponsored by Amoco.

Mr. Russ Mallatt, Dr. James Marum, Mr. John Lamping, Ms. Carol Cummings and others from Amoco attended meetings of the Joint Commission and the scientific sessions. They were always helpful and supportive of the Commission's work and never intruded on the design or conduct of the program.

We have, through this cooperative effort, obtained more detailed and more useful knowledge of the effects of this oil spill than of any other large oil spill in history. A major reason for this is that the biological communities present before the spill had been studied in great detail by French scientists.

Today many of the areas impacted by the spill appear to the casual observer to be recovered from the effects of the oil. However, investigations have shown that differences still exist between some of the current ecosystems and those present prior to the spill. Hopefully other studies will continue to watch and document the recovery processes.

These studies have added substantially to man's knowledge about oil spills. We can only hope that others will follow and build on the understanding of oil spill effects accumulated through these studies.

Lucien Laubier
Wilmot Hess
Joseph Angelovic

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PART I

Physical, Chemical, and Microbiological Studies After the AMOCO CADIZ Oil Spill

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MICROBIAL HYDROCARBON DEGRADATION WITHIN SEDIMENT IMPACTED BY THE AMOCO CADIZ OIL SPILL

by

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INTRODUCTION

The wreck of the AMOCO CADIZ in March 1978 released over 210,000 tons of oil into the marine environment. As much as one third of the spilt oil may have been washed into the intertidal zone. The spill occurred during storm surges, thereby spreading the oil throughout the intertidal zone. Two years after the AMOCO spill, the wreck of the tanker TANIO resulted in another oil spill that contaminated much of the same Brittany shoreline impacted by the AMOCO CADIZ. This study was undertaken to determine the fate of petroleum hydrocarbons within surface sediments along the Brittany coast with reference to the role of microorganisms in the oil weathering process.

METHODS

Sampling Regime

Duplicate samples were collected at intertidal sites along the Brittany coast which had received varying degrees of oiling from the AMOCO CADIZ spillage (Fig. 1). The sampling sites included the salt marsh at Ile Grande, a beach near Portsall in the vicinity of the wreck site, a mudflat in Aber Wrac'h, a beach at St-Michel-en-Grève near where a large bivalve kill had been reported, a relatively lightly oiled reference site at Trez Hir and a site at Tregastel which was not oiled by the AMOCO CADIZ spill, but was later oiled by the spill from the tanker TANIO (Table 1). Surface sediment samples (upper 5 cm) were collected with a 3 cm diameter soil corer.

Samples were placed in metal cans for hydrocarbon analyses and in Whirlpak bags for microbial analyses. Samples were collected during December, 1978; March, 1979; August, 1979, November, 1979, March 1980, July, 1980 and June, 1981; 9, 12, 17, 20, 24, 28 and 39 months after the spillage, respectively. During November, 1979 sediment samples were also collected at four offshore sites in the Bay of Morlaix.

Samples for microbiological analyses were processed within four hours of collection. For hydrocarbon analyses, samples were frozen and shipped to Energy Resources Company (ERCO) for extraction and analysis

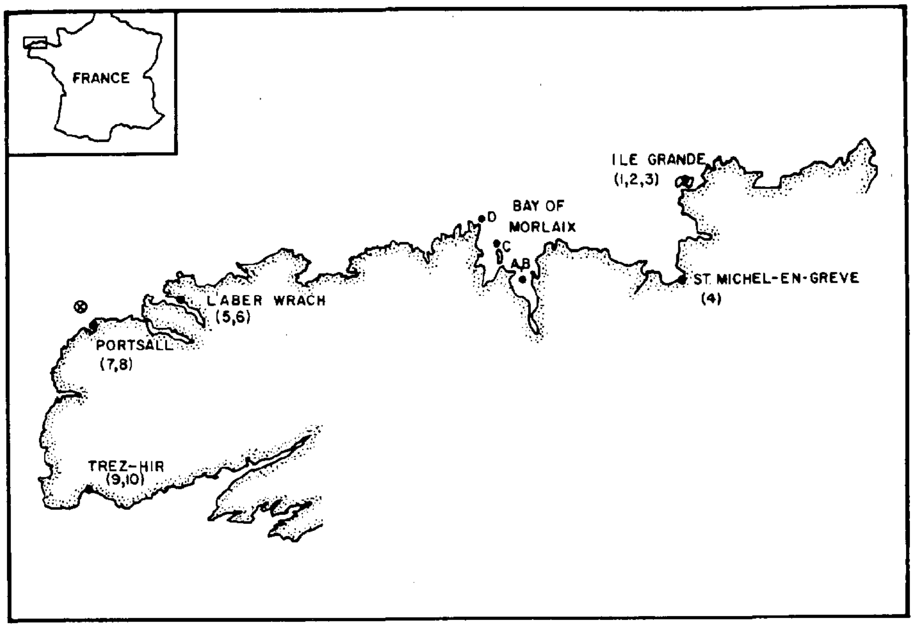


FIGURE 1. Location of intertidal and subtidal sampling sites.

TABLE 1 - Description of sampling sites.

Site	Description
1	Ile Grande - sandy - low energy - NE of bridge - relatively unoiled.
2	Ile Grande - sandy - low energy - SW of bridge - near end of excavation area.
3	Ile Grande - soil - heavily oiled - amid Juncus - above excavation area.
4	St-Michel-en-Grève - sandy - high energy - near low tide mark.
5	Aber Wrac'h - mud - 100m offshore at Perros.
6	Aber Wrac'h - mud - 200m offshore at Perros.
7	Portsall - sandy - high energy - near wreck site - below high tide line.
8	Portsall - sandy - high energy - near wreck site - near rocks - 100m below high tide line.
9	Trez Hir - sandy - moderate energy - reference site - below high tide.
10	Trez Hir - sandy - moderate energy - reference site - 20m below high tide line.
11	Tregastel - sandy - low energy - Tanio spill site - 20m below high tide line.
12	Tregastel - sandy - low energy - Tanio spill site - 50m below high tide line.

by silica gel column chromatography, weight determination, glass capillary gas chromatography and mass spectrometry.

Enumeration of Microbial Populations

Total numbers of microorganisms per gram dry weight of sediment were determined by direct count procedures. Portions of collected sediment samples were preserved with formalin. Microorgansims in the preserved samples were collected on a 0.2 mm pore size Nuclepore filter which had been stained with irgalan black. The microorganisms were stained with acridine orange and viewed using an Olympus epifluorescence microscope. Cells staining orange or green were counted in 20 randomly selected fields and the mean concentration determined.

Hydrocarbon utilizing microorganisms were enumerated using a three tube Most Probable Number (MPN) procedure. Serial dilutions of sediment samples, prepared using Rila marine salts solutions, were inoculated into sealed serum vials containing 10 ml Bushnell Haas broth (Difco) and 50 ml of Arabian crude oil spiked with ^{14}C hexadecane (sp. act. 1 mCi/ml). After 14 days incubation at 15°C, the $^{14}CO_2$ (if any) in the head space was collected by flushing and trapping in oxifluor CO_2 and quantitated by liquid scintillation counting. Vials showing $^{14}CO_2$ production (counts significantly above background) were scored as positive and the Most Probable Number of hydrocarbon utilizers per gram dry weight calculated from standard MPN tables.

Biodegradation Potentials

Portions of sediment samples were placed into serum vials containing 10 ml Bushnell Haas broth and 50 ml light Arabian crude oil spiked with either ^{14}C hexadecane, ^{14}C pristane, ^{14}C naphthalene, ^{14}C benzanthracene or ^{14}C 9-methylanthracene. After 14 days incubation, microbial hydrocarbon degrading activities were stopped by addition of KOH. The $^{14}CO_2$ produced from mineralization of the radiolabelled hydrocarbon was determined by acidifying the solution, flushing the headspace, trapping the $^{14}CO_2$ in oxifluor CO_2 and quantitating the ^{14}C by liquid scintillation counting. The residual undegraded hydrocarbons and biodegradation products were recovered by extraction with hexane. The ^{14}C in each solvent extract was determined and fractionated, using silica gel column chromatography, into undegraded hydrocarbon fractions (hexane + benzene eluates) and degradation product fractions (methanol eluate + residual non-eluted counts). A 0.75 cm diameter X 10 cm column packed with 70-230 mesh silca gel 60 was used. Radiolabelled material in each fraction was quantitated by liquid scintillation counting. Sterile controls were used to correct for efficiency of recovery and fractionation. Triplicate determinations were made for each sample and radiolabelled hydrocarbon substrate combination. The percent hydrocarbon mineralization was calculated as $^{14}CO_2$ produced (above sterile control)/^{14}C hydrocarbon added. The percent hydrocarbon biodegradation was calculated as $^{14}CO_2$ produced + ^{14}C methanol fraction + ^{14}C residual (all above sterile control)/^{14}C hydrocarbon added. Carbon balances generally accounted for approximately 90% of the radiolabelled carbon added to the sediment (except for naphthalene where volatility losses prevented efficient recovery).

Chemical Hydrocarbon Analyses Performed at ERCO

For hydrocarbon analyses the samples were thawed, dried with methanol and extracted by high energy shaking with a mixture of methylene chloride-methanol (9:1). The extract was fractionated into an aliphatic (f_1) fraction and an aromatic (f_2) fraction using silica gel/alumina column chromatography. A 1 cm diameter X 25 cm column (1 cm alumina on top of 15 cm silica gel) was used. The f_1 fraction was eluted with 18 ml hexane; the f_2 fraction subsequently was eluted with 21 ml of a 1:1 mixture of hexane-methylene chloride. After reducing the volume of solvent by evaporation, the gross amount (weight) of hydrocarbon in each fraction was determined gravimetrically from an evaporated and dried aliquot of the extract. The extracts were subjected to quantitative glass capillary-gas chromatographic (GC) analysis. Selected aromatic fractions also were analysed by combined glass capillary gas chromatographic/mass spectrometric (GC/MS) analysis for qualitative identification of individual compounds and quantification of minor components. Participation in an intercalibration exercise under the direction of the National Analytical Laboratory indicated that these analyses were at the state of the art with repeatable ± 20% detection of hydrocarbons in the ng g^{-1} dry weight sediment range. The details of GC and GC/MS analysis employed are as follows:

GC: Hewlett Packard 5840A reporting GC with glass; splitless injection inlet system; 30 m glass capillary column coated with SE-30 (≅ 100,000 theoretical plates); FID detector; temperature programmed at 60-275°C min^{-1}; helium carrier gas 1 ml min^{-1}; transmission of integrated peak areas and retention time through HP 18846A digital communications interface to a PDP-10 computer for storage, retention index and concentration calculations. Deuterated anthracene (f_2) and androstane (f_1) were used as internal standards and response factors were determined with known concentrations of the reported compounds. GC analysis was used to quantitate components of the f_1 fraction.

GC/MS: Hewlett Packard 5985 quadrapole system (GC/MS Computer); mass spectrometer conditions: ionization voltage=70 eV, electron multiplier voltage=2200 V, scan conditions 40 amu to 500 amu at 225 amu s^{-1}. Quantification of components of the f_2 fraction was accomplished by mass fragmentography wherein the stored GC/MS data is scanned for parent ions (m^+). The tabulated total ion currents for each parent ion is compared with deuterated anthracene (internal standard) and an instrumental response factor applied. Authentic polynuclear aromatic hydrocarbon standards were used to determine relative response factors (when no standard was available a response factor was assigned by extrapolation).

In vitro Biodegradation

Sediment was collected at sites 6 and 7 in November, 1979 for _in vitro_ biodegradation experiments. Replicate one hundred gram portions of sediment were placed into 250 ml flasks to which 50 ml of a sterile solution containing 0.5% KNO_3 + 0.5% KH_2PO_4 and 0.5 ml of light Arabian crude oil were added. The flasks were agitated on a rotary shaker at

100 RPM. After two, four, and six weeks of incubation at 15°C, the oil remaining in replicate flasks (two at each sampling time) was extracted and analysed as described below.

Additionally, replicate 100 g portions of sediment were placed into 1 liter stainless steel buckets. The containers were continuously flushed with a solution of Rila marine salts supplemented with 10 ppm KNO_3 + 10 ppm KH_2PO_4. The height of the water level was adjusted to be 3 cm above the surface of the sediment layer. The flow rate was adjusted to 10 ml/h. After two, four, and six weeks of incubation at 15°C the oil remaining in replicate sediment portions was extracted and analysed as described below.

Analyses of <u>in vitro</u> Experiments

Residual oil was recovered from samples by extraction with sequential portions of diethyl ether and methylene chloride. The sediment was shaken at 200 RPM with repetitive portions of solvent. The extracts were subjected to column chromatography to split the extracts into aliphatic (f_1) and aromatic (f_2) fractions. Columns were prepared by suspending silica gel 100 (E. M. Reagents, Darmstadt, W. Germ.) in CH_2Cl_2 and transferring the suspension into 25 ml burets with teflon stopcocks to attain a 15 ml silica gel bed. The CH_2Cl_2 was washed from the columns with three volumes of pentane. Portions of the extracts in pentane were applied to the columns, drained into the column bed, and allowed to stand for three to five minutes. The aliphatic fraction (f_1) was eluted from the column with 25 ml pentane. After 25 ml pentane had been added to the column, 5 ml of 20% (v/v) CH_2Cl_2 in pentane was added and allowed to drain into the column bed. Fraction f_1 was 30 ml. The aromatic fraction (f_2) was eluted from the column with 45 ml of 40% (v/v) CH_2Cl_2 in pentane.

The fractions of each extract were then concentrated to about 5 ml at 35°C and transferred quantitatively to clean glass vials. Fractions f_1 and f_2 were prepared for analysis by gas chromatography or gas chromatography mass spectrometry. An internal standard, hexamethyl benzene (Aldrich Chem. Co., Milwaukee, WI.), was added to each sample. In fraction f_1, hexamethyl benzene (HMB) was present at 12.6 ng/ml; in fraction f_2, HMB was present at 25.2 ng/ml.

Fraction f_1 was analyzed by GC on a Hewlett-Packard 5840 reporting GC with FID detector. The column was a 30 m, SE54 grade AA glass capillary (Supelco, Bellefonte, PA.). Conditions for chromatography were injector, 240°C; oven 70°C for 2 min. to 270°C at 4°C/min. and hold for 28 min.; FID, 300°C; and carrier, He at 25 cm/sec. A valley-valley intergration function was used for quantitative data acquisition. Response factors were calculated using <u>n</u>-alkanes, (C_{10}-C_{28}), pristane and phytane standards.

Fraction f_2 was analyzed with a Hewlett-Packard 5992A GC-MS. Conditions for chromatography were injector, 240°C; oven 70°C for 2 min. to 270°C at 4°C/min. and hold for 18 min. Data was acquired using a selected ion monitor program. Thirteen ions were selected for representative aromatic compounds. The ions monitored were 128, 142, 147, 156, 170, 178, 184, 192, 198, 206, 212, 220, and 226. The representative compounds were naphthalene, methyl naphthalene, HMB as an interanal standard, dimethyl naphthalene, trimethyl naphthalene, phenanthrene, dibenzothiophene, methyl phenanthrene, methyl dibenzothiophene, dimethyl phenanthrene, dimethyl dibenzothiophene, trimethyl phenanthrene, and trimethyl dibenzothiophene, respectively. The dwell time per ion was 10 msec. Instrument response factors were calculated by injecting known quantities of unsubstituted and C_1 and C_2 substituted authentic aromatic hydrocarbons and determining the integrated response for each compound. These values were used to extrapolate for quantitation of isomers and C_3 substituted compounds.

For analysis of the polar fraction including microbial degradation products, three samples were selected for analysis by the University of New Orleans Center for Bio-organic Studies. The samples were: 1) flow through, 6 week incubation from site 6; 2) flow through, 6 week incubation from site 7; 3) agitated flask, 6 week incubation from site 7. Frozen samples were sent for analysis. At the Center for Bio-organic Studies the samples were extracted with successive portions of CH_3OH, CH_3OH/CH_2Cl_2 and CH_2Cl_2. The extracts were fractionated using silica gel and the f_3 fraction was collected, methylated and analysed by high resolution GC-MS.

RESULTS AND DISCUSSION

The enumeration of hydrocarbon utilizing microorganisms indicated that numbers of hydrocarbon utilizers in the intertidal sediments increased significantly in response to hydrocarbon inputs (Table 2). Site 3, which is covered with seawater only at times of extreme high tide, showed very high populations of hydrocarbon utilizing microorganisms even three years after the AMOCO CADIZ spillage. Sites 5 and 6 (located within Aber Wrac'h) and Sites 7 and 8 (located near Portsall) showed variable, but apparently elevated, numbers of hydrocarbon utilizers for up to two years following the spill. It appears that hydrocarbons contained within the mud sediments of Aber Wrac'h continued to exert a selective pressure on the microbial community that favored elevated populations of hydrocarbon utilizers for a longer period of time than sites on high-energy sand beaches. Site 2 showed evidence that the TANIO spill impacted the Ile Grande region. This site did not show elevated numbers of hydrocarbon utilizers in December 1978 or at later sampling times as a result of the AMOCO CADIZ spill, but in July of 1980, several months after the wreck of the TANIO, numbers of hydrocarbon utilizers were greatly elevated. A year later, however, the numbers of hydrocarbon utilizers had returned to background levels at this site. The unoiled control sites 9 and 10 and sites 1 and 4, which were impacted by the AMOCO CADIZ spill, did not show any evidence of elevated hydrocarbon-utilizing populations during the sampling period. Similarly, the offshore sites A-D in the Bay of Morlaix did not appear to be elevated at the time of sampling in November 1979. Sites 11 and 12 were added following the wreck of the TANIO and showed obviously elevated populations of hydrocarbon utilizers that persisted for over a year.

TABLE 2. MPN-Hydrocarbon Utilizers.

(# X 10^3/g dry wt.)

Site	2-78	3-79	8-79	11-79	3-80	7-80	6-81
1	0.2	0.5	1	0.7	5	16	1
2	5	7	1	14	30	45000	1
3	2200	14000	41000	13000	160000	48000	24000
4	2	0.4	2	7	1	4	5
5	8	18	8	450	19	10	15
6	9	390	20	27	190	11	17
7	40	1900	1	2	12	2	2
8	57	350	150	8	3	1	10
9	0.7	0.4	3	1	4	1	4
10	0.1	0.2	4	1	2	2	3
11	-	-	-	-	19000	140000	24000
12	-	-	-	-	920000	140000	24000
A	-	-	-	66	-	-	-
B	-	-	-	32	-	-	-
C	-	-	-	13	-	-	-
D	-	-	-	13	-	-	-

The elevation in hydrocarbon utilizing populations, when detected, represented a shift within the microbial community. There generally was no evidence that total microbial biomass increased as a result of oiling although there generally was a tenfold variation in the microbial biomass between different sampling times (Table 3).

TABLE 3. Direct Count.

(# X 10^8/g dry wt.)

Site	12-78	3-79	8-79	11-79	3-80	7-80	6-81
1	3	1	4	3	1	1	3
2	4	2	16	7	3	40	2
3	10	6	220	18	24	40	38
4	2	1	3	0.4	0.5	0.6	2
5	3	2	19	12	17	2	15
6	6	7	150	20	27	24	26
7	3	1	8	1	1	1	2
8	1	4	1	1	2	2	4
9	1	0.5	2	1	0.3	1	4
10	0.5	0.4	13	1	0.4	2	7
11	-	-	-	-	5	1	4
12	-	-	-	-	39	36	40
A	-	-	-	15	-	-	-
B	-	-	-	16	-	-	-
C	-	-	-	3	-	-	-
D	-	-	-	10	-	-	-

The microbial hydrocarbon biodegradation potential measurements showed that following the AMOCO CADIZ oil spillage, indigenous microbial populations in the sediment at all sampling sites were capable of degrading both aliphatic and aromatic components of crude oil (Tables 4-8). The variability in the results is not indicated in these tables, but the standard error was less than 4% for the percentage degraded and less than 10% for the percentage mineralized in all cases. The biodegradation potentials indicated that n-alkanes were preferentially degraded and that pristane was degraded at approximately half the rate of n-hexadecane. For aliphatic hydrocarbons approximately 30% of the amount of hydrocarbon biodegraded was converted to CO_2 (mineralized). Methodological difficulties in handling naphthalene made it difficult to assess the true extent of biodegradation for this compound. It is apparent, though, that the indigenous microbial populations were capable of degrading light aromatic hydrocarbons. The rates of degradation of the 3- and 4-ringed polynuclear aromatic hydrocarbons were lower than for branched and straight chained aliphatic hydrocarbons. In the case of the polynuclear aromatic hydrocarbons, a very low proportion of the amount of hydrocarbon degraded was converted to CO_2.

TABLE 4. Hexadecane biodegradation showing % degraded and (% mineralized).

Site	12-78	3-79	8-79	11-79	3-80	7-80	6-81
1	40	41	21	17	10	25	17
	(8)	(11)	(1)	(15)	(2)	(12)	(10)
2	43	38	26	22	25	19	6
	(11)	(13)	(8)	(13)	(17)	(12)	(7)
3	45	46	29	23	51	19	33
	(15)	(15)	(8)	(18)	(39)	(14)	(26)
4	36	48	21	25	8	26	17
	(14)	(13)	(7)	(14)	(6)	(19)	(10)
5	42	46	25	35	32	24	23
	(14)	(14)	(13)	(14)	(20)	(18)	(15)
6	34	47	29	26	36	18	20
	(11)	(12)	(11)	(20)	(18)	(11)	(13)
7	31	45	13	31	2	20	28
	(10)	(13)	(3)	(21)	(1)	(14)	(19)
8	40	43	21	35	3	17	34
	(15)	(11)	(5)	(19)	(2)	(12)	(20)
9	28	32	22	35	7	27	34
	(12)	(3)	(3)	(14)	(3)	(15)	(24)
10	37	30	21	45	8	22	25
	(10)	(3)	(10)	(32)	(3)	(14)	(17)

TABLE 5. Pristane biodegradation showing % degraded and (% mineralized).

Site	12-78	3-79	8-79	11-79	3-80	7-80	6-81
1	18	22	12	17	18	17	27
	(3)	(3)	(1)	(3)	(2)	(3)	(3)
2	23	22	16	15	17	24	24
	(3)	(4)	(3)	(5)	(3)	(3)	(1)
3	19	21	16	14	18	20	24
	(2)	(4)	(3)	(5)	(5)	(3)	(6)
4	26	23	16	18	17	19	20
	(3)	(4)	(2)	(4)	(1)	(3)	(2)
5	21	28	21	17	16	22	25
	(3)	(6)	(4)	(5)	(4)	(3)	(6)
6	21	30	19	19	17	20	21
	(3)	(6)	(3)	(5)	(4)	(4)	(3)
7	25	24	16	25	12	23	23
	(3)	(4)	(1)	(4)	(1)	(2)	(4)
8	31	23	21	18	20	21	23
	(3)	(4)	(1)	(5)	(1)	(2)	(4)
9	27	20	22	19	17	22	24
	(3)	(2)	(1)	(5)	(2)	(2)	(7)
10	29	-	21	20	18	21	20
	(2)	(-)	(3)	(5)	(2)	(2)	(8)

TABLE 6. Biodegradation of naphthalene showing % degraded and (% mineralized).

Site	3-79	8-79	11-79	3-80
1	3(2)	2(1)	3(31)	1(1)
2	9(7)	5(3)	2(2)	2(2)
3	12(10)	5(3)	2(2)	6(6)
4	7(6)	1(1)	5(5)	1(1)
5	8(6)	1(1)	7(7)	7(7)
6	11(10)	1(1)	1(1)	2(2)
7	10(9)	1(1)	6(6)	1(1)
8	9(7)	1(1)	7(7)	1(1)
9	1(1)	2(1)	1(1)	1(1)
10	-	2(1)	1(1)	1(1)

TABLE 7. Biodegradation of 9-methylanthracene showing % degradation and (% mineralization).

Site	3-79	8-79	11-79	3-80	7-80	6-81
1	10	-	1	5	6	9
	(0)	(0)	(0)	(0)	(0)	(0)
2	19	8	6	8	10	9
	(0)	(0)	(0)	(0)	(0)	(0)
3	18	18	6	7	7	10
	(0)	(0)	(0)	(0)	(0)	(0)
4	23	2	2	1	10	6
	(0)	(0)	(0)	(0)	(0)	(0)
5	17	4	2	7	21	6
	(0)	(0)	(0)	(0)	(0)	(0)
6	19	1	3	4	11	5
	(0)	(0)	(0)	(0)	(0)	(0)
7	15	7	3	2	9	5
	(0)	(0)	(0)	(0)	(0)	(0)
8	21	2	4	1	6	5
	(0)	(0)	(0)	(0)	(0)	(0)
9	15	11	1	4	11	4
	(0)	(0)	(0)	(0)	(0)	(0)
10	-	6	3	13	5	4
	(-)	(0)	(0)	(0)	(0)	(0)

TABLE 8. Biodegradation of benzanthracene showing % degradation and (% mineralization).

Site	12-78	3-79	8-79	11-79	3-80	7-80	6-81
1	5	21	18	2	5	10	13
	(0)	(0)	(0)	(0)	(0)	(0)	(0)
2	8	17	10	-	4	2	6
	(0)	(0)	(0)	(0)	(0)	(0)	(0)
3	11	15	7	7	8	2	12
	(0)	(0)	(0)	(-)	(0)	(0)	(0)
4	4	5	6	2	6	3	5
	(0)	(0)	(0)	(0)	(0)	(0)	(0)
5	8	13	14	2	10	11	8
	(0)	(0)	(0)	(0)	(0)	(0)	(0)
6	4	8	11	2	4	1	6
	(0)	(0)	(0)	(0)	(0)	(0)	(0)
7	11	3	6	7	4	3	6
	(0)	(0)	(0)	(0)	(0)	(0)	(0)
8	6	5	5	4	1	7	4
	(0)	(0)	(0)	(0)	(0)	(0)	(0)
9	2	8	5	7	2	13	5
	(0)	(0)	(0)	(0)	(0)	(0)	(0)
10	1	-	14	8	11	12	4
	(0)	(-)	(0)	(0)	(0)	(0)	(0)

Based on the changes in the composition of the microbial community, as evidenced by elevations in numbers of hydrocarbon utilizing microorganisms and based on the microbial biodegradation potentials, it can be stated that biodegradation appears to have been a very important process that had the potential for significantly altering the composition of the hydrocarbon mixture that impacted the sediments of the Brittany Coast following the AMOCO CADIZ spill. With time the residual hydrocarbon mixture should contain increasingly high proportions of complexed branched and condensed-ring hydrocarbon compounds that are degraded relatively slowly by the indigenous microorganisms.

The weight of the extractable hydrocarbons confirmed the occurrence of contaminating hydrocarbons at site 2 in July 1980, presumably as a result of the TANIO spillage (Table 9). Similarly, high concentrations of hydrocarbons were found in at Sites 11 and 12 which were closer to the TANIO wreck. The levels of hydrocarbons at Site 3 remained high throughout the sampling program. Sites 1, 4, 9, and 10 showed a general lack of significant hydrocarbon concentration that would be indicative of petroleum pollution. Sites 5, 6, 7, and 8, in contrast, showed somewhat elevated hydrocarbon concentrations.

TABLE 9. Weights of extractable hydrocarbons (ug/g).

Site		12-78	3-79	8-79	11-79	3-80	7-80	6-81
1	f_1	27	9	5	17	8	20	4
	f_2	57	5	16	58	37	25	22
2	f_1	52	21	42	147	50	2370	9
	f_2	53	11	47	136	50	2160	29
3	f_1	272	2232	121092	108000	33000	16600	3680
	f_2	338	2537	70329	95833	22500	32400	8080
4	f_1	21	22	9	20	35	12	8
	f_2	57	17	11	19	29	17	49
5	f_1	122	140	56	213	65	68	21
	f_2	103	82	99	233	73	156	42
6	f_1	178	458	177	536	874	109	56
	f_2	226	416	209	556	830	281	75
7	f_1	91	72	152	80	58	23	15
	f_2	75	59	123	63	39	26	29
8	f_1	179	382	164	243	98	34	31
	f_2	148	298	135	194	83	24	59
9	f_1	7	32	77	20	21	43	13
	f_2	3	34	78	32	21	36	25
10	f_1	8	29	13	36	23	23	21
	f_2	11	20	29	96	31	48	74
11	f_1	-	-	-	-	515000	60800	320
	f_2	-	-	-	-	512000	36300	440
12	f_1	-	-	-	-	-	73200	67
	f_2	-	-	-	-	-	14300	109
A	f_1	-	-	-	102	-	-	-
	f_2	-	-	-	88	-	-	-
B	f_1	-	-	-	210	-	-	-
	f_2	-	-	-	210	-	-	-
C	f_1	-	-	-	13	-	-	-
	f_2	-	-	-	21	-	-	-
D	f_1	-	-	-	21	-	-	-
	f_2	-	-	-	10	-	-	-

The detailed gas-chromatographic and mass-spectral analyses of the samples collected at each site indicated a lack of significant petroleum hydrocarbons throughout the study at Sites 1, 4, 9, and 10 (Tables 10, 13, 18, 19). Site 2 showed some evidence of weathered hydrocarbons in 1978 and a significant input of fresh petroleum hydrocarbons in July 1980 (Table 11). Site 3 had significant concentrations of weathered petroleum origin throughout the study (Table 12). Sites 5 and 6 showed an alteration of the hydrocarbon mixture with time that indicated the occurrence of biodegradation (Tables 14, 15). Samples at Sites 7 and 8 continued to show the presence of a relatively unweathered hydrocarbon mixture up to two years following the AMOCO CADIZ spill (Tables 16, 17). It appears that undegraded hydrocarbons were seeping into the surface sediments at Site 8 and it is postulated that either shifts in the sediment were repeatedly exposing hydrocarbons that had been protected from microbial degradation and/or that some oil continued to be washed ashore from the sunken AMOCO CADIZ vessel. Site 11 showed clear evidence of heavy oiling from the TANIO spill which persisted for a year following the spill (Table 20). The offshore sites sampled in November 1979 in the Bay of Morlaix failed to show the presence of AMOCO CADIZ oil.

TABLE 10. Hydrocarbon concentration ng/g.

SITE 1

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	0	0	1	2	0	-	2
15	20	0	125	250	23	1	123
16	2	0	14	12	3	3	9
17	65	15	438	86	156	5	91
pristane	62	22	27	76	43	30	102
18	3	2	3	5	8	4	10
phytane	11	2	2	5	6	3	2
19	0	2	2	3	7	3	5
20	5	2	2	4	7	3	4
21	6	1	3	4	8	2	7
22	6	2	3	4	8	3	9
23	9	2	4	6	9	5	18
24	9	2	3	5	8	2	24
25	17	2	5	14	9	13	34
26	8	2	2	2	6	3	28
27	10	1	4	10	7	6	35
28	7	1	1	2	5	2	21
29	18	5	6	10	10	18	38
30	8	1	7	6	3	4	35
alkanes: isoprenoids	1.2	0.9	20.0	2.3	5.6	6.0	2.3
pristane: phytane	5.6	7.8	13.5	-	7.3	1.6	50.0

TABLE 11. Hydrocarbon concentration ng/g.

SITE 2

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	0	3	6	0	0	11700	3
15	44	115	331	154	39	18200	111
16	0	11	18	0	4	19800	11
17	65	40	169	68	18	22000	93
pristane	27	38	160	1100	6	19800	46
18	8	9	8	181	5	22600	10
phytane	17	126	32	151	15	25900	15
19	30	26	5	35	2	23400	6
20	18	16	9	9	8	18600	9
21	14	40	18	15	5	16700	13
22	16	29	7	10	9	12800	14
23	14	11	8	38	8	10900	23
24	12	11	7	6	13	10000	22
25	45	70	30	64	146	7120	35
26	22	12	6	10	16	6450	30
27	34	15	23	36	24	6320	33
28	53	40	6	29	10	4780	15
29	51	47	9	39	53	6040	36
30	31	95	71	116	55	4380	20
alkanes: isoprenoids	-	1.5	2.5	0.3	3.2	1.3	3.5
pristane: phytane	0.4	0.8	5.1	7.3	0.4	0.8	3.0

TABLE 12. Hydrocarbon concentration ng/g.

SITE 3

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	19	-	3525	1040	1390	-	-
15	-	-	11025	0	633	-	-
16	17	121	9350	0	400	-	-
17	32	12	9550	3440	200	-	-
pristane	213	809	145150	47900	104	-	-
18	48	86	20250	3600	106	-	-
phytane	985	3088	275900	130000	673	-	-
19	255	948	63075	29200	283	825	-
20	149	247	36000	11900	0	-	1270
21	31	241	17975	6210	508	2103	2380
22	25	12	8875	1440	130	-	3050
23	38	56	11100	0	0	-	3280
24	54	48	7925	3510	0	-	4640
25	-	-	12600	21300	2340	-	5700
26	-	160	14900	2540	329	-	5190
27	172	898	45250	0	1160	516	10800
28	81	934	18600	0	109	-	2460
29	41	2025	31250	2090	3330	1692	12120
30	-	400	75775	0	118	13900	3120
alkanes: isoprenoids	0.1	-	0.1	0.1	0.8	-	-
pristane: phytane	0.5	0.3	0.5	0.4	0.2	-	1.9

TABLE 13. Hydrocarbon concentration ng/g.

SITE 4

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	0	0	0	0	-	1	2
15	0	2	0	0	3	13	8
16	0	2	0	0	5	8	7
17	18	4	4	4	12	15	8
pristane	60	6	0	2	3	4	3
18	37	3	0	2	10	6	8
phytane	153	18	0	8	8	6	11
19	68	9	2	5	9	7	6
20	37	10	2	3	8	7	6
21	30	8	4	4	12	5	9
22	21	5	1	3	7	1	8
23	27	6	2	6	8	7	17
24	20	4	0	3	5	3	17
25	23	6	1	3	14	3	27
26	42	7	0	3	4	6	16
27	37	7	10	7	17	11	48
28	60	9	3	3	3	3	7
29	47	19	9	18	28	22	61
30	43	1	30	1	50	2	12
alkanes: isoprenoids	0.2	0.7	-	0.6	2.8	4.1	2.3
pristane: phytane	0.4	0.4	-	-	0.4	0.6	0.3

TABLE 14. Hydrocarbon concentration ng/g.

SITE 5

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	19	37	2	16	-	5	3
15	20	65	167	59	11	35	29
16	-	-	18	0	-	12	8
17	25	112	122	0	95	18	585
pristane	24	150	11	885	8	175	108
18	16	50	17	0	4	2	4
phytane	158	293	43	158	12	36	20
19	64	140	12	16	4	3	8
20	43	5	17	19	16	7	9
21	57	50	23	208	8	13	17
22	39	18	19	14	6	18	11
23	25	36	23	30	20	18	20
24	4	30	15	18	9	13	20
25	22	5	39	115	99	111	100
26	46	5	11	24	21	11	21
27	51	93	30	87	48	29	62
28	70	64	6	13	7	13	10
29	96	136	17	113	130	86	122
30	17	57	80	10	20	0	23
alkanes: isoprenoids	0.3	0.5	5.1	0.3	5.5	2.7	4.4
pristane: phytane	0.2	0.5	0.3	12.0	-	0.7	5.4

TABLE 15. Hydrocarbon concentration ng/g.

SITE 6

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	-	82	0	10	-	4	2
15	8	61	28	111	68	28	30
16	-	-	0	0	-	9	9
17	23	117	14	164	200	6	101
pristane	102	125	15	71	70	0	12
18	-	8	0	28	-	6	7
phytane	360	489	135	289	225	23	22
19	101	121	20	20	-	0	15
20	35	12	0	42	-	2	10
21	86	180	59	223	35	31	30
22	-	149	4	48	38	9	6
23	39	58	16	81	80	28	61
24	5	-	5	37	27	5	10
25	109	159	80	128	484	47	85
26	128	151	10	50	53	6	30
27	68	126	88	135	255	48	124
28	97	56	13	105	28	12	21
29	159	319	55	200	590	122	245
30	36	140	254	342	30	18	55
alkanes: isoprenoids	0.1	0.3	0.2	0.8	0.9	1.5	2.7
pristane: phytane	0.3	0.3	0.1	0.3	-	0.0	0.5

TABLE 16. Hydrocarbon concentration ng/g.

SITE 7

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	9	4	9	0	2	14	43
15	7	7	43	11	11	29	77
16	51	10	58	18	32	29	64
17	109	20	96	27	63	38	85
pristane	154	24	102	25	47	35	7
18	121	33	113	36	39	41	64
phytane	256	65	159	43	86	43	39
19	54	46	139	55	44	47	65
20	122	36	124	23	56	35	67
21	83	25	106	30	49	29	42
22	108	24	94	30	58	0	45
23	84	23	86	28	59	24	43
24	61	18	80	46	47	24	54
25	111	27	89	42	62	25	32
26	105	27	62	25	42	22	35
27	54	20	110	48	30	17	35
28	126	42	52	15	28	14	17
29	92	28	62	28	49	26	44
30	112	36	356	152	29	37	29
alkanes: isoprenoids	0.6	0.7	1.0	1.4	1.1	1.6	3.3
pristane: phytane	0.6	0.4	0.6	0.6	0.6	0.8	0.2

TABLE 17. Hydrocarbon concentration ng/g.

SITE 8

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	160	34	16	24	-	8	12
15	368	77	66	73	7	21	80
16	518	87	47	73	22	23	29
17	640	113	80	104	44	31	64
pristane	941	427	98	135	41	38	108
18	818	219	70	108	50	34	40
phytane	1839	955	116	171	74	54	46
19	1122	352	85	158	57	54	42
20	849	203	81	97	51	35	29
21	530	121	62	66	44	28	27
22	430	135	50	64	38	24	30
23	314	85	47	59	34	32	36
24	272	85	42	68	30	43	21
25	54	190	52	42	56	18	25
26	489	234	33	46	28	19	28
27	328	197	71	31	26	41	22
28	431	292	24	19	11	12	11
29	362	326	15	30	63	27	33
30	315	411	170	10	218	14	29
alkanes: isoprenoids	0.7	0.3	1.1	1.1	1.0	1.1	1.2
pristane: phytane	0.5	0.4	0.8	0.8	0.6	0.7	2.3

TABLE 18. Hydrocarbon concentration ng/g.

SITE 9

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	0	0	0	0	-	28	-
15	0	0	3	8	-	42	-
16	0	0	3	3	-	21	-
17	3	11	5	22	8	36	19
pristane	1	4	6	2	-	0	1
18	3	6	3	5	6	9	9
phytane	2	4	2	3	-	0	5
19	4	3	4	4	8	6	12
20	4	3	0	6	11	6	12
21	4	6	4	6	11	4	15
22	4	7	3	5	9	6	12
23	4	9	3	10	8	7	19
24	4	8	2	5	4	4	15
25	7	13	8	15	7	62	29
26	8	23	1	3	1	2	15
27	8	12	3	11	3	14	44
28	11	34	0	3	1	0	12
29	11	22	0	12	15	24	65
30	6	8	13	1	1	3	25
alkanes: isoprenoids	1.8	2.0	1.6	7.1	-	-	5.7
pristane: phytane	0.6	0.2	2.6	0.9	-	-	-

TABLE 19. Hydrocarbon concentration ng/g.

SITE 10

C-#	12-78	3-79	8-79	11-79	3-80	7-80	6-81
14	0	1	2	-	-	25	7
15	0	5	33	3	-	42	747
16	1	5	29	3	-	19	17
17	3	11	53	14	8	41	228
pristane	1	3	7	3	-	7	4
18	3	7	9	10	4	8	10
phytane	2	0	4	2	-	2	5
19	3	11	5	6	1	7	11
20	4	8	4	6	3	6	8
21	3	4	5	6	4	246	14
22	2	10	5	5	3	6	15
23	2	8	7	7	4	10	31
24	1	4	5	5	2	1	21
25	3	226	13	24	7	55	76
26	2	13	6	7	2	2	33
27	1	11	22	21	5	31	137
28	1	11	8	7	5	7	23
29	0	5	38	33	14	53	194
30	0	3	23	3	-	4	34
alkanes: isoprenoids	1.9	-	9.0	-	-	4.0	63.6
pristane: phytane	0.5	-	1.9	-	-	3.5	0.8

TABLE 20. Hydrocarbon concentration ng/g.

SITE 11

C-#	3-80	7-80	6-81
14	605000	73200	69
15	661000	88200	285
16	625000	89100	83
17	636000	93600	110
pristane	342000	66900	418
18	643000	110700	76
phytane	231000	53400	372
19	629000	129000	80
20	680000	142000	128
21	724000	132000	122
22	719000	141000	141
23	719000	142000	196
24	724000	140000	209
25	685000	133000	202
26	718000	155000	224
27	669000	167000	207
28	627000	192000	108
29	620000	168000	152
30	479000	190000	318
Alkanes:Isoprenoids	0.2	2.4	0.3
Pristane:Phytane	0.7	1.3	1.1

The significant features of the chemical changes that were observed included a marked decrease in the proportion of n-alkanes relative to isoprenoid hydrocarbons, the transient occurrence of an increase in unresolved hydrocarbons within the first year following the AMOCO CADIZ spillage, and the decreased importance of unsubstituted polynuclear aromatic hydrocarbons relative to dibenzothiophenes and the comparable or substituted forms of the polynuclear aromatic hydrocarbons (Figs. 2 and 3).

The *in vitro* hydrocarbon biodegradation experiments confirmed the fact that the indigenous microbial populations were capable of rapid and extensive degradation of Arabian crude oil. Much greater rates of biodegradation were observed in agitated compared to flow through experiments (Figs. 4-9). Both the *in vitro* experiments and the analysis of field experiments support the hypothesis that mixing energy has a very significant effect on the rates of hydrocarbon biodegradation. Rates of biodegradation appear to be environmentally influenced by the turbulence of mixing which can ensure a continued supply of nutrients and oxygen as well as dispersing the oil so as to establish a favorable surface area to volume ratio for rapid microbial hydrocarbon biodegradation. The similarity of changes observed in the composition of the hydrocarbon mixture *in vitro* compared to the analysis of field samples also suggests that nutrients were not a limiting factor that determined the rates of hydrocarbon biodegradation.

The analysis of the polar fractions from the *in vitro* experiments showed some surprising results (Table 21). There was a lack of oxygenated aromatic compounds. It had been predicted that there would be a greater accumulation of polar products from aromatic biodegradation since less CO_2 was being produced than from aliphatic biodegradation where a significant proportion of the hydrocarbon that was biodegraded was released as CO_2. There were significant accumulations of polar compounds that appear to be biodegradation products of aliphatic hydrocarbons, especially as C_{16}-C_{18} acids. Interestingly, the major polar products included unsaturated acids. As a rule, the predominant biochemical pathway for the biodegradation of straight chained hydrocarbons does not involve the formation of unsaturated compounds, although a biochemical pathway has recently been elucidated for some bacteria that does introduce a double bond into the hydrocarbon. It appears that the microbial populations indigenous to the sediment of the Brittany Coast possess such a biochemical capability.

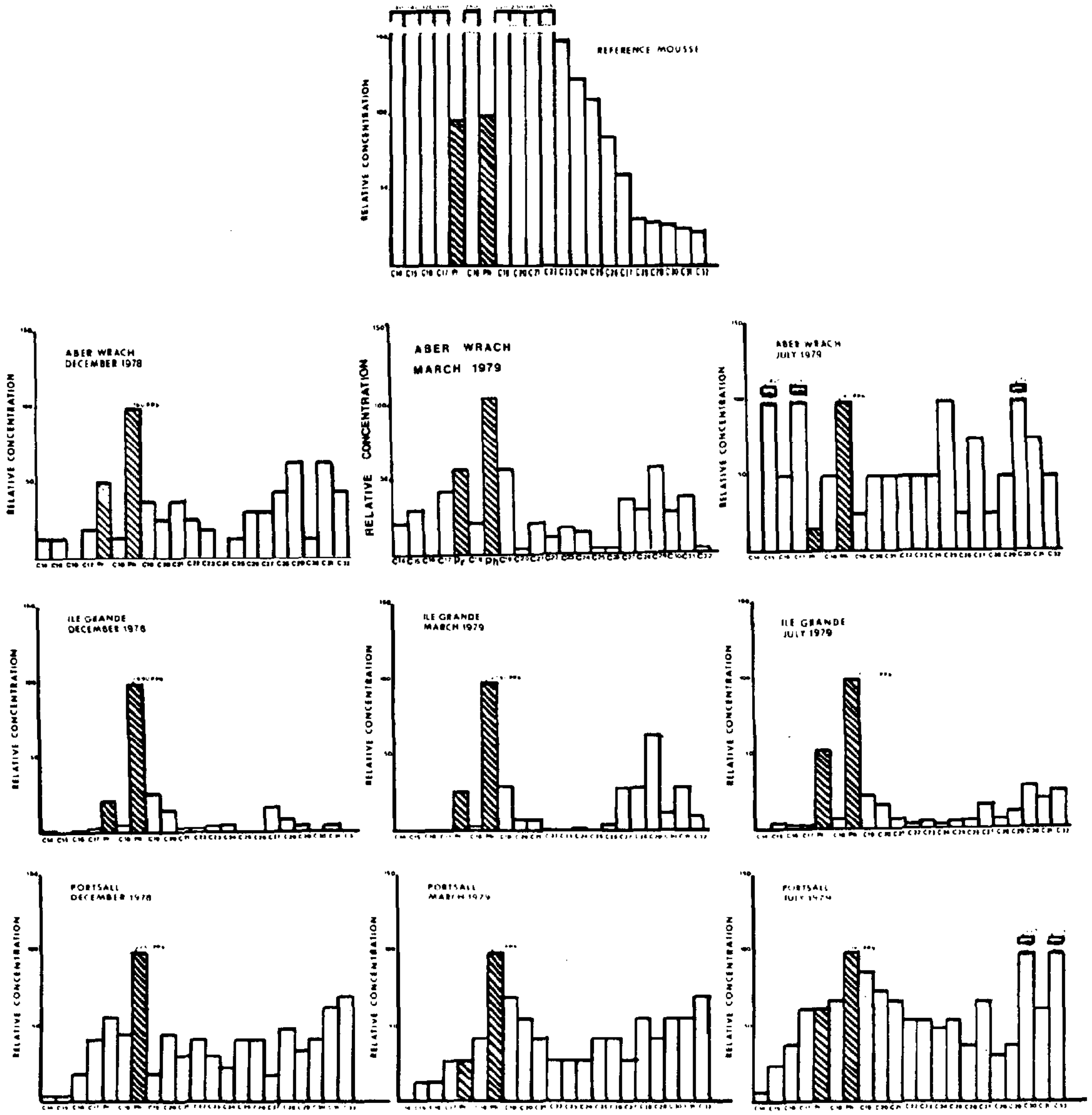


FIGURE 2. Changes in the relative concentrations of aliphatic hydrocarbons at sites 3, 5, and 7.

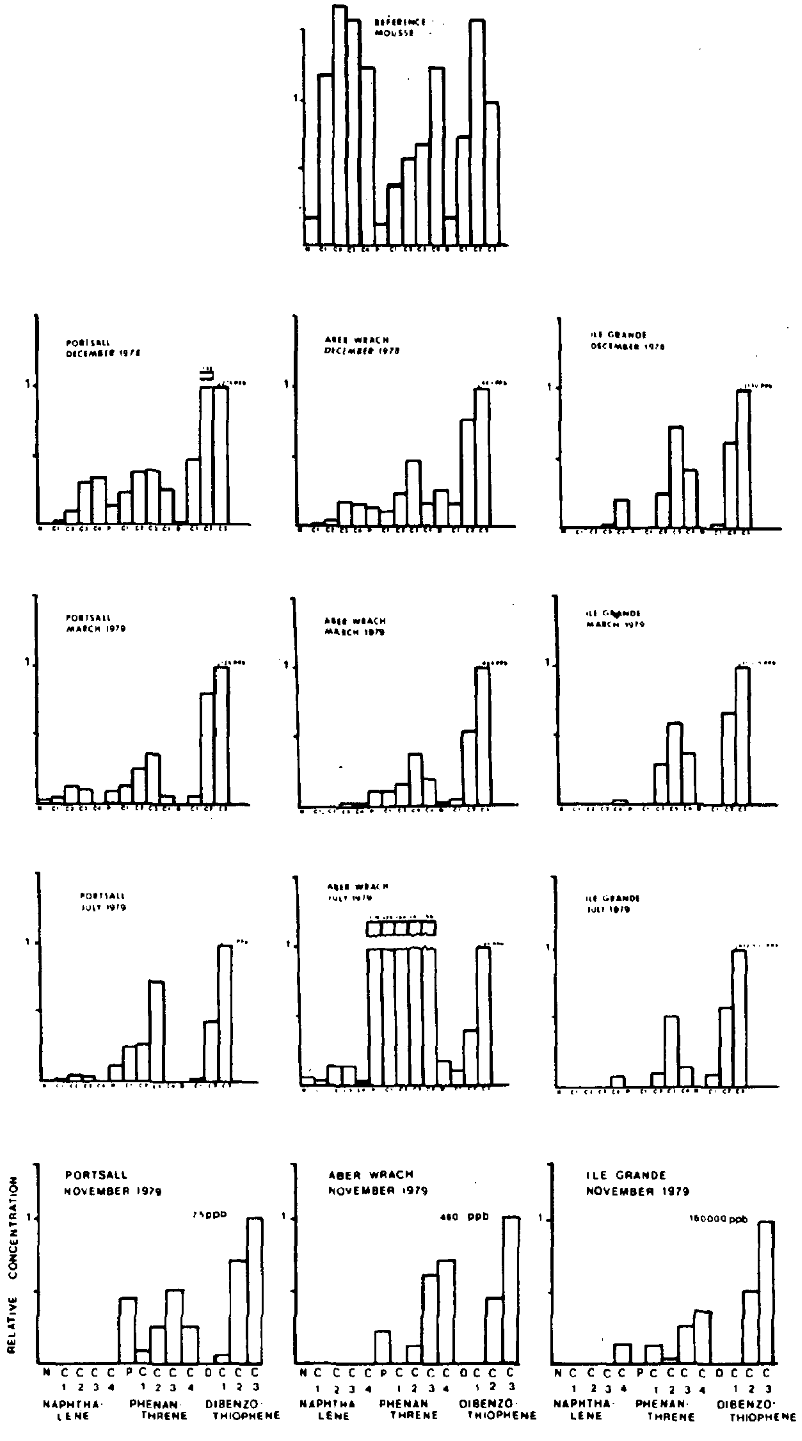
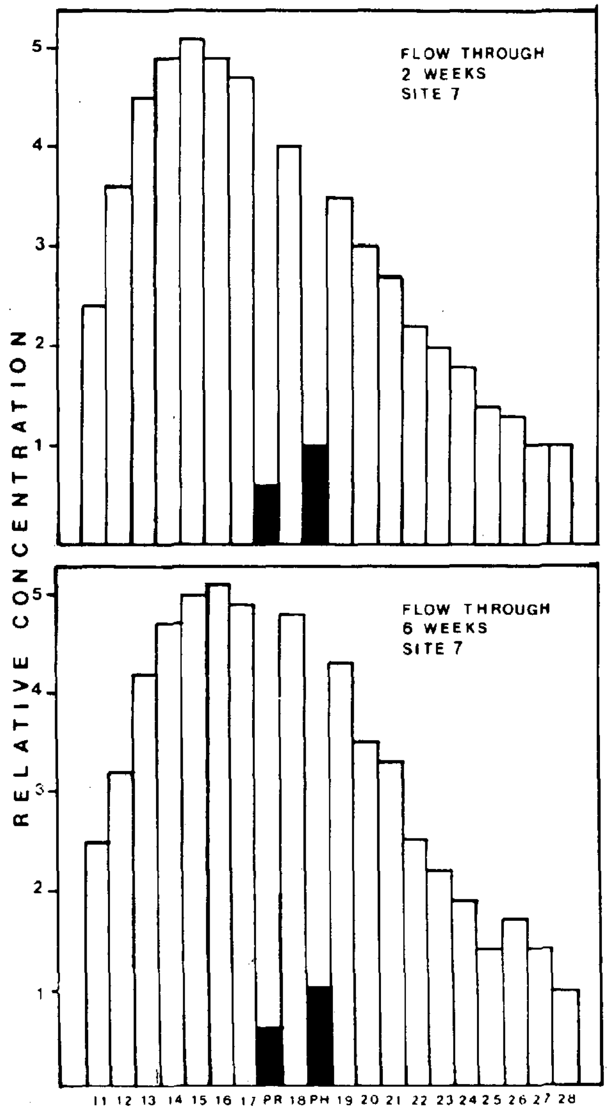


FIGURE 3. Changes in the relative concentrations of aromatic hydrocarbons at sites 3, 5, and 7.



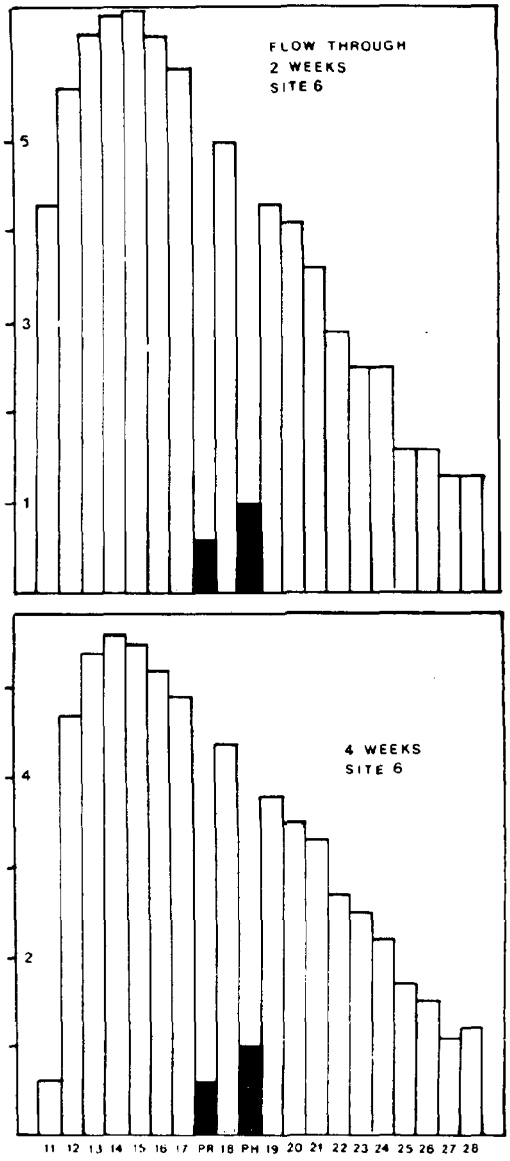
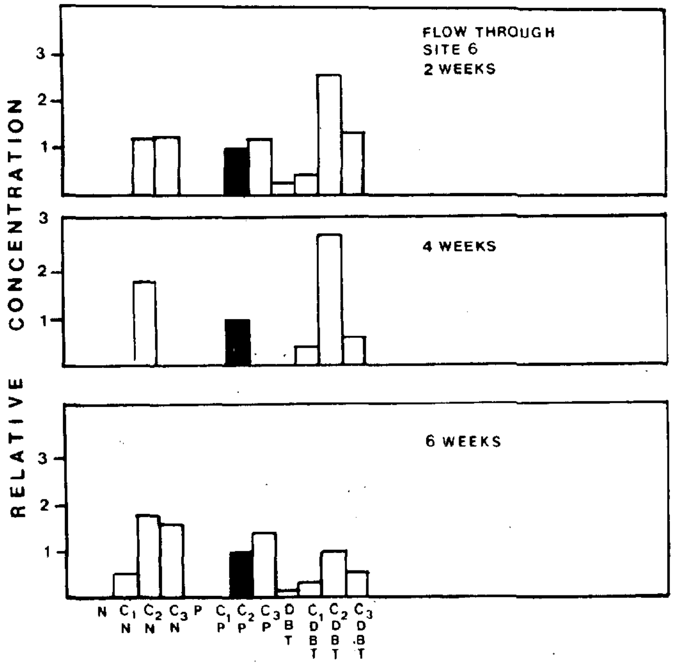


FIGURE 4. (Left column) Changes in the relative concentrations of aliphatic hydrocarbons in flow-through experiment with sediment from site 7.

FIGURE 5. (Right column) Changes in the relative concentrations of aliphatic hydrocarbons in flow-through experiment with sediment from site 6.



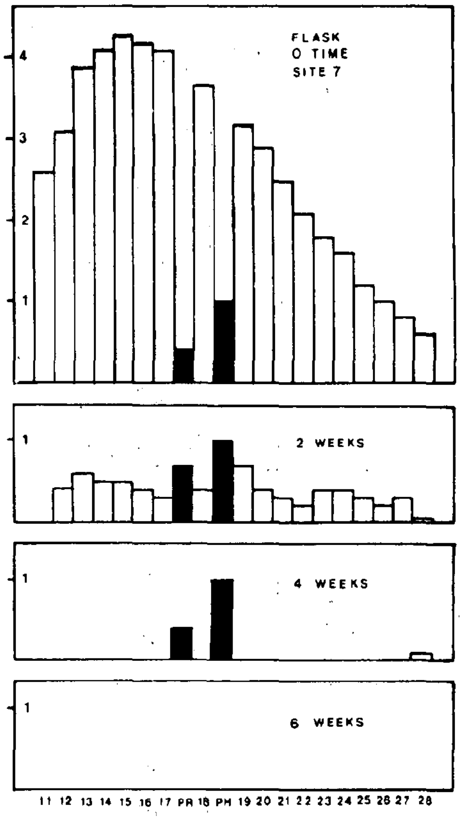
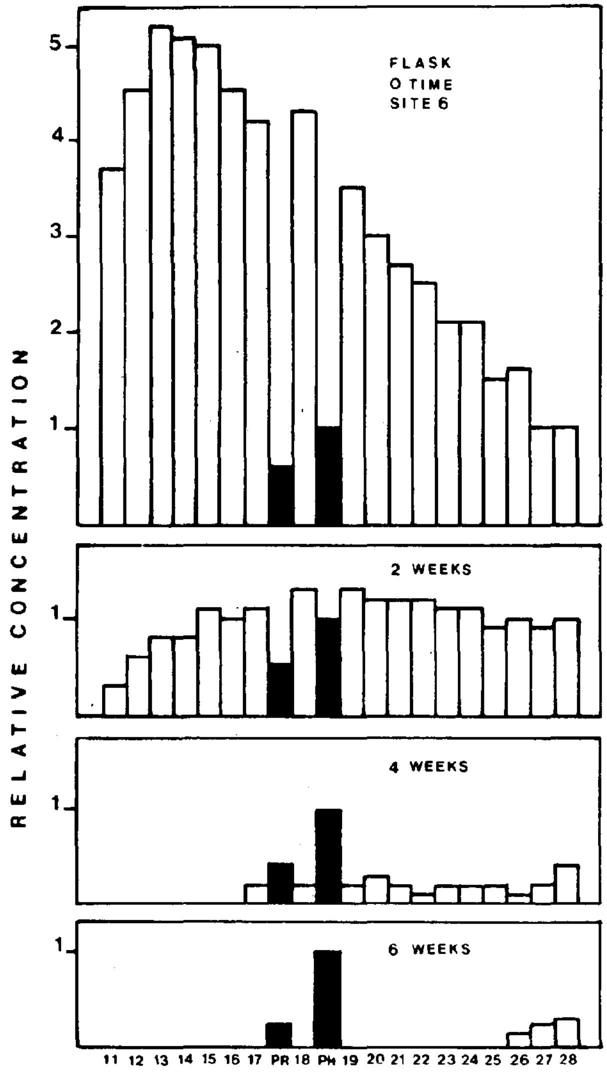


FIGURE 6. (Left column) Changes in the relative concentrations of aromatic hydrocarbons in flow-through experiment with sediment from site 6.

FIGURE 7. (Right column) Changes in the relative concentrations of aliphatic hydrocarbons in flask experiment with sediment from site 7.



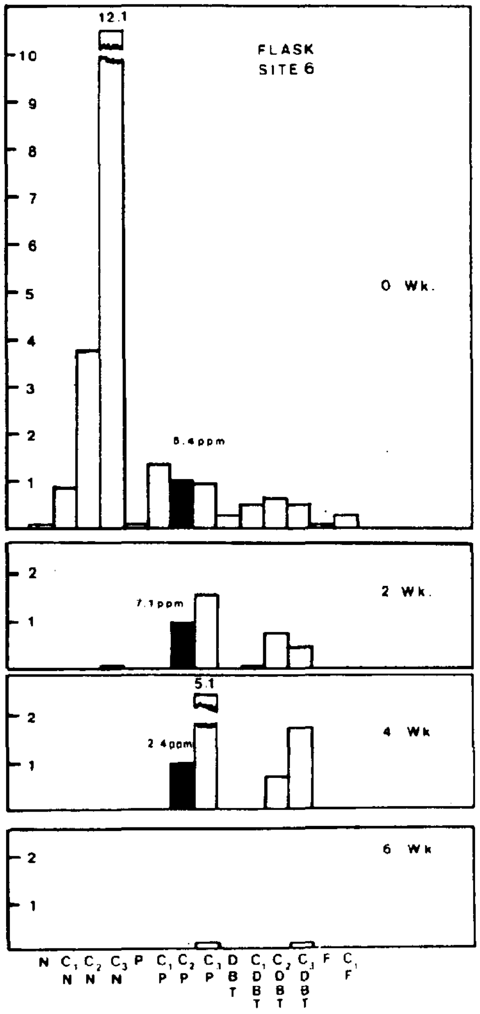


FIGURE 8. (Left column) Changes in the relative concentrations of aliphatic hydrocarbons in flask experiment with sediment from site 6.

FIGURE 9. (Right column) Changes in the relative concentrations of aromatic hydrocarbons in flask experiment with sediment from site 6.

TABLE 21. Concentrations and Identities of Polar Compounds ng/g

	SITE 7 FLASK	SITE 6 FLOW THROUGH	SITE 7 FLOW THROUGH
dodecanoic acid	16.5	2.3	4.7
tetradecanoic acid	22.2	13.7	24.5
methyltetradecanoic acid	15.0	19.7	43.3
pentadecanoic acid	10.5	13.5	24.0
hadecenoic acid	47.0	129.0	217.0
hexadecanoic acid	73.9	109.0	157.0
isoheptadecanoic acid	8.3	7.8	11.9
heptadecanoic acid	3.2	8.6	4.3
octadecenoic acid	59.3	76.4	99.2
octadecanoic acid	44.4	34.2	44.6
nonadecanoic acid	10.1	25.4	-
eicosanoic acid	9.8	25.4	55.8
tetracosanic acid	14.5	3.0	13.8
hexacosanic acid	7.7	1.4	10.8
octacosanic acid	15.8	13.3	15.6
isocyclopropaneoctanoic acid	15.5	25.5	42.2
methyloctahydrophenanthrene-carboxylic acid (tent.)	21.3	2.0	-

CONCLUSIONS

Microbial degradation appears to have played a very important role in the weathering of oil spilled from the AMOCO CADIZ. Microbial hydrocarbon degradation potentials are in general agreement with the observed changes in the composition of oil stranded within the littoral zone. The chemical evolution of the hydrocarbon mixture within intertidal sediments led to a relative enrichment in isoprenoid alkanes, a transient complex unresolved mixture, and a relative enrichment of dibenzothiophenes and alkylated phenanthrenes.

There was a general, but variable decline in concentrations of hydrocarbons over the three year period following the AMOCO CADIZ spill within Aber Wrac'h. The concentrations of hydrocarbons also declined at sites that were regularly covered by tides. At the one site in Ile Grande, which is not subject to daily tidal washing, the concentrations of hydrocarbons remained high even three years following the spill. At nearby sites within the Ile Grande salt marsh, which were physically cleansed of AMOCO CADIZ oil, there was little chemical or microbial evidence of any impact from the AMOCO CADIZ spill at any of the sampling times. The incurrence of oil from the TANIO wreck was apparent even at sites that had been oiled as a result of the AMOCO CADIZ spill.

The microbial population levels generally reflected the relative degrees of persistence of petroleum hydrocarbons. The microbial community at all of the sites studied had essentially the same potential capability for degrading hydrocarbons and as such the differences in the hydrocarbon concentrations and composition recovered from the field samples probably reflect the initial rates of oiling and environmental influences. The indigenous microbial community retained the capability of responding to a second incursion of oil resulting from the TANIO spill.

Both the field experiments and the *in vitro* studies suggest that mixing energy, related to nutrient and oxygen availability, was extremely important in permitting the high rates of observed oil weathering. The occurrence of both saturated and unsaturated acids in the sediments studied *in vitro* suggest that several biochemical pathways were active in the biodegradation of the aliphatic hydrocarbon fraction. The hydrocarbon biodegradation potential suggested that relatively high concentrations of oxygenated aromatic hydrocarbons should accumulate, but for unexplained reasons the analyses of the polar fraction generally failed to show such accumulations.

LABORATORY SIMULATION OF THE MICROBIOLOGICAL DEGRADATION OF CRUDE OIL IN A MARINE ENVIRONMENT

by

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This study essentially intends to quantify the biodegradation process of a crude oil in optimum conditions compatible with the marine environment.

Experiments were conducted in the Laboratory reactors (batch and continuous cultures), with perfect monitoring of all physicochemical parameters such as pH (pH 8.1), temperature at 20°C, mixing rate 600 rpm, and aeration velocity (1 liter of air/liter of medium per hour).

The composition of the mineral medium was defined by taking the mean composition of salts in the Atlantic Ocean as a basis, and enriching it with nitrogen (235 mg.1-1), phosphorus (26.7 mg. 1-1) and iron (0.4 mg.1-1).

In order to reproduce conditions prevailing at sea as closely as possible, in which the evaporation of light products is not negligible Arabian Light Crude was employed (ALC 240+) from which all fractions distilling below 240°C were removed by low pressure distillation.

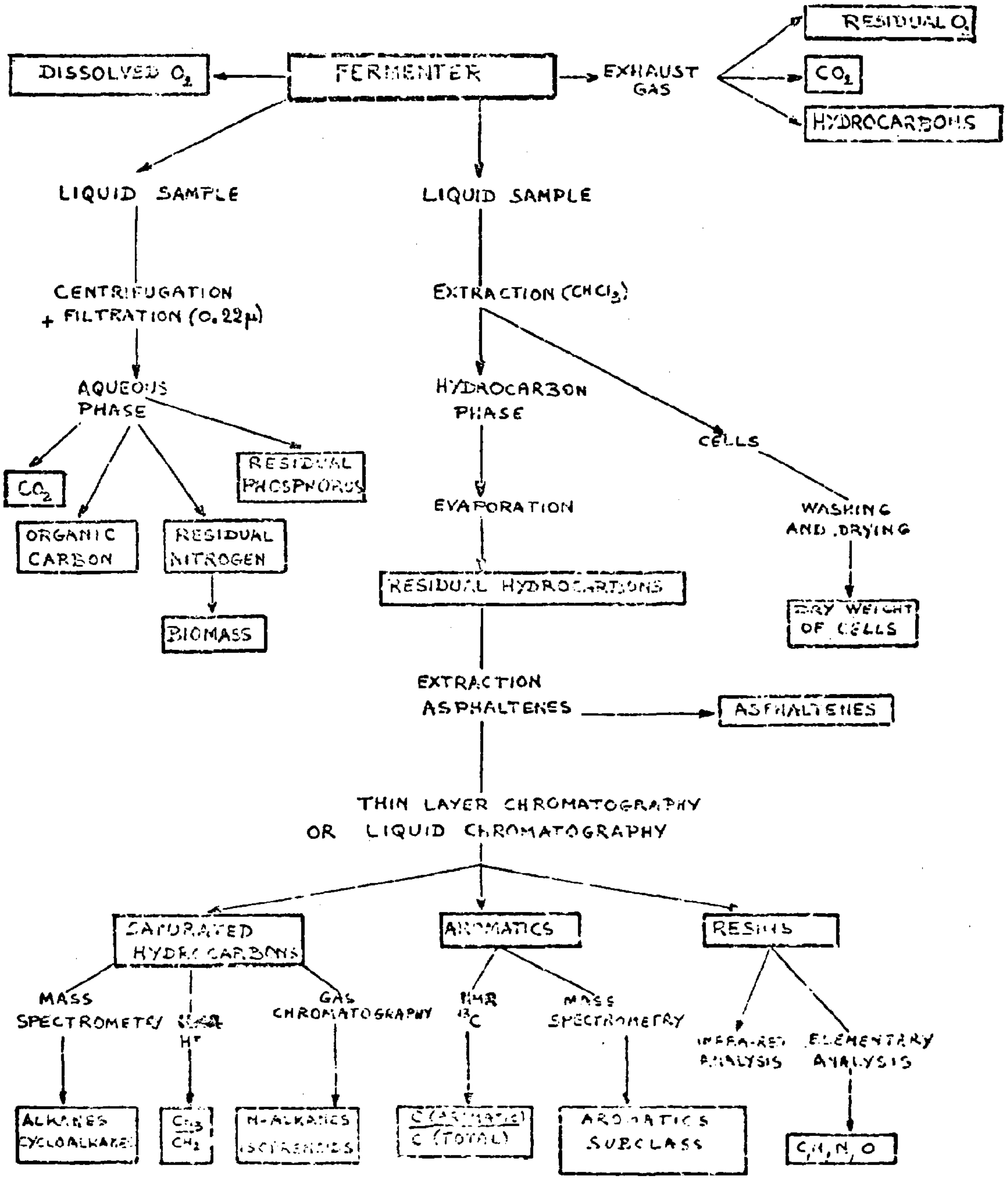
The analytical methodology employed to observe the crude oil biodegradation process is shown schematically in the following figure.

The gas flow was passed through a trap containing CCl4, which retained the evaporated hydrocarbons, and then through a second trap containing a known quantity of 1 N KOH, which retained the carbon dioxide. The hydrocarbons were then determined by infrared spectrometry. The CO2 produced was determined by titrimetry.

Liquid samples were taken during fermentation.

The first sample was centrifuged to separate the hydrocarbon phase from the aqueous phase, which was then filtered (filter pore diameter 0.22 µ) to eliminate fine particles in suspension. The following were analyzed in this perfectly clarified aqueous phase:

- total organic carbon (Dohrman DC.50 instrument),
- dissolved CO2 using the Warburg equipment,



FERMENTER
DISSOLVED O_2
EXHAUST GAS
RESIDUAL O_2
CO_2
HYDROCARBONS
LIQUID SAMPLE
LIQUID SAMPLE
CENTRIFUGATION + FILTRATION (0.22µ)
EXTRACTION ($CHCl_3$)
AQUEOUS PHASE
HYDROCARBON PHASE
CELLS
CO_2
RESIDUAL PHOSPHORUS
ORGANIC CARBON
RESIDUAL NITROGEN
BIOMASS
EVAPORATION
WASHING AND DRYING
RESIDUAL HYDROCARBONS
DRY WEIGHT OF CELLS
EXTRACTION ASPHALTENES
ASPHALTENES
THIN LAYER CHROMATOGRAPHY OR LIQUID CHROMATOGRAPHY
SATURATED HYDROCARBONS
AROMATICS
RESINS
MASS SPECTROMETRY
NMR H^+
GAS CHROMATOGRAPHY
NMR ^{13}C
MASS SPECTROMETRY
INFRA-RED ANALYSIS
ELEMENTARY ANALYSIS
ALKANES CYCLOALKANES
$\frac{CH_3}{CH_2}$
N-ALKANES ISOPRENOIDS
$\frac{C\ (AROMATIC)}{C\ (TOTAL)}$
AROMATICS SUBCLASS
C, H, N, O

FIGURE 1

- residual phosphorus,
- residual ammoniacal nitrogen and the intracellular nitrogen concentration (Kjeldahl's method); these two analyses served to determine the quantity of biomass formed.

The residual hydrocarbons were extracted from a second liquid sample. Asphaltenes were precipitated from the hydrocarbon residue using hot heptane for one hour, dried and weighed.

The residue obtained after evaporation of the heptane was processed to separate the three main families of hydrocarbons in crude oil: saturates, aromatics and resins, by thin layer chromatography (50 mg samples) or liquid chromatography (samples weighing about 1 g).

The sum of the weights of the three fractions thus recovered, using liquid chromatography, compared with the initial rate of the hydrocarbons deposited on the column, always accounted for a proportion between 90 and 100 %.

The loss percentage increased when the test samples were taken at increasingly long culture times, hence with samples that underwent the longest biodegradation times. These losses are likely to be due largely to the retention of polar compounds of the resins on the column, compounds that are formed during oxydation reactions, or possibly by biochemical co-oxydation, and whose concentration increases with biodegradation time.

Using the different fractions obtained (saturates, aromatics, resins), we performed more detailed analyses by gas phase chromatography (Varian 3700 chromatograph) equipped with "Splitless" injection and flamme ionization detector), a combination of gas phase chromatography and mass spectrometry (Varian CH5DF spectrometer), proton NMR that yielded the fraction of hydrogen belonging to methyl groups in the saturates family, ^{13}C NMR, which yields the percentage of aromatic carbon in comparison with total carbon in the aromatic fraction, and by infrared spectrometry on the resins.

1. BATCH CULTURES

1.1. Biodegradation of hydrocarbon families and sub-families in ALC 240^+.

We selected a mixed culture of bacteria from samples of muds and sludges collected on places hit by crude oil spills.

The experiment was conducted with ALC 240^+ in an initial concentration of 2.65 $g.l^{-1}$, over a period of 48 hours.

Of the 2.65 $g.l^{-1}$ of initial hydrocarbons, 1.08 $g.l^{-1}$ were consumed, representing 41 % degradation. It appears clearly that the saturates fraction is most sensitive to biodegradation, because 67 % of this fraction were consumed, whereas only 27 % of the aromatics fraction were degraded. The quantity of hydrocarbons evaporated was negligible.

From the standpoint of reproducibility of results, a previous experiment yielded the following results: hydrocarbons consumed 44 %, saturates degraded 63.1 %, aromatics disappeared 48.6 %.

The saturated hydrocarbons were most rapidly biodegraded. At the end of the culture, the disappearance of aromatic compounds is accompanied by an enrichment of the aqueous phase in organic carbon, the concentration of which may reach 250 $mg.l^{-1}$. This observation tends to show that a large part of the aromatics are only partly oxidized before passing into the aqueous phase.

The resins were only slightly attacked if at all, and the asphaltene concentrations at the start and end of the batch culture were absolutely comparable, demonstrating total insensitivity of these substances to biochemical processes.

The determination of n-alkanes (C_{14}-C_{35}) and detectable isoprenoids (C_{16}-C_{23}) by gas phase chromatography showed that these compounds disappeared almost totally by the end of the culture.

The mass spectrometry analysis of the "saturates" fraction showed that the alkanes were mainly biodegraded, as 88.9 % disappeared at the end of the culture. This enables us to postulate that, in addition to the n-alkanes and isoprenoids, which only account for 14.8 % of the "saturates" fraction, the bulk of the iso-alkanes present in the crude oil was consumed by microorganisms.

Among the naphtenic compounds, the 1- and 2-cycle naphtenes were mainly consumed, with respective biodegradation rates of 44 and 47 %.

Proton NMR analyses giving the CH_3/CH_2 ratio, conducted on the saturates, failed to indicate any significant difference between the start and end of the batch culture.

With respect to the "aromatics" fraction, the action of microorganisms mainly affected the mono- and di-aromatic compounds. At the end of the culture, all the mono- and di-aromatics with a number of carbons less than 16 had disappeared.

Among the mono-aromatics, the substances most sensitive to microbial action were the alkylbenzenes, of which 67.7 % disappeared at the end of the culture, and the benzocycloparaffins, with a consumption rate of 46.2 %. The differences measured for benzodicycloparaffins were not sufficiently wide to be meaningful.

As for di-aromatic compounds, the microorganisms displayed a very clear effect on the residual concentration of naphtalenes, of which 50 % disappeared after 48 hours of culture.

Through a second experiment, we investigated the changes in composition of the aromatics fraction, by drawing a distinction between sulfucompounds and other aromatics.

Apart from those with a rough formula C_nH_{2n}-10S, the sulfur-containing compounds were not attacked by bacteria. The aromatics/sulfur-compounds ratio of 0.98 before biodegradation decreased to 0.82 after biodegradation, showing that it was mainly the non-sulfur-containing aromatics (mono- and di-) that disappeared. In addition, the weight percentage of sulfur in the aromatics fraction increased with time from 4.05 to 4.15, confirming the enrichment of this fraction in sulfur-containing subtances.

The ^{13}C NMR analyses used to quantify the aromatics C/total C ratio failed to reveal any significant difference before (43.4 %) an after (43.7 %) biodegradation.

With respect to the resins, part of the polar compounds of this fraction formed during biodegradation remained absorbed on the liquid chromatography column, and consequently the analyses performed on the eluted resin fraction were not truly representative. This retention of polar compounds of the liquid chromatography column was confirmed by elemental analysis, showing oxygen to drop from 2.75 % (by weight) at the start of the batch culture to 2.35 % at the end of the culture.

The determination of molecular weights of the resins yielded the following results: 690 at the start of the batch culture, 740 at the end of the batch, namely very slightly differing molecular weights.

1.2. Examination of oxidation products.

Analyzing the aqueous phase sampled at the end of the batch culture (volume sampled = 1 liter), centrifuged and filtered, we found a total organic carbon concentration of 260 $mg.l^{-1}$.

We carried out an esterification (BF_3-CH_3OH) of the compounds of this aqueous phase. The organic extract was evaporated and weighed. The weight of the extracted compounds, related to one liter of culture, was 120.2 mg. In the acidified residual aqueous phase, initial extraction with CH_2Cl_2, followed by a second extraction with benzene, yielded a new organic phase that contained polar compounds such as alcohols, ketones and phenols, which represented 7.58 mg/l of aqueous phase after evaporation.

Identification by GC/MS coupling of compounds separated by gas phase chromatography was difficult because of the presence of a strong background of poorly resolved constituents, which could be hydrocarbons. Despite these problems, we succeeded in identifying normal and iso acidic compounds in the aqueous phase, in the form of their corresponding esters, obtained after esterification of the aqueous phase.

The GC/MS coupling enabled us to observe the masses m/e = 74 characteristic of n-esters, and m/e = 88 characteristics of iso-esters.

1.3. Changes in microbial flora with time.

Three samples were taken during the batch culture, the first at the start of growth, the second during the active biodegradation phase (after 15 hours of culture), corresponding to consumption of the saturated hydrocarbons, and the third after 25 hours, in the slowdown period of the biodegradation process, corresponding to microbial attack of the aromatics.

In the three different stages investigated, different dominant strains were found, belonging to two genera only, Pseudomonas and Moraxella, confirming that changes in the crude oil during a biodegradation process are accompanied automatically by changes in the microbial flora. At the start and middle of the batch culture, we chiefly identified bacteria of the genus Moraxella, indicating that these strains are perfectly adapted to the hydrocarbons present at that particular time in the culture and, being dominant, they therefore naturally and preferentially consumed the hydrocarbons of the "saturates" fraction, as these types of constituents were biodegraded during this period. At the end of the culture, however, when the "aromatics" fraction was attacked by the microorganisms, only the Pseudomonas strains were dominant.

This investigation again confirms that, to observe a significant degradation of hydrocarbons in a crude oil containing a wide variety of compounds, a mixed culture of bacteria is certainly more effective than a pure bacteria, of which the metabolism is only adapted to a given type of constituent.

1.4. Toxicity analysis of oxidation products.

During the different ALC 240^+ crude oil biodegradation experiments, we always observed a substantial rise in total organic carbon (TOC) concentration in the aqueous phase with the passage of time, with a regular final concentration around 200 mg.l^{-1}.

We decided to evaluate the potential toxicity of these solubilized products in the aqueous phase, enriched mainly in aromatics and oxidation products of certain hydrocarbons present in the crude oil.

In particular, the mutagenicity of two samples was determined by the Ames test, the procedure of which is described in detail in Mutation Research 1975, 31, pp. 347-364. The first sample was taken at the start of the culture (with a TOC of 30 mg.l^{-1}), and the second at the end of the batch culture (sample with a TOC of 210 mg.l^{-1}).

The correlation between carcinogenic properties and mutagenic properties of 300 compounds was pointed out in Proc. Natl. Acad. Sci., (USA), 1975, 72, pp. 5135-5139.

The principle of the Ames test is to measure the mutagenic properties of compounds that may be carcinogenic in Salmonella bacteria.

The two samples were tested in a range from 0.1 to 500 µl on three of the five strains used in the Ames test (TA.1538, TA.98 and TA.100) in order to detect the mutagenicity of products such as HAP, for example.

No mutagenic activity was detected in these two samples.

It was shown finally that neither of these two samples had any toxic effect on the three strains tested (TA.1538, TA.98 and TA.100).

1.5. Study of the biodegradation of a mixture of pure hydrocarbons.

The mixture of pure hydrocarbons consisted of two n-alkanes, hexadecane and octacosane, one isoprenoid, pristane, a two-ring naphtene, decaline, two mono-aromatics, p-cymene and dodecylbenzene, one di-aromatic, dimethyl-naphtalene, one tri-aromatic, phenanthrene, and two sulfur-containing aromatics, benzothiophene and dibenzothiophene.

Experiments were conducted in batch culture in the same conditions as those described for Arabian Light Crude. The mixed culture of bacteria used was the mixture of the strains Pseudomonas and Moraxella isolated and purified, described in Section 1.3. By sucessive cultures in flasks with the pure hydrocarbon mixture as the only carbon substrate, the mixed culture was progressively adapted to grow on these ten hydrocarbon compounds.

The culture was carried out in batch for 61 ½ hours, and we observed the changes in the biomass, total hydrocarbons, and each compound, and also the organic substances that passed into the aqueous phase.

Following a lag phase of about 10 hours, a growth acceleration phase was observed up to the 25th hour, then a linear phase from the 25th to the 35th hour, and finally the slowdown of bacterial growth. At the end of the batch, the dry cell weight was 0.5 $g.l^{-1}$. The biodegradation process of total hydrocarbons perfectly matched the microorganism growth pattern. After 61 ½ hours, 82.8 % of the hydrocarbons were degraded.

It appears that the three most volatile compounds, paracymene, decaline and benzothiophene, could not be found after extraction, from the very outset of the experiment. These three products must therefore disappear chiefly during extract evaporation operations. However, a small proportion passes very rapidly into the aqueous phase in the marine environment, because the latter contained oxidation products of p-cymene among others, as well as benzothiophene.

The two n-alkanes, n-hexadecane and octacosane, and the dodecylbenzene were consumed first. For these three products, which practically disappeared by the end of the batch, their respective biodegradation rates after 37 ½ hours only of culture were 93 %, 87.5 % and 80 %. Pristane only started being attacked after 24 ½ hours, and was 69.2 % consumed at the end of the culture. During the last 20 hours, while practically no alkanes or alkylbenzene remained in the reactor, dimethylnaphtalene was biodegraded (disappearance rate 67 %). Phenanthrene and dibenzothiophene were consumed very little if at all.

These results perfectly confirm those found with Arabian Light Crude, which showed that alkanes and isoprenoids were attacked first, followed by mono- and di-aromatics. Similarly, it was observed that triaromatics and sulfur-containing aromatics were only slightly sensitive or insensitive to the action of microorganisms. The fact that dodecylbenzene disappeared fairly rapidly is explained by the presence of the linear chain which, like the n-alkanes, is readily accessible to bacteria.

The total organic carbon concentration (TOC) measured in the medium was 385 $mg.l^{-1}$. After esterification and evaporation of the organic extract, esters and some other polar compounds were found in a concentration of 221.5 $mg.l^{-1}$.

We carried out analyses by gas phase chromatography and GC/MS coupling in an attempt to identify these products.

Since many products were present in trace amounts, and several of them were eluted simultaneously and combined in a single peak, we encountered considerable difficulty in identifying them on the mass spectrometer.

2. CONTINUOUS CULTURES

In continuous culture, since this technique serves to check the concentrations of all the nutritive elements at all times, and to adjust these concentrations to limit thresholds, thus closely approaching conditions encountered at sea, we attempted to quantify the nitrogen, phosphorus and oxygen requirements for the biodegradation of given quantities of hydrocarbons present in ALC 240^+.

The following operating conditions were used:

● Dilution rate	$D = 0.04\ h^{-1}$
● Temperature	20°C
● Reactor volume	2 liters
● Agitation	520 rpm
● Ph of culture	8.1
● GHSV	1

(except for quantification of the oxygen requirements, where the GHSV was varied from 1 to 0.25).

● ALC 240^+ concentration entering reactor always about	$2.5\ g.l^{-1}$

For a given concentration of an element (nitrogen, phosphorus or oxygen) entering the reactor, the experimental time was about one week. Upon each alteration in operating conditions, it was necessary to wait another week for equilibrium to be re-established.

When the residual concentration of nitrogen was in excess, the bacterial consumption of this element per mg of hydrocarbons degraded ranged from 0.1 to 0.11 mg. However, when the nitrogen reached a limit with residual contents around 1 mg/liter, the nitrogen requirements dropped to 0.07 mg.

The same occurence was observed with phosphorus. In conditions of non-limitation, the biochemical consumption of phosphorus, around 0.012 to 0.013 mg/mg of hydrocarbons consumed, declined to only 0.005 mg/mg of hydrocarbons consumed when the residual concentration of elemental P reached a limit ($< 1\ mg.l^{-1}$).

With respect to oxygen, microorganism requirements fluctuated between 1.4 and 1.9 mg oxygen per mg of biodegraded hydrocarbons, for residual dissolved oxygen concentrations between 50 and 7 % of the saturation value.

THE *AMOCO CADIZ* ANALYTICAL CHEMISTRY PROGRAM

by

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INTRODUCTION

All fate and effects studies of oil spills in the marine environment depend on analytical chemical information concerning the distribution and composition of the spilled oil. This includes petroleum hydrocarbon concentrations and compositions in water, sediment, and tissue samples. In turn, this information can be used to deduce the nature of the weathering process (including evaporation, dissolution, and biodegradation), biological assimilation and depuration, and the mass budget of the oil. Thus the analytical chemistry component of the AMOCO CADIZ research program provides crucial information to many other components of the program in the investigation of the time-dependent fate and effects of this spill.

During the six weeks following the grounding of the supertanker AMOCO CADIZ on March 16, 1978, oil came ashore along 320 kilometers of the Brittany coastline (Gundlach and Hayes, 1978). Various shoreline types were impacted (e.g., rocky shores, sand flats, coastal embayments, tidal mud flats and salt marshes). During the early stages of the spill, oil was transported offshore and deposited in the benthic environment. The fate of petroleum residues deposited in these impacted areas was and continues to be affected by coastal processes which dictate such factors as wave energy and sediment transport, and create environments of differing substrate character (e.g., grain size), chemical status (oxidizing versus reducing), and biological activity (e.g., microbiological biomass). All of these factors and others (e.g., light intensity) combine to determine the weathering characteristics of the residual petroleum assemblage.

Biological populations initially impacted by the spilled oil may be subject to chronic exposure to petroleum hydrocarbons associated with (and released from) the substrate to which they are closely linked, or they may undergo rapid or slow depuration of initial residues if no longer exposed to oil, via transplantation or due to flushing by "clean" seawater. Such differential exposure histories have been previously observed to profoundly affect the spilled oil residual body burdens in marine organisms (Boehm et al., 1982).

Although oil spills have received increasing attention from the scientific community during the past decade, there have been few opportunities to examine the chemical compositional changes in beached or sedimented oil in a variety of coastal environments, over a significant period of time and to examine uptake (impact) and depuration (recovery) of petroleum by marine organisms. A detailed examination of the chemical changes in oiled substrate suggests both the anticipated residence time of deposited oil, and the potential for biological damage of the petroleum residues. Rashid (1974) examined compositional changes of Bunker C oil from the ARROW spill in Nova Scotia at different coastal locations. Other than this study only site-specific studies of the geochemistry of petroleum weathering (e.g., Mayo et al., 1978; Blumer et al., 1973; Teal et al., 1978) have been undertaken.

Uptake and depuration by organisms have been the subjects of many laboratory experiments (e.g. Neff et al., 1976; Roesijadi et al., 1978) but relatively few real spill scenarios (e.g. Boehm et al., 1982; Grahl-Nielsen et al., 1978).

This report is intended to present an overview of the chemistry program along with enough supporting data and interpretations for each program element to make this a self contained document. After a methods section, a summary of the general findings is presented. Discussions of the analytical chemical and biogeochemical findings of each of the six specific investigations follow; the last section draws conclusions from the study as a whole. Much of the raw analytical data has been omitted here for brevity. Tabulations of analytical data are available either from the individual principal investigators or from the chemistry group. This data has formed the basis of several publications to date (Calder and Boehm, 1981, Boehm et al., 1981, Atlas et al., 1981, Winfrey et al. 1981) as well as several manuscripts in preparation. Additional interpretative details are found in these manuscripts.

METHODS AND MATERIALS

As part of the NOAA/CNEXO research program to examine the long-term fates and effects of the spill, we obtained samples of frozen intertidal surface sediment, benthic sediment, sediment cores, oysters, flatfish and macroalgae from a number of U.S. and French investigators (Table 1).

TABLE 1. Summary of AMOCO CADIZ chemistry program.

1 - Chemical Composition, Weathering, and Concentrations in Surface Intertidal Sediments (Atlas; Calder): 1978-1981

2 - Chemical Composition, Weathering, and Concentrations in Subtidal Sediment (Marchand, Courtot): 1978-1979

3 - Chemical Composition, Weathering, and Concentrations in Intertidal Cores (Ward): 1978-1980

4 - Chemical Concentrations and Composition of Oil in Oysters and Flatfish from Abers (Neff): 1978-1980

5 - Chemical Concentrations and Composition of Oil in Variety of Fish and Oyster Tissues (Michel): 1978-1979

6 - Chemical Concentrations and Composition of Oil Associated with Seaweeds (Topinka): 1978-1980

2.1 Sediments and Sediment Cores (Extraction and Processing)

Samples of surface sediment or specific depth interval sections of sediment cores were solvent-extracted and fractionated according to an ambient temperature solvent drying and solvent extraction procedure based on that of Brown et al. (1980) as revised by Atlas et al. (1981) and Boehm et al. (1981). The procedure, involving methanol drying and ambient temperature extraction with a methylene chloride/methanol azeotrope, is illustrated in Figure 2.1. The concentrated extract is displaced with hexane and charged to a glass absorption chromatography column (1 cm i.d.) containing 10 g fully activated (150°C) 80-100 mesh silica gel topped with 1 g 5% deactivated alumina and 1 g activated (i.e. acid washed) copper powder. The column, which is wet packed in methylene chloride, is rinsed with this solvent followed by hexane. A 0.5 ml volume of extract is charged to the column and eluted with hexane (17 ml, f_1), hexane: methylene chloride (21 ml, f_2), and methanol (20 ml, f_3). The fractions are collected separately, reduced in volume, desulfurized using an activated (1 N HCl) copper powder slurry, and an aliquot weighed on a Cahn electrobalance. The f_1 and f_2 fractions are then analyzed by fused silica capillary gas chromatography (FSCGC flame ionization detector) and a selected set further scrutinized by gas chromatographic mass spectrometry. FSCGC analysis determined the overall composition of the sample by appraisal of the distribution of resolved (peaks) and unresolved (hump) features, as well as the specific quantities of individual n-alkane (C_{10} to C_{32}) and isoprenoid (C_{15} to C_{20}) compounds. GC/MS/computer analyses focused on the list of saturated and aromatic compounds presented in Table 2 to confirm the identities of compounds or to quantify minor, but important "marker" compounds.

Details of the GC and GC/MS analytical procedures are presented in Table 3.

Quantification of GC traces was according to the internal standard method wherein quantities of individual hydrocarbons are computed. Several other GC-derived parameters were routinely calculated on sample data. One of these was the n-alkane to isoprenoid ratio (ALK/ISO) in the $C_{13} - C_{19}$ range:

$$\text{ALK/ISO} = \frac{\text{n} - \text{C}_{14} + \text{n-C}_{15} + \text{n-C}_{16} + \text{n C}_{17} + \text{n-C}_{18}}{1380 + 1450 + 1650 + 1710 + 1812^{a}}$$

This ratio, beginning at $\sim$7 in the reference oil is quickly decreased due to preferential bacterial degradation of n-alkanes versus the branched isoprenoids.

The carbon preference index (CPI), the ratio of odd chain alkanes to even chain alkanes in the n-C_{26} to n-C_{31} range, is defined as follows:

$$\text{CPI} = \frac{2(\text{n-C}_{27} + \text{n-C}_{29})}{\text{n-C}_{26} + 2\text{n-C}_{28} + \text{n-C}_{30}}$$

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[a] GC retention indices of isoprenoids: 1450 = farnesane, 1710 = pristane, 1812 = phytane.

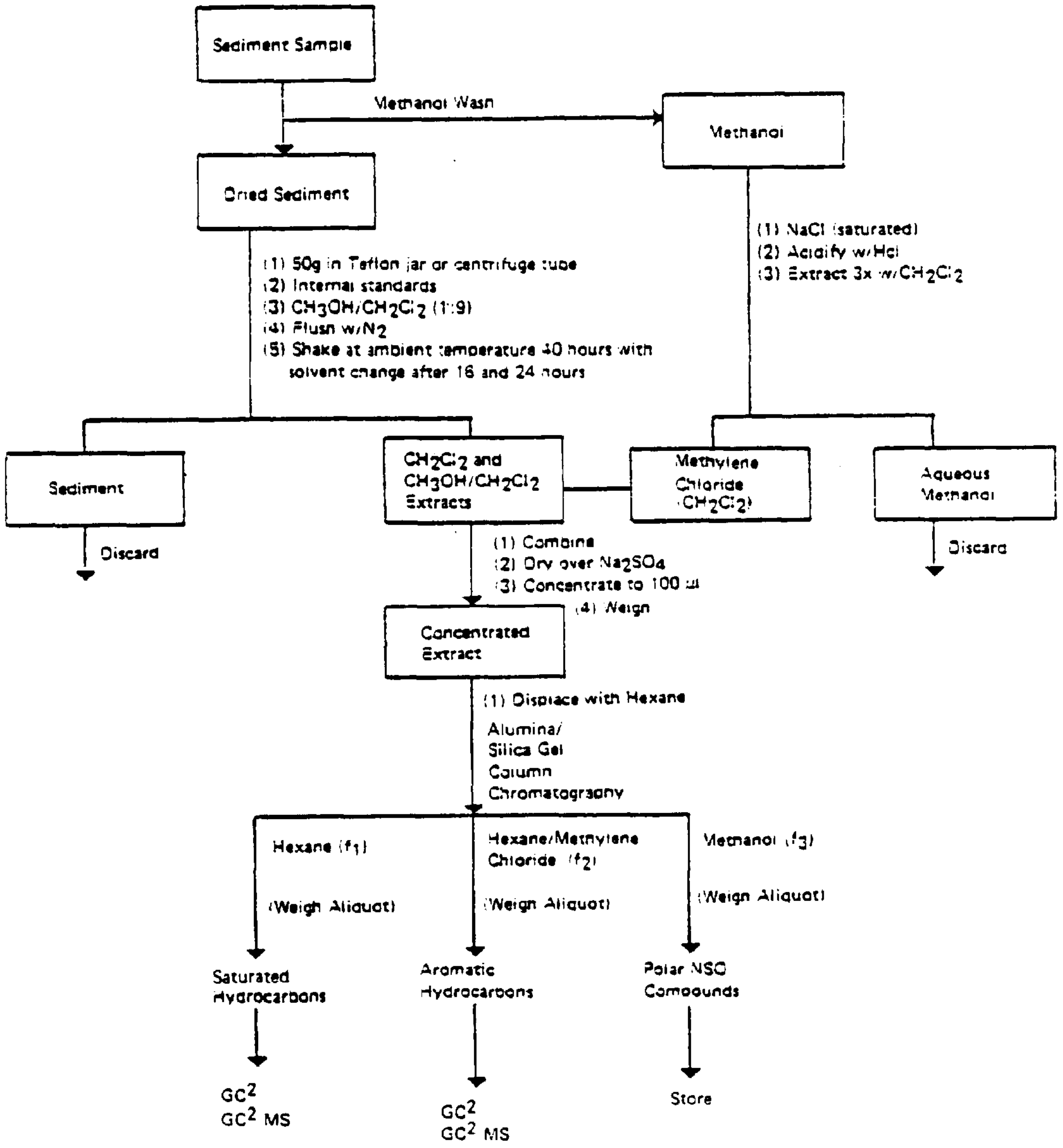


FIGURE 2.1. Analytical scheme for sediment samples.

TABLE 2. Focus of GC/MS analyses.

- Saturated hydrocarbons
  - Pentacyclic triterpanes (hopanes)
- Aromatic hydrocarbons
  - Alkylated benzenes (C_4, C_5, C_6)
  - Naphthalene and alkylated naphthalenes (C_1, C_2, C_3, C_4)
  - Fluorene and alkylated fluorenes (C_1, C_2, C_3)
  - Phenanthrenes and alkylated phenanthrenes (C_1, C_2, C_3, C_4)
  - Fluoranthene
  - Pyrene
  - Benzanthracene
  - Chrysene
  - Benzofluoranthenes
  - Benzo(a)pyrene
  - Benzo(e)pyrene
  - Perylene
- Aromatic heterocyclics
  - Dibenzothiophene and alkyl dibenzothiophenes (C_1, C_2, C_3)

TABLE 3. GC and GC/MS conditions.

	GC	GC/MS/COMPUTER
A. Instrument	HP 5840A	HP 5985
B. Column	SE-30 (saturates)	SE-52
1. Liquid phase	SE-52 (aromatics)	
2. Type	Fused silica (J&W Scientific)	Fused silica (J&W Scientific)
3. Diameter	0.25 id	0.25 id
4. Length	30 m	30 m
5. Carrier	Helium @ 1 ml/min	Helium @ 1 ml/min
C. Temperatures		
1. Oven	40-290 @ 3°/min	40-290 @ 3°/min
2. Injector	250° C	250° C
3. Detector	300° C (FID)	300 (ion source)
D. Ionization voltage	-	70ev
E. Electron multiplier voltage	-	2200 volts
E. Scan conditions	-	40-500 amu @ 225 amu/sec (1 scan/2 seconds minimum of 5 spectra per peak)

The CPI ranges from values of 1, where oil is present, to values greater than 1 if odd chain biogenic terrigenous n-alkanes dominate the higher boiling n-alkanes.

Quantification of aromatic hydrocarbons was accomplished using the technique of mass fragmentography wherein the computer stored raw GC/MS data is searched for parent ions (m+) and the total ion currents for these ions is integrated and tabulated. Retention times of the parent ion mass fragmentograms obtained were compared with authentic standards. The total ion current for each parent ion is compared with that for the internal standard (deuterated anthracene) and instrumental response factors applied. Where authentic polynuclear aromatic hydrocarbon (PAH) standards were not available for relative response factor determination, a response factor was assigned by extrapolation.

All of the above techniques were applied successfully to the analyses of replicates of a NOAA intercalibration sediment sample, Duwamish I, prior to commencement of the program and to Duwamish II during the program. Additionally, the EPA "megamussel" intercalibration sample was successfully analyzed for PAH levels.

2.2 Plant and Animal Tissues

All specimens of wet tissue, freeze dried tissue, and plant material were thawed and homogenized, or in the case of the seaweed tissue were cut into small pieces, prior to placement in a digestion flask. The samples were added to 250 ml Teflon screw top jars. The digestion, extraction, and fractionation schemes were similar to those developed by Warner (1976) except that the digestion was performed using a 0.5 N KOH/distilled water/distilled methanol system heated in a boiling water bath for 4 hours to achieve complete digestion and hence release of hydrocarbons from the cellular matrix (Boehm et al., 1982). Internal standards were added prior to digestion and carried through the entire procedure (Fig. 2.2) (f_1 = androstane; f_2 = deuterated anthracene or phenanthrene).

The digestate was extracted three times with distilled hexane in the jar, the mixture being centrifuged between extractions. The extracts were combined, concentrated to 0.5 ml, weighed on a Cahn electrobalance, and fractionated on an alumina over silica gel column (see previous section). Two fractions corresponding to the saturated or f_1 (hexane eluate) and the aromatic/olefinic or f_2 (hexane:methylene chloride eluate) hydrocarbons were obtained for gas chromatographic and combined gas chromatographic/mass spectrometric analyses (GC/MS).

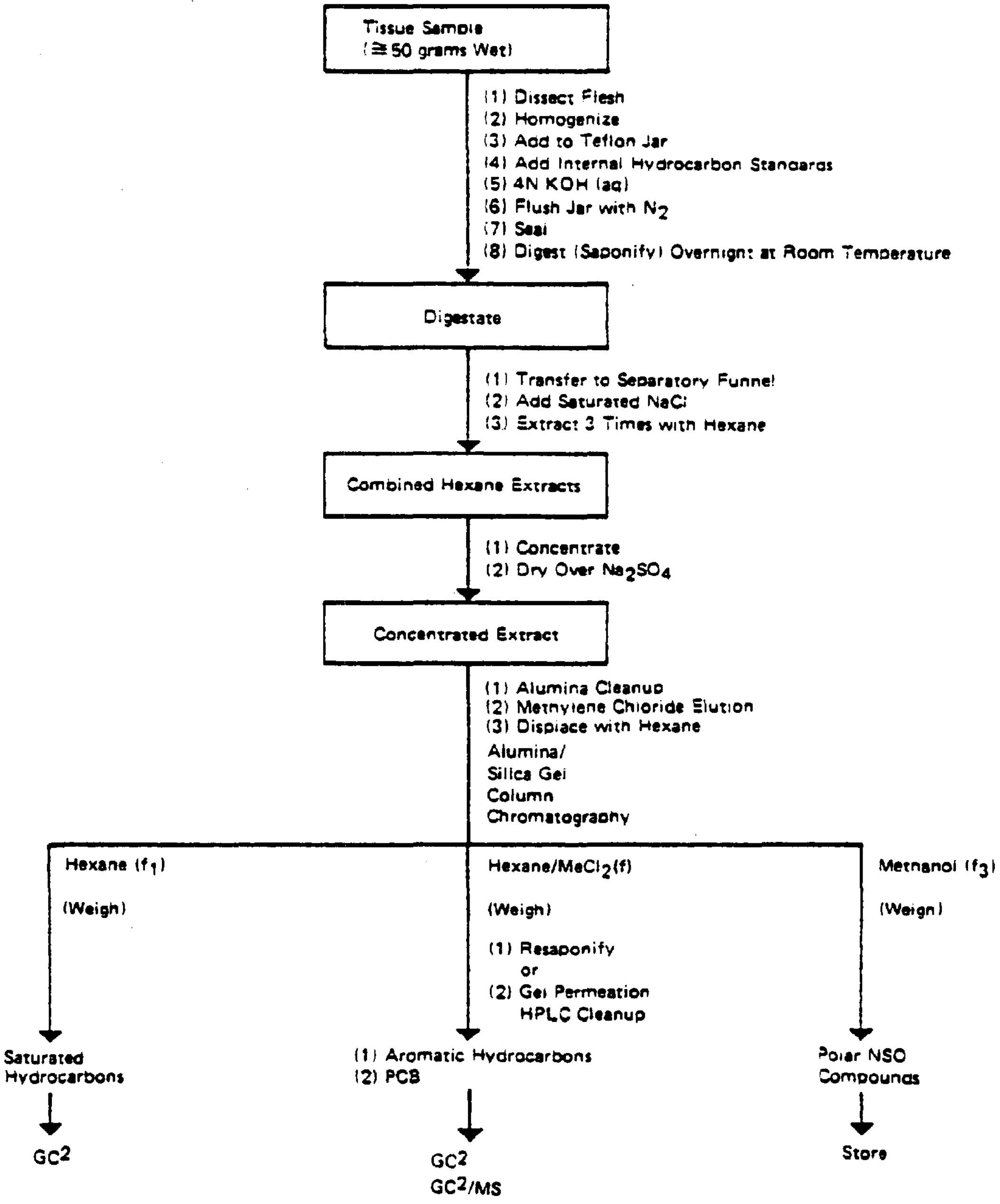


Figure 2.2. Analytical scheme for tissue samples (after Warner, 1976; Boehm et al., 1982).

3. RESULTS AND DISCUSSION

3.1 Overall Findings

Several general trends in the data presented in the following sections should be noted here along with several considerations of the use of marker compounds as "fingerprints" to trace aged AMOCO CADIZ oil in environmental samples.

3.1.1 Weathering of AMOCO CADIZ Oil

The chemical composition of spilled oil from the tanker changed markedly over the first days to weeks, both at sea and once associated with sediment (Atlas et al., 1981; Calder and Boehm, 1981; Boehm et al., 1981). The changes are well documented in Figures 3.1 and 3.2 and are summarized in Table 3A. For comparison, the background saturated and aromatic hydrocarbon composition of sediment samples is illustrated. The non-impacted sediments contain: 1) an unresolved complex mixture (UCM) of hydrocarbon material in both fractions, 2) terrigenous n-alkanes (odd chain) in the saturated fraction, and 3) pyrogenic PAH compounds in the aromatic fraction. Weathered AMOCO CADIZ oil is identified as such in the sections that follow based on the following:

1) The presence of large UCM in f_1 and f_2 fractions with residual triterpenoid peaks.

2) The presence of isolated isoprenoid hydrocarbon compounds in the resolved (peak) part of the GC trace (in samples during the first year post-spill only).

3) The dominance of alkylated phenanthrene (C_2, C_3, C_4), dibenzothiophene, naphthalene, and fluorene (in earlier samples) compounds in the aromatic fraction and a dominance of these aromatics versus pyrogenic PAH (i.e. fluoranthene, pyrene, benzanthracene, chrysene, benzopyrenes, etc.).

3.1.2 Persistence of Marker Compounds

The most persistent compounds in the saturated (f_1) fraction are the pentacyclic triterpanes (PCT); in the aromatic (f_2) fraction the alkylated phenanthrenes (P) and dibenzothiophenes (DBT) are most persistent. To examine the PCT compound distribution, GC/MS analysis of the f_1 fraction was necessary (e.g. Figs. 3.3 and 3.4). This results in a "terpanogram" yielding information on the relative concentration of eight PCT compounds used by several investigators as indicators of presence and origin of petroleum (e.g. Dastillung and Albrecht 1976; Pym et al., 1975).

Two PCT time series (Fig. 3.5) reveal that the PCT fingerprint is rather constant throughout the December 1978 to March 1980 time period

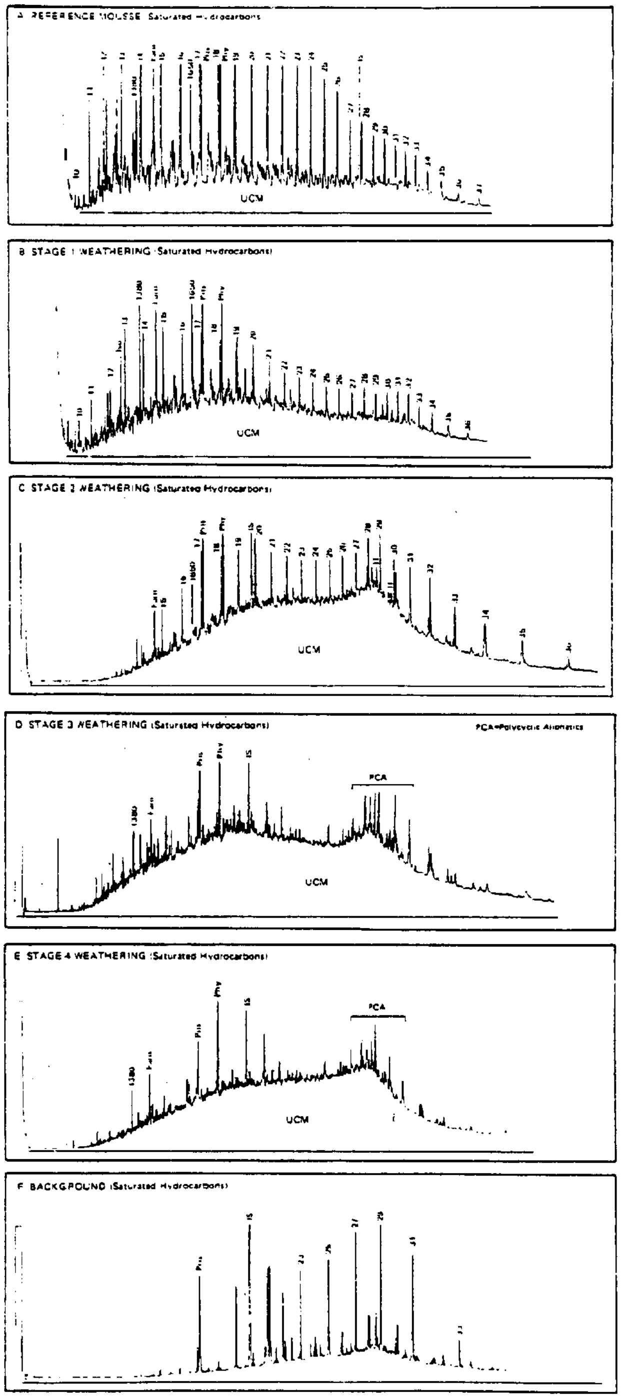


FIGURE 3.1. Weathering patterns of saturated hydrocarbons in AMOCO CADIZ oil.

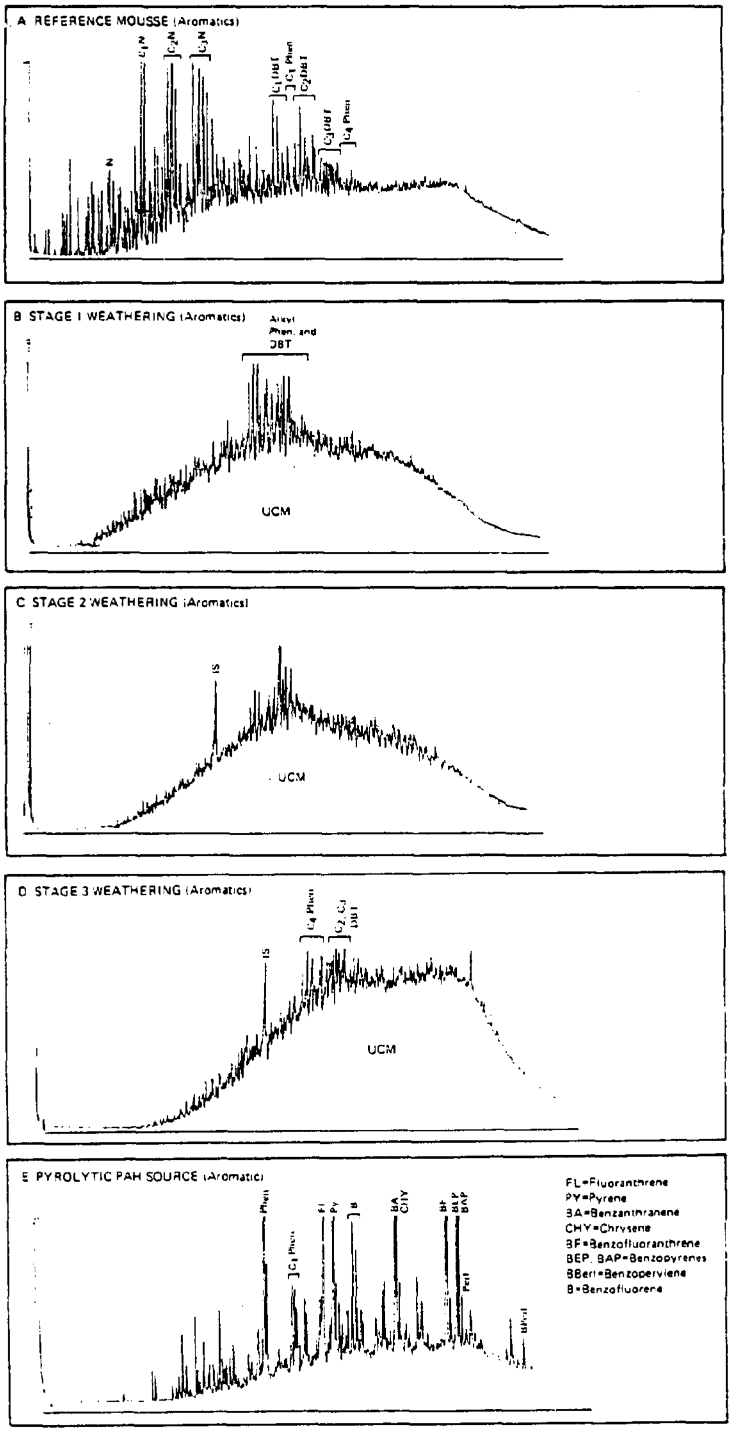


FIGURE 3.2. Weathering patterns of aromatic hydrocarbons in AMOCO CADIZ oil.

TABLE 3A. Weathering of AMOCO CADIZ oil.

RAPID

1. Loss of volatile ($<$n-C_{15}) hydrocarbons due to evaporation of:

   a. alkanes
   b. aromatics - benzenes, naphthalenes, biphenyl (one- to two-ring aromatics)

2. Relative and/or absolute increase in unresolved complex mixture.

MODERATE

1. Microbial degradation of n-alkanes; preferential attack of n-alkanes versus branched alkanes (i.e., decrease in ratio of alkanes to isoprenoids).

2. Loss (to solution or other processes) of most resolved saturated hydrocarbon GC traces.

3. Emergence of triterpanes as major molecular markers in saturated fraction.

4. Increase in UCM, with formation of secondary (bimodal) UCM distribution.

5. Loss of fluorenes (two aromatic rings, one saturated ring) and alkyl naphthalenes.

6. Increase in abundance of polar fraction.

LONG

1. Persistence of alkylated phenanthrenes and alkylated dibenzothiophenes.

2. Increase in polar fraction.

3. Loss of long chain n-alkanes and isoprenoids.

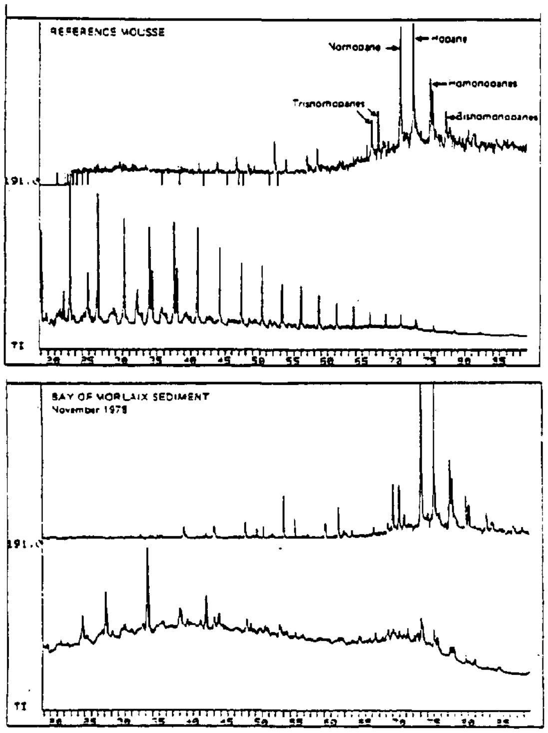


FIGURE 3.3. GC/MS selected ion searches for pentacyclic triterpanes (hopanes) in AMOCO CADIZ reference and November 1978 weathered oil in sediments.

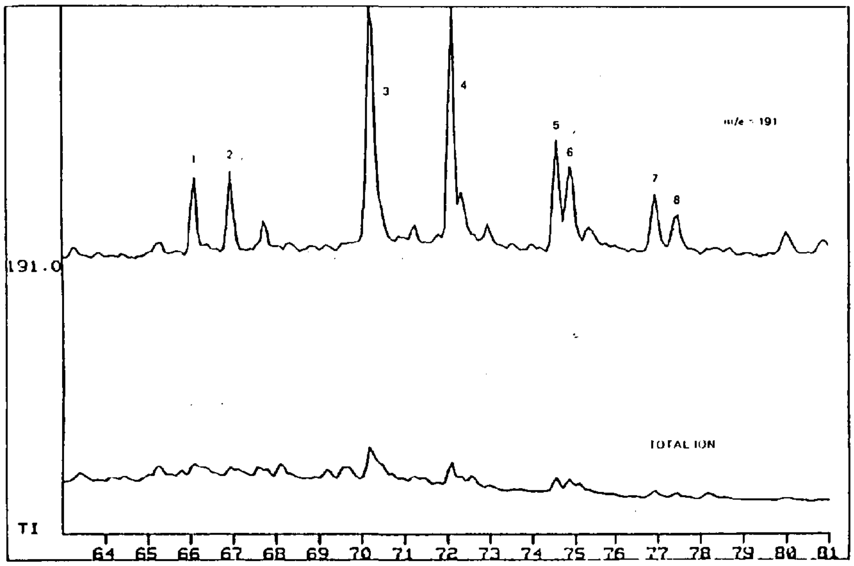
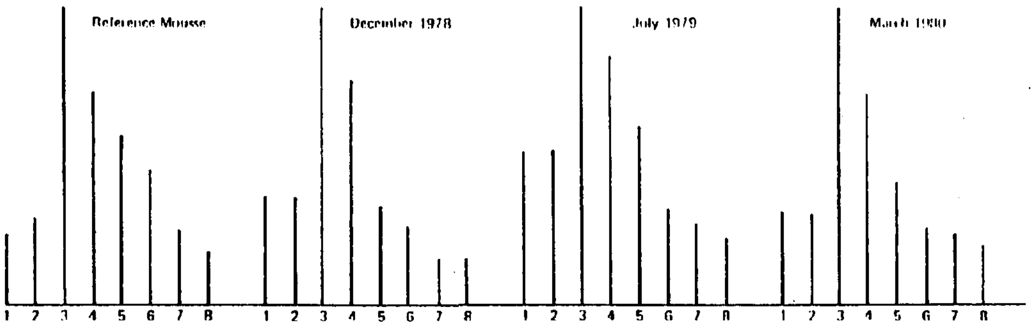
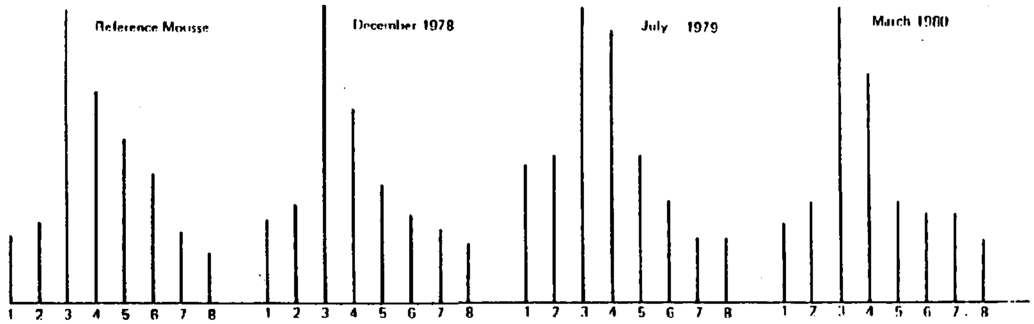


FIGURE 3.4. Triterpane (m/e 191) mass chromatogram of weathered oil. 1,2 = Trisnorhopane ($C_{27}H_{46}$), 3 = Norhopane ($C_{29}H_{50}$), 4 = Hopane ($C_{30}H_{52}$), 5,6 = Homohopanes ($C_{31}H_{54}$), 7,8 = Bishomohopanes ($C_{23}H_{56}$).



STATION 3-ILE GRAND MARSH



STATION 6- ABER WRAC'H

FIGURE 3.5. 191 terpanograms. (1,2 = Trisnorhopanes, 3 = Norhopane, 4 = Hopane, 5,6 = Homohopanes, 7,8 = Bishomohopanes)

at the two stations. Note that the PCT fingerprints of AMOCO CADIZ and TANIO oils are quite distinct (Fig. 3.6), notably in the ratios of compounds 1, 2 and 5, 6. Thus it appears that PCT fingerprints offer a good means to trace AMOCO oil in highly weathered samples when most other identifiable molecular characteristics have been lost.

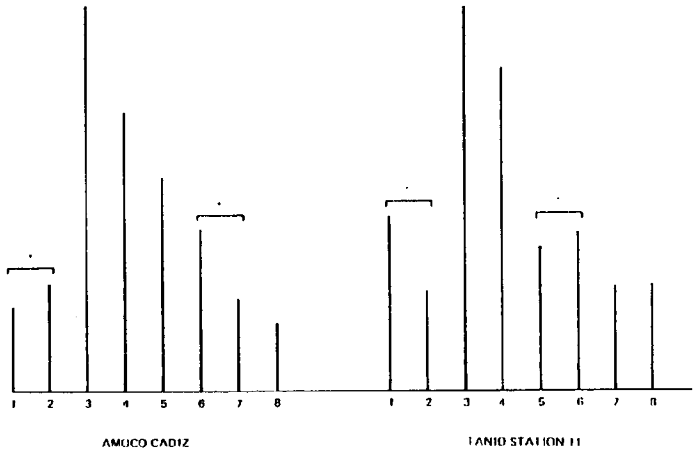


FIGURE 3.6. 191 terpanograms of crude oils.

As the most persistent aromatic compounds, the P and DBT compounds (mainly C_2, C_3, and C_4) mark AMOCO CADIZ oil in tissue (oysters) and sediments through mid-1980. The final (June 1981) sediment sampling failed to reveal significant P and DBT levels in any of the stations. The latest (1981) status of the oyster P and DBT levels is unknown. However, through most of the data to be discussed, the P and DBT compounds dominate the f_2 distribution. The ratios of C_2P/C_2DBT and C_3P/C_3DBT, used by Overton et al. (1981) to differentiate oils, in this spill remain in the 0.3-0.6 range. The use of this ratio is discussed in the text.

3.1.3 Residues in Tissues

As stated, the P and DBT compounds are most readily associated with oyster tissue samples in the two-year period following the spillage. The branched alkanes (isoprenoids) also persist throughout this period.

3.1.4 Environmental Variability

A major question in oil spill studies and for that matter environmental studies in general is the question of patchiness of pollutant distributions and the variability due to patchiness in chemical measurements. To shed some light on this subject two sets of measurements are available. Two principal investigators (Atlas and Ward) obtained samples at the same time and location in several instances, Atlas sampling the top 3-5 cm, Ward sampling an entire sediment core but subdividing the top 0-5 cm section. The total hydrocarbon values (Table 4) reveal wide disparities where contamination is very heavy (pooling of oil in the Ile Grande) but reasonable to excellent agreement in most cases. (Note also that additional replicate analyses are available for sediment samples in Section 3.2 as well).

TABLE 4. Analysis of sampling variability.

		TOTAL HYDROCARBONS (f_1 + f_2) (μg/g)	
STATION	DATE	ATLAS (SURFACE)	WARD (0-5 cm)
Ile Grande	12-78	650/1300[a]	1,100
	3-79	4,700	700
L'Aber Wrac'h	12-78	400/400[a]	770
	3-79	870	1,100
	7/8-79	390	290
	11-79	1,100	1,100

[a] Replicate samples.

3.2 Surface Sediments (Atlas, University of Louisville)

The frequency of sampling for surface sediment (0-3 cm) is shown in Table 5. Ten primary locations were sampled repeatedly (Fig. 3.7). Results of total hydrocarbon determinations for the ten stations, as these concentrations varied with time, are presented in Figures 3.8 through 3.12. Also included in these figures are source evaluations for each sample hydrocarbon assemblage, based on GC information. The biogenic (B) category indicates that terrigenous odd chain n-alkanes dominate the f_1 GC trace. The pyrogenic (P) category signifies an important abundance of combustion-related polynuclear aromatic hydrocarbons (PAH) in the f_2 fraction as well as the presence of some unresolved material (UCM) in both the f_1 and f_2. In those samples labeled B or B/P the primary sources of hydrocarbons are as indicated although a small fraction of the hydrocarbons may consist of petroleum. Figure 3.13 summarizes these source criteria. Only GC/MS analysis of each sample would definitely eliminate the small chance of a false negative (i.e. not finding AMOCO oil where there were traces).

The error bars in the figures indicate that two determinations were made for the December 1978 samples (Table 6). All other determinations were based on one replicate. Note that the coefficient of variation ranges from .01 (1%) to .94 (94%). The higher variability is observed in samples with the lowest and highest (~1000 ppm) absolute concentration levels, the former due to natural patchiness, the latter owing to "pooling" of oil in heavily impacted stations.

GC/MS results are available for stations 3, 5 and 7 throughout the study period and are presented graphically in Figures 3.14 through 3.33. These semi-log plots illustrate quantitatively the aromatic composition of all samples normalized to C_3 dibenzothiophene or where C_3DBT is absent to pyrene. C_3DBT was used to normalize the data as it is assumed that these compounds are the slowest to weather of all of the aromatic hydrocarbons.

All AMOCO CADIZ-impacted stations illustrate a normal weathering sequence (i.e. see Fig. 3.1). However, fresh inputs of petroleum were observed to impact the region of stations 7 and 8 in the form of tar chips during November 1979 and stations 2, 11 and 12 in the form of oil from the TANIO spill in August of 1980 (Fig. 3.34).

Although a wide range of residual oil concentrations appear in the various samples, several trends in the data seem apparent. Stations 1, 9, and 10 remain unimpacted by the spill throughout the study. Station 2 remains unimpacted until a secondary petroleum input influences its hydrocarbon chemistry in November of 1979 (the timing of the secondary tar impact at stations 7 and 8 also is probably related to leakage from the sunken tanker) and again in August of 1980, the latter relating to the TANIO spill, also readily detected at Stations 11 and 12 at this time. Through March of 1980 weathered AMOCO CADIZ oil is readily detected at Stations 3, 4, 5, 6, 7 and 8. However, the results of the August 1980 samplings indicate that inputs of non-AMOCO CADIZ hydrocarbons (i.e. background) at Stations 6 and 8 become dominant. At stations 3 and 7 where GC/MS data exists, the main AMOCO CADIZ aromatic

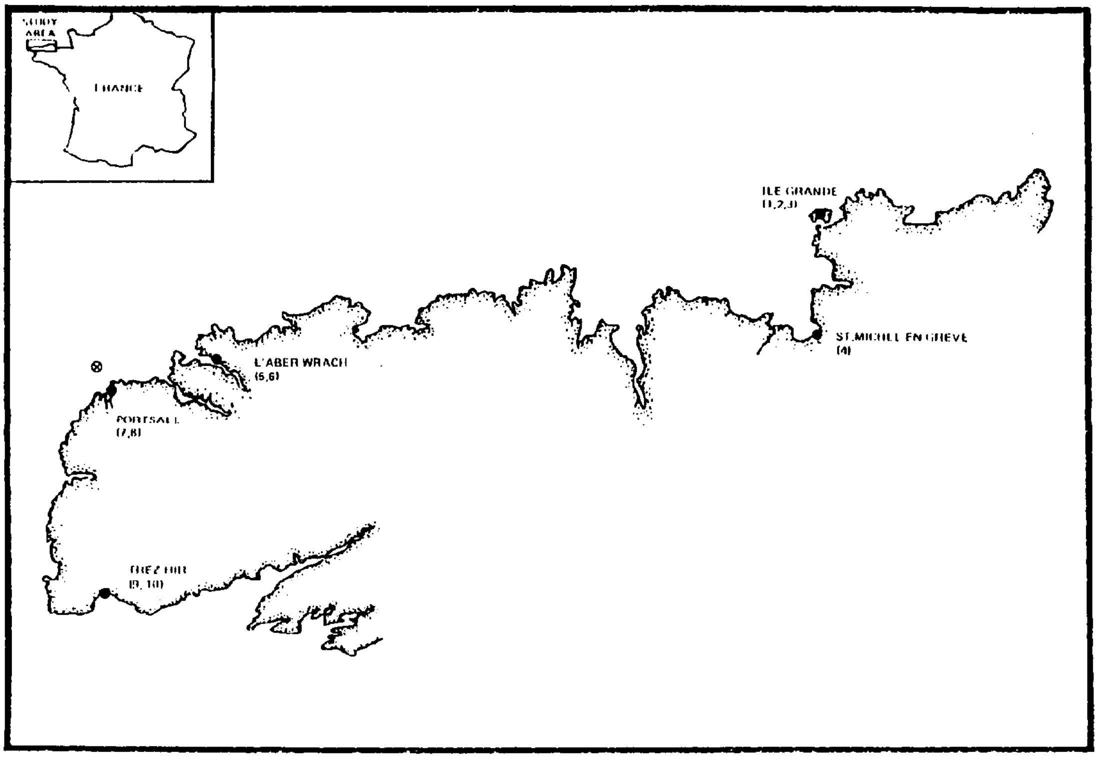


FIGURE 3.7. Surface sediment sampling locations (Atlas).

TABLE 5. AMOCO CADIZ chemistry program; surface sediments (Atlas).

Frequency	
April 1978-October 1978 (Calder)	
December 1978	20
March 1979	10
July 1979	12
November 1979	15
March 1980	11
May 1981	12
Total	80

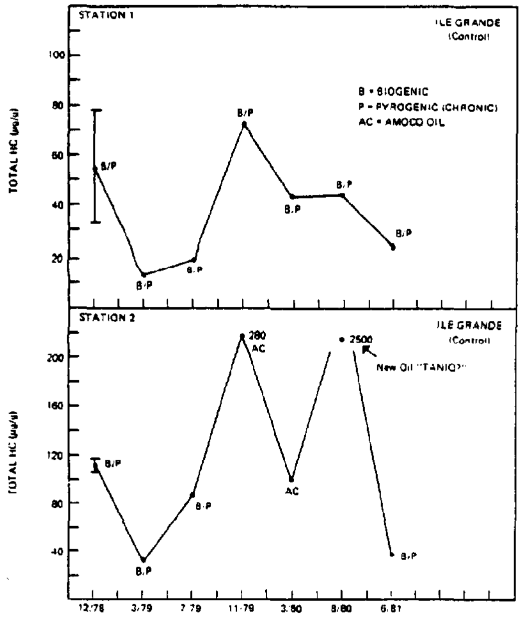
Locations

Ten Primary Stations

1,2,3	Ile Grande
4	St. Michel-en-Greve
5,6	L'Aber Wrac'h
7,8	Portsall
9,10	Trez-Hir
11-14	Other Impact Stations

GC/MS

Stations 3 (Ile Grande), 5 (L'Aber Wrac'h), 7 (Portsall)



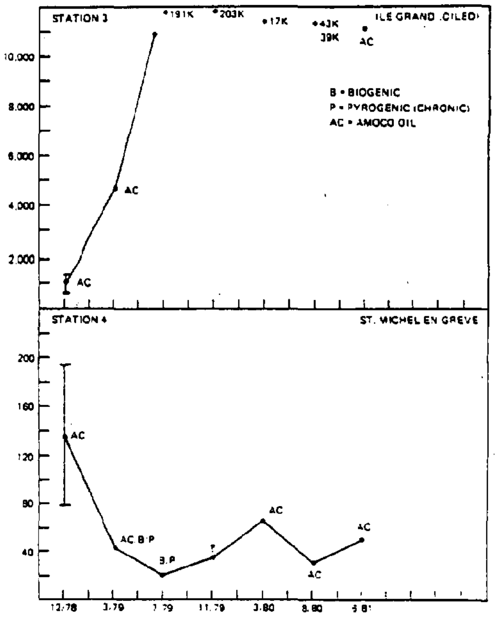
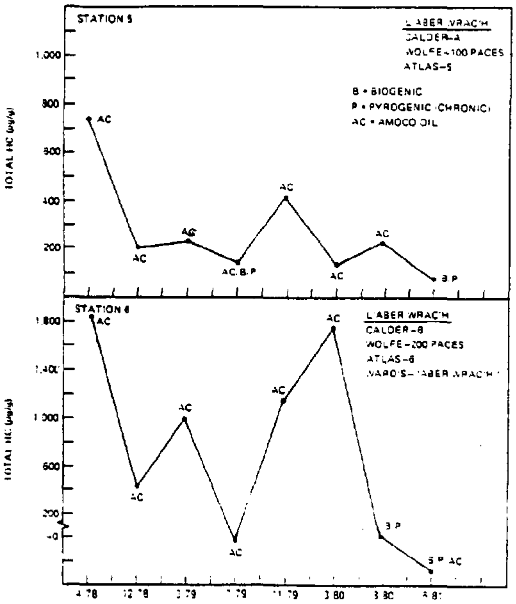


FIGURE 3.8. (Left) Ile Grande (control) sediment time series.
FIGURE 3.9. (Right) Ile Grande (oiled) and St. Michel-en-Greve time series (note scale difference between the two plots).



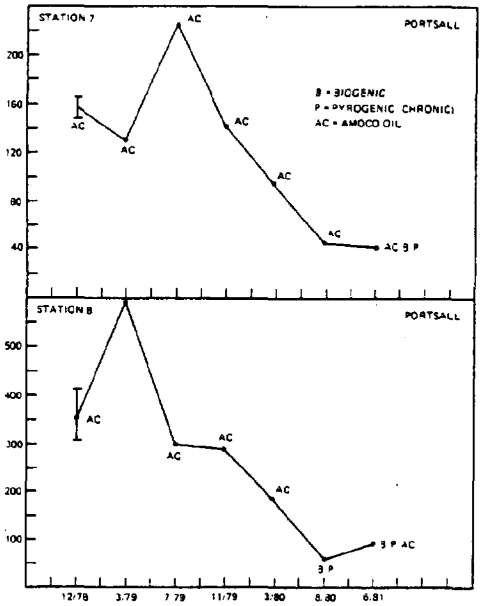


FIGURE 3.10. Aber Wrac'h sediment time series (left).
FIGURE 3.11. Portsall sediment time series (right).

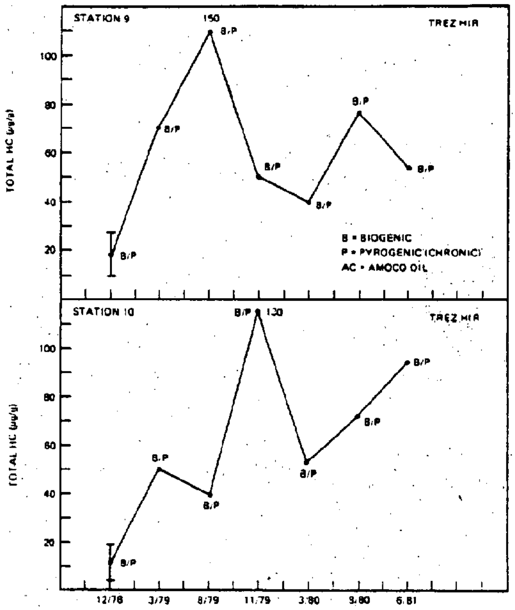


FIGURE 3.12. Trez Hir sediment time series.

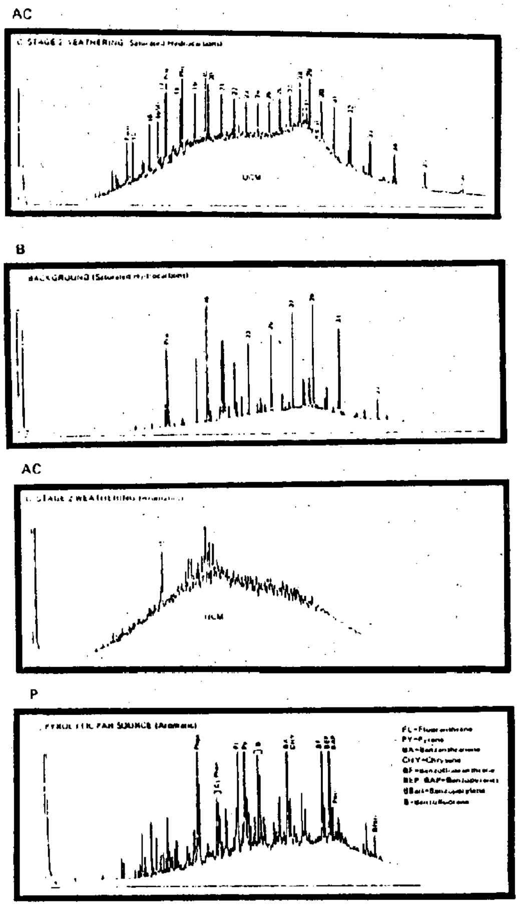


FIGURE 3.13. Hydrocarbon compositions forming the basis of source classification categories.

TABLE 6. Replication of hydrocarbon concentration data (based on December 1978 analyses of two replicates).

STATION	$\bar{x}$	$\sigma/\bar{x}$
1	56	0.57
2	113	0.08
3	1,000	0.52
4	135	0.60
5	401	0.01
6	217	0.06
7	159	0.10
8	358	0.21
9	18	0.71
10	11	0.94

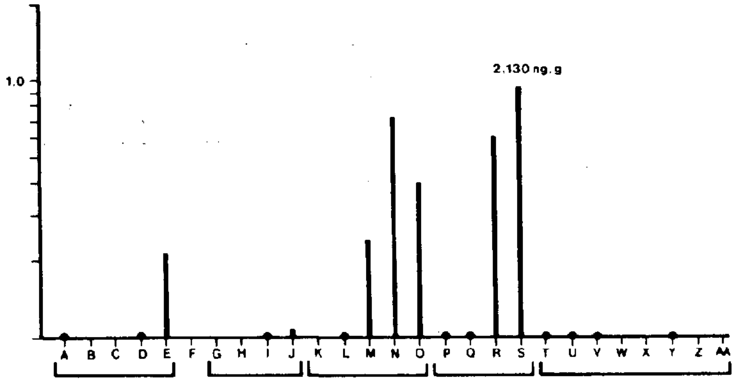
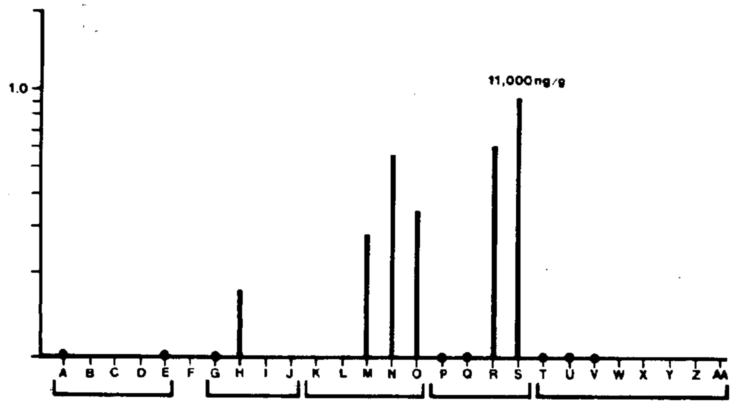
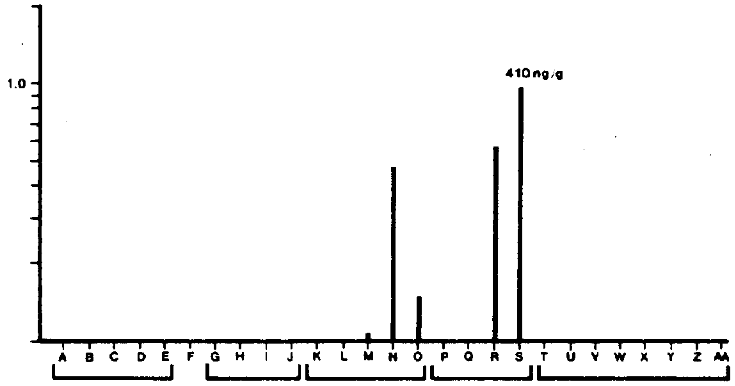


FIGURE 3.14. Aromatic hydrocarbons, station 3, December 1978; normalized to C_3DBT.
(A = napthelenes, B = C_1N, C = C_2N, D = C_3N, E = C_4N, F = biphenyl, G = fluorenes, H = C_1F, I = C_2F, J = C_3F, K = phenanthrenes, L = C_1Ph, M = C_2Ph, N = C_3Ph, O = C_4Ph, P = dibenzothiophenes, Q = C_1DBT, R = C_2DBT, S = C_3DBT, T = fluorene, U = pyrene, V = benzo(a)anthracene, W = chrysene, X = benzofluoranthene, Y = benzo(a)pyrene, Z = benzo(e)-pyrene, AA = perylene)





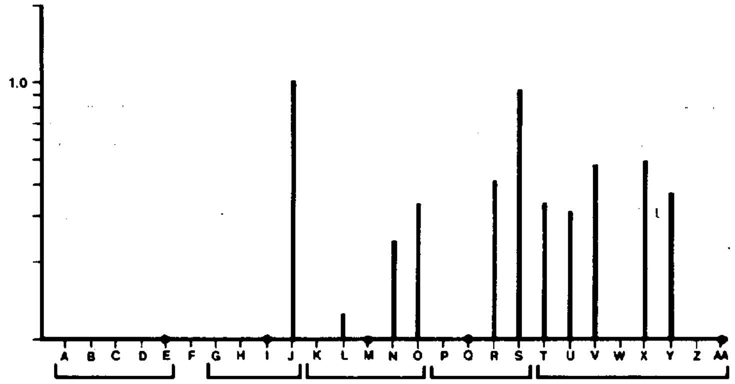
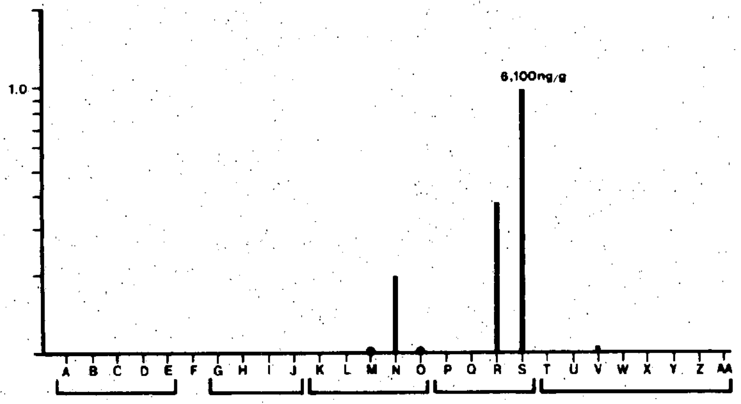


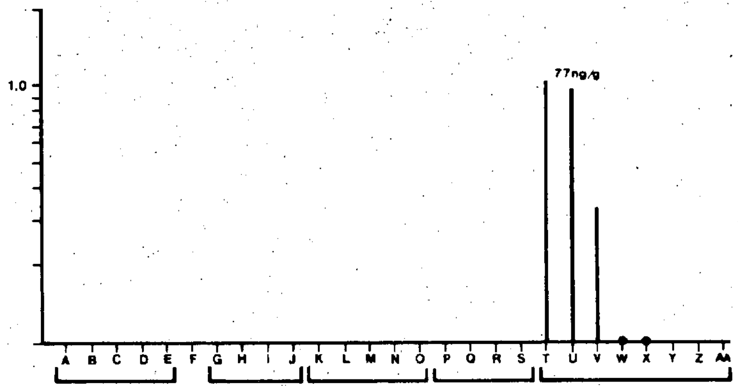
FIGURE 3.15. (Top) Aromatic hydrocarbons, station 3, March 1979; normalized to C_3DBT.

FIGURE 3.16. (Middle) Aromatic hydrocarbons, station 3, July 1979; normalized to C_3DBT.

FIGURE 3.17. (Bottom) Aromatic hydrocarbons, station 3, November 1979; normalized to C_3DBT.

(See Figure 3.14 for key.)





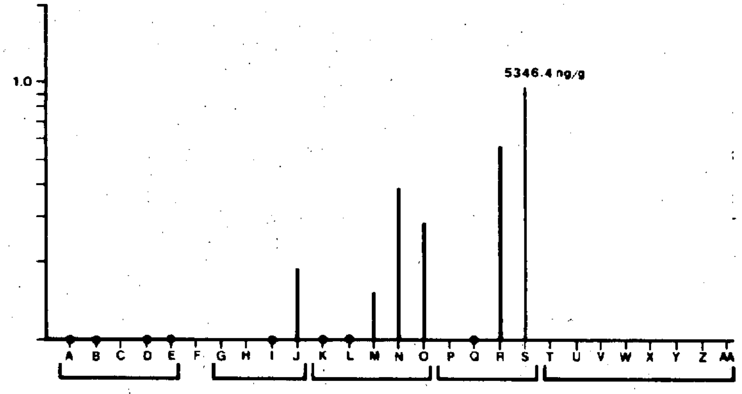
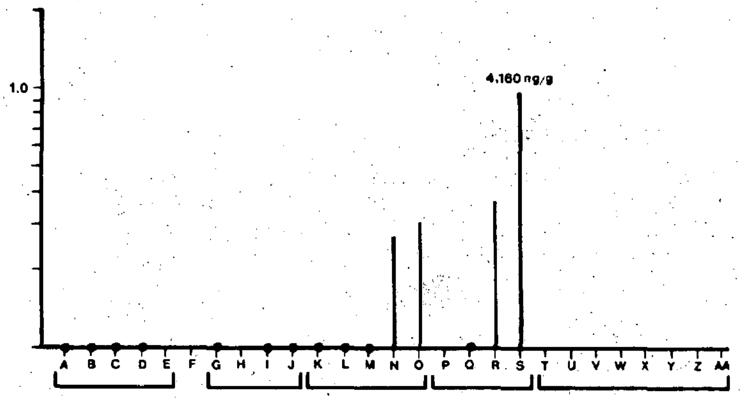


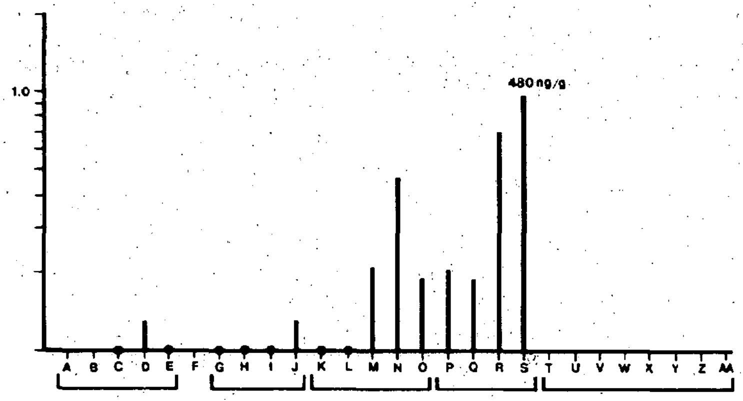
FIGURE 3.18. (Top) Aromatic hydrocarbons, station 3, March 1980; normalized to C_3DBT.

FIGURE 3.19. (Middle) Aromatic hydrocarbons, station 3, June 1981; normalized to C_3DBT.

FIGURE 3.20. (Bottom) Aromatic hydrocarbons, station 5, April 1978; normalized to C_3DBT.

(See Figure 3.14 for key.)





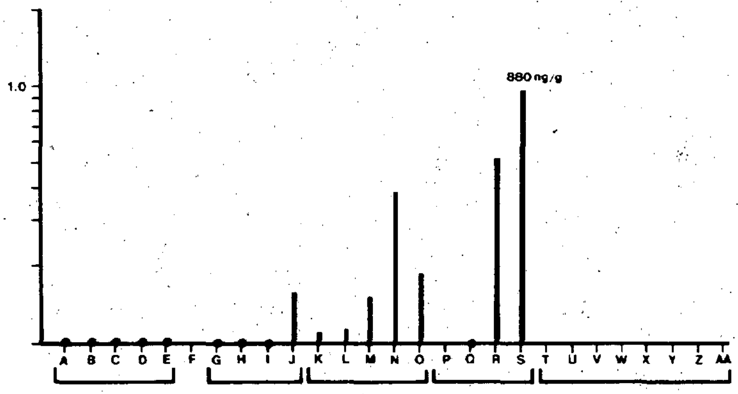
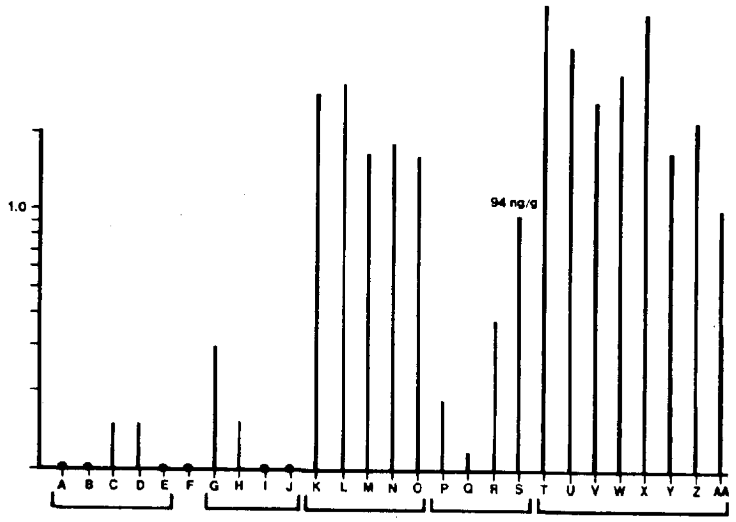


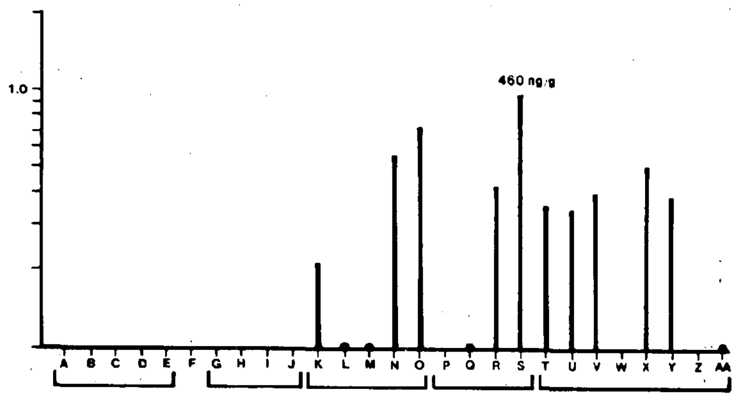
FIGURE 3.21. (Top) Aromatic hydrocarbons, station 5, October 1978; normalized to C_3DBT.

FIGURE 3.22. (Middle) Aromatic hydrocarbons, station 5, December 1978; normalized to C_3DBT.

FIGURE 3.23. (Bottom) Aromatic hydrocarbons, station 5, March 1979; normalized to C_3DBT.

(See Figure 3.14 for key.)





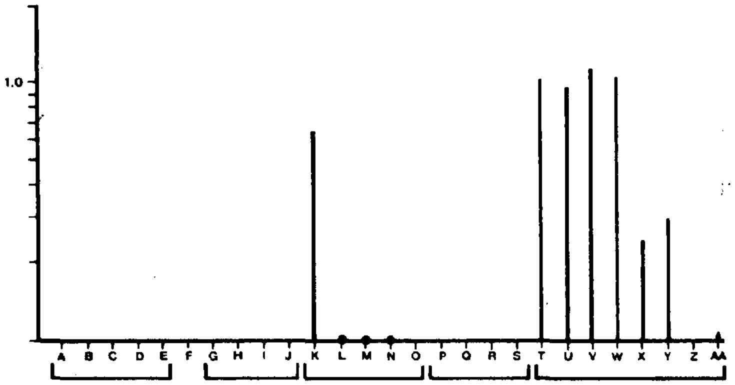
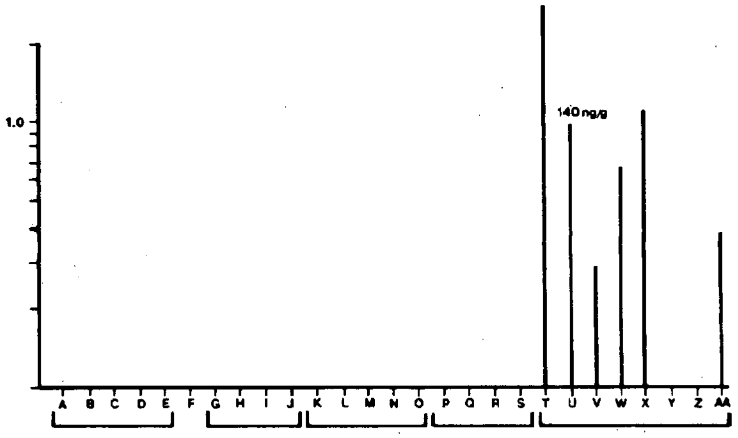


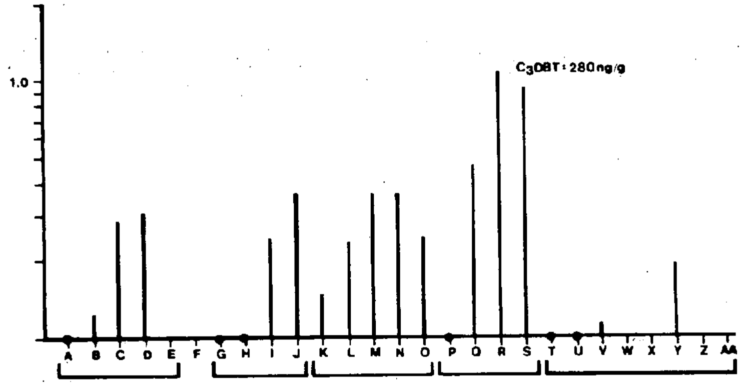
FIGURE 3.24. (Top) Aromatic hydrocarbons, station 5, July 1979; normalized to C_3DBT.

FIGURE 3.25. (Middle) Aromatic hydrocarbons, station 5, November 1979; normalized to C_3DBT.

FIGURE 3.26. (Bottom) Aromatic hydrocarbons, station 5, March 1980; normalized to C_3DBT.

(See Figure 3.14 for key.)





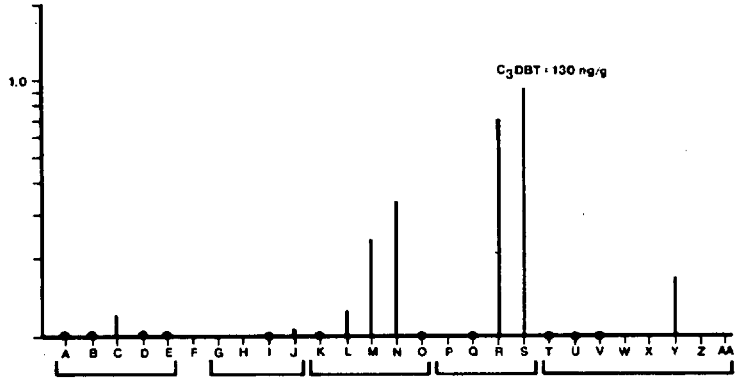
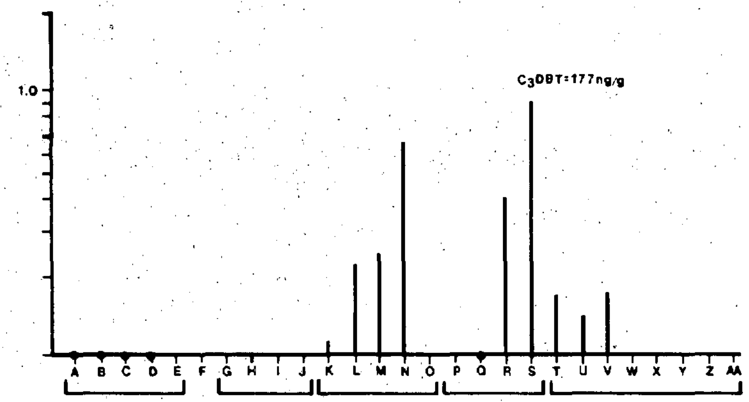


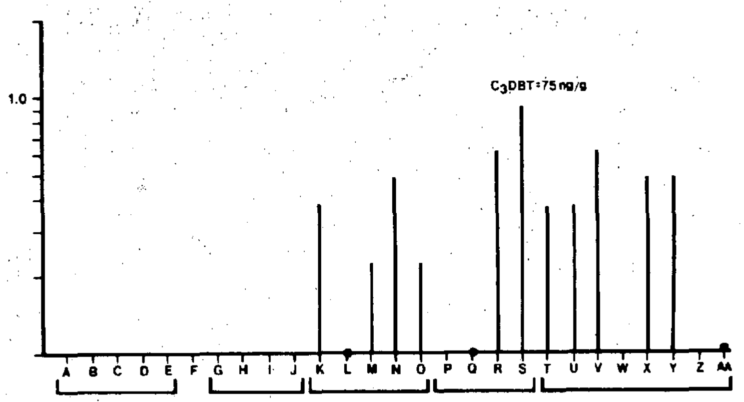
FIGURE 3.27. (Top) Aromatic hydrocarbons, station 5, June 1981; normalized to pyrene.

FIGURE 3.28. (Middle) Aromatic hydrocarbons, station 7, December 1978; normalized to C_3DBT.

FIGURE 3.29. (Bottom) Aromatic hydrocarbons, station 7, March 1979; normalized to C_3DBT.

(See Figure 3.14 for key.)





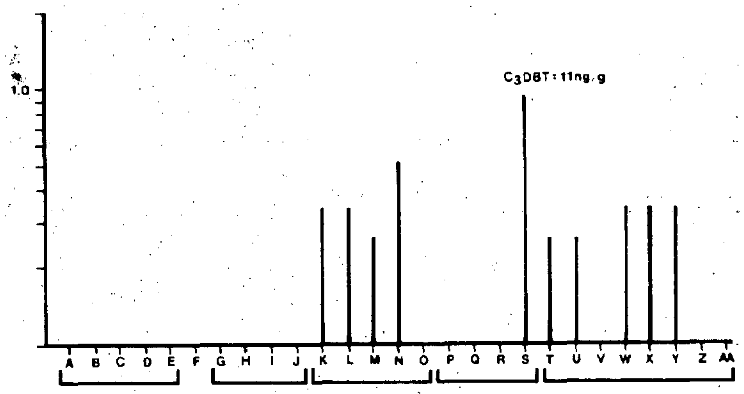


FIGURE 3.30. (Top) Aromatic hydrocarbons, station 7, July 1979; normalized to C_3DBT.

FIGURE 3.31. (Middle) Aromatic hydrocarbons, station 7, November 1979; normalized to C_3DBT.

FIGURE 3.32. (Bottom) Aromatic hydrocarbons, station 7, March 1980; normalized to C_3DBT.

(See Figure 3.14 for key.)

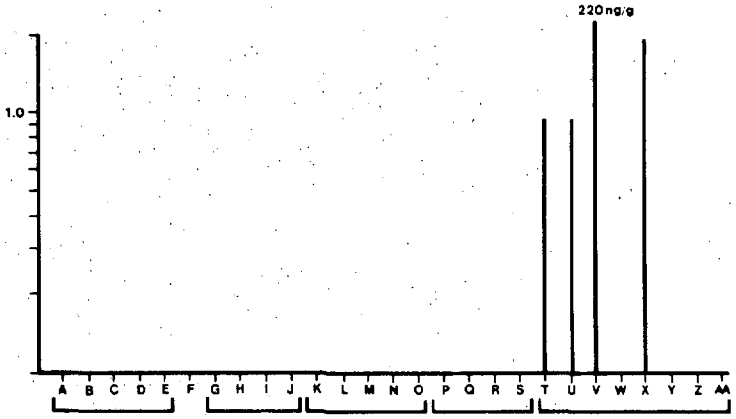


FIGURE 3.33. Aromatic hydrocarbons, station 7, June 1981; normalized to pyrene. (See Figure 3.14 for key.)

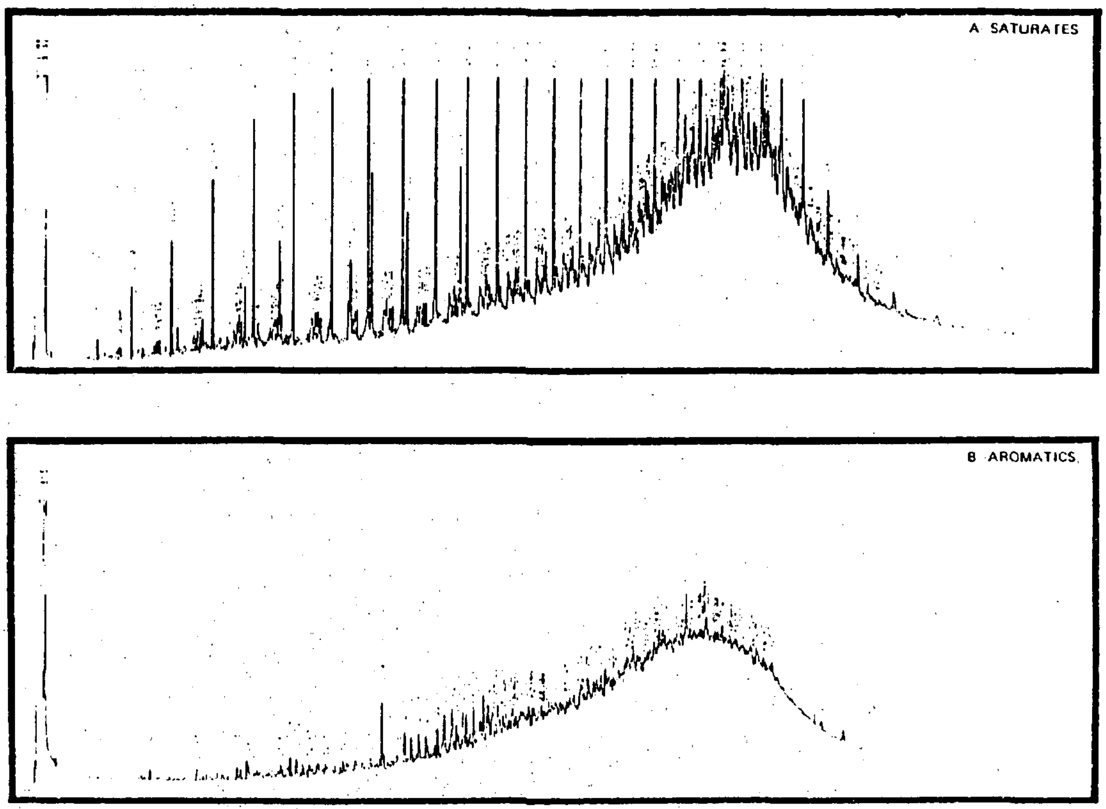


FIGURE 3.34. TANIO oil.

marker compounds, the C_3 dibenzothiophenes, and C_3 and C_4 phenanthrenes, persist but the pyrogenic PAH compounds have replaced any AMOCO CADIZ oil traces at Station 5 in L'Aber Wrac'h.

The last sampling, June 1981, reveals total disappearance of traces of AMOCO CADIZ aromatic marker compounds at stations 3, 5, and 7. By June 1981 the only unequivocal presence of AMOCO oil is seen at station 3 in Ile Grande, although it has been extremely weathered. Only pentacyclic triterpanes can be linked to the residual AMOCO oil. GC patterns suggest that petroleum still affects stations 4, 6, 7, and 8, but in only minor quantities relative to other inputs.

Thus, for the most part, less than three and one half years has been required to allow normal background inputs to resume their sedimentary dominance at all but the most heavily impacted (in terms of post cleanup oil concentrations) and lowest energy (i.e. most protected from waves) environments (i.e. station 3 in the Ile Grande).

Further interpretive details are presented in Atlas et al. (1981).

3.3 Offshore Sediments (Marchand, CNEXO)
L'Aber Benoit Sediments (Courtot, U. West Brittany)

In this phase of the analytical chemical program the levels, the persistence, and the precise chemical nature of petroleum hydrocarbons in the offshore sediments of the Bays of Morlaix and Lannion were examined as well as those of L'Aber Benoit sediments (November 1978 only). A summary of the samples analyzed appears in Table 7 and in Figure 3.35.

TABLE 7. AMOCO CADIZ chemistry program; 2. Offshore surface sediments (Marchand) and Aber Benoit sediments (Courtot).

Frequency:	
April 1978	6
July 1978	14
November 1978	13+7
February 1979	13
TOTAL	53

Locations:
Aber Benoit (November 1978)
Baie de Morlaix
Baie de Lannion

GC/MS:
Four Time Series (18 Samples)

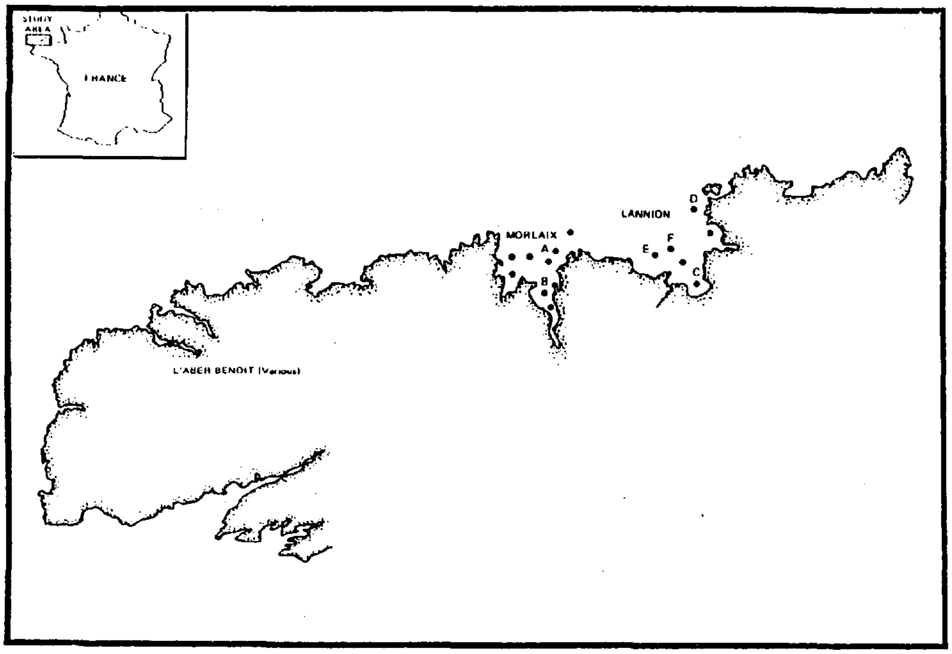


FIGURE 3.35. Offshore surface sediment and l'Aber Benoit sampling locations (Marchand, Courtot).

Hydrocarbon concentrations and source classifications for the entire data set are shown in Table 8. Individual aromatic hydrocarbon determinations by GC/MS appear for several time series in Tables 9 through 13 and for two of the L'Aber Benoit samples in Table 14.

An instructive way of viewing the time series information is presented in Figures 3.36 and 3.37. At both the Terenez/ Morlaix and Ile Grande time series, concentrations increased between April and July 1978. In the case of the Terenez samples, the increase is due to offshore transport of weathered oil as evidenced by 1) an increase in absolute concentrations, 2) a decrease in the ALK/ISO ratio, and 3) an increase in phenanthrenes (total P, C_1, C_2, C_3, C_4P) and dibenzothiophenes, without an accompanying increase in the pyrogenic PAH compounds (m/e 202). However, the Ile Grande benthic samples show an increase in total hydrocarbons along with increases in the aromatics including the pyrogenic PAH. This latter finding indicates that both petroleum hydrocarbons and combustion-related PAH material are being transported to and deposited in the offshore sediments near Ile Grande by a similar mechanism, most likely in association with suspended matter from riverine plumes. Figure 3.38, a plot of phenanthrene and its alkyl homologues at the Morlaix Station (Station B), reveals that while the source of the phenanthrenes is petroleum in July 1978, as evidenced by the greater abundance of alkylated compounds versus the parent (unsubstituted) compounds, the input in February of 1979 is largely pyrogenic (i.e. greater amounts of parent phenanthrene). This illustrates both the usefulness of detailed GC/MS-derived data and their subsequent presentation in alkyl homologue distribution plots.

TABLE 8. AMOCO CADIZ sediment sample, source classification (GC) (offshore sediments).

SAMPLE NO.	F_1	(ug/g)	F_2	(ug/g)
AC100	4/5B	84.1	3/4	90.4
AC369	3/5B	252.9	3	3,172.5
AC426	4/5B	35.5	3	133.6
AC103	4/2/5B	200.6	4/2	397.0
AC365	4/5B	107.9	4/2	503.4
AC429	5B/4	37.3	5/3	31.6
AC42	1	109.0	2/1	123.7
AC138	2/4	408.8	2/4	502.8
AC371	4/2	19.4	2/4	97.7
AC453	2/4	142.5	2/4	36.0
AC56	2	47.2	2	86.0
AC139	4	5.9	4/2	17.2
AC381	2	54.4	2	119.3
AC458A	4	5.0	5	8.1
AC458B	4	4.4	5	7.9
AC127	2	36.9	2/4	58.5
AC370	4/2	18.6	3/4/2	48.8
AC452	2/4	13.8	4	41.5
AC132	2/4	165.8	4/2	211.7
AC362	2/4	24.5	4/2	44.7
AC141	2	15.6	2	39.2
AC396	4/5B	6.0	3/4	13.6
AC432	4	35.4	4/2	26.9
AC134	5B/4	78.3	2/4	171.9
AC451	4/2	42.2	4/2	50.7
AC44	1	38.5	2	69.1
AC121	2	17.5	2	44.8
AC384	2	32.1	2/1	173.6
AC112	2/4	122.5	2	239.3
AC389	2/4	56.4	2/4	151.1
AC445	2/4	29.0	2/4	64.8
AC107	2	53.2	2/4	176.7
AC376	2/4	5.9	2/4	8.9
AC436	2	27.7	2	60.6
AC53	1	28.9	2	31.5
AC118	2/4	82.2	2/4	97.2
AC377	4	31.9	4	132.1
AC438	2/4	15.8	4	32.1
AC51	1/2	96.4	1/2	102.4
AC114	2/4	56.4	2/4	249.6
AC379	2/4	27.2	2/4	80.7
AC440	2	103.5	2/4	104.5
AC48	2/1	21.0	4	14.3
AC125	2	59.1	2	27.8
AC378	2	63.3	2	181.0
AC479	2	36.5	2	37.9

AC40 and 50 series sampled 4/78
AC100 series sampled 7/78
AC300 series sampled 11/78
AC400 series sampled 2/79

L'Aber Benoit Sediments:

ABT	2	158.2	2	180.8
ACC	4	25.1	4	29.3
AB25	2	29.2	2	29.7
AB29	4	13.6	4	17.5
AB16	2	22.2	2	28.6
AB21	2	455.1	2	440.7
AB4	2	75.6	2	76.9

TABLE 9. Terenez/Morlaix time series (station A).

SAMPLE	DATE	ALIPHATICS (µg/g) TOTAL	ALIPHATICS (µg/g) RESOLVED	ALK/ISO	AROMATICS (µg/g) TOTAL	AROMATICS (µg/g) RESOLVED
AC 42	4/78	109.0	6.1	0.79	123.7	7.4
AC 138	7/78	408.8	11.7	0.10	502.8	17.2
AC 371	11/78	19.4	1.3	0.10	97.7	2.4
AC 453	2/79	142.5	2.8	0.51	36.0	1.6

	N	C_1N	C_2N	C_3N	C_4N	F	C_1F	C_2F	C_3F	P	C_1P	C_2P	C_3P	C_4P
AC 42	nd	10.3	40.9	115.5	161.0	16.8	20.1	54.3	198.9	125.7	95.4	153.3	274.8	138.3
AC 138	2.9	6.3	62.5	423.2	730.7	nd	37.4	230.2	905.8	20.4	151.4	593.5	1678.9	882.9
AC 371	nd	nd	nd	nd	nd	nd	nd	4.4	88.0	7.4	9.0	12.4	41.9	27.6
AC 453	2.1	4.6	9.7	10.6	18.8	nd	1.0	7.8	14.8	5.2	5.9	15.0	22.0	21.9

	DBT	C_1DBT	C_2DBT	C_3DBT	FL	PYR	CHRY	BF	B(e)P	B(a)P	PERL
AC 42	19.2	113.6	714.4	1088	195.0	171.4	265.7	298.9	83.5	64.8	24.5
AC 138	12.4	383.9	3363.0	6388	3.0	47.1	77.4	120.8	87.2	45.4	20.3
AC 371	nd	4.0	54.8	179.0	9.1	7.6	16.4	20.9	9.8	3.7	1.8
AC 453	2.3	6.7	63.8	93.5	4.8	4.2	9.6	9.1	6.0	3.4	1.5

KEY: nd = none detected.
N = napthalene, C_1-C_4N = alkylated naphthalenes, F = fluorene, C_1F-C_3F = alkylated fluorenes, P = phenanthrene, C_1-C_4P = alkylated phenanthrenes, DBT = Dibenzothiophene, C_1DBT-C_2DBT = alkylated dibenzothiophenes, Fl = fluoranthene, PYR = pyrene, CHRY = Crysene, BF = benzofluoranthene, B(e)P = Benzo(e)pyrene, B(a)P = Benzo(a)pyrene, PERL = perylene.

TABLE 10. Morlaix time series (station B).

SAMPLE	DATE	ALIPHATICS (µg/g) TOTAL	ALIPHATICS (µg/g) RESOLVED	ALK/ISO	AROMATICS (µg/g) TOTAL	AROMATICS (µg/g) RESOLVED
AC 103	7/78	200.6	12.6	2.5	397.0	7.4
AC 365	11/78	107.9	5.3	5.0	503.0	7.5
AC 429	2/79	37.3	2.0	0.02	31.6	11.9

	N	C_1N	C_2N	C_3N	C_4N	F	C_1F	C_2F	C_3F	P	C_1P	C_2P	C_3P	C_4P
AC 103	17	18.9	48.6	135.9	220.7	16.3	23.8	72.8	301.0	117.8	95.4	160.1	312.5	273.9
AC 365	9	14.4	33.8	55.0	111.2	9.9	14.9	36.9	81.3	114.2	65.3	58.9	143.3	103.6
AC 429	6	10.0	19.7	28.0	50.2	16.8	11.6	23.3	7.4	185.6	108.6	52.6	26.0	34.9

	DBT	C_1DBT	C_2DBT	C_3DBT	FL	PYR	CHRY	BF	B(e)P	B(a)P	PERL
AC 103	15.2	100.8	772.3	1348	240.2	203.8	345.2	350.7	146.4	166.3	87.0
AC 365	13.2	32.7	235.2	440.3	202.2	175.6	254.0	324.6	115.0	130.9	65.4
AC 429	11.3	7.7	10.3	5.0	325.9	302.9	207.4	202.5	112.5	115.0	37.7

KEY: nd = none detected.
N = napthalene, C_1-C_4N = alkylated naphthalenes, F = fluorene, C_1F-C_3F = alkylated fluorenes, P = phenanthrene, C_1-C_4P = alkylated phenanthrenes, DBT = Dibenzothiophene, C_1DBT-C_2DBT = alkylated dibenzothiophenes, Fl = fluoranthene, PYR = pyrene, CHRY = Crysene, BF = benzofluoranthene, B(e)P = Benzo(e)pyrene, B(a)P = Benzo(a)pyrene, PERL = perylene.

TABLE 11. St. Michel en Greve/Lannion time series (station C).

		ALIPHATICS (µg/g)			AROMATICS (µg/g)	
SAMPLE	DATE	TOTAL	RESOLVED	ALK/ISO	TOTAL	RESOLVED
AC 44	4/78	38.5	1.9	0.38	69.1	8.6
AC 121	7/78	17.5	0.2	0.09	44.3	0.6
AC 384	11/78	32.1	0.5	0.14	173.6	1.6

	N	C_1N	C_2N	C_3N	C_4N	F	C_1F	C_2F	C_3F	P	C_1P	C_2P	C_3P	C_4P
AC 44	8.6	6.3	12.6	25.0	51.7	nd	3.3	12.4	89.5	10.9	29.8	48.3	115.0	79.4
AC 121	6.2	4.8	10.2	14.0	12.4	1.6	2.2	13.7	57.5	19.8	17.7	55.6	75.4	33.9
AC 384	2.4	2.1	6.3	59.6	121.1	nd	4.2	35.8	73.1	2.8	28.7	80.6	76.6	36.4

	DBT	C_1DBT	C_2DBT	C_3DBT	FL	PYR	CHRY	BF	B(e)P	B(a)P	PERL
AC 44	8.7	26.6	253.5	378.9	17.2	10.2	22.7	31.4	21.5	11.1	6.9
AC 121	3.0	21.2	211.1	348.7	22.1	17.1	34.3	35.1	15.3	10.8	3.2
AC 384	3.3	86.2	337.8	322.5	3.0	3.5	7.5	6.7	3.8	1.5	0.5

KEY: nd = none detected.
N = napthalene, C_1-C_4N = alkylated naphthalenes, F = fluorene, C_1F-C_3F = alkylated fluorenes, P = phenanthrene, C_1-C_4P = alkylated phenanthrenes, DBT = Dibenzothiophene, C_1DBT-C_2DBT = alkylated dibenzothiophenes, Fl = fluoranthene, PYR = pyrene, CHRY = Crysene, BF = benzofluoranthene, B(e)P = Benzo(e)pyrene, B(a)P = Benzo(a)pyrene, PERL = perylene.

TABLE 12. Ile Grande time series (station D).

		ALIPHATICS (µg/g)			AROMATICS (µg/g)	
SAMPLE	DATE	TOTAL	RESOLVED	ALK/ISO	TOTAL	RESOLVED
AC 48	4/78	21.0	0.3	3.4	14.3	0.6
AC 125	7/78	59.1	0.9	0.6	27.8	0.3
AC 378	11/78	63.3	1.9	0.4	181.0	12.9
AC 439	2/79	36.5	0.8	5.4	37.0	0.1

	N	C_1N	C_2N	C_3N	C_4N	F	C_1F	C_2F	C_3F	P	C_1P	C_2P	C_3P	C_4P
AC 48	nd	nd	nd	nd	nd	nd	nd	9.9	25.1	14.9	16.5	20.2	35.2	21.3
AC 125	11.8	5.1	143.7	686.2	825.1	12.8	68.0	286.0	674.3	63.3	309.9	539.7	729.0	229.0
AC 378	4.2	3.1	112.2	472.5	569.0	nd	20.2	152.4	386.7	13.9	127.8	251.7	473.0	227.9
AC 439	0.8	<1.0	<1.0	1.0	nd	nd	nd	nd	nd	3.7	5.9	5.1	4.5	2.4

	DBT	C_1DBT	C_2DBT	C_3DBT	FL	PYR	CHRY	BF	B(e)P	B(a)P	PERL
AC 48	nd	13.2	80.7	145.6	19.8	18.3	34.1	17.2	16.7	6.1	44.7
AC 125	1033	785.4	2420	2917	55.2	39.4	107.7	99.0	52.7	16.0	6.1
AC 378	27.3	274.0	1481	1945	3.8	9.4	na	15.0	19.3	2.2	2.0
AC 439	nd	2.5	12.4	15.5	<1.0	<1.0	na	1.0	1.0	1.0	nd

KEY: nd = none detected.
na = not analyzed.
N = napthalene, C_1-C_4N = alkylated naphthalenes, F = fluorene, C_1F-C_3F = alkylated fluorenes, P = phenanthrene, C_1-C_4P = alkylated phenanthrenes, DBT = Dibenzothiophene, C_1DBT-C_2DBT = alkylated dibenzothiophenes, Fl = fluoranthene, PYR = pyrene, CHRY = Crysene, BF = benzofluoranthene, B(e)P = Benzo(e)pyrene, B(a)P = Benzo(a)pyrene, PERL = perylene.

TABLE 13. Lannion I and II time series.

LANNION I TIME SERIES (STATION E)

SAMPLE	DATE	N	C_1N	C_2N	C_3N	C_4N	F	C_1F	C_2F	C_3F	P	C_1P	C_2P	C_3P	C_4P
AC 107	7/78	nd	8.7	32.8	87.4	167.8	4.5	10.1	34.1	95.3	22.8	41.3	60.9	117.7	71.1
AC 436	2/79	1.6	1.9	2.9	1.9	nd	nd	nd	nd	nd	3.7	5.9	5.1	2.3	nd

	DBT	C_1DBT	C_2DBT	C_3DBT	FL	PYR	CHRY	BF	B(e)P	B(a)P	PERL
AC 107	9.9	75.4	295.6	416.4	23	19.6	na	42.6	20.6	19.4	9.5
AC 436	nd	1.5	22.4	40.2	1.6	1.9	na	12.3	3.7	2.5	2.0

LANNION II TIME SERIES (STATION F)

SAMPLE	DATE	N	C_1N	C_2N	C_3N	C_4N	F	C_1F	C_2F	C_3F	P	C_1P	C_2P	C_3P	C_4P
AC 118	7/78	nd	nd	9.5	16.3	26.3	1.0	2.2	5.0	18.6	7.9	9.3	19.8	37.1	21.0
AC 377	11/78	5.8	4.1	7.3	6.1	nd	1.5	1.3	2.3	9.0	12.5	7.3	7.8	17.9	7.8

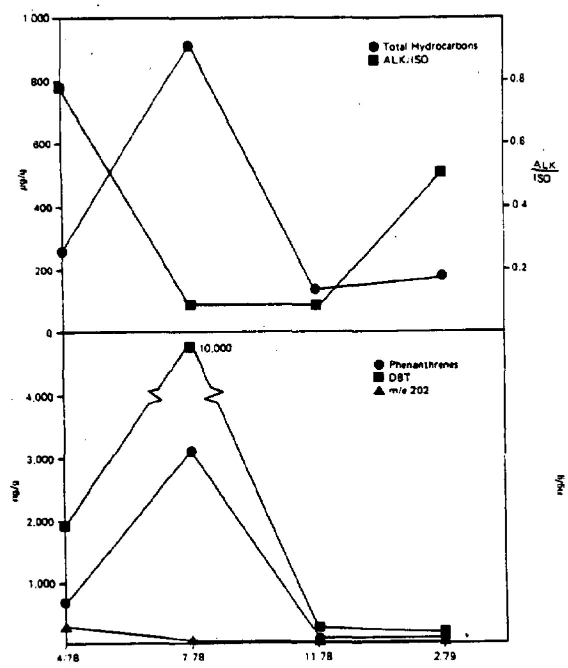
	DBT	C_1DBT	C_2DBT	C_3DBT	FL	PYR	CHRY	BF	B(e)P	B(a)P	PERL
AC 118	nd	10.2	90.1	181.2	10.6	8.5	na	22.6	12.8	6.7	2.2
AC 377	nd	nd	nd	nd	nd	nd	na	nd	nd	nd	nd

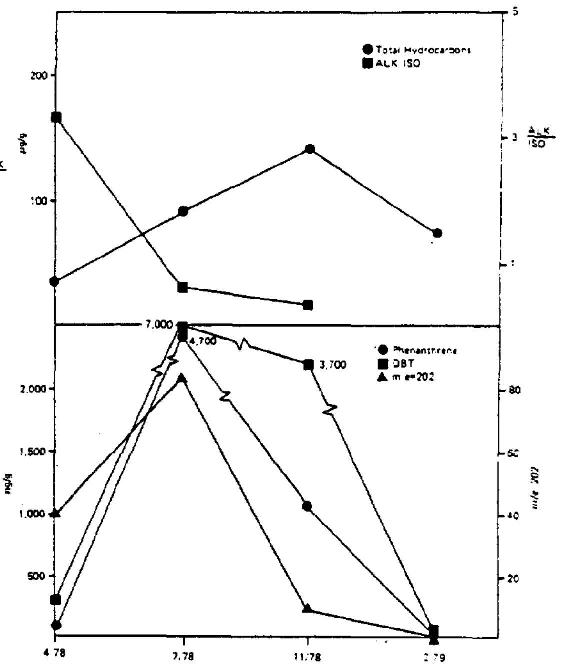
KEY: nd = none detected.
na = not analyzed.
N = napthalene, C_1-C_4N = alkylated naphthalenes, F = fluorene, C_1F-C_3F = alkylated fluorenes, P = phenanthrene, C_1-C_4P = alkylated phenanthrenes, DBT = Dibenzothiophene, C_1DBT-C_2DBT = alkylated dibenzothiophenes, Fl = fluoranthene, PYR = pyrene, CHRY = Crysene, BF = benzofluoranthene, B(e)P = Benzo(e)pyrene, B(a)P = Benzo(a)pyrene, PERL = perylene.

TABLE 14. L'Aber Benoit GC/MS results.

	AB 25	AB 21
N	nd	3
C_1N	nd	11
C_2N	4	104
C_3N	30	220
C_4N	55	307
F	-	4
C_1F	4	25
C_2F	11	113
C_3F	59	311
P	3	14
C_1P	30	50
C_2P	54	440
C_3P	84	800
C_4P	54	450
DBT	5	29
C_1DBT	34	200
C_2DBT	166	1350
C_3DBT	195	2000
Fluoranthene	6	19
Pyrene	4	23
Benzanthracene	nd	69
Chrysene	15	53
Benzofluoranthene	11	43
Benzo(e)pyrene	7	24
Benzo(a)pyrene	4	12
Perylene	2	10
F_1 (Total)	29	460
F_2 (Total)	30	440

KEY: nd = none detected.





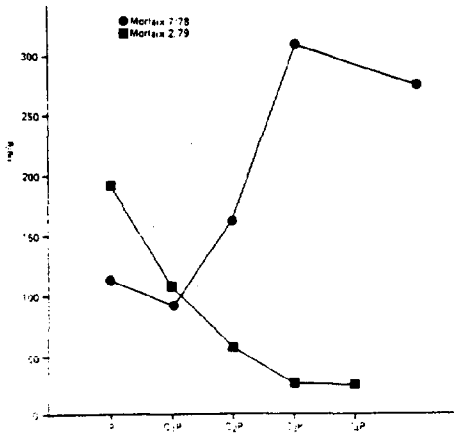


FIGURE 3.36. (Upper left) Terenez/Morlaix sediment time series.
FIGURE 3.37. (Upper right) Offshore Ile Grande sediment time series.
FIGURE 3.38. (Bottom) Alkyl homologue distributions of phenanthrene series.

3.4 Sediment Cores (Ward, Montana State University)

An extensive series of sediment cores was obtained and analyzed by GC and selected samples analyzed by GC/MS for detailed aromatic hydrocarbon profiles. Samples were analyzed in support of anaerobic petroleum biodegradation experiments (e.g. Winfrey et al., 1981). Three impacted sites and three control sites representative of beach, aber (estuarine) and marsh environments were selected (Table 15, Fig. 3.39).

TABLE 15. AMOCO CADIZ chemistry program, intertidal cores (Ward).

Frequency:	
December 1978	16
March 1979	28
August 1979	16
November 1979	10
May 1980	12
Total	82

Locations:

Oiled:
- AMC-4 (Portsall) - beach
- L'Aber Wrac'h - estuary
- Ile Grande (South-Oiled) - marsh

Unoiled:
- Ile Grande (North-Control) - marsh
- Trez Hir - beach
- Aber Ildut - estuary

Other Stations:
- Station 11
- Station 12
- Baie de Morlaix
- Port de Concarneau

GC/MS:

Several selected cores

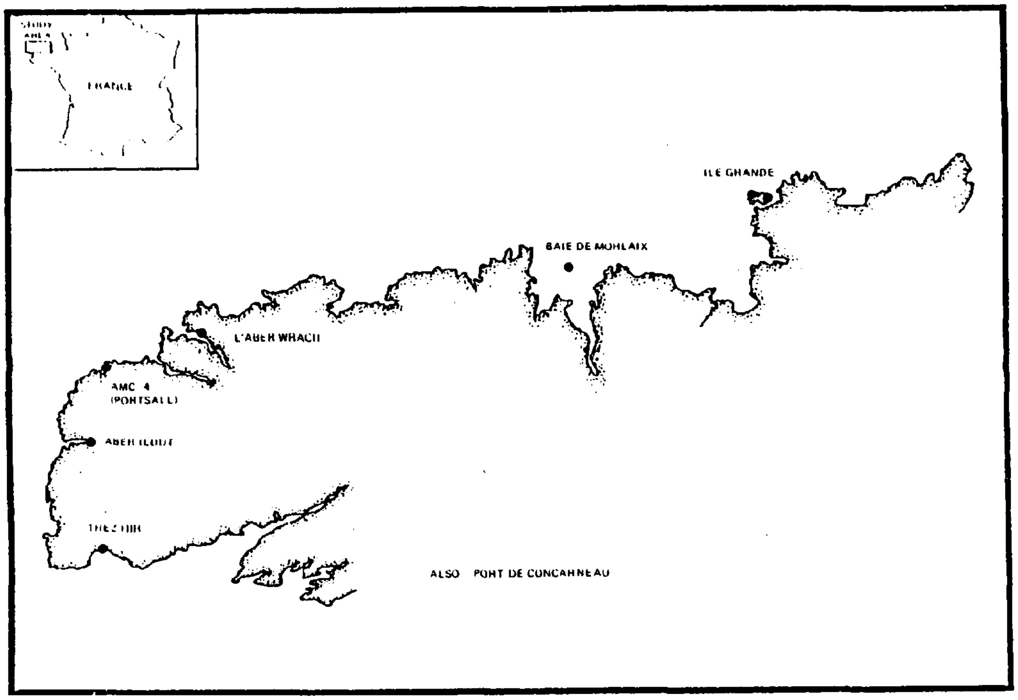


FIGURE 3.39. Sediment core sampling locations (Ward).

The basic set of data, where GC and GC/MS data exist, is illustrated by the data in Table 16. However, secondary data products are presented here to illustrate the basic findings of this program segment.

Illustrative GC traces from March of 1979 are shown in Figures 3.40 and 3.41. While the hydrocarbon composition of the control estuary (L'Aber Ildut) is comprised mainly of biogenic compounds the marsh mudflat and beach both contain anthropogenic inputs. The Ile Grande "control" has been impacted by the AMOCO oil as its GC profiles closely resemble those for weathered oil. However, the Trez Hir (beach) site consists mainly of compounds of a pyrogenic origin. The impacted sites all illustrate AMOCO oil in various states of weathering, the Ile Grande (marsh) site containing the best "preserved" oil. This is also indicated by secondary treatment of some of the core aromatic data (Fig. 3.42) where oil in both L'Aber Wrac'h and Ile Grande appears to be less weathered at depth.

Data from the three impacted cores and the control core are shown in Figures 3.43. 3.52, 3.58, and 3.63. Each figure depicts the depth of penetration of AMOCO CADIZ oil throughout the December 1978 to May 1980 time period. The C_3P/C_3DBT ratio is presented as is the level of the non-petrogenic fluoranthene + pyrene (m/e = 202) total.

Accompanying these figures are graphs of the down-core variations in gross hydrocarbon parameters (i.e. f_1, f_2, ALK/ ISO ratio) and detailed aromatic compound families. Figures 3.44 to 3.51 depict details of the L'Aber Wrac'h cores, Figures 3.53 to 3.57 the Ile Grande

TABLE 16. L'Aber Wrac'h sediment core (March 1979).

		ALIPHATICS (ug/g)			AROMATICS (ug/g)	
SAMPLE	DATE	TOTAL	RESOLVED	ALK/ISO	TOTAL	RESOLVED
0-5 cm	3/79	530.0	27.8		565.0	16.4
5-10	3/79	113.7	8.4		318.4	19.4
10-15	3/79	95.4	2.8		118.1	4.0
15-20	3/79	4.4	1.1		46.5	4.4
20-25	3/79	10.1	1.6			

	N	C_1N	C_2N	C_3N	C_4N	F	C_1F	C_2F	C_3F	P	C_1P	C_2P	C_3P	C_4P
0-5	20.4	23.0	42.5	292.2	288.9	18.9	53.7	137.2	434.4	100.6	200.5	294.0	516.6	120
5-10	2.6	11.9	50.3	181.6	426.7	13.4	40.8	226.3	440.2	156.5	185.0	278.4	348.7	102
10-15	4.9	10.7	21.8	87.4	159.1	9.3	24.5	91.2	176.1	134.1	101.3	165.6	153.6	117
15-20	5.8	11.5	20.5	36.7	78.0	10.6	13.0	14.3	86.0	146.1	80.7	50.5	21.3	35
20-25	nd	2.6	14.3	17.9	nd	6.9	7.0	25.0	25.5	100.0	67.4	29.3	10.1	26

	DBT	C_1DBT	C_2DBT	C_3DBT	FL	PYR	CHRY	BF	B(e)P	B(a)P	PERL
0-5	40.0	87.5	1466	1598	116.6	157.7	170.4	136.3	89.0	64.8	24.5
5-10	24.3	135.6	1360	830.7	132.8	172.2	185.2	219.7	124.2	128.2	33.7
10-15	17.4	93.7	498.4	491.6	156.4	178.6	174.4	230.9	118.6	137.0	36.2
15-20	10.7	16.7	32.3	65.2	164.3	155.7	169.6	215.3	100.2	122.3	29.5
20-25	5.0	8.0	10.1	5.0	98.9	74.9	93.2	76.7	94.3	105.6	24.4

KEY: nd = none detected.
N = napthalene, C_1-C_4N = alkylated naphthalenes, F = fluorene, C_1F-C_3F = alkylated fluorenes, P = phenanthrene, C_1-C_4P = alkylated phenanthrenes, DBT = Dibenzothiophene, C_1DBT-C_2DBT = alkylated dibenzothiophenes, Fl = fluoranthene, PYR = pyrene, CHRY = Crysene, BF = benzofluoranthene, B(e)P = Benzo(e)pyrene, B(a)P = Benzo(a)pyrene, PERL = perylene.

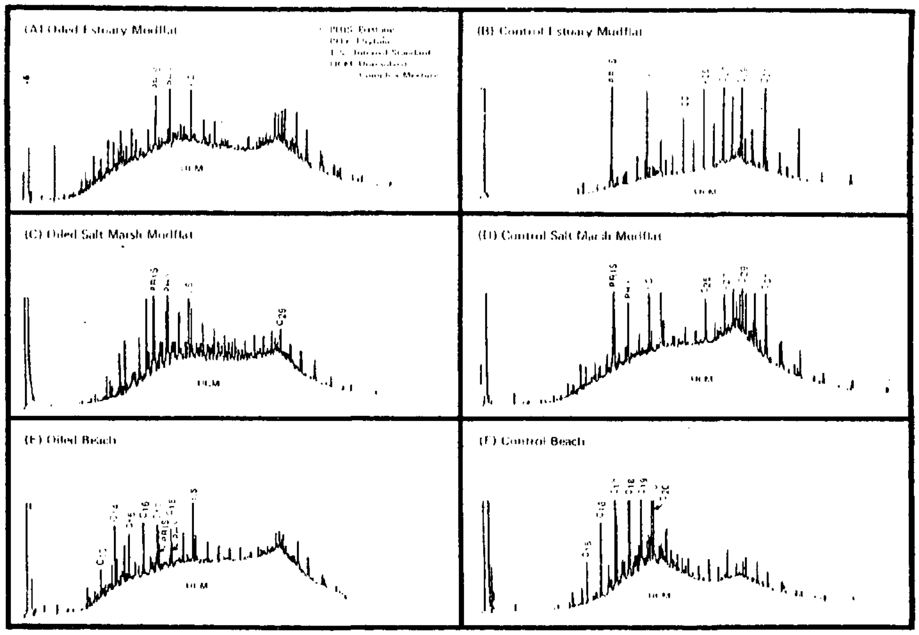
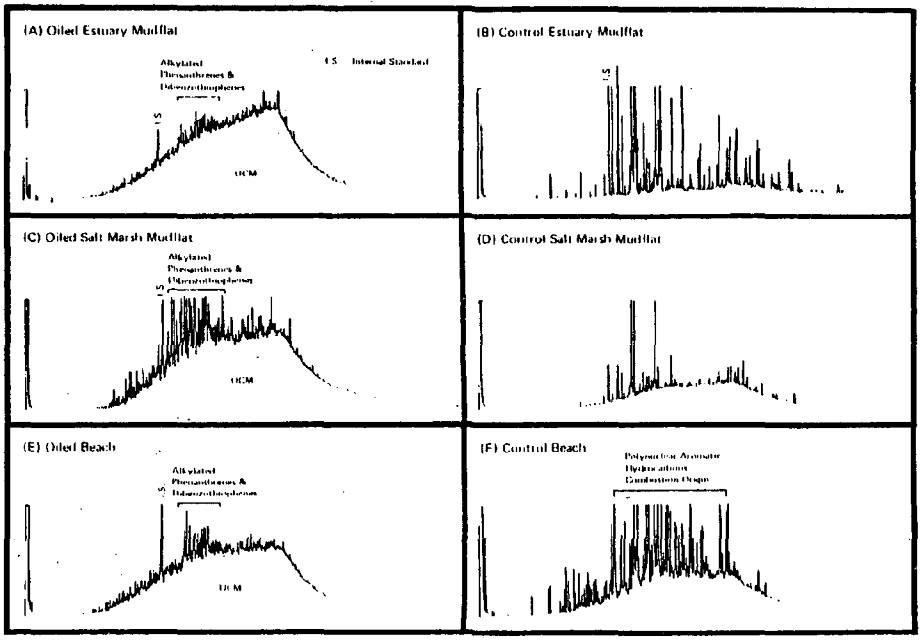


FIGURE 3.40. Saturated hydrocarbons in sediments from oiled and control sites.



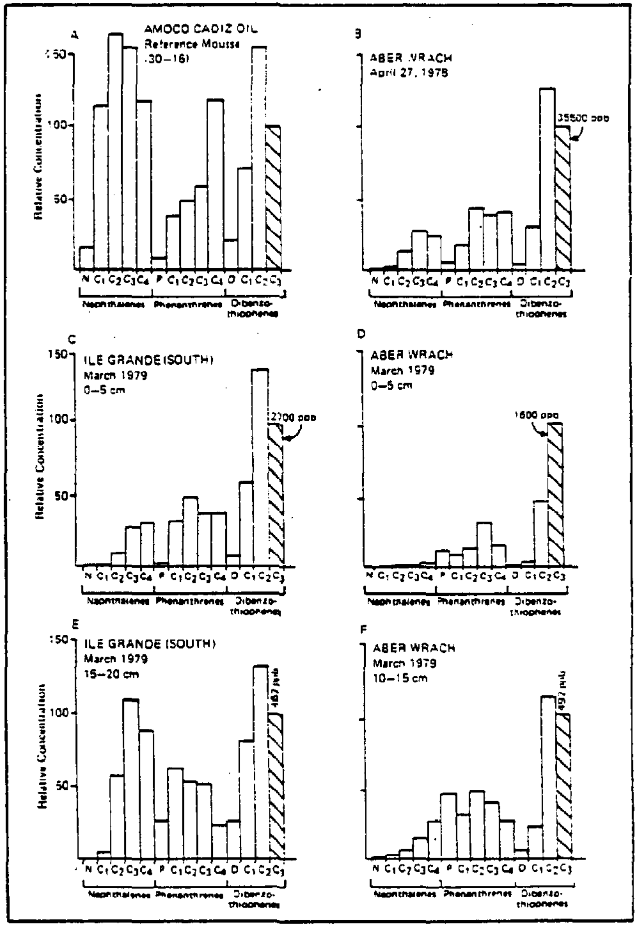


FIGURE 3.41. (Top) Aromatic hydrocarbons in sediments from oiled and control sites.

FIGURE 3.42. (Bottom) Comparative aromatic compound concentration profiles derived from GC/MS-histogram presentation.

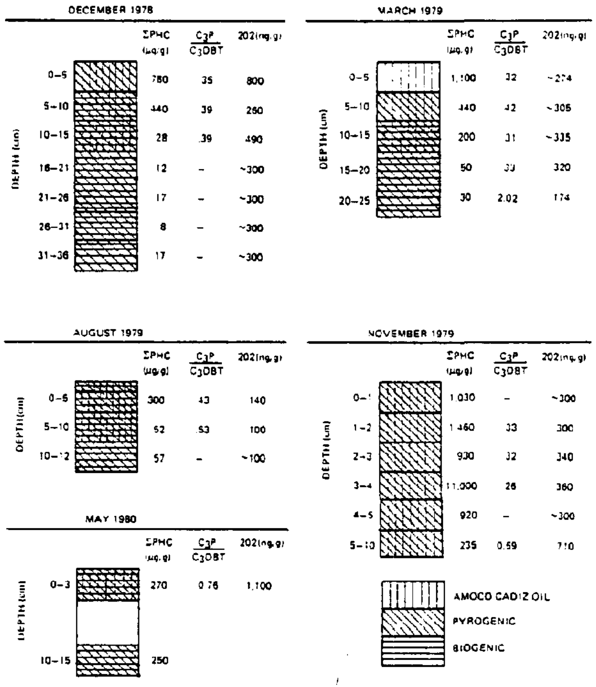
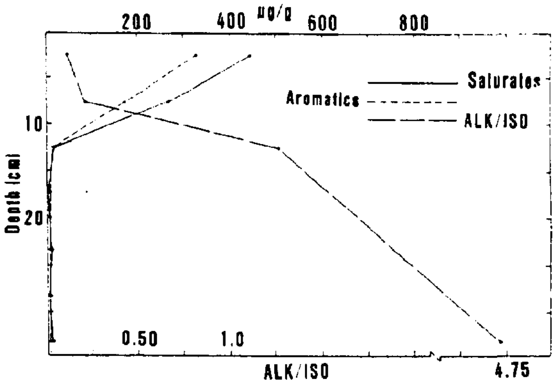


FIGURE 3.43. Aber Wrac'h sediment cores.



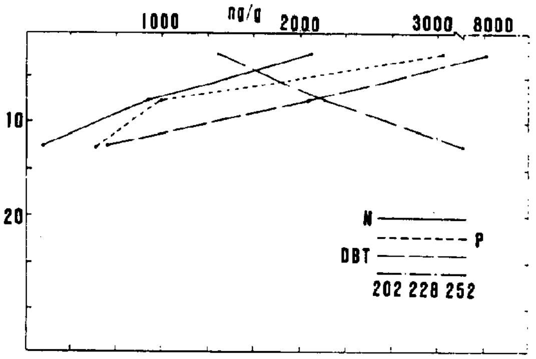
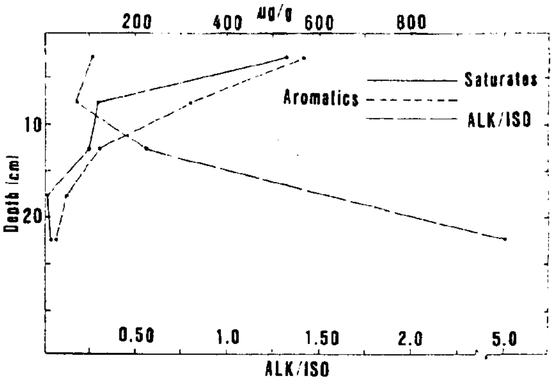


FIGURE 3.44. (Left) Aber Wrac'h core, December 1978.
FIGURE 3.45. (Right) Aber Wrac'h core, December 1978.



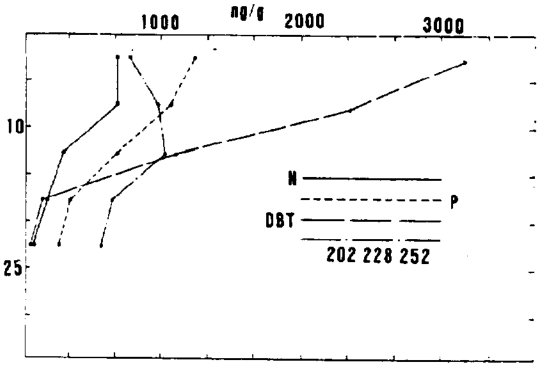
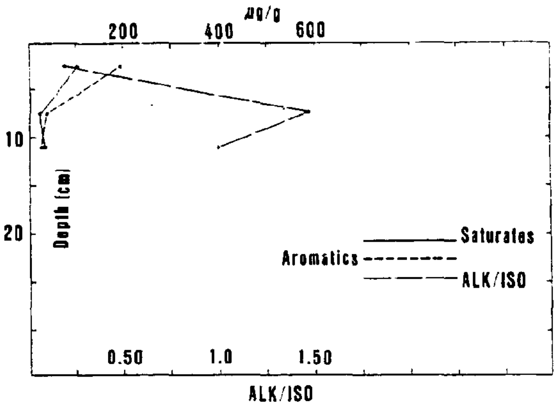


FIGURE 3.46. (Left) Aber Wrac'h core, March 1979.
FIGURE 3.47. (Right) Aber Wrac'h core, March 1979.



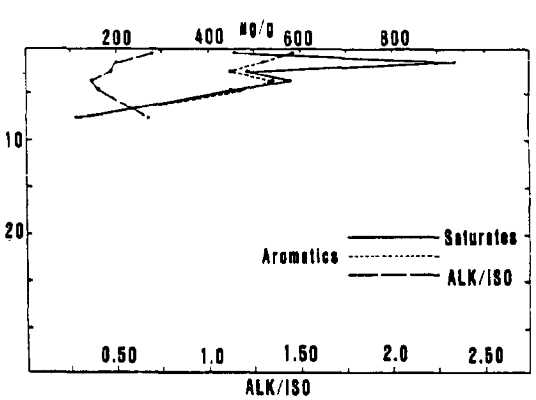
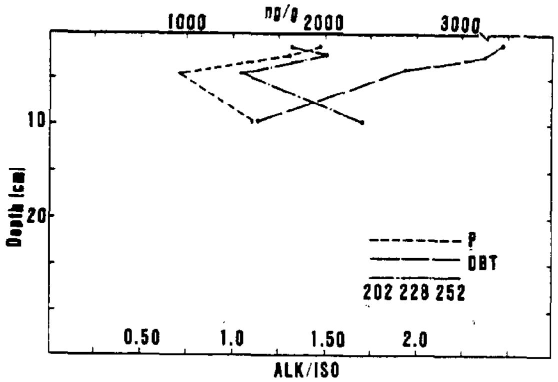


FIGURE 3.48. (Left) Aber Wrac'h core, August 1979.
FIGURE 3.49. (Right) Aber Wrac'h core, November 1979.



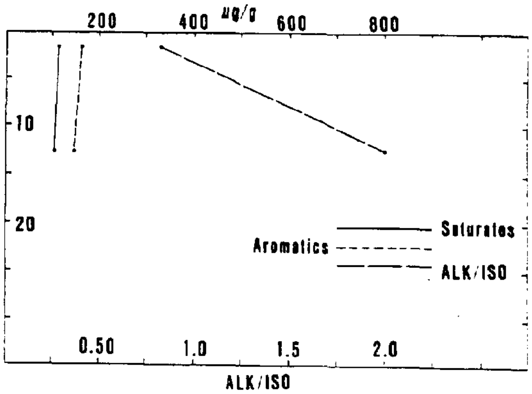
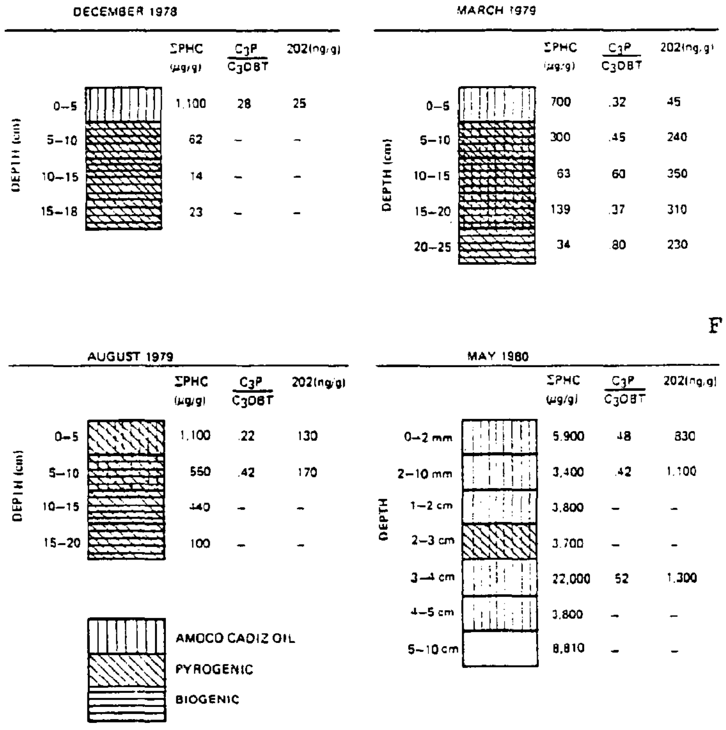


FIGURE 3.50. (Left) Aber Wrac'h core, November 1979.
FIGURE 3.51. (Right) Aber Wrac'h core, May 1980.



DECEMBER 1978

DEPTH (cm)	ΣPHC (µg/g)	$\frac{C_3P}{C_3DBT}$	202(ng/g)
0–5	1,100	28	25
5–10	62	–	–
10–15	14	–	–
15–18	23	–	–

MARCH 1979

DEPTH (cm)	ΣPHC (µg/g)	$\frac{C_3P}{C_3DBT}$	202(ng/g)
0–5	700	.32	45
5–10	300	.45	240
10–15	63	60	350
15–20	139	.37	310
20–25	34	.80	230

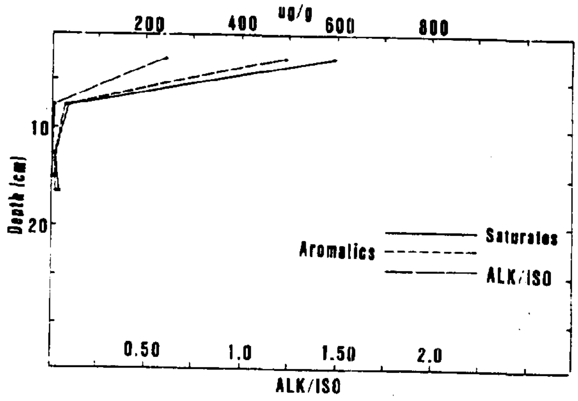
AUGUST 1979

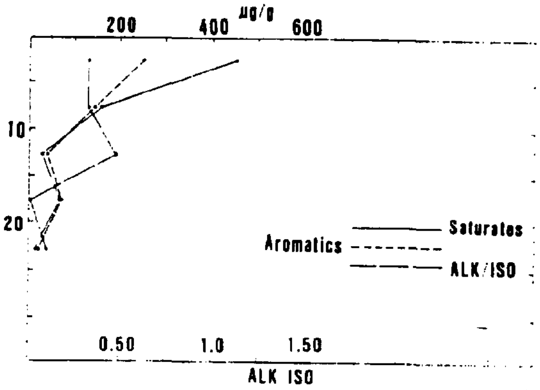
DEPTH (cm)	ΣPHC (µg/g)	$\frac{C_3P}{C_3DBT}$	202(ng/g)
0–5	1,100	.22	130
5–10	550	.42	170
10–15	440	–	–
15–20	100	–	–

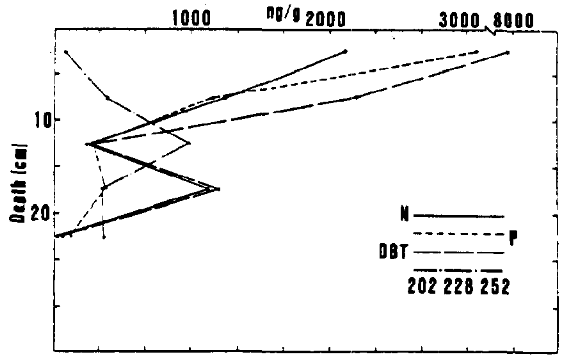
MAY 1980

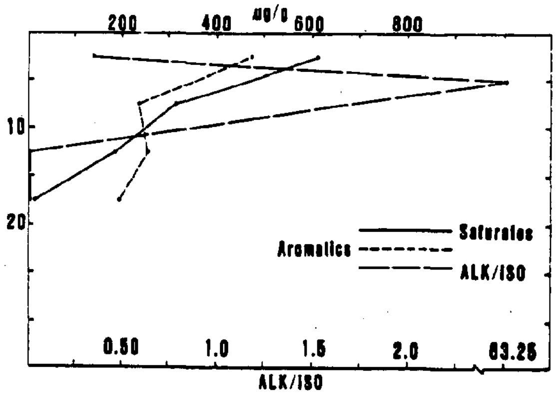
DEPTH	ΣPHC (µg/g)	$\frac{C_3P}{C_3DBT}$	202(ng/g)
0–2 mm	5,900	48	830
2–10 mm	3,400	.42	1,100
1–2 cm	3,800	–	–
2–3 cm	3,700	–	–
3–4 cm	22,000	52	1,300
4–5 cm	3,800	–	–
5–10 cm	8,810	–	–

FIGURE 3.52. Ile Grande (oiled) sediment cores.









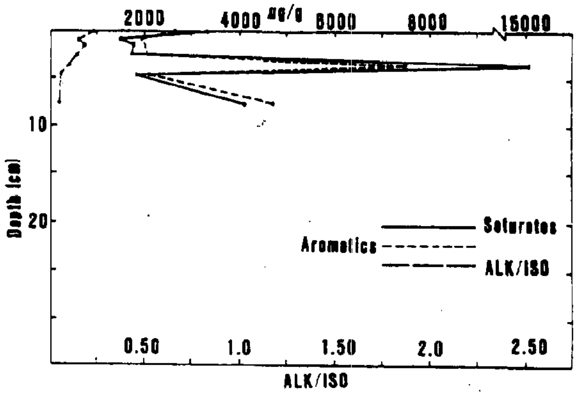
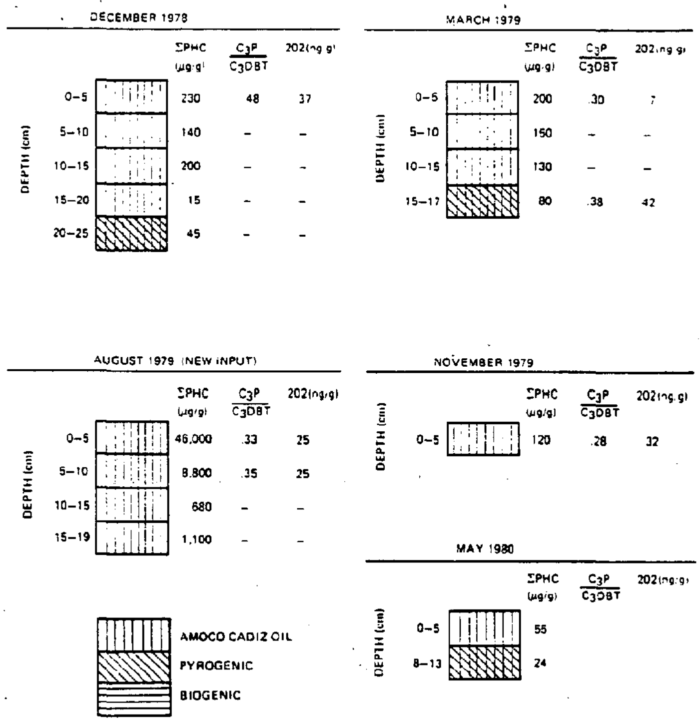


FIGURE 3.53. (Upper left) Ile Grande south core, December 1978.
FIGURE 3.54. (Upper right) Ile Grande south core, March 1979.
FIGURE 3.55. (Middle left) Ile Grande south core, March 1979.
FIGURE 3.56. (Middle right) Ile Grande south core, August 1979.
FIGURE 3.57. (Bottom) Ile Grande south core, May 1980.

FIGURE 3.58. AMC-4 sediment cores.



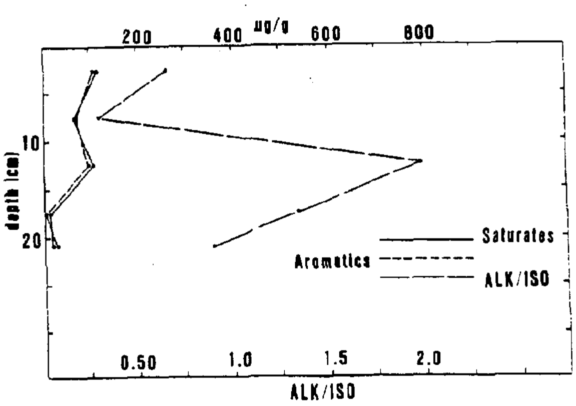
cores, Figures 3.59 to 3.62 the AMC-4 cores and Figure 3.64 a L'Aber Ildut core. Most of the cores were subdivided into sections of 3-5 cm in depth. However, two finer subdivisions from L'Aber Wrac'h - November 1979 (1 cm segments down to 5 cm), Ile Grande - May 1980 (top 10 mm subdivided plus 1 cm sections down to 5 cm) were made.

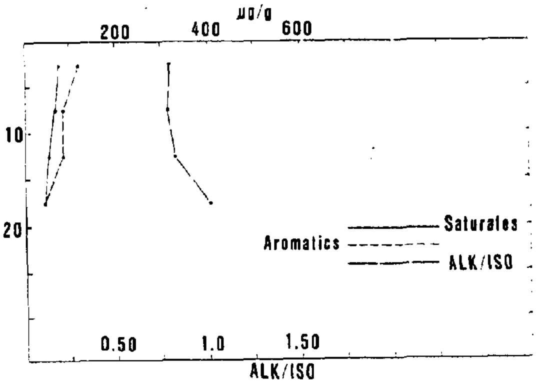
Penetration of oil was observed down to 10-15 cm in L'Aber Wrac'h sediments with concentrations decreasing with depth when viewed in 5 cm sections. Note however, that while petroleum aromatics were decreasing in concentration with depth, the pyrogenic PAH compounds increased with depth. Finer subdivisions of the core indicate greater variation within the core than the 5 cm sections would indicate (Fig. 3.52).

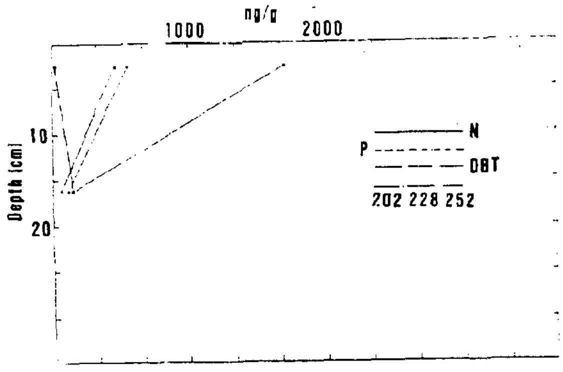
An increase in vertical penetration of oil was observed for the Ile Grande site between December 1978 and March 1979. A fresher layer of oil is found at the 15-20 cm depth (see Figs. 3.53 and 3.54) where naphthalenes, dibenzothiophenes, and to a lesser extent phenanthrenes, are more abundant than in surrounding layers. The gross hydrocarbon concentration changes at this level are not nearly as dramatic as are the petroleum aromatics, thus confirming that the "bulge" in Figure 3.42 is due to the less weathered nature of the buried oil. The finely divided May 1980 core (Fig. 3.52) indicates a higher petroleum content probably owing to a secondary input or to sampling variability which resulted in much higher levels (5-10 parts per thousand) during May 1980. The down core distribution of hydrocarbons is quite non-uniform as well with a preserved layer of fresher oil at 3-4 cm.

The AMC-4 cores appear dominated by well mixed AMOCO oil throughout the 0-20 cm depths. Lesser amounts of pyrogenic PAH vis-a-vis the Aber and marsh sediments are due to the sandy nature of the AMC-4 samples. A new large input of oil is seen in August 1979 resulting in some down-core concentration variation.

Chemical descriptions of the "control" site cores are shown in Figure 3.63. Note that non-petroleum PAH are widely observed in these sediments and that non-AMOCO CADIZ-impacted sediments contain 50-300 ppm of chronic hydrocarbon pollutants.







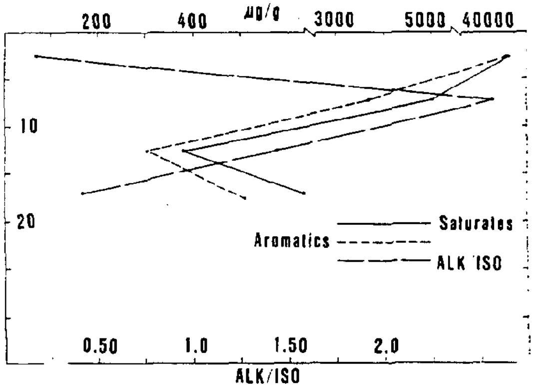


FIGURE 3.59. (Upper left) AMC-4 core, December 1978.
FIGURE 3.60. (Upper right) AMC-4 core, March 1979.
FIGURE 3.61. (Lower left) AMC-4 core, March 1979.
FIGURE 3.62. (Lower right) AMC-4 core, August 1979.

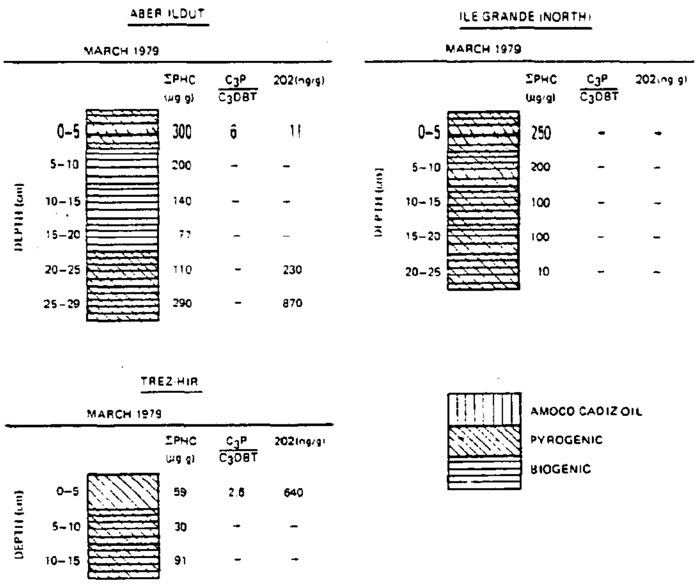


FIGURE 3.63. Miscellaneous sediment cores.

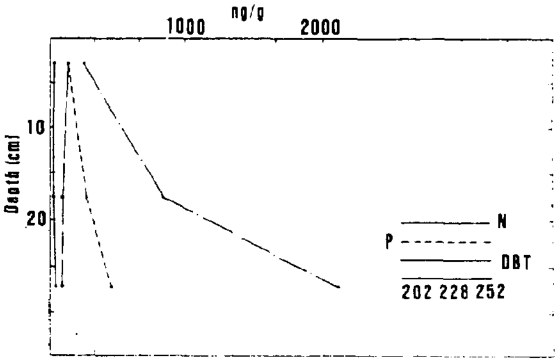


FIGURE 3.64. Aber Ildut, March 1979.

3.5 Oysters and Plaice (Neff, Battelle)

Samples of oysters and plaice from several impacted regions (L'Aber Wrac'h, L'Aber Benoit and Baie de Morlaix) and two supposedly unimpacted locations (Brest and Loctudy) were analyzed (Table 17, Figure 3.65).

The results of the oyster time series analyses are summarized in Table 18. Both the "gross" hydrocarbon parameters as well as the petroleum-associated aromatic hydrocarbons are presented. Though not "clean", the control (Brest) oysters are several times lower in gross concentration throughout the time period and an order of magnitude lower in aromatic hydrocarbon content than either of the impacted sites. It is not apparent if the levels have decreased substantially in either of the Abers, though aromatic levels are 3 to 4 times lower a year and a half after the spill. For comparison, levels of several of the non-petrogenic PAH components (i.e. m/e 252) are presented.

TABLE 17. AMOCO CADIZ chemistry program; oysters and plaice (Neff).

Frequency:

December 1978	4
April 1979	6
July 1979	7
February 1980	9
June 1980	11
Total	37

Location:

L'Aber Benoit - Oysters; Plaice Muscle/Liver
L'Aber Wrac'h - Oysters; Plaice Muscle/Liver
Loctudy - Plaice Muscle/Liver; Oysters (7/79 only)
Brest - Oysters
Baie de Morlaix - Oysters (7/79 only)

GC/MS

L'Aber Benoit Oysters
L'Aber Wrac'h Oysters
Control Oysters

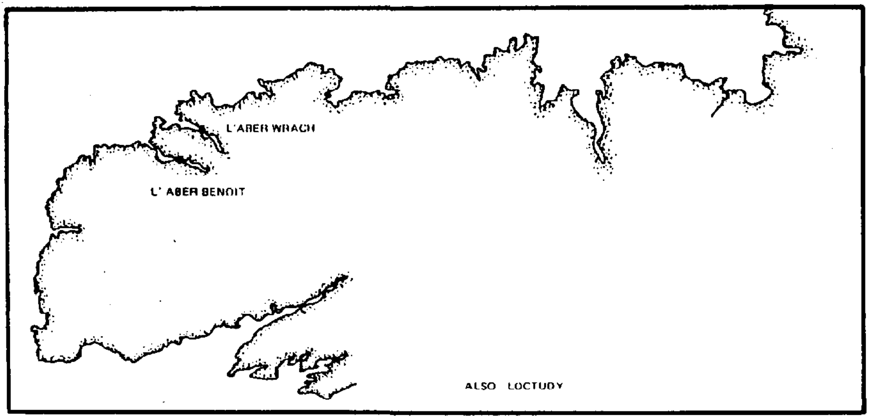


FIGURE 3.65. Oysters and Plaice sampling locations.

TABLE 18. Petroleum hydrocarbons in oysters (<u>Crassostrea gigas</u>).

LOCATION	DATE	PETROLEUM HYDROCARBONS (ug/g)	P[a] (ug/g)	DBT[b] (ug/g)	252[c] (ug/g)
L'Aber Wrac'h	12/78	660	12	22	0.04
	4/79	1,200	15	12	0.02
	7/79	590	5	10	0.03
	2/80	820	10	16	0.60
	6/80 (#1)	440	4	6	0.40
	6/80 (#2)	560/570[d]	-	-	-
Brest (control)	12/78	260	4	4	0.07
	4/79[e]	1,100	11	10	0.01
	7/79	91	0.3	0.3	-
	2/80	150	0.4	1.1	0.6
	6/80	93	0.6	0.7	0.2
L'Aber Benoit	12/78	690	-	-	-
	4/79	800	15	15	1.0
	7/79	-	-	-	-
	2/80	430	14	9	1.1
	6/80	520	3	5	0.2

---

[a] Sum of phenanthrene and alkyl phenanthrenes.

[b] Sum of dibenzothiophenes and alkyl dibenzothiophenes.

[c] Sum of m/e = 252.

[d] Replicate analyses.

[e] Origin of sample unclear.

The GC traces for the impacted oysters are consistent throughout the study. The aromatic hydrocarbons (Figs. 3.66, 3.67) are dominated by the alkylated dibenzothiophenes and alkylated phenanthrenes throughout. The alkyl naphthalenes and fluorenes, significant in December of 1978, are removed from the tissues by June 1980. Aromatic hydrocarbons in the control oysters (Fig. 3.67), while less concentrated, are dominated by the same compound series, though the compositions in the controls remain consistent with time (i.e. no loss of fluorenes or naphthalenes). GC/MS traces of the oysters confirm the importance of the dibenzothiophene series (Fig. 3.68).

Saturated hydrocarbon GC traces are illustrated in Figures 3.69 and 3.70 for impacted and control oysters respectively. The saturates of the L'Aber Wrac'h samples are dominated by branched alkanes (e.g. isoprenoids) and a large low boiling UCM (C_{11} - C_{20}). The UCM in the controls is less pronounced yet significant, and while the isoprenoids are abundant indicating some weathered petroleum, a higher boiling smooth n-alkane distribution (i.e. paraffins, n-C_{20} - n-C_{30}) is of equal importance. Figures 3.71 to 3.76 show some representative aromatic and saturated fraction data from oyster samples taken from L'Aber Wrac'h and the control station.

The results of the plaice analyses are summarized in Table 19. The absolute concentration data does not address the source of the observed levels which for the most part are not linked to AMOCO CADIZ oil. The muscle tissues exhibit some petroleum-like GC traces including some UCM material and smooth n-alkane distributions with the presence of UCM material primarily responsible for the higher levels shown in Table 19. Liver tissue in all samples is much higher in absolute hydrocarbon content (Figs. 3.77 and 3.78). The f_1 (saturated) traces are characterized by a high molecular weight UCM (cycloalkanes), and an n-alkane distribution in the C_{22} to C_{23} region, while the f_2 traces are characterized by polyolefinic material, including the biosynthesized compound squalene. These f_1 and f_2 distributions are characteristic of fish livers from many geographic regions (Boehm, 1980; Boehm and Hirtzer, 1981) and are probably not related to any particular spill event.

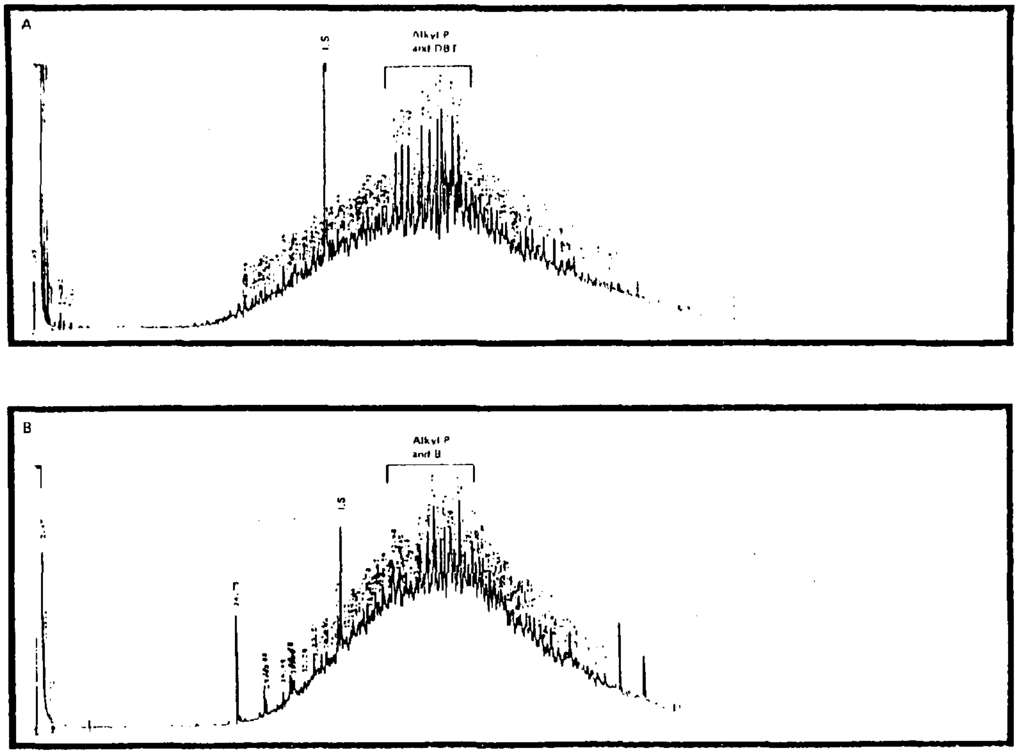


FIGURE 3.66. Aber Wrac'h impacted oysters - aromatic hydrocarbons; A - December 1978; B - June 1980.

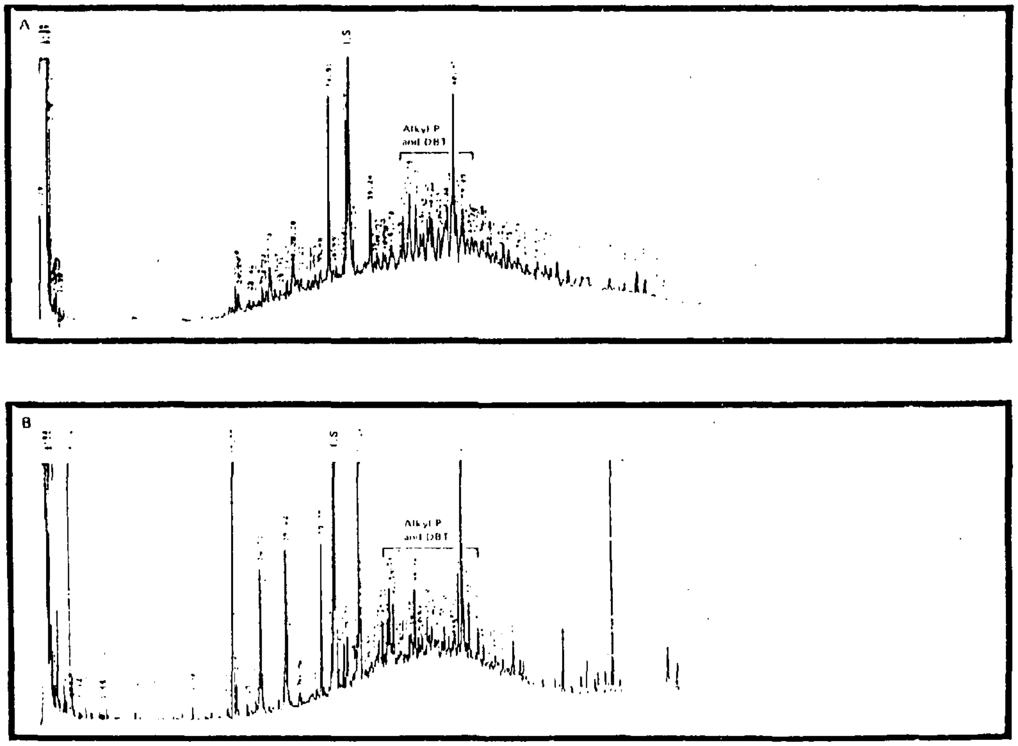


FIGURE 3.67. Brest control oyster - aromatic hydrocarbons; A - December 1978; B - June 1980.

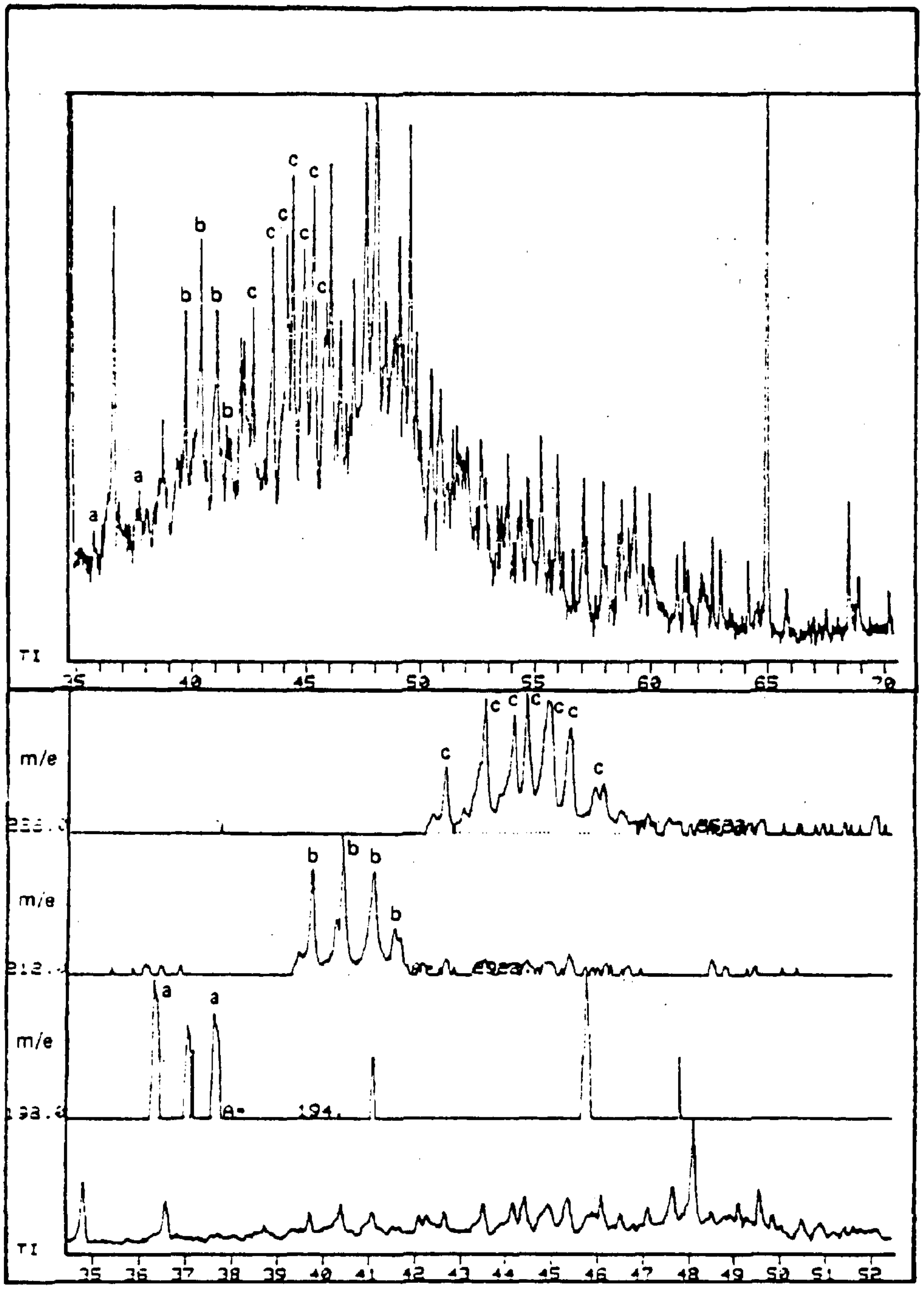


FIGURE 3.68. Section of GC/MS total ion chromatogram of aromatic fraction of oyster sample illustrating major alkyl dibenzothiophene (DBT) components (a = C_1DBT: b - C_2DBT; C = C_3DBT).

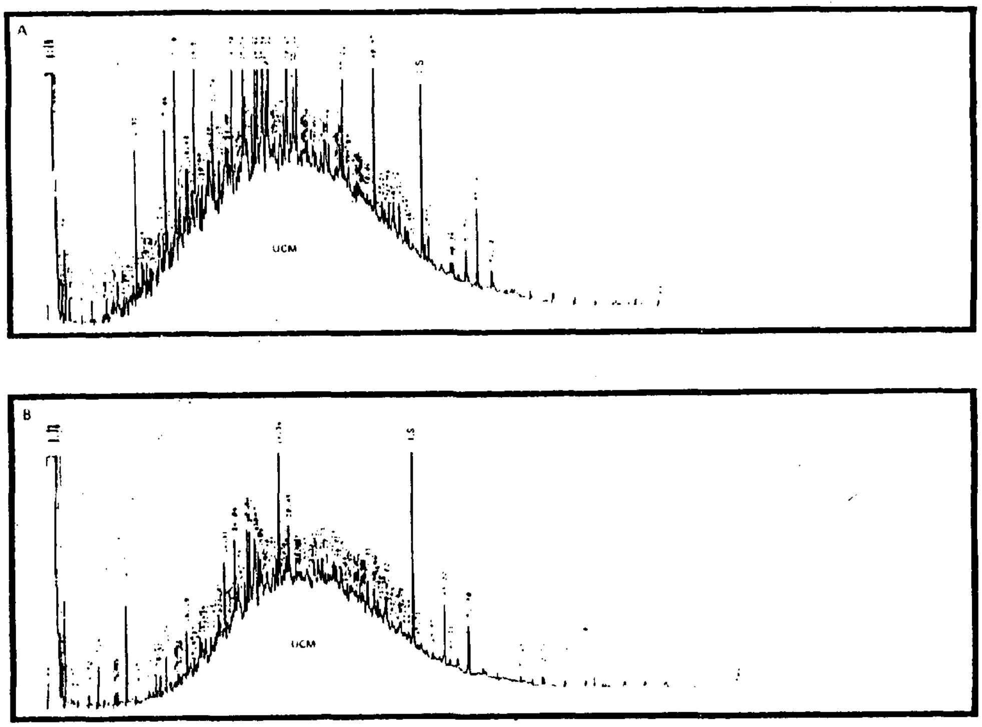


FIGURE 3.69. Aber Wrac'h impacted oysters - saturated hydrocarbons; A - December 1978; B - June 1980.

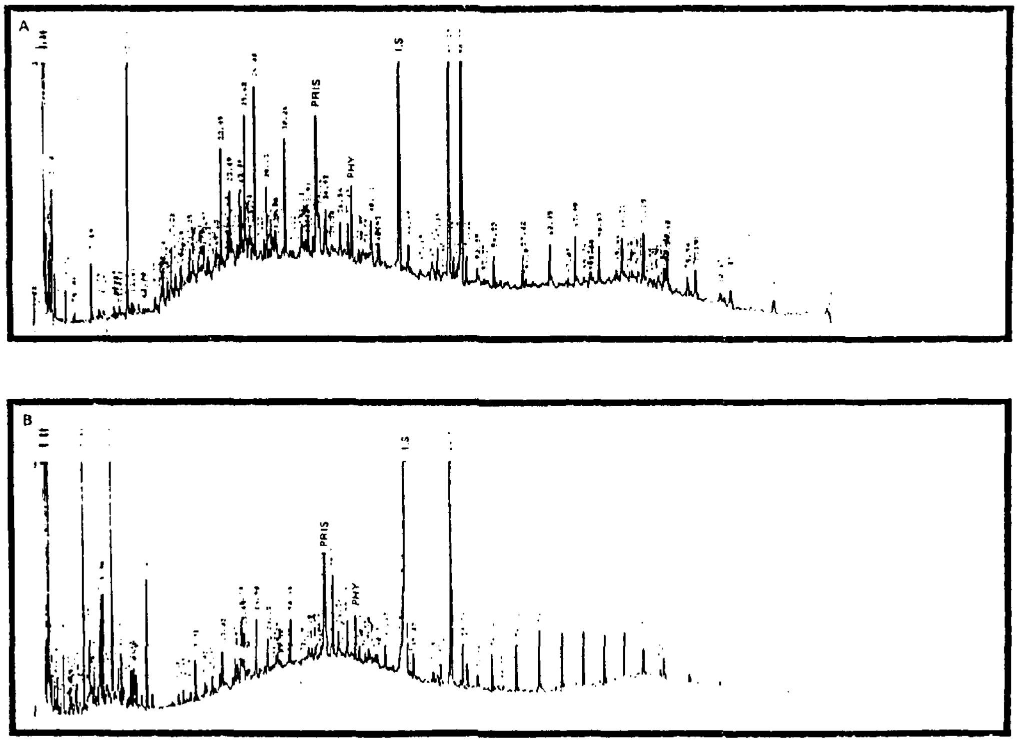
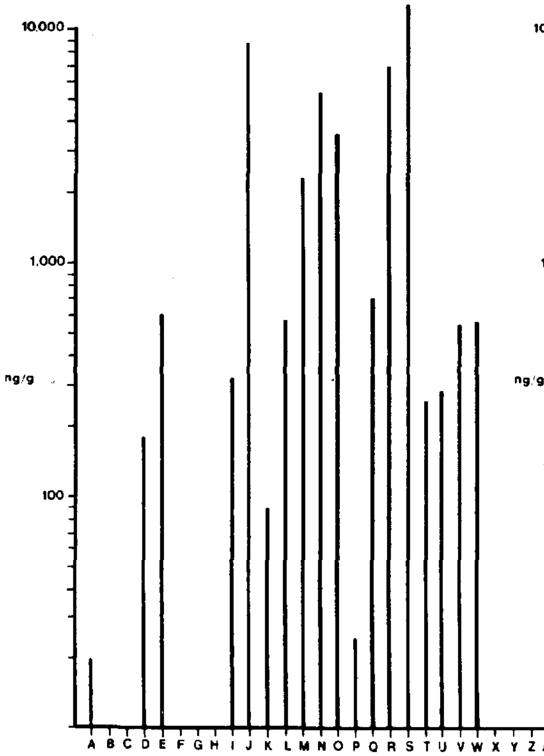


FIGURE 3.70. Brest control oysters - saturated hydrocarbons; A - December 1978; B - June 1980.



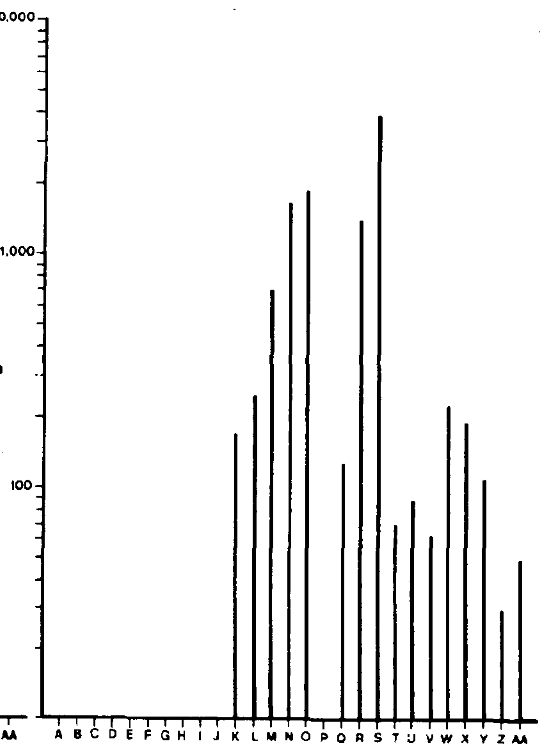
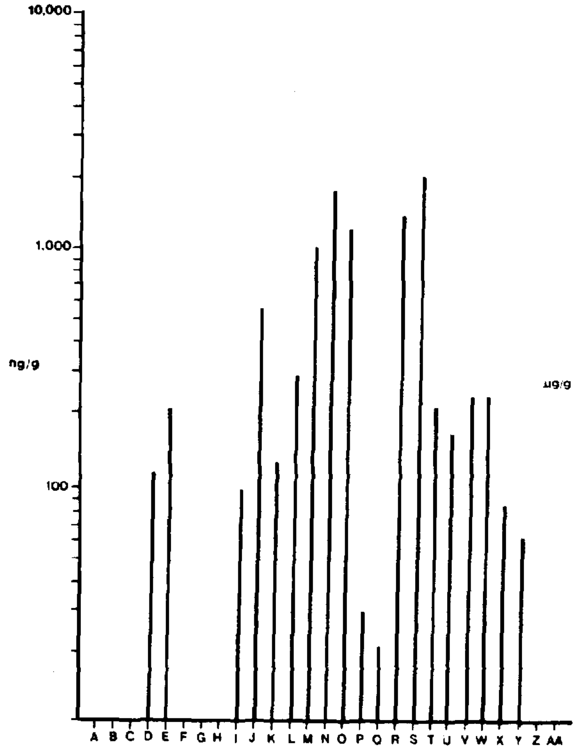


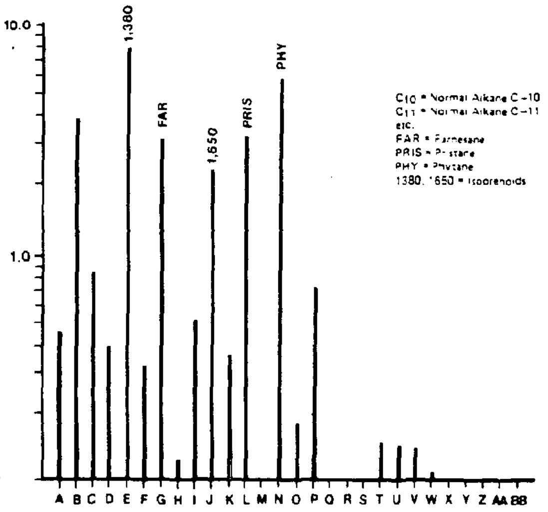
FIGURE 3.71. (Left) Aber Wrac'h, <u>Crassostrea gigas</u>, aromatic hydrocarbons, December 1978. (See Figure 3.14 for key.)

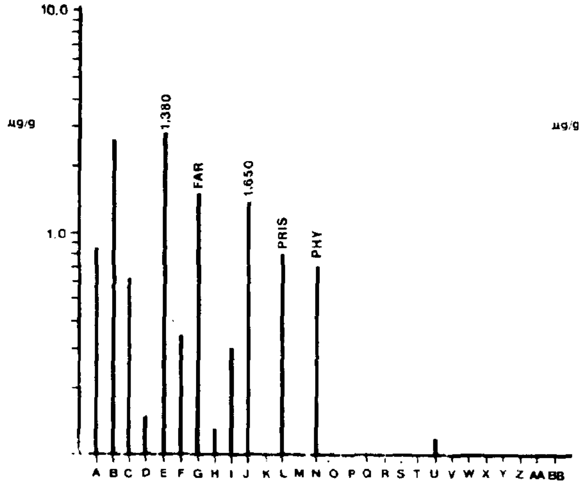
FIGURE 3.72. (Right) Aber Wrac'h, <u>Crassostrea gigas</u>, aromatic hydrocarbons, June 1980. (See Figure 3.14 for key.)

TABLE 19. Summary of Plaice hydrocarbon data.

STATION	DATE	TISSUE	HYDROCARBONS (µg/g dry wt)
L'Aber Wrac'h	5/79	Muscle	90
	7/79	Muscle	33
	2/80	Muscle	77
	6/80	Muscle	186
	7/79	Liver	1,350
	2/80	Liver	1,200
	6/80	Liver	1,640
L'Aber Benoit	5/79	Muscle	147
	7/79	Muscle	17
	2/80	Muscle	104
	6/80	Muscle	48
	7/79	Liver	1,030
	2/80	Liver	1,860
	6/80	Liver	2,500
Loctudy	4/79	Muscle	
	2/80	Muscle	41
	6/80	Muscle	38
	2/80	Liver	1,300
	6/80	Liver	1,900







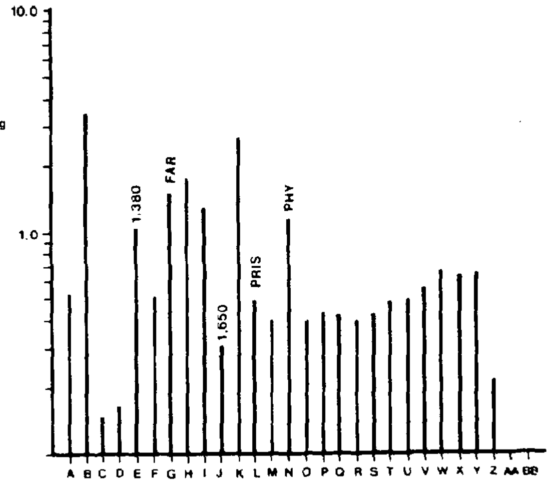


FIGURE 3.73. (Upper left) Brest, *Crassostrea gigas*, aromatic hydrocarbons, December 1978. (See Figure 3.14 for key.)

FIGURE 3.74. (Upper right) Aber Wrac'h, *Crassostrea gigas*, saturated hydrocarbons, December 1978.

FIGURE 3.75. (Lower left) Aber Wrac'h, *Crassostrea gigas*, saturated hydrocarbons, June 1980. (See Figure 3.74 for key.)

FIGURE 3.76. (Lower right) Brest, *Crassostrea gigas*, saturated hydrocarbons, December 1978. (See Figure 3.74 for key.)

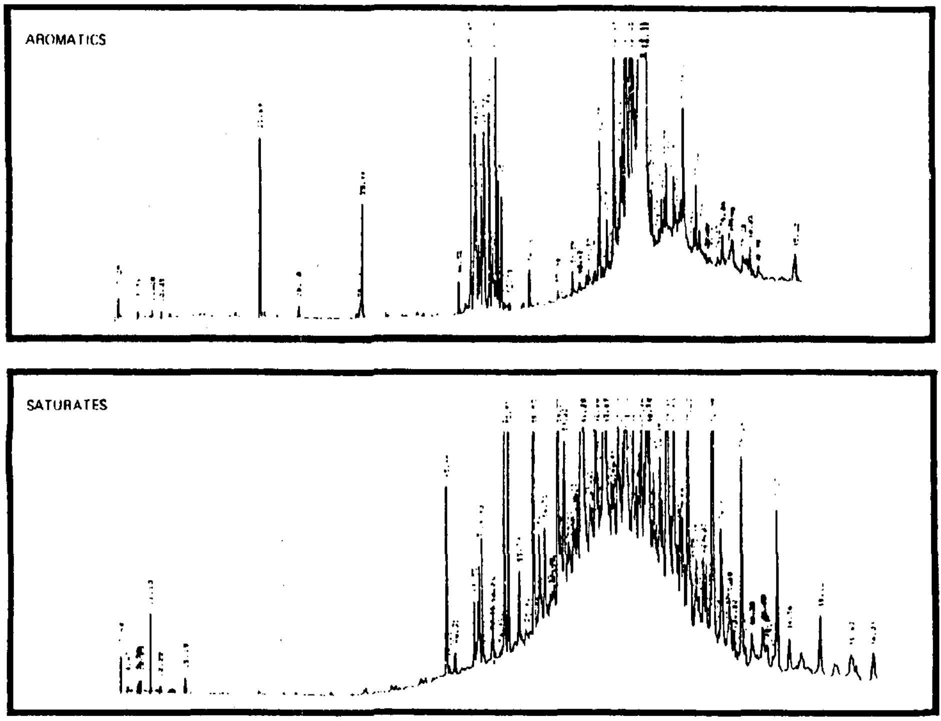


FIGURE 3.77. Plaice Liver, control.

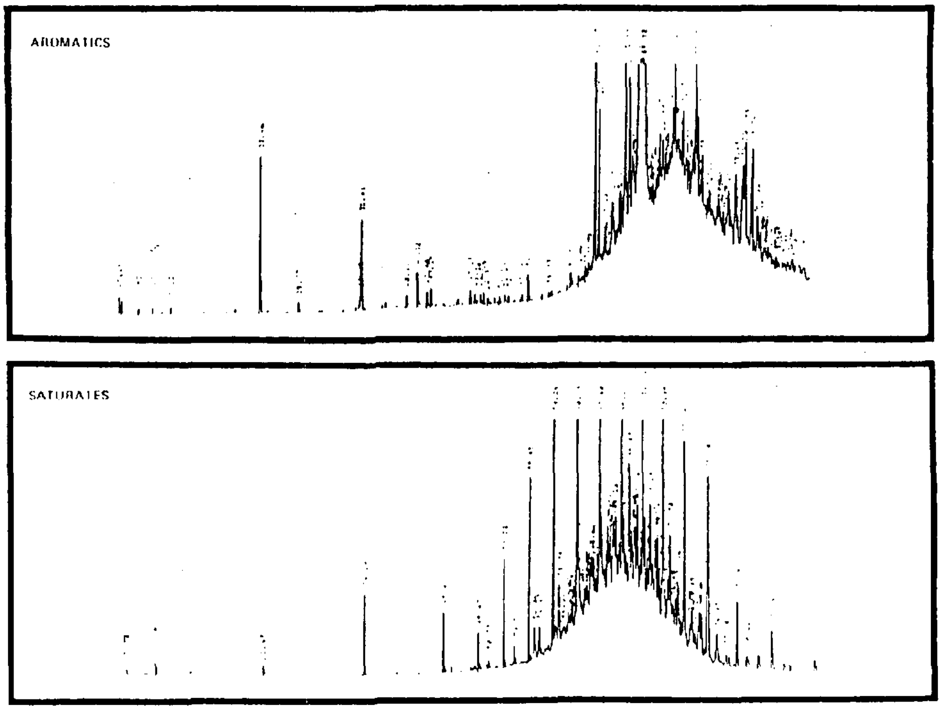


FIGURE 3.78. Plaice Liver, oil-impacted.

3.6 Oysters and Fish (Michel, ISTPM)

An analytical chemical program in support of the early post-spill (March 1978 - March 1979) programs of the Institute Scientifique et Technique des Peches Maritimes (ISTPM) was undertaken (Tables 20 to 22 and Fig. 3.79). Samples of freeze-dried oysters and fish (various species) were analyzed by GC and several samples by GC/MS. The results of the analyses are tabulated in Tables 23 to 25. Based on the nature of the GC traces, sources of observed hydrocarbon distributions are derived: fresh AMOCO CADIZ oil, weathered oil, and biogenic hydrocarbons. Often combined sources are apparent (e.g. weathered oil/biogenic hydrocarbons).

Two oyster time series, summarized in Table 26, indicate that initial heavy oil impacts on the tissues are reduced over time but certainly not eliminated. GC traces illustrating the change in aromatic hydrocarbon composition with time (Fig. 3.80) show that again the alkylated phenanthrene (P) and dibenzothiophenes (DBT) dominate the assemblage through February of 1979.

Fish tissues do not reveal significant oil impacts. For the most part the hydrocarbons consist mainly of biogenic compounds (e.g. olefins) with an occasional UCM and again the presence of DBT and P compounds probably, though not definitely, related to AMOCO CADIZ oil (see Table 24).

An attempt at decontamination via oyster transplantation yielded lower levels of hydrocarbons (Table 23; sample 143) indicating that once removed from a polluted substrate the oysters can depurate their oil burden significantly.

Thus the oysters exhibit similar area-wide uptake of AMOCO oil, initially at the 3000 ppm level, rapidly reduced to the 300-700 ppm level and to the 50-200 ppm level a year after the spill. However, identifiable oil residues remain. Fish samples show only sporadic uptake of any oil indicating that the oil has not significantly impacted coastal fish, or that once impacted the fish rapidly depurate and/or metabolize petroleum.

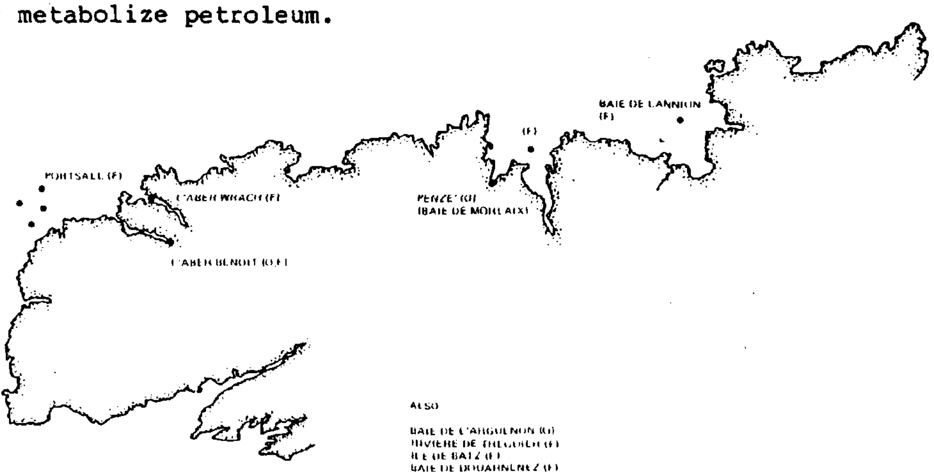


FIGURE 3.79. Oysters and fish sampling locactions.

TABLE 20. AMOCO CADIZ chemistry program, freeze-dried fish and oysters (Michel, ISTPM).

Frequency	
March 1978	1 Oyster
April 1978	1 Oyster + 7 Fish
May 1978	1 Oyster + 6 Fish
June 1978	3 Oysters + 1 Fish
July 1978	4 Oysters
September 1978	4 Oysters
October 1978	3 Oysters + 4 Fish
November 1978	3 Oysters
December 1978	4 Oysters + 5 Fish
January 1979	1 Oyster
February 1979	2 Oysters
March 1979	3 Oysters
TOTAL	30 + 23 = 53

Locations
Various

TABLE 21. ISTPM oyster sample summary.

No.	Date	Sampling Location
5	5.4.1978	Aber Benoit - Prat ar Coum
71	23.3.1978	Baie de L'Arguenon
143	24.5.1978	Essai de decontamination
176	22.6.1978	Baie de Morlaix - Penze R.G. (Le Ven)
178	20.6.1978	Aber Benoit - Prat ar Coum
184	22.6.1978	Baie de Morlaix - Calot (transfert)
212	20.7.1978	Aber Benoit - Prat ar Coum
223	21.7.1978	Baie de Morlaix - Le Frout (Le Ven)
234	18.7.1978	Baie de Morlaix - Penze R G (Le Ven)
242	18.7.1978	Baie de Morlaix - Penze (B.I. Brannelec)
295	20.9.1978	Baie de Morlaix - Penze R D (Cablet)
297	20.9.1978	Baie de Morlaix - Penze R G (Le Ven)
311	20.9.1978	Baie de Morlaix - Penze R D (Gallion)
327	18.9.1978	Aber Benoit (Garo - Hanssen)
349	19.10.1978	Aber Benoit (Garo - Hanssen)
357	19.10.1978	Baie de Morlaix - Penze R D (Kerarmel)
359	19.10.1978	Baie de Morlaix - (B I Brannelec)
399	16.11.1978	Baie de Morlaix - Penze R D (V. Bernard)
400	16.11.1978	Baie de Morlaix - (B I Brannelec)
406	16.11.1978	Baie de Morlaix - Penze (Cadoret)
420	15.12.1978	Baie de Morlaix - Penze R G (Le Ven)
436	15.12.1978	Baie de Morlaix - Penze R D (Cadoret)
440	15.12.1978	Baie de Morlaix - Penze R G (Vallegant)
442	15.12.1978	Baie de Morlaix - R D Ile Noire (Kerarmel)
446	31.1.1979	Baie de Morlaix - Penze R D (V. Bernard)
471	27.2.1979	Baie de Morlaix - Penze R D (Gallion)
473	27.2.1979	Baie de Morlaix - Penze R D (Vallegant)
514	30.3.1979	Baie de Morlaix - Penze R D (Cadoret)
517	30.3.1979	Baie de Morlaix - Penze R D (Ker Armel)
518	30.3.1979	Baie de Morlaix - Penze R D (Cadoret)

TABLE 22. ISTPM fish sample summary.

No.	Nature	Date	Sampling Location
36-3	lieu jaune	13.04.1978	Portsall (3' W Amoco)
40-7	roussette	13.04.1978	Roscoff (Bank ar Forest)
41-3	lieu noir	13.04.1978	Portsall (8' N Amoco)
58-1	lieu jaune	13.04.1978	Portsall (2' E Amoco)
93-2	mulet	27.04.1978	Portsall
100	flet	24.04.1978	Baie de Lannion
118	lieu jaune	29.04.1978	Portsall (3' E Amoco)
151	maquereau	23.05.1978	Baie de Douarnenez
170-1	lieu jaune	11.05.1978	Riviere de Trequier
170-2	lieu jaune	11.05.1978	Riviere de Trequier
198	tacaud	16.05.1978	Baie de Lannion
200	lieu jaune	16.05.1978	Baie de Lannion
203	maquereau	3.05.1978	Baie de Lannion
209	mulet	30.06.1978	Aber Wrac'h
377-2	plie	26.10.1978	Ile de Batz
379-3	mulet	24.10.1978	Baie de Morlaix
415-2	sole	6.12.1978	Baie de Lannion
419-2	grondin	6.12.1978	Baie de Lannion
446-4	plie	15.10.1978	Baie de Morlaix
449-2	sole	15.10.1978	Baie de Morlaix
454-1	plie	5.12.1978	Aber Benoit
454-6	sole	5.12.1978	Aber Benoit
455-4	plie	20.12.1978	Aber Wrac'h

TABLE 23. Results of ISTPM oyster analyses.

Sample No.[a]	Total Hydrocarbons ($f_1 + f_2$; ug/g dry wt)	Source (from GC)[b]
5	2700	1
71	610	2/1
143	180	2/3
176	530	2
178*	1600	2
184	380	2
212	270	2
223	400	2/3
234	640	2
242	80	2/3
295	340	2
297	270	2
311	290	2/3
327	630	2
349	420	2
357	320	2
359	50	2
399	100	2
400	95	2
406	70	2
420	150	2
436*	1000	2
440	90	3/2
442	150	2/3
446*	105	3/2
471	50	3/2
473	140	2
514	140	2
517	170	2
518	140	2

* GC/MS results available (Table 25).

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[a] See Table 21 for location and data of each sample number.

[b] 1 = fresh AMOCO CADIZ oil
2 = weathered oil
3 = biogenic hydrocarbons

TABLE 24. Results of ISTPM fish analyses.

Sample No.[a]	Total Hydrocarbons (f_1 + f_2; ug/g dry wt)	Source (from GC)[b]
36-3	15	3
40-7	39	3
41-3	29	3
58-1	18	3
93-2	45	2/3
100	70	3/2
118	45	3/2
151*	170	3/2
170-1	18	3
170-2*	31	2/3
198	31	3/2
200	69	2/3
203	37	3
209	30	3
377-2	8	3
379-3	25	3
415-2	154	3/2
419-2	15	3
449-2	13	3
454-1	21	3
454-6	20	
455-4	6	

* GC/MS results available (Table 25).

---

[a] See Table 22 for location and data of each sample number.

[b] 1 = fresh AMOCO CADIZ oil
2 = weathered oil
3 = biogenic hydrocarbons

TABLE 25. GC/MS results of selected analyses of oyster and fish tissues (ng/g).

	Oysters			Fish	
	#178	#436	#446	#151	#170-2
N	nd	nd	nd	nd	2
C_1N	nd	nd	nd	nd	nd
C_2N	nd	nd	nd	nd	nd
C_3N	290	nd	52	nd	nd
C_4N	1400	nd	150	nd	nd
N	2190	nd	202	nd	2
P	220	30	85	17	40
C_1P	1400	nd	220	30	70
C_2P	4600	130	350	30	60
C_3P	9500	200	1300	20	50
C_4P	10000	100	1300	10	40
P	25720	460	3170	107	260
DBT	180	nd	16	nd	2
C_1DBT	1400	nd	100	nd	24
C_2DBT	6400	320	480	nd	120
C_3DBT	9600	580	1410	nd	100
DBT	17580	900	2006	nd	246
m/e 202	700	100	320	30	41
m/e 228	900	30	330	nd	20
m/e 252	500	nd	400	nd	nd

N = naphthalenes
P = phenanthrenes
DBT = dibenzothiophenes
202 = fluoranthene + pyrene
228 = benzanthracene + chrysene
252 = benzofluoranthenes + benzopyrenes

TABLE 26. Petroleum hydrocarbons in oysters.

LOCATION	DATE	ΣPETROLEUM (ppm)
Aber Benoit	April 5, 1978	2,700
	June 20, 1978	1,600
	July 20, 1978	270
	September 18, 1978	620
	September 19, 1978	410
Baie de Morlaix	June 2, 1978	530
	July 1978	70-600
	October 19, 1978	60-230
	February 27, 1979	240
	March 30, 1979	150

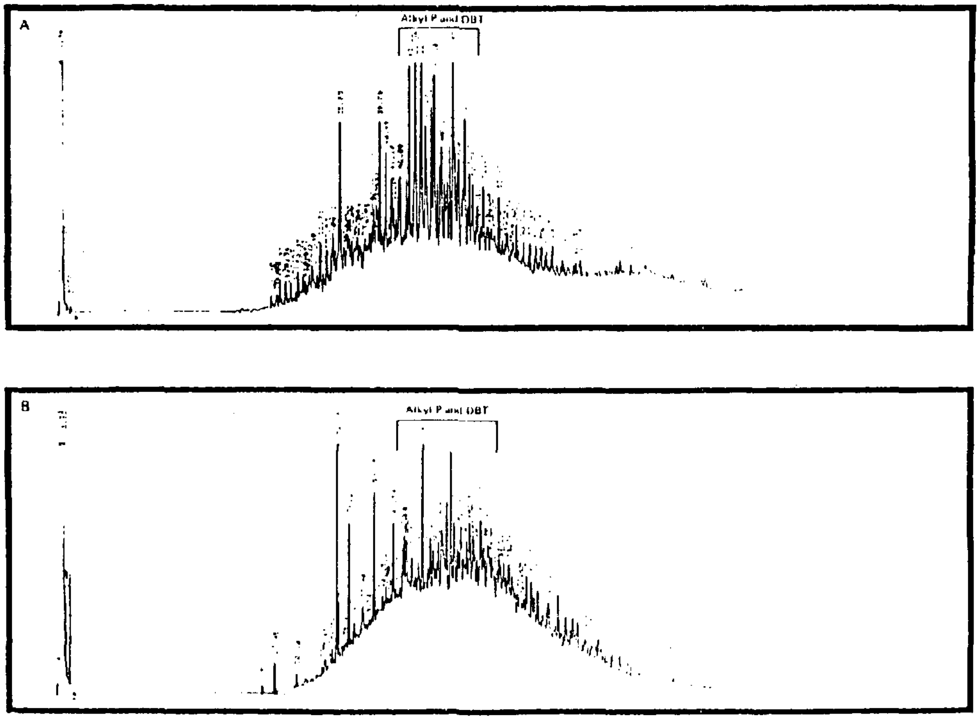

FIGURE 3.80. Baie de Morlaix impacted oysters - aromatic hydrocarbons; A - 5 April 1978; B - 27 February 1979.

3.7 Seaweed and Sediments (Topinka, Bigelow Laboratory for Ocean Sciences)

In support of an investigation on the impact of the spill on macroalgal population recovery and growth, a series of plant and adjacent sediment samples was analyzed by GC to determine if and to what extent AMOCO oil was associated with the plants (Table 27).

The data presented in Table 28 in conjunction with a consideration of Figures 3.81 and 3.82 illustrate that while several of the plant samples do contain weathered oil (see Fig. 3.81) the n-alkane, pentadecane ($n\text{-}C_{15}$), is the most abundant biogenic component in all samples. The distribution of biogenic components in general (Fig. 3.82) can be seen as contributing markedly to the total hydrocarbon levels even in the "oil-impacted" tissues.

GC/MS results of an "oil impacted" plant's aromatic hydrocarbon fraction (Table 29) indicate that again the P and DBT family series are the most abundant aromatic compounds present. In this sample the P compounds are, in total, more abundant than the DBT series, but the C_3DBT are still the most abundant group (8400 ppb).

TABLE 27. AMOCO CADIZ chemistry program; seaweed samples (Topinka).

Frequency	
June 1979	15 Plant + 7 Sediment
August 1979	2 Plant
May 1980	3 Plant
Summer 1980	12 Plant
TOTAL	32
Locations	
Various	
GC/MS	
One Seaweed Sample	

TABLE 28. Summary of analytical results; seaweeds, summer 1980.

	Gravimetric		GC	
Sample	f_1 (μg/g)	f_2 (μg/g)	n-C_{15} (μg/g)	Status
HC-4-1	41	74	38.5	1/2
HC-4-2	10	16	10.7	2
HC-4-3	11	14	4.8	2
HC-5-1	31	29	5.0	1/2
HC-5-2	61	72	35.0	1/2
HC-5-3	61	52	25.0	1/2
HC-5-4	11	12	21.3	2
HC-5-5	39	23	58.0	2
HC-5-6	17	16	25.3	2
HC-5-7	10	7	7.2	2
HC-5-8	12	9	34.0	2
HC-5-8 (Repeat)	8	12	9.0	2
HC-5-9	5	15	3.7	2

Status codes: 1 = weathered petroleum
2 = biogenic

TABLE 29. GC/MS results of seaweed aromatic fraction analysis (sample HC-5; Tregolonou, *Fucus vesiculosis*; 4 June 1979).

Compound	Concentration (ng/dry weight plant)
C_3-fluorene	610
Phenanthrene (P)	420
C_1P	920
C_2P	2400
C_3P	6400
C_4P	5300
ΣP	15,440
Dibenzothiophene (DBT)	40
C_1DBT	300
C_2DBT	4000
C_3DBT	8400
Σ DBT	12,740
m/e 202 (fluoranthene + pyrene)	930
m/e 252 (benzofluoranthenes + benzopyrenes)	1800

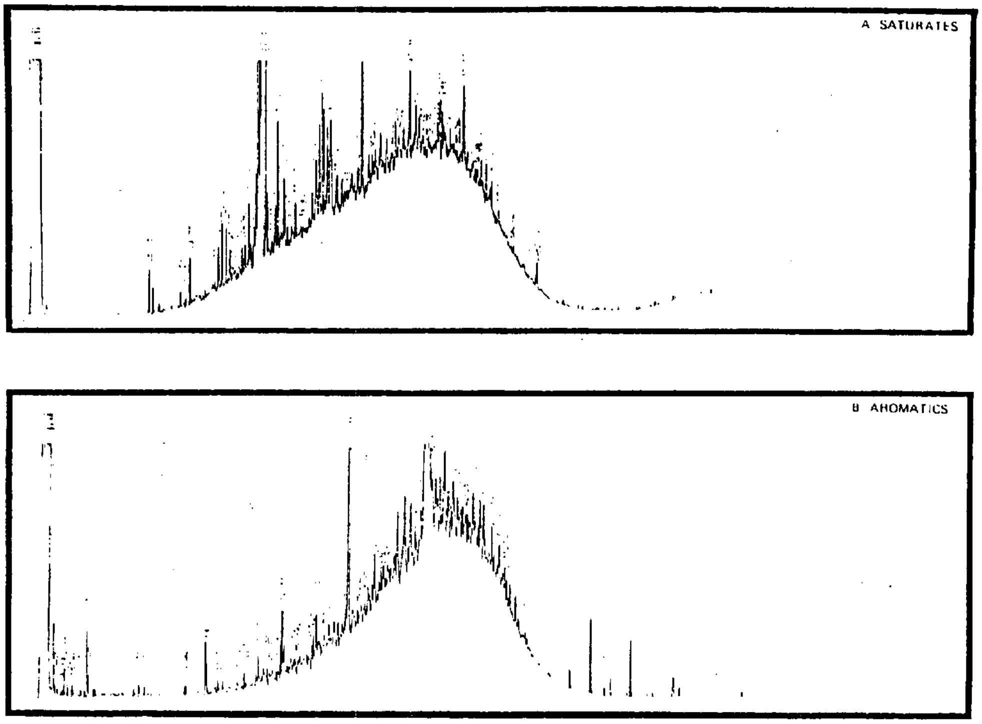

FIGURE 3.81. HC-4-1 seaweed hydrocarbons - oil-impacted.

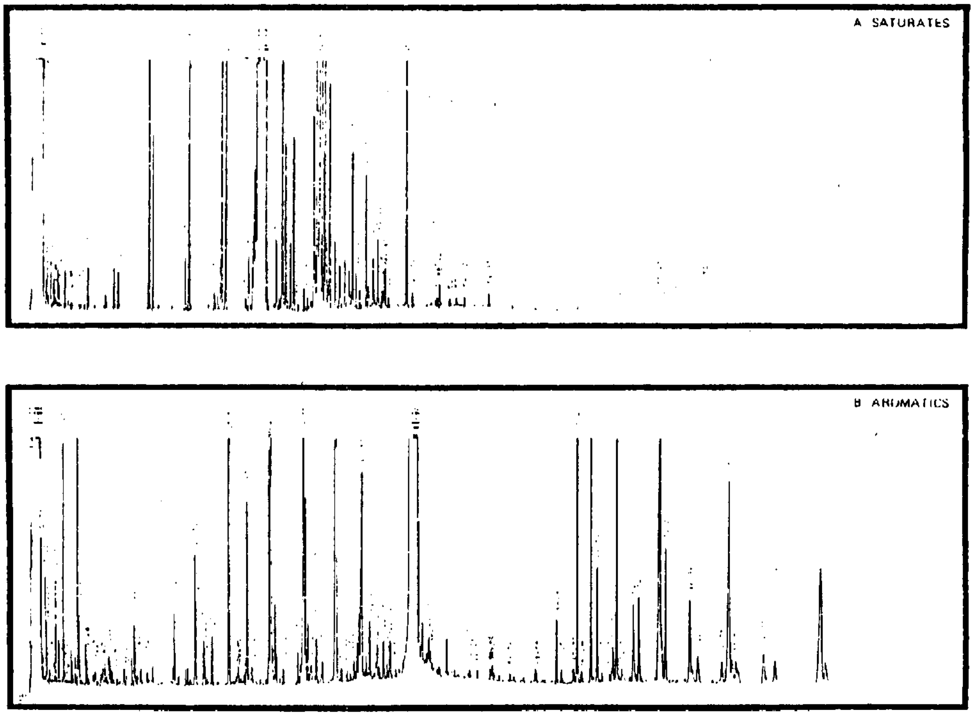

FIGURE 3.82. Seaweed hydrocarbons control.

CONCLUSIONS

A number of specific conclusions concerning the levels of AMOCO CADIZ petroleum hydrocarbons in various environmental compartments, the changing chemistry of the hydrocarbon assemblages, and the persistence of petroleum in these compartments are presented here.

1) Upon introduction into the environment, the oil weathered rapidly with evaporation and biodegradation changing the oil's chemistry markedly even prior to landfall.

2) Oil impacted a variety of intertidal sedimentary types and a number of secondary impacts were noted at many stations.

3) Oil was buried in most sedimentary environments with burial and/or penetration down to 15 cm in fine-grained sediments and deeper (∿20-30 cm) in sandy sediment.

4) Oil remained less biodegraded in sandy beach environments than in fine-grained sediments in which heavily biodegraded oil was characteristic.

5) The presence of UCM material, pentacyclic triterpanes, and alkylated phenanthrene and dibenzothiophene compounds remain as characteristic chemical features of AMOCO CADIZ oil in sediments.

6) Less weathered oil appeared to be buried (10-20 cm) in fine-grained sediments as evidenced in samples taken one year after the spill.

7) Offshore sediments were impacted after the shoreline impact, probably through processes involving beaching, sorption on intertidal sediments, and offshore transport of these sediments. Samples taken after the spill in April 1978 do not reveal AMOCO CADIZ oil, thus indicating a lag (weeks to months) in offshore deposition.

8) Surface intertidal sediments taken in June 1981 show that "normal" background inputs, both of biogenic and chronic pollutant origins, have replaced AMOCO CADIZ oil as major components of the hydrocarbon geochemistry. Only at the most impacted stations at Ile Grande marsh and within the sandy beach sediment at AMC-4 (Portsall) do identifiable AMOCO residues persist. At Ile Grande the aromatic marker compounds are absent, but hopanoid compounds (triterpanes) and a large UCM persist.

9) Oysters were initially heavily impacted by oil (several thousand ppm) and after two years (June 1980) traceable AMOCO CADIZ residues are still evidenced by homologous series of isoprenoid alkanes, phenanthrenes and dibenzothiophenes. Petroleum residues persist approximately at the 100 ppm level.

10) Fish do not appear to have been directly impacted (chemically) by the spillage to any significant extent.

11) Compositional profiles traceable to AMOCO CADIZ oil are likely to "disappear" from all sediments within another year (i.e. 1982; four years after the spill) although this should be confirmed by direct measurements and attention to molecular marker compound distributions.

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STUDIES OF HYDROCARBON CONCENTRATIONS AT THE ILE GRANDE AND BAIE DE LANNION STATIONS POLLUTED BY THE WRECK OF THE AMOCO CADIZ

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INTRODUCTION

A study of the hydrocarbon concentrations in district no. 7 has been made since December 1978 in collaboration with the Marine Station of Endoume (Mesdames Vacelet, Plante, and Lecampion). The first series of analyses was made outside of the CNEXO-NOAA framework, while our second study was supported by them. Results of the two studies are herein combined.

METHODS

Nature of the Samples

Samples were collected at sites indicated in Figure 1. Sample sites A, D, and F are located in a very polluted zone; B, C, and E are located in a zone where the pollution level is lower since a dam was erected under the bridge to prevent the spreading of oil. Subscripts indicate specific areas samples: 1 = marsh, 2 = tidal creek, and 3 = upper mud flat (see Figs. 1 and 2).

Samples were collected in December 1978, March 1979, November 1979, and May 1980, using a plexiglass corer (ID = 26 mm). The 5 mm superficial layer was subsampled with a steel spatula. Sediments were immediately frozen, flown to Marseilles, and kept at -30°C.

Analytical Techniques

To yield the maximum amount of information, we have chosen the systematic soxhlet extraction following Farrington's method. This method is expensive and time-consuming but, for the biologists, it is the only one which gives satisfactory results. Moreover, reproducibility was tested several times and was found to be satisfactory.

To avoid the very long separation of alumina- or silica-packed columns, we developed a micromethod of separation using Sep-Pak of Waters. This rapid technique is described in Analytica Chemica Acta. The general analytical scheme is as follows:

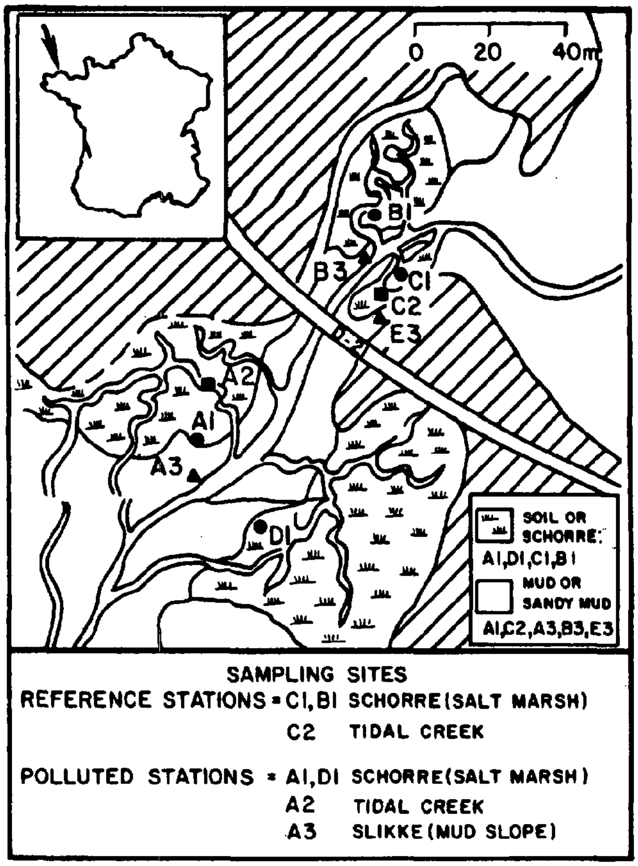


FIGURE 1. Ile Grande marsh sampling sites.

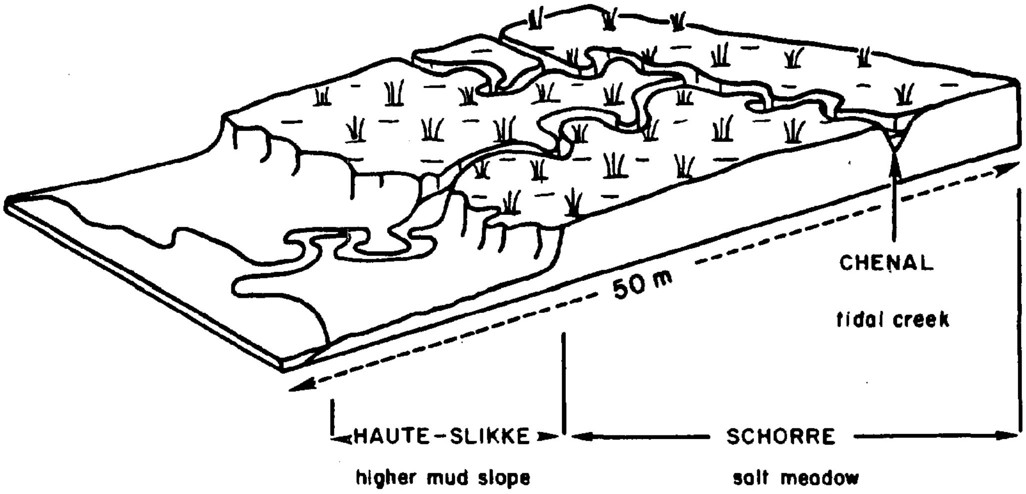
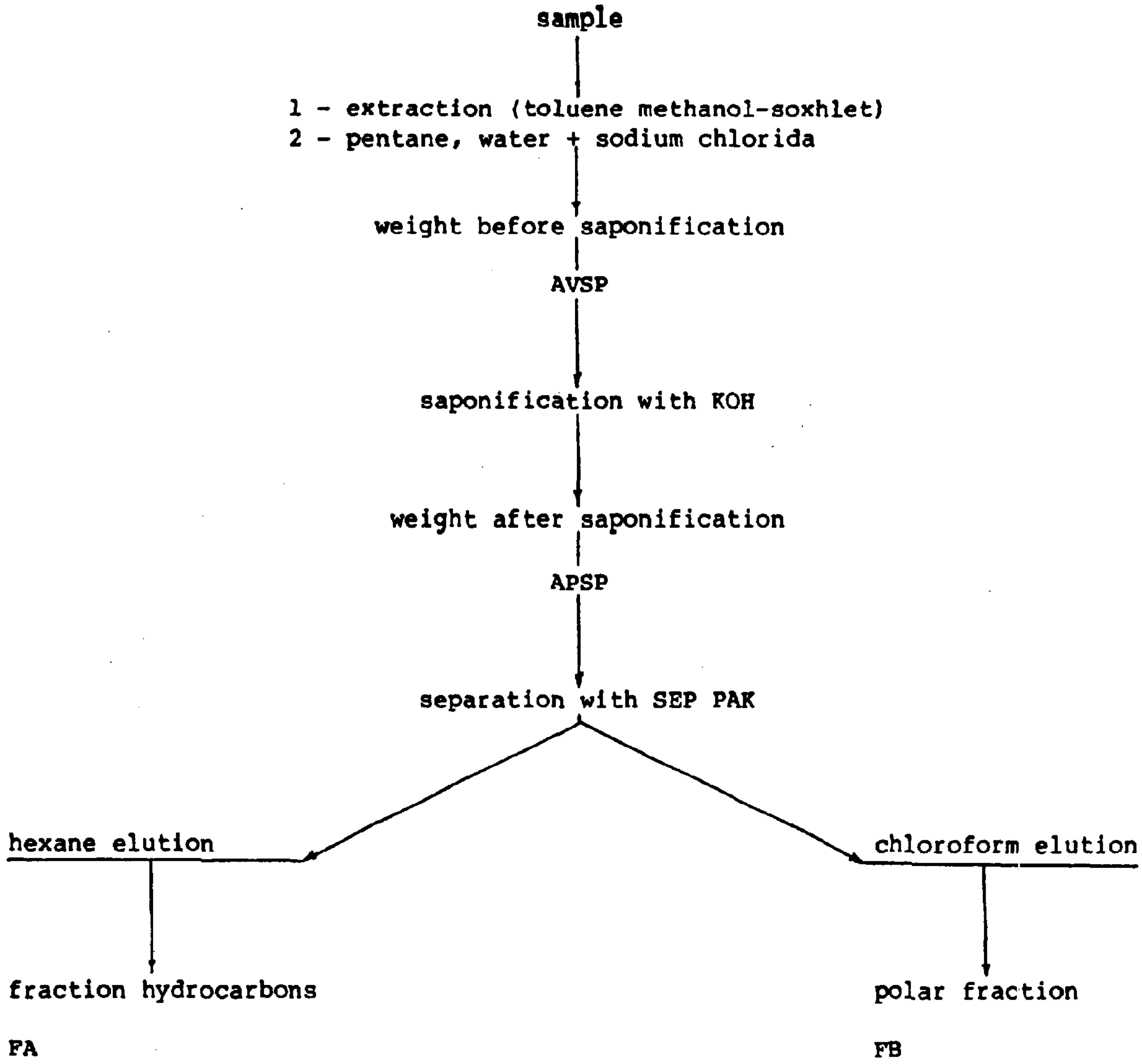


FIGURE 2. Detailed map of the sampling area.



Each fraction was weighted by gravimetry (Balance: Perkin Elmer AD2Z, 1/10 of ug). In each case, the fraction FA was chromatographed on a capillary column (OV1 or SE52) with a Girdel or a Carlo Erba Fractovap 4160. The fractions FA and FB were also analyzed by high-pressure liquid chromatograph (column RP 18, radial pressure, Waters-type detector). Since the FA fraction may contain saturated, unsaturated, or aromatic hydrocarbons, fluorescence spectrometry (Perkin Elmer 3000) was used especially during the 1980 survey when biodegradation rates were higher. Infrared spectroscopy (Perkin Elmer 125 and 225) was also used systematically to show the absence of carbonyl functions on the FA fractions. During the 1980 campaign, it appeared that some of the chromatograms of the FA fractions were not sufficiently resolved due to the absence of saturated hydrocarbons. However, as they represented a substantial weight (A_1 = 257 g; A_2 = 14 g), NMR spectroscopy (C 13 and proton 250 Mz Cameca) was used to denote the presence of heavy-weight compounds with fused rings (aromatic and nonaromatic). The structure of these complex mixtures was not elucidated at this time, but could be studied in the future.

RESULTS

Results are presented in Tables 1, 2, and 3. The weight of the compounds AVSP (before saponification) and APSP (after saponification) are indicated. The value of the ratio AVSP/APSP is characteristic of the capacity of the medium to be biodegraded. A value close to 1 shows poor bacterial activity. As the value increases, better biodegradation is indicated. This fact is due to the functionalized intermediate compounds made by the bacteria and extract in basic medium.

The fraction FB is constituted by unsaponifiable compounds. Comparison of FA and FB is not interesting. Only the comparison of FB values for different sampling times is relevant. When biodegradation increases (less linear hydrocarbons), the FB fraction decreases. At A1 and A2 station (in very polluted zones), the concentrations of hydrocarbons (linear or substituted) were about null in 1980, but complex organic compounds of heavy molecular weight, which are extractable with hexane (since present in the FA fraction), remain in the sediment. The molecular structure of these compounds has not been established (the petroleum-type asphaltenes are not extracted at the very beginning by pentane).

CONCLUSIONS

Station C_1

In 1980, in spite of the presence of 0.54 g in the FA fraction, its chromatogram was no longer characteristic of a petroleum-polluted zone (C17, C18, C19 predominant). However, light petroleum pollution is indicated at 20 cm below the surface.

Station B_2

In 1980, various deposits did not allow correct sampling; therefore, only 1978 and 1979 values were determined. In 1979, there seemed to an indication of return to normal state, especially at the rhizome level (bottom).

Station A_1

In 1980, half of the hydrocarbons of 1979 remained. This fraction did not show the presence (by chromatogram) of linear or substituted hydrocarbons; however, its weight cannot be explained only by the unbiodegradable cyclanes or aromatics (33 g).

TABLE 1. Hydrocarbon content of Ile Grande marsh sediments.

STATIONS		SAMPLE WET WEIGHT (g)		AV-SP (g/kg)		AP-SP (q/kg)		AV/AP		FB (q/kq)		TOTAL HYDROCARBONS (FA) (q.kq)	
		12/78	11/79	12/78	11/79	12/78	11/79	12/78	11/79	12/78	11/79	12/78	11/79
Marsh													
A_1	su	13.40	25.10	73.60	43.48	67.60	39.58	1.09	1.10	35.70	16.93	32.97	18.84
	rh	68.70	99.50	2.00	0.92	1.37	7.10	1.47	1.26	0.90	2.99	0.47	3.68
D_1	su	12.55	14.60	173.90	243.45	162.50	210.50	1.07	1.15	67.90	102.12	94.60	94.51
	rh	59.15	90.10	19.78	1.98	16.50	0.96	1.20	2.06	8.40	0.46	8.13	0.23
	ca	24.25	74.80	1.89	0.24	1.48	0.19	1.26	1.26	1.00	0.06	0.40	0.10
B_1	su	13.30	10.85	8.00	10.29	5.15	8.58	1.34	1.14	4.00	5.56	1.90	1.75
	rh	53.50	97.80	2.12	1.99	1.30	0.89	1.63	2.24	1.06	0.58	0.17	0.10
C_1	su	15.35	19.85	13.74	2.43	12.27	1.93	1.12	1.26	8.10	1.19	4.17	0.43
	rh	58.50	132.50	2.41	0.15	1.59	0.12	1.52	1.25	1.33	0.05	0.26	0.03
Marsh Channel													
A_2	ps	51.20	6.10	18.52	21.49	16.70	12.41	1.11	1.73	9.00	4.25	7.69*	6.59
	zr	83.50	120.60	1.78	0.95	1.42	0.60	1.25	1.58	0.91	0.23	0.48	0.29
	ca	23.10	139.20	0.63	0.17	0.31	0.07	2.03	2.43	0.21	0.02	0.10	0.03
C_2	su	10.50	15.90	14.07	6.32	8.27	4.28	1.70	1.48	5.00	2.17	3.26	1.41
	zr	39.80	41.20	3.07	1.89	2.26	0.94	1.36	2.01	1.50	0.66	0.77	0.09
	cs	18.65	76.90	0.60	0.92	0.45	0.40	1.33	2.3	0.22	0.25	0.23	0.05
Upper Mud Flat													
A_3	ps	37.80	1.05	11.48	16.91	11.44	12.91	1.00	1.31	5.88	8.	5.56*	3.45
	cs		106.50		7.43		6.66		1.12		3.54		2.40
B_3	ps		7.50		6.18		2.66		2.32		1.34		0.52
	cs		112.30		3.43		1.76		1.95		1.14		0.19
Arabian light				100 g		94 g		1.07		32%		68%	

AV-SP = Before saponification
AP-SP = After saponification
Weight in g per kg of dry sediment.
*These concentrations were evaluated from sediment samples scraped by a spatula to about 0.05 cm depth.

AV/AP = Ratio
FB = Fraction B separated by Sep-Pak, HC Cl, elution
FB = Fraction A, hexane elution:total hydrocarbons
ps = Surface crust

TABLE 2. Hydrocarbon concentrations with depth.

BIOTOPE AND STATIONS	DEPTH OF SAMPLE (cm)	TOTAL HC; g/kg OF DRY SEDIMENT			
		12/78	3/79	11/79	5/80
Marsh					
	ps				39.87
A_1	0- 3	32.97		18.84	14.98
	3-14	0.47		3.68	0.03
	34-37				0.04
D_1	0- 3	94.68		94.51	230.60
	3-14	8.13		0.23	17.78
	14-26	0.48		0.10	0.18
B_1	0- 3	1.90		1.75	
	3-14	0.17		0.10	
	ps				1.50
C_1	0- 3	4.17		0.43	0.54
	3-14	0.26		0.03	0.15
	14-32				0.05
Channel					
	ps				14.20
A_2	0- 3	7.69	10.78	6.59	2.57
	3- 9	0.48	0.22	0.29	1.14
	9-28	0.10		0.03	0.08
	28-36				0.08
	ps				0.07
C_2	0- 3	3.26		1.41	
	3- 9	0.77		0.09	
	9-28	0.23		0.05	
Upper Mud Flat					
	ps	5.56		3.45	0.27-15
A_3	0- 3		24.95	2.40	0.60
	3- 9		0.65		0.50
E_3	0- 3				0.89
	3-11				0.08
	11-20				0.08
	20-27				2.81
	ps			0.52	
B_3	0- 3			0.19	0.56
	3-15				0.22
	15-19				0.16

ps = surface crust, sampled by scraping

TABLE 3. Chromatographic analysis of the saturated fraction of hydrocarbons from Ile Grande sediments.

STATIONS		C_{17}/Pr		C_{18}/Ph		Pr/Ph		Predominance x	
		12/78	11/79	12/78	11/79	12/78	11/79	12/78	11/79
Marsh									
A_1	su	1.	0.5	0.77	0.58	0.54	0.67	1.	xx
	rh	0.48	0.5	0.41	0.21	0.61	0.35	1.	xx
D_1	su	0.60	0.2	0.47	0.11	0.60	0.54	1.	xx
	rh	1.24	1.5	0.93	1.92	0.75	0.66	1.	1.40
	ca	3.30	2.	0.78	1.92	0.17	0.83	xx	xx
B_1	su	xx	4.43	xx	2.62	xx	0.54	xx	1.57
	rh	0.21	1.31	1.75	4.	7.80	3.2	2.37	xx
C_1	su		1.42		6.20		3.80		1.11
	rh		2.80		6.67		1.66		1.05
Marsh Channel									
A_2	ps		0.35		0.13		0.56		xx
	zr		0.50		0.26		0.58		1.25
	ca		1.00		3.50		2.20		1.03
C_2	su	0.13	0.19	0.39	0.44	3.50	23.33	0.99	1.84
	zr	0.13	1.25	0.26	2.5	2.28	4.	1.06	1.48
	cs	2.33	3.33	5.30	3.5	2.	0.75	0.89	1.30
A_3	ps		4.10		6.00		1.25		1.03
	cs		0.09		<0.06		<0.85		xx
B_3	ps		0.53		0.53		0.94		1.04
	cs		0.29		0.14		4.72		1.60
Arabian light		10.30		4.70		0.48		0.88	

$$x \text{ predominance} = \frac{2(C_{23} + C_{25} + C_{27})}{C_{22} + 2C_{24} + 2C_{26} + C_{28}} \text{ aliphatic hydrocarhons}$$

xx = Nonextractable
su = Superficial part; some centimeters
rh = Rhizosphere
cs = Sandy layer

Pr = Pristane
Ph = Phytane
zr = Reduced zone
ca = Clay layer

Station D_1

This site is subjected to a flowing stream which must have facilitated the deposit of hydrocarbons in large quantity, since between 1978 and 1980, an important increase was measured (95 g in 1978 to 230 g in 1980).

Station C_2

This site indicates that substantial biodegradation is in progress.

Station A_2

In the FA fraction, we again notice the presence of organic compounds which are neither linear nor substituted. The ratio FA 1978/FA 1980 is very close to that of station A1.

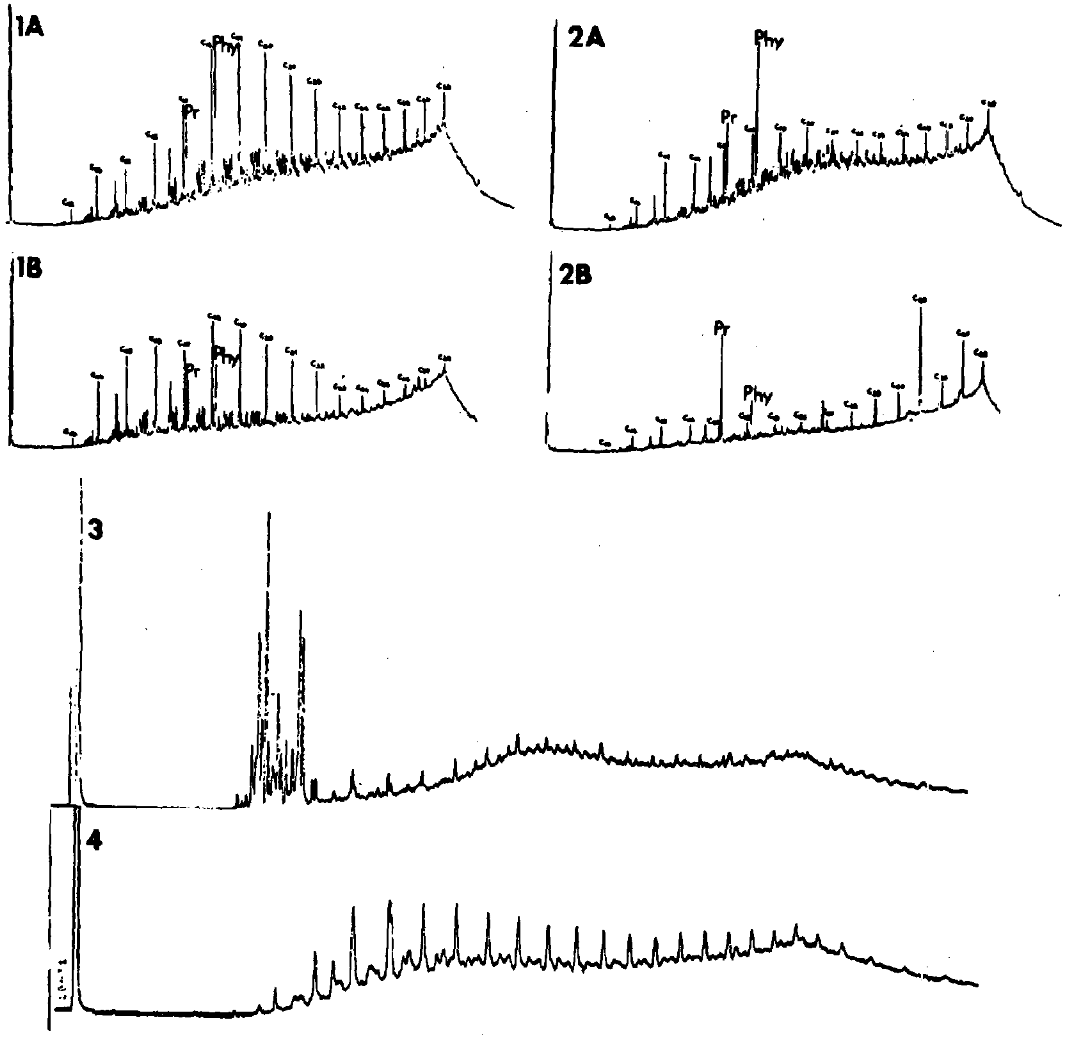
Stations B_3 and E_3

These are reference stations in the less polluted zone. Some remaining pollution is visible, indicating that the dam was not able to prevent oil from spreading to this side of the bridge. In 1980 at B_3, the chromatogram of the FA fraction did not indicate petroleum type, and there was a return to normal levels. At E_3 in 1980, some minor petroleum pollution remained.

Station A_3

There are large variations in the hydrocarbon concentrations, primarily at the sediment surface. Two separate samplings in 1980 (5 mm depth) yielded 0.27 g and 15 g of oil. However, at -10 cm, the values became equal to 0.50 g. The analysis of the 15-g fraction shows that linear and substituted hydrocarbons have disappeared.

Our results are in good agreement with those noted by biologists. The less polluted zones are returning to a normal state, but a seemingly important residue remains in very polluted sites after linear, substituted, and light aromatic hydrocarbons have disappeared.



Chromatogram 1) Station A_3, March 1979. (A) surface, (B) bottom.
2) Station A_2, March 1979. (A) surface, (B) bottom.
3) Station A_3, 1980, surface skim.
4) Station D, 1980, 0-3 cm surface.

EVOLUTION OF THE HYDROCARBONS PRESENT IN THE SEDIMENTS OF THE ABER WRAC'H ESTUARY

by

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Following the Amoco Cadiz accident, the Institut Francais du Petrole analyzed various samples in an effort to learn the physico-chemical characteristics of the oil pollutant released, and to observe its evolution over time. These studies dealt principally with samples of "chocolate mousse" and of beach-sand type surface sediments taken at various depths.

In March 1978 a study of the physico-chemical evolution of the hydrocarbons trapped in the subtidal sediments of the Aber Wrac'h estuary was undertaken with the collaboration of the Centre Océanologique de Bretagne.

INITIAL CHARACTERIZATION OF THE POLLUTANT

The description of the Amoco Cadiz's cargo was undertaken on the basis of samples of foam ("chocolate mousse") taken at Portsall on March 22 and 23, 1981. This method of identification is based on the work of Pelet and Castex, and operates according to a breakdown by chemical family, which has already been used by J. Roucache to make a geochemical study of the organic matter extracted from sediments.

The pollutant was identified as a mixture of two crude oils -- Iranian light and Arabian light -- physico-chemical characteristics of which are fairly similar ; they contain 45 to 47 % saturated hydrocarbons, 31 to 34 % aromatic hydrocarbons, 16 to 17 % polar compounds, and 4 to 5 % asphalt compounds (Fig. 1). The ratio of saturated hydrocarbons to aromatic hydrocarbons is on the order of 1.3.

The saturated fraction is constituted of normal paraffins, isoparaffins of isoprenoid compounds (pristane, phytane, etc...) and cyclic and polycyclic alkanes or cycloparaffins. With gas-phase chromatography, n-alkanes in the sample taken at Portsall 23 March follow a regular distribution curve ; her top corresponds to the n-C15 and n-C16, after which it tapers off regularly to the n-C35 (Fig. 1). It is likely, incidentally, that compounds over n-C35 exist, but they have not yet been detected.

Ratios of pristanes/n-C17 and phytanes/n-C18 in crude oil are, respectively, 0.37 and 0.51. To take into account evaporation phenomena and compare with evolved samples, this oil sample was topped at 340°C.

An unresolved complex mixture (UCM) appears under the n-alkanes, constituted of isoparaffins and cycloparaffins. Alkane (n+iso)

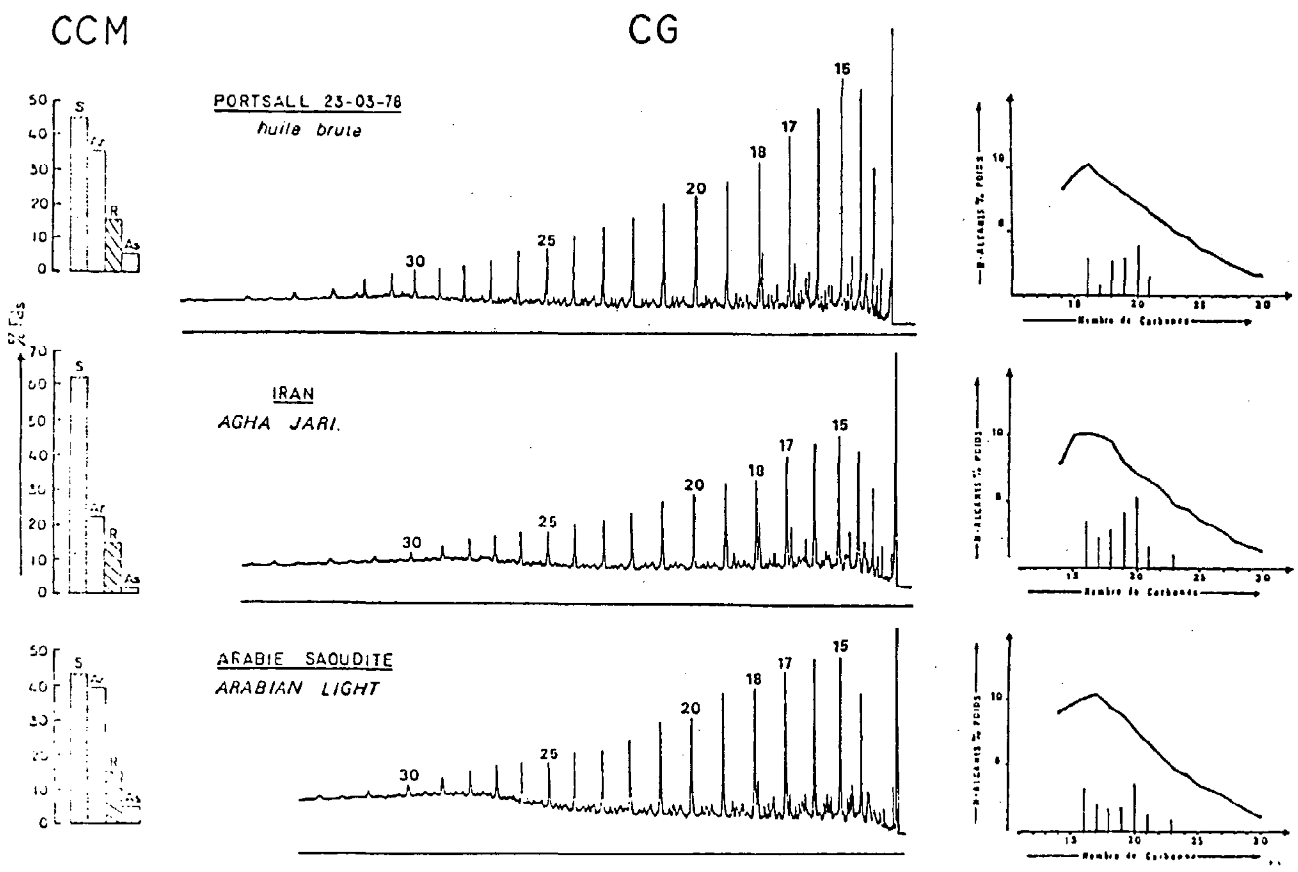


FIGURE 1. Saturated hydrocarbons : Portsall 23.03.78; Iranian light and Arabian light.

contents determined by mass spectrometry are on the order of 53 %. Cyclane contents decrease gradually from 14.2 % for the single-membered cycloparaffin rings, to 2.3 % for six-membered rings.

The combination of gas-phase chromatography with mass spectrometry (GPC/MS) made it possible to distinguish the components of the aromatic fraction : monoaromatic, diaromatic, and triaromatic hydrocarbons.

Aromatic sulfur compounds of the thiophenic type are the benzothiophenes, dibenzothiophenes, and naphthobenzothiophenes.

Polar compounds (resins) contain oxygenated functions (principally hydroxyls and carboxyls) shown by infra-red spectrometer analysis. Elemental analysis of this resin fraction made it possible to assess contents of the following elements :

C = 78 %
H = 8.9 %
N = 1.5 %
O = 4.5 %

In this case, the oxygen content is not calculated by subtracting the total of the other contents from 100 %, but is titrated by the Unterzaucher method.

Asphaltene compounds, separated from the oil by cold-hexane precipitation, are known to have very complex laminated structures.

Metal (such as nickel and vanadium) and sulfur contents were determined on a dry extract before deasphalting (Fig. 2). Métals are essentially present in heavy fractions of crude (resins and asphaltenes) as chelate compounds. Their contents range from 14 to 16.5 ppm for nickel and from 45 to 60 ppm for vanadium, with a ratio Ni/V of 0.27 to 0.31. Sulfur contents are on the order of 2.35 % by weight.

These determinations will eventually serve as a point of departure in following the pathways of the pollutant. It is to be noted that this study is not intended to cover the light evaporated and/or dissolved third of the cargo, of which about 25,000 tons is light aromatic hydrocarbons. Benzene, toluene, and xylene are estimated at respectively 3,300, 4,600, and 3,000 tons.

EVOLUTION OF THE PHYSICO-CHEMICAL PARAMETERS OF THE HYDROCARBONS

We followed the chemical evolution of the hydrocarbons in three different types of samples taken from three areas of pollution :

- subtidal sediments (Aber Wrac'h) ;
- water surface-stable emulsions (hydrocarbons with water, or "chocolate mousse") ;
- intertidal sediments (beaches).

The Subtidal Sediments of the Aber Wrac'h

A detailed study of these sediments was made by DUCREUX and MARCHAND (1979) (collaboration IFP/COB). The evolution of the

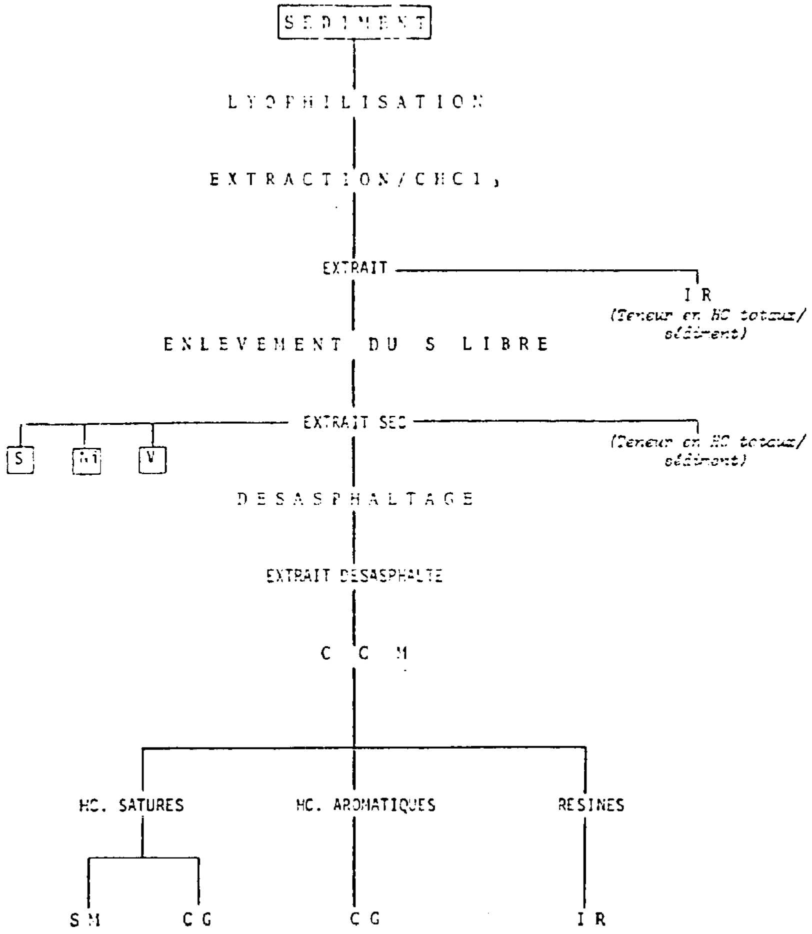


FIGURE 2. The program of analysis.

hydrocarbons trapped in the subtidal sediments of the Aber Wrac'h was followed from March 31, 1978 to October 22, 1979 at nine stations (Fig. 3) in the estuary, taking into account the fairly pronounced marine character of the environment and the sandy, muddy, or sandy/muddy nature of the sediments.

Over a second period, from January 17, 1980 to June 24, 1980, three study sites, Stations 5, 6 and 8, which were deemed representative of the different evolutionary patterns of the hydrocarbons, were again put into operation. The studies undertaken at these three stations only, are included in this report. To simplify the evolutions observed in the Aber Wrac'h, the figures included here are limited to samplings taken on March 31, 1978, November 22, 1978, June 20, 1979, January 17, 1980, and June 24, 1980.

Station 5

A reduction was noted in the pollution of this station, situated in the outer area of the Aber where the marine environment is particularly pronounced (Fig. 4). It was very slight, however, since the concentrations observed in June 1980 were over 670 mg of hydrocarbons per kilogram of sediment. This may be explained by the slightly muddy character of the sediment.

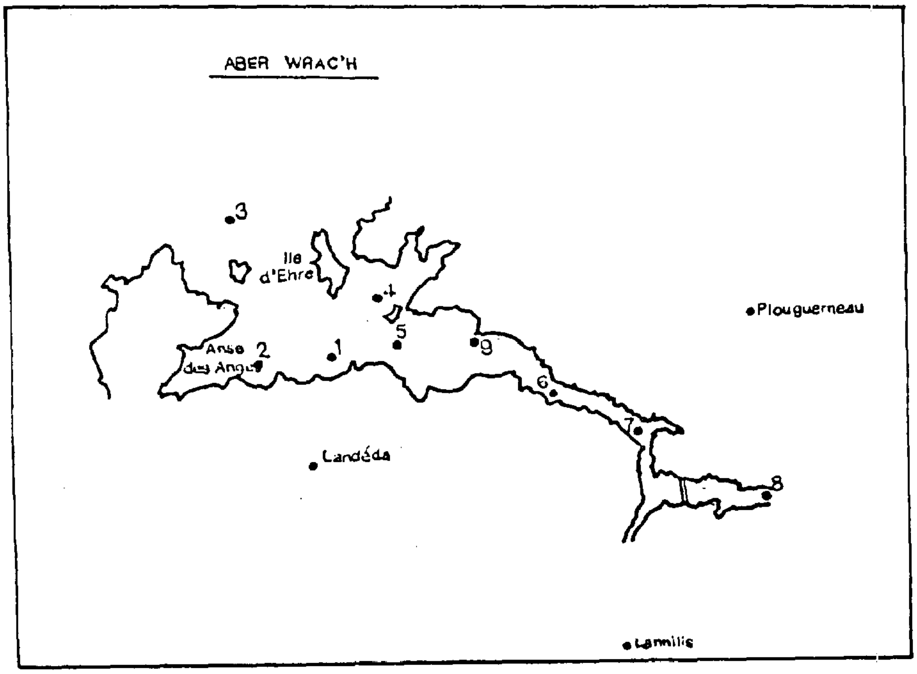


FIGURE 3. The Aber Wrac'h.

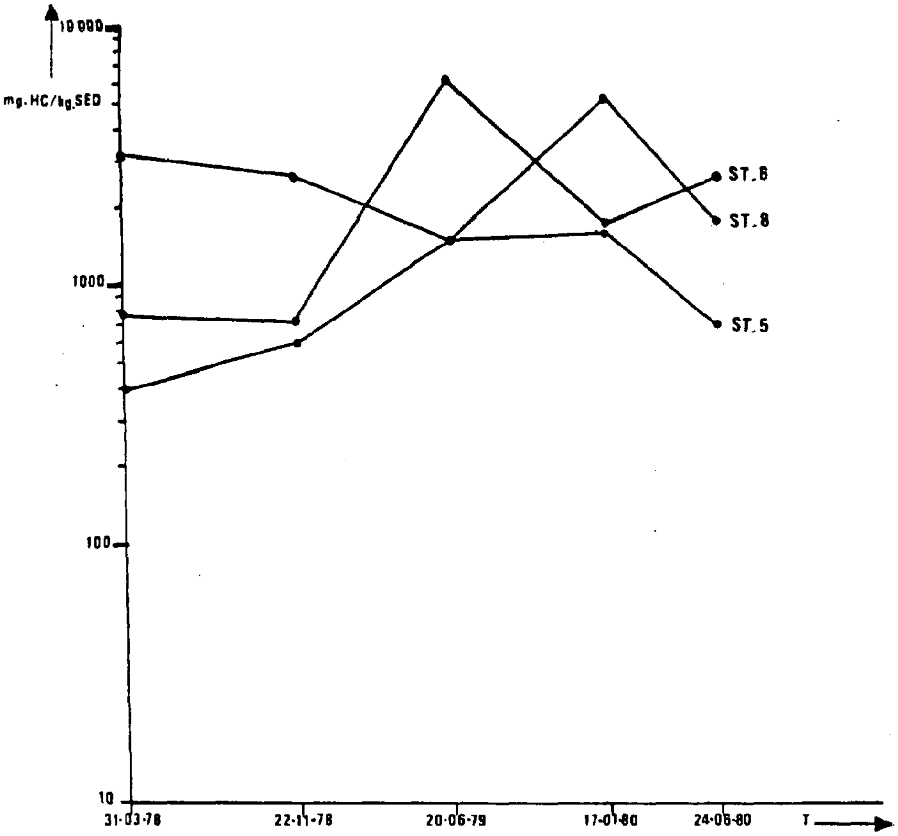


FIGURE 4. Evolution of hydrocarbon contents in the sediments of the Aber Wrac'h.

The relative contents of saturated and aromatic hydrocarbons decreased perceptibly (Fig. 5) in nearly constant proportions, the ratio SAT/ARO decreased slightly probably because of a significant increase in resins -- from 17 to 40 % by weight. After January 1980, however, contents of all these substance remained about the same.

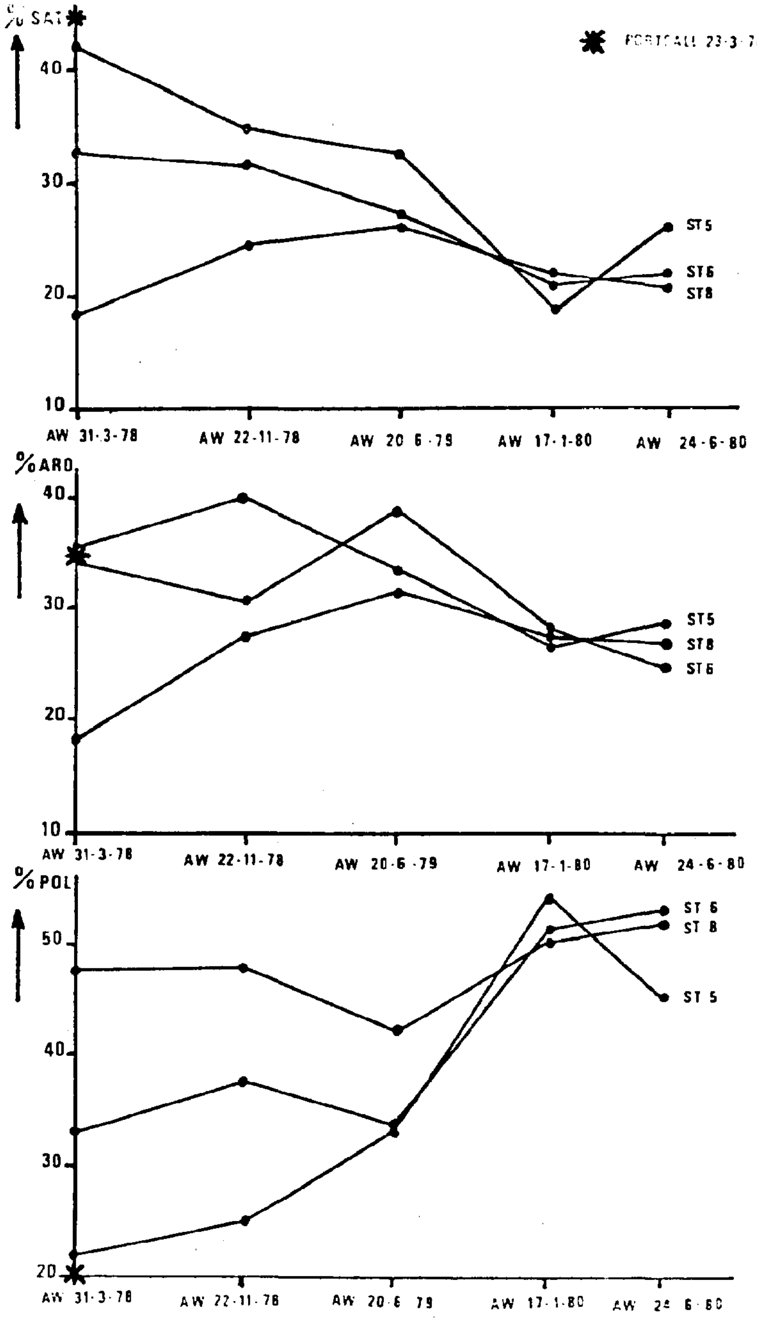
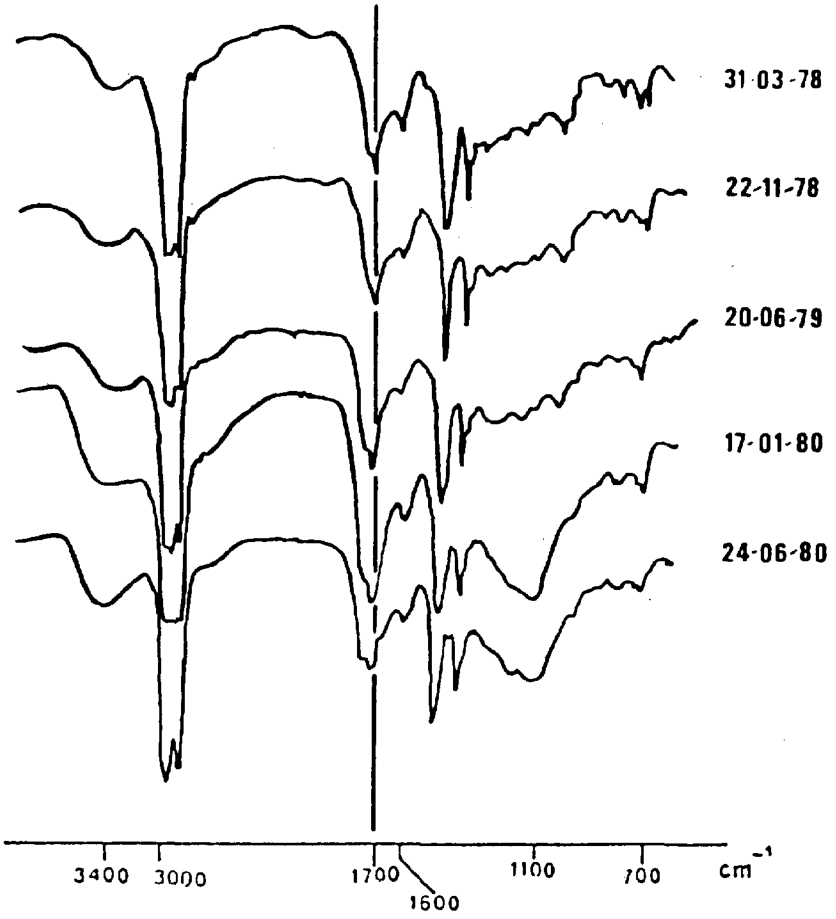


FIGURE 5. Evolution of the various chemical families (saturated hydrocarbons, aromatic hydrocarbons, polar compounds).

The increase in resin contents results from an oxidation degradation of the crude in the medium, reflected in a perceptible upward curve of the absorption bands under infra-red at the level of the hydroxyls (3,600 to 2,900 cm^{-1}) and the carbonyls -- including esters, and a great predominance of Carboxylic acids (1,740 →

1,700 cm^{-1}) (Figure 6). The elemental analyses of the resins confirm this tendency toward oxidation over time (oxygen levels in March 1978 were 5.4 % ; in June 1980, 7 %).



Station 5 - FIGURE 6. Infra-red spectrometry of the resins.

The distribution of saturated hydrocarbons determined by gas phase chromatography (Fig. 7) demonstrates the evolution which led to the degradation of the n-alkanes (5.99 % to 3.33 %) to n-C30 (Table 1). It should be noted that by March 31, 1978, this degradation appears to have begun. Thus there was an increase in the ratios of pristane/n-C17 and phytane/n-C18 (Table 1). The isoprenoids degraded less easily than the n-alkanes (TISSOT and al.), but by June 1980 these compounds could no longer be detected. As degradation advanced, the contribution of the biogenic hydrocarbons increased, and n-alkanes of odd carbon numbers between n-C25 and n-C35 -- characteristic of the cuticle waxes of higher plants (Eglington and Hamilton - 1963) -- appeared, confirming the terrestrial contribution to the contents of organic matter in the sediments.

Moreover, the disappearance of the n-paraffins points up a relative enrichment in the unresolved complex mixture of the isoalkane and cycloalkane compounds, and generally in the compounds around n-C30. It was possible to demonstrate these last compounds by GPC/MS by measuring the masses : m/e 217 being characteristic of the tetracyclic

STATIONS	Prélèvements	HC.SAT. % Pds	HC.ARO. % Pds	RESINES % Pds	ASPHAL. % Pds	SAT. / ARO.	PRIST. / PHYT.	PRIST / n-C17	PHYT / n-C18	n-alc. % Pds	S % Pds	Ni µg/g	V µg/g	Ni / V
PORTSALL	23.03.78 huile brute	45,12	34,55	15,70	4,60	1,30	0,81	0,37	0,51	22,11	-	-	-	-
PORTSALL	23.03.78 340+	37,30	34,10	24,30	4,32	1,09	0,44	0,37	0,51	29,05	2,38	14	45	0,31
5	1*	42,40	35,80	17,20	4,60	1,18	0,80	1,20	1,55	5,99	2,30	13	45	0,29
	2	36,30	37,70	17,00	8,90	0,96	0,67	1,60	2,14	3,33	3,57	35	50	0,70
	3	35,00	40,20	20,00	4,80	0,87	-	-	-	-	2,70	20	40	0,50
	4	31,50	32,00	30,60	5,90	0,98	-	-	-	-	3,55	39	40	0,97
	5	33,00	33,70	27,80	5,50	0,98	-	-	-	-	2,65	21	43	0,49
	6	23,80	29,50	39,30	7,40	0,81	-	-	-	-	2,62	27	45	0,60
	7	18,80	26,70	40,00	14,50	0,70	-	-	-	-	2,22	23	50	0,70
	8	26,00	28,70	36,90	8,40	0,91	-	-	-	-	-	-	-	-
6	1	32,70	34,30	24,60	8,41	0,95	0,50	1,62	2,16	4,09	-	-	-	-
	2	34,00	35,20	20,10	10,60	0,96	0,65	1,46	2,02	3,91	4,80	400	800	0,50
	3	31,60	30,80	29,50	8,00	1,03	-	-	-	-	-	-	-	-
	4	30,50	37,10	27,80	4,60	0,82	-	-	-	-	2,60	18	44	0,41
	5	27,40	39,00	29,10	4,50	0,70	-	-	-	-	2,32	10	35	0,29
	6	20,40	29,30	37,20	13,10	0,70	-	-	-	-	2,62	6	17	0,38
	7	20,40	28,40	37,00	14,20	0,72	-	-	-	-	2,28	24	48	0,50
	8	22,00	24,80	43,40	9,80	0,88	-	-	-	-	2,90	18	34	0,53
8	1	18,40	18,10	38,10	9,40	1,02	0,22	0,50	0,96	9,74	-	-	-	-
	2	29,90	29,20	25,50	15,40	1,02	0,42	2,10	2,67	3,95	-	-	-	-
	3	24,60	27,70	36,20	11,50	0,89	-	-	-	-	3,80	-	-	-
	4	27,00	31,00	32,20	9,80	0,87	-	-	-	-	3,90	40	67	0,60
	5	26,20	31,70	37,30	4,90	0,83	-	-	-	-	2,86	14	65	0,21
	6	19,20	22,70	40,90	17,20	0,85	-	-	-	-	4,21	36	95	0,38
	7	22,00	27,60	34,90	15,50	0,80	-	-	-	-	2,00	24	45	0,53
	8	20,80	27,20	36,60	15,40	0,91	-	-	-	-	3,32	22	39	0,56

* SAMPLING DATES : 1 = 31.03.78 2 = 02.05.78 3 = 22.11.78 4 = 22.02.79
5 = 20.06.79 6 = 22.10.79 7 = 17.01.80 8 = 24.06.80

TABLE 1. Evolution of the physico-chemical parameters.

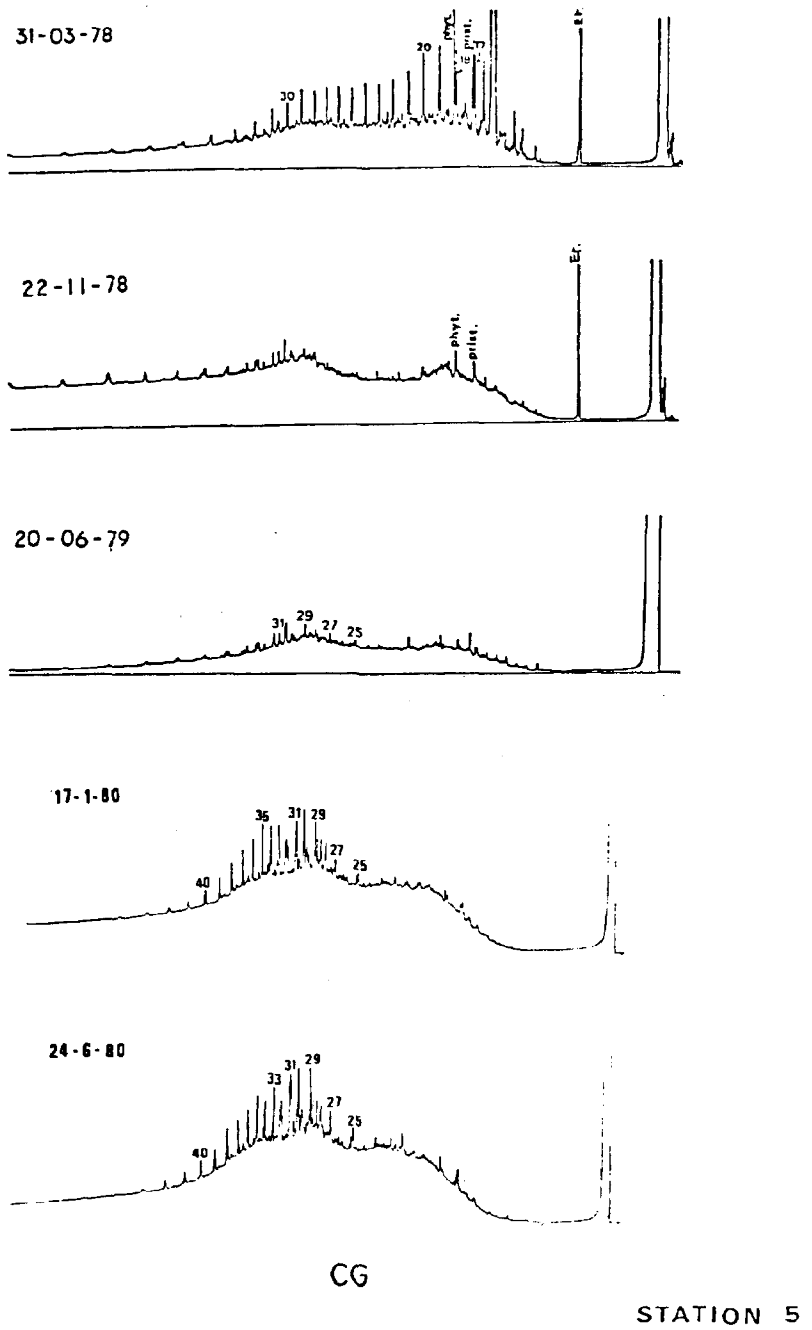


FIGURE 7. Evolution of the saturated hydrocarbons.

sterane compounds (CnH2n-6) eluted between n-C26 and n-C31, and m/e 191 characteristic of pentacyclic triterpane compounds (CnH2n-8) eluted over n-C30 (Fig. 8 and 9). This relative enrichment in cycloparaffins is confirmed by the mass spectrometry analysis of the fraction (Hood and O'Neal - 1958). Among the saturated hydrocarbons, (n+iso)-alkanes show a rapid diminution after March 31, 1978, and a stabilization thereafter ; cycloparaffins, principally 2-, 3- and 4-membered rings show an enrichment, and 5- and 6-membered rings are fairly stabilized (Fig. 10).

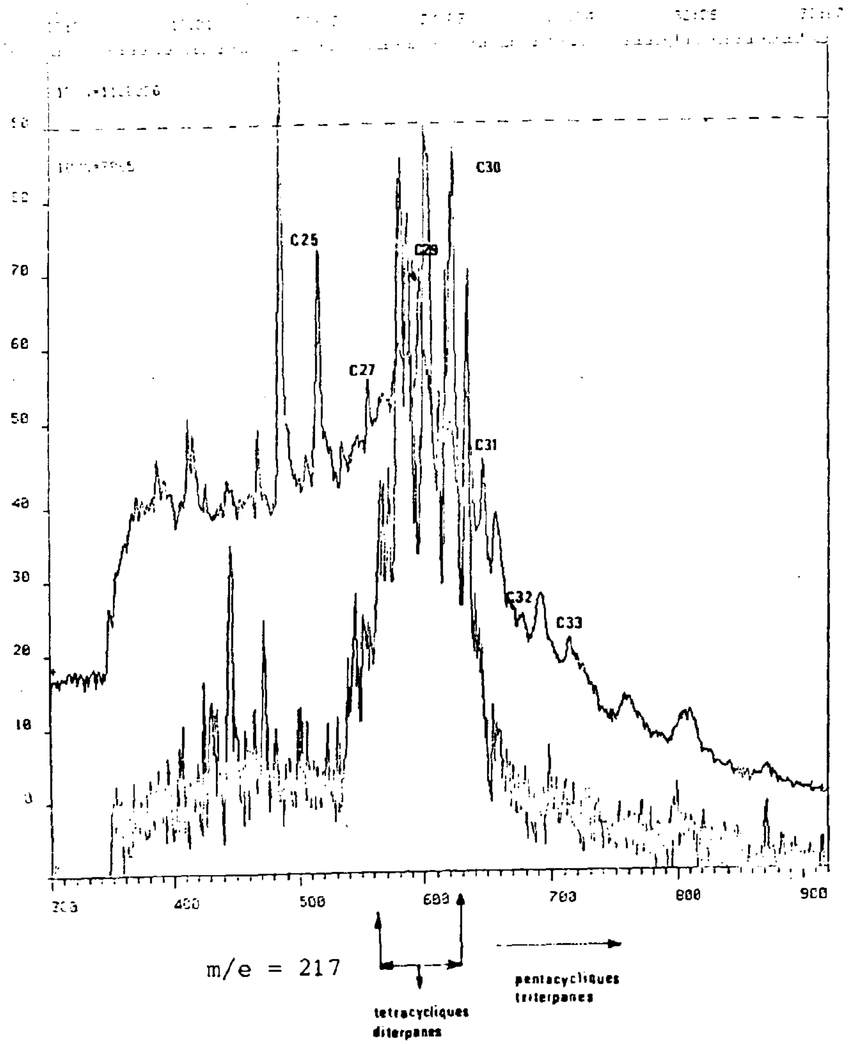


FIGURE 8. Combination of gas-phase chromatography and mass spectrometry of a saturated hydrocarbon fraction.

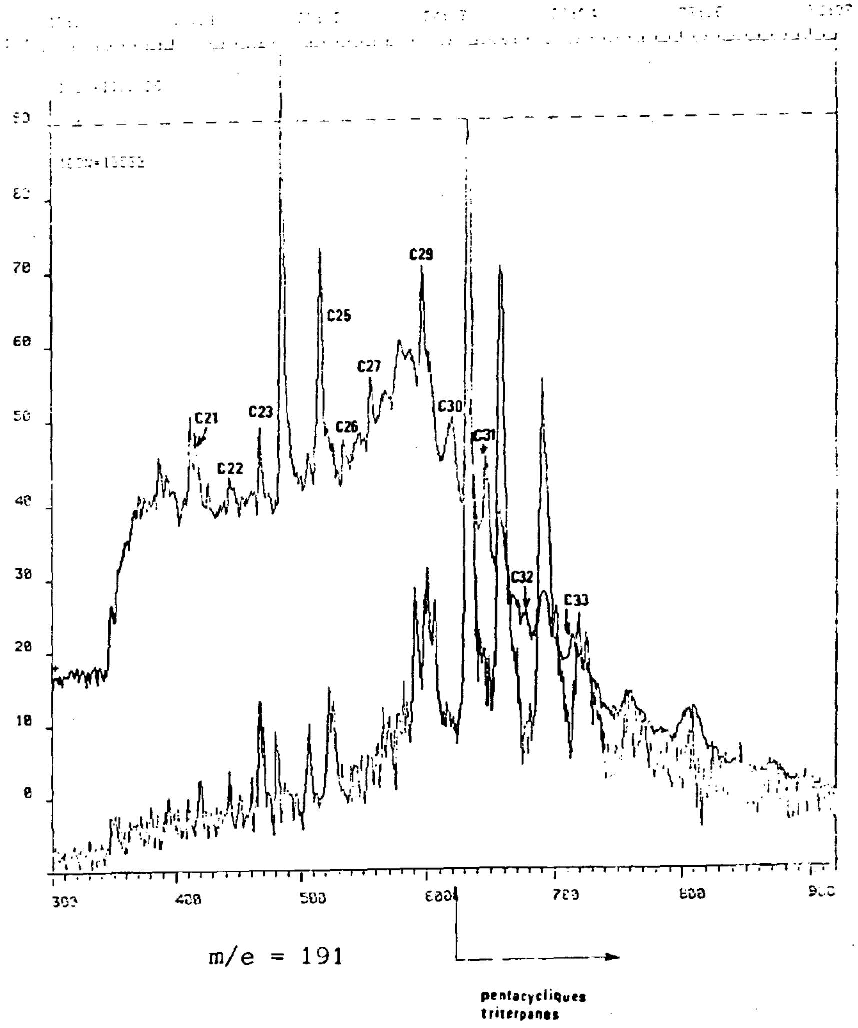


FIGURE 9. Combination of gas-phase chromatography and mass spectrometry of a saturated hydrocarbon fraction.

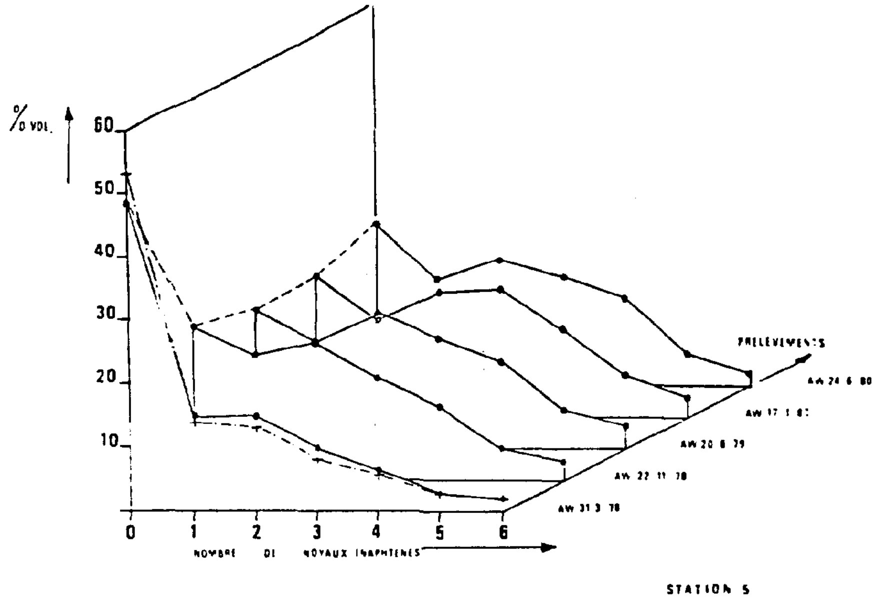


FIGURE 10. Distribution of cycloparaffins by mass spectrometry.

In the aromatic fraction, chromatographic profiles showed :

- a loss in light hydrocarbons as from March 31, 1978 ;
- a positive response in flame photometric detector (FPD), specific sulfur detector, throughout the period studied, which is significant, given the presence of sulfurous aromatic hydrocarbons (thiophenics), and indicative of extensive continual pollution (Fig. 11) ;
- a persistence of dibenzothiophene despite a decrease until June 1980 ;
- an unresolved complex mixture similar to that of the saturated hydrocarbons.

Metal (nickel and vanadium) and sulfur contents assessed through various samplings (Table I) confirm the persistence of the pollution, and do not indicate any significant evolution. The divergences observed may be attributed to variations in concentration of the two crudes present in the polluting mixture. It is to be noted that it is not possible to date to establish the cause of a relatively high peak at the level of the n-C16 in some saturated hydrocarbon chromatogram. A similar peak appeared at Station 6.

Station 6

Decontamination is virtually nil here. In June 1979 A maximum of over 6,000 mg of hydrocarbons/kg of sediment was registered ; by June 1980 hydrocarbon contents were still over 1,200 mg of hydrocarbons/kg of sediment (Fig. 4). This is true of all stations where the marine character of the environment is least pronounced -- that is, in the upper reaches of the Aber.

As can be generally observed in the stations, the most striking evolution is the degradation of the n-alkanes to n-C30. They appear to evolve very rapidly, since the very first sampling taken at this station on March 31, 1978 indicated that the ratios of pristane/n-C17,

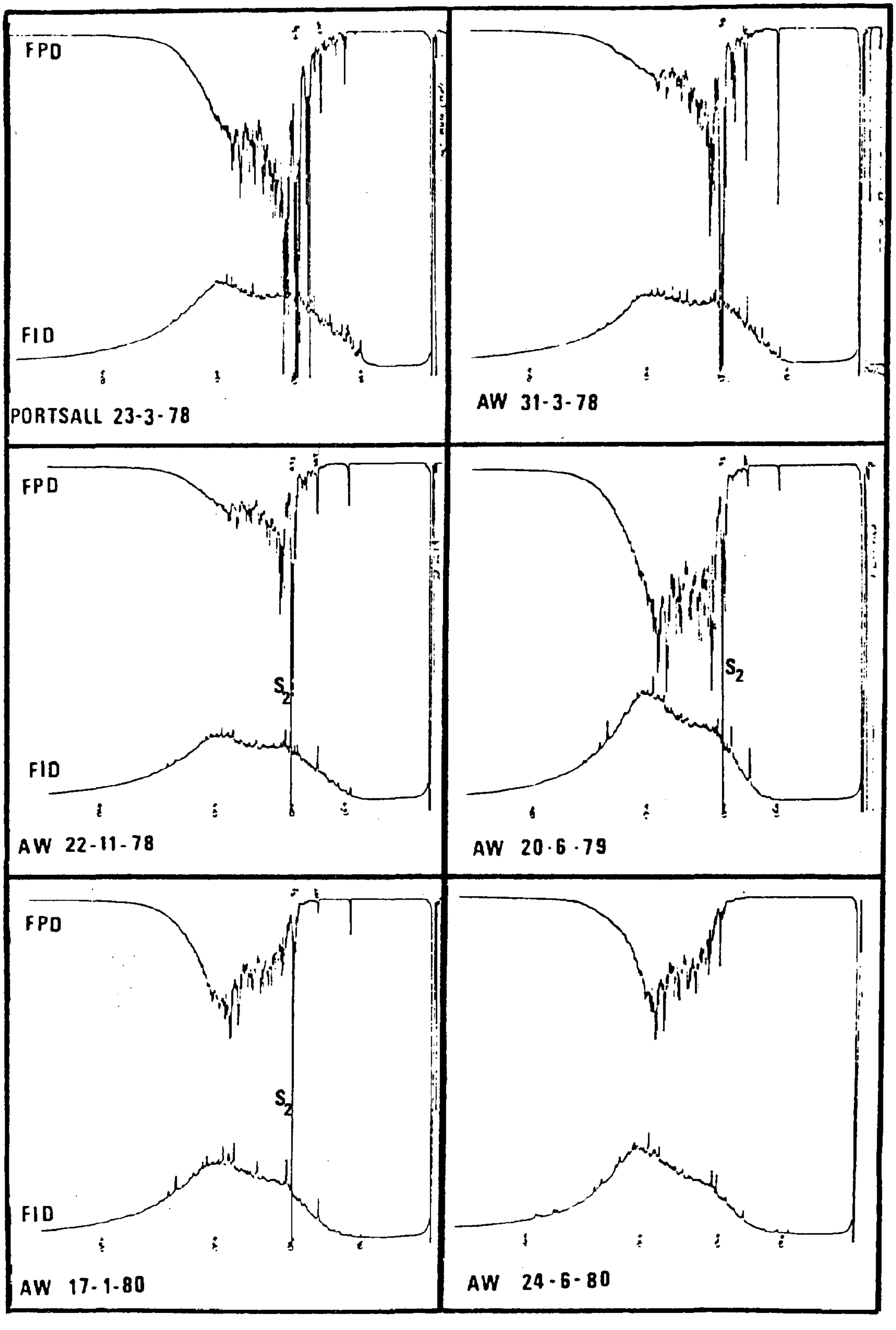


FIGURE 11. Evolution of aromatic hydrocarbons.

and phytane/n-C18 were among the highest and that this degradation was among the most advanced (Table I). In figure 5, a continual decrease in saturated hydrocarbons is noted over time (33 % on March 31, 1978 -- 22 % on June 24, 1980). In spite of a maximum aromatic hudrocarbons content observed in June 1979, they underwent the same type of evolution (34.3 % on March 31, 1978 → 24.8 % on June 24, 1980). The result is an increase in the relative contents of resins ; but it should be noted that in March 1978, polar compound contents (24.6 %) were higher at this station, which is located in the outer part of the Aber, than at Station 5 (17.2 %) where the marine character is more pronounced. This level held true until June 1979, when the increase became the same as at Station 5.

This phenomenon can be explained by the presence of polar compounds of terrestrial origin deposited by the river which flows into the Aber. This contribution of autochthonous organic matter is seen in the presence of n-alkanes of odd carbon numbers (n-C25, 27, 29, 31, 33) in the aliphatic fraction, all the more accentuated by the significant acceleration in the degradation of the normal fossil paraffins (Fig. 12) The result is a relative enrichment in cyclic satured hydrocarbons (cycloparaffins) -- particularly of 3-, 4-, and 5-membered rings around n-C30 -- which are less easily degradable.

Chromatogram profiles of the aromatic hydrocarbons indicate how polluted the station is, principally in the FDP response (Fig. 13), where the persistence of dibenzothiophene and its alkylate derivatives (and also of naphthobenzothiophenes) may be noted. The stability of these compounds made it possible for us to use these chromatograms as the "fingerprints" of the pollution.

As a matter of fact, according to the DGMK report, biogenic hydrocarbons of terrestrial origin (recent sediments) are poor in aromatic compounds (< 5 %), and particularly low in sulfurous compounds of the thiophenic type.

The evolutions observed in the resins are similar to those at Station 5 ; an oxidizing degradation increases the oxygen contents (8.5 % in June 1980), and an upward curve is noted in the infra-red absorption bands of the hydroxyls and carbonyls, the latter of which are predominantly carboxylic acid compounds (Fig. 14). Metal and sulfur contents remain fairly constant over time.

Station 8

This station was distinguished in being the farthest from the sea, in an area of sandy mud. Hydrocarbon contents show that the decontamination process at this station was virtually nil (Fig. 4).

An anomaly is noted in January 1980, when the hydrocarbon contents went over 5,000 mg/kg of sediment. As we shall see below, this is due to pollution from petroleum cuts or fuel oil which was later deposited on top of the pollution under study (Fig. 15 and 16). Althrough information is lacking on some samples, which were too small, it can be seen that metal and sulfur contents remained very stable over time.

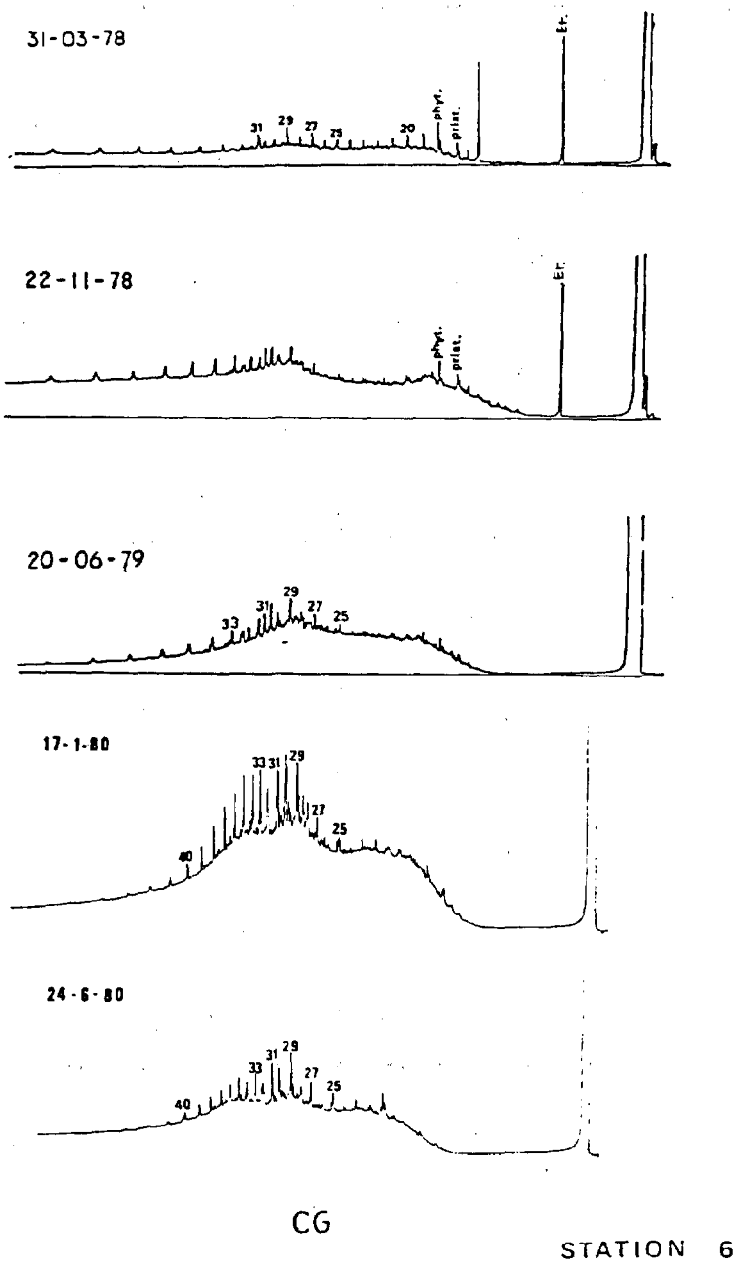
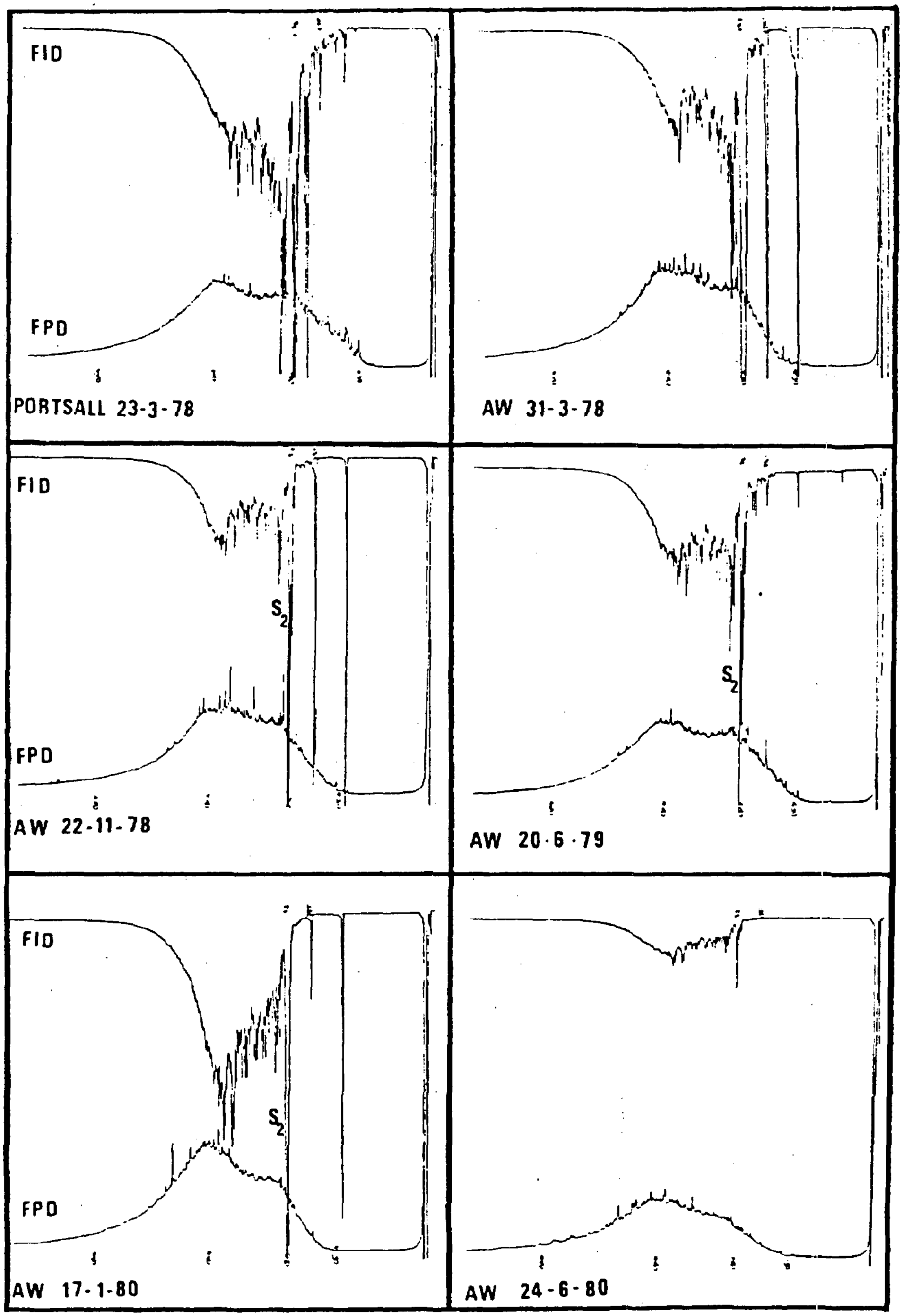


FIGURE 12. Evolution of the saturated hydrocarbons.

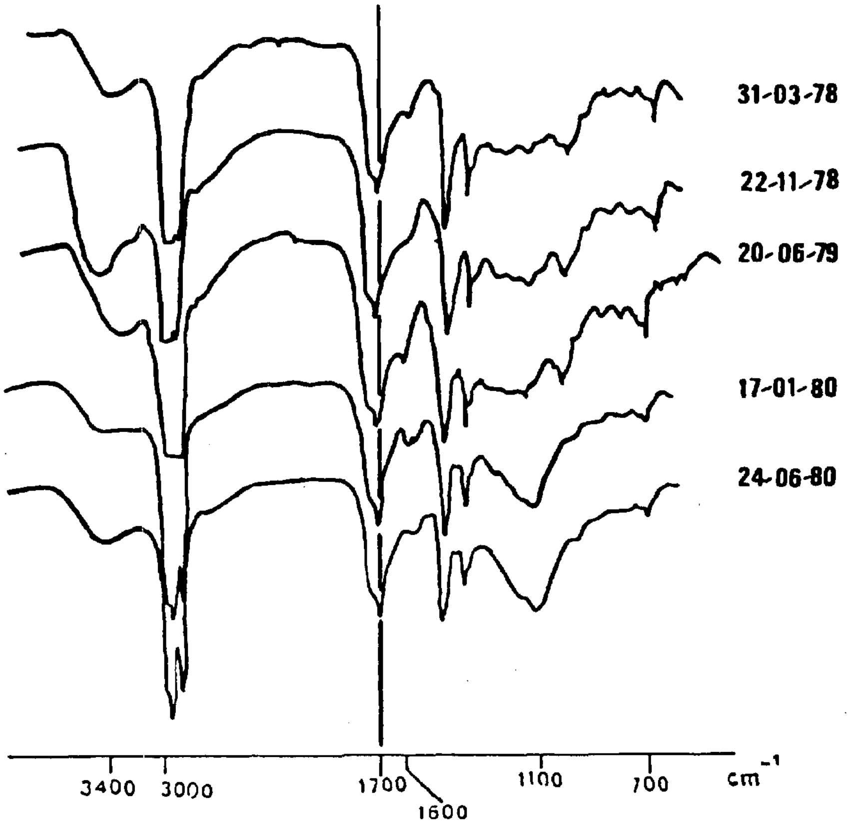
Examination of the chromatograms of the "saturated" fractions shows a quantity of biogenic hydrocarbons of terrestrial origin which is not negligible, and which is characteristic of the upstream stations on the Aber which receive large quantities of deposits from the soils. The distribution of n-alkanes is in fact typical of that observed in the extracts from recent sediments in the predominance of odd-carbon-numbered n-alkanes from n-C25 through n-C35 (Fig. 16).

These quantities of biogenic hydrocarbons present in the aliphatic hydrocarbons are demonstrated in the ratio R29-31 developed



STATION 6

FIGURE 13. Evolution of aromatic hydrocarbons.



STATION 6

FIGURE 14. Infra-red spectrometry of the resins.

by Tissot et al ; they were calculated for the earliest samples only :

$$R\ 29\text{-}31 = \frac{2(C29 + C31)}{C28 + 2C30 + C32}$$

The relationship shows that in this case, the n-C29 and n-C31 predominate. Where the value is over 1, the odd-numbered carbons predominate. In Table 2, a compilation of these values for all the stations, it is seen that R is much higher than 1 at Stations 6 and 8.

Even in March 1978, this presence of natural compounds modified the distribution of hydrocarbons by family :

- saturated hydrocarbon contents were low ;
- polar-compound contents (resins and asphaltenes) high.

While the ratio of SAT/ARO decreased slightly, as at Stations 5 and 6 (Fig. 17), degradation of n-alkanes and isoprenoids (pristane and phytane) was observed throughout the period, showing clearly which polycyclic alkanes are most resistant to degradation.

The chromatograms of the aromatic hydrocarbons have very marked profiles under photometric detection (FPD), and a persistence in the unresolved complex mixture (FID) (Fig. 16). Comparison of the results of the GPC with those of the high resolution MS undertaken on the reference samples of March 23, 1981 at Portsall, and the sampling taken

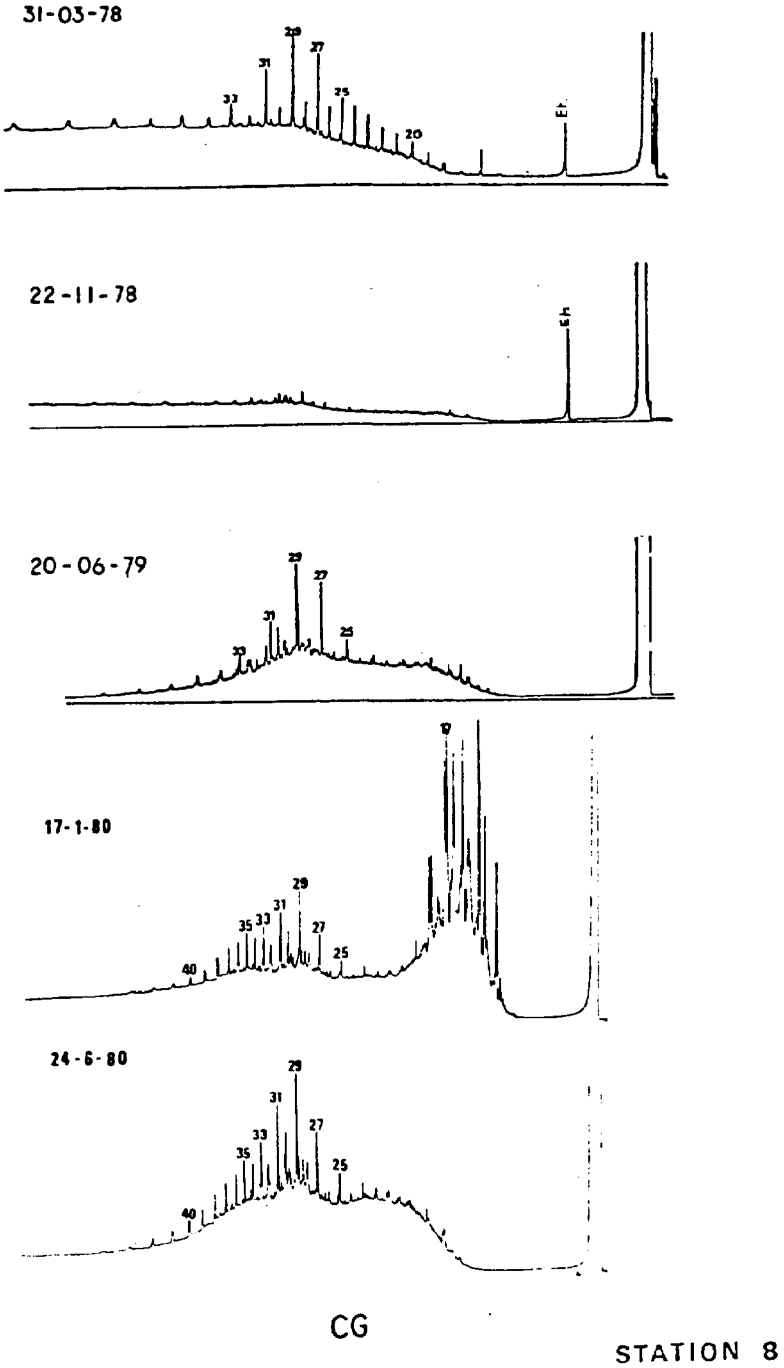
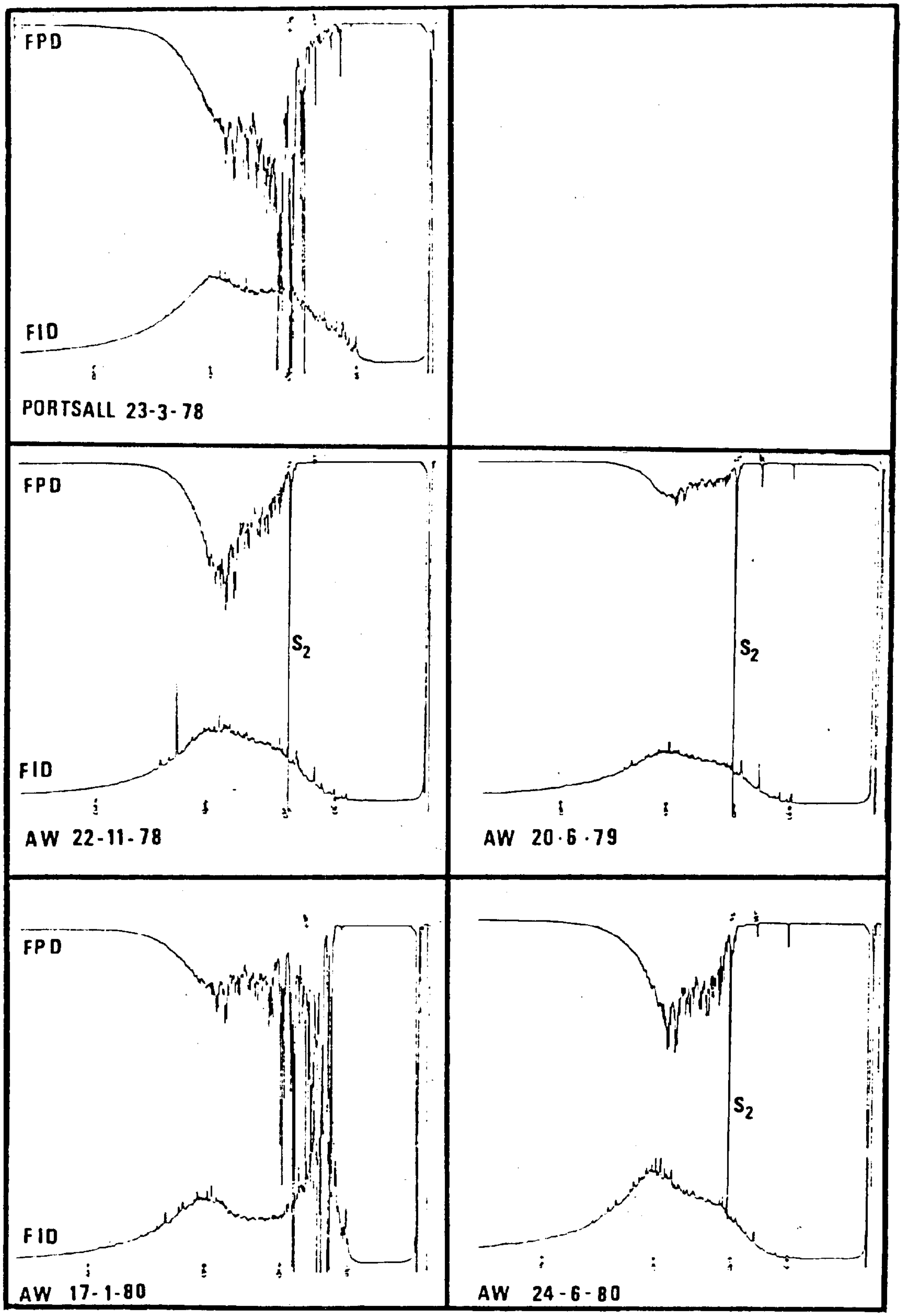


FIGURE 15. Evolution of the saturated hydrocarbons.

on June 24, 1980 at this station, shows a degradation (or dissolution) of the alkylated cycloparaffins to C3 and C4. Concerning the thiophenic derivatives, some benzothiophenic alkyls disappear to become C5, while the initial alkylate derivatives of dibenzothiophene become C3 ; the naphthobenzothiophenes persisted.

In the sampling taken in January 1980, an abnormally high response is noted -- by Flame Ionization Detection (FID) and by Flame



STATION 8

FIGURE 16. Evolution of aromatic hydrocarbons.

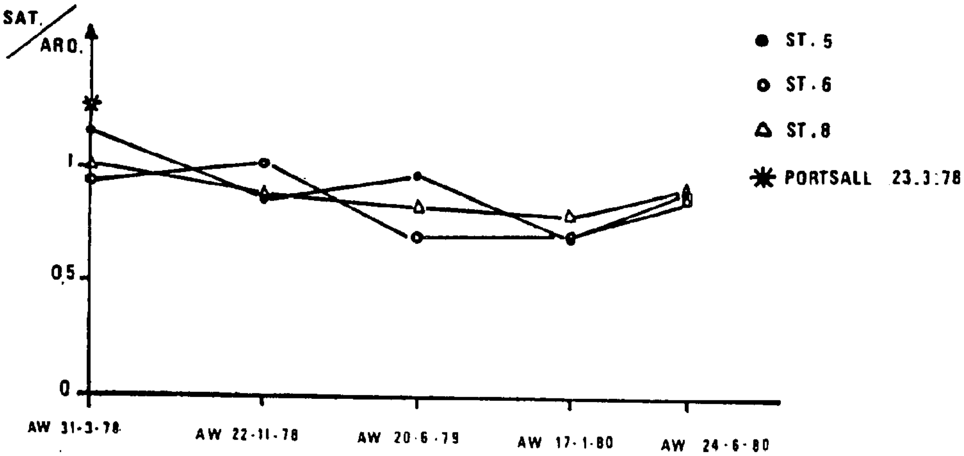


FIGURE 17. Evolution of the ratio of saturated hydrocarbons to aromatics.

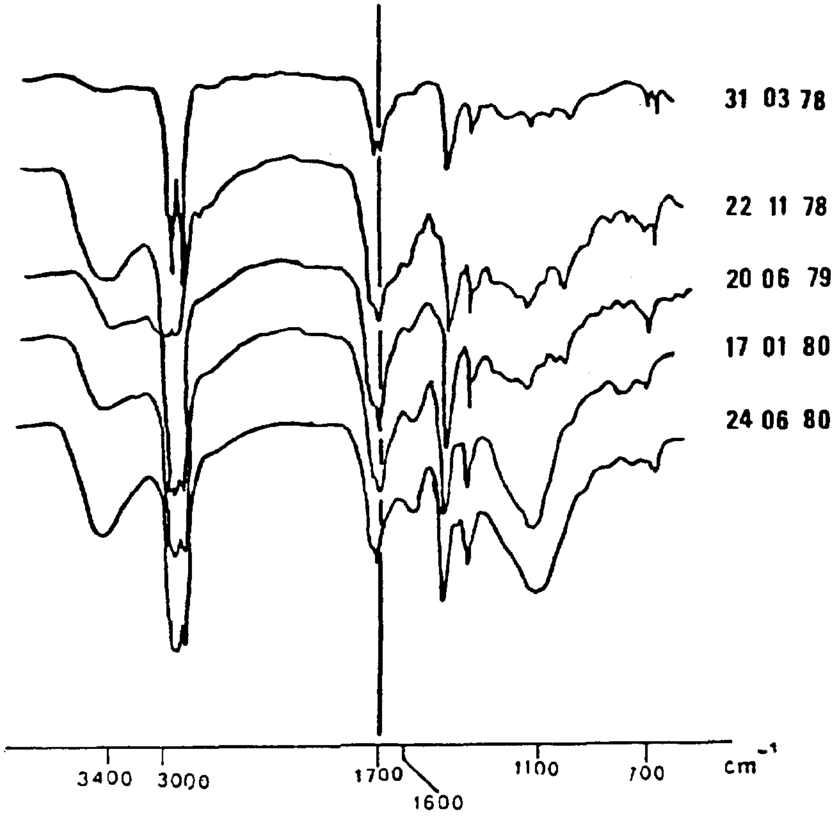
TABLE 2. Values of the R29-31 ratio.

$$\text{Valeurs de } R_{29-31} = \frac{2\,(C_{29} + C_{31})}{C_{28} + 2C_{30} + C_{32}}$$

Station	31.03.78	2.05.78
1	1,02	1,23
2	1,13	-
3	1,10	1,05
4	1,36	1,07
5	1,23	1,22
6	2,34	1,35
7	1,26	1,59
8	4,00	2,78
9	1,06	1,12

Photometry Detection (FPD) as well. This is caused by the presence of a light cut (gasoline, fuel oil, etc...) deposited on top of the Amoco Cadiz pollution (Fig. 16).

Here again the infra-red resin spectrum shows an increase in absorption of hydroxyls and the transformation of esters to acids (Fig. 18). The oxygen content of these polar compounds (7.4-7.5 %) confirms the degradation by oxidation.



Station 8

FIGURE 18. Infra-red spectrometry of the resins.

Stable Hydrocarbon/Water Emulsions or "Chocolate Mousse"

When petroleum spreads over a marine environment, the movement of the waves, the wind, and the currents causes a very rapid emulsion with the sea water which is called "chocolate mousse". These stable emulsions are constituted by dispersing sea water in the hydrocarbons (inverse emulsions). This type of emulsion was sampled during the first month after the accident (Fig. 19, Table 3).

A program of analysis which is different from that used for the sediments and sands was employed (Fig. 20). After purifying the emulsions (of sand, algae, etc...), they were distilled to measure the water contents of those emulsions with boiling points below and those above 340°C. This method is described by Pelet and Castex. The results are assembled in Table 4.

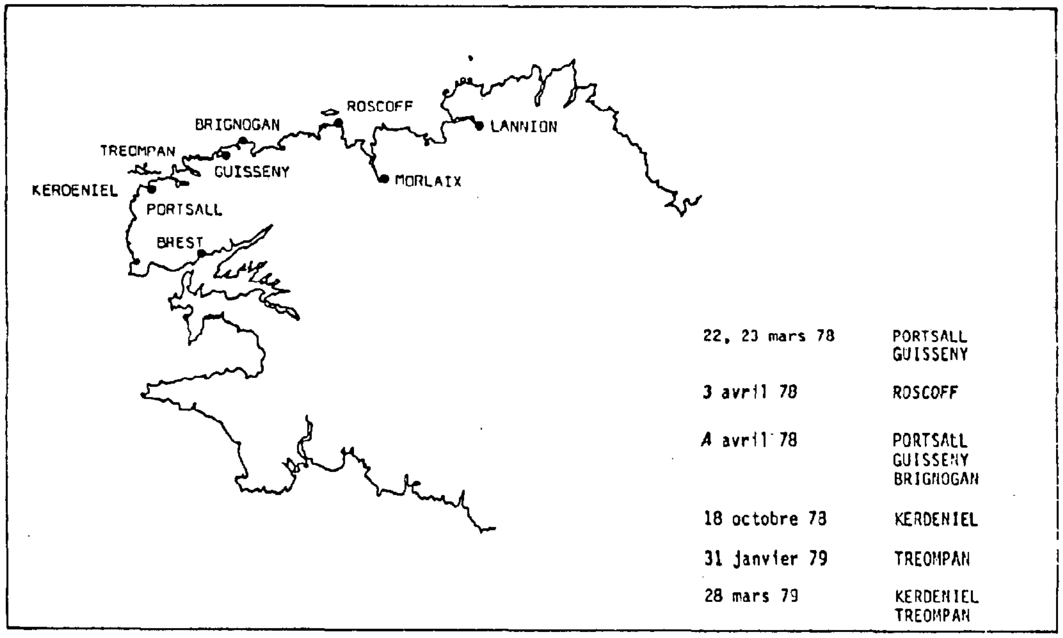


FIGURE 19. Sampling stations.

TABLE 3. Characteristics of the samples.

Lieux	Dates	Caractéristiques des prélèvements
PORTSALL	22.03.78	Emulsion ("mousse au chocolat") déposée sur sable et rochers, recueillis à la limite des eaux libres.
PORTSALL	23.03.78	
GUISSENY	23.03.78	
ROSCOFF	3.04.78	
PORTSALL	4.04.78	
GUISSENY	4.04.78	
BRIGNOGAN	4.04.78	Emulsion déposée sur des rochers, sans trou d'eau à la limite des points hauts de marée.
KERDENIEL (Prof. = 35 cm)	18.10.78	Sables de plages pollués
TREOMPAN	31.01.79	
TREOMPAN	28.03.79	
KERDENIEL	28.03.79	
KERDENIEL (Prof. = 25 cm)	28.03.79	

Moreover, on these fractions a simulated distillation curve was plotted (TBP or True Boiling Point) by gas phase chromatography according to the method of Petroff et al. (1981).

Results of the distillation (Table 4) and the TBP on the PI-340°C (Table 5) indicate a loss by evaporation and dissolution of about 7-8 % of the light hydrocarbons between March 22, 1978 and April 4, 1978.

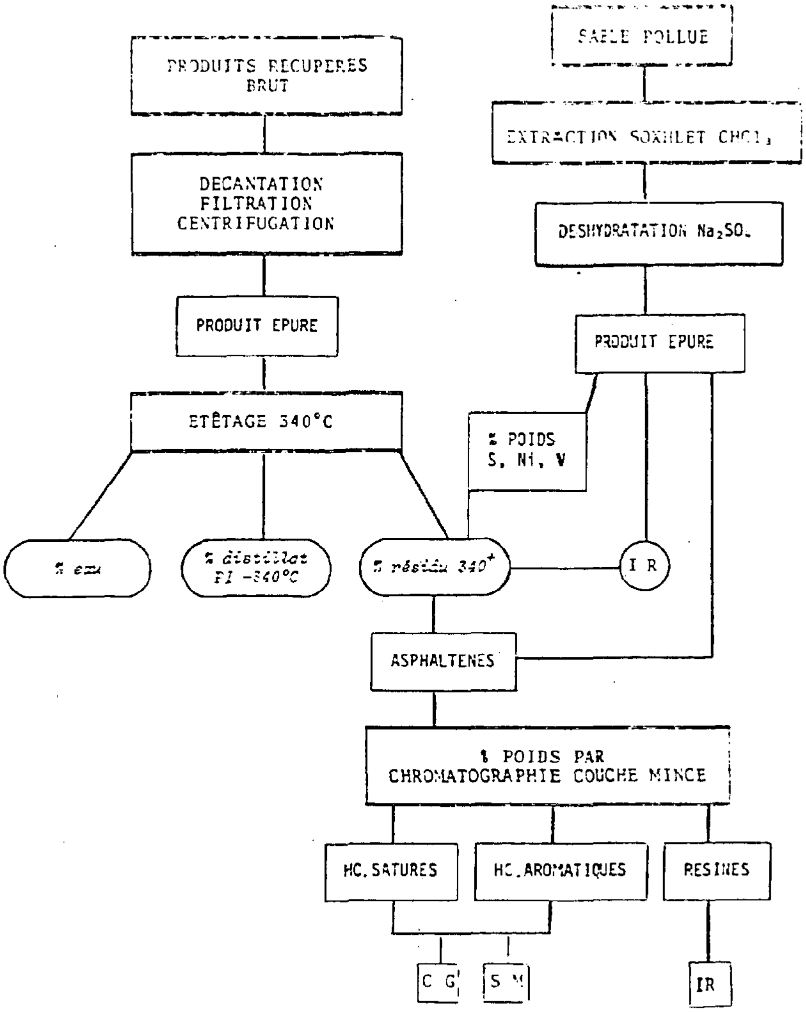


FIGURE 20. The program of analysis.

TABLE 4. Results of distillations of samples of emulsified crude.

Prélèvements / Teneurs	PORTSALL	PORTSALL	GUISSENY	ROSCOFF	PORTSALL	GUISSENY	BRIGNOGAN
	22 mars 78	23 mars 1978		3 avril 78	4 avril 1978		
% poids eau dans émulsion	71,7	68,7	67,7	51	60	57	57
% poids HC PE < 340°C	24,3	20,0	8,9	13,15	15,95	13,05	8,7
% poids HC PE > 340°C	75,7	80,0	91,1	84,05	86,95	91,3	86,10

Two samples (Guisseny, March 23, 78, and Brignogan, April 4, 1978), lost considerably more. The latter was initially the point of highest contents. Its chromatographic profile (TBP) is also very different from that of the samples taken at Portsall on March 22 and 23 (Fig. 21).

TABLE 5. Simulated distillations of PI-340°C.

% poids cumulés	Températures °C						
	PORTSALL 22.3.	PORTSALL 23.3	GUISSENY 23.3	PORTSALL 4.4	GUISSENY 4.4	ROSCOFF 3.4	BRIGNOGAN 4.4
1	162,2	199,1	223,6	216,3	210,2	227,3	239,2
5	193,4	218,6	248,2	233,4	229,7	245,5	172,9
10	207,8	228,7	260,8	246,7	245,9	257,8	285,9
20	224,6	245,0	273,8	264,2	266,2	271,4	300,5
30	237,0	256,1	285,9	275,8	279,9	284,8	310,5
40	251,4	271,7	292,4	286,3	290,7	292,9	319,6
50	267,9	281,7	300,6	294,7	300,9	301,6	326,5
60	282,7	290,6	306,5	303,5	308,9	308,9	235,0
70	295,2	301,3	317,0	313,2	319,2	318,8	343,6
80	310,6	313,2	326,0	322,9	329,9	328,1	353,9
90	330,6	331,3	343,0	340,7	346,8	343,6	371,0
100	392,1	390,3	400,9	459,8	439,3	422,3	460,0

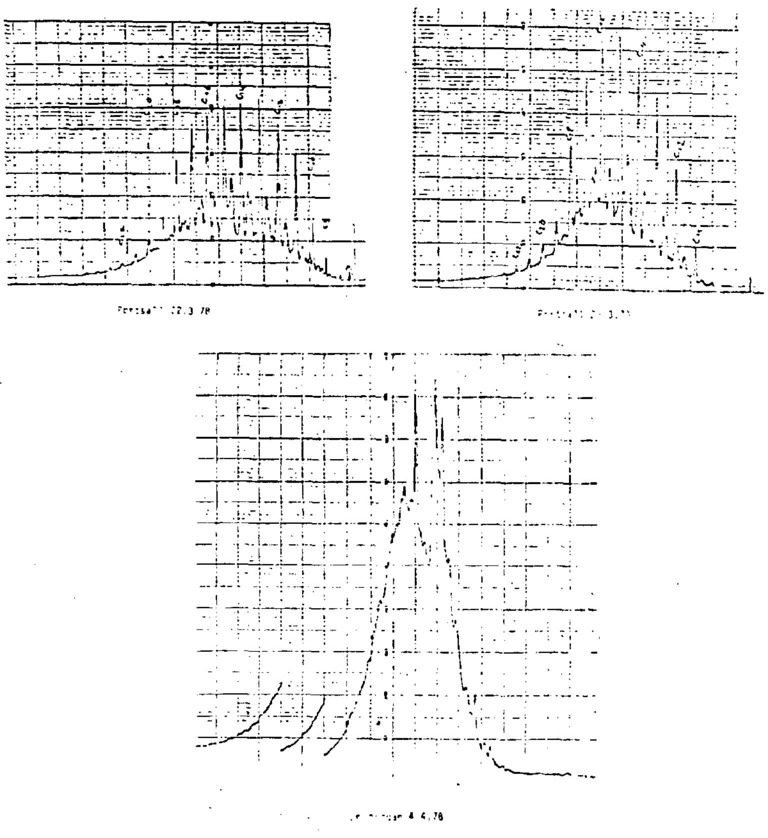


FIGURE 21. "TBP" PI-340°C.

The chromatogram of the saturated hydrocarbons shows that the n-paraffins had disappeared (Fig. 22). Although there are divergences in such parameters as metal and sulfur contents, the infra-red spectrum definitely confirms that it is crude from the Amoco Cadiz. We believe that these differences may be due to the fact that we were dealing with oil sheets that had been treated to a greater or lesser degree. These observations should be compared with those made by Aminot et al. at the same time at a station just offshore from this one, which showed an abnormal loss of dissolved oxygen. He explained it as in-situ biodegradation of the hydrocarbons, which appears to be the only logical explanation.

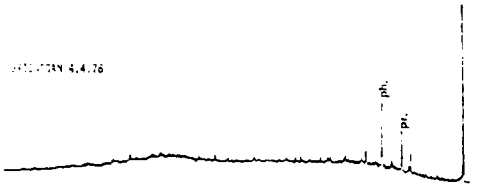


FIGURE 22. Chromatogram of the saturated hydrocarbons taken at the Brignogan station April 4, 1978.

Distillation of the 340+ fractions shows little in the way of interpretable differences (Table 6). The sulfur and metal (nickel and vanadium) contents show, by their stability, how important these compounds are as pollution markers (Table 7).

TABLE 6. Simulated distillations of 340+ residues.

% poids distillés	Températures °C						
	PORTSALL 22.3	PORTSALL 22.3	GUISSENY 22.3	PORTSALL 4.4	GUISSENY 4.4	ROSCOFF 3.4	BRIGNOGAN 4.4
1	291,4	283,8	281,8	305,1	303,2	295,8	266,6
5	346,0	325,2	327,1	338,9	342,9	336,1	318,1
10	370,2	346,9	349,6	359,4	367,9	360,8	342,2
20	408,8	383,3	386,2	394,2	405,3	399,5	386,3
30	446,0	420,3	422,6	431,3	443,8	439,5	421,7
40	484,1	457,5	458,5	463,7	478,3	472,7	458,8
50	526,9	497,8	497,2	499,3	514,6	515,7	503,6
60		543,4	540,2	539,3	558,6		560,1
	54,35 % à 547,3	61 % à 547,5	62,1 % à 548,6	62,3 % à 549,6	61,8 % à 568,6	56,8 % à 548	61,7 % à 569,4

The breakdown by chemical family is shown in Table 8. It appears that the evolution of the crude in all of the emulsions is a slow process. A slight decrease in the ratio of saturated to aromatic

TABLE 7. Sulfur, nickel and vanadium contents.

			S % pds	Ni µg/g	V µg/g	Ni/V
Résidus 340+	PORTSALL	22.03.78	2,33	16,5	62	0,27
	PORTSALL	23.03.78	2,38	14,0	45	0,31
	GUISSENY	23.03.78	2,38	16	50	0,32
	ROSCOFF	3.04.78	2,22	14	48	0,29
	PORTSALL	4.04.78	2,30	16	58	0,28
	GUISSENY	4.04.78	2,18	20	65	0,31
	BRIGNOGAN	4.04.78	2,30	22	68	0,32

TABLE 8. Evolution by chemical family.

	Familles Prélèvements		% pds H.C. saturés	% pds HC aromat.	% pds Résines	% pds Asphaltènes	SAT/AROS.
Résidus 340+	PORTSALL	22.03.78	38,06	35,66	21,71	4,57	1,06
	PORTSALL	23.03.78	37,28	34,12	24,28	4,32	1,09
	GUISSENY	23.03.78	41,69	34,11	16,40	7,80	1,22
Brut total	PORTSALL	22.03.78	47,65	31,29	16,77 (21,05)	4,28	1,52
	PORTSALL	23.03.78	45,12	34,55	15,70 (20,30)	4,60	1,30
	GUISSENY	23.03.78	39,45	31	24,90 (29,50)	4,60	1,27
	PORTSALL	4.04.78	46,75	30,12	19,18 (23,13)	3,95	1,55
	GUISSENY	4.04.78	46,38	31,76	17,65 (21,86)	4,21	1,46
	BRIGNOGAN	4.04.78	34,10	31,50	25,60 (34,40)	8,80	1,08
	ROSCOFF	3.04.78	43,69	34,01	16,96 (22,30)	5,34	1,28
Extraits de sables	KERDENIEL (35 cm)	18.10.78	40,00	33,50	19,50 (26,50)	7,00	1,19
	TREOMPAN	31.01.79	33,90	39	19,20 (27,1)	7,90	0,87
	TREOMPAN	28.03.79	31,10	38,90	22,60 (30,0)	7,40	0,80
	KERDENIEL	28.03.79	38,40	36,90	18,10 (24,70)	6,60	1,04
	KERDENIEL (25 cm)	28.03.79	27,60	26,30	35,90 (46,10)	10,20	1,05

hydrocarbons is seen, however, as well as a slight relative increase in the polar compounds. The infra-red spectra of these compounds show, in fact, a slight upward curve in the absorption band of the carbonyls between the end of March and early April 1978 (Fig. 23). These results corroborate those of Roussel and Gautier at Antifer.(1979).

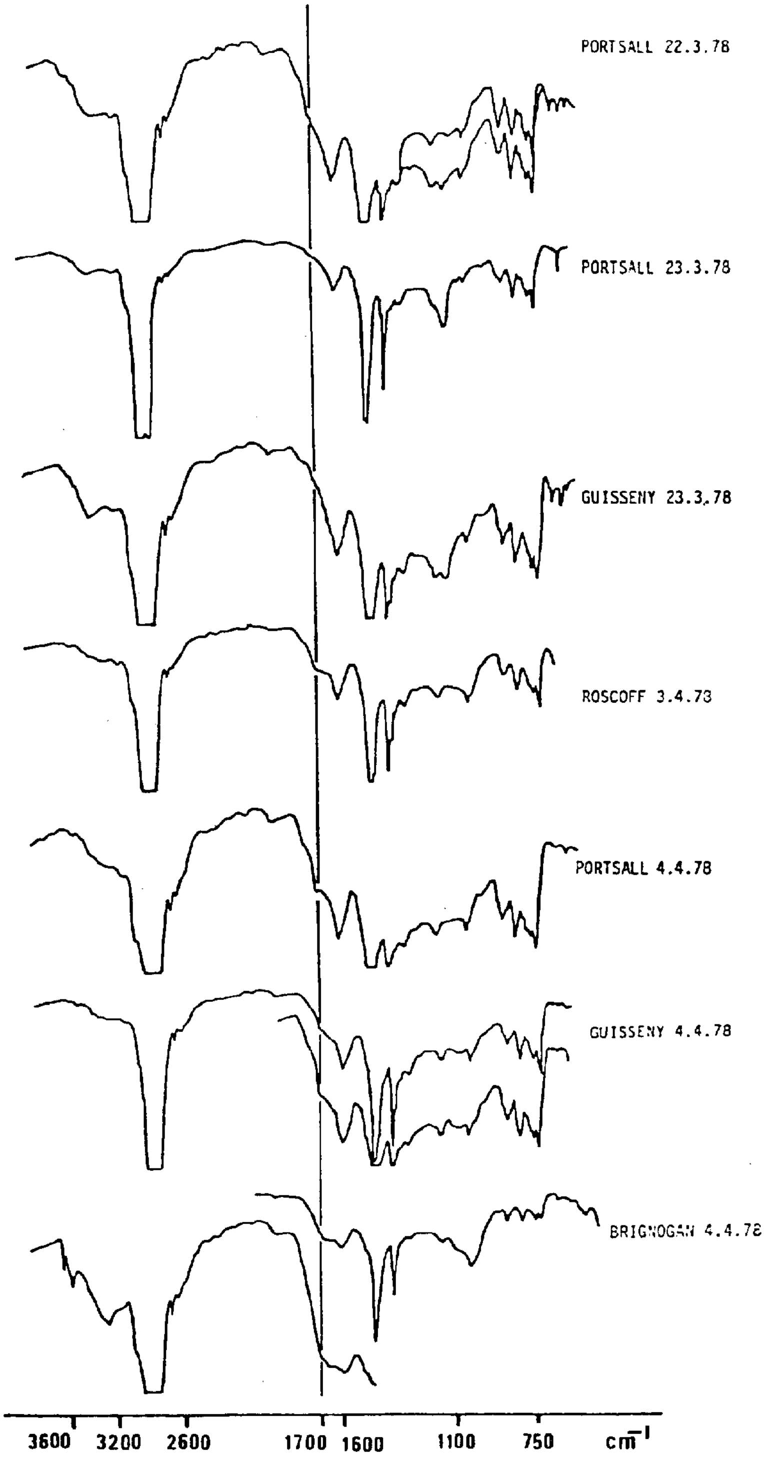


FIGURE 23. Infra-red spectrometry of samples of crude (R 340+) and extracts.

The pathways followed by the crude are shown in the triangular diagram of the saturated and aromatic hydrocarbons, and the polar compounds (resins + asphaltenes) (Fig. 24).

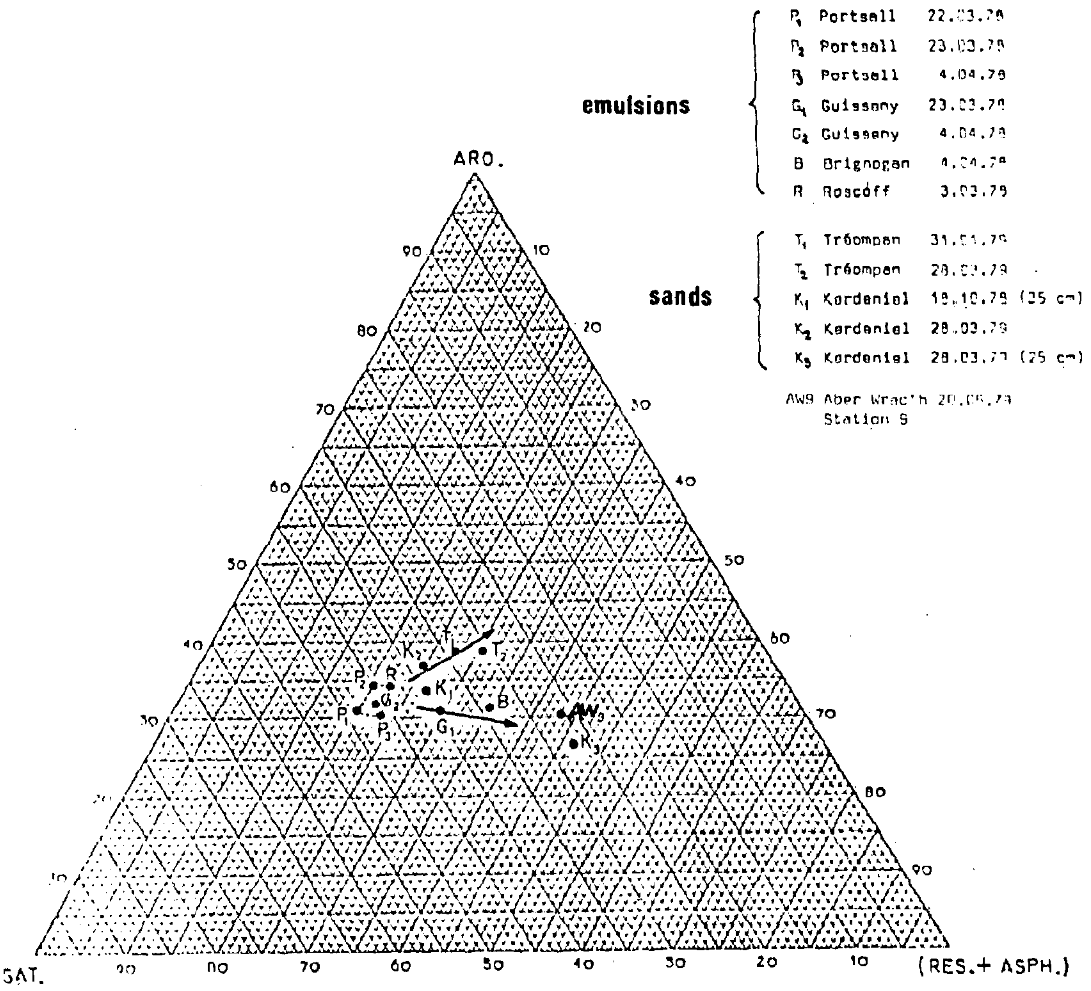


FIGURE 24. Triangular diagram of the distribution by chemical family.

For the sake of comparison, we have added the corresponding values in samples of polluted sands and a subtidal sediment taken in the Aber Wrac'h (AW 9).

Intertidal Sediments - Polluted Beach Sands

The program of analysis employed for the study of these sands is identical to that used for the subtidal sediments of the Aber Wrac'h (Fig. 2). The characteristics of these samples are seen in Table 3.

The sulfur contents of these polluted sands (2.5-2.6 %) are all slightly higher than those of the emulsions -- even the most advanced (2.3-2.4 %), but are about the same as those in samples taken from the Aber Wrac'h. This evolution is explained by the disappearance of certain chemical species which are easily degradable and/or soluble, such as n-alkanes, light aromatics, etc... leaving a higher relative concentration of sulfurous species, which are more resistant to degradation. Metal concentrations (nickel and vanadium), and their ratio, did not vary significantly through the one-year pariod of the study (Table 9). This behavior was noted above in the discussion of the Aber Wrac'h samples.

TABLE 9. Sulfur, nickel and vanadium contents.

			S % pds	Ni µg/g	V µg/g	Ni/V
Ext. $CHCl_3$	KERDENIEL (35 cm)	18.10.78	2,55	14	45	0,31
	TREOMPAN	31.01.79	2,65	17,5	83	0,21
	TREOMPAN	28.03.79	2,66	19	65	0,29
	KERDENIEL	28.03.79	2,69	20	85	0,23
	KERDENIEL (25 cm)	28.03.79	-	18	65	0,28

As we did with the samples of emulsified mousse, as described above (Fig. 24), we recorded the saturated and aromatic hydrocarbons, and the polar compounds (resins and asphaltenes) on the triangular diagram (Table 8).

The most notable evolution took place in the latest samplings of polluted sand. But it is difficult to isolate the factors contributing to evolution in the beach sands : time, extent of dispersion of the crude, how long the oil was on the sea, the support material (sand, mud, rocks). This is all the more true of a sampling taken a year after the catastrophe, which may have undergone a very complex history of burial before being picked up again by the water during a storm or a spring tide. Even with these reservations, however, it seems that the triangular diagram shows that the crude follows several pathways in its evolution :

- a very short and stable pathway, as we saw above in emulsions on free water ;
- a pathway in which a relatively slow disappearance of saturated hydrocarbons (n-alkanes) and aromatics (Mono- and diaromatics) results in a moderate increase in polar products, when the crude is trapped in sand ;
- an evolving pathway followed by crude which is trapped in more or less muddy subtital sediments of the Aber Wrac'h, as demonstrated above.

The chromatograms of the saturated hydrocarbons in the polluted samples taken in 1979 all show a general degradation of n-paraffins to n-C30, confirmed by an increase in the ratios of isoprenoids to n-alkanes (n-C17 and n-C18). This degradation seems slower in polluted beach sands than in the sediments of the Aber Wrac'h.

The mass spectrometry study of the (n+iso) distribution, and that of the 1- to 6-membered rings of cycloparaffins confirms this evolution (Table 10, Fig. 25). But a slight alteration is seen in surface samples (Kerdeniel, March 28, 1979), which may be explained by the reemergence of masses of only slightly degraded crude, which have been trapped in sand, during a storm.

TABLE 10. Distribution of the cycloparaffins by the mass spectrometry.

Prélèvements	% vol. paraffines (n + iso)	% vol de cyclanes à					
		1 cycle	2 cycles	3 cycles	4 cycles	5 cycles	6 cycles
PORTSALL 23.03.78	52,92	14,20	13,07	8,20	5,87	2,81	2,33
GUISSENY 23.03.78	55,66	14,29	13,04	8,05	5,30	2,07	1,59
ROSCOFF 3.04.78	53,13	14,09	13,50	9,17	6,22	2,69	1,19
PORTSALL 4.04.78	47,49	14,44	14,80	10,16	7,46	3,31	2,34
GUISSENY 4.04.78	46,33	16,88	15,78	10,43	7,73	2,29	0,55
BRIGNOGAN 4.04.78	31,90	22,15	19,72	12,24	8,52	3,64	1,83
TREOMPAN 31.01.79	35	17,12	18,13	13,20	9,74	4,26	2,54
TREOMPAN 28.03.79	30,93	17,75	19,42	14,24	10,21	4,59	2,86
KERDENIEL 28.03.79	44,52	15,58	16,17	10,92	7,52	3,11	2,18
KERDENIEL 28.03.79 (25 cm prof.)	31,88	11,23	15,97	16,07	13,99	6,80	4,06

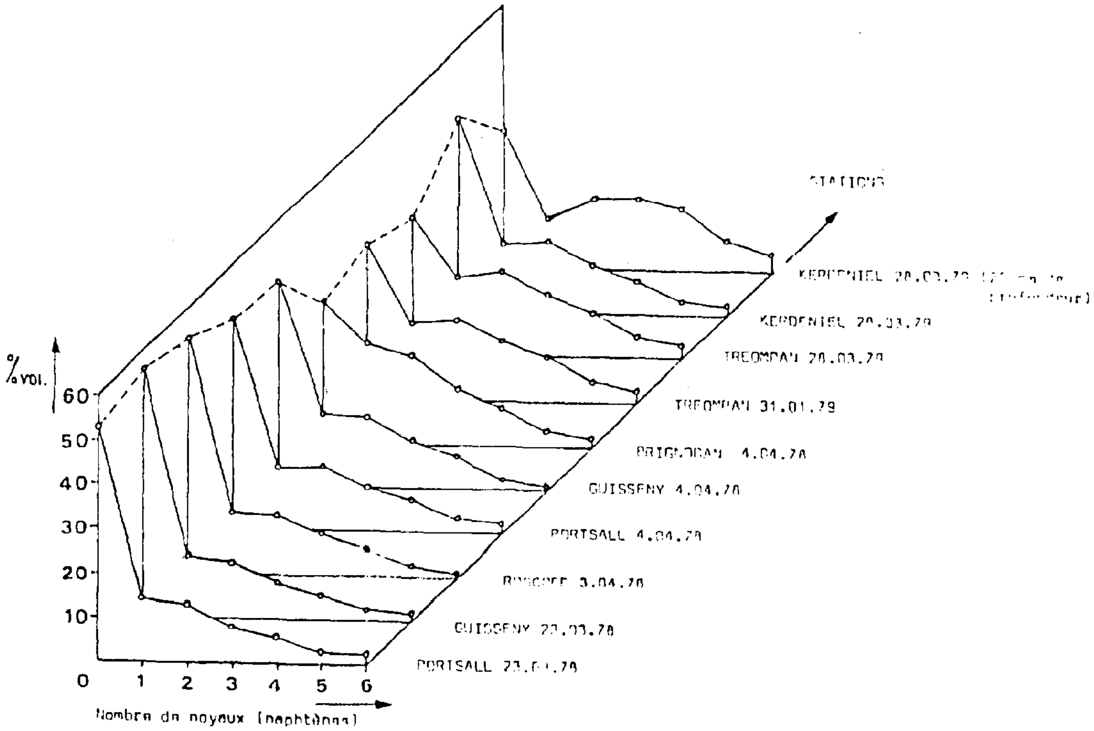


FIGURE 25. Distribution of the cycloparaffins by the mass spectrometry.

It should be noted that a sample taken at the same time and same place, but at a depth of 25 cm, shows an advanced stage of degradation, comparable to that observed in samples from the Aber Wrac'h at the same time.

Compared with these subtidal sediments, oxidation degradation of the trapped crude in beach sands is slight and slow. This is seen in the infra-red spectra of the resins, where the absorption bands of the carbonyls are less marked (Fig. 26).

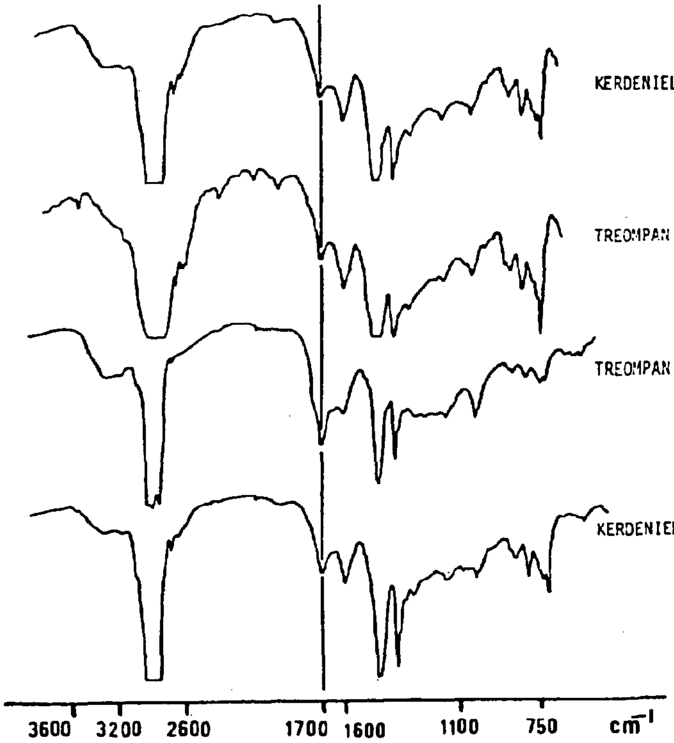


FIGURE 26. Infra-red spectrometry of the resins.

CONCLUSION

The samples studied fall into three categories :

- subtidal sediments (Aber Wrac'h) ;
- oil/water emulsions or "chocolate mousse" ;
- intertidal sediments (beach sands).

In the slightly muddy sediments of fine sand in the stations located in the outer part of the Aber Wrac'h, where the marine character is pronounced, a decrease in global contents of extractable compounds is observed, whereas in those located in the upper part of the Aber, the decontamination process is slow, probably inhibited by the muddy nature of the sediments.

The degradations observed in these sediments results in :

- the progressive disappearance of saturated hydrocarbons, principally the normal paraffins ;
- the disappearance of the light aromatic hydrocarbons ;
- the oxidation of the polar compounds (esters, acids, etc...).

The compounds which persist are :

- the saturated polycyclic hydrocarbons and the heavy aromatics ;
- sulfurous aromatic hydrocarbons of the thiophenic type ;
- resins and asphaltenes, resulting in stable metal (nickel and vanadium) and sulfur contents.

In the sediments samples in the area of the Aber, terrigenic deposits are superposed on the Amoco Cadiz crude, resulting in :

- an increase in polar compounds -- resins and asphaltenes. The increase in asphaltene contents is due to the presence of pigment (green) of chlorophyllaceous origin ;
- the appearance of n-alkanes of odd carbon numbers (n-C25 through n-C33).

The most striking evolution in the "chocolate mousse" samples is the loss of light hydrocarbons due to evaporation and dissolution. Volatile compounds under C14 were not considered in this report.

The samples of polluted sand taken from the beaches a year after the accident show a degradation phenomenon principally affecting the saturated hydrocarbons, and among these, principally the n-paraffins. There is an increase in the contents of polar compounds. But our information is not adequate to state at what point in the history of these samples, the degradation was most intense.

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THE *AMOCO CADIZ* OIL SPILL
DISTRIBUTION AND EVOLUTION OF OIL POLLUTION IN MARINE SEDIMENTS

by

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INTRODUCTION

In March 1978, the supertanker AMOCO CADIZ was stranded on shallow rocks off Portsall (north Brittany), 2.5 km from the coast. Two hundred twenty-three thousand tons of a mixture of Arabian light crude oil (100,000 t) and Iranian light crude oil (123,000 t) flowed into the sea without interruption from 17 March to 30 March. The maximum extent of the oil slicks is presented in Figure 1. At this point, about 360 km of coastline were polluted by the oil.

The analyses of oil in seawater, measured by UV fluorescence spectroscopy (Marchand and Caprais, 1981), revealed that the oil spill

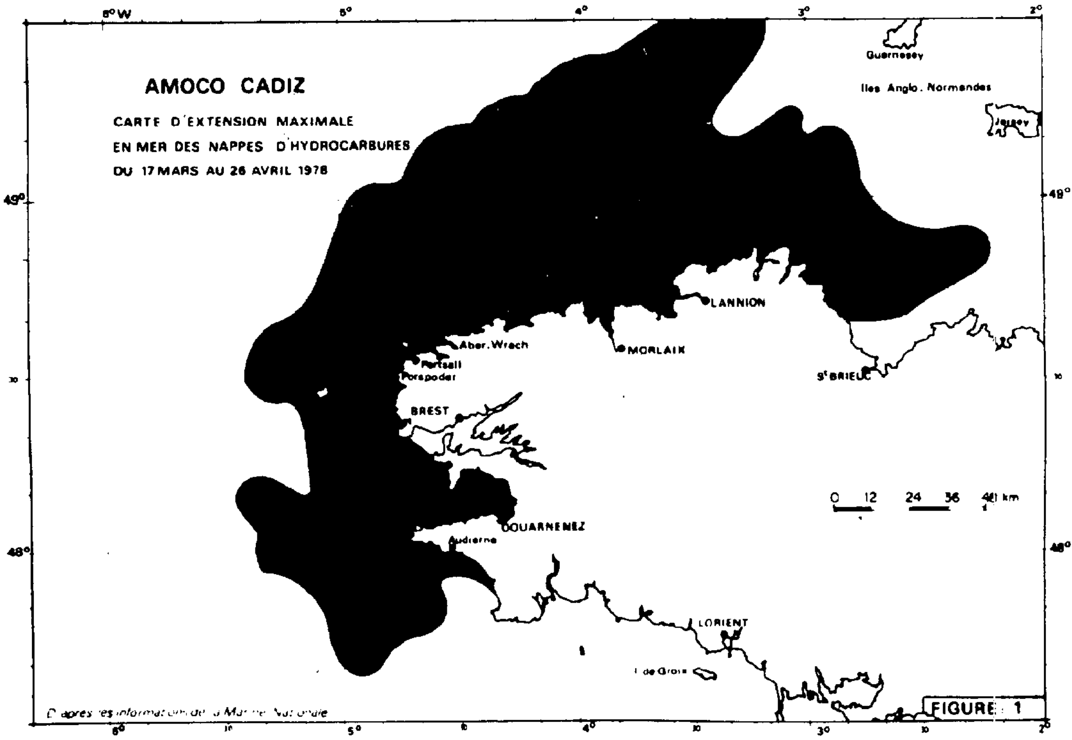

FIGURE 1. Maximum extent of oil slicks on the sea surface, 17 March to 26 April 1978.

affected a very large section of the western English Channel. One month after the AMOCO CADIZ wreck, the most polluted areas were located in the coastal zones, such as the Aber zone (38.9 ± 6.7 µg/l), the Bay of Morlaix (11.5 ± 5.1 µg/l), and the Bay of Lannion (10.7 ± 3.0 µg/l). Analysis of samples from various depths revealed that the contamination extended throughout the water column. The 49°N parallel roughly constituted the northern limit of pollution. Beyond this limit, oil in surface seawater was not observed (1.6 ± 0.5 µg/l). In March 1979, one year after the AMOCO CADIZ stranding, hydrocarbon concentrations returned to a normal level (below 2.0 µg/l); however, some residual traces of pollution were still observed near the Abers and at the bottom of the Bay of Lannion (about 2.0 µg/l).

We also began a chemical follow-up study of oil pollution in marine sediments. Some data have already been presented during the international symposium held in Brest (France) in November 1979 (CNEXO, 1981; Ducreux and Marchand, 1981; Marchand, 1981; Marchand and Caprais, 1981). In this document, results of our study are presented in three parts: (1) oil pollution in sediments collected from the western English Channel one month after the wreck, (2) specific study in the Bays of Morlaix and Lannion to determine the distribution of oil pollution in surface sediments and at various depths, and the evolution of oil contamination over one year, and (3) specific study of the Aber Wrac'h to determine oil evolution from 1978 to 1981.

MATERIAL AND METHODS

Surface marine sediments were collected in the western English Channel with a Shipek grab. In coastal areas, small Ekman and Hamon grabs were used. The samples, after freezer storage, were dried by using a Soxhlet apparatus or by stirring with chloroform. The organic extract was concentrated to dryness, then dissolved with 10 ml of carbon tetrachloride. A first indication of petroleum pollution in sediment was obtained through a direct analysis of nonpurified extracts by IR spectrophotometry (Perkin Elmer 397). Quantitative measurements were carried out at 2920 cm^{-1} corresponding to the presence of hydrocarbons and polar compounds. The data also reflect coextracted natural substances (fats, fatty acids, etc.) from sediments. The IR spectrophotometer was calibrated with a mixture of Arabian and Iranian light crude oils.

Hydrocarbons were analyzed after cleanup of organic extracts on activated Florisil (200°C) in glass columns (15 cm x 0.6 cm i.d.). Hydrocarbons were eluted with 15 ml of carbon tetrachloride and measured by IR spectrophotometry. For some samples, organic carbon was determined with an auto-analyzer LECO WR-12. In a joint study with the French Petroleum Institute concerning the Aber Wrac'h sediments (Ducreux and Marchand, 1981), we compared the gravimetric determinations and the IR spectrophotometric analysis of nonpurified organic extracts. Results of the two methods are similar (Fig. 2). We also compared the IR spectrophotometric results obtained on nonpurified and purified organic extracts from some Aber Wrac'h sediments. In this case, correlation was significant (Fig. 3).

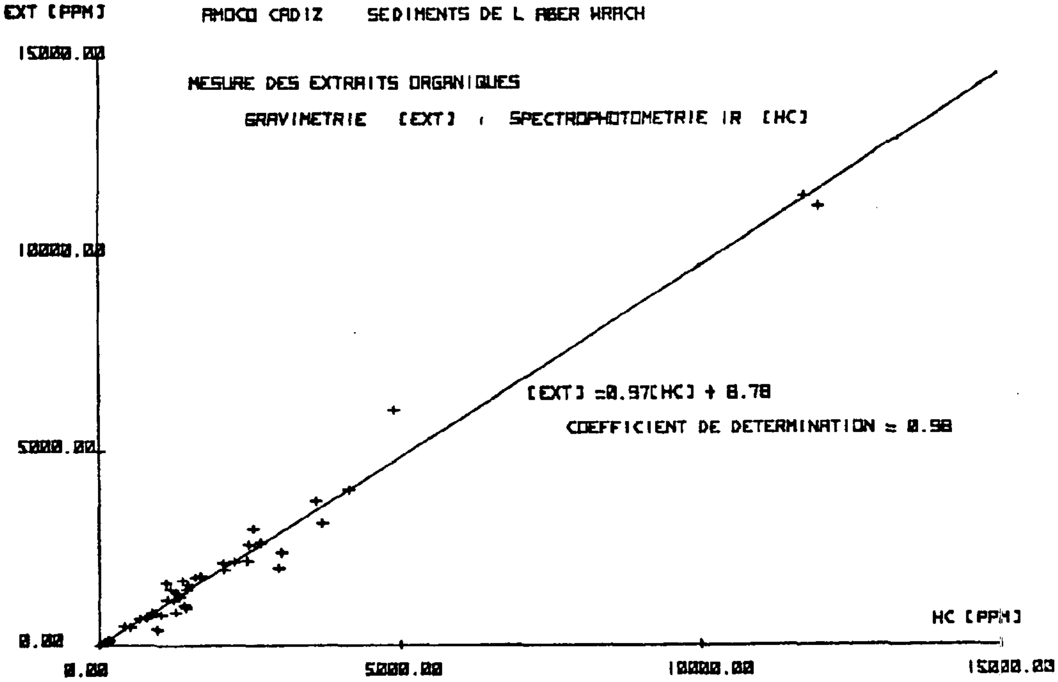

FIGURE 2. Correlation between gravimetric determinations and IR spectrophotometric measurements for organic extracts.

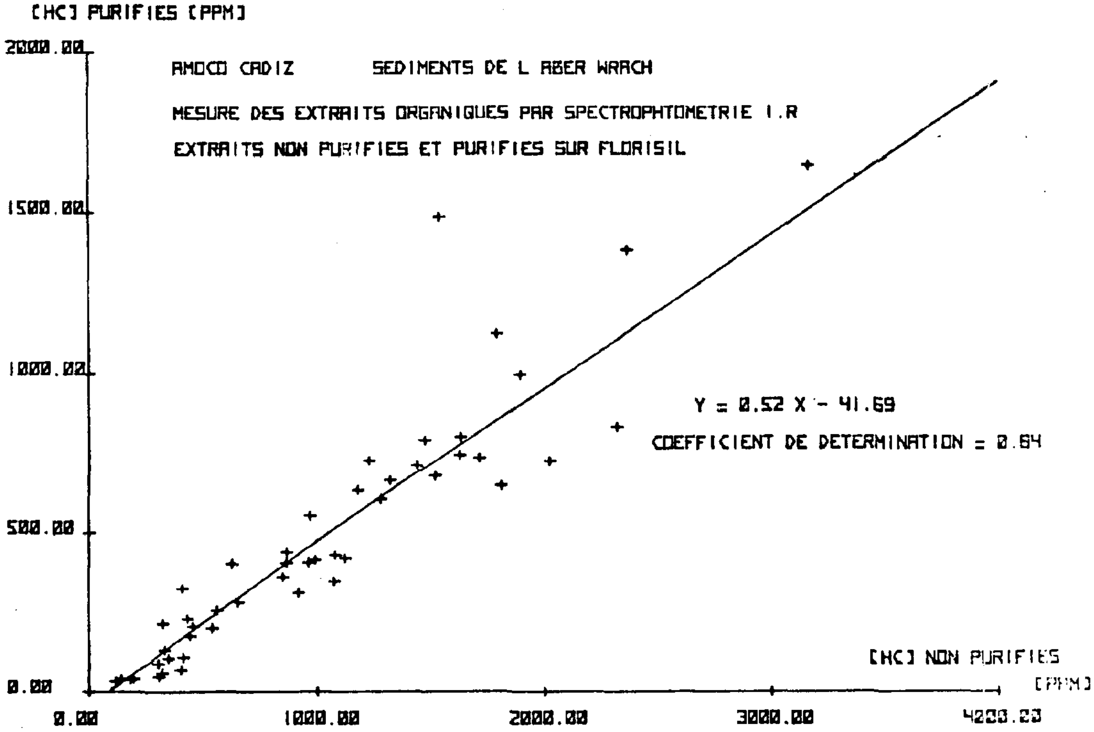

FIGURE 3. Correlation between IR spectrophotometric measurements for nonpurified and purified organic extracts.

OIL POLLUTION IN THE WESTERN ENGLISH CHANNEL (APRIL 1978)

One month after the wreck, sediments were collected during an oceanographic cruise (R/V SUROIT) to assess sea bottom contamination of the western English Channel. The sampled sediments were coarse- to medium-grained calcareous sands (more than 70% $CaCo_3$). In the Bays of Morlaix and Lannion and near the Aber zone, the content of calcium carbonate in the sands was somewhat lower (50-70% $CaCO_3$). Organic carbon content was generally low, from 0.02 to 0.6 percent ($\bar{m}$ = 0.18% ± 0.13%). The oil concentrations in the sediment ranged from 10 to 1,100 ppm (nonpurified organic extracts) (Marchand and Caprais, 1981). Generally, the zone of contaminated sediments reflected offshore and coastal areas impacted by the drifting slicks (Fig. 4). The pollution of the sea bottom was a result of the diffusion of oil into the water column. Off Sept-Iles, a gradient was observed from the coast to the open sea (210, 52, 42, 34 ppm). At the 49°N parallel, from west to east, one could observe an increasing and decreasing gradient (21, 19, 48, 102, 54, 52, 24 ppm). The highest petroleum accumulation in marine sediments were located in the coastal and sheltered zone of the Abers (100 to more than 10,000 ppm) and in the Bays of Morlaix and Lannion (10 to more than 1,500 ppm) (Fig. 5).

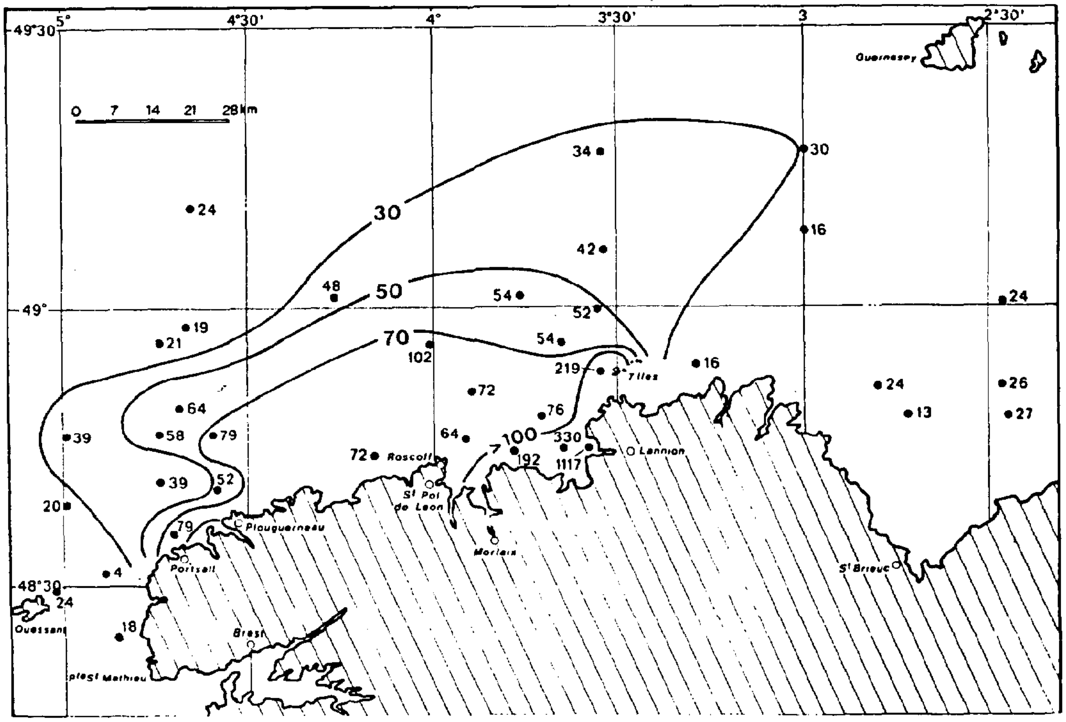

FIGURE 4. Oil pollution in marine sediments (April 1978). Concentrations expressed in ppm.

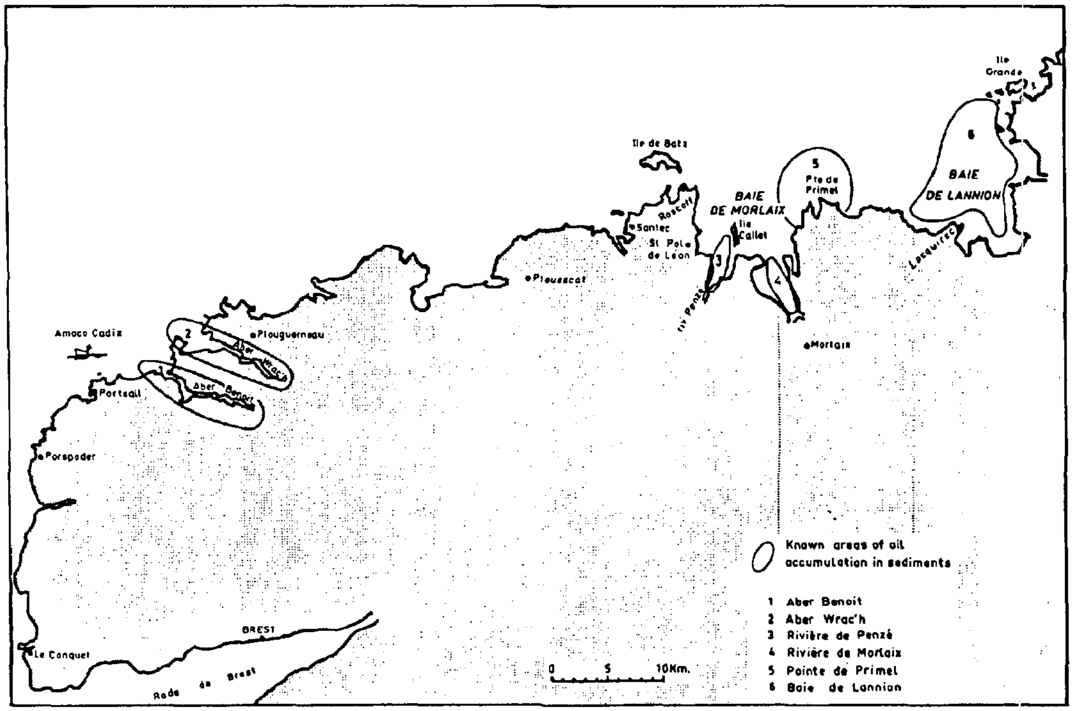

FIGURE 5. Known coastal areas of oil accumulation in sediments.

BAYS OF MORLAIX AND LANNION (JULY 1978 TO FEBRUARY 1979)

A survey was undertaken from July 1978 to February 1979 to follow oil degradation within bottom sediments of the Bays of Morlaix and Lannion. In August 1978, specific sampling of some stations was made by the BRGM (Bureau de Recherche Geologique et Miniere). At these stations (Fig. 6), several-meter-long cores were taken by vibracoring to ascertain the vertical distribution of oil in sediments.

Oil Pollution in Surface Sediments

The sedimentary description of sediments collected from July 1978 to February 1979 is given by Beslier (1981) and Beslier et al. (1981). The weathering of hydrocarbons in some polluted samples was studied by Boehm et al. (1981). In July 1978, the hydrocarbon concentrations (determinations made on purified organic extracts) ranged from 8 ppm to more than 1,500 ppm. Average concentrations are presented in Table 1. Complete data are given by Marchand and Caprais (1981).

We also used the parameter of total hydrocarbons/organic carbon (HC/OC) to demonstrate pollution of surface sediments. This ratio (HC/OC x 10^4) ranged from 48 to 6,065. Marchand and Roucache (1981) showed in a study of another oil spill in Brittany (BOHLEN wreck) that a

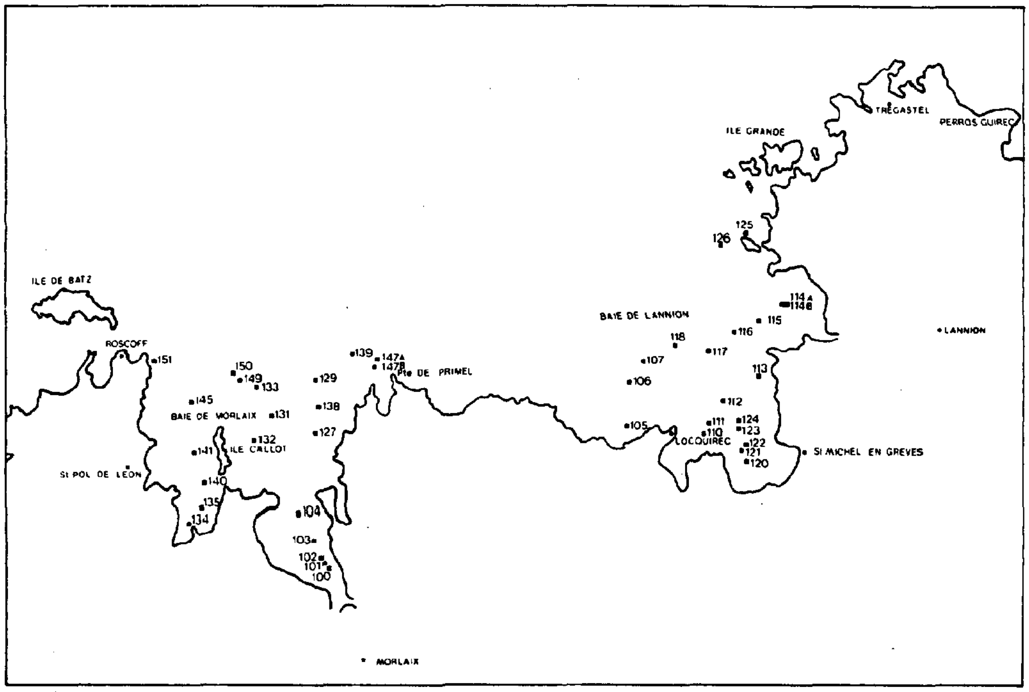

FIGURE 6. Sampling stations in the Bays of Morlaix and Lannion.

TABLE 1. Average hydrocarbon concentrations in surface sediments in the Bays of Morlaix and Lannion (July 1978).

AREA	SEDIMENT	Number of observations	Hydrocarbon concentrations (ppm)
BAY OF MORLAIX		25	311 ± 418
. Morlaix river	coarse sand to sandy mud	6	267 ± 88
. East area around Primel	coarse sand to fine sand	8	600 ± 656
. Central area West area	coarse sand to muddy sand	7	116 ± 100
. Penzé river	coarse sand to muddy sand	4	145 ± 96
BAY OF LANNION		22	188 ± 213
. North West area	fine sand	2	41 ± 43
. South Ile Grande marsh	coarse sand	2	298 ± 140
. Central area	coarse sand to fine sand	9	182 ± 138
. South East area	fine sand	9	204 ± 298

ratio of more than 100 is an index of oil pollution in surface sediments (Table 2). In this study, only four samples had a ratio less than 100; 80 percent of collected samples had a ratio more than 200; and 28 percent had a ratio more than 1,000. This simple parameter confirmed that the surface sediments were highly contaminated.

Three sedimentary oil accumulation areas were recognized in the Bay of Morlaix: the Morlaix River, the Penze River, and the east area of the bay around Primel. In the Bay of Lannion, pollution was located beyond a line joining Beg An Fry and the Ile Grande marsh (Fig. 5). On the whole, from July 1978 to February 1979, the decontamination process was related to two essential factors: sediment type and the energy level of the geographic zone. Among muddy and fine-grained sands, a slow decrease in oil content was observed, whereas in areas more exposed to high energy conditions, as around Primel in the eastern part of the Bay of Morlaix, the fine- and coarse-grained sand bottom was rapidly cleaned (Table 3).

TABLE 2. Ratio of hydrocarbons/organic carbon (HC/OC) in unpolluted and polluted surface sediments (from Marchand and Roucache, 1981).

LOCATION	Number of samples	HC (ppm)	$\frac{HC}{OC}\times 10^{4}$	REFERENCES
UNPOLLUTED SEDIMENTS				
English Channel (France)				
- estuary of Seine	3	30 - 40	16 - 31	TISSIER, 1974
- bay of Veys	2	31 - 51	26 - 53	TISSIER & OUDIN, 1973, 1975
Iroise sea and bay of Audierne (France)	23	3.6-109.5	21 - 70	MARCHAND & ROUCACHE, 1981
NW Atlantic	9	1.3 - 19	10 - 41	FARRINGTON & TRIPP, 1977
Gulf of Mexico	60	1.5-11.7	9 - 23	GEARING *et al.* 1976
POLLUTED SEDIMENTS				
English Channel (France)				
- Estuary of Seine	3	230 - 920	232 - 430	TISSIER, 1974
- Estuary of Seine	3	70 - 170	276 - 283	TISSIER & OUDIN, 1973, 1975
N-W Atlantic (coastal zone)	6	113 - 2900	120 - 180	FARRINGTON & TRIPP, 1977
Bay of Narragansett (USA)	4	350 - 3560	313 - 724	FARRINGTON & QUINN, 1973
Bay of Narragansett (USA)	8	520 - 5410	1350-15590	VAN VLEET & QUINN, 1977
Bay of Morlaix and Lannion	45	9 - 1547	48 - 6065 $\bar{m}$: 951±1237 ±σ	present study

TABLE 3. Average hydrocarbon concentrations (ppm) in the sediments in the Bays of Morlaix and Lannion from July 1978 to February 1979.

AREAS	July 1978	November 1978	February 1979
Bay of Morlaix	311 ± 418	168 ± 246	172 ± 262
- Morlaix river	267 ± 88	185 ± 82	169 ± 80
- Penzé river	155	22	97
- East area (Primel)	600 ± 656	53 ± 54	19 ± 21
Bay of Lannion	281 ± 262	130 ± 113	126 ± 110

Oil Pollution in Core Sediments

In August 1978, 24 several-meter-long vibracores were taken to ascertain the vertical distribution of oil incorporated into bottom sediments. Complete data concerning sedimentary characteristics and oil chemical analysis are presented by Marchand and d'Ozouville (1981). A comparison of grain size analyses of surface (Hamon) grab and vibracore samples revealed some differences. The vibracoring technique disturbed the first 10 cm of surface sediment layer as expressed by a shortage of fine particles. In another respect, we noticed that hydrocarbon levels in surface layer sediments were low, from 1 to 50 ppm ($\bar{m} = 22 \pm 19$ ppm), except for two stations in the Bay of Morlaix (AF 132: 100 ppm; AF 139: 174 ppm). These values were lower than those reported for surface sediments collected by the Hamon grab in July and November 1978 (Table 4). These observations are an indirect confirmation that oil is inclined to be adsorbed on fine sedimentary particles.

Hydrocarbon analyses of deep layers in the sediment cores did not reveal any significant vertical penetration of oil. Organic extract (EXT) and/or hydrocarbon (HC) concentrations very often remained homogeneous in the whole of the cores. The most important levels were found in the first 20-30 centimeters, corresponding to the layer sampled by the Hamon grab. A diving survey undertaken during the same time by d'Ozouville et al. (1979) gave the same conclusion: the depth of oil penetration was usually given to less than 7 cm, possibly related to the depth of biological reworking. Table 5 presents some results showing the absence of a deep diffusion of oil into bottom sediments. Reference data relating hydrocarbon content to sediment type for 17 cores are presented in Table 6.

TABLE 4. Comparison between hydrocarbon concentrations (ppm) observed in surface sediments according to sampling techniques.

STATION	HAMON GRAB		VIBRATORY BORING
	July 1978	November 1978	August 1978
102	291	276	36
103	392	228	49
112	914	-	50
114	207 - 367	25	4
122	79	-	22.5
124	72	-	16
132	292	56	100
138	1547	130	29
139	60	-	174
151	211	79	4
154	-	-	8
227	-	-	< 5
$\bar{m}$	403 ± 447	132 ± 100	41 ± 6

TABLE 5. Hydrocarbon concentrations (ppm) in some sediments cores from the Bays of Morlaix and Lannion.

Station / Depth (cm)	A F 112	A F 122	A F 132	A F 139
0 - 10		22.5	100	174
10 - 20	50		< 5	
20 - 30			< 2	11
30 - 40			< 2	19
40 - 50	4	< 5		< 2
50 - 60				
60 - 70	< 2	< 5		

TABLE 6. Average concentrations of organic extracts (EXT) and hydrocarbons (HC) in sediment cores collected from the Bays of Morlaix and Lannion.

Sample	Depth of coring (m)	Sediment	(n)	(EXT) ppm	(n)	(HC) ppm
AF 102	1.5	mud	(8)	231 ± 80	(7)	26 ± 15
AF 103	7.2	mud	(14)	220 ± 73	(11)	23 ± 23
AF 104	3.2	medium to coarse sand and maërl	(8)	16 ± 15		-
AF 112	4.0	fine to coarse sand		-	(6) (*)	< 2 - 4
AF 114	1.3	medium to coarse sand and maërl		-	(5)	< 2 - 4
AF 122	6.8	fine sand		-	8 (*)	< 2
AF 124	5.5	fine to coarse sand		-	8 (*)	< 2 - 6
AF 129	2.1	medium to fine sand	(8)	14 ± 9		-
AF 132	1.5	maërl and silt	(3) (*)	26 ± 4	3 (*)	< 2
AF 138	2.1	fine sand	7 (*)	14 ± 7		-
AF 139	2.7	fine to coarse sand		-	6 (*)	< 2
AF 147	2.0	medium to fine sand	(9)	10 ± 10		-
AF 151	2.0	fine to coarse sand		-	(8)	< 2 - 5
AF 165	2.1	medium to fine sand	(7)	13 ± 3		-
AF 186	2.3	coarse to fine sand	(7)	26 ± 6		-
AF 191	2.5	medium to fine sand	(7)	10 ± 6		-
AF 237	2.3	fine sand to sandy mud		-	(6)	< 5

(*) : excepted surface layer,
(n) : number of observations,
(EXT) : IR spectrophotometric determinations of non-purified organic extracts,
(HC) : IR spectrophotometric determinations of purified organic extracts on florisil.

ABER WRAC'H (1978 TO 1981)

The two Abers (Benoit and Wrac'h), located 8 km east of Portsall, were heavily impacted during the spill. These Abers are small estuaries, 10-15 km long and about 1 km wide. A study from March 1978 to March 1979 (Marchand and Caprais, 1981) revealed that the bottom sediments throughout the Abers were heavily polluted. Concentrations were more than 100 ppm and, as in a muddy area in the Aber Benoit, sometimes reached higher than 10,000 ppm. After one year, Marchand and Caprais (1981) showed that the natural decontamination process was related to the nature of the sediment and the energy level of the geographic zone. The fine- and medium-grained sands located in the exposed, downstream part of the Aber Benoit were well decontaminated (average hydrocarbon content reduced from 700 to 27 ppm). On the other hand, in mud-dominated areas, the sediment acted as an oil trap (oil content above 10,000 ppm) and decontamination was not observed. For the Aber Benoit, oil pollution of mud-dominated zones such as Loc Majan will be long term.

In the Aber Wrac'h, the evolution of oil pollution in the bottom sediments has been followed since March 1978. The location of sampling stations is presented in Figure 7; analyses for hydrocarbon and organic carbon concentrations are presented in Tables 7 and 8, respectively. Organic carbon concentrations ranged from 0.08 to 3.32 percent. Sediments are much more homogeneous than those of the Aber Benoit. Composition, with the exception of station 3 located at the mouth of the Aber, varied from slightly muddy to muddy sands.

FIGURE 7. Sampling stations in the Aber Wrac'h.

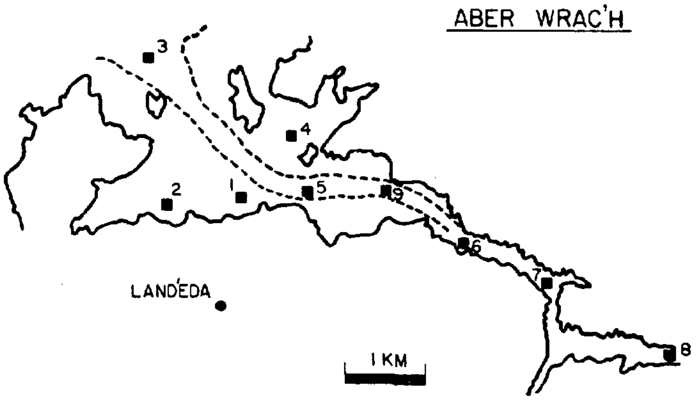

TABLE 7. AMOCO CADIZ oil pollution in the sediments of the Aber Wrac'h. Data collected from 1978 to 1981. OC = organic carbon; $CaCO_3$ = carbonate calcium; EXT = gravimetric determination of organic extract; OIL = IR spectrophotometric determination of nonpurified organic extract; HC = hydrocarbons, IR spectrophotometric determination of purified organic extract; (S) = surface; (P) = 10-15 cm depth.

DATE	T (months)	SAMPLING STATION	1	2	3	4	5	6	7	8	9
March 31, 1978	0,5	OC (%)	0.53	2.42	0.23	0.96	1.06	1.03	0.66	1.94	0.78
		EXT (ppm)	2130	11220	711	2185	3160	765	–	397	1951
		OIL (ppm)	2051	12000	773	2450	3706	839	2259	953	2063
May 5, 1978	1.5	$CaCO_3$ %	–	-	–	–	18.8	20.8	7.0	8.3	13.3
		OC	0.66	-	0.34	1.59	0.71	0.78	0.61	0.55	0.80
		EXT	2400	-	800	11490	2000	1660	1190	460	2160
		OIL	3023	-	1020	11750	2970	1380	1236	508	2236
November 22, 1978	8.25	EXT	990	4030	130	3740	2600	710	1600	680	856
		OIL	1439	4144	148	3598	2481	781	1105	671	872
February 22, 1979	11.25	OC	0.56	1.53	–	0.75	0.85	1.12	1.00	0.60	–
		EXT	1740	2660	80	1340	1360	3020	1050	870	1780
		OIL	1589	2679	113	1301	1268	2556	1410	1266	1677
June 20, 1979	15.25	EXT	1550	1200	80	480	1450	6070	1260	1450	850
		OIL	1458	1124	74	412	1178	4900	1325	1445	928
October 22, 1979	19.25	OIL	715	1374	80	1237	1047	2712	2496	485	569
January 20, 1980	22.25	OIL	1408	2567	-	1796	1473	1861	1399	5694	1097
June 1980	27	OC	0.44	1.74	0.08	1.15	0.81	2.40	1.90	1.67	1.00
		OIL	442	1892	42	1225	1075	1788	1280	1628	866
		HC (ppm)	175	995	-	724	348	1122	602	798	540
January 1981	34	OC	0.83	0.84	-	0.90	0.70	2.70(S) 2.03(P)	3.32(S) 2.19(P)	0.34(S) 2.30(P)	1.01
		OIL	321	562	-	347	456	2320(S) 1709(P)	3167(S) 2369(P)	1178(S) 1121(P)	324
		HC	62	255	-	105	205	831(S) 731(P)	1644(S) 1383(P)	630(S) 420(P)	214
March 16, 1981	36	OIL	432	1078(S) 303(P)	25	970(S) 627(P)	653(S) 1471(P)	1517(S) 2020(P)	180	940(S) 1807(P)	919(S) 1437(P)
		HC	229	431(S) 90(P)	-	551(S) 403(P)	281(S) 787(P)	674(S) 722(P)	40	415(S) 642(P)	312(S) 707(P)
June 23, 1581	39,25	OIL	308	851(S) 404(P)	-	413(S) 195(P)	864(S) 1541(P)	1623(S) 1320(P)	540(S) 961(P)	117(S) 331(P)	411
		HC	50	362(S) 71(P)	-	108(S) 42(P)	406(S) 1486(P)	740(S) 660(P)	200(S) 417(P)	35(S) 131(P)	326

TABLE 8. Organic carbon content of the Aber Wrac'h sediments. (n) = number of observations.

SAMPLING STATION.	n	OC(%) $\bar{m}$ ± s
1	5	0.60 ± 0.15 (25 %)
2	4	1.63 ± 0.65 (40 %)
3	3	0.22 ± 0.13 (59 %)
4	5	1.07 ± 0.32 (30 %)
5	5	0.83 ± 0.15 (17 %)
6	6	1.16 ± 0.88 (54 %)
7	6	1.50 ± 1.14 (76 %)
8	6	1.22 ± 0.87 (71 %)
9	4	0.90 ± 0.12 (14 %)

In this discussion, three areas of Aber Wrac'h are described in terms of oil degradation:: (1) the mouth of the estuary (fine-grained sands, station 3), (2) the downstream part (stations 1, 2, 4, and 5), and (3) the upstream part (stations 6, 7, 8, and 9). The evolution of oil pollution in sediments from 1978 to 1981 for each station is given in Figure 8; the change in average oil concentrations for each of the three defined areas is given in the Table 9 and Figure 9. In March 1978, 15 days after the AMOCO CADIZ wreck, concentrations ranged from 773 ppm to 12,000 ppm. At the mouth of the Aber which is well exposed to high marine energy, hydrocarbon content dropped from 773 ppm in March 1978 to 25 ppm in March 1981, illustrating a fairly rapid decontamination of these fine-grained sands.

Since the sediments of the Aber Wrac'h are relatively homogeneous, the decontamination process is mainly related to the energy level of the zone. In the downstream part of the Aber, the sediments were more polluted in March 1978 (average about 5,000 ppm) than the sediments collected in the upstream part (average about 1,500 ppm). For the first 39 months after the spill, a natural but slow decontamination was observed with some temporary increases in January 1980 (about 1,800 ppm) and March 1981 (about 780 ppm). In June 1981, the average residual oil content was about 600 ppm. In the upper part of the Aber, the sediments were initially less polluted, but since this is a low-energy area, a decrease in hydrocarbon content was not observed until January 1981. As had been observed in the downstream portions of the Aber, significant increases in hydrocarbon levels were observed during January 1980 in upstream areas. Since March 1981, oil levels have decreased; average residual oil concentrations were about 670 ppm.

Three years after the wreck, the petroleum pollution of the Aber Wrac'h sediments seems to be relatively uniform. Figure 10 gives the observed decontamination rate of the sediments. At first approximation, station 3 (located at the mouth of the Aber) is well decontaminated (3% of the initial residual oil level observed in March 1978). However, sediments within Aber Wrac'h remain quite polluted with about 20 percent of the residual oil content still remaining in March 1978.

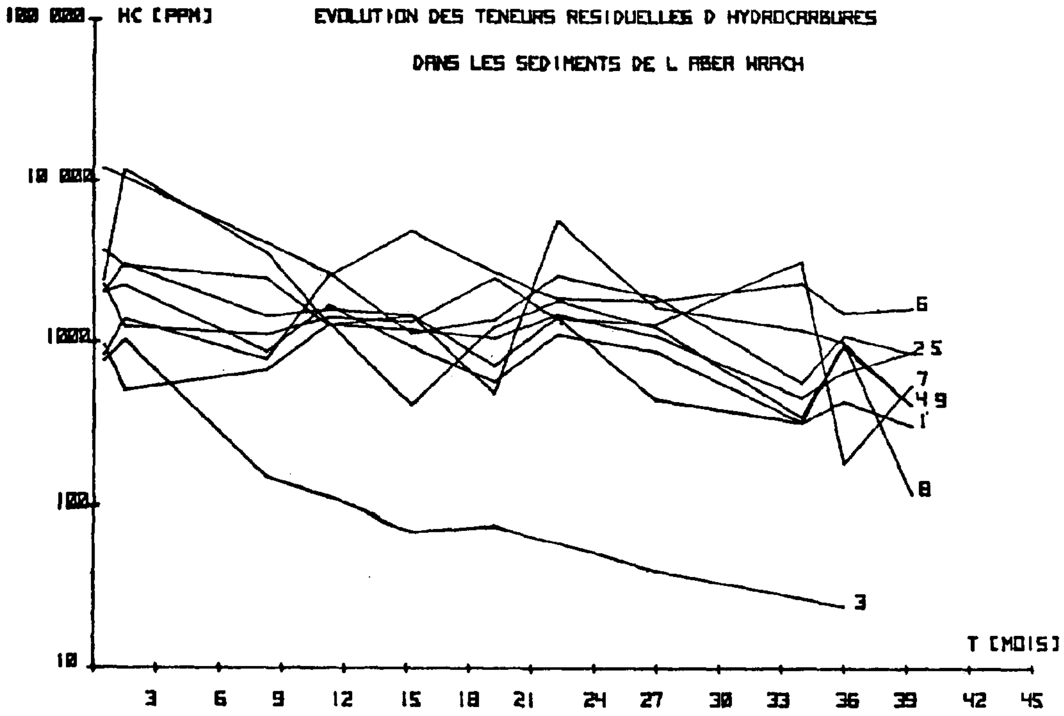

FIGURE 8. Evolution of residual oil pollution in Aber Wrac'h sediments.

TABLE 9. Evolution of oil pollution (ppm) in the Aber Wrac'h sediments from 1978 to 1981.

DATE T (months) LOCATION	March 31 1978 0.5	May 5, 1978 1.5	Nov.22, 1978 8.25	Febr.22, 1979 11.25	June 20, 1979 15.25	Oct.22, 1979 19.25	Jan.20 1980 19.25	June 1980 22.25	January 1981 34	March 16 1981 36	June 23 1981 39.25
Month (st. 3)	773	1020	148	113	74	80	-	42	-	25	-
Downstream part (st. 1,2,4,5)	5051 ±4685	5914 ±5053	2915 ±1203	1709 ±662	1043 ±445	1093 ±285	1811 ±531	1158 ±595	421 ±110	783 ±295	609 ±290
Upstream part (st. 6,7,8,9)	1528 ±736	1340 ±708	857 ±184	1727 ±578	2149 ±1846	1565 ±1202	2512 ±2143	1390 ±409	1747 ±1250	901 ±550	672 ±657
All stations of the Aber Wrac'h (st. 1,2,4,5,6, 7,8,9)	3290 ±3631	3300 ±3839	1886 ±1358	1718 ±575	1596 ±1377	1329 ±847	2161 ±1493	1274 ±489	1084 ±1085	842 ±413	640 ±472

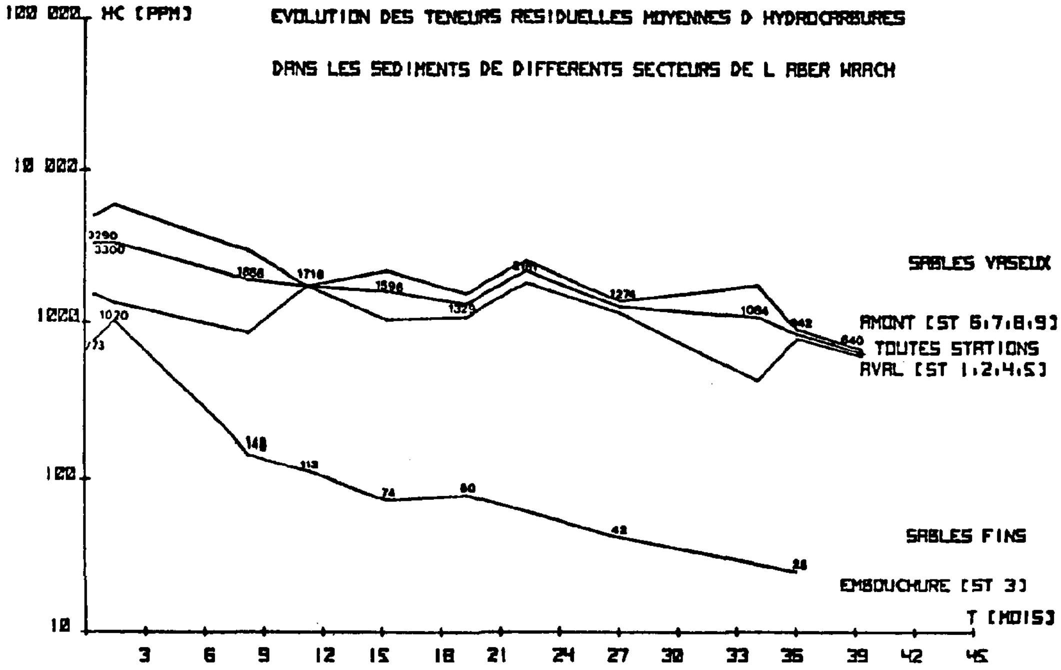

FIGURE 9. Evolution of average residual oil pollution in sediments from the Aber Wrac'h.

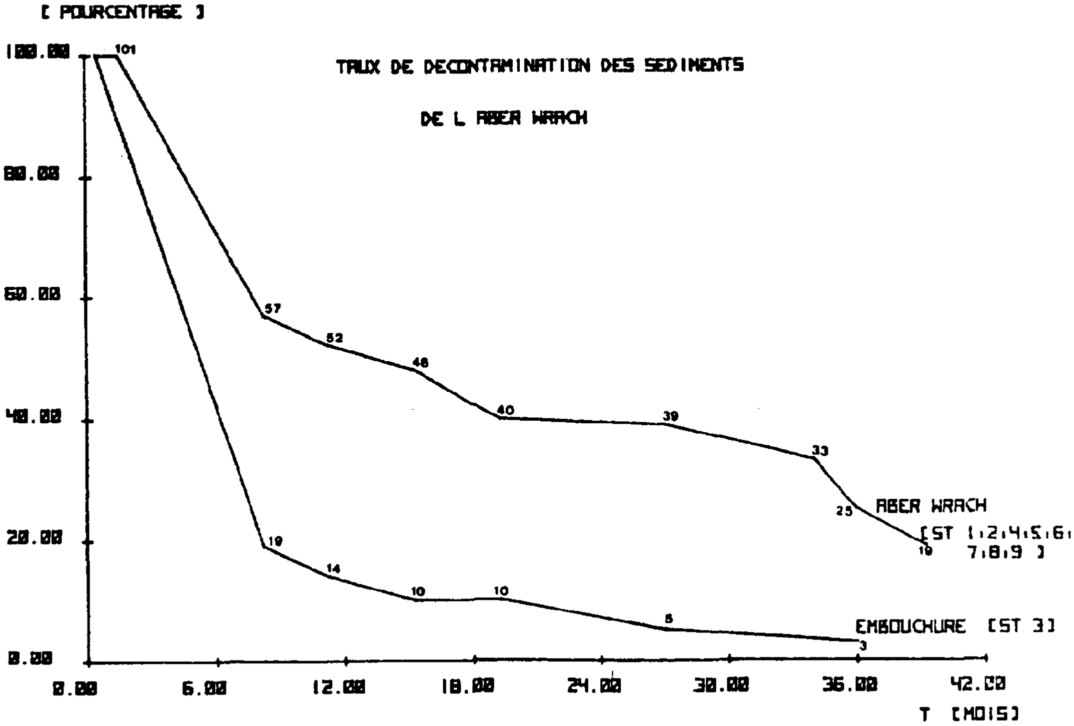

FIGURE 10. The rate of oil decontamination in sediments from the Aber Wrac'h.

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AMOCO CADIZ POLLUTANTS IN ANAEROBIC SEDIMENTS: FATE AND EFFECTS ON ANAEROBIC PROCESSES

by

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INTRODUCTION

It was estimated that much of the oil spilled after the wreck of the AMOCO CADIZ impacted intertidal and subtidal sediments (Hann, et al, 1978; Gundlach and Hayes, 1978). Considerable differences exist between sediment and aquatic environments which could have dramatic effects on the persistence of spilled oil and its effects on the native biology of coastal environments. Recent investigations have shown that intertidal and subtidal sediments are anaerobic except in the initial few millimeters near the surface (Sørensen, et al, 1979; Revsbech, et al, 1980a, b). Since oxygen is known to be of extreme importance in the microbial biodegradation of hydrocarbons (Atlas, 1981; Hambrick, et al, 1980; DeLuane, et al, 1981; Ward and Brock, 1978) it is likely that the persistence of hydrocarbons would be much greater in anoxic sediments. This could create a source of relatively unweathered petroleum for secondary pollution events. One of the major objectives of this study was to investigate the extent of pollution by AMOCO CADIZ oil in anaerobic coastal sediments. Evidence for weathering and potential biodegradation of sediment hydrocarbons under aerobic and anaerobic conditions was also obtained in chemical and microbiological studies.

Sediments are also important sites where extensive mineralization of organic matter and recycling of nutrients occurs (Fenchel and Jørgensen, 1977). The effect of oil on sediment microorganisms and processes has been examined in some studies (Walker, et al, 1975; Knowles and Wishart, 1977) but only a few studies have examined the effects on mineralization (Griffiths, et al, 1981, 1981 (in press)). Because of the extreme thinness of the oxygenated zones of coastal sediments anaerobic processes are important in mineralization and nutrient recycling (Sørensen, et al, 1979). The effects of oil on anaerobic processes have not been studied. Many studies on sediment chemistry and microbiology support the model for anaerobic microbial food chains in marine sediments presented in Figure 1 (see Mah, et al, 1977; Fenchel and Jørgensen, 1977; Rheeburg and Heggie, 1977; Bryant, 1976). Within anaerobic zones polymeric organic matter is fermented principally to H_2, CO_2 and acetic acid. Acetate and H_2 are the main energy sources for specialized anaerobic bacteria which terminate the food chain. The activity of these terminal groups is thought to be important in influencing fermenting bacteria to produce mainly acetate, H_2 and CO_2 (Bryant, 1976). The importance of these energy sources in marine sediments has

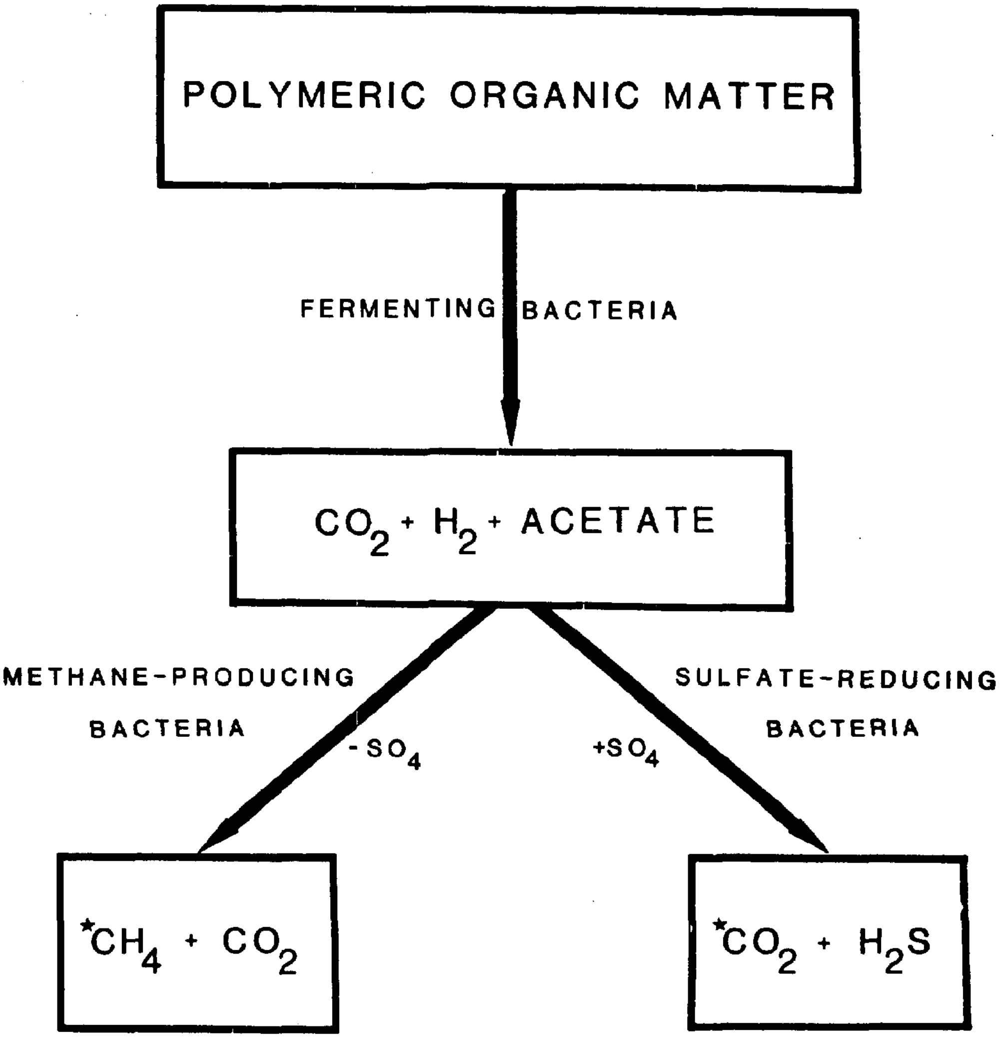

Figure 1. Simple model for anaerobic microbial food chains in marine sediments. The model does not include minor electron sink fermentation products or other possible anaerobic groups such as denitrifying bacteria. The asterisk indicates the major product from the methyl position of acetate.

only been confirmed in this and other recent studies (Winfrey and Ward, submitted; Sørensen, et al, 1981; Banat and Nedwell, personal communication). Sulfate-reducing and methane-producing bacteria share the potential to utilize these fermentation products and may compete in marine sediments. Detailed investigations to characterize anaerobic processes in Brittany sediments were made as a part of this study, but discussion here is beyond the scope of this report. In summary, methanogenesis is only significant in the competition for acetate and H_2 in sediments where sulfate is depleted (e.g., deep subtidal sediments, Winfrey, et al, in press). In intertidal sediments where sulfate is high at all depths (see below), methanogenic bacteria may be restricted to energy sources other than acetate or H_2 (such as methylamines), and sulfate reduction dominates as the significant terminal process (Winfrey and Ward, submitted). As another major objective of this study we examined terminal processes of the anaerobic food chain, which should indicate the activity of the overall food chain, for evidence of changes due to AMOCO CADIZ oiling. The processes investigated were those which dominated the food chains, principally sulfate reduction and acetate metabolism.

METHODS

Location of Sampling Sites

Sites were selected to represent beach, estuary and salt marsh sediments in the lower intertidal region which were significantly oiled by AMOCO CADIZ pollutants. Similar sites in areas unoiled or lightly polluted were selected as controls. Locations are shown in Figure 2. Observations on the chronology of oil movements were used to determine the extent of oil impact and times at which oiling first occurred (Centre National pour l'Exploitation des Océans, 1979; Gundlach and Hayes, 1978). The oiled beach site was between stations B and C at AMC-4 (Gundlach and Hayes, 1978). This site was opposite the wreck and was heavily oiled immediately after the spill. The control beach site was 100 m east of the west access to the beach at Trez-hir. This beach faces the Bay of Brest and was not reported to have been oiled. The oiled estuary site was a mudflat at Aber Wrac'h, 200 m south of the stone wall at EPA-7 (Calder, et al., 1978). AMOCO CADIZ oil was detected in this area in medium thickness two days after the oil spill. The control estuary site was a mudflat on the south bank of Aber Ildut approximately 3 km west of Breles. Oiling at this site was prevented by two booms extended across the mouth of the Aber. The oiled marsh site was an intertidal mudflat in Ile Grande near AMC-18 (Gundlach and Hayes, 1978). Thick accumulations of AMOCO CADIZ oil reached this site by the eighth day following the spill. The control marsh was a natural mudflat in the Ile Grande marsh, just south of the main intertidal channel. A barricade at the bridge adjoining east and west marsh areas prevented oiling at this site.

Sample Collection and Processing

Sediment cores were collected with hand-pushed plexiglass tubes (60 cm x 37 mm ID), stoppered, and transferred to the lab in an upright position. Cores for sediment hydrocarbon analysis were kept frozen until processing. Processing of samples for biological activities was done at the Centre Océanologique de Bretagne in Brest or at the Station Biologique at Roscoff within 8 h after collection. All manipulations for analysis of biological activity were carried out using strict an-

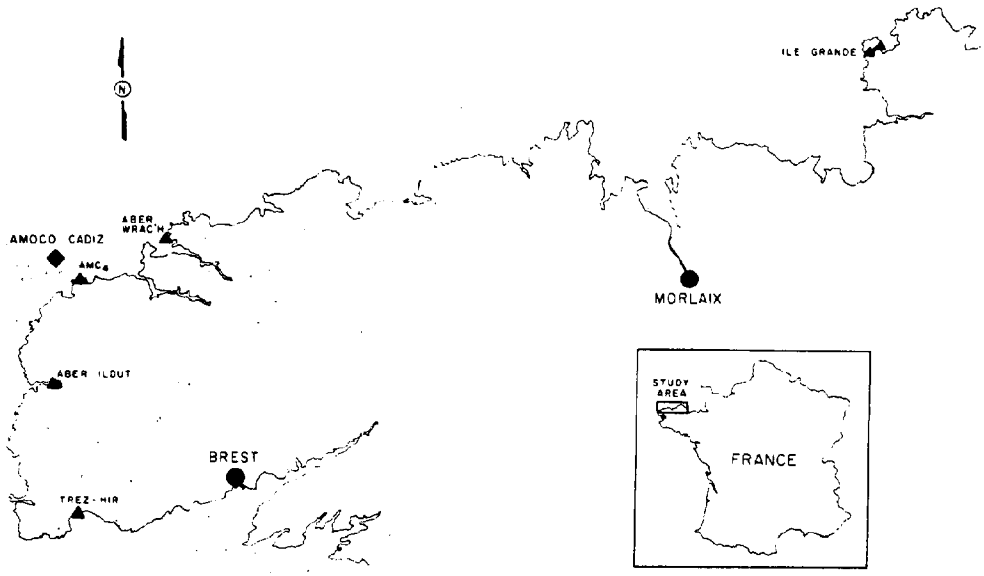

Figure 2. Locations of sampling sites.

aerobic techniques designed for cultivation of bacteria with extreme sensitivity to oxygen (Hungate, 1969). Cores were sectioned into desired intervals and subcores removed by a No. 4 cork-borer or by a 3 ml syringe with the end of the barrel cut off (50.3 mm^2). The 0-3 cm interval was used for all the oil and mousse addition experiments. For these experiments an anoxic slurry was made by mixing the core section with 20% (V/V) anoxic artifical seawater (ASW, Burkholder, 1963). In experiments on hydrocarbon metabolism slurries of other depth intervals were made in the same way. Subsamples (2.0-2.5 ml) from core sections or slurries were transferred to 2 dram glass vials (Acme Vial and Glass Co.) and sealed under a stream of helium. The helium was passed over heated copper filings to remove any traces of oxygen. Vials were sealed with 00 butyl rubber stoppers (A.H. Thomas). Unless noted below, all isotope additions (1.0 ml) were taken from sterile anoxic stock solutions with a 1 ml helium flushed glass syringe (Glaspak). Mousse, oil and hydrocarbon additions were added to vials containing sediment under a flow of helium gas using a 1 ml pipet 12 hours before microbial activities were assayed. Benzene and toluene were added with a 5 µl syringe (Hamilton).

Measurement of Microbial Activities

All incubations were done at ambient temperatures (20-24°C). Comparisons between different samples were made by a two sample t test, using the ANOV1 program of MSUSTAT (Lund, 1979).

Sulfate Reduction

Sulfate reduction assays were set up using a modification of the technique of Ivanov (1964). Each vial of sediment received approximately 1 μCi of $Na_2{}^{35}SO_4$ in 1 ml of anoxic ASW. Samples were mixed and incubated for 2.0 h. The reaction was stopped by the addition of 0.5 ml of 2% zinc acetate followed by 0.2 ml of formalin. Samples were assayed and rates determined in the Montana State University lab as described by Jørgensen (1978). $H_2{}^{35}S$ was distilled to traps containing 2% zinc acetate. Radioactivity was determined by counting the zinc acetate trap (5 ml) in 10 ml Aquasol (New England Nuclear) on a Beckman LS-100C liquid scintillation counter. Correction for quenching was by the channels ratio method.

Methane Production

Methane production was measured by quantifying the increase in methane in the head-space of vials containing sediment. A 0.2 ml gaseous subsample was removed at desired intervals and analyzed by flame ionization gas chromatography (see below).

Acetate Metabolism

Acetate metabolism was measured by adding approximately 0.5 μCi of [2-^{14}C]-acetate in 1.0 ml of sterile anoxic sulfate-free ASW. Samples were mixed and incubated for 2.0 h unless otherwise stated. The reaction was stopped by the addition of 0.2 ml formalin. $^{14}CO_2$ and/or $^{14}CH_4$ were measured in samples of the gas headspace (see below).

Hydrocarbon Metabolism

All radiolabelled hydrocarbons except benzene and toluene were diluted in benzene to the desired activity. The radioisotopes were added to vials and the benzene allowed to evaporate completely before addition of sediment and anaerobic tubing as described above. Anoxic ASW (1.0 ml) was added to each sample to mix sediment and radioisotopes. Radiolabelled benzene and toluene were dissolved in anoxic ASW and added (1.0 ml) after anoxic tubing of sediment. When indicated, samples were incubated in darkness by wrapping with aluminum foil or electricians tape. In one experiment samples contained in anaerobically sealed tubes were incubated within an anaerobic chamber (Gaspak) with a H_2 + CO_2 atmosphere. During long term incubations gaseous metabolities ($^{14}CO_2$ and/or $^{14}CH_4$) were quantified in samples of the gas headspace as described below. After incubation was completed, samples were poisoned by addition of 0.5 ml 10% formalin. Carbon dioxide was reabsorbed by addition of 2 ml 2N NaOH. The sediment was extracted four times by vortex mixing with 6 ml methylene chloride: methanol (9:1) followed by centrifugation to break the emulsion. Solvent fractions were removed from beneath the aqueous phase and pooled together with three 6 ml rinses of the original sample vial. Anhydrous sodium sulfate was added to dry the sample. The extract was concentrated to 0.1 ml by evaporation and the volume increased by addition of 0.7 ml of hexane. This sample was separated into saturate (f_1), aromatic (f_2)

and methanolic (f_3) fractions by silica gel chromatography as described for oil samples below. Each solvent fraction was concentrated by rotary evaporation to 4 ml and radioactivity of a portion of the extract was determined in Aquasol as described above. Using these methods [1-^{14}C]-hexadecane and [1-^{14}C]-heptadecene standards were recovered in the f_1 fraction, whereas [1(4, 5, 8)-^{14}C]-naphthalene was recovered in the f_2 fraction. $^{14}CO_2$ was transferred from the extracted sediment after acidification and distillation to a CO_2 absorbant trap (Carbosorb, Packard). This trap was combined with Aquasol and radioactivity determined as described above.

Gas Measurements

Gas subsamples (0.2 to 1.0 ml) were removed from the headspace of incubation vials using a helium flushed 1 ml glass syringe (Glaspak) fitted with a Mininert pressure-lock syringe valve (Supelco). $^{14}CH_4$ and $^{14}CO_2$ were measured by gas chromatography- gas proportional counting using the method of Nelson and Zeikus (1974) as modified by Ward and Olson (1980). This method ensured the specific detection of these gaseous metabolites. All numerical results were based on amounts clearly above detection limits and quantifiable by integration using a Spectra-Physics Minigrator. $^{14}CO_2$ values were corrected for CO_2 solubility and bicarbonate equilibrium as described by Stainton (1973). Methane concentrations were quantified on a Varian 3700 flame ionization gas chromotograph as described by Ward and Olson (1980). All values for gas analyses are reported on a per vial basis.

Chemical Analysis of Oils

Similar methods were used for the analysis of sediment hydrocarbons (ERCO) or oils (MSU) although the specific details differed. Sediment hydrocarbon samples were solvent extracted and fractionated according to an analytical scheme patterned after that of Brown et al (1980). Hydrocarbons were separated from methanol-dried samples by high-energy shaking with methylene chloride:methanol (9:1), fractionated into saturate, aromatic/unsaturate and methanolic fractions by alumina/silica gel column chromatography, and analysed by gas chromatography, mass spectrometry and/or mass fragmentography as described by Boehm, et al (1981).

AMOCO CADIZ mousse, fresh or evaporated crude oil, and extracts from ^{14}C-hydrocarbon experiments (see above) were analyzed as follows. Each sample (a 25 μl aliquot of each oil sample diluted in 0.5 ml of hexane, or extracts described above) was loaded onto a glass column (1 cm ID x 20 cm long) packed with 40-140 mesh silica gel (Baker Chemical Co.). Saturate components (f_1) were removed from the column by eluting with 200 ml of hexane. The aromatic fraction (f_2) was then eluted with 200 ml of a solution of hexane and methylene chloride (70/30 V/V). The methanolic fraction (f_3) was then eluted with 150 ml of methanol. Each fraction was concentrated to 3-4 ml by flash evaporation and further concentrated to less than 1 ml under a stream of nitrogen. The volume of each fraction was adjusted to one ml with the appropriate solvent. A 0.2 µl subsample was injected into a Varian 3700 flame ionization gas chromatograph equipped with a 30 m glass WCOT column packed with SE-54 (Supelco). Operating conditions were as follows: column temperature programmed from 60 C to 260 C at 3 C/min with a 30 min hold at 260 C; injection temperature: 250 C; detector temperature 250 C; carrier gas: 1 ml helium/min with a helium make-up gas of 29 ml/min. Results were recorded on a Spectra-Physics model 4100 recording integrator. Hydrocarbons labelled in Figure 9 were identified by comparison of retention times to those of pure hydrocarbon standards.

Sediment Chemistry

Eh

Duplicate sediment cores for Eh measurements were collected using a plexiglass tube (30 cm x 25 mm ID) which had been split lengthwise and taped together. Upon returning to the laboratory, one half of the core liner was removed, and fresh sediment exposed 1 cm at a time by slicing the core lengthwise with a spatula. Eh measurements were taken by pressing a combination platinum electrode (Orion) into the freshly exposed sediment surface. All Eh values are reported relative to the normal H_2 electrode.

pH

pH was determined using a VWR pH Master pH meter and glass combination electrode.

Interstitial Water

Sediment porewater was obtained using the porewater squeezer of Kalil (1974). After appropriate dilutions were made, sulfate was measured by the turbidometric method of Tabatabai (1974) and chloride was measured by silver nitrate titration (Am. Pub. Health Assoc., 1976).

Methane

Dissolved methane was quantified by killing a 2 ml subcore in a sealed vial by the addition of 0.5 ml of formalin and mixing on a vortex mixer to strip the dissolved methane into the headspace. A gas subsample was then analyzed by flame ionization gas chromatography as described above.

Radioisotopes, Chemicals, and Oils

The following radioactive chemicals were used (radiochemical purity in parentheses): $Na_2{}^{35}SO_4$, 738 mCi/mmole (on 3/5/79), Na-[2-^{14}C]-acetate, 44 mCi/mmole, and [ring-1-^{14}C]-toluene, 3.4-5.2 mCi/mmole (97-

99%) from New England Nuclear; n-[1-^{14}C]-hexadecane, 54 mCi/mmole (97-99%), [1(4, 5, 8)-^{14}C]-naphthalene, 5 mCi/mmole (97-98%), [U-^{14}C]-benzene, 101 mCi/mmole (98-99%), [7, 10-^{14}C]-benzo[a]pyrene, 60.7 mCi/m mole, (99%), and [methyl-^{14}C]-toluene, 30 mCi/mmole (96-99%) from Amersham Corp.; n-[1-^{14}C]-heptadecane, 16 mCi/mmole (>99%), and [1-^{14}C]-heptadecene, 18.5 mCi/mmole (97%) from ICN.

AMOCO CADIZ mousse was obtained from the NOAA National Analytical Facility and was collected at Ploumanach on April 30, 1978 (44 days after the spill). Light Arabian crude oil (SX#0308) was obtained from Exxon Corp., Baytown, Texas. The crude oil was weathered by evaporation at 25 C for 8 and 48 h. All oil samples were stored at 4 C until used.

RESULTS

Physical-Chemical Comparison of Sites

Beach cores consisted of medium grained sand, while estuary and marsh cores consisted of fine grained silt and clay. It was possible to determine Eh profile for muddy sediments (Fig. 3). In all sediments conditions became more reducing with depth. The steepest Eh profile was observed in the Ile Grande oil site where a brown layer approximately 2 mm thick covered black sediment. Marsh mudflat sediments

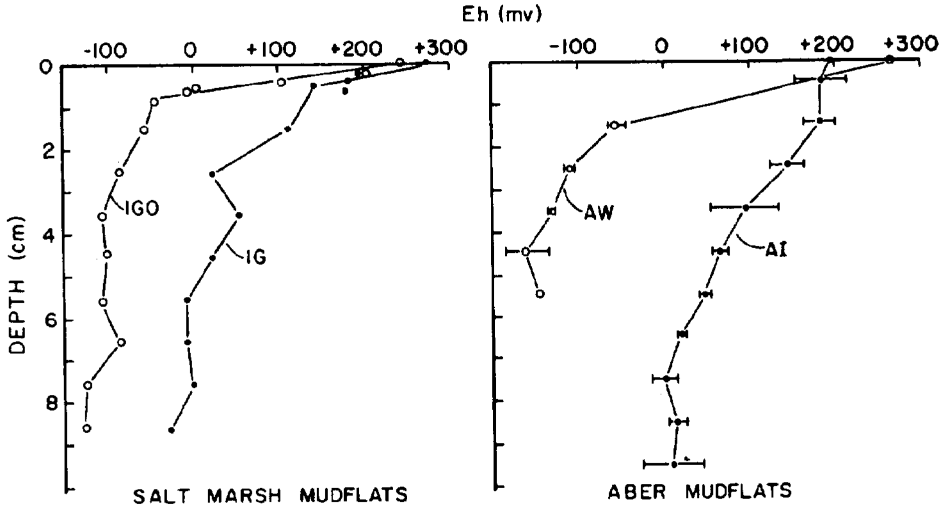

Figure 3. Eh profiles in muddy sediments. Bars indicate the range of measurements on duplicate cores. pH increased with depth from 7.5 to 8.2 in Ile Grande, from 6.7 to 7.2 in Aber Ildut, from 5.9 to 7.5 in Aber Wrac'h and from 7.0 to 8.1 in the Ile Grande oiled site.

showed steeper Eh profiles than estuarine sediments and oiled sediments were more reducing and showed steeper Eh profiles than unoiled sediments of the same type. The large Eh change with depth (300mv over 1 cm (Ile Grande oiled site) or 2 cm (Aber Wrac'h) suggested that sediments below these depths are likely anoxic.

Chloride (data not presented) was relatively constant at all depths in all sites and was near seawater chloride levels (20,000 mg/liter). The concentrations of dissolved sulfate and methane with depth in each site are reported in Table 1. No major differences in sulfate concentration with depth or between cores were observed, and levels were similar to seawater values (approximately 800 mg$SO_4^{=}$-S/1). Methane

TABLE 1. Sediment Chemistry[a]

SULFATE CHEMISTRY[b]

DEPTH (cm)	AMC$_4$	TREZ-HIR	ABER WRAC'H	ABER ILDUT	ILE GRANDE (oiled)	ILE GRANDE (control)
0-5	720	720	860	840	840	760
5-10	850	770	900	830	790	800
10-15	820	820	870	970	750	760
15-20	1070	1190	820	810	740	790
20-25	--	--	860	920	800	710

METHANE CHEMISTRY[c]

DEPTH (cm)	AMC$_4$	TREZ-HIR	ABER WRAC'H	ABER ILDUT	ILE GRANDE (oiled)	ILE GRANDE (control)
0-5	0.17	0.16	0.74	1.57	3.36	0.24
5-10	0.52	0.84	0.80	0.89	2.89	1.09
10-15	0.64	0.67	0.79	0.66	3.34	0.26
15-20	--	--	0.99	0.93	4.26	0.35
20-25	--	--	0.50	0.81	4.36	0.36

[a] Sediments collected in March 1979

[b] Results expressed in μg $SO_4^{=}$-S/ml porewater

[c] Results expressed in μmoles CH_4/1 sediment

concentrations were extremely low (less than 5 µmoles/l) and relatively constant with depth. Methane concentrations were significantly higher at the Ile Grande oiled site ($p < .001$).

Sediment Hydrocarbons

Sediments collected during December 1978 and March 1979 were analyzed for hydrocarbon content (Table 2) and type (Figs. 4 and 5). Surface sediments (0-5 cm) at all sites oiled with AMOCO CADIZ oil exhibited a composition indicative of highly weathered oil residues. The saturate fractions were comprised of a degraded hydrocarbon assemblage with greater degradation in estuary and marsh mudflat samples than in the beach sample as evidenced by the relative dominance of the branched isoprenoid hydrocarbons (Fig. 4). Residual alkylated phenanthrenes, and dibenzothiophenes in the aromatic/unsaturate fractions (Fig. 5) also indicated the presence of weathered petroleum. All samples known to be impacted by AMOCO CADIZ oil exhibited a characteristic unresolved complex mixture (UCM) in both saturate and aromatic/unsaturate fractions indicative of weathered petroleum (Farrington and Meyers, 1975).

Qualitative and quantitative differences existed between oiled and unoiled control sediments in the 0-5 cm depth interval. Hydrocarbon content was always higher in oiled sediments (Table 2). The control estuary sediment exhibited a small UCM and hydrocarbons indicative of biogenic origin in the saturate (odd chain alkanes n-C_{23} to n-C_{31}) and aromatic/saturate (polyolefinic material) fractions. The control marsh sediment exhibited a mixture of hydrocarbons of biogenic (odd-chain alkanes n-C_{25} to n-C_{31}) and petroleum (UCM) origin, with low concentrations of residual aromatic/unsaturate hydrocarbons. The control beach sediment exhibited a n-alkane series (n-C_{15} to n-C_{20}) and UCM in the saturate fraction, and polynuclear aromatic components originating from combustion of fossil fuel (eg., nonalkylated 3-5 ringed polynuclear aromatics) (Youngblood and Blumer, 1975).

The types and amounts of hydrocarbons were consistent with the known degree of impact from the AMOCO CADIZ oil spill. It is clear that in control beach and marsh sediments impact by hydrocarbons of petroleum or other anthropogenic sources had occurred.

Evidence for degraded Amoco Cadiz oil at various sediment depths is summarized in Table 2. At the beach station AMC-4, AMOCO CADIZ oil was evident in the hydrocarbon assemblage down to the 10-15 cm interval in a sample collected in December 1978, and to the 15-20 cm interval in a sample collected in March 1979. At Aber Wrac'h there was evidence of AMOCO CADIZ oil to the 10-15 cm interval at both collection dates. The amount of oil decreased with depth as evidenced by the total hydrocarbon concentration and the increasing contribution of native sediment hydrocarbons (e.g., plant derived saturate and aromatic/saturate compounds) which dominated in the deepest layers as in the entire Aber Ildut core (see Figs. 4, 5). Similar results were found at the Ile Grande oiled site where AMOCO CADIZ oil was detected in the 5-10 cm layer on both sampling dates and biogenic compounds dominated deeper layers.

TABLE 2. Preliminary Results of Total Hydrocarbon Levels in Brittany Sediments (AC=AMOCO CADIZ Oil Indicated by GC-MS Data)

Sediment Type	Depth Interval (cm)	Total Hydrocarbons (µg/g)		
		Oiled		Control
		12/78	3/79	3/79
Beaches		AMC-4		Trez-Hir
	0-5	295 AC	217 AC	110
	5-10	158 AC	181 AC	46
	10-15	244 AC	162 AC	130
	15-20	72	128 AC	
	20-22	123		
Abers		Aber Wrac'h		Aber Ildut
	0-5	977 AC	1095 AC	690
	5-10	590 AC	630 AC	530
	10-15	47 AC	307 AC	305
	15-20	80	118	204
	20-25	33	103	115
	25-30	25		346
	30-35	45		
Salt Marshes		Ile Grande		Ile Grande
	0-5	1137 AC	863 AC	465
	5-10	144 AC	439 AC	365
	10-15	28	134	217
	15-20	32	220	154
	20-25		74	54

Evidence for Weathering of Sediment Hydrocarbons

It was evident that oil was present at depths where extremely reducing conditions indicated the lack of oxygen (Aber Wrac'h and the Ile Grande oiled site), as well as in surface sediments which were more likely exposed to oxygen. This provided an opportunity to compare weathering patterns in sediments with markedly different exposure to oxygen. Since the actual amount of oil could vary between sites, evidence of weathering was sought by comparing the relative amounts of hydrocarbons extracted from single samples known to be polluted with AMOCO CADIZ oil. Because of the rapid and extensive biodegradation which apparently followed the AMOCO CADIZ spill (Atlas, et al, 1981; Ward et al, 1980) the comparison of n-alkanes to the more recalcitrant isoprenoid alkanes of similar volatility was not possible as an index of biodegradation. By the first sampling date (December 1978), n-C17/

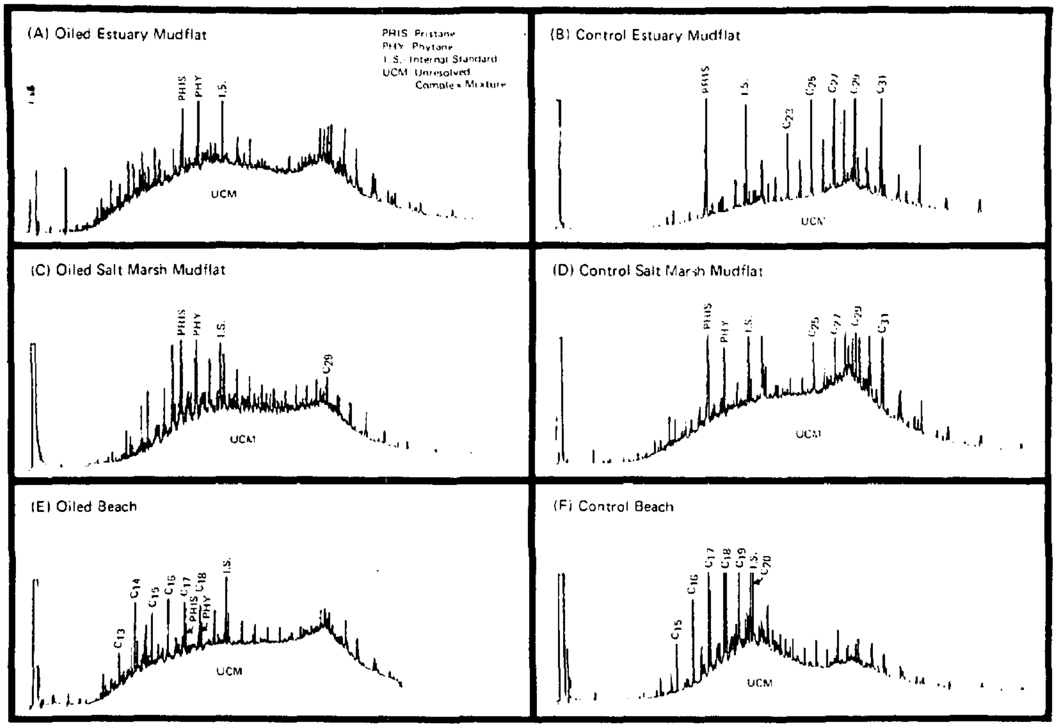

FIGURE 4. Gas chromatograms of saturate fraction of hydrocarbons extracted from Brittany sediments collected in March 1979 (0-5 cm depth interval).

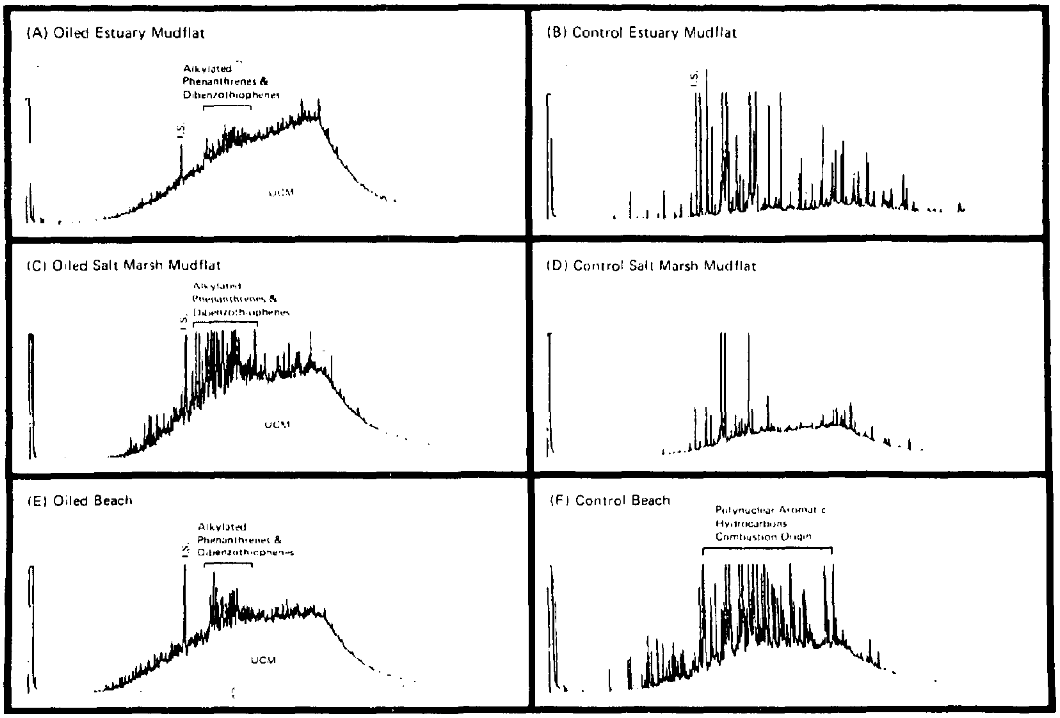

FIGURE 5. Gas chromatograms of aromatic/unsaturate fraction of hydrocarbons extracted from Brittany sediments collected in March 1979 (0-5 cm depth interval).

pristane and n-C18/phytane which were 3.3 and 2.8 in the reference mousse, were 0.07-0.58 and 0.024-0.36, respectively. Becase of the relative persistence of aromatic hydrocarbons (Atlas, et al, 1981; Ward et al, 1980), alkylated naphthalenes, phenanthrenes and dibenzothiophenes remained in Brittany sediments at levels sufficient to study weathering patterns in a long term time/depth series. The loss of these compounds relative to the more persistant C_3-alkylated derivatives of dibenzothiophene (C_3-DBT) is thought to be an index of weathering (Grahl-Nielsen, et al, 1978). There is no published evidence that changes in aromatic compounds relative to C_3-DBT are due to biodegradation, but these compounds are subject to microbial attack (Atlas, 1981).

The ratios of aromatic compounds to C_3-DBT showed different changes with time depending on type of sediment and sediment depth (Table 3). In the AMC-4 0-5 cm interval there was a systematic decrease in nearly all ratios from December 1978 to March 1979 to November 1979. In muddy sediments decreases were less rapid and extensive. By March 1979, C_3- and C_4- naphthalenes, C_3- and C_4- phenanthrenes and C_1-DBT showed relative decreases in the Aber Wrac'h 0-5 cm interval. An Aber Wrac'h core collected in November 1979 was subdivided at one centimeter intervals at the depths of greatest change in Eh. In the 1-2, 2-3, and 3-4 cm intervals naphthalenes were almost entirely depleted. Relative decreases in all except C_3- and C_4- phenanthrenes were noted compared to earlier samples, with no great differences among these depth intervals. Phenanthrenes were enriched relative to C_3-DBT by May 1980 in the 0-3 cm interval. Ratios of aromatic hydrocarbons to C_3-DBT were generally greater in deeper layers (5-10, 10-15 cm) than in surface layers on any given date. Nevertheless, the data suggest that decreases in naphthalenes and DBT's occurred in the 5-10 cm interval in the one year between December 1978 and November 1979, despite the relative constancy of C_3-DBT over the time period. Results for Ile Grande were similar to those for Aber Wrac'h but the relative amounts of most components were greater. Between December 1978 and March 1979, there was a relative enrichment of all components except C_4-naphthalene. In March 1979, ratios were higher at 5-10 cm than at 0-5 cm. Finer resolution around the depths of greatest Eh change was attempted on samples collected in May 1980. There was evidence of decreases in naphthalenes compared to earlier samples at all depths, even though the concentration of C_3-DBT was very high in all samples. Relative decreases in C_1-phenanthrenes and DBT's were noted in the 0-2 mm and 2-10 mm intervals. Except for C_4-phenanthrenes, ratios were always higher in the 3-4 cm interval.

Hydrocarbon Biodegradation

In initial experiments, various ^{14}C-labelled aliphatic or aromatic hydrocarbons were incubated with surface sediments under aerobic conditions, or with subsurface sediments under conditions designed to prevent the exposure of obligately anaerobic bacteria to oxygen. The success of anaerobic methods was evidenced by the reduction of $^{35}SO_4^=$ to

TABLE 3. Aromatic Hydrocarbons in Brittany Sediments Oiled by the Spill

STATION DATE	DEPTH	RATIOS TO C_3-DIBENZOTHIOPHENES									ng/g C_3-DBT
		NAPTHTHALENES			PHENANTHRENES				DBT'S		
		C_2	C_3	C_4	C_1	C_2	C_3	C_4	C_1	C_2	
AMC-4											
12/78	0-5cm	.17	.52	.67	.28	.29	.48	.36	.35	1.08	785
3/79	0-5cm	.02	.21	.38	.04	.23	.30	.17	.32	.97	735
11/79	0-5cm	.01	.01	.01	.10	.20	.28	.08	.01	.38	400
ABER WRAC'H											
12/78	0-5cm	.03	.19	.29	.05	.16	.35	.18	.16	.84	3,976
	5-10cm	.02	.51	.54	.16	.34	.39	.24	.35	1.14	811
	10-15cm	.05	.17	.20	.28	.42	.39	.36	.19	.90	275
3/79	0-5cm	.03	.18	.18	.13	.18	.32	.08	.01	.92	1,598
	5-10cm	.06	.22	.51	.22	.33	.42	.12	.16	1.64	831
		.04	.18	.32	.21	.34	.31	.24	.19	1.01	492
11/79	1-2cm	0	0	0	.10	.08	.33	.28	.01	.63	2,400
	2-3cm	0	0	.01	.05	.10	.32	.23	.01	.59	2,200
	3-4cm	0	0	0	.02	.05	.05	.26	0	.44	1,800
	5-10cm	0	.02	.09	.14	.23	.59	.53	.06	0.60	860
5/80	0-3cm	0	0	.04	.13	.20	.76	1.00	.04	.42	1,700
ILE GRANDE OILED											
12/78	0-5cm	.04	.22	.32	.13	.22	.28	.22	.16	.91	2,809
3/79	0-5cm	.10	.36	.37	.24	.38	.32	.22	.54	1.37	2,666
	5-10cm	.34	.88	.76	.46	.42	.46	.27	.63	1.44	745
5/80	0-2mm	0	.01	0	.05	.24	.48	.56	.09	.63	16,000
	2-10mm	0	0	.05	.06	.19	.42	.45	.07	.62	12,000
	3-4cm	.007	.07	.18	.09	.28	.52	.35	.15	.82	82,000

$H_2{}^{35}S$, and in some cases the generation of $^{14}CH_4$ within two hours of incubation (Winfrey and Ward, submitted). In long-term incubations no $^{14}CO_2$ or $^{14}CH_4$ was detected in formalin-killed controls. In most cases significant amounts of $^{14}CO_2$ + $^{14}CH_4$ were detected in vials incubated aerobically (Table 4). Anaerobic incubation severely reduced the amount of radiolabelled gases formed. However, small amounts of these metabolites were formed from n-hexadecane, n-heptadecane, heptadecene, ring or methyl labelled toluene, and benzene after lengthy anaerobic incubation. The amount of gaseous metabolites formed did not exceed 5% of the added radiolabel and reproducibility was poor. Repeated efforts by two individuals experienced in cultivation of methanogenic bacteria led to the same observations. Additions of $FeCl_3$ or KNO_3 (1 ml of 0.5% (w/v) solutions in anoxic ASW replaced the 1 ml addition of anoxic ASW) did not stimulate the formation of $^{14}CO_2$ and $^{14}CH_4$ from n-hexadecane or heptadecene in Aber Wrac'h 5-10 cm sediment.

The possibility of initial accidental exposure to oxygen during tubing of samples was investigated by late addition of ^{14}C-toluene which was soluble in water and could be added as an anoxic solution well after any oxygen initially present should have been consumed during dark incubation. Revsbech, et al, (1980b) have shown that oxygen consumption in intertidal sediments occurs in a matter of minutes following darkening to eliminate photosynthesis. As shown in Figure 6, ring-^{14}C-toluene was readily metabolized to $^{14}CO_2$ when added either at the time of anaerobic tubing or 38 hours after dark anaerobic incubation began. Similar results were found for [methyl-^{14}C]-toluene.

It was conceivable that the radiolabelled gases might have been produced from contaminants rather than from the hydrocarbons themselves. When an attempt was made to recover the added ^{14}C in long-term radiolabelling experiments with [1-^{14}C]-heptadecane and [1-^{14}C]-heptadecene, it was noted that the total amount of $^{14}CO_2$ + $^{14}CH_4$ produced during anaerobic incubations was similar to the level of impurities measured in ^{14}C-labelled hydrocarbons recovered from formalin controls or from unpurified stocks of added radioisotopes (Table 5). Stock solutions were chromatographically separated into f_1, f_2 and f_3 components which were then tested separately as sources of ^{14}C-gases in dark anaerobic incubations with anaerobic sediments. The results of such experiments are presented in Figure 7. The repurified f_1 fractions of [1-^{14}C] hexadecane and [1-^{14}C]-heptadecane were clearly significant sources for production of $^{14}CO_2$ during dark anaerobic incubations with a slurry of Ile Grande oiled 3-6 cm sediment. Increases in $^{14}CO_2$ with time following a lag of 5-15 days also suggested that oxidation did not result from any oxygen which might have been introduced accidentally during tubing. Similar results were observed with repurified f_1 of [1-^{14}C]-heptadecene.

A final control was run to test the possibility that slow diffusion of oxygen through the vessels containing incubating samples could account for the observed metabolism. Dark anaerobic incubations of repurified f_1 of [1-^{14}C]-hexadecane and [1-^{14}C]-heptadecane were carried out with a slurry of mud from the 3-6 cm interval of Aber Wrac'h sediment. The individual vials were incubated inside an anaerobic

TABLE 4. $^{14}CO_2$ + $^{14}CH_4$ produced on 223 Day Incubation of Oiled Sediments with ^{14}C-Hydrocarbons (12/78).

	% OF ADDED $^{14}C^a$								
	ABER WRAC'H			ILE GRANDE			AMC-4		
HYDROCARBON	0-5cm AEROBIC	5-10cm ANOXIC	10-15cm ANOXIC	0-2cm AEROBIC	2-7cm ANOXIC	7-12cm ANOXIC	0-5cm AEROBIC	8-13cm ANOXIC	13-18cm ANOXIC
1-^{14}-Hexadecane	33	3	?[b]	45	?	?		?	?
1-^{14}C-Heptadecane	25	1	0	34	0	?	20	0.5	0.6
1-^{14}C-Heptadecene	28	0	?	34	0	0	72	0.4	1.4
Ring-^{14}C-Toluene	27	2	1.4	34	2.3	2.9	28	0	?
Methyl-^{14}C-Toluene	18	?	?	29	5	1.4	23	?	?
1-(4,5,8)-^{14}C-Naphthalene	72	0	0	78	0	0			
U-^{14}C-Benzene	26	0	2	45	0	0	25	0	0
7,10,-^{14}C-Benzo(a)pyrene	3	0	0	0	0	0		0	0

[a] Initial levels ranged from 0.22-2.2 x 10^6 DPM/vial

[b] Indicates that $^{14}CO_2$ was apparently present but was not quantifiable because levels were near detection limits

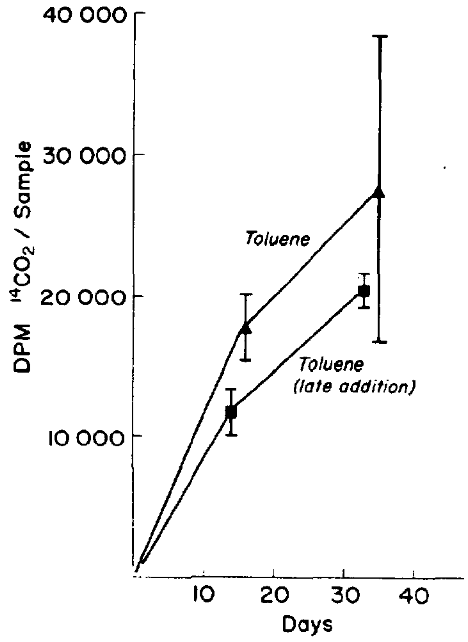

FIGURE 6. $^{14}CO_2$ production from [ring-^{14}C]-toluene during dark anaerobic incubation with sediments from the Aber Wrac'h 5-10 cm interval. Radiolabel was added at the beginning of the incubation period or 38 hours after incubation began (late addition). Bars indicate one standard deviation.

TABLE 5. Recovery of ^{14}C following long term incubation with sediment collected at Aber Wrac'h, March 1979

COMPOUND/DEPTH	% RECOVERED IN					
	CO_2	CH_4	CO_2+CH_4	F_1	F_2	F_3
1-^{14}C-Heptadecane						
Mean of Controls	--	--	--	98.6	0.5	0.8
0-5cm Aerobic	6.0	3.0	(9.0)	89.4	0.5	1.2
5-10cm Anoxic	3.1	0.3	(3.4)	95.7	0.4	0.5
10-15cm Anoxic	3.9	--	(3.9)	94.9	0.5	0.6
1-^{14}C-Heptadecene						
Mean of Controls	--	--	--	91.5	2.9	5.6
0-5cm Aerobic	3.5	6.2	(9.7)	84.0	2.9	3.5
5-10cm Anoxic	3.9	1.2	(5.1)	89.0	2.2	3.6
Unpurified Radioisotope				87.8	5.6	6.6
Repurified Radioisotope				99.4	0.06	0.5

chamber sealed under a H_2 + CO_2 atmosphere. As shown in Table 6 these incubation conditions did not prevent the slow, albeit variable, generation of $^{14}CO_2$.

Effects of Oiling on Anaerobic Process

Evidence for effects due to AMOCO CADIZ oiling was first sought by comparing anaerobic processes at sites oiled or not oiled by this spill. Sulfate reduction dominated methane production at all sites (the ratio of sulfate reduction rate to the sum of sulfate reduction and methane production rate ranged from 0.951-0.998, even though methane production may have been overestimated). Thus, major changes in the type of terminal process were not evident during the period of our experimentation. The potential use of acetate by either of these two

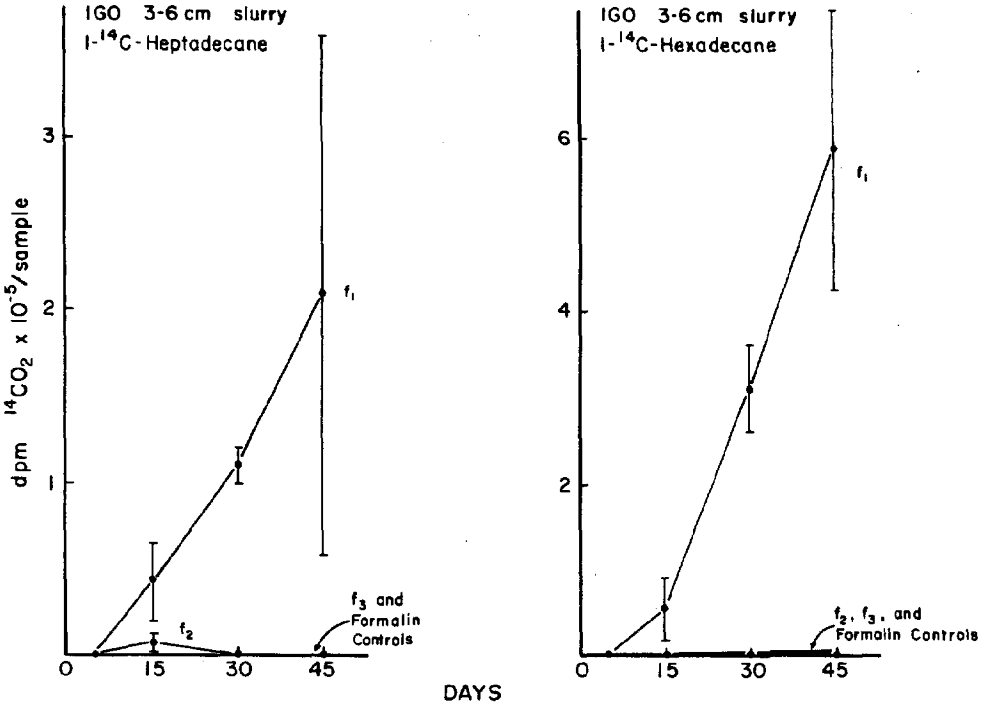

Figure 7. $^{14}CO_2$ production during dark anaerobic incubations of repurified f_1, f_2 and f_3 fractions of radiolabelled hydrocarbons with a slurry made from the 3-6 cm interval of Ile Grande oiled sediment. Bars indicate one standard deviation.

TABLE 6. Recovery of ^{14}C following dark incubation with a 3-6 cm Aber Wrac'h sediment slurry in double anaerobic incubator (± 1SD)

COMPOUND	% RECOVERED IN			
	CO_2	F_1	F_2	F_3
1-^{14}C-Hexadecane	3.94±3.48	96.1±3.48	0	0
1-^{14}C-Heptadecane	16.4±23.6	83.4±23.6	0	0.2

groups makes [2-^{14}C]-acetate metabolism a useful means of differentiating the importance of bacterial processes which accomplish its oxidation to $^{14}CO_2$ (sulfate reduction) or its conversion to $^{14}CH_4$ (methane production, see Fig. 1). [2-^{14}C]-acetate was metabolized only to $^{14}CO_2$ at all sites except in the surface layer of the oiled Ile Grande site where small amounts of $^{14}CH_4$ were detected on one occasion (March, 1979). This observation, however, was not repeated at later sampling dates. Rates of sulfate reduction were highest in the surface layer and decreased with depth in all sites (Fig. 8). Rates in the 0-3 cm intervals were higher in oiled compared to control Aber mudflat sediments ($p = .004$). In the beach and salt marsh mudflat sediments, rates were higher in control sites than in oiled sites ($p < .001$). It is not possible, however, to attribute differences to the presence of AMOCO CADIZ oil since other differences between sites (e.g., amount of organic loading) could also explain differences in sulfate reduction.

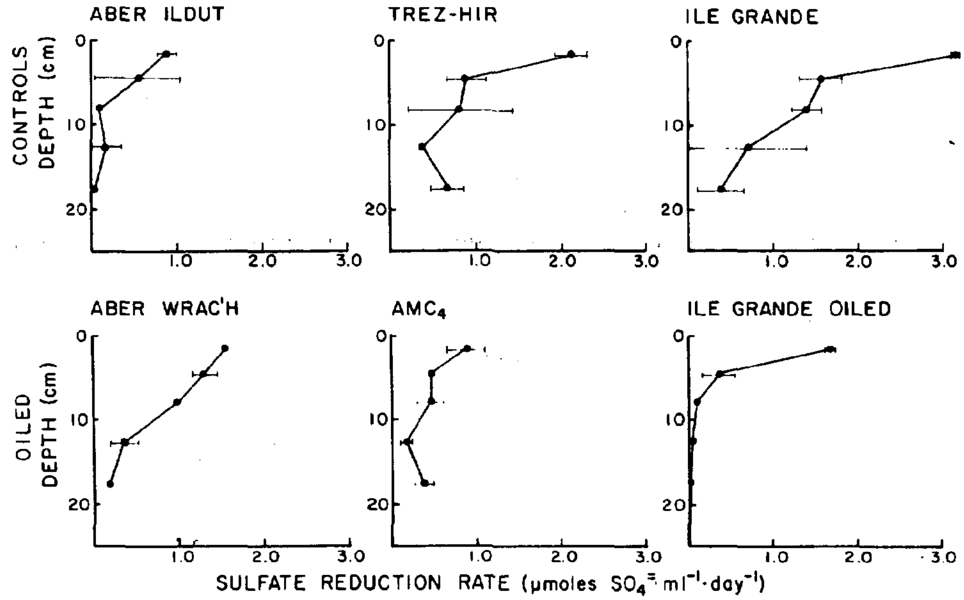

Figure 8. Depth profiles for rates of sulfate reduction in oiled and control sediments. Bars indicate one standard deviation.

To more directly observe the effect of oiling on microbial activities in sediments, AMOCO CADIZ mousse was added to Ile Grande sediments, and activities compared between mousse-treated and untreated sediments. Gas chromatograms of the saturate and aromatic fractions of this mousse are shown in Figures 9 and 10, respectively. These tracings can be compared to fresh light Arabian Crude in Figures 9 and 10. In the saturate fraction of the mousse, no hydrocarbons below C-12 were detected. In the aromatic fraction all predominant compounds were gone and only a UCM remained. Thus, extensive weathering of the low molecular weight compounds in the mousse had occured before collection. Extensive biodegradation of aliphatic compounds was not suggested as evidenced by the dominance of normal compared to isoprenoid alkanes.

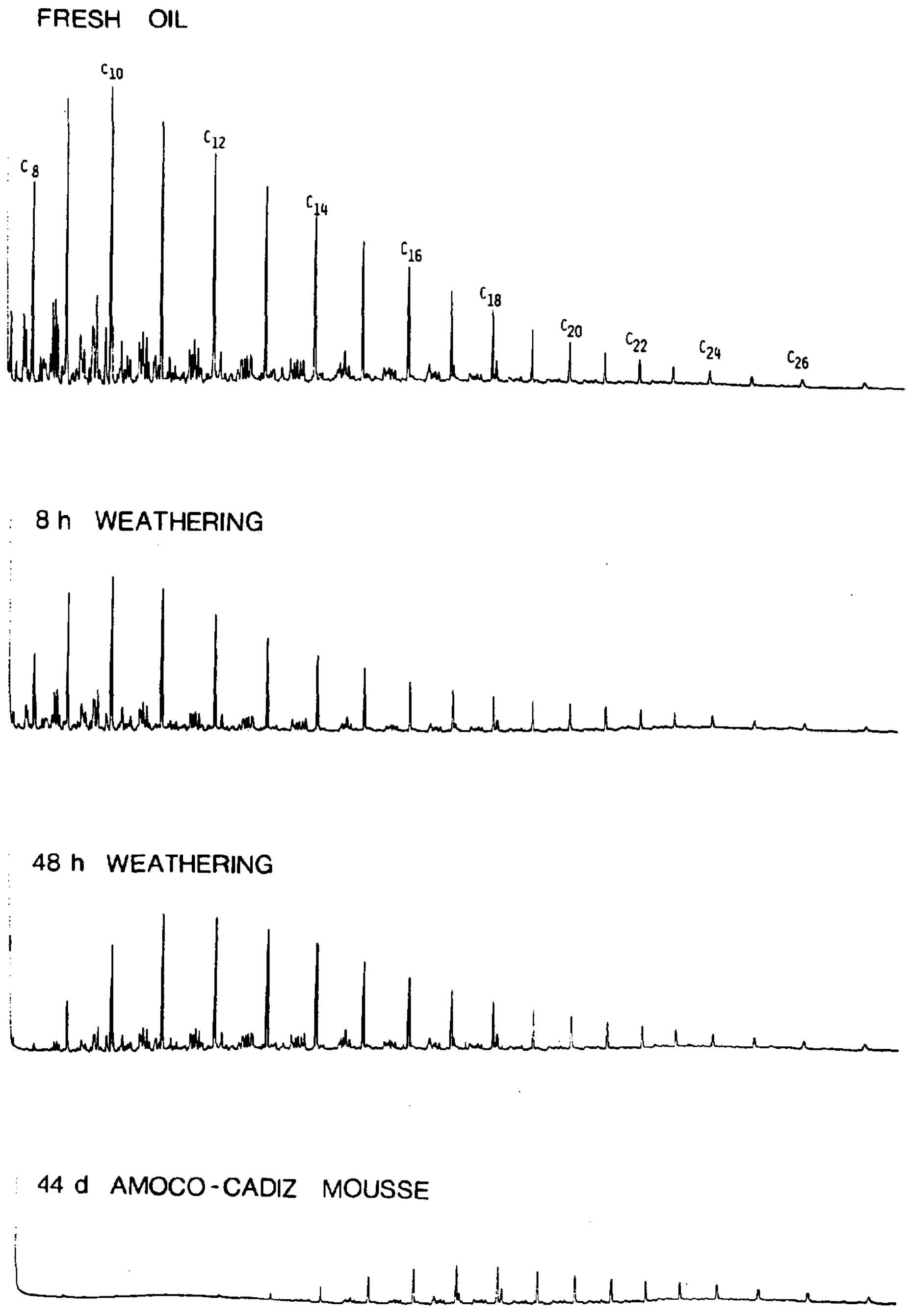

Figure 9. Gas chromatograms of saturate fraction of light Arabian crude oils and Amoco Cadiz mousse.

Table 7 shows the effect of the addition of 5% and 25% mousse (v/v) on sulfate reduction in sediments from the oiled and unoiled site at Ile Grande. A slight inhibition of sulfate reduction rate was observed at the control site, while a slight stimulation was observed at the oiled site. These rates, however, were not significantly different from the unoiled controls ($p \geqq 0.08$). No significant differences in methane production between control sediments and sediments treated with

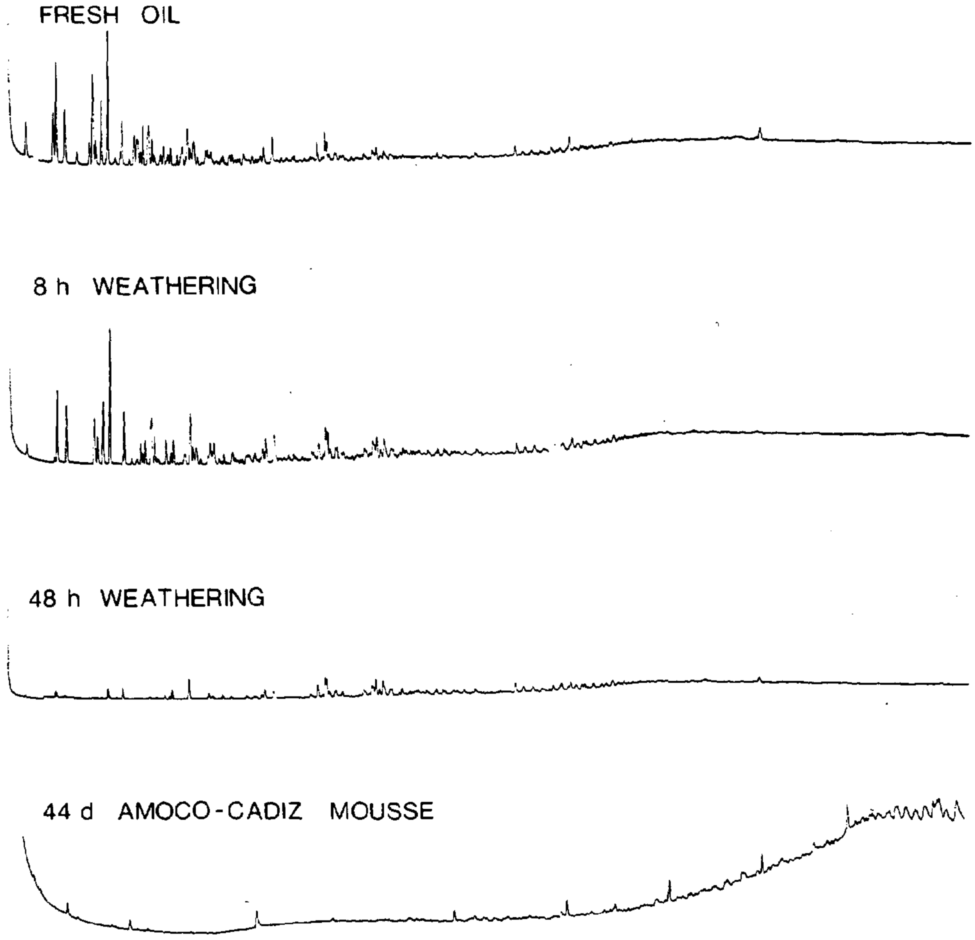

Figure 10. Gas chromotograms of aromatic fraction of light Arabian crude oils and Amoco Cadiz mousse.

mousse were observed at either site (data not shown). Table 8 shows the effects of mousse additions on [2-^{14}C]-acetate oxidation to $^{14}CO_2$. No $^{14}CH_4$ was detected in any of the samples. At the unoiled control site, the addition of 5% mousse decreased the amount of $^{14}CO_2$ produced in 2h by 70% ($p = 0.02$), while an 86% inhibition was observed with the additions of 25% mousse ($p = 0.01$). At the oiled site at Ile Grande, mousse additions appeared to inhibit $^{14}CO_2$ production from [2-^{14}C]-acetate although these differences were not significantly different from the control ($p \geqq 0.25$).

The effect of fresh and slightly weathered light Arabian crude oil on microbial activities in sediments was also examined because of the probable chemical differences between the highly weathered mousse and the oil which impacted the sites studied. The effect of 0.05% benzene and toluene was also examined. They are highly volatile aromatic compounds with known inhibitory effects (Robertson et al, 1973). Figure 9 shows chromatograms of the saturate fraction of the fresh and weathered light Arabian crude. The fresh oil contained large amounts of low

TABLE 7. Effect of AMOCO CADIZ Mousse on Sulfate Reduction in Marsh Sediments[a]

Addition	Ile Grande Control Rate[b]	Ile Grande Control % of Control	p	Ile Grande Oiled Rate[b]	Ile Grande Oiled % of Control	p
Control	4.99	100%	-	1.64	100%	-
5% Mousse	3.16	63	.08	2.36	144	.15
25% Mousse	3.53	71	.14	1.67	102	.95

[a] Sediment samples were collected in November, 1979.

[b] Rates are the mean of 3 replicates and expressed as µmoles $SO_4^{=}$-S/ml/d.

TABLE 8. Effect of Mousse on [2-^{14}C]-Acetate Metabolism to $^{14}CO_2$ in Marsh Sediments

Addition[a]	Ile Grande Control $^{14}CO_2$[b]	Ile Grande Control % of Control	p	Ile Grande Oiled $^{14}CO_2$[b]	Ile Grande Oiled % of Control	p
Control	513	100%	-	184	100%	-
5% Mousse	156	30%	.02	115	63%	.41
25% Mousse	72	14%	.01	78	42%	.25

[a] Sediment samples were collected in November, 1979.

[b] Mean of 3 replicates expressed as DPM x 10^{-3}.

molecular weight compounds which decreased relative to less volatile n-alkanes (e.g., C-24) in the 8h and 48h weathered oil. Octane and other compounds of similar volatility were nearly depleted after 48h of evaporative weathering. Figure 10 shows the aromatic fractions of each of the oils used. After 8h of evaporative weathering, toluene and several other volatile aromatic compounds were significantly reduced, and after 48h most of the predominant volatile aromatics were evaporated.

Table 9 reports the effect of these oil and aromatic additions on sulfate reduction rates in Ile Grande surface sediments. At the unoiled control site, all oil additions decreased the rate of sulfate reduction although not significantly below the control ($p \geqq 0.16$). The greatest inhibition was observed for toluene and benzene additions ($p = 0.10$ and 0.09 respectively). At the oiled site, rates under all conditions were not significantly different from the control rate. The effect of the oil, benzene, and toluene additions on methanogenesis (results not shown) were variable. None of the additions resulted in a rate of methanogenesis that was significantly different than the rate without additions.

TABLE 9. Effect of Hydrocarbons on Sulfate Reduction in Marsh Sediments[a]

	Ile Grande Control			Ile Grande Oiled		
Addition	Rate[b]	% of Control	p	Rate[b]	% of Control	p
Control	2.27	100%	-	1.01	100%	-
10% Fresh Oil	1.67	73	0.95	0.85	84	0.08
10% 8 h Weathered Oil	1.57	71	0.16	0.87	86	0.11
10% 48 h Weathered Oil	1.82	82	0.97	1.07	106	0.48
.05% Toluene	1.42	64	0.10	1.11	110	0.23
.05% Benzene	1.32	59	0.09	1.13	112	0.15

[a] Sediment samples were collected in April, 1980.

[b] Rates are the mean of 3 replicates and expressed as µmoles $SO_4^=$-S/ml/d.

The effect of the oil and aromatic additions on [2-^{14}C]-acetate oxidation to $^{14}CO_2$ in Ile Grande is shown in Table 10. At both the oiled and unoiled site, the amount of $^{14}CO_2$ produced in 2 h was significantly reduced ($p \leqq 0.01$) with all of the additions. In general, the magnitude of inhibition was greater at the control site (76-97%) than at the oiled site (51-93%). In both sites inhibition was greatest with the unweathered oil and decreased with the extent of weathering of the oils.

TABLE 10. Effect of Hydrocarbons on $[2\text{-}^{14}C]$-Acetate Metabolism to $^{14}CO_2$ in Sediments[a]

Addition	Ile Grande Control $^{14}CO_2$[b]	Ile Grande Control % of Control	p	Ile Grande Oiled $^{14}CO_2$[b]	Ile Grande Oiled % of Control	p
Control	358	100%		244	100%	
Fresh Oil	10	3	0.00	17	7	<0.001
8 h Weathered Oil	44	12	0.01	66	27	<0.001
48 h Weathered Oil	87	24	0.01	119	49	<0.001
Toluene	29	8	0.002	86	35	<0.001
Benzene	31	9	0.002	65	27	<0.001

[a] Sediment samples collected in April, 1980.

[b] Mean of 3 replicates expressed as DPM x 10^{-3}.

DISCUSSION

The major objectives of this study were to address the fate and effects of hydrocarbons from the AMOCO CADIZ spill in anaerobic sediments. It is first necessary to consider the chemistry of the various intertidal sediments with respect to exposure to oxygen. A great deal has been learned recently due to the application of microelectrodes to the study of oxygen distribution and dynamics in marine subtidal and submerged intertidal sediments (Revsbech, et al, 1980a, b). A variety of sediments (including medium—grained sands) exhibited very narrow oxygenated intervals ranging from 2-10 mm below the sediment water interface. Oxygen depletion has been measured to occur above the vertical discontinuity of Eh in coastal sediments (Revsbech, et al, 1980a). Thus the Eh profile of a sediment may serve as a conservative estimate for the aerobic/anoxic boundary. Eh profiles then indicate that anoxic conditions were likely below 1 cm in the Ile Grande oiled site and below 2-3 cm in Aber Wrac'h sediment. The real depth of oxygen penetration at any given time is likely to be less. Changes in color from brown to black at about 2 mm in the Ile Grande oiled sediment may indicate an extremely narrow aerobic zone. Similar color changes in Aber Wrac'h at about 2-3 cm may indicate that net oxygen penetration in this sediment is deeper, but oxidants other than oxygen could keep Eh higher. The Eh profiles were measured on undisturbed sediments and probably reflect the chemistry of sediments under relatively calm conditions. Mixing which occurs as a result of tidal or storm driven wave action might alter the depth to which oxygen can penetrate sediments. This should vary with the nature of the sediment so that muds should be less affected than sandy sediments on high-energy beaches. Over seasonal time intervals, it is likely that the aerobic/anoxic boundaries predicted by Eh profiles are preserved in the muddy sediments sampled. However, it is likely at the oiled beach AMC-4 that erosion and deposition created considerable instability in the depth of oxygen penetration and could even have caused vertical redistribution of sediments (Gundlach and Hayes, 1978). It is also possible that oxygen could be introduced to depths below the lower boundary of its diffusion by sediment infauna which can burrow into anaerobic sediments.

The vertical distribution of the dominant anaerobic process, sulfate reduction, indicated maximum activity in the surface 0-3 cm interval at all stations. The obligately anaerobic sulfate-reducing and methane-producing bacteria were also present in maximum number in the 0-3 cm depth interval (Winfrey and Ward, submitted). These observations suggest that at least portions of the 0-3 cm interval at all sites were sufficiently anoxic to allow survival and activity of obligately anaerobic microorganisms.

A survey of the various sediments sampled confirmed the presence of AMOCO CADIZ pollutants in oiled sites. Although control sites were not polluted by the AMOCO CADIZ spill, each contained some hydrocarbons of anthropogenic origin. The extent of oiling was greatest at the sediment surface where anaerobic processes were greatest. Oiling decreased with depth, but there was clear evidence of AMOCO CADIZ hydrocarbons in sediments likely to be free from exposure to oxygen. Sediments below the aerobic/anoxic boundary and above the deepest level of penetration of AMOCO CADIZ pollutants provided an environment suitable for the enrichment of anaerobic hydrocarbon-degrading microorganisms, and appropriate for comparison to aerobic surface sediments to study the differences in weathering in situ due to different exposures to oxygen.

Because of the rapid biodegradation of aliphatic components and relative enrichment of aromatic components of the spilled oil (Atlas, et al, 1981; Ward, et al, 1980), ratios of naphthalenes, phenanthrenes and dibenzothiophenes to the more persistent C_3-DBT were used as an index of weathering. This index should be independent of absolute amounts of oil within sediment samples which could vary due to patchy distribution of oil. Changes in sediment aromatic hydrocarbons occurred in all sediments and at all sediment depths where comparisons were made for one year or longer. The greatest and most rapid changes were noted in surface sediments of the beach station AMC-4 where most compounds had decreased by one year after the spill and extensive losses had occurred by about 20 months after the spill. This seems consistent with the expected mixing and oxygenation of this high energy beach sediment. In muddy sediments, slower changes in relative amounts of aromatic compounds were noted. Extensive losses were observed mainly among the naphthalenes and DBT's. This may have been related to the low energy nature of these sediments and/or the corresponding lack of oxygenation indicated by reducing conditions. Decreases in these compounds were, however, noted at depths below the minimum Eh 20-26 months after the oil spill. It is difficult to rule out exposure to oxygen in these subsurface muddy-sediment environments. The degree of storm driven mixing and/or irrigation by sediment fauna are unknown and might be significant over a 12-18 month time course. More than two years after the AMOCO CADIZ spill many well resolved aromatic compounds persisted (e.g., phenanthrenes); however, the slow disappearance of some aromatic hydrocarbons (e.g., naphthalenes) from subsurface muds may indicate that these resolved compounds will not persist indefinitely.

The potential biodegradation of hydrocarbons measured using ^{14}C-labelled hydrocarbons was much lower under anaerobic than under aerobic conditions. For example, 33% of added [1-^{14}C]-hexadecane was converted to ^{14}C-gases aerobically, whereas only 3% was converted anaerobically after 233 days in Aber Wrac'h sediment (Table 4). Due to the expected high oxygen demand of the surface sediments, it is likely that O_2 depletion occurred rapidly in vials incubated aerobically. Thus, it is not surprising that complete conversion of added ^{14}C-hydrocarbons to ^{14}C-gases did not occur in long term aerobic incubations. Since the added radiolabelled hexadecane (4.2 μg/sample) exceeded the indigenous hexadecane measured in these sediments (16-174 ng/sample), potential rates of aerobic and anaerobic metabolism can be calculated by multiplying the percentage conversion by the amount of hexadecane added and dividing by the number of days incubation and dry weight of the sample. The maximum level of ^{14}C-gases produced under aerobic conditions was detected at the earliest analysis time (66 days). This leads to a calculated rate of 13.8 ngm/gm-dry weight/day. The true potential rate is probably greater due to incomplete exposure of the entire sample to oxygen and to oxygen depletion. Calculations using rates of oxygen consumption for European coastal sediments suggest that oxygen should have been completely consumed within the first ten days of incubation. The corresponding rate would be 91 ngm/gm-dry weight/day, approximately one-fifth of the potential rate reported by Atlas and Bronner (1981). ^{14}C-gases increased with time during anaerobic incubation. The corresponding potential anaerobic rate of hexadecane metabolism after 233 days incubation of 0.3 ngm/gm-dry weight/day is 46 times slower than the measured potential aerobic rate and over 300 times slower than the aerobic rate calculated from reasonable assumptions about the conditions under which aerobic controls were run. These results are similar to other reports which demonstrate the severe limitations on hydrocarbon metabolism imposed by reduced amounts or lack of oxygen (Ward and Brock, 1978; Hambrick, et al, 1980; DeLaune, et al, 1981). It was interesting that no evidence was obtained for anaerobic naphthalene oxidation. Obvious problems of naphthalene volatility may have decreased the amount actually added to vials thereby lowering the sensitivity for detecting its oxidation.

As in earlier studies (Ward and Brock, 1978), it was not possible to eliminate metabolism of hydrocarbons under stringent anaerobic conditions. It was important to investigate the possibility that slow anaerobic oxidation might occur in order to predict whether or not hydrocarbons buried in permanently anoxic sediments persist indefinitely. Controls were run against the possibility of initial accidental inclusion of oxygen, photosynthetic oxygen production, oxygen leakage into experimental vials during incubation, and against the possibility that compounds contaminating radiolabelled hydrocarbons were the sources of ^{14}C-gases. The results of these experiments were consistent with the conclusion that slow anaerobic oxidation of some petroleum hydrocarbons may be occurring in anoxic sediments polluted with AMOCO CADIZ oil.

A second major objective of this work was to study the effects of AMOCO CADIZ oil on the dominant anaerobic processes within sediments. As mentioned above, oiling was heaviest in the surface sediments where anaerobic processes occurred at maximum rates. We were unable to monitor any immediate effects of the AMOCO CADIZ spill because our first sampling trip was in December 1978, 9 months after the spill. We were also limited by the lack of any data on the microbial activities in our sampling sites prior to the oil spill. However, by examining unoiled sites, we were able to see if any major alterations in chemistry and activities had occurred in the oiled sediments.

Comparisons between oiled and unoiled sites revealed lower rates of sulfate reduction in oiled beach and marsh sediments. It is not possible, however, to attribute these differences to the presence of AMOCO CADIZ oil because 1) inhibition was not observed at all sites where oiling occurred, 2) the magnitude of differences observed was small (largest difference was 51% of the control), and 3) other differences between sites which could influence sulfate reduction rate (e.g., organic loading) were unknown. It is also difficult to interpret whether rates measured in control sites were typical of unpolluted sediments, as hydrocarbon analyses revealed a previous history of oiling in control sediments. No differences in methane production or acetate metabolism were noted. Although preliminary results demonstrated small amounts of $^{14}CH_4$ production from [2-^{14}C]-acetate at the Ile Grande oiled site (Winfrey and Ward, 1981), this observation was not confirmed in subsequent work. Sulfate reduction always dominated methane production and acetate was metabolized only to $^{14}CO_2$. The lack of profound differences in comparative experiments performed 9-18 months following the spill, suggest that no long-term effects on anaerobic processes occurred in these sediments.

We examined the effect of an unweathered oil on anaerobic processes in order to determine whether any short-term effects of oiling might have occurred. A fresh light Arabian crude oil did not significantly effect sulfate reduction or methane production, but inhibited acetate oxidation 93-97% in oiled and control sediments from Ile Grande. The ability of the crude oil to inhibit acetate oxidation was reduced in oil samples which had been evaporated to increasing degrees. Highly volatile molecules such as toluene and benzene caused 91-92% inhibition in control sediments and 65-73% inhibition in oiled sediments. The data suggest that volatile components of the oil may be responsible for inhibitions to acetate oxidation. However, mousse collected near Ile Grande 44 days after the AMOCO CADIZ spill also inhibited acetate oxidation at the Ile Grande control site. This mousse sample had lost most of its more volatile components, but was apparently not extensively altered by biodegradation. Inhibitions were always greater at the control site than at the oiled site. Thus, the oiled area of the marsh appears to have become less sensitive to the effects of additional heavy oiling. This may be a result of an adaptation of microorganisms to the presence of oil, or to a selection of oil resistant populations.

It is not surprising that hydrocarbons did not directly inhibit sulfate reduction since this process appears to be active in oil formation waters (Bailey, et al., 1973; ZoBell, 1958). However, it was surprising that compounds which inhibited acetate oxidation did not directly inhibit sulfate reduction, as other investigations have pointed to the importance of acetate as a substrate for sulfate reduction (Winfrey and Ward, submitted; Sϕrensen et al, 1981; Banat and Nedwell, personal communication).

The chemistry of hydrocarbons present in the various sediments one year after the spill indicated the presence of oil highly altered by evaporation and biodegradation. The levels observed in the environment were also lower (0.1-1 mg/g) than the levels added in our experiments to simulate heavy oiling (50-250 mg/g). It is possible that a temporary inhibition of acetate oxidation could have resulted from very heavy oiling of relatively fresh oil. Such conditions could have existed at all polluted sites immediately following the AMOCO CADIZ spill, although rapid loss of volatile compounds probably occurred between spillage and beaching of oil (Dowty, et al, 1981; Ward, et al, 1980). Any inhibitory effect would then have been reduced as cleanup or transport of hydrocarbons out of the sediments decreased hydrocarbon amount, and as evaporation, dissolution and biodegradation altered the remaining sediment hydrocarbons. By the time site comparison experiments could be performed, recovery from any negative effects which might have occurred had apparently taken place. The inhibitory effects on acetate oxidation we observed may be significant in extremely cold regions where slow rates of evaporation would occur.

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PART II

Biological Studies
After the AMOCO CADIZ Oil Spill

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REPONSES DES PEUPLEMENTS SUBTIDAUX A LA PERTURBATION CREEE PAR L'*AMOCO CADIZ* DANS LES ABERS BENOIT ET WRAC'H

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ABSTRACT

During three years after the Amoco-Cadiz oil-spill, the succession in time of different ecological groups with regard to excess of organic matter, allow to define chronological process. First, total disappearance of sensible and tolerant species by toxicity. When pollution is stabilized, we describe appearance, development and regression of an opportunist fauna, finally the excessive development of tolerant species before return to a new equilibrium. This temporal succession is studied along two different gradient of decreasing hydrodynamism, the abers, where the chemical decontamination and the biological process are not synchronized. Near three years after the oil-spill most communities are still perturbated and unbalanced. Patterns of temporal evolution and succession is discussed.

INTRODUCTION

Les deux Abers, Benoît et Wrac'h, sont situés à proximité de l'échouage de l'Amoco-Cadiz (Fig. 1). C'est l'aire qui a été la plus affectée par la marée noire. Depuis, de nombreuses études ont concerné ces abers et l'analyse des successions écologiques au sein des communautés de l'endofaune s'est avérée très intéressante dans le cas d'études à moyen et long terme. Sont apparues des fluctuations temporelles non saisonnières mais évolutives, qu'il est possible de synthétiser trois ans après l'échouage.

Après la destruction quasi-complète des communautés d'origine, la recolonisation passe par le développement transitoire d'une faune spéciale, caractérisant un excès de matière organique sur les fonds sédimentaires. Notre approche consiste d'abord à reconnaître les groupes taxonomiques, constitués par les espèces de polluosensibilité équivalente en face d'un excès de matière organique. Leur apparition successive dans le temps, leur disparition, constituent les paramètres obligatoires de cette approche dynamique. Ce type d'analyse montre comment une perturbation biologique peut persister longtemps dans l'écosystème, alors que les facteurs écologiques semblent normaux. Le long du même gradient écologique spatial, l'étude comparée des deux Abers peut montrer des différences dans les vitesses de retour à un nouvel équilibre.

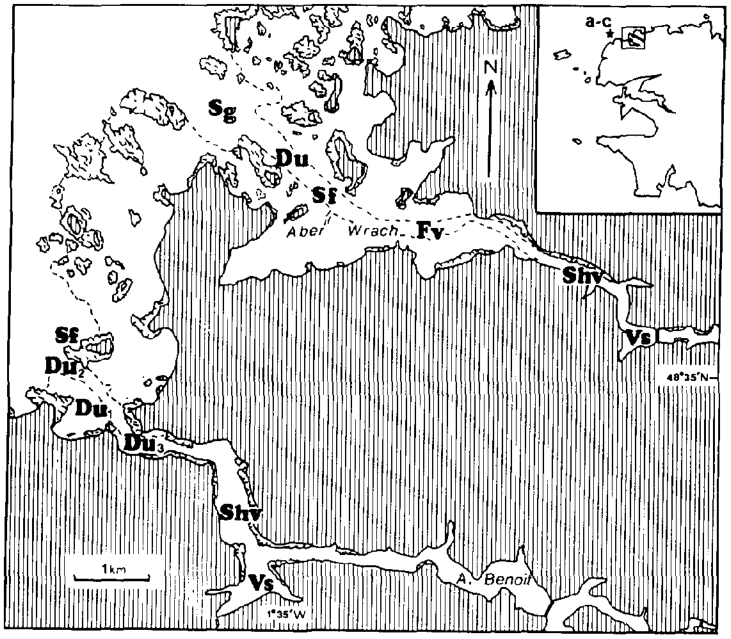

FIGURE 1. Localisation de l'épave de l'AMOCO-CADIZ (A-C) par rapport aux deux abers, où sont représentées les différentes unités biosédimentaires.

METHODES

La macrofaune qui vit dans les aires sédimentaires est étudiée le long des chenaux subtidaux de ces deux Abers. Cinq prélèvements sont réalisés à chaque station avec une benne "Aberdeen". Ces échantillons sont répétés trois fois dans l'année (hiver, printemps, été). Les stations -plus de quinze dans chaque Aber- sont représentatives des différents peuplements. Leur distribution spatiale de l'aval vers l'amont est la suivante (Gentil et Cabioch, 1979 ; Glémarec et Hussenot, 1981) : sables grossiers (SG), sables dunaires fins et moyens (DU), sables fins et envasés (FV), sables hétérogènes envasés (SHV) et vases sableuses (VS).

Cette distribution (Fig. 1) correspond à un hydrodynamisme décroissant et, inversement, à une accumulation croissante d'hydrocarbures dans les sédiments (Marchand et Caprais, 1981). Dans l'Aber Benoît, la répartition est en partie différente parce que les sables dunaires sont très développés. Il y a une vaste accumulation en aval (DU_1) sous la pointe de Corn ar Gazel et, au milieu du chenal, deux unités quelque peu différentes, DU_2 et DU_3, la dernière est en contact avec les sables hétérogènes envasés (SHV). Les sables fins n'existent pas dans le chenal, mais sont localisés en aval dans une aire protégée entre des plateaux rocheux, collectant les particules fines qui peuvent sortir dans l'aber.

RESULTATS

Tout d'abord -phase première de Marchand (1981)- la toxicité des hydrocarbures a induit de lourdes mortalités au sein de toutes les communautés (Chassé, 1978, Cabioch *et al.*, 1979). D'autres espèces, plus opportunistes, s'installent dans une deuxième phase (Glémarec et Hussenot, 1981 ; Le Moal et Quillien-Monot, 1981).

Les nouvelles populations caractérisent un excès de matière organique, dans le cas d'effluent urbain arrivant en mer par exemple. Après différents travaux sur ces problèmes (Pearson et Rosenberg, 1978 ; Bellan *et al.*, 1980 ; Glémarec et Hily, 1981) il est possible de regrouper ces espèces en fonction de leur polluosensibilité.

Groupe I : espèces sensibles, largement dominantes en conditions normales. Elles diffèrent selon chaque type de peuplement. Dans les sables dunaires fins et moyens par exemple, les Amphipodes sont nombreux (*Bathyporeia* spp., *Ampelisca* spp.) ; ils meurent rapidement dans tous les cas de marée noire (Chassé *et al.*, 1967 ; Cabioch *et al.*, 1980 ; Sanders, 1978 ; Pfister, 1980).

Groupe II : espèces tolérantes, toujours en petites quantités. Elles ne fluctuent pas significativement dans le temps. La majorité d'entre elles sont des prédateurs : *Nephtys hombergii, Morphysa bellii, Glycera* spp., *Platynereis dumerilii* ...

Groupe III : espèces sensibles* qui disparaissent tout d'abord puis réapparaissent en élargissant leur répartition écologique par rapport aux conditions normales : *Spio* spp., *Notomastus latericeus, Phyllodoce* spp., *Nereis diversicolor* ...

Groupe IV : espèces opportunistes, essentiellement des Polychètes Cirritulidés et Capitellidés (*Chaetozone setosa, Heterocirrus* spp., *Polydora* spp., *Cirratulus cirratulus, Andouinia tentaculata, Capitomastus minimum* ...). A l'intérieur de ce groupe une succession écologique a pu être précédemment définie (Glémarec et Hussenot, 1981 ; Le Moal, 1981).

Groupe V : espèces opportunistes, très peu nombreuses (2 ou 3) qui restent seules mais sont présentes en densités considérables là où la pollution est maximale : *Capitella capitata, Capitellides giardi, Scolelepis fuliginosa,* Oligochètes ...

Ces différents groupes peuvent coexister et le long du gradient de pollution organique, six étapes peuvent être définies (Fig. 2a) :

Etape 1 : groupes I, II et III sont en plus faibles densités qu'en conditions normales, mais il n'y a aucun changement qualitatif.

Etape 2 : l'écosystème est déséquilibré et le groupe III est dominant.

Etapes 4 et 6 : elles sont définies respectivement par la prolifération des groupes IV et V.

Etapes 3 et 5 : elles correspondent à des écotones ; le groupe II est seul car la compétition entre groupes est affaiblie.

Ce diagramme (adapté de Glémarec et Hily, 1981) est statique. Il illustre par exemple la disposition d'auréoles concentriques de pollution décroissante en face d'un effluent urbain arrivant en mer. La perturbation créée par l'AMOCO-CADIZ apporte une nouvelle dimension temporelle à ce diagramme. Nous proposons de décrire l'installation progressive des différents groupes (Fig. 2b) et la régression de cette faune spéciale de substitution (Fig. 2c).

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* à la toxicité des hydrocarbures.

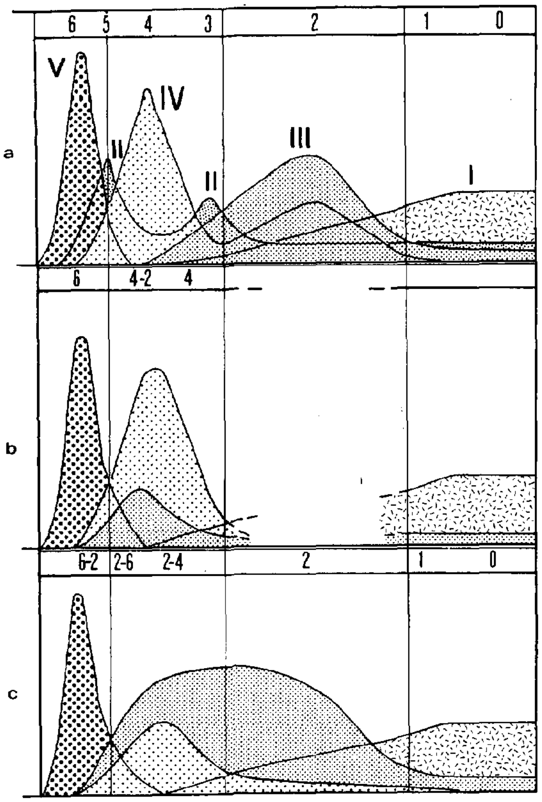

FIGURE 2a. Importance relative des différents groupes et définitions des étapes 0 à 6 le long du gradient d'excès en matière organique.

2b. Installation de la faune opportuniste (groupes IV et V) après stabilisation de la pollution.

2c. Evolution régressive de la faune opportuniste et développement du groupe III.

Trois mois après la perturbation (t_3), la population est largement stabilisée, c'est le début de la deuxième phase de Marchand. A t_8, tous les peuplements sont détruits (Fig. 3 et 4). On notera que dans l'Aber Wrac'h, quelques espèces tolérantes ou du groupe IV meurent ultérieurement (t_{21}). Dans l'Aber Wrac'h, les sables grossiers d'aval ne sont pas affectés (étape 0, cf. Tableau 1), le peuplement montre seulement quelques fluctuations saisonnières. Partout ailleurs les hydrocarbures sont stockés dans les sédiments en fonction de l'hydrodynamisme décroissant de l'aval vers l'amont, et il est possible de décrire l'installation des différents groupes d'espèces le long du même gradient.

Dans les sables dunaires (DU_1) de l'Aber Benoît (Tableau II), la teneur d'hydrocarbures est partout inférieure à 50 ppm et la communauté montre une légère baisse des densités (étape 1) et une étape 2 de déséquilibre apparaît, de façon fugace. Pour tous les autres sédiments des deux abers, là où la teneur en hydrocarbures est toujours supérieure à 1 000 ppm (Marchand et Caprais, 1981), leurs peuplements sont très appauvris.

TABLEAU I. L'évolution des peuplements dans l'ABER WRAC'H est illustrée par l'utilisation des étapes définies sur la Figure 2.

t	8	13	17	21	25	29	33
VS	-	-	6	5	4	2	2-6
SHV	4	4	4-2	4-2	4	-	2-4
FV	4	4	4-2	3	4	3	3
SF	4	4	4-2	2-4	4-2	2-4	2-4
DU	4	4	4-2	2-4	4-2	1	2
SG	0	0	0	0	0	-	-

TABLEAU II. L'évolution des peuplements dans l'ABER BENOIT est illustrée par l'utilisation des étapes définies sur la Figure 2.

t	8	13	17	21	25	29	33
VS	6	6	6-2	2-6	6-2	6-2	6-2
SHV	4-2	4-2	2-4	2-4	2	6	2-4
DU_3	4-2	4-2	6-2	2-6	2	2	2
SF	-	4	4	2	2-4	4-2	2-4
DU_2	4	1	1	1	1	1	2
DU_1	1	1	1	2	0	0	0

A partir de t_8 le groupe IV est prédominant sur les groupes I et III (étape 4). Dans les aires envasées d'amont, les peuplements sont très sinistrés et le groupe V prolifère (étape 6). Néanmoins, dans l'Aber Benoît, les peuplements de la partie moyenne (DU_3 et SHV) montrent déjà la prédominance du groupe III sur le groupe I, ce qui définit une étape intermédiaire 4-2 qui apparaît à t_8 dans l'Aber Benoît mais seulement à t_{17} dans l'Aber Wrac'h. Le long du gradient spatial d'excès en matière organique de l'aval vers l'amont, les étapes successives 6 et 4 illustrent l'apparition successive des groupes V et IV, les étapes intermédiaires 6-2 et 4-2 celle du groupe III toujours dominé par les groupes V ou IV.

Les conditions hivernales, par leurs tempêtes fréquentes, brassent et oxygènent les sédiments. Après le premier hiver (t_{12}), Marchand et Caprais notent la décontamination chimique dans l'Aber Benoît au niveau des sables dunaires DU_1 et DU_2, puisque la teneur en hydrocarbures est inférieure à 100 ppm. Cependant, dans la majorité des autres sédiments, cette teneur, à t_{12}, est toujours supérieure à 1 000 ppm et peut atteindre plus de 10 000 ppm dans les vases et dans les sables hétérogènes envasés. C'est seulement durant le deuxième hiver (t_{21}) que la décontamination peut être prouvée par des données biologiques (Tableau I) dans l'ensemble de l'Aber Wrac'h. La Figure 2c illustre cette régression des groupes opportunistes V et IV, l'extension puis finalement la disparition du groupe III avec les différentes étapes :

étape 2-6 : le groupe III domine les groupes V et IV.
étape 2-4 : le groupe III domine les groupes IV et V.

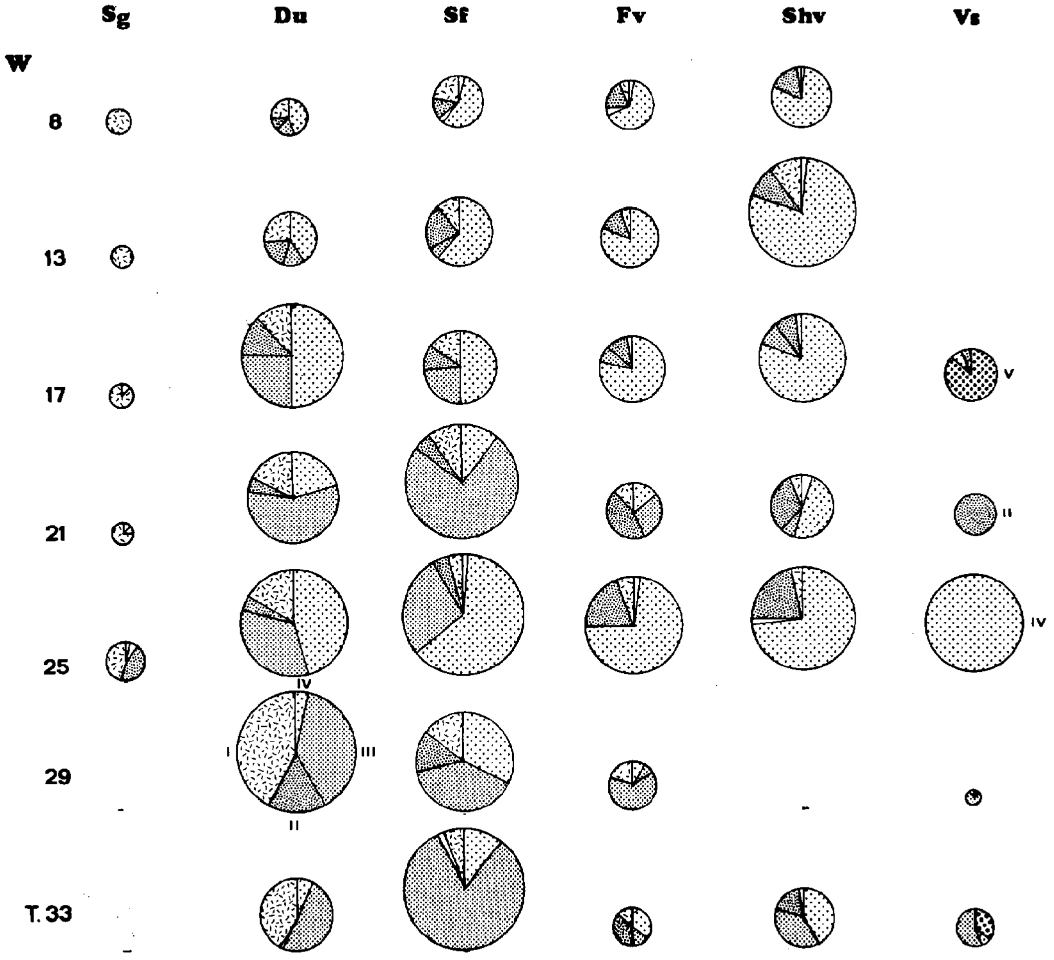

FIGURE 3. Evolution temporelle de la densité des peuplements et de l'importance relative des différents groupes de I à V, ceci dans les différentes unités biosédimentaires de l'Aber Wrac'h, de l'aval à gauche vers l'amont à droite.

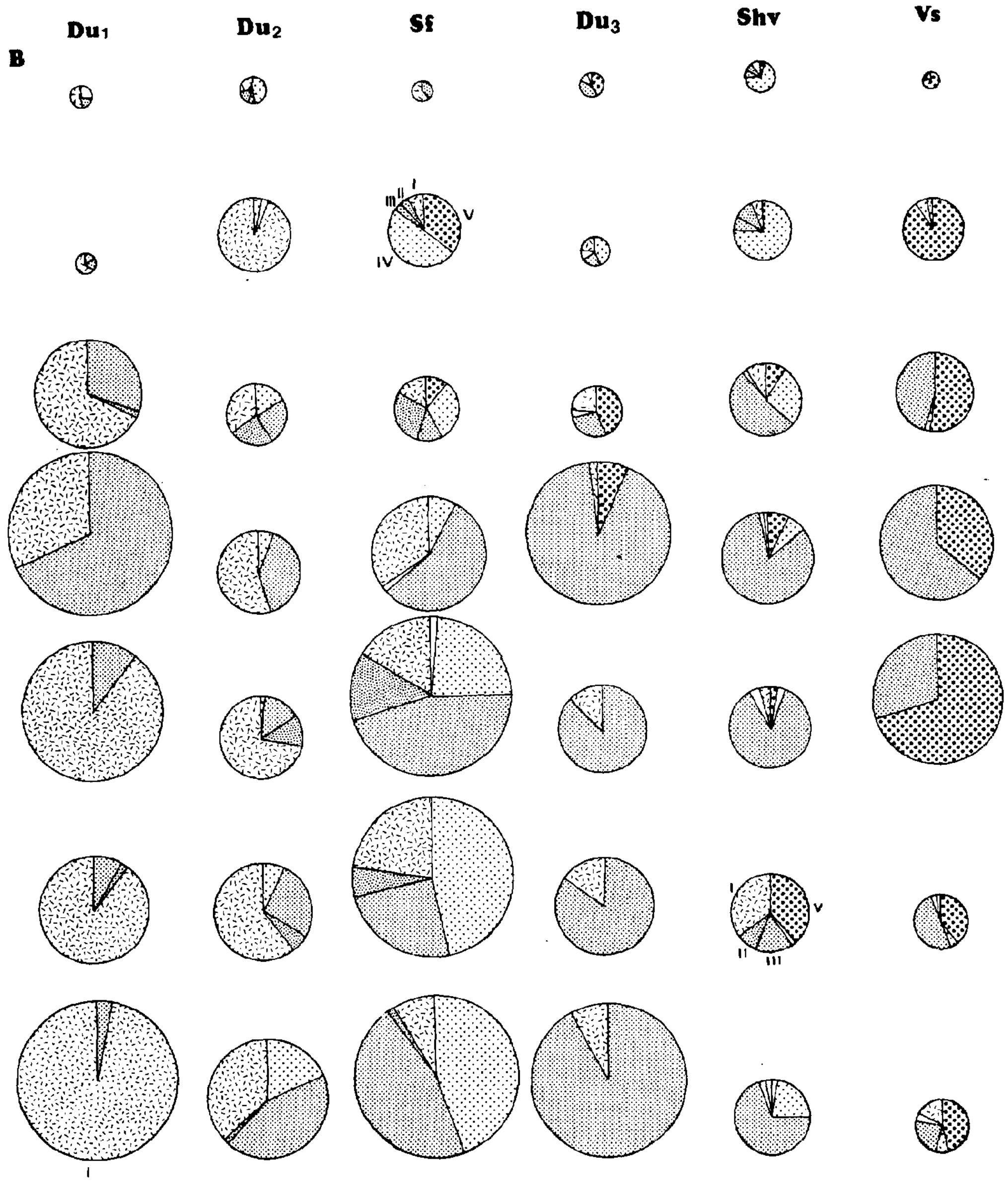

FIGURE 4. Evolution temporelle de la densité des peuplements et de l'importance relative des différents groupes de I à V, ceci dans les différentes unités biosédimentaires de l'Aber Benoît, de l'aval à gauche vers l'amont à droite.

étape 2 : le groupe III domine les groupes I et IV.
étape 1 : le groupe I domine le groupe III, le groupe IV est encore présent, les densités totales sont encore plus faibles qu'en conditions normales.

Ces quatre étapes prouvent que l'écosystème est encore déséquilibré. Le début de l'évolution régressive de cette faune de substitution semble simultané dans l'Aber Wrac'h. Dans l'Aber Benoît, cette évolution apparaît différemment au sein des peuplements, plus rapidement là où les sédiments sont bien oxygénés, après t_8 pour les sables dunaires (DU_2), après t_{13} pour les sables hétérogènes envasés (SHV), après t_{17} pour les sables fins, les DU_3 et les vases sableuses (VS). Deux ans après la perturbation (t_{25}), les sables dunaires (DU_1) présentent un peuplement qui semble normal (étape 0), tous les autres peuplements sont en déséquilibre (étapes 1, 2 et 2-4) et, dans les aires envasées, la décontamination commence à peine (étape 6-2). Ensuite les processus dynamiques sont plus lents et, au cours du troisième hiver (t_{33}), les sables dunaires (DU_2 et DU_3) atteignent l'étape 2, les sables fins et les sables hétérogènes envasés l'étape 2-4.

Les dragages mécaniques qui ont été préconisés pour résorber les poches de vase et d'hydrocarbures ont eu lieu en avril 1980, à proximité des sables hétérogènes envasés. Leur peuplement témoigne d'un accroissement passager de la perturbation, étape 6 à t_{29}. Il faut moins de deux mois en effet pour que prolifère une nouvelle génération de *Capitella capitata*. Cette étape 6 est apparue comme une élévation dans l'évolution logique de l'écosystème ; elle est provoquée par une intervention anthropique.

Il est important de noter que les conditions hydrodynamiques ne sont pas aussi efficaces dans l'Aber Wrac'h que dans l'Aber Benoît, ce qui se traduit par une décontamination qui n'est pas simultanée dans les abers. L'évolution régressive de la faune opportuniste n'est pas aussi rapide dans l'Aber Wrac'h et nous pouvons observer qu'au même moment (t_{25} par exemple), les mêmes peuplements restent plus perturbés que dans l'Aber Benoît :

	DU_2	SHV	SF	VS
Abers Benoît	1	2	2-4	6-2
Wrac'h	4-2	4	4-2	4 .

A t_{33} cette différence disparaît :

	DU_2	SHV	SF	VS
Abers Benoît	2	2-4	2-4	6-2
Wrac'h	2	2-4	2-4	2-6

DISCUSSION

Indépendemment des fluctuations cycliques annuelles, l'évolution temporelle des différents groupes le long des gradients spatiaux que constituent les abers, montre une évolution acyclique et des processus chronologiques tout à fait similaires de ceux décrits par Le Moal (1981) dans la zone intertidale de ces abers. Ils sont résumés sur la Figure 5a. Il y a d'abord régression quasi-totale des populations initiales durant la première période t_0-t_8. Le groupe II ne fluctuant pas significativement ou étant seulement dominant lorsque les autres groupes disparaissent (étapes 3 et 5), n'est pas représenté sur les figures.

Entre t_8 et t_{13}, c'est le commencement de la seconde phase et la faune opportuniste s'installe. Le groupe IV est abondant partout à partir de t_8 dans l'Aber Wrac'h, ses densités sont maximales au printemps, c'est-à-dire à t_{13} et t_{25}. Son importance est décroissante dans les peuplements de l'Aber Benoît après t_8, t_{13} ou t_{17} selon l'hydrodynamisme.

Le groupe V apparaît de façon significative seulement dans les vases sableuses de l'Aber Benoît, mais, sur un plan général, le développement de la faune opportuniste IV et V est maximal entre t_8 et t_{20}.

Le groupe III réapparaît à t_{13} et son développement est très important partout durant le deuxième hiver. De façon simultanée, le groupe I réapparaît, un an après la marée noire, mais après deux années son importance est encore limitée par le développement anormal du groupe III qui semble entrer en compétition.

Avec le développement des groupes I ou III, essentiellement après t_{20}, commence donc la phase de reconstitution. A côté de ce schéma général d'évolution, nous avons regroupé les différents scénarios d'évolution temporelle : celui des vases sableuses de l'Aber Benoît est évoqué plus haut (Fig. 5b), celui des vases sableuses de l'Aber Wrac'h montre d'abord la prédominance du groupe V, remplacé ensuite par celui du groupe IV (Fig. 5c). Pour les autres sédiments de l'Aber Wrac'h, il y a deux pics successifs du groupe IV séparés par un maximum du groupe III à t_{21}. Le cas aberrant des sables hétérogènes envasés de l'Aber Benoît est illustré par la recrudescence du groupe V, après celui du groupe IV.

Pour l'ensemble des sédiments dunaires des deux abers, les Figures 5d, e et f, illustrent les différentes possibilités où la compétition entre les groupes I et III apparaît de façon tout à fait évidente.

Ces scénarios sont établis sur les densités relatives des différents groupes, mais la communauté sera jugée en état d'équilibre lorsque les caractéristiques essentielles (A = abondance relative des espèces ; S = nombre d'espèces ; B = biomasse) restent relativement inchangées, hormis les fluctuations saisonnières. Qualitativement, l'ensemble de ces peuplements est encore en déséquilibre et l'analyse simultanée des trois paramètres S, A, B, suggère des faits complémentaires qui méritent d'être suivis dans le temps. On notera que c'est dans le cas des sédiments d'aval de l'Aber Benoît que l'on est encore le plus éloigné d'une certaine stabilisation de ces trois facteurs. Au contraire, c'est dans le cas des sédiments envasés que l'équilibre semble atteint le plus vite.

Nous avons déjà exposé (Glémarec *et al.*, 1981) comment le modèle méthodologique, mis au point dans le cas des effluents urbains arrivant en mer, pouvait être utilisé dans le cas de catastrophes pétrolières, et l'échelle de temps des phénomènes observés semble avoir été la même dans le cas du Tanio (Aelion et Le Moal, 1981).

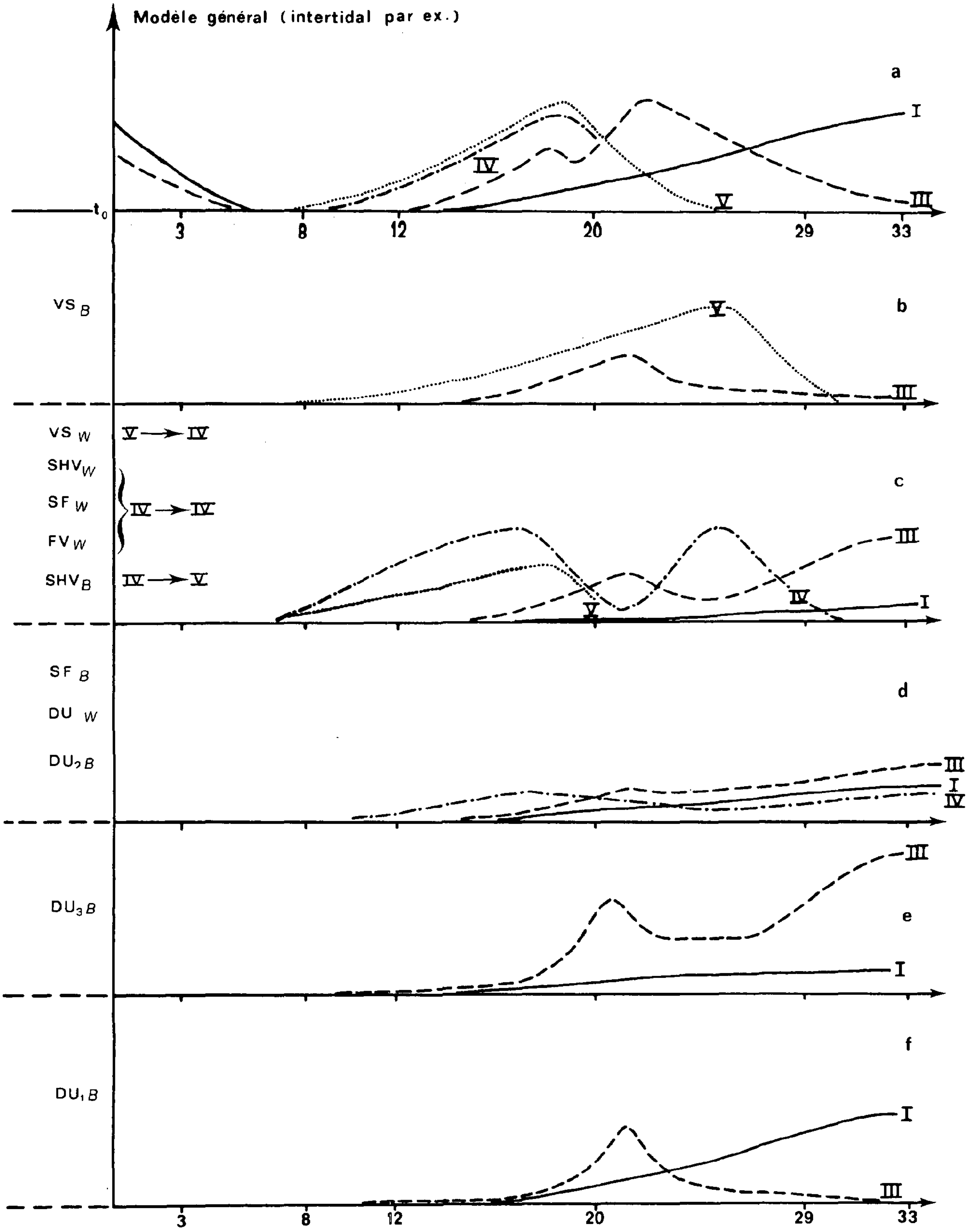

FIGURE 5. Principaux modèles de succession temporelle des différents groupes I à V au sein des peuplements des Abers Wrac'h (W) et Benoît (B). Le modèle général (Fig. 5a) est synthétique ; il illustre les trois phases après une telle perturbation : mortalité, substitution, reconstitution.

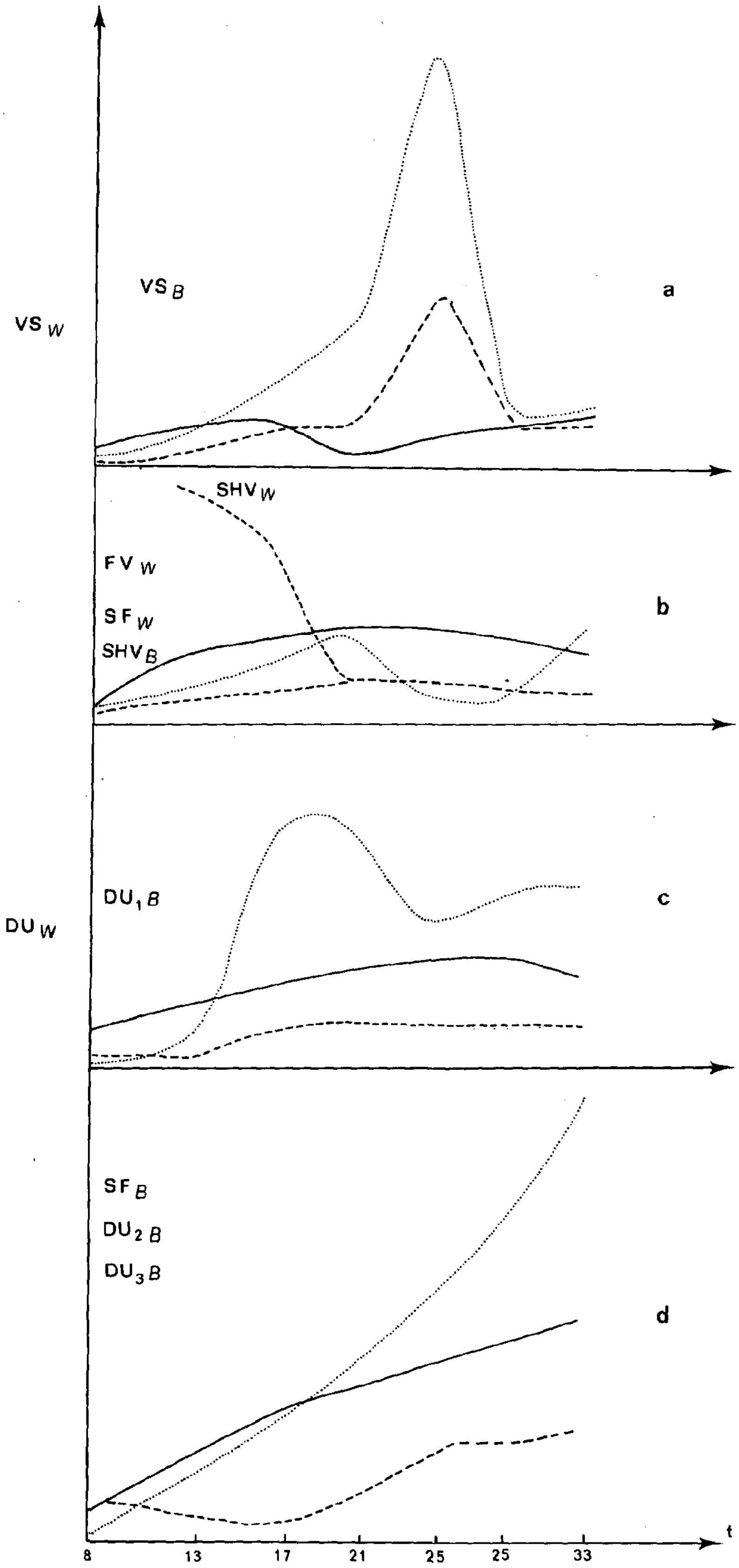

FIGURE 6. Evolution des paramètres synthétiques, nombre d'espèces S en trait plein, A abondance des individus en pointillé, B biomasse en tireté. La stabilisation simultanée dans le temps de ces trois paramètres n'est pas acquise dans le cas des peuplements d'aval de l'Aber Benoît.

L'accident de l'Amoco-Cadiz s'est révélé pour nous une expérience d'écologie expérimentale tout à fait exceptionnelle. Elle permet d'apporter des éléments de réflexion quant aux mécanismes qui mettent en place de telles séquences, problème posé récemment par Connell et Slatyer (1977).

Dans la succession décrite, les premiers stades correspondent à des espèces à vie courte, de type opportuniste, capables de supporter une proportion de matière organique encore importante dans les sédiments. Cette première phase de recolonisation par substitution est mieux expliquée par les caractéristiques biologiques des espèces en cause que par quelque propriété émergente de la communauté toute entière (Sousa, 1980). Si ces premières espèces ne facilitent pas le retour des espèces caractéristiques des stocks ultérieurs (modèle de facilitation), il leur est difficile d'inhiber la réapparition des espèces à stratégie différente qui s'installent plus lentement, mais de façon plus durable. Les phénomènes de compétition existant, peu à peu les premières espèces disparaissent. Ce type de succession secondaire peut correspondre au modèle de tolérance de Connell et Slatyer, dont il n'existe jusqu'ici que peu d'exemples connus. Les premières étapes ont donc été relativement rapides, pour les suivantes c'est plus long. La reconstitution, si elle est qualitative, doit aussi être quantitative, énergétique.

L'approche que nous avons développée semble plus efficace que l'étude dynamique de certaines populations. Elle s'est inspirée de la recherche des indicateurs biologiques bien connus en milieu terrestre et d'eau douce. Même si les paramètres écologiques abiotiques semblent normaux, ces bioindicateurs peuvent révéler des perturbations dans les écosystèmes, qu'il est impossible de détecter par une analyse des paramètres physiques.

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LES EFFETS DES HYDROCARBURES DE L'AMOCO-CADIZ SUR LES PEUPLEMENTS BENTHIQUES DES BAIES DE MORLAIX ET DE LANNION D'AVRIL 1978 A MARS 1981

par

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1) INTRODUCTION

Les premières nappes d'hydrocarbures de l'Amoco-Cadiz atteignent le littoral de la région de Roscoff et les côtes orientales de la baie de Morlaix le 21 mars, quatre jours après l'échouage du pétrolier sur les roches de Portsall. Alors que les masses d'eau chargées en hydrocarbures plus toxiques transitent rapidement sur l'ensemble de la région, des particules oléosédimentaires contaminent les fonds sublittoraux. Elles se déposent préférentiellement dans les zones calmes et forment localement des poches de mazout résiduel; en baie de Morlaix, on note leur présence le 3 avril dans les sables fins peu envasés de la Pierre Noire par 20 mètres de profondeur et le 13 avril dans les chenaux des rivières de Morlaix et de Penzé (CABIOCH et al., 1978). Durant la même période ce phénomène est observé par MARCHAND et CAPRAIS (1981) en baie de Lannion.

La connaissance de la composition et de la distribution des communautés benthiques de cette région depuis 1968 (CABIOCH) et celle de la dynamique du peuplement des sables fins de la Pierre Noire engagée depuis 1977 (DAUVIN, 1979) permettent une meilleure évaluation des conséquences de la pollution sur le macrobenthos subtidal.

Ces études, financées par la NOAA (contrat 78/5830, 80/6145), entreprises immédiatement après la catastrophe, ont déjà fait l'objet de publications : DAUVIN (1979 a, b et sous presse), CABIOCH et al. (1980, 1981 et sous presse).

2) LES PEUPLEMENTS ETUDIÉS

En Manche les séquences bio-sédimentaires sont principalement sous le contrôle de l'intensité des courants de marée; en conséquence de l'entrée vers le fond des baies, les peuplements des sédiments grossiers sont progressivement relayés par des peuplements de sédiments fins plus ou moins envasés.

Certains termes de cette séquence ont une distribution spatiale discontinue; tel est le cas des peuplements des sables fins séparés par de vastes étendues de nature différente.

Les unités cénotiques correspondant aux principaux maillons de cette succession bio-sédimentaire ont été régulièrement échantillonnées :

- les sables grossiers à *Venus fasciata* - *Amphioxus lanceolatus* de la baie de Morlaix (au large de la Pointe de Primel; carte 1, P.P.), peu classés avec pour mode la classe 1000 à 5000 microns (55 à 70% du sédiment total) : relevés trimestriels d'août 1977 à août 1980;

- le maerl envasé de Trébeurden en baie de Lannion (carte 1, L6), très peu classé, avec pour mode la classe 250 à 500 microns (7 à 58% du sédiment total) : relevés trimestriels d'avril 1978 à mai 1979;

- les sables fins faiblement envasés à *Abra alba* - *Hyalinoecia bilineata* des baies de Morlaix et de Lannion (carte 1, P.N., L7, L8), bien classés avec pour mode la classe 125 à 250 microns (42 à 62% du sédiment total) : relevés mensuels d'avril 1977 à mars 1981 (P.N.) et trimestriels d'avril 1978 à février 1981 (L7 et L8);

- les sables très fins peu vaseux à *Tellina fabula* - *Abra alba* en baie de Lannion (sous Saint-Efflam; carte 1 : L1, L2, L3, L4 et L5), très bien classés avec pour mode la classe 63 à 125 microns (70 à 78% du sédiment total) : relevés trimestriels d'avril 1978 à février 1981 sauf L4 d'avril 1978 à mai 1979;

- les vases sableuses à *Abra alba* - *Melinna palmata* de la rivière de Morlaix (carte 1 : R.M.) où domine la classe des particules inférieures à 63 microns (47 à 74% du sédiment total) accompagnée d'une importante fraction de sables très fins et fins : relevés trimestriels d'août 1977 à février 1981.

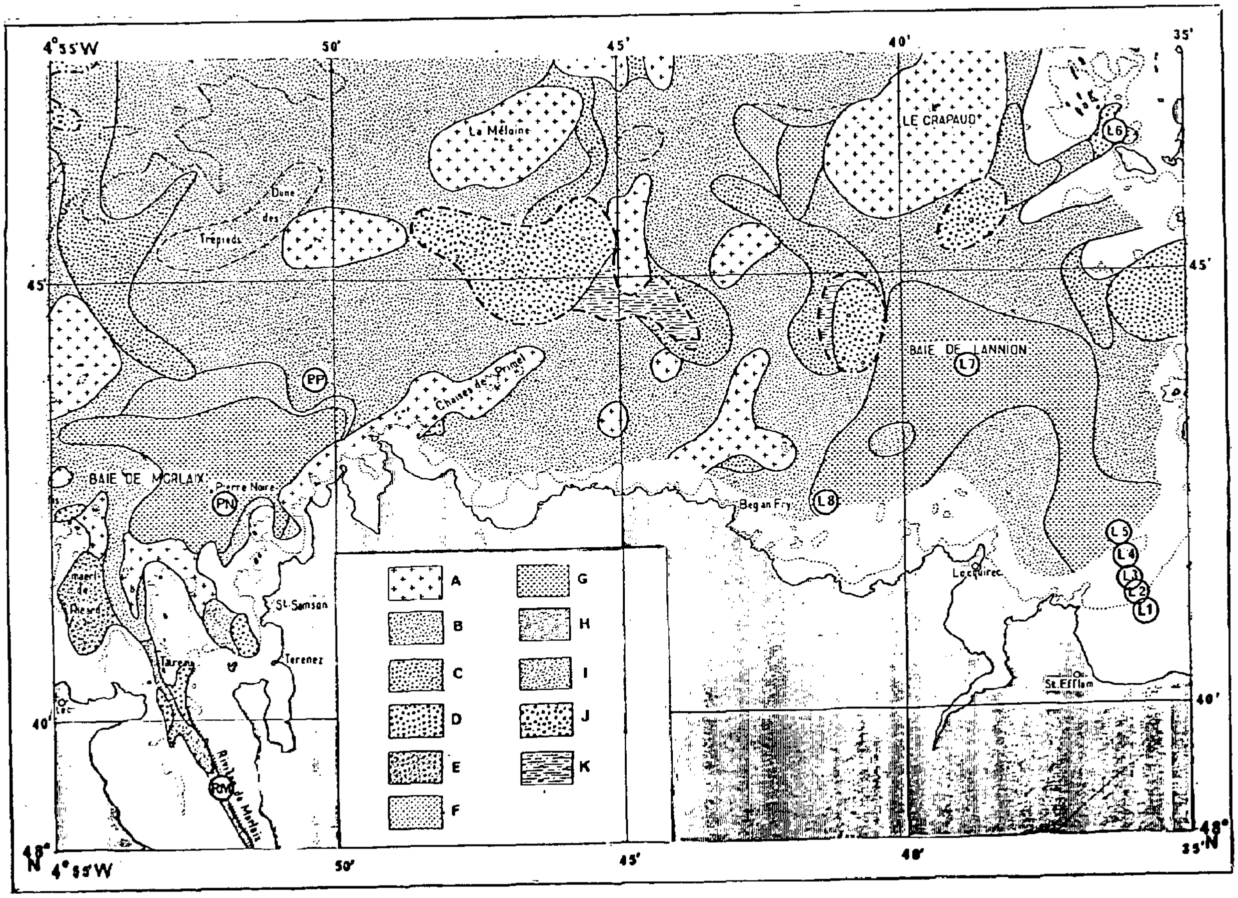
Les stations de la baie de Morlaix ont été étudiées par J.C. DAUVIN depuis 1977; Ch. RETIÈRE et V. RIVAIN ont observé les stations de la baie de Lannion avec la contribution de D. ARCHAMBAULT pour l'étude de la fin du 3ième cycle annuel. L'échantillonnage a été effectué parallèlement au moyen d'une benne Smith Mc Intyre et d'une benne Hamon (relevés : 10 prélèvements à la benne Smith Mc Intyre et 4 ou 5 à la benne Hamon; surface échantillonnée $1m^2$ ou plus) ; le tamisage a été réalisé sur une maille circulaire de 1mm.

3) RÉSULTATS

Nous avons suivi l'évolution des paramètres écologiques richesse spécifique, densité et biomasse. Les richesse spécifique et densité sont actuellement connues pour la totalité des sites et jusqu'au printemps de 1981. Il en est de même pour les biomasses relatives aux stations de Primel et de la rivière de Morlaix; par contre à la Pierre Noire elles ont été mesurées entre avril 1977 et novembre 1978, puis calculées pour les autres relevés ; elles n'ont pas encore été quantifiées pour les stations de la baie de Lannion.

3.1) Caractère du stress

Les effets du stress n'ont pu être évalués avec précision que sur



Carte 1 - Répartition des stations d'échantillonnages en baie de Morlaix et en baie de Lannion.

A	: fonds rocheux
B - C	: communauté des sédiments grossiers à *Amphioxus* - *Venus fasciata*, relativement indépendante de l'étagement (C : faciès d'épifaune à *Sabellaria spinulosa*).
D - E	: communauté du maërl (D : faciès à *Lithothamnium corallioïdes* var. *corallioides*; E : faciès à *L. corallioïdes* var. *minima*).
F	: communauté des sables dunaires fins à *Abra prismatica*- *Glycymeris glycymeris*.
G à I	: communauté des sédiments fins à *Abra alba* (G : faciès sableux à *Hyalinoecia bilineata;* H : faciès envasé à *Melinna palmata;* I : faciès hétérogène envasé à *Pista cristata*).
J	: communauté des cailloutis et graviers prélittoraux côtiers : faciès à *Ophiothrix fragilis* et à Bryozoaires dominants dans l'encroûtement.
K	: communauté des cailloutis et graviers prélittoraux du large : faciès d'ensablement à *Porella concinna*.

les peuplements de la baie de Morlaix pour lesquels nous disposions d'observations juste avant la pollution.

Richesse spécifique. (fig. 1)

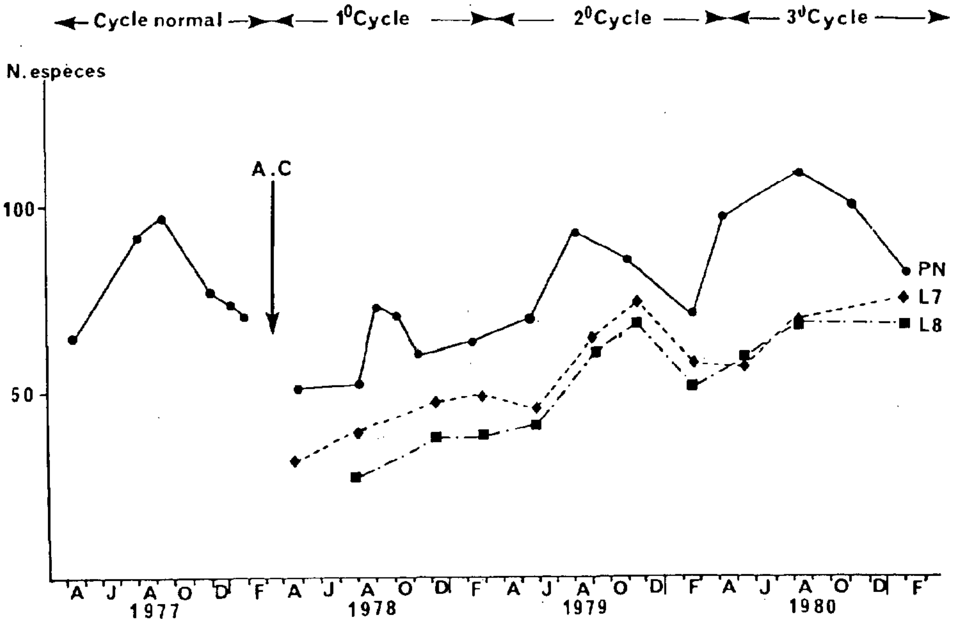


Figure 1 - Peuplement des sables fins à *Abra alba* - *Hyalinoecia bilineata* : évolution de la richesse spécifique des relevés (4 prélèvements à la benne Hamon) d'avril 1977 à février 1981 (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

A la Pierre Noire la richesse spécifique totale passe de 84 espèces le ler mars à 61 le 3 avril; cette brusque diminution est due pour une grande part à une forte réduction du nombre d'espèces d'amphipodes (20 le 18 janvier, 24 le ler mars, 10 le 3 avril et 7 le 25 avril).

Par contre dans les peuplements de Primel et de la rivière de Morlaix la richesse spécifique totale se maintient, la chute du nombre d'espèces d'amphipodes étant compensée par l'accroissement concomitant de celui des polychètes.

Les effets du stress sur le groupe des amphipodes sont d'autant plus intenses que les espèces avaient, avant la pollution un haut degré de constance dans le peuplement; ils concernent les espèces suivantes :

Pierre Noire	Rivière de Morlaix	Primel
Ampelisca armoricana	*Ampelisca armoricana*	*Ampelisca armoricana*
Ampelisca brevicornis	*Ampelisca brevicornis*	-
Ampelisca spinipes	-	*Ampelisca spinipes*
Ampelisca tenuicornis	*Ampelisca tenuicornis*	-
Ampelisca typica	-	-
Cheirocratus intermedius	*Cheirocratus intermedius*	-
Photis longicaudata	-	-
Pariambus typicus	-	-
Corophium crassicorne	-	-
Melita obtusata	-	-
Melita gladiosa	-	-
Megaluropus agilis	-	-
	Ampelisca spinimana	-

Dès 1972, les travaux de SANDERS et al. ont mis en évidence le caractère d'espèces "tests" que pouvaient présenter les amphipodes vis-à-vis de ce type de pollution. Dans le même esprit les études expérimentales de LINDEN (1976), LEE, WELCH et NICOL (1977), LEE et NICOL (1978a et b) ont montré l'extrême sensibilité des amphipodes aux hydrocarbures. Cette sensibilité est confirmée par les observations *in-situ* de SANDERS et al. (1980), DEN HARTOG et JACOBS (1980), ELKAIM (1980) et ELMGREN et al. (1980) relatives aux marées noires de West Falmouth en 1969 aux Etats-Unis, de l'Amoco-Cadiz sur les côtes de Bretagne et de la Tsesis en mer Baltique.

Cependant des recherches de CABIOCH et al. en baie de Morlaix (1981) il ressort que certaines espèces appartenant à d'autres groupes zoologiques réagissent plus ou moins intensément aux effets du stress; il en est ainsi des polychètes *Phyllodoce kosteriensis*, *Terebellides stroemi* et de nombreuses espèces de micro-mollusques. Les fortes mortalités subies, en baie de Lannion, par les populations de mollusques, surtout celle de *Pharus legumen* et de l'oursin *Echinocardium cordatum*, viennent à l'appui de ces observations. Il convient de noter, qu'en règle générale, ce sont les annélides polychètes qui constituent le noyau d'espèces le plus résistant (tabl. i).

L'amplitude du stress est donc fonction de la composition originelle des peuplements et varie fortement d'une unité cénotique à l'autre.

Densités et biomasses

Les travaux de DAUVIN montrent qu'à cet égard les effets du stress sont particulièrement dévastateurs sur les peuplements de sables fins de la Pierre Noire (1979). Les densités passent de 8707 à 1808 individus par m^2 et la biomasse de 5.0 à 2.5 g. par m^2 soit une réduction respective de 80 et 50 % des valeurs initiales; elle est essentiellement due à l'élimination des ampeliscidés *Ampelisca armoricana* et *Ampelisca tenuicornis* et à la diminution des effectifs des populations d'*Ampelisca sarsi*. Ces trois espèces représentaient à elles seules 90 à 99 % des densité et biomasse moyennes annuelles du peuplement. (fig. 2)

Sur les peuplements des sables grossiers de Primel et des vases sableuses de la rivière de Morlaix, les effets du stress, en terme de densité et de biomasse, sont peu marqués.

En conclusion, il apparait clairement que les effets du stress sont sélectifs au niveau :

- des espèces et groupes zoologiques : les amphipodes et plus spécialement les ampeliscidés se révèlent extrêmement sensibles;

- des peuplements : la communauté infralittorale des sables fins à *Abra alba* - *Hyalinoecia bilineata* est la plus intensément perturbée.

3.2) Evolution à long terme des peuplements.

Les peuplements des sédiments fins sablo-vaseux subissent les perturbations les plus significatives; dans cet exposé, l'étude de leur dynamique fera donc l'objet d'une attention toute particulière.

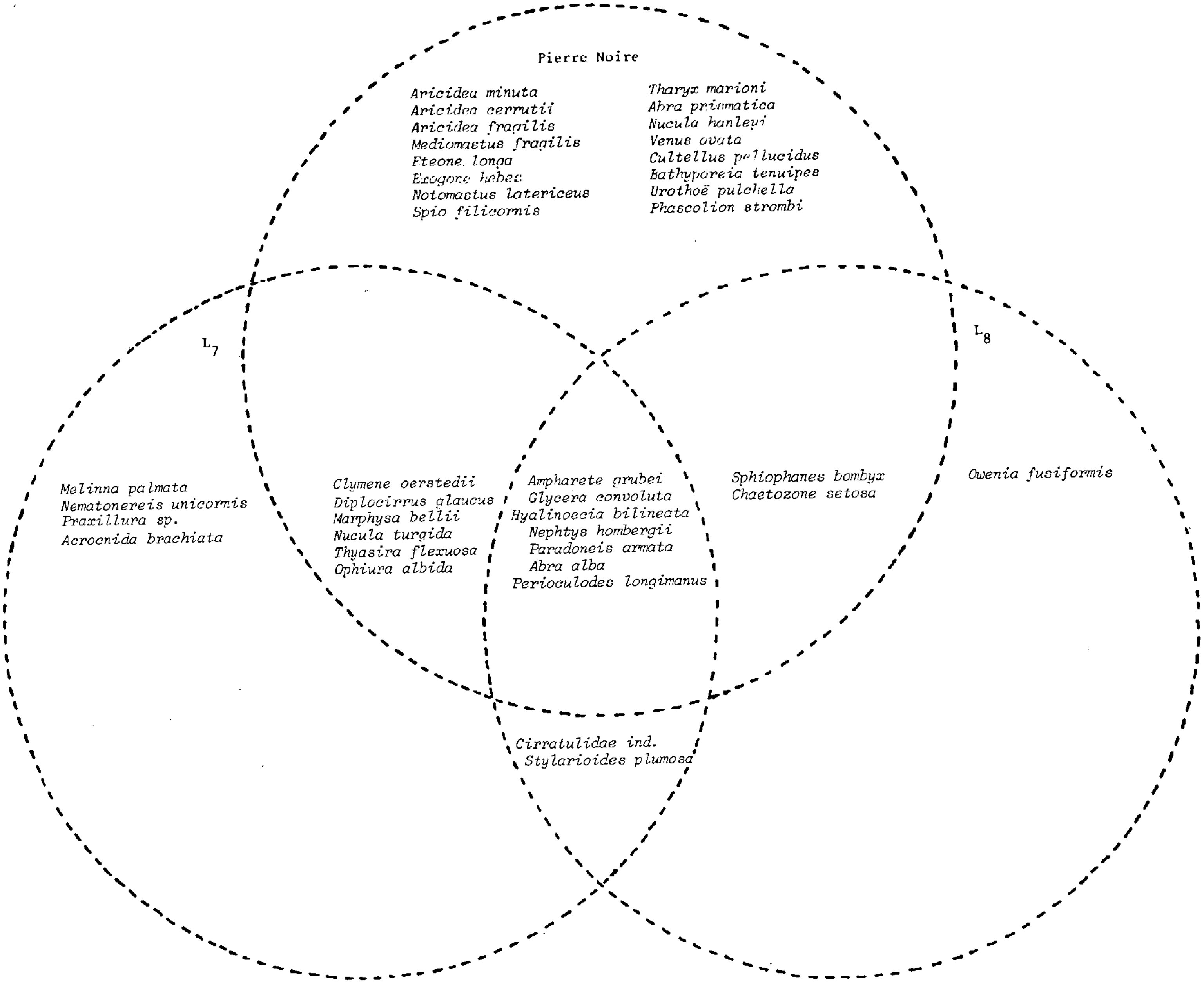


Tableau 1 - Espèces dont la constance est supérieure ou égale à 90% sur la durée totale des observations d'avril 1978 à février 1981 aux stations Pierre Noire, L_7 et L_8.

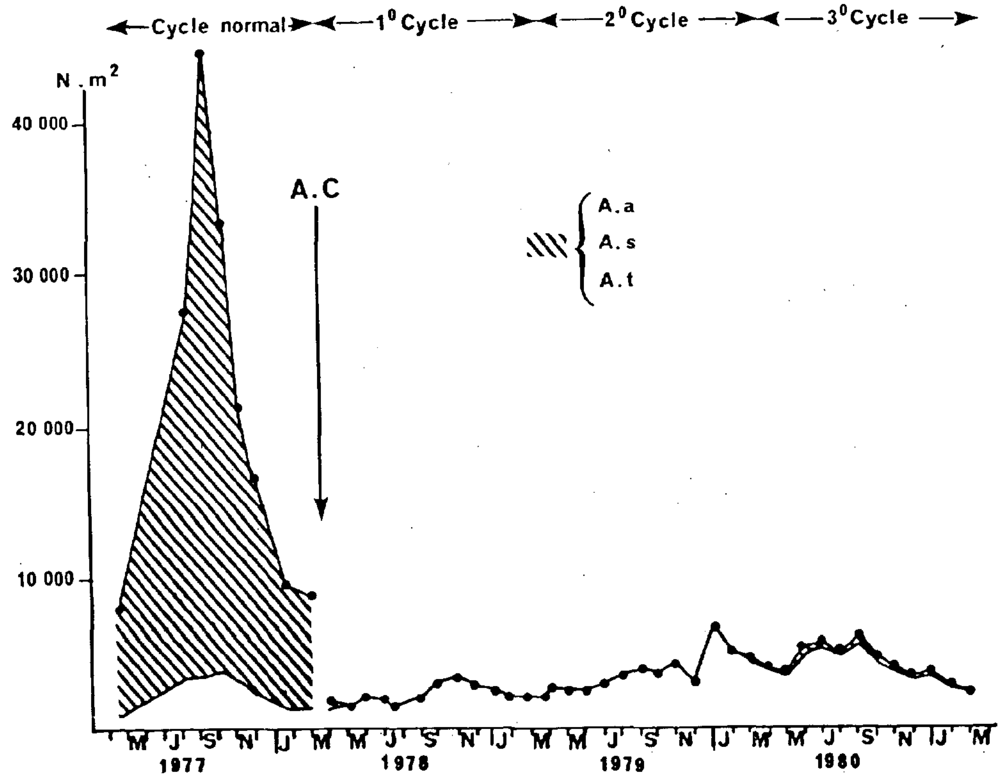


Figure 2 - Peuplement des sables fins à *Abra alba - Hyalinoecia bilineata* de la Pierre Noire : évolution de la densité totale d'avril 1977 à février 1981 avec mise en évidence de la part des trois espèces d'*Ampelisca* très dominantes (A.a. : *Ampelisca armoricana;* A.s. : *Ampelisca sarsi;* A.t. : *Ampelisca tenuicornis*). (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

3.2.1) Peuplement des sédiments grossiers à *Venus fasciata - Amphioxus lanceolatus*.

Pollution (tabl. 2)

Dès le 27 avril les sédiments sont contaminés par les hydrocarbures; aux fortes teneurs enregistrées jusqu'en novembre (231 ppm) succède, en fin d'hiver, une phase de dépollution rapide.

Richesse spécifique (fig. 3)

Le fléchissement des valeurs de la richesse spécifique au cours du premier cycle annuel suivant la pollution semble surtout lié à la disparition de crustacés et de polychètes; cependant, de 1978 à 1980, on note globalement, abstraction faite des fluctuations saisonnières, un accroissement graduel auquel contribuent largement les amphipodes (48 taxons en août 1978 contre 80 en août 1980; 4 espèces d'amphipodes en août 1978 contre 19 en août 1980).

Densités et biomasses

Les densités très faibles varient de 100 à 290 individus par m^2; maximales en août, minimales en février, les valeurs sont du même ordre de grandeur d'une année à l'autre.

	avril 1978	août 1978	nov. 1978	fév. 1979	mai 1979	août 1979	nov. 1979	fév. 1980	mai 1980	août 1980	nov. 1980	fév. 1981
PRIMEL	250	81	231	< 10	< 10	< 10	< 10	16	28	28	33	< 10
PIERRE NOIRE	34	18	57	< 10	juin 1979 463	14	< 10	58	< 10	44	< 10	52
RIVIERE DE MORLAIX	10	297	333	44	3152	136	36	48	60	12	20	?
L_1	18	22	10	37	22	< 10	16	16	< 10	12	41	20
L_2	-	-	58	102	91	< 10	66	58	< 10	12	12	20
L_3	-	132	18	254	269	< 10	83	49	28	12	12	20
L_4	22	13	< 10	26	14	-	-	-	-	-	-	-
L_5	-	69	< 10	132	318	< 10	165	< 10	< 10	12	28	< 10
L_6	26	114	162	63	43	74	12	28	< 10	12	28	28
L_7	-	31	49	74	138	< 10	165	20	< 10	12	28	20
L_8	-	1015	44	34	120	83	91	16	< 10	12	12	< 10

Tableau **2** - Evolution des teneurs en hydrocarbures mesurées en spectrophotométrie aux infra-rouges selon la méthodologie suivie par BESLIER *et al.* (1980) exprimées en milligramme par kilogramme de sédiment sec ou ppm (données BESLIER, 1981).

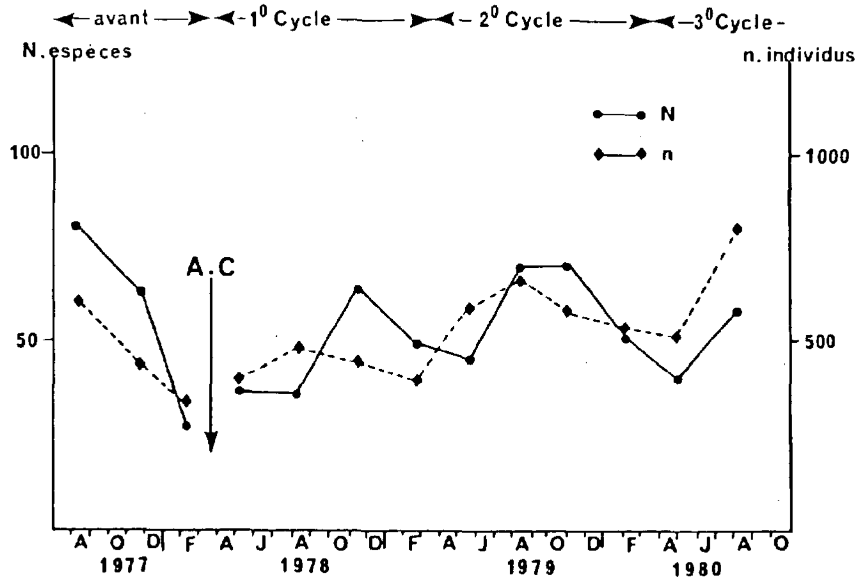


Figure 3 - Peuplement des sables grossiers à *Venus fasciata* - *Amphioxus lanceolatus* : évolution de la richesse spécifique des relevés et du nombre d'individus (10 prélèvements à la benne Hamon) d'août 1977 à août 1980 (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

Les biomasses des différentes composantes faunistiques sont très inégales; par exemple, le lamellibranche *Glycymeris glycymeris* représente 75 % de la biomasse totale du peuplement. Après avoir dépassé 25 g. par m^2 en août et novembre 1977, les valeurs n'oscillent ensuite qu'entre 8 et 12 grammes. Toutefois en novembre 1979, à la suite d'une récolte plus importante de *Glycymeris glycymeris*, elle culmine à près de 24 g. par m^2 rejoignant les valeurs données par HOLME (1953) et RETIERE (1979) pour des peuplements analogues de la Manche occidentale.

3.2.2) Peuplement de maerl envasé

Pollution

La texture hétérogène des sédiments a favorisé le piégeage et la rétention des particules oléosédimentaires pendant une longue période.

Richesse spécifique

Cette biocénose définie par CABIOCH (1968) comme un maerl à *Lithothamnium corallioïdes* a évolué vers un nouveau faciès, vraisemblablement sous l'effet d'un ensablement lié à des extractions industrielles; elle est actuellement très comparable à celle du maerl de Ricard, en baie de Morlaix : maerl à *Lithothamnium corallioïdes* var. *minima*. Le caractère cénotique dominant est son extrême appauvrissement; aucune espèce d'épifaune sessile n'est en effet présente dans nos échantillons prélevés après la pollution, ce qui atteste sans doute une profonde perturbation.

Nous n'avons récolté dans ces fonds qu'un petit nombre d'espèces de l'endofaune. On passe toutefois de 23 espèces en avril 1978 à 34 en février 1979 et parmi les plus abondantes il convient de citer trois annélides polychètes : *Goniada maculata, Staurocephalus kefersteini, Heteromastus filiformis* et deux péracarides : *Nototropis swammerdani* et *Perioculodes longimanus*. De plus il faut noter que les deux espèces électives du faciès hétérogène envasé, *Sthenelais boa* et *Pista cristata*, présentes dans les échantillons d'avril 1978, ont ensuite disparu.

Densités

L'évolution de la densité globale du peuplement reflète principalement celle de quelques populations d'annélides polychètes, en particulier *Goniada emerita* et *Staurocephalus kefersteini* dont les recrutements sur maille de 1 mm s'observent de façon synchrone en automne. La première espèce bénéficierait de la proximité de biotopes servant de "réservoirs" à partir desquels se réaliserait la dispersion des larves pélagiques.

En conclusion il est important de rappeler que depuis 1968, le peuplement macrobenthique de ces fonds a évolué, vraisemblablement sous l'effet d'un ensablement lié à l'extraction du maerl. Ne disposant d'aucun état de référence juste avant la catastrophe il est extrêmement difficile de connaitre la part qui revientà la pollution par les hydrocarbures, dans la modification de cette communauté. Pour cette raison son suivi écologique a été rapidement abandonné.

3.2.3) Peuplements des sédiments fins à *Abra alba*

3.2.3.1) Peuplement des sables fins à *Abra alba - Hyalinoecia bilineata*

Pollution

Après une phase initiale de forte pollution, que nous n'avons pu mesurer, les teneurs en hydrocarbures (mesurées en spectrophotométrie aux infra-rouges, exprimées en mg par kilogramme de sédiment sec ou ppm) atteignent 1000 ppm à la station L8 au cours de l'été 1978 alors qu'elles ne dépassent pas 50 ppm aux deux autres stations (L7 et P.N.), puis elles diminuent fortement au cours de l'automne dans l' ensemble des stations. Ensuite une remobilisation, lors des tempêtes automnales et hivernales, des stocks d'hydrocarbures piégés en zone intertidale entraine une augmentation des teneurs en hydrocarbures au printemps 1979 sur l'ensemble des stations, (ces teneurs dépassent 200 ppm à la station de la Pierre Noire); elles décroissent rapidement ensuite, sauf en L8 où elles se maintiennent à des valeurs voisines de 100 ppm jusqu'à l'automne 1979. Enfin, dans l'ensemble des stations, après une phase de recontamination au cours de l'hiver 1979-1980, les teneurs deviennent inférieures à 50 ppm à partir de mai 1980.

Richesse spécifique

Après le stress on note dans les trois stations un enrichissement progressif en espèces qui aboutit en 1980, à la Pierre Noire, à des valeurs du même ordre qu'avant la pollution. Cette remontée provient d'une part de la réapparition d'espèces éliminées lors du stress, d'autre part de l'augmentation de la fréquence de quelques espèces de polychètes et de mollusques. Les mêmes phénomènes s'observent en baie de Lannion (L7 et L8), mais avec des valeurs inférieures. La comparaison des présences d'espèces dans les trois stations apporte à cet égard des informations significatives dans le cas des amphipodes, groupe le plus affecté par le stress initial.

Certaines espèces, qui ont survécu en baie de Morlaix, sont absentes en baie de Lannion immédiatement après la marée noire, mais elles y apparaissent au cours du premier cycle annuel suivant le stress *(Leucothoe incisa, Urothoe grimaldii, Tryphosites longipes, Bathyporeia tenuipes)*, du deuxième cycle *(Ampelisca sarsi)* ou du troisième *(Bathyporeia elegans, Synchelidium maculatum)*. Cette constatation témoigne de leur présence probable en baie de Lannion avant la pollution et renforce l'hypothèse d'une plus grande intensité de l'impact initial dans cette baie par rapport à la baie de Morlaix (CABIOCH *et al.*, 1981).

Des espèces éliminées temporairement par le stress en baie de Morlaix et qui s'y réinstallent au cours du premier cycle perturbé *(Megaluropus agilis, Ampelisca spinipes)* ou de deuxième *(Cheirocratus intermedius)* (DAUVIN, 1981) ne sont rencontrées dans les sables fins plus longuement pollués de la baie de Lannion qu'au cours du troisième cycle. Ces introductions comme les précédentes, interviennent généralement plus tôt en L7 qu'en L8, station la plus polluée des deux.

Les espèces dont on est certain de l'"insularité" figurent parmi celles qui se réinstallent plus tardivement dans l'une ou l'autre des baies *(Ampelisca tenuicornis, A. brevicornis, A. armoricana)* ou qui n'ont pas encore réapparu *(Photis longicaudata)*. A l'opposé, *Ampelisca spinipes*, espèce non insulaire, commune dans les sables grossiers avoisinant la zone polluée, repeuple les sables fins de la Pierre Noire dès le premier cycle.

En conclusion, les effets éliminateurs du stress, plus ou moins accusés selon les localités d'un même type de peuplement semblent accompagnés de réimplantations d'autant plus tardives que la pollution résiduelle du sédiment est durable et intense. La conjugaison de l'insularité géographique des populations de certaines espèces et de leur mode direct de reproduction a aussi un effet retardateur.

Densités et biomasses

En baie de Morlaix, à la station de la Pierre Noire la densité moyenne du peuplement passe de 19450 individus par m^2 lors du cycle normal à 2135 au cours du premier cycle après la pollution. Cette très grande différence est le fait de la disparition et de la forte réduction d'effectifs de trois espèces d'*Ampelisca* largement dominantes avant la pollution. Durant les deuxième et troisième cycles annuels les densités moyennes atteignent les valeurs respectives de 3650 et 4110 individus par m^2.

Parallèlement à la forte décroissance des densités, la biomasse subit une réduction de près de 50 % pendant le premier cycle annuel après la pollution (4.4 g. par m^2 contre 8.1). Dès le second cycle elle retrouve des valeurs comparables à celles du cycle normal (8.0 g. par m^2) et les dépasse même au cours du troisième cycle annuel (8.7 g. par m^2).

Ainsi, trois ans après le stress, bien que les densités soient encore inférieures, d'environ 80 %, à celles observées avant la pollution, les valeurs de biomasse sont du même ordre de grandeur que celles du cycle normal. En effet les espèces subsistant après le stress, dont les densités sont passées de 2100 à 3860 individus par m^2 du premier au troisième cycle annuel après la pollution, ont des poids individuels moyens très supérieurs à celui des ampeliscidés.

En baie de Lannion l'évolution de la densité globale du peuplement est fortement marquée par les variations d'abondance, d'ailleurs difficiles à interpréter, d'une seule espèce, *Paradoneis armata*. La densité de celle-ci diminue progressivement à la station L8 pour atteindre 500 individus par m^2 en janvier 1981 et se maintient à un niveau compris entre 500 et 600 individus par m^2 à la station L7 pendant la période d'observation. Il faut toutefois noter qu'au cours des trois cycles annuels la densité correspondant à l'ensemble des autres espèces tend à s'accroître.

Les réintroductions d'espèces temporairement éliminées apportent très peu à cette croissance de la densité à moyen terme après le le stress. Elle résulte principalement de quelques cas de recolonisation par des espèces réduites en effectifs pendant le premier cycle perturbé et de prolifération d'espèces non affectées par la pollution. Les autres espèces poursuivent des cycles annuels peu différents du cycle normal.

Les recolonisations significatives sont le fait de trois espèces : *Ampelisca sarsi, Ampharete acutifrons, Nephtys hombergii*. Alors que la communauté des sables fins de la Pierre Noire a pu héberger jusqu'à 40.000 *Ampelisca* par m^2, l'espèce subsistante, *A. sarsi* ne recolonise cette vaste niche écologique vacante qu'à une cadence restreinte (fig. 4) de par la conjonction de sa distribution "insulaire" et de ses caractères biologiques (reproduction directe printanière et estivale, femelles porteuses de 8 à 20 embryons seulement et ne se reproduisant qu'une fois, vie brève ne dépassant guère un an (DAUVIN, 1979)). Limitée par l'insularité au seul potentiel reproducteur de sa population résiduelle, l'espèce multiplie cependant son effectif maximum annuel par un facteur de 5 à 9 d'une année sur l'autre ce qui témoigne du succès de la reproduction directe. *Ampharete acutifrons*, (fig. 5) insulaire, de durée de vie inférieure à deux ans, à larve presque immédiatement benthique, suit un schéma de recolonisation lente du même type. Par contre, le repeuplement de *Nephtys hombergii* (vie longue, non insularité, larves pélagiques pendant plus d'un mois) s'effectue rapidement (fig. 6); dès 1980, les effectifs estivaux, qui ne dépassaient pas 30 individus par m^2 en 1978, atteignent 170 par m^2, valeur supérieure à celle observée avant pollution (90 par m^2).

En ce qui concerne les proliférations, la brève poussée d'*Heterocirrus alatus* à l'automne de 1978 (fig. 7) est suivie, au cours du deuxième et du troisième cycle par des accroissements importants de *Chaetozone setosa*, (Fig. 7), *Spio filicornis, Scoloplos armiger, Thyasira flexuosa, Abra alba*. Ainsi se dessine probablement un premier élément d'une série de "successions" (PEARSON & ROSENBERG, 1978), phénomène moins immédiat et moins accusé ici que sur les fonds sublittoraux bien plus pollués des Abers (GLÉMAREC & HUSSENOT, 1981; GLÉMAREC & HUSSENOT, sous presse).

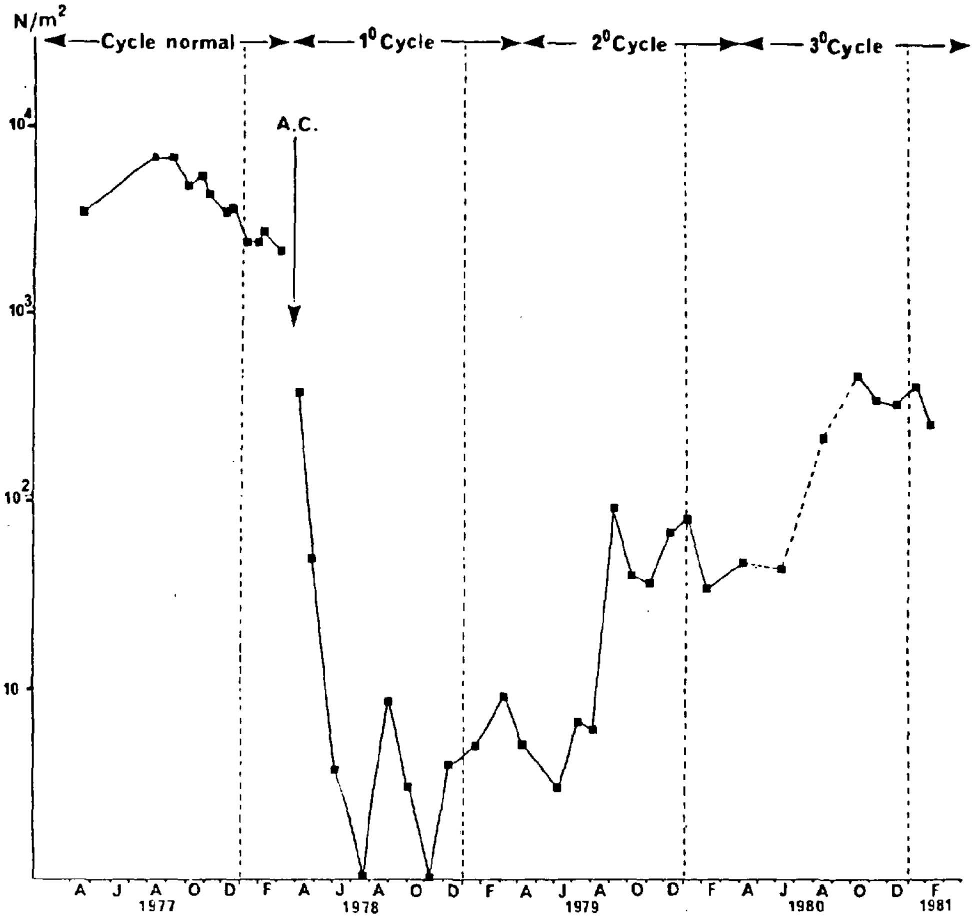


Figure 4 - Peuplement des sables fins à *Abra alba - Hyalinoecia bilineata* de la Pierre Noire : évolution de la densité d'*Ampelisca sarsi* d'avril 1977 à février 1981 (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

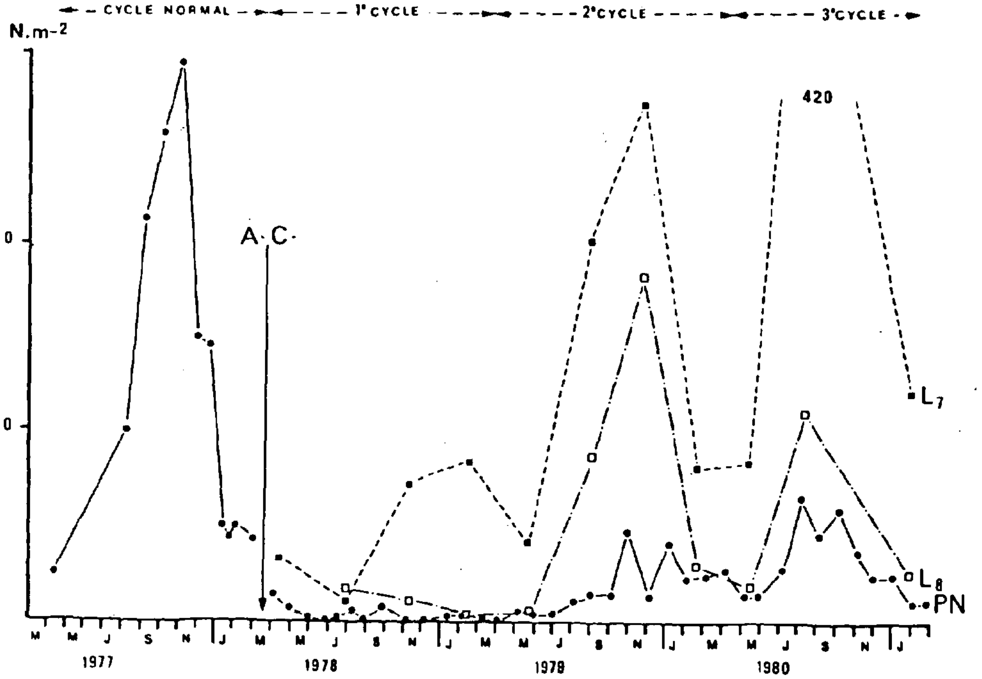


Figure 5 - Peuplement des sables fins à *Abra alba - Hyalinoecia bilineata* : évolution de la densité d'*Ampharete grubei* d'avril 1977 à février 1981 (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

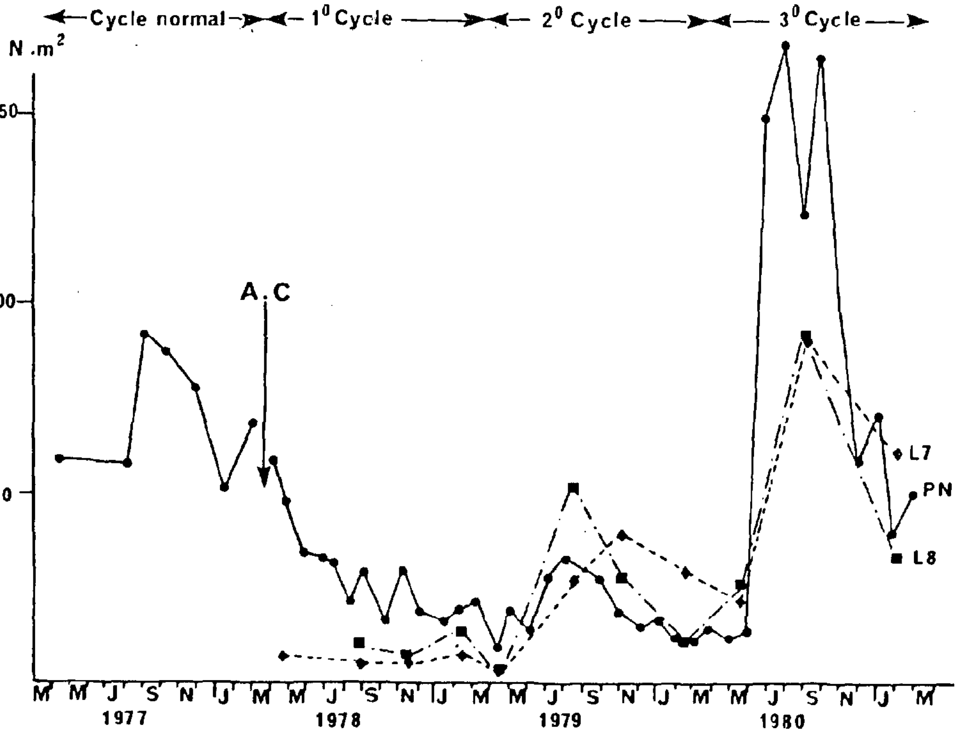


Figure 6 - Peuplement des sables fins à *Abra alba - Hyalinoecia bilineata* : évolution de la densité de *Nephtys hombergii* d'avril 1977 à février 1981 (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

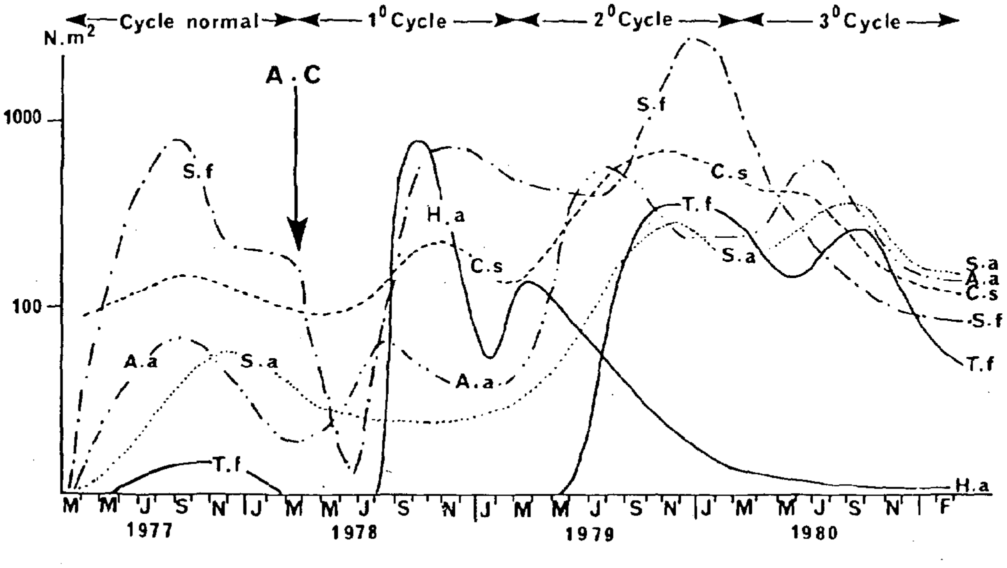


Figure 7 - Peuplement des sables fins à *Abra alba - Hyalinoecia bilineata* : évolution schématique de la densité d'*Abra alba* (A.a.), de *Chaetozone setosa* (C.s.), d'*Heterocirrus alatus* (H.a.), de *Scoloplos armiger* (S.a.), de *Spio filicornis* (S. f.) et de *Thyasira flexuosa* (T.f.). (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

3.2.3.2) Peuplement des sables très fins à *Tellina fabula* - *Abra alba*

Pollution

Les teneurs en hydrocarbures après un pic passager en août 1978 (premières mesures) redeviennent fortes de février à mars 1979 (valeurs comprises entre 80 et 300 ppm). A la faveur d'une recontamination du sédiment au cours de l'automne 1979, les teneurs dépassent de nouveau 50 ppm en novembre (70 à 165 ppm) elles se maintiennent à ce niveau en L2 et L3 au cours de l'hiver 1980, puis redeviennent inférieures à partir de mars 1980 dans l'ensemble des trois stations.

Richesse spécifique et densités*

On sait indirectement que ce peuplement a subi une perturbation considérable lors du stress; les immenses quantités d'*Echinocardium cordatum* et de mollusques de diverses espèces, rejetés morts sur la grève de St-Efflam en mars-avril 1978 en témoignent (CHASSÉ & GUENOLÉ-BOUDER, 1981). Les phénomènes observés à la suite du stress présentent de grandes analogies avec ceux que nous venons de décrire : croissance à moyen terme de la richesse spécifique (fig. 8) et de la densité totale, phénomènes de recolonisation.

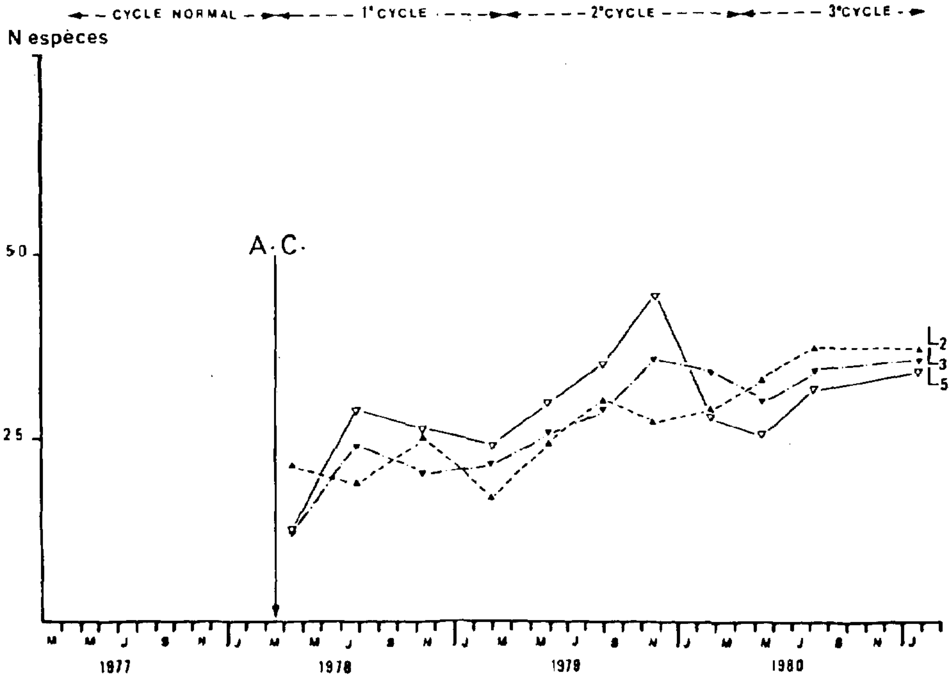


Figure 8 - Peuplement des sables très fins à *Tellina fabula* - *Abra alba* : évolution de la richesse spécifique des relevés (4 prélèvements à la benne Hamon) d'avril 1978 à février 1981 (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

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*Les résultats du suivi de la station L1 dont le peuplement présente un caractère nettement intertidal ne sont pas intégrés dans ce travail qui a pour objet l'étude des communautés subtidales. De même le suivi de la station L4 a été abandonné en mai 1979, le dépouillement des données n'apportait pas d'informations complémentaires de celles recueillies aux stations L3 et L5.

La croissance de la densité est principalement liée à la prolifération, depuis la perturbation, du Capitellidae *Mediomastus fragilis* (fig. 9) dont les effectifs à la station L3 passent de 100 individus par m^2 en avril 1978 à plus de 7000 par m^2 en mai 1980.

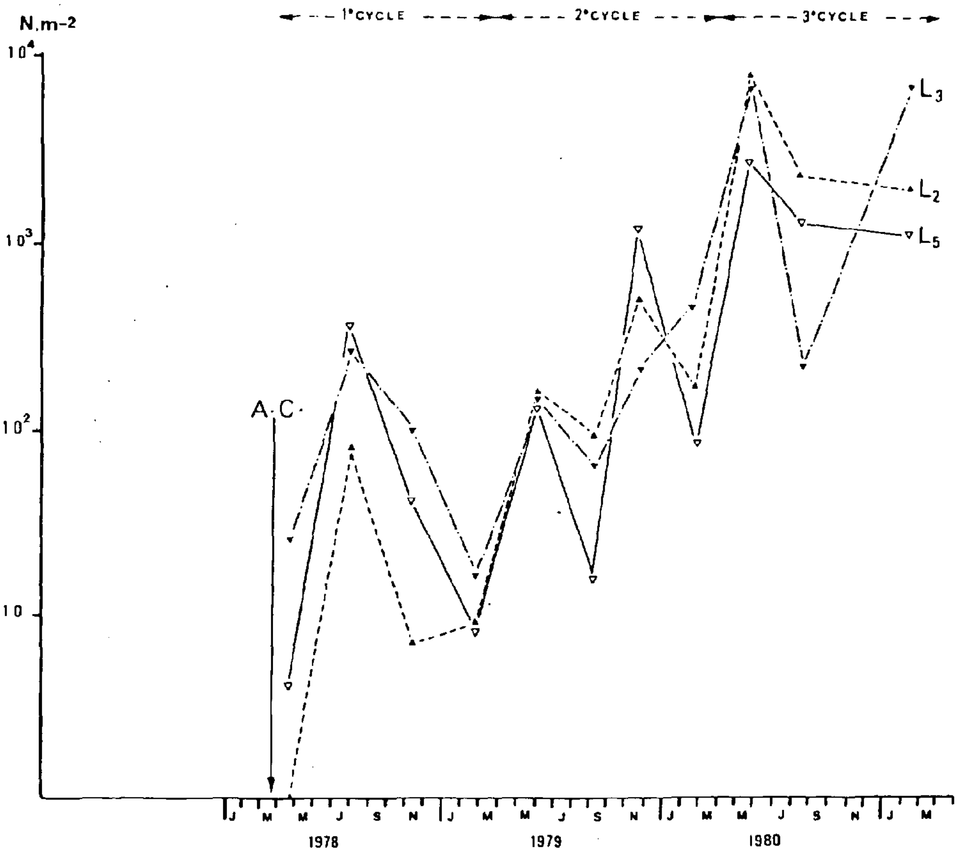

Figure 9 - Peuplement des sables très fins à *Tellina fabula - Abra alba :* évolution de la densité de *Mediomastus fragilis* d'avril 1978 à février 1981 (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

Les recolonisations significatives sont ici le fait de trois espèces : *Nephtys hombergii, Glycera convoluta* et *Tellina fabula;* mais alors que les deux premières voient leurs effectifs augmenter progressivement d'année en année (respectivement de 63 et 80 individus par m^2 en 1978 à 162 et 360 en 1981) l'abondance de *Tellina fabula* qui n'a pas dépassé 250 individus par m^2 en 1978 et 1979, s'élève brusquement à plus de 1000 individus par m^2 pendant le troisième cycle.

3.2.3.3) Peuplement des vases sableuses à *Abra alba - Melinna palmata*

Pollution

Les teneurs en hydrocarbures sont très élevées jusqu'en juillet 1979 (elles dépassent toujours 100 ppm sauf en février 1979 et elles atteignent même 3000 ppm en mars 1979); ensuite on observe une dépollution graduelle.

Richesse spécifique (fig. 10)

D'avril 1978 à avril 1980 la richesse spécifique est stable; elle s'accroît considérablement au cours de l'été 1980, diminue ensuite et se maintient durant l'hiver suivant à un niveau plus élevé que celui des hivers précédents.

L'augmentation de la richesse spécifique provient à la fois de la réintroduction d'espèces d'amphipodes éliminées lors du stress et de l'accroissement du nombre d'espèces de polychètes.

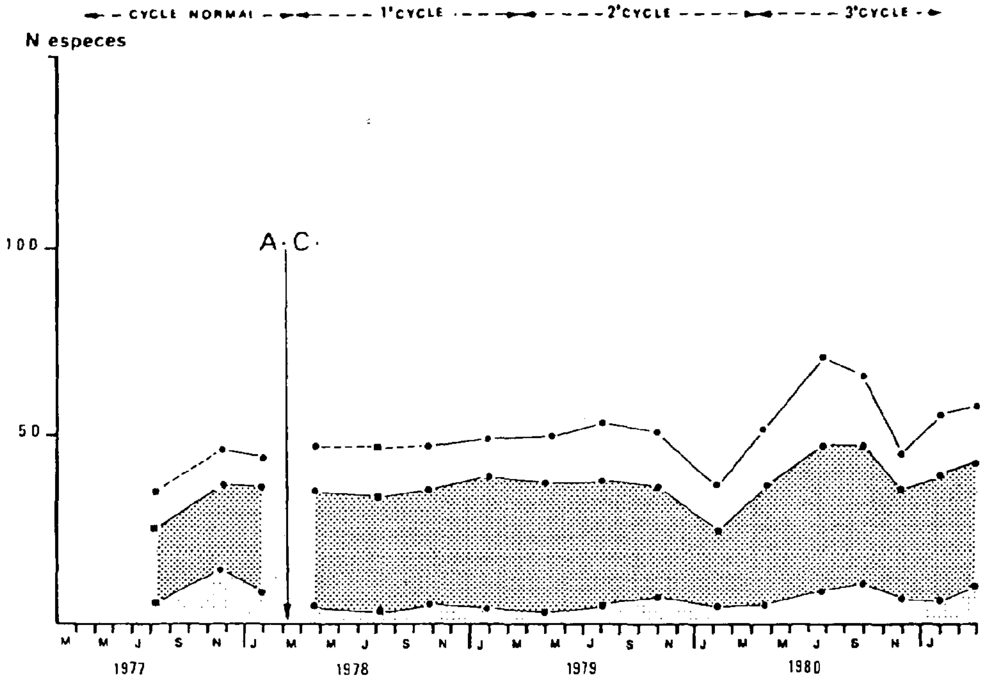

Figure 10 - Peuplement des vases sableuses à *Abra alba* - *Melinna palmata* : évolution de la richesse spécifique des relevés (10 prélèvements à la benne Smith Mc Intyre) d'août 1977 à mars 1981.

Les réapparitions d'amphipodes se réalisent de manière graduelle : les premiers exemplaires de *Cheirocratus intermedius* et *Ampelisca brevicornis* sont récoltés plus d'un an après leur disparition, ceux d'*Ampelisca tenuicornis* seulement au cours du second cycle; les espèces *Ampelisca armoricana* et *Ampelisca spinimana* n'ont pas encore été retrouvées.

En ce qui concerne les polychètes on observe conjointement la capture plus fréquente d'espèces sporadiques avant la pollution et l'intrusion d'espèces constantes, pour la plupart, dans le peuplement des sables fins à *Abra alba* - *Hyalinoecia bilineata* de la Pierre Noire.

Densités et biomasses

La densité qui n'est pas modifiée lors du stress croît au cours du premier cycle annuel perturbé : 4467 individus par m^2 en moyenne contre 2855 durant le cycle normal avant la pollution. Cette différence est due essentiellement aux fluctuations d'effectifs d'espèces présentes, avant la pollution, en densités faibles (*Mediomastus fragilis*, fig. 12 et *Tharyx marioni*, fig. 13) ou fortes (*Chaetozone setosa*, fig. **11**).

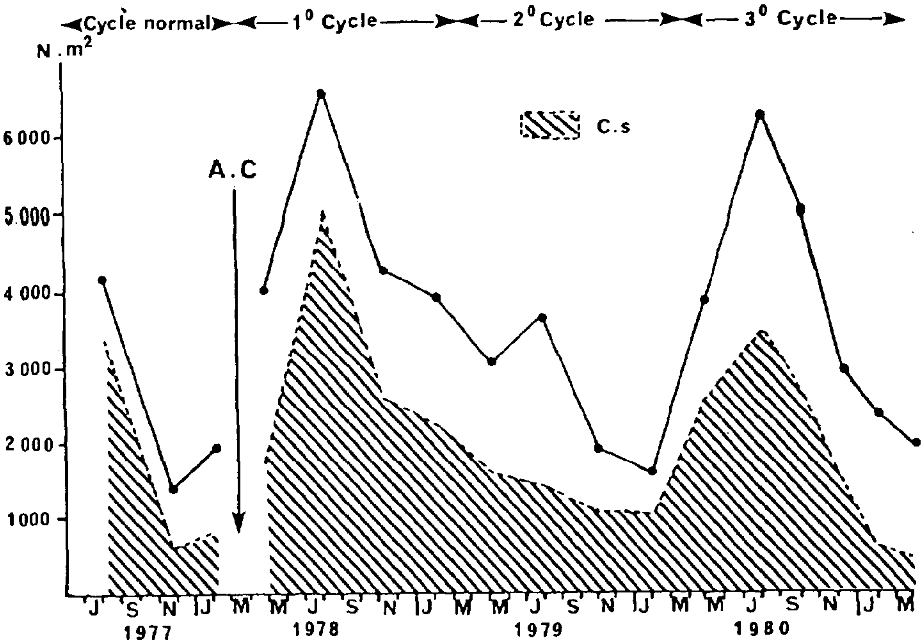

Figure 11 - Peuplement des vases sableuses à *Abra alba - Melinna palmata :* évolution de la densité totale d'août 1977 à mars 1981 avec mise en évidence de la part de *Chaetozone setosa* (C.s.) (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

Au cours du second cycle, la densité redevient voisine de celle du cycle normal, puis augmente à nouveau durant le troisième cycle : 3724 individus par m^2; cette dernière valeur s'explique par le haut niveau d'abondance de *Mediomastus fragilis* et *Tharyx marioni*, par une élévation de la densité d'autres espèces de polychètes notamment *Lanice conchilega* et *Melinna palmata* et enfin par la recolonisation de *Nephtys hombergii* et *Ampelisca tenuicornis*.

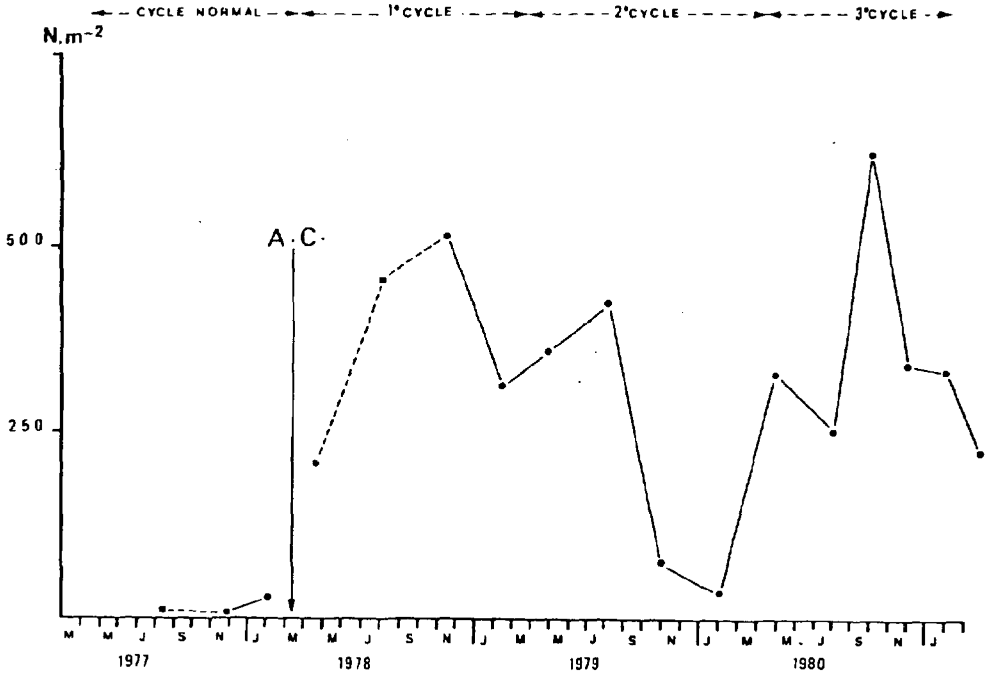

Figure 12 - Peuplement des vases sableuses à *Abra alba - Melinna palmata* : évolution de la densité de *Mediomastus fragilis* d'août 1977 à février 1981 (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

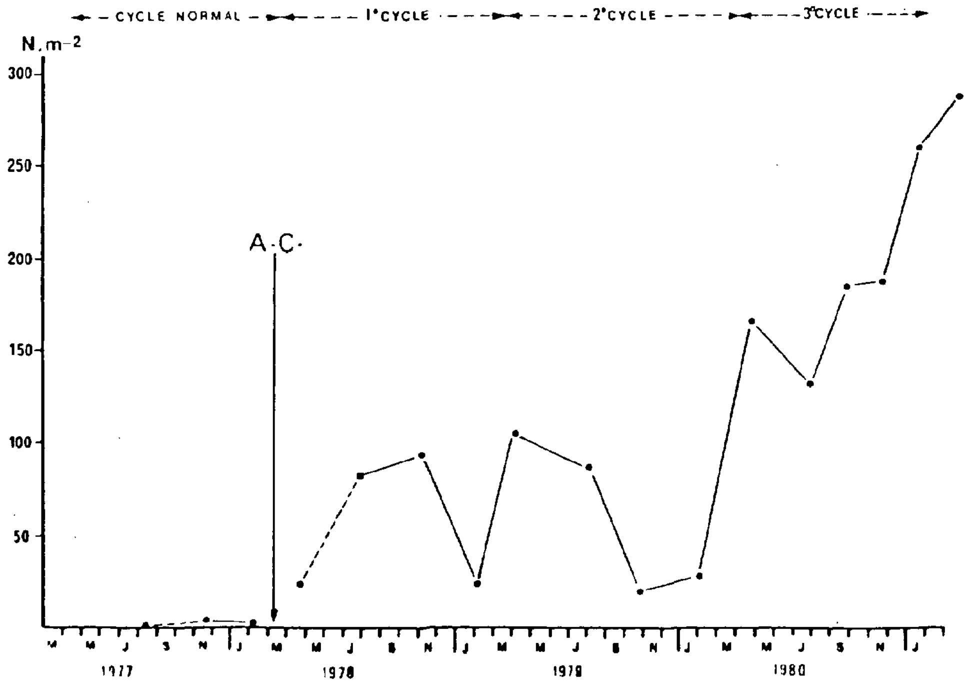

Figure 13 - Peuplement des vases sableuses à *Abra alba - Melinna palmata :* évolution de la densité de *Tharyx marioni* d'août 1977 à février 1981 (A.C. : début de la pollution par les hydrocarbures de l'"Amoco Cadiz").

L'accroissement des biomasses moyennes correspondant aux quatre cycles annuels d'observations est lié surtout à l'installation progressive de *Lanice conchilega* dans le peuplement. De 1977 à 1980 les valeurs relatives aux observations effectuées entre les mois d'août et avril sont respectivement 9.0, 13.0 et 12.4 g. par m^2; pour la période comprise entre août 1980 et mars 1981 la biomasse moyenne atteint 16.8 g. par m^2.

Les valeurs moyennes de densité (3425 individus par m^2) et de biomasse (12.9 g. par m^2) s'accordent avec celles données par les auteurs travaillant sur des peuplements analogues (RETIERE, 1979).

Au terme de l'étude des peuplements de sables fins vaseux il importe de souligner que les modalités quantitatives des divers phénomènes qui se succèdent dans le peuplement à *Abra alba - Melinna palmata* de la rade de Morlaix diffèrent profondément de celles observées dans le peuplement à *Hyalinoecia bilineata* de la Pierre Noire. Dans le premier, les espèces sensibles aux hydrocarbures sont en effet peu représentées avant la pollution, si bien que les mortalités initiales sont faibles et n'altèrent que légèrement la structure du peuplement, contrairement à la modification structurale considérable intervenue à la Pierre Noire. L'évolution ultérieure des deux peuplements à moyen et long terme offre un contraste d'une autre nature. La communauté subsistante de la Pierre Noire poursuit une dynamique de reconstitution, dans un milieu benthique rapidement décontaminé, et naturellement oligotrophe. Au contraire, le peuplement de la rade de Morlaix vit dans un milieu benthique naturellement eutrophe, plus durablement chargé d'hydrocarbures et de matières organiques. On assiste alors à un développement plus important des populations de détritivores, déjà abondants en conditions naturelles (*Chaetozone setosa)* et aussi à de véritables proliférations d'espèces réellement opportunistes (*Mediomastus filiformis* et *Tharyx marioni* sp.), présentes seulement à l'état latent au cours du cycle normal précédant la pollution.

4) CONCLUSIONS GÉNÉRALES

Le recul qu'apportent trois années d'observations permet de distinguer parmi les phénomènes qui ont affecté les peuplements subtidaux de la région de Roscoff ceux liés au stress proprement dit de ceux qui se sont succédés au cours des cycles annuels suivants et d'en interpréter les différentes modalités :

- l'élimination des espèces lors du stress est sélective; elle est fonction à la fois de l'éco-éthologie des espèces et des concentrations du milieu en hydrocarbures toxiques dissouts. Cette phase de mortalité est relativement limitée dans le temps (quelques semaines);

- l'intensité des perturbations dues au stress varie d'une communauté à l'autre le long du gradient édaphique et à l'intérieur de la même unité de peuplement. Alors que les peuplements des sédiments grossiers et des sables vaseux ont été peu modifiés qualitativement et quantitativement par le stress, ceux des sables fins et très fins ont été intensément perturbés. Ces divers degrés d'altération sont fonction du nombre et de l'abondance des espèces sensibles aux hydrocarbures; dans le cas extrême du peuplement des sables fins à *Abra alba - Hyalinoecia bilineata* on assiste à une réduction respective de 80 et 50 % des valeurs initiales de densité et de biomasse. Toutefois il convient de noter que ce n'est pas nécessairement sur le peuplement où le stress a été le plus dévastateur que les effets secondaires sont les plus marqués;

- dans les biotopes où les effets du stress se sont faits sentir sévèrement les valeurs de la richesse spécifique, de la densité et de la biomasse restent faibles pendant le premier cycle annuel après la pollution; on n'enregistre pas, au cours de cette période, de mortalités massives d'adultes mais l'absence de recrutement chez un certain nombre d'espèces freine considérablement la recolonisation du milieu. Dans la plupart des cas, durant cette première phase, les effets sont proportionnels aux quantités d'hydrocarbures résiduels;

- le deuxième cycle annuel marque globalement le début de la réintroduction des espèces éliminées et de la recolonisation des fonds par celles dont les populations ont été affectées par le stress. Ces phénomènes s'accélèrent et s'accentuent au cours du troisième cycle. La vitesse de réintroduction et le taux de recolonisation de certaines d'entre elles sont d'ailleurs limités par le caractère insulaire de leur distribution et l'absence de phase pélagique;

- les cycles de densité de la plupart des espèces qui n'ont pas été affectées par le stress se déroulent à peu près normalement; par contre un petit nombre d'espèces regroupant surtout des *Capitellidae* et *Cirratulidae* prolifèrent soit au cours du premier cycle, soit plus tardivement, constituant probablement les amorces d'une succession écologique;

- trois ans après la pollution par les hydrocarbures la richesse spécifique du peuplement le plus perturbé , c'est-à-dire celui des sables fins peu envasés, a retrouvé son niveau initial et bien que sa densité soit toujours beaucoup plus faible qu'elle ne l'était auparavant les valeurs de biomasse sont à nouveau tout à fait comparables à celles de 1977. Il semble donc que ce peuplement évolue vers un "nouvel équilibre";

En outre, de cette étude se dégagent un certain nombre d'enseignements :

- les cartes bio-sédimentaires, support des études dynamiques, constituent un état de référence dont l'intérêt est évident dans le cas de pollutions accidentelles du type "Amoco-Cadiz";

- sur l'ensemble du secteur touché par la pollution les premières investigations doivent être engagées très rapidement; au sein des communautés présumées les plus sensibles il est indispensable de sélectionner plusieurs stations, le suivi de certaines d'entre elles pouvant être abandonné à la lumière des premiers résultats;

- le suivi écologique doit s'étaler sur une période suffisamment longue pour qu'au delà du bruit de fond des fluctuations naturelles à plus ou moins long terme on puisse percevoir les phénomènes réellement dépendants de la perturbation.

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ETUDE EXPERIMENTALE D'UNE POLLUTION PAR HYDROCARBURES DANS UN MICROECOSYSTEME SEDIMENTAIRE. I : EFFET DE LA CONTAMINATION DU SEDIMENT SUR LA MEIOFAUNE

par

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RESUME

Les conséquences de deux niveaux de contamination par hydrocarbures ont été analysées, par rapport à un témoin, dans des microécosystèmes expérimentaux en circuit clos contenant 100 litres de sable fin sublittoral. L'évolution des caractéristiques du peuplement de méiofaune (Nématodes et Copépodes) a été choisie pour caractériser l'impact des hydrocarbures. Les densités des Nématodes augmentent sensiblement par rapport au témoin pendant les deux premiers mois de la pollution puis regressent lentement sans qu'il soit possible de distinguer les effets d'une forte pollution de ceux d'une faible pollution. Les densités des Copépodes harpacticoïdes sont d'autant plus faibles que le sédiment est plus contaminé. Le rapport Nématodes/Copépodes paraît être un indice significatif du degré de pollution.

La composition faunistique des Nématodes est profondément modifiée dans le module le plus pollué après 3 mois d'expérience. Cette dégradation se manifeste par une chute brutale de la biomasse et de la diversité spécifique. Des petites espèces opportunistes connues pour leur association avec l'enrichissement en matière organique, deviennent dominantes. Le module faiblement pollué ne présente aucune dégradation interprétable, par rapport au témoin, des paramètres du peuplement.

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INTRODUCTION

Les conséquences des pollutions sur l'environnement sont souvent difficilement interprétables car leur dilution dans un milieu complexe peut provoquer des altérations, plus ou moins discernables des fluctuations naturelles, apparaissant immédiatement après contamination ou différées dans le temps.

L'expérimentation en laboratoire de la toxicité des polluants sur des organismes isolés de leur environnement naturel a montré souvent ses limites car elle ne prend pas en compte les effets cumulatifs. Par contre, les simulations sur des microécosystèmes complexes appelés microcosmes ou mesocosmes selon leur taille, permettent de mieux cerner les conséquences des perturbations des écosystèmes et souvent de compléter les observations réalisées dans le milieu naturel. L'utilisation des microcosmes permet en outre d'intégrer les interactions entre les niveaux trophiques d'organisation des écosystèmes par exemple et de réaliser des manipulations et des replications.

Quelques rares simulations au laboratoire de contaminations par hydrocarbures ont jusqu'ici été réalisées soit pour envisager les vitesses de dégradation des hydrocarbures en milieu sédimentaire complexe (Johnston, 1970; Delaune et coll. 1980; Wade & Quinn 1980) soit pour comprendre les effets sur les organismes dans les différents niveaux d'organisation de l'écosystème (Lacaze 1979; Elmgren et coll. 1980; Grassle et coll. 1981; Elmgren & Frithsen, sous presse).

A la suite de la pollution pétrolière de l'"Amoco Cadiz" sur les côtes de Bretagne Nord (Manche occidentale), nous nous sommes attachés parallèlement à l'étude *in situ* des conséquences de la contamination des sables fins sublittoraux (Boucher 1980 et 1981; Boucher, Chamroux et Riaux 1981), à réaliser une simulation du phénomène dans des microécosystèmes en circuit clos.

MATERIEL ET METHODES

Trois modules expérimentaux en circuit clos dont le principe a été fourni dans Boucher et Chamroux (1976) ou Mével (1979) sont utilisés (Figure n° 1). Chaque bac comporte trois compartiments dont les niveaux sont régulés par contacteur électrique (500 litres d'eau de mer du large). Le compartiment principal comporte 100 litres de sable réparti sur un double fond sur une surface de 0,41 m^2 et une hauteur de 25 cm environ, et percolé par différence de niveau entre les compartiments à une vitesse de filtration de l'ordre de 15 l/m^2/heure. Le sédiment est prélevé à la benne Smith-McIntyre en milieu sublittoral par - 19 mètres de profondeur (Station de la Pierre Noire). Sa médiane est de 136 ± 5 μ. La température pendant le durée de l'expérience a varié entre 10 et 15°C. Les trois mesocosmes ont été nourris tous les deux jours par des casaminoacides de DIFCO à des doses correspondant à 50 g d'Azote/an/m^2.

L'un des problèmes importants à résoudre était le mode d'introduction des hydrocarbures dans les modules expérimentaux. Respectivement 100, 10 et 0 g. d'hydrocarbures Arabian light, étêtés à 240°C,

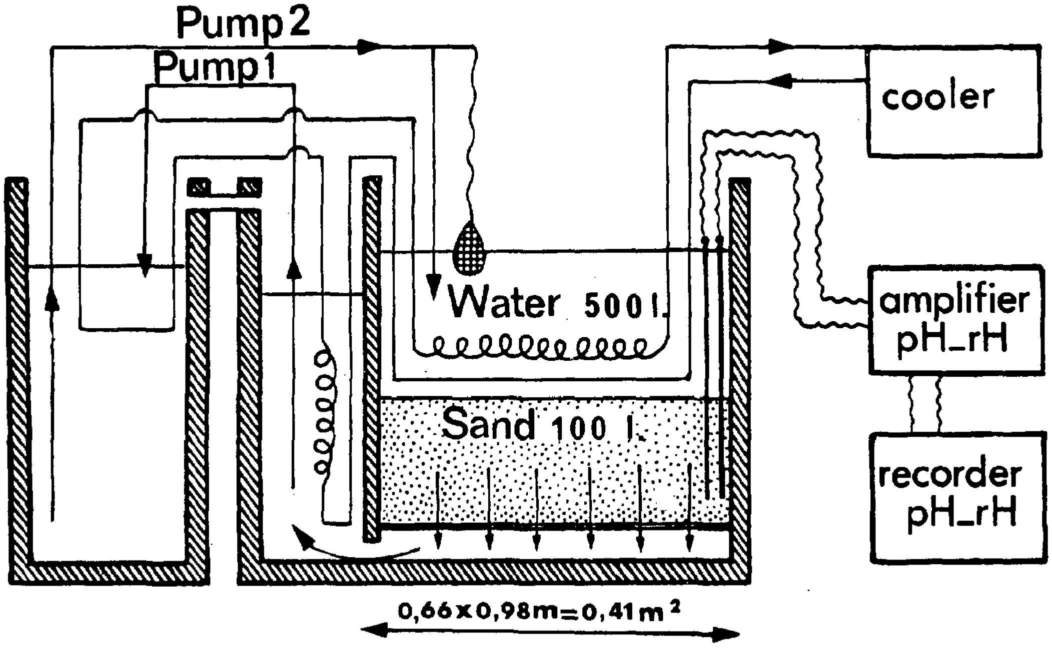

FIGURE 1. Schéma d'un bac expérimental ou mesocosme utilisé pour les simulations de pollution par hydrocarbures (Volume de sable : 100 litres, Volume d'eau : 500 litres).

(fournis par l'IFP) ont été mélangés à 1 kg de sable sec et homogénéisés avec 100 ml de tetrachlorure de Carbone. Après évaporation totale, le sédiment ainsi traité a été ajouté respectivement dans chacun des trois modules appelés : Bac fortement pollué, Bac faiblement pollué et Témoin. Le coulage des hydrocarbures a été ainsi quasi immédiat et l'essentiel des particules contaminées s'est réparti à la surface du substrat. Une faible fraction est restée à la surface de l'eau contenue dans le compartiment à sable du bac le plus pollué pendant quelques jours.

Les prélèvements dans chacun des mesocosmes ont été réalisés à l'aide de tubes de carottages en plexiglass de 5,72 cm². Trois prises simultanées ont été effectuées pour les hydrocarbures et les comptages de méiofaune avec une fréquence hebdomadaire puis mensuelle, pendant plus de six mois du 17 mars 1981 au 29 septembre 1981. Chaque carottage a été fractionné en trois niveaux : 0-4; 4-8; 8-12 centimètres pour analyse de la répartition verticale des paramètres.

Les hydrocarbures ont été extraits au tetrachlorure de Carbone à partir de 10 grammes de sédiment séché à 60°C à l'étuve. Après passage sur Fluorisil, destiné à éliminer les fractions oxydées et les hydrocarbures endogènes, l'extrait a été lu au spectrophotomètre infrarouge UNICAM SP 1100, à une longueur d'onde comprise entre 2500 et 3000 cm^{-1}, avec calage du pic caractéristique à 2925 cm^{-1}. Les résultats ont été exprimés en ppm (mg/kg sable PS) d'après l'abacle réalisée sur l'Arabian light IFP.

La filtration sur Fluorisil provoque une rétention sur le filtre de l'ordre de 60.4% du poids du produit d'origine.

Les organismes de la méiofaune ont été fixés au formol 4%, colorés au rose bengal et triés après passage sur tamis de 40 µ et élutriation. Des lots de 100 Nématodes ont été mesurés et identifiés pour la détermination de la biomasse et de la composition spécifique.

RESULTATS

Evolution des hydrocarbures

Les quantités d'hydrocarbures introduites dans les modules faiblement et fortement pollués correspondent à des teneurs initiales théoriques de 223 et 2236 ppm puisque seuls les quatre premiers centimètres du sédiment (soit 17.7 kg) sont contaminés et que le passage de l'extrait tetrachlorure sur Fluorisil entraîne une perte de 60.4% au dosage.

En effet, l'analyse de la répartition verticale des hydrocarbures dans la colonne sédimentaire en utilisant ce type de dispositif expérimental révèle une pénétration quasiment nulle du polluant sous la surface. Seule la tranche 0-4 centimètres contient des hydrocarbures en quantité notable et une pénétration limitée dans la tranche 4-8 centimètres n'apparaît épisodiquement qu'à partir du 106ème jour. Le témoin n'a jamais montré la moindre trace d'hydrocarbures.

La figure n° 2 fournit l'évolution des teneurs au cours du temps dans les bacs faiblement et moyennement pollués.

L'évolution des teneurs dans le module faiblement pollué indique une dégradation extrêmement faible au cours des six mois d'expérience avec une hétérogénéité des teneurs mesurées très légèrement plus accentuée en début d'expérience.

Par contre, l'évolution des teneurs dans le module fortement pollué met en évidence une très forte hétérogénéité des concentrations jusqu'au 23ème jour de prélèvement qui reflète la répartition en aggrégats des hydrocarbures à la surface du substrat ainsi qu'il a été possible de l'observer en plongée *in situ* sur les sables d'origine de la Pierre Noire. Cette hétérogénéité tend à se réduire ensuite considérablement du fait de la bioturbation. Le pic d'abondance des hydrocarbures observé au 23ème jour reste compatible avec les incertitudes de l'intervalle de confiance à la moyenne. Il est lié probablement aussi au délai nécessaire au coulage de toutes les particules mazoutées ayant partiellement tendance à flotter à la surface de l'eau du compartiment principal en début d'expérience.

La disparition des hydrocarbures entre 23 et 93 jours semble satisfaisante puisqu'elle indique une évolution de 2979 ± 1672 ppm à 248 ± 52 ppm soit 38.5 mg HC/kg sable/jour (24 mg HC/kg sable/jour si l'on tient compte d'une valeur initiale théorique de 2236 ppm. Il est cependant impossible de considérer cette valeur comme un taux de dégradation réaliste du fait de l'hétérogénéité des teneurs initiales mesurées mais aussi d'une remontée incompréhensible des teneurs en hydrocarbures au 106ème et 124ème jour.

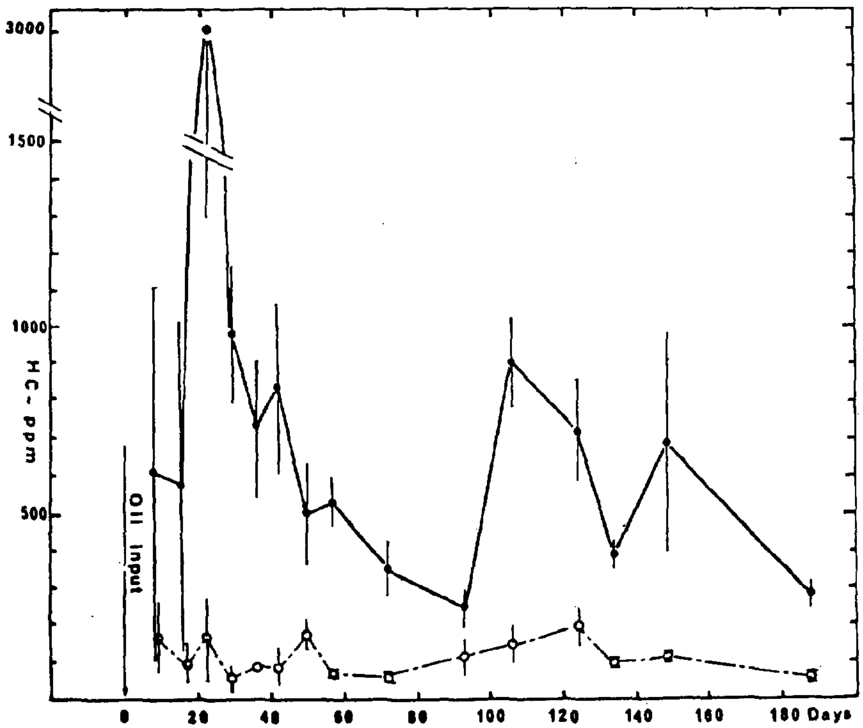

FIGURE 2. Evolution des teneurs en hydrocarbures et de leur écart à la moyenne, dosées par la méthode infra-rouge après passage sur Fluorisil, dans le sable des modules fortement pollués (100 g HC : ● —— ●) et faiblement pollués (10 g HC : o –·–·o). Les hydrocarbures sont presque toujours concentrés dans les quatre premiers centimètres du sédiment.

La difficulté d'interprétation des résultats des dosages effectués par infrarouge après passage de l'extrait au CCl_4 sur Fluorisil montre donc l'extrême hétérogénéité de la répartition des hydrocarbures dans le sédiment, même à l'échelle de quelques dizaines de centimètres. Il apparaît difficile de réaliser des calculs de biodégradation dans des microcosmes où l'on ne prélève qu'un faible aliquot du volume de sable contaminé.

Evolution des densités de la Méiofaune

Les densités initiales du Méiobenthos dans le mesocosme témoin et dans ceux contaminés par les hydrocarbures étaient comparables (non significativement différentes au niveau 5% par le test de Kruskall Wallis). Les valeurs initiales trouvées de 1218 ± 13 Nématodes/10 cm^2 et de 198 ± 26 Copépodes harpacticoides/10 cm^2, deux groupes qui constituent la quasi totalité des organismes méiobenthiques recensés, peuvent être favorablement comparées avec la densité relevée dans le milieu naturel à la même date (1357 Nématodes et 105 Copépodes/10 cm^2) dans les douze premiers centimètres du sédiment en mars 1981.

Bien que l'analyse de la répartition verticale montre qu'environ 70% des nématodes et 44% des copépodes sont concentrés dans les quatre premiers centimètres du sédiment, les impératifs de temps de tri ont conduit à estimer les densités seulement dans les quatre premiers centimètres.

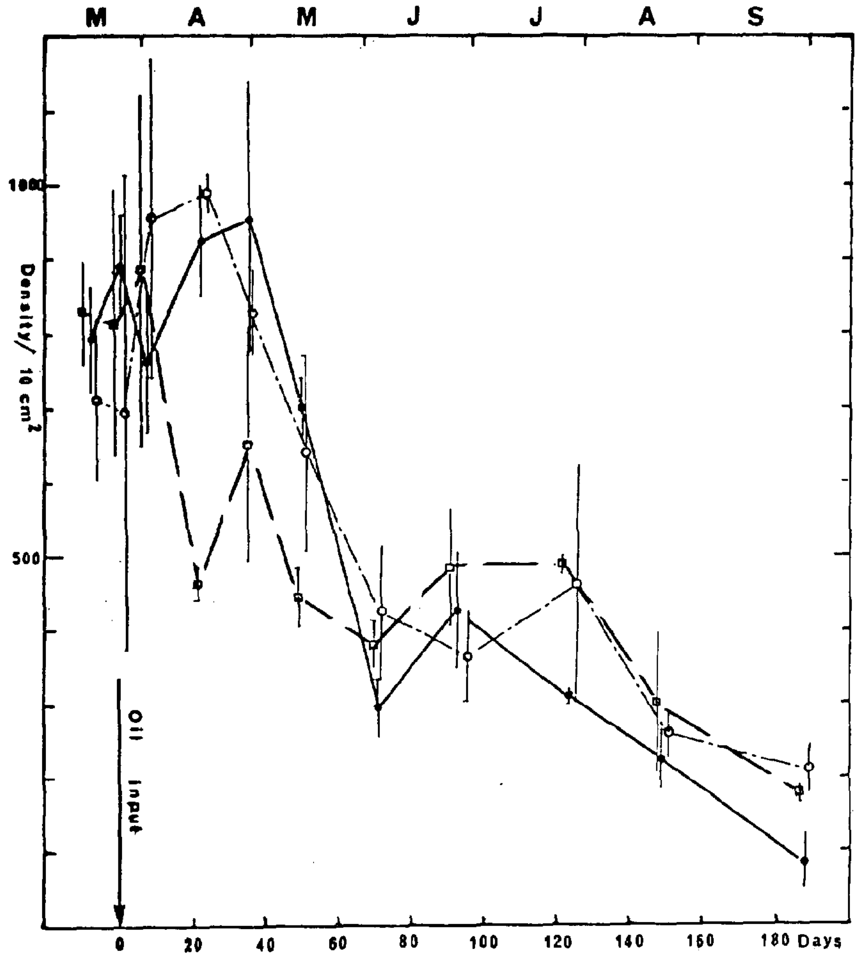

FIGURE 3. Evolution des densités (et de leur écart à la moyenne) des Nématodes dans les quatre premiers centimètres du sédiment pendant une période de six mois. Témoin : 0 -- 0; Module faiblement pollué : o -.- o; Module fortement pollué : ● —— ●.

Quel que soit le module considéré, les densités de nématodes ont tendance à décroître en circuit clos (Fig. 3). Cependant, il est possible de distinguer : - une période initiale de deux mois environ où les valeurs trouvées dans les bacs pollués sont généralement plus fortes que dans le témoin;

- une période ultérieure où les densités dans ces modules contaminés ont tendance à être légèrement plus faibles par rapport au témoin.

Il apparaît donc que la phase de pollution primaire serait caractérisée par une prolifération des nématodes (1,5 à 2 fois le niveau du témoin) mais que rapidement apparaîtrait un déclin lent (0,5 à 0,8 fois la valeur du témoin dans le bac le plus pollué, 0,7 fois à une valeur comparable au témoin dans le bac faiblement pollué). Ces observations sont compatibles avec celles relevées dans le milieu naturel (Elmgren 1980 a; Boucher et al. 1981).

L'évolution des Copépodes harpacticoïdes (Fig. 4) montre au contraire un effet dépressif des hydrocarbures sur le niveau de densité du peuplement. Les abondances observées dans le témoin restent toujours supérieures à celles des bacs pollués malgré des fluctuations importantes des densités au cours de l'expérience. Dans le bac le plus pollué, les densités de Copépodes deviennent faibles après le 21ème jour.

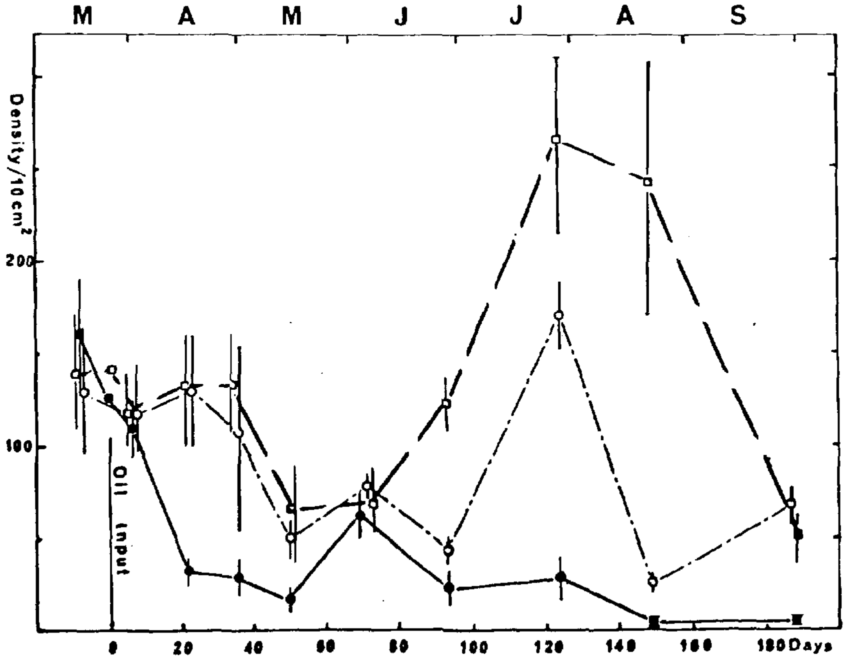

FIGURE 4. Evolution des densités (et de leur écart à la moyenne) des Copépodes harpacticoïdes dans les quatre premiers centimètres du sédiment pendant une période de six mois. Témoin : O----O; Module faiblement pollué : o -.- o; Module fortement pollué : ● —— ●.

Evolution du rapport Nématodes/Copépodes

L'utilisation de ce rapport dans les études de pollution vient récemment d'être proposée par Raffaelli et al (1981). Cette proposition séduisante dérive de deux considérations :

- d'une part les Copépodes sont apparemment plus sensibles que les Nématodes au stress des pollutions;

- d'autre part l'utilisation de la méiofaune dans les études d'impact ne se justifie que si celle-ci répond avant que les effets ne deviennent visibles sur la macrofaune. L'un des obstacles majeurs à l'interprétation de cet indice réside dans le fait que celui-ci est corrélé négativement avec la médiane granulométrique du sédiment. L'expérimentation permet d'éliminer ce facteur qui obscurcit l'interprétation des résultats.

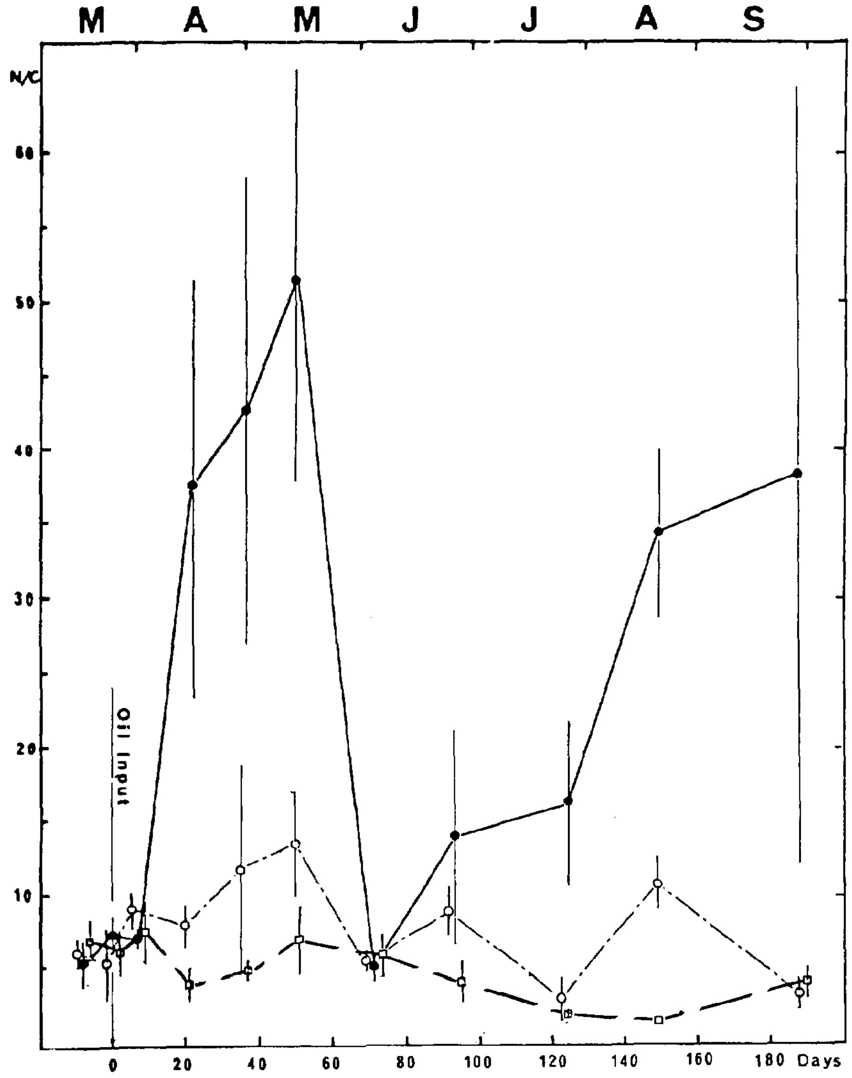

FIGURE 5. Evolution du rapport Nématodes/Copépodes dans les quatre premiers centimètres du sédiment pendant les six mois d'expérience. Témoin : O---O; Module faiblement pollué : o-·-o; Module fortement pollué : ●——●.

La figure 5 montre l'évolution de ce rapport dans les trois modules expérimentaux. Les valeurs sont d'autant plus fortes que le bac est plus pollué par les hydrocarbures. Ainsi le témoin présente toujours des valeurs faibles comprises entre 1.07 et 12.40 (moyenne : 5.04 ± 0.50 par 33 mesures en six mois).

Le bac faiblement pollué présente des valeurs légèrement plus fortes et plus variables comprises entre 1.64 et 20.07 (moyenne : 7.81 ± 0.78 par 32 mesures). Enfin le module fortement pollué présente des valeurs nettement plus élevées mais fluctuantes. Deux périodes correspondant à des fortes valeurs peuvent être distinguées entre 22 et 50 jours (N/C = 37 à 51) et entre 124 et 188 jours (N/C = 16 à 71) séparées par une période de faible valeur à 71 jours (N/C = 5 à 14).

La sensibilité de cet indice semble donc se confirmer puisqu'un accroissement sensible est discernable après 25 jours d'expérience dans le bac fortement pollué du fait de la quasi disparition des Copépodes, alliée à une prolifération des Nématodes. La généralisation de son utilisation demande cependant de préciser les modalités de ces fluctuations dans différentes conditions expérimentales en tenant compte des changements de la composition spécifique aussi bien des Nématodes que des Copépodes et de leur niveau de compétition pour la nourriture par exemple (Warwick 1981). Il est probable que le retour de ce rapport à des valeurs faibles dans le bac le plus pollué correspond à un changement de la composition faunistique des Copépodes.

Evolution des paramètres Abondance, Biomasse et Diversité.

La plupart des études utilisant les modifications de la macrofaune pour mettre en évidence l'impact des pollutions préconisent l'emploi de paramètres simples tels le nombre d'espèces, l'abondance et la biomasse (Pearson et al. 1978; Glémarec et al.1981 entre autres). Cette approche pose pour l'instant de sérieux problèmes méthodologiques en ce qui concerne la Méiofaune. Les progrès réalisés dans la systématique des groupes dominants des Nématodes et des Copépodes permettent d'envisager raisonnablement la détermination de routine du nombre d'espèces dans un échantillon représentatif de la population (100 à 300 individus) malgré la grande diversité de ces groupes. Il n'en est pas de même en ce qui concerne l'évolution de la biomasse du méiobenthos dans un échantillon donné. En effet, les méthodes actuellement utilisées posent généralement l'hypothèse que la biomasse moyenne ne varie pas au cours du temps, ce qui n'est bien sûr pas le cas. Elles consistent par conséquent soit à évaluer les biovolumes à la chambre claire d'un microscope en utilisant des formules d'équivalence (Andrassy 1956; Wieser 1960; Juario 1975), soit à peser à la microbalance de précision un unique lot de quelques centaines à quelques milliers d'individus (De Bovée 1981; Guille et al. 1968).

Les résultats présentés dans cet article doivent être considérés comme la mise au point d'une méthode d'évaluation des indices volumiques sur la méiofaune qui permet d'analyser rapidement chaque prélèvement et évite les incertitudes des méthodes de pesée. Chacun des lots de 100 specimens montés entre lame et lamelle pour la détermination a été grossi cent fois grâce à un projecteur de profil. Les contours de chaque individu identifié ont été tracés puis analysés à la table digitalisante d'un analyseur d'images. Le volume a été calculé et exprimé en poids sec en utilisant une valeur de densité de 1,13 (Wieser 1960) et un rapport Poids sec/Poids frais = 0,25.

Les autres paramètres classiquement utilisés tels que nombre d'espèces (S), Indice de diversité de Fisher et al. (α), Indice de diversité de Shannon (H) et Equitabilité de Pielou (J) ont été aussi calculés au temps zéro, 36 jours (avant la chute de densité) et 93 jours (après la chute de densité) de l'expérience dans chacun des trois modules sur des échantillons de 100 individus (Tableau I).

Dans le bac témoin, l'évolution du poids sec individuel n'indique pas de fluctuations significatives puisque les limites des intervalles de confiance de la moyenne se recoupent (0.088 à 0.207 µg PS/individu).

Modules	Indices	TO	36	93
Témoin	N	633	653	485
	PS	0.125 ± 0.015 (97)	0.150 ± 0.057 (97)	0.101 ± 0.012 (101)
	B	79.3	98.1	49.1
	S	29	33	33
	α	13.71	17.19	17.19
	H	3.97	4.62	4.46
	J	0.82	0.92	0.88
Pollution faible	N	1007	831	365
	PS	0.130 ± 0.028 (100)	0.071 ± 0.0009 (90)	0.103 ± 0.035 (101)
	B	131.2	59.1	37.7
	S	29	34	34
	α	13.71	18.15	18.15
	H	4.46	4.35	4.15
	J	0.92	0.86	0.82
Pollution forte	N	1016	955	418
	PS	0.107 ± 0.015 (101)	0.125 ± 0.029 (95)	0.019 ± 0.003 (90)
	B	108.9	119.6	7.8
	S	23	28	17
	α	9.35	12.91	5.88
	H	3.71	4.05	2.19
	J	0.82	0.84	0.54

TABLEAU I. Evolution des densités (N), du poids sec moyen (PS), de la biomasse (B), du nombre d'espèces pour 100 individus (S) et des Indices de diversité du peuplement de Nématodes au cours du temps (zéro, 36 et 93jours). α = Indice de Fisher et al.; H = Indice de Shannon; J = Equitabilité de Pielou.

La biomasse est sensiblement plus forte au temps zéro et surtout à 36 jours qu'à 93 jours essentiellement du fait de densités plus fortes. Le nombre d'espèces identifiées est assez constant (29 à 33) ainsi que les divers indices de diversité.

Dans le bac faiblement pollué, le poids sec individuel après 93 jours n'est pas significativement différent de celui de l'état initial. La décroissance de la biomasse 131,2 à 37,7 µg PS) est surtout liée à la chute des densités (1007 à 365). Le nombre d'espèces recensé a tendance à légèrement augmenter (29 à 34) ce qui provoque une augmentation parallèle de l'indice de Fisher et al. (13,71 à 18,15). La diminution lente de l'indice de Shannon et de l'équitabilité indique l'apparition d'une hiérarchisation plus marquée au cours du temps.

Dans le bac fortement pollué, l'évolution des densités est similaire à celle du module faiblement pollué. Après 36 jours, les valeurs des paramètres demeurent comparables à celles de l'état initial. Après 93 jours, par contre, le poids sec moyen chute fortement (0.019 ± 0.003 µg PS) d'où une réduction brutale de la biomasse (7.8 µg/10 cm^2). Celle-ci est liée au remplacement du peuplement d'origine par quelques espèces de petite taille caractéristiques des milieux riches en matière organique (*Leptclaimus tripapillatus* Boucher 1977, *Monhystera aff. disjuncta* Bastian 1865; *Monhystera pusilla* Boucher 1977) et mises en évidence dans des expériences préalables d'eutrophisation (Boucher 1979).

DISCUSSION

Ces simulations de pollutions en microécosystèmes sédimentaires soulignent la difficulté d'interpréter les phénomènes de dégradation des hydrocarbures dans les sédiments. La disparition de ceux-ci n'est pas détectable pendant la durée de l'expérience dans le module faiblement pollué; elle est anarchique dans le bac fortement contaminé. Il ne semble donc pas possible de caractériser aisément une pollution par le niveau du polluant dans le milieu avec la méthode employée.

L'utilisation d'organismes sensibles au polluant (indicateurs biologiques) intégrant l'ensemble des conséquences du stress paraît plus fiable pour caractériser un impact. Elle suppose, pour être efficace, que ceux-ci répondent avant que la perturbation devienne évidente. Si certains organismes de la macrofaune benthique répondent de façon nette au stress primaire de la pollution par hydrocarbures (Dauvin 1979 a et b et 1981) la durée des cycles (1 à 10 ans) rend problématique l'analyse des effets différés (Chassé et al. 1981).

Du fait de la rapidité de reproduction, la méiofaune, dont les Nématodes et les Copépodes constituent l'essentiel des organismes, est un matériel prometteur pour comprendre les mécanismes régissant la destructuration et la restructuration d'un écosystème.

Ces expériences de contamination brutale par hydrocarbures ne suggèrent pas un effet très important sur le niveau des densités des Nématodes. Leur augmentation entre 21 et 50 jours ne semble pas liée à un développement d'opportunistes nécrophages comme le suggère Chassé (1978) puisque la composition faunistique reste très comparable à celle du témoin. La chute des densités observée ultérieurement dans le bac le plus pollué est conforme aux résultats obtenus expérimentalement *in situ* par Bakke et al. (1980) ou en mesocosmes par Elmgren et al. (1980 b). Elle s'accompagne d'un changement très perceptible de la composition faunistique avec réduction du nombre d'espèces et diminution de la biomasse.

Contrairement à l'idée généralement admise, le groupe des Nématodes peut donc constituer un indicateur biologique fiable des modifications de l'écosystème (Platt & Warwick, 1980) puisque leurs possibilités adaptatives permettent à certaines espèces de se maintenir quelles que soient les conditions de milieu, à d'autres de proliférer en quelques semaines pour occuper la niche laissée vide. La détermination expérimentale de groupes de Nématodes à comportement similaire vis-à-vis de l'eutrophisation ou des pollutions, apparaît donc comme une voie de recherche prometteuse pour caractériser l'état ou la dynamique des écosystèmes perturbés.

SUMMARY

Experimental study of hydrocarbon pollution in a sand microecosystem
I. Effect of the sediment contamination on meiofauna.

The effects of hydrocarbon pollution, at two different intensities with respect to a control, on the microecosystems were studied using recirculating experimental tanks containing 100 liters of subtidal fine sand. Changes in the population characteristics of meiofauna (nematodes and copepods) were chosen to follow the effects of oil pollution. Irrespective of the intensity of pollution, the density of nematodes in the experimental tanks increased at a significantly higher rate than in the control tank during the first two months after pollution and then decreased slowly. The density of harpacticoid copepods was negatively related to the intensity of oil pollution. It appears that the nematodes/copepods ratio would be an useful indicator of the degree of oil pollution.

After 3 months of experimental duration, the faunal composition of the nematodes in the highly polluted tank was drastically modified. This change is evident from a sharp fall in biomass and species diversity; small opportunistic nematode species, known for their association with eutrophicated environment, became dominant. Changes in the meiofauna population parameters in the slightly polluted experimental tank did not show any significant variation from those in the control tank.

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EVOLUTION A MOYEN-TERME DU MEIOBENTHOS ET DU MICROPHYTOBENTHOS SUR QUELQUES PLAGES TOUCHEES PAR LA MAREE NOIRE DE L'*AMOCO-CADIZ*

par

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ABSTRACT

The ecological follow-up undertaken after the Amoco-Cadiz oil spill, on the beaches Brouennou and Corn ar Gazel (mouth of Aber Benoit) and Kersaint (near Portsall), was continued untill november 1980. Chlorophyll pigments have suffered little quantitatively from the direct effect of pollution, but the study of temporal variations in the meiofaunal densities revealed disturbances in seasonal cycles. Other factors, e.g. hydrodynamic fluctuations and macrofaunal predators, could act as regulating mechanisms on the evolution of the populations.

The effects of pollution are particularly obvious in some faunistic imbalances, as the study of harpacticoid copepods showed. However, particular evolutionary trends between and within ecological groups of species implied that recovery was nearly complete, at least on exposed beaches.

The conclusions drawn to date are tentative because of the lack of reference data, and it is intended to continue the survey annually in spring.

Key words : Pollution, Amoco-Cadiz, Chlorophyll pigments, Meiofauna, harpacticoids, Beaches.

RESUME

Le suivi écologique mensuel entrepris, à la suite de la catastrophe de l'Amoco-Cadiz, sur les plages de Brouennou et Corn ar Gazel, à l'entrée de l'Aber Benoît, et de Kersaint près de Portsall, a été maintenu jusqu'en novembre 1980. Alors que les pigments chlorophylliens ne semblent pas avoir souffert de l'action directe de la pollution, l'étude des variations temporelles de la densité de la méiofaune révèle une perturbation des cycles saisonniers. D'autres facteurs, tels que l'hydrodynamisme et les prédateurs de la macrofaune, peuvent intervenir en tant que mécanismes régulateurs.

Les effets de la pollution sont surtout sensibles au niveau de certains déséquilibres faunistiques, comme le montre l'étude des Copépodes Harpacticoïdes. Cependant, une certaine évolution des groupes écologiques permet de penser qu'un processus de retour à l'état initial est en cours d'achèvement, du moins sur les plages de mode battu.

En fait, l'absence d'états de références nous oblige encore à la prudence dans l'interprétation des résultats, et il est envisagé une poursuite des recherches sous forme de "veille" écologiques.

Mots-clés : Pollution, Amoco-Cadiz, Pigments chlorophylliens, Méiofaune, Harpacticoïdes, Plages.

INTRODUCTION

Dans le cadre du suivi écologique entrepris, à la suite du naufrage de l'"AMOCO-CADIZ", par les Laboratoires de l'Institut d'Etudes Marines de l'Université de Bretagne Occidentale, la méiofaune *sensu lato* et le microphytobenthos de la zone intertidale ont été l'objet d'une étude réalisée par le Laboratoire d'Océanographie biologique.

Après une recherche de site effectuée sur la côte nord-Finistère au cours des mois de septembre et octobre 1978, deux plages à la sortie de l'Aber Benoît (Fig. 1), Corn ar Gazel au SW et Brouennou au NE, ont été retenues pour cette étude. Ces deux plages, situées dans une zone particulièrement éprouvée par la pollution due aux hydrocarbures de l'"AMOCO-CADIZ", sont également étudiées au point de vue physico-chimique et au point de vue de la macrofaune (Le Moal, 1981) dans le cadre de ce suivi. Il a malheureusement été impossible de trouver dans cette région une plage écologiquement homologue mais non polluée afin de servir de témoin. Une étude parallèle, mais portant uniquement sur la méiofaune *sensu stricto*, a été réalisée sur la plage de Kersaint (près de Portsall).

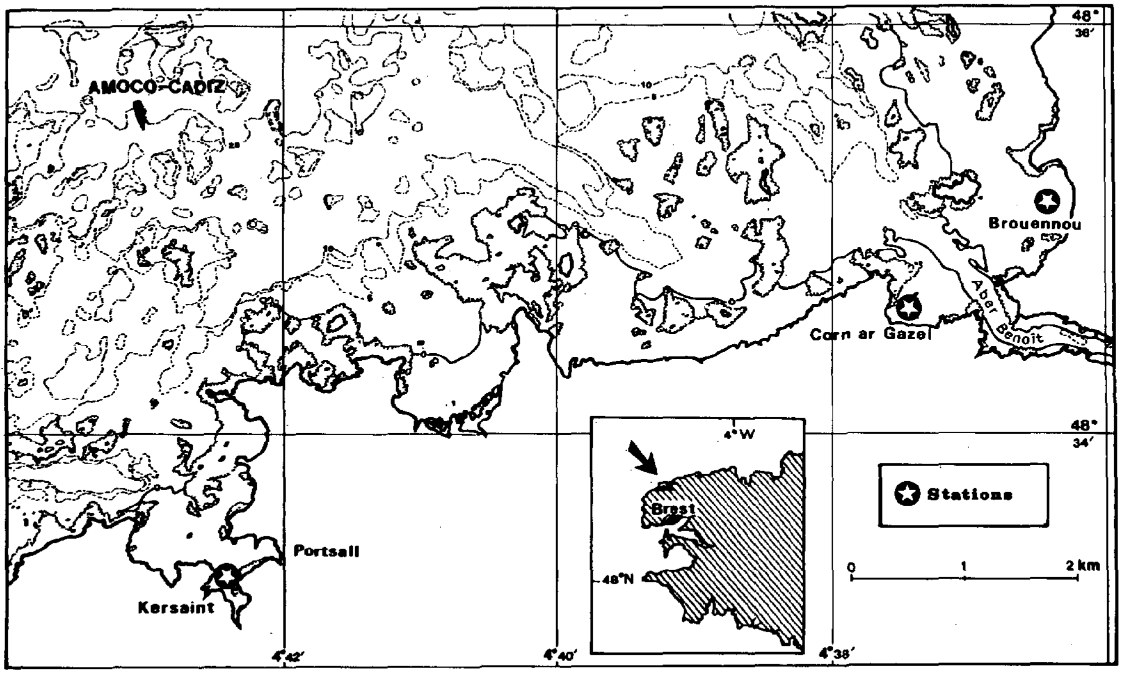

FIGURE 1. Localisation des stations.

Sur chacune des plages, une station (Quadrat) située en dessous de la mi-marée, dans l'étage médiolittoral, est l'objet de prélèvements mensuels depuis mars 1978 pour la plage de Kersaint, novembre 1978 pour les plages de Brouennou et Corn ar Gazel. Sur cette dernière, deux prélèvements "de référence" ont pu être réalisés le 17 mars 1978, avant l'arrivée de la nappe d'hydrocarbures.

Une première publication (Bodin et Boucher, 1981) faisait état des résultats acquis en juillet 1979. La présente note les complète par les données obtenues jusqu'en novembre 1980 et tente une réflexion sur l'ensemble de ce suivi écologique.

Les techniques de prélèvement et de traitement des échantillons, ainsi que les principaux paramètres édaphiques, ont déjà été exposés dans la première publication, nous ne les reprenons donc pas ici*. Nous rappelons simplement quelques caractéristiques granulométriques essentielles sous forme de courbes pondérales cumulatives (Fig. 2). De plus, nous présentons le profil topographique de deux des plages prospectées (Fig. 3) et les variations temporelles de la température et de la vitesse du vent (Fig. 4).

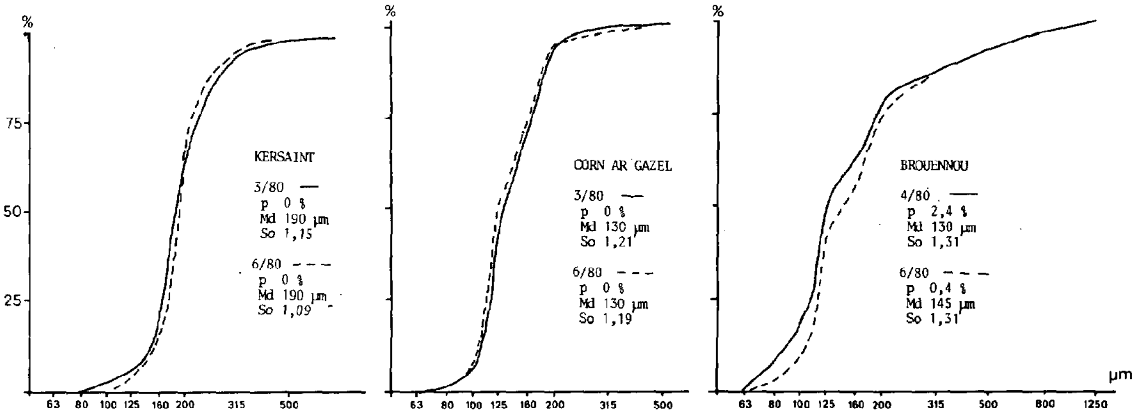

FIGURE 2. Granulométrie : courbes pondérales cumulatives.
Teneur en pélites (p %), Médiane (Md), Indice de triage (So).

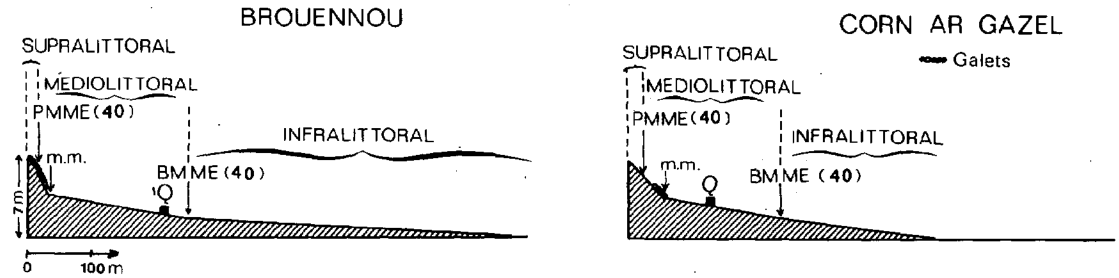

FIGURE 3. Profil topographique des plages. Limites des étages bathymétriques. Emplacement des quadrats (Q).

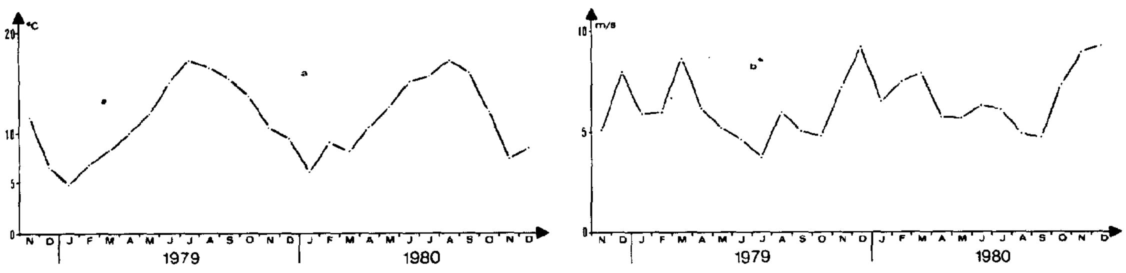

FIGURE 4. Température de l'air (a), vitesse du vent (b) : variations des moyennes mensuelles.

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*Une correction doit cependant être apportée (Bodin et Boucher, 1981, p. 328) : à la place de P.M.M.E. il faut lire B.M.M.E., et à la place de B.M.M.E. il faut lire B.M.V.E.

RESULTATS

Pigments chlorophylliens

Un dépouillement additionnel de carottes pour les prélèvements antérieurs à septembre 1979 modifie légèrement les chiffres précédemment obtenus et publiés (Bodin et Boucher, 1981).

Le nombre de carottes utilisé a permis l'utilisation de tests statistiques : test U de Mann-Whitney et test de Kruskall-Wallis ; hypothèse nulle rejetée au niveau 5 %.

Brouennou

Dans les premiers centimètres d'épaisseur du sédiment on observe une décroissance très rapide des teneurs en pigments chlorophylliens, ce qui nous a permis de limiter l'étude aux quatre premiers centimètres.

Pour la chlorophylle *a*, ce gradient est très marqué (on retrouve en moyenne 12 % de la teneur superficielle sous 4 cm d'épaisseur) et régulièrement observé dans les prélèvements (à l'exception des mois de décembre 1978 et 1979).

L'hétérogénéité spatiale est importante et, de ce fait, les teneurs moyennes calculées pour les deux premières couches (0-0,2 cm et 0,2-1 cm) ne sont pas significativement différentes pour la majorité des prélèvements mensuels, alors que la présence du film superficiel, plus riche en chlorophylle *a*, est constatée dans 85 % des carottes.

Pour les phéopigments, le gradient est moins accentué (26 % de la teneur superficielle sont présents en moyenne sous 4 cm d'épaisseur) et moins fréquemment observé (absent en novembre et décembre 1978, de décembre 1979 à février 1980 et de juillet à septembre 1980) que dans le cas de la chlorophylle *a*, ce qui peut être dû en partie à ses plus faibles teneurs et donc à la moindre précision du dosage. L'enrichissement superficiel en phéophytine n'est rencontré que dans 63 % des carottes.

La chlorophylle *a* est le pigment largement dominant surtout au sein des deux premiers centimètres d'épaisseur, là où se limitent les variations temporelles. Sa teneur relative élevée (71 % de la somme C*a* + Phéo) est un indice de la présence d'une active population de microphytes dans cette zone correspondant à l'épaisseur maximale de la couche oxygénée.

L'amplitude des variations temporelles est maximale au niveau de la couche superficielle et s'atténue rapidement dans l'épaisseur du sédiment.

La chlorophylle *a* présente, les deux années, un cycle annuel de type saisonnier (Fig. 5a).

Dans la couche superficielle, le minimum de décembre est suivi par un fort accroissement pendant les mois d'hiver ; il aboutit à un "plateau printanier" entre mars et juillet 1979 (22,1 µg/g) et entre février et juillet 1980 (15,6 µg/g, en excluant le mois de juin). Entre juillet et août, une nette décroissance est observée ; elle est suivie par un "plateau automnal" entre août et novembre 1979 (14 µg/g). Au cours de ces deux cycles il faut noter le minimum estival esquissé en juin 1979, très prononcé en juin 1980, phénomène déjà observé en zone intertidale (Colijn et Dijkema, 1981) mais non explicité.

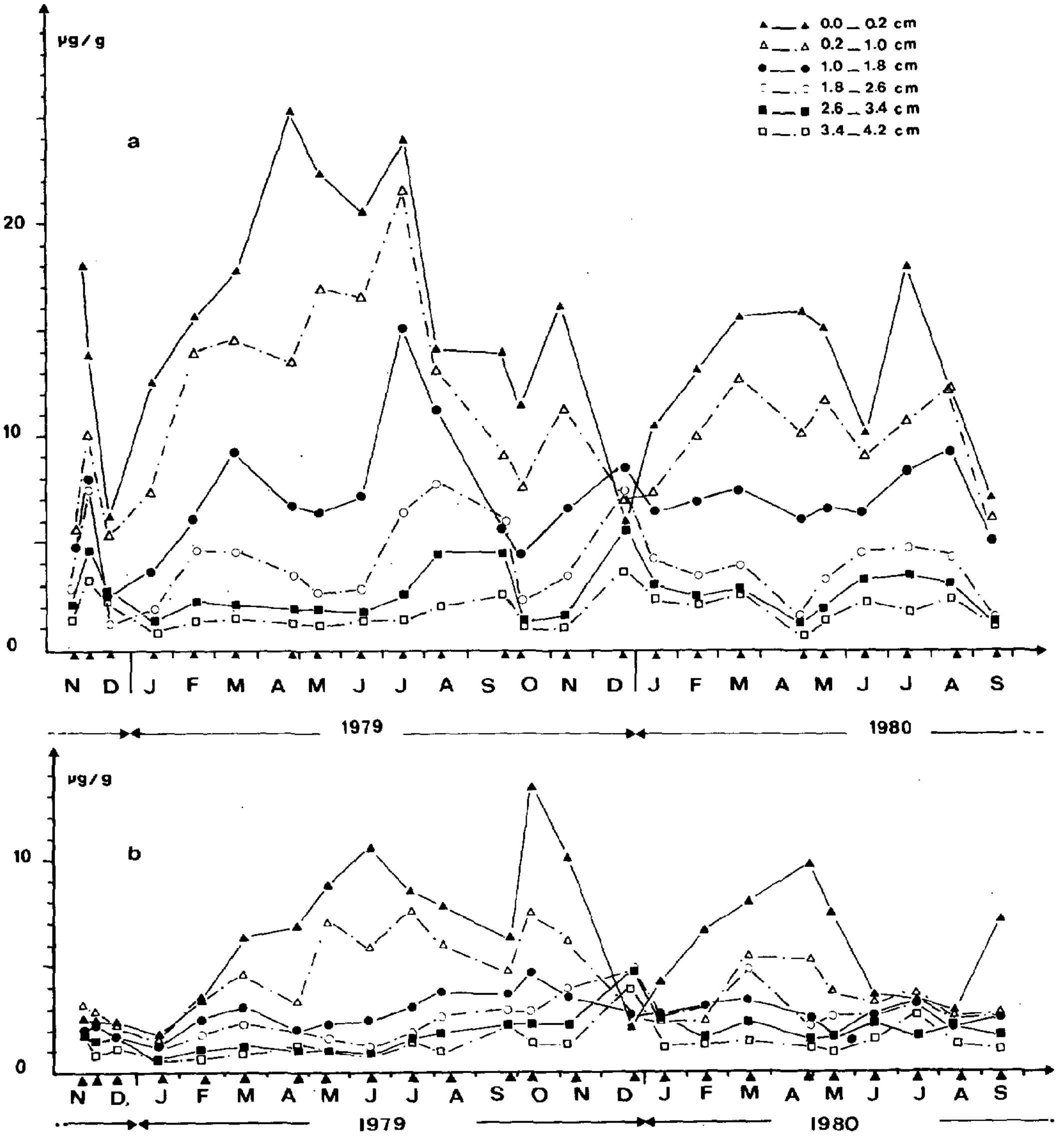

FIGURE 5. Variation des moyennes mensuelles de la chlorophylle *a* (a) et de la phéophytine (b) à Brouennou.

Dans la couche sous-jacente les fluctuations sont similaires. Le plateau printanier, situé entre février et août, correspond à une teneur moyenne de 15,8 µg/g en 1979 et de 11 µg/g en 1980 et le plateau automnal, entre septembre et novembre 1979, à une teneur moyenne de 9,4 µg/g.

Les deux cycles annuels de la phéophytine (Fig. 5b) diffèrent essentiellement par le minimum estival très accusé en 1980, les teneurs moyennes des plateaux, atteints de mars à novembre 1979 (8,8 µg/g) et de février à mai 1980 (8,5 µg/g), étant semblables.

Les variations temporelles de ces deux pigments sont parallèles, sauf au cours de l'automne où elles tendent à s'inverser, faisant diminuer la teneur relative en chlorophylle *a*.

Les fortes diminutions de concentration sont accompagnées d'une disparition ou d'une atténuation du gradient dans l'épaisseur du sédiment. Cette homogénéisation des couches superficielles, très apparente en décembre 1978 et août 1979, moins prononcée en décembre 1979 et juin 1980, peut être attribuée à une érosion de la pellicule superficielle et à un brassage du sédiment sous l'action des forces hydrodynamiques.

C'est à la suite de ces décroissances que se situent les plus forts accroissements relatifs (100 % entre décembre 1978 et janvier 1979, 74 % entre décembre 1979 et janvier 1980, 77 % entre juin et juillet 1980). Ces accroissements sont du même ordre de grandeur en hiver et en été, et il semble donc que les facteurs climatiques (température, éclairement) ne soient pas limitant.

La comparaison des résultats obtenus en 1979 et en 1980 montre pour la chlorophylle *a*, qu'il n'y a pas de différences significatives entre les moyennes mensuelles de ces deux années des mois de janvier à mars, tandis que celles-ci sont toujours plus élevées en 1979 du mois d'avril au mois de juillet.

Corn ar Gazel

La distribution des pigments au sein des 12 premiers centimètres de sédiment s'étant révélée très homogène, nous avons étudié trois couches successives de 4 cm d'épaisseur.

Les teneurs moyennes en chlorophylle *a* et en phéophytine varient peu entre les trois couches (variation environ de 10 %). On reconnaît cependant, surtout pour la chlorophylle *a*, deux types de distribution : dans le premier type, la teneur est maximale dans la couche superficielle puis décroît régulièrement, dans le deuxième type la teneur est maximale dans la couche intermédiaire (4-8 cm).

Sur l'ensemble des prélèvements, la teneur moyenne mensuelle en chlorophylle *a* du sédiment est la plus faible dans la couche la plus profonde. Entre les deux premières couches, comme à Brouennou, la différence observée n'est pas, le plus souvent, significative du fait de l'hétérogénéité spatiale ; elle est cependant corroborée par la fréquence, dans le prélèvement mensuel, de chacun des deux types de distribution verticale.

La teneur relative en chlorophylle *a* du sédiment ne varie pas entre les trois couches.

Les teneurs moyennes mensuelles en chlorophylle *a* (Fig. 6a) de la couche superficielle (0-4 cm) s'accroissent de janvier à novembre 1979. Après la brutale diminution de décembre 1979, les moyennes mensuelles mesurées en 1980 sont, à l'exception du mois d'avril, toujours inférieures à celles de l'année précédente. Dans la couche sous-jacente la variation des teneurs moyennes mensuelles présente la même tendance, mais son amplitude est plus faible.

Pour la phéophytine on remarque une élévation en automne (octobre 1979 - septembre 1980) (Fig. 6b) des teneurs moyennes des deux premières couches et, pour l'ensemble des deux années, une augmentation des moyennes mensuelles au cours de l'année 1980.

S'il n'y a pas de cycle de type saisonnier apparent au niveau des variations des teneurs moyennes mensuelles au sein de chacune des couches sédimentaires étudiées, un tel cycle se présente (plus nettement pour la chlorophylle *a*) sous la forme d'une succession régulière des deux types de distribution verticale. Un enrichissement subsuperficiel est constaté pendant l'hiver (de novembre à avril) alors qu'en été c'est la couche superficielle qui est la plus riche en pigments, ce qui peut être dû à la différence saisonnière de stabilité sédimentaire.

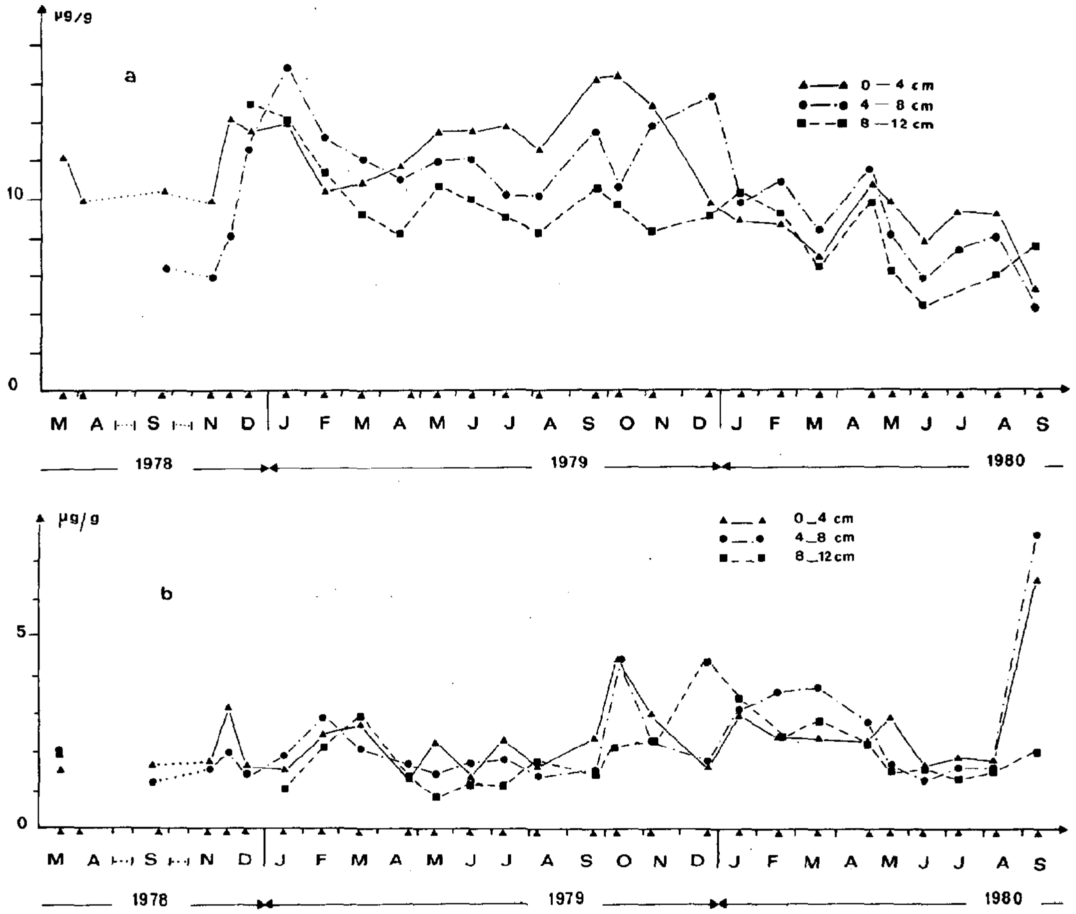

FIGURE 6. Variation des moyennes mensuelles de la chlorophylle *a* (a) et de la phéophytine (b) à Corn ar Gazel.

Discussion

La comparaison des deux plages montre que les teneurs pigmentaires dans les couches superficielles, au moment du minimum hivernal, sont très peu différentes (10 µg/g environ). Elles peuvent être assimilées à la valeur intrinsèque de Hartwig (1978) et résultent ici de l'identité des médianes granulométriques. De la même façon, la faible différence existant au niveau des valeurs moyennes du taux de chlorophylle *a* (81 % et 71 %) et de l'indice de diversité pigmentaire (voisin de 2) dans la couche superficielle, peut être reliée à l'identité du taux de pélites.

La différence de nature et d'action des forces hydrodynamiques agissant sur la stabilité et l'oxygénation du sédiment se reflète dans la différence observée pour la distribution des pigments dans l'épaisseur du sédiment (Fig. 7a, b, c), ainsi que l'ont constaté de nombreux auteurs depuis Steele et Baird (1968).

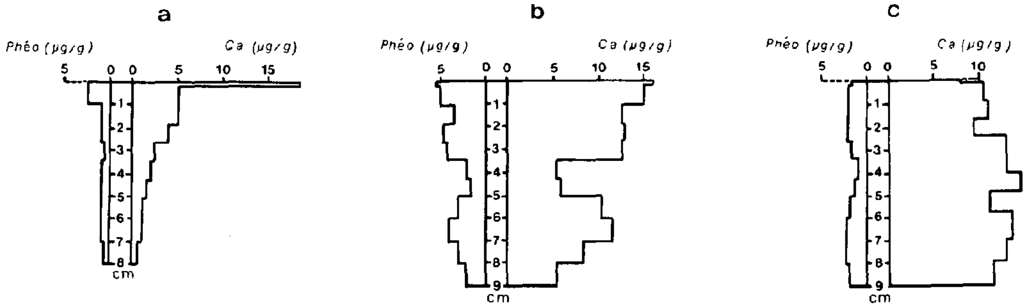

FIGURE 7. Répartition de la chlorophylle *a* et de la phéophytine à Brouennou (a) et à Corn ar Gazel (b, c) dans l'épaisseur du sédiment.

Sur la plage de Corn ar Gazel, la distribution homogène des différentes caractéristiques pigmentaires, mise en place par un brassage sous l'action des vagues, peut se maintenir dans un milieu interstitiel présentant de bonnes conditions d'oxygénation (Gargas, 1970 ; McIntyre *et al.*, 1970 ; Hunding, 1971) assurées par l'existence de houle et de courants de marée. Dans ces milieux instables où les microphytes sont passivement distribués, l'essentiel de la flore est généralement constitué par de petites Diatomées liées aux grains (Amspoker, 1977).

Sur la plage de Brouennou la stabilité de la surface sédimentaire permet le développement dans la zone photique d'un film superficiel, tandis que, sous les deux premiers centimètres, en milieu non oxygéné, on observe un enrichissement relatif en pigments de dégradation, ceci pouvant être dû à la combinaison entre la migration des formes mobiles vers la couche superficielle et la mort des cellules en milieu fortement réduit (Gargas, 1970).

La teneur en pigments chlorophylliens du sédiment est en moyenne deux fois plus élevée à Brouennou qu'à Corn ar Gazel lorsque sont considérées les pellicules superficielles ; elle est au contraire deux fois plus faible lorsque les dix premiers centimètres sont pris en compte. Il est donc important de préciser l'épaisseur utilisée pour l'évaluation de la biomasse. Pour une comparaison avec la méiofaune, à Brouennou, ce sont les valeurs mesurées dans le premier centimètre, zone où se concentrent les méiobenthontes, qui représentent la quantité de nourriture disponible et traduisent la stabilité de cette zone.

L'étude des variations saisonnières, dans le cas de sédiments instables comme à Corn ar Gazel, montre qu'un cycle quantitatif n'est pas apparent sur une année et c'est essentiellement l'instabilité sédimentaire qui limite la biomasse des microphytes, la production dans la zone photique devant être essentiellement exportée après érosion et remise en suspension.

Lorsque la surface sédimentaire est stable, comme cela est le cas à Brouennou, il apparaît au contraire un cycle quantitatif reproductible. Toutes les études menées en zone intertidale montrent ce type de cycle annuel dès que le sédiment présente une fraction fine importante (signe de sédiment stable) (Cadée et Hegeman, 1977 ; Admiraal et Peletier, 1980 ; Colijn et Dijkema, 1981). Ce type de cycle se rencontre également en milieu infralittoral (Boucher, 1975). La biomasse pigmentaire est constante au cours des plateaux de printemps et d'automne, ce qui laisse présumer soit d'une productivité plus faible qu'en hiver, soit de l'établissement d'un seuil régit par les conditions moyennes de stabilité sédimentaire d'une part, et d'activité de nutrition du maillon secondaire d'autre part. La première hypothèse n'est pas confirmée par les différentes études menées en milieu intertidal. On ne peut l'étayer, en effet, ni par une photoinhibition (Cadée et Hegeman, 1974 ; Colijn et Van Buurt, 1975), ni par un effet limitant des concentrations en sels nutritifs (Admiraal, 1977), ni par une saturation de la zone photique (Admiraal et Peletier, 1980), car la constitution de denses colonies de microphytes n'est pas suggérée par les teneurs observées (lors de la formation de croûtes de microphytes, des teneurs supérieures à 100 µg sont mesurées ; Plante-Cuny *et al.*, 1981). La deuxième hypothèse est en accord avec l'observation de la tendance à un accroissement en phéophytine au cours de cette période.

La comparaison des résultats obtenus au cours de ces deux années successives (Tableau 1) montre, à Brouennou comme à Corn ar Gazel, une diminution globale au cours de la deuxième année des teneurs en chlorophylle *a*, alors que la teneur en phéopigments reste inchangée ou est en augmentation. Cette variation résulte soit d'un effet secondaire de la pollution due aux hydrocarbures de l'"AMOCO-CADIZ", soit de la variation naturelle pluriannuelle (Cadée et Hegeman, 1974).

TABLEAU 1. Valeurs moyennes au sein de chaque tranche de sédiment calculées pour les périodes de janvier à septembre 1979, 1980, et pour la durée totale de l'étude.
Ca : chlorophylle *a* (µg/g) - Phéo : phéophytine (µg/g)
Ca % : Ca × 100 / (Ca + phéo) - DI : indice de diversité pigmentaire

Epaisseur (cm)	BROUENNOU											
	JANV. 1979 - SEPT. 1979				JANV. 1980 - SEPT. 1980				NOV. 1978 - SEPT. 1980			
	Ca	Phéo	Ca %	DI	Ca	Phéo	Ca %	DI	Ca	Phéo	Ca %	DI
0 -0,2	18,6	6,7	73	1,99	13,2	6	69	2,3	14,9	6,1	71	2,14
0,2-1,0	14,1	4,9	74	2,26	10,1	3,6	74	2,49	11,1	4,2	72,5	2,44
1 -1,8	8,1	2,9	74	2,76	7,1	2,8	72	2,72	7,2	2,8	72	2,81
1,8-2,6	4,5	2,0	69	3,20	3,6	3	54,5	3,19	4,1	2,7	61	3,19
2,6-3,4	2,6	1,3	67	3,62	2,6	2	56,5	3,67	2,7	1,8	60	3,64
3,4-4,2	1,5	1	60	3,97	2,0	1,7	54	3,97	1,9	1,6	54	3,89

Epaisseur (cm)	CORN AR GAZEL											
	JANV. 1979 - SEPT. 1979				JANV. 1980 - SEPT. 1980				NOV. 1978 - SEPT. 1980			
	Ca	Phéo	Ca %	DI	Ca	Phéo	Ca %	DI	Ca	Phéo	Ca %	DI
0- 4	12,9	2	87	2,24	8,4	2,75	76	2,40	11	2,05	82	2,29
4- 8	12,2	1,5	87	2,30	8,2	3,0	74	2,51	10,7	1,9	78	2,41
8-12	10	1,5	87	2,42	7,55	2,1	78	2,64	9,1	1,8	82	2,54

Les conditions météorologiques (facteurs climatiques et hydrodynamiques) ne peuvent être retenues comme facteurs déterminants de cette variation, n'ayant pas été plus particulièrement défavorables au cours de la deuxième année, si ce n'est en automne, alors que les deux plages, pourtant d'exposition différente, ont réagi similairement et ceci dès le mois d'avril.

La reprise des activités de grazing est un des facteurs biologiques qui intervient au niveau de l'évolution saisonnière et qui peut expliquer la différence observée entre les deux années. Il est possible en effet de rapporter ces variations de la biomasse pigmentaire à l'évolution de la macrofaune au sein du processus de décontamination (Le Moal, 1981). Ainsi, la réapparition de l'Amphipode *Bathyporeia* sur la plage de Corn ar Gazel peut être pour partie responsable de la diminution de la teneur en chlorophylle *a*, son activité de "brouteur" étant reconnue (Sundbäck et Persson, 1981).

Méiofaune : résultats quantitatifs

La Figure 8 et le Tableau 2 montrent l'évolution temporelle de la densité (nombre d'individus/10 cm^2 de surface) des Copépodes Harpacticoïdes (échelle × 100), des Nématodes et de la méiofaune totale (*sensu stricto*) dans les différents prélèvements recueillis aux trois stations prospectées. Les Tableaux 3 et 4 indiquent l'évolution temporelle (en pourcentage de la méiofaune totale) des autres groupes du méiobenthos vrai et de la méiofaune temporaire. Il est évident que cette évolution est différente d'une station à l'autre.

Brouennou

La densité moyenne (8 033 ind./10 cm^2) y est extrêmement élevée en comparaison des données de la littérature pour la zone intertidale (Hicks, 1977), et la méiofaune est composée essentiellement de Nématodes. Les variations saisonnières sont assez nettes et à peu près conservées d'une année à l'autre, avec des minima en février-mars et en septembre (1979) ou juillet (1980), et des maxima en avril-mai et en décembre-janvier ou octobre (1980). Cependant, la densité moyenne des Harpacticoïdes est nettement plus faible durant la seconde période (1979-80) : 282 ind./10 cm^2 (contre 593 précédemment). Durant cette seconde période, le rapport Nématodes/Copépodes oscille entre 11 (juin 1980) et 172 (janvier 1980).

Les autres groupes du méiobenthos vrai représentent un pourcentage relativement modeste de la méiofaune (maximum : 26,7 % en octobre 1979). De plus, ce pourcentage est en régression : il ne dépasse pas 3,5 % depuis juillet 1980.

Les Annélides constituent l'essentiel du méiobenthos temporaire, ce qui correspond aux données de la macrofaune (Le Moal, 1981).

Corn ar Gazel

La densité moyenne de la méiofaune (3 013 ind./10 cm^2) y est beaucoup plus faible qu'à Brouennou. De plus, c'est à cette station que la différence avec la période étudiée précédemment est la plus nette du point de vue quantitatif : la densité moyenne passe de 4 697 à 1 666 ind./10 cm^2. Cette régression n'est pas le fait des

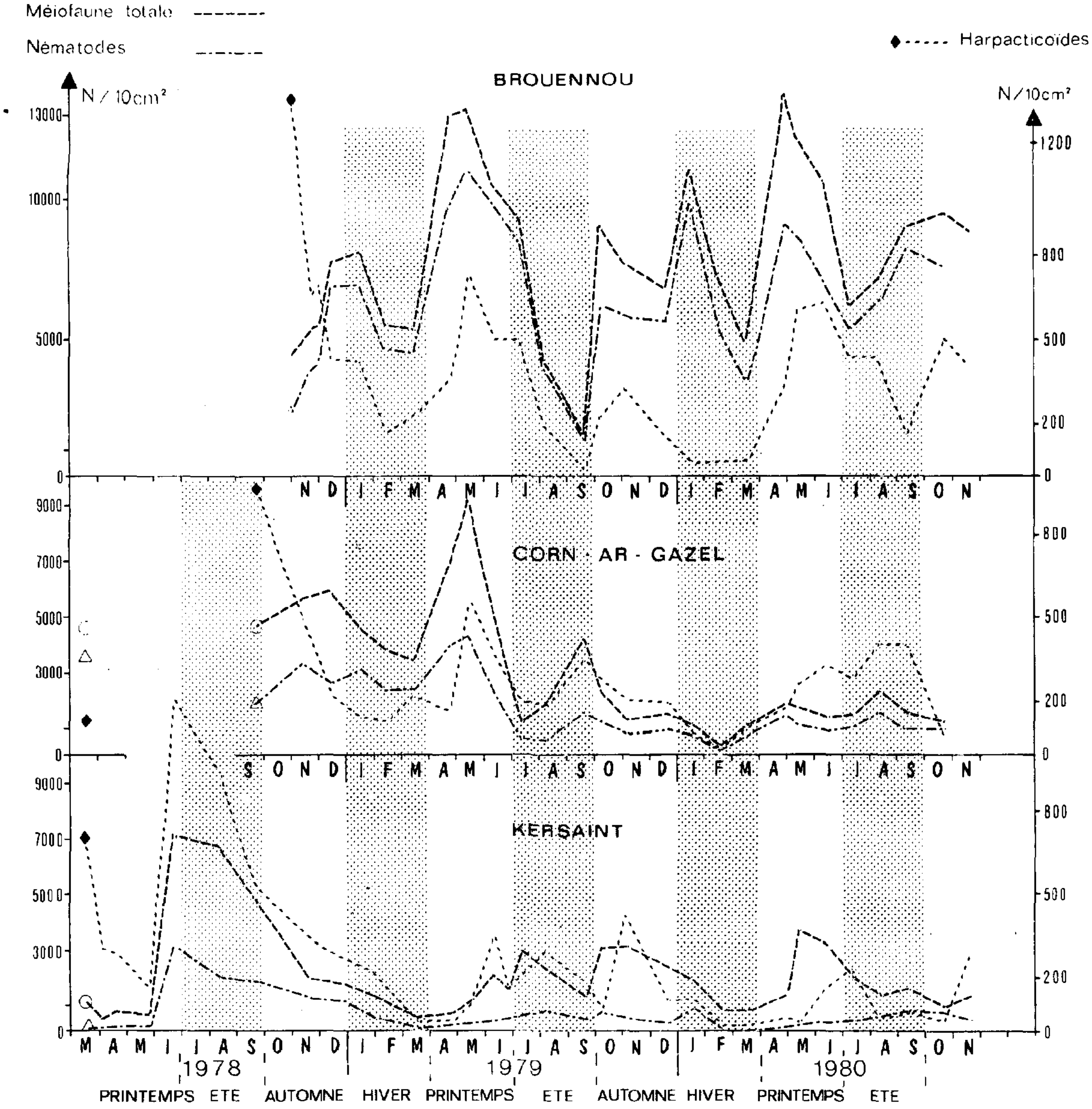

FIGURE 8. Evolution temporelle des densités de la méiofaune aux trois stations.

Copépodes Harpacticoïdes, mais celui des Nématodes et des autres groupes : de 53,8 % en août 1979, ces derniers ne constituent plus que 11,5 % du méiobenthos vrai en août 1980.

Les variations saisonnières sont encore plus ou moins marquées durant la seconde période, avec un minimum classique en février mais aussi un maximum en septembre (1979) et deux légers pics en avril et août 1980). Dans ce biotope mieux oxygéné, le rapport Nématodes/Copépodes varie dans des limites plus étroites : 2,3 (septembre 1980) à 15 (octobre 1980).

Parmi la méiofaune temporaire, les Amphipodes constituent un groupe particulièrement intéressant à cette station où ils avaient subi de lourdes pertes dès le début de la marée noire : ils ont été absents durant tout l'hiver 1979-80, mais ont été régulièrement

TABLEAU 2. Evolution temporelle des densités de la méiofaune aux trois stations (N/10 cm^2).

	1979						1980										
<u>BROUENNOU</u>		6/8	21/9	8/10	5/11	20/12	17/1	19/2	18/3	30/4	14/5	12/6	10/7	11/8	11/9	23/10	20/11
Nématodes		3928	1312	6163	5840	5611	9960	5179	3444	9155	8624	6995	5293	6368	8261	7531	7904
Harpacticoïdes		200	33	224	325	149	58	63	68	336	611	630	440	432	160	512	400
Méiofaune totale		4257	1574	9038	7654	6781	11022	7102	4858	13721	12104	10667	6140	7141	8968	9499	8859
<u>CORN AR GAZEL</u>		7/8	20/9	9/10	6/11	21/12	18/1	18/2	19/3	29/4	13/5	13/6	11/7	12/8	12/9	22/10	
Nématodes		585	1596	1184	845	917	776	213	796	1480	1124	867	1021	1579	924	968	
Harpacticoïdes		189	351	272	209	200	83	32	123	180	260	332	283	409	407	65	
Méiofaune totale		1870	4166	2283	1264	1444	1073	305	1181	1818	1726	1387	1434	2313	1527	1198	
<u>KERSAINT</u>	11/7	7/8	20/9	8/10	6/11	21/12	18/1	18/2	19/3	29/4	14/5	13/6	11/7	12/8	12/9	22/10	20/11
Nématodes	603	727	427	719	472	348	832	104	83	200	336	341	376	599	783	660	433
Harpacticoïdes	208	297	181	82	425	112	123	31	27	56	39	160	232	48	77	39	289
Méiofaune totale	2954	2309	1252	3068	3101	2352	1859	704	759	1259	3623	3168	1903	1295	1529	865	1256

TABLEAU 3. Evolution temporelle des autres groupes de la méiofaune et des nauplii (%).

	1979						1980										
<u>BROUENNOU</u>		6/8	21/9	8/10	5/11	20/12	17/1	19/2	18/3	30/4	14/5	12/6	10/7	11/8	11/9	23/10	20/11
ROTIFERES		+	+	12,1	2,9	7,2	+	0,5	+	0,9	3,4	0,5	+	+	+	+	
TARDIGRADES		0,8	+	+	+	+	0,6	0,7	1,3	3,5	3,1	0,5	+	+	+	+	0,6
GASTROTRICHES			+	+		+			+	+				0,5	+	+	+
OSTRACODES		+		+	+	+		+	+	+	+	+	+	+	+	+	+
TURBELLARIES	}	5,9	10,4	14,6	9,2	5,4	1,5	1,5	1,8	2,9	1,8	1,6	1,0	0,8	0,6	+	
DIVERS							5,3	20,0	15,1	16,2	7,9	20,5	0,7	0,7	2,9	1,2	0,5
TOTAL		6,7	10,4	26,7	12,1	12,6	7,4	22,7	18,2	23,5	16,2	23,1	1,7	2,0	3,5	1,2	1,1
NAUPLII		0,8	0,7	0,9	4,0		0,7	2,5	8,5	6,4	5,4	3,3	1,6	0,7	0,8	12,6	4,4
<u>CORN AR GAZEL</u>		7/8	20/9	9/10	6/11	21/12	18/1	18/2	19/3	29/4	13/5	13/6	11/7	12/8	12/9	22/10	
ROTIFERES		2,3	0,9	+	+	+	+	+		+	1,3	+	+	0,8	0,8	+	
TARDIGRADES		1,2	1,0	+	0,7	2,0	1,5	6,6	3,1	+	+			+			
GASTROTRICHES			6,9	10,9	4,8	14,1	12,3	4,3	12,5	4,1	4,9	4,5	3,6	9,3	4,7	2,6	
OSTRACODES		2,4	2,7	1,2	0,7	+	+	4,3	2,0	1,1	1,7	0,6	+	+	+	0,8	
TURBELLARIES	}	47,9	39,0	21,9	4,4	3,1	1,4	3,0	1,3	1,0	1,7	1,8	1,9	1,4	1,4	7,4	
DIVERS							3,3	2,3	2,0	0,7	1,0	0,6	0,6	+	+	+	
TOTAL		53,8	50,5	34,0	10,6	19,2	18,5	20,5	20,9	6,9	10,6	7,5	6,1	11,5	6,9	10,8	
NAUPLII		2,9	2,1	0,7	0,6	+		+	0,6	1,5	3,2	+	+	+	+	+	
<u>KERSAINT</u>	11/7	7/8	20/9	8/10	6/11	21/12	18/1	18/2	19/3	29/4	14/5	13/6	11/7	12/8	12/9	22/10	20/11
ROTIFERES	0,9	1,1	1,6	1,0	2,0	4,5	1,3	+	11,9	+	+	2,7	+	1,0	1,4	1,4	4,6
TARDIGRADES	7,4	5,7	8,1	5,6	2,6	1,3	7,6	+	5,4	2,9	1,3	2,0	5,1	2,3	8,9	1,8	7,4
GASTROTRICHES	14,3	1,6	+	7,2	+	1,0	11,9	2,3	2,7	3,1	5,9	3,2	3,1	16,4	4,2	6,1	5,4
OSTRACODES	13,8	13,8	8,0	2,4	0,9	1,4	2,6	23,4	13,9	18,7	4,2	18,7	39,3	20,3	3,0	2,0	3,9
TURBELLARIES	}34,8	31,9	30,3	56,0	64,5	71,0	20,4	46,2	43,7	18,8	30,9	18,1	5,4	0,9	11,5	1,4	1,1
DIVERS							3,5	5,8	3,4	25,5	44,6	27,7	2,8	3,5	8,5	2,4	3,2
TOTAL	71,2	54,1	48,0	72,2	70,0	79,2	47,3	77,7	81,0	69,0	86,9	72,4	55,7	44,4	37,5	15,1	25,6
NAUPLII	0,8	+	1,6	1,1	+	0,5		+	4,2	9,6	2,1	11,2	10,2	3,8	3,2		13,9

TABLEAU 4. Evolution temporelle de certains groupes du méiobenthos temporaire (%).

	1979						1980										
<u>BROUENNOU</u>		6/8	21/9	8/10	5/11	20/12	17/1	19/2	18/3	30/4	14/5	12/6	10/7	11/8	11/9	23/10	
Annélides		0,8	2,8	1,5	3,3	1,8	0,8	0,7	0,7	0,7	2,2	2,6	3,1	1,4	0,6	+	
Gastéropodes							+					+			+		
<u>CORN AR GAZEL</u>		7/8	20/9	9/10	6/11	21/12	18/1	18/2	19/3	29/4	13/5	13/6	11/7	12/8	12/9	22/10	
Annélides		3,8	+	+	1,3		+				3,8	3,5	+	+	+		
Tanaïdacés		+	+	+	3,3	1,3		+	+		2,3	+	0,8	+	+	+	
Cumacés		+	+								+	1,4		0,5	3,9	+	
<u>KERSAINT</u>	11/7	7/8	20/9	8/10	6/11	21/12	18/1	18/2	19/3	29/4	14/5	13/6	11/7	12/8	12/9	22/10	20/11
Annélides	+	0,9	+	0,8	+	1,7	1,7	+	+	+	+	+	+	+	+		+
Tanaïdacés	0,5	1,3	2,2	0,9	0,6	0,8	0,6	2,7	2,0	1,0	+	1,4	1,5	+	1,0	+	+
Gastéropodes													1,8	1,3	2,9	3,8	1,7
Cumacés		+		+		+				+	+		+	+			

présents de mai à octobre 1980, confirmant la réinstallation de ce groupe très important au niveau de la macrofaune de Corn ar Gazel (Le Moal, 1981). Les Cumacés ont à peu près le même comportement que les Amphipodes : presque toujours présents entre mai et octobre 1980, ils atteignent 3,9 % de la population totale en septembre.

Kersaint

Suivie mensuellement depuis le 17 mars 1978, la méiofaune de cette station présentait une véritable explosion démographique en juin, juillet et août 1978. Ce phénomène ne s'est pas reproduit par la suite, et l'on est revenu à des variations saisonnières faiblement accentuées, avec un minimum en février-mars (comme aux deux autres stations) et un maximum en octobre-novembre 1979 (comme à Brouennou) et en mai-juin 1980. La densité moyenne (2 063 ind./10 cm^2) est la plus faible des trois, ce que laissaient prévoir les caractéristiques du sédiment. L'évolution temporelle de la méiofaune de cette station a été marquée par une inversion du rapport Nématodes/Copépodes à partir de mai 1978, date depuis laquelle les Nématodes sont devenus prépondérants et le sont restés : depuis le mois de juillet 1979, ce rapport oscille entre 1,1 (décembre 1979) et 16,9 (novembre 1980). La densité moyenne des Harpacticoïdes est d'ailleurs passée de 366 ind./10 cm^2, entre mars 1978 et juin 1979, à 157 entre juillet 1979 et novembre 1980, en raison principalement du pic "anormal" de juin 1978.

C'est à Kersaint que les autres groupes du méiobenthos vrai sont proportionnellement les plus importants : ils constituent près de 87 % de la population en mai 1980, et les valeurs dépassant 70 % ne sont pas rares. Les Ostracodes (tous à des stades très jeunes) constituent 39,3 % de la population en juillet 1980, et la proportion des Gastrotriches s'élève à 16,4 % en août de la même année ; mais le groupe le plus important et le plus régulièrement présent est celui des Turbellariés.

Parmi le méiobenthos temporaire, les Tanaïdacés sont toujours présents (2,7 % au maximum en février 1980), les Annélides deviennent de plus en plus rares à partir de février 1980, alors qu'au contraire les Gastéropodes réapparaissent depuis juillet 1980 (3,8 % de la méiofaune totale en octobre).

Discussion

En l'absence de données antérieures au 17 mars 1978, il est bien difficile de dire qu'elle est la période la plus proche de la "normale" du point de vue quantitatif. Les fortes densités observées durant la première période à Corn ar Gazel et Kersaint pourraient correspondre à une phase d'eutrophisation "anormale" consécutive à l'accumulation de matière organique dans le sédiment, accumulation résultant elle-même de la pollution par les hydrocarbures. Dans ces biotopes à "haute énergie", l'hydrodynamisme intense a pu provoquer un retour à l'oligotrophie durant la seconde période, alors qu'à Brouennou la stabilité du milieu maintenait une certaine eutrophisation. Malheureusement, nous ne disposons pas de données sur la teneur en matière organique des sédiments pour étayer cette hypothèse.

On peut aussi expliquer, du moins en partie, les chutes de densités de la seconde période par la réinstallation dans le biotope de prédateurs provisoirement éliminés par l'arrivée des hydrocarbures ; en tout cas, cette réinstallation est évidente au niveau des Amphipodes.

Evolution comparée de la méiofaune et du microphytobenthos

La comparaison des résultats obtenus, aux deux stations de Brouennou et Corn ar Gazel, pour les pigments chlorophylliens de la couche superficielle (0-1 cm) et la méiofaune, montre que l'amplitude des variations et les valeurs maximales de la densité et de la teneur pigmentaire sont plus élevées à Brouennou, biotope le plus stable. A cette station, des relations de type trophique entre microphytes et méiofaune sont fortement suggérées. On observe en effet un relais entre la phase d'accroissement des pigments chlorophylliens (décembre à mars) et celle de la méiofaune (avril-mai), relais suivi d'une phase d'équilibre relatif. L'accroissement des pigments chlorophylliens apparaît donc en hiver, alors que l'activité des méiobenthontes est ralentie et leur densité en diminution. Les facteurs climatiques n'étant pas ici limitants pour les microphytes, on est en droit de penser que c'est une diminution du "grazing" qui favorise leur accroissement.

A Corn ar Gazel, l'amplitude des variations saisonnières des pigments chlorophylliens et de la méiofaune, surtout depuis juin 1979, est fortement limitée par l'action de l'hydrodynamisme. Il est possible d'établir une coïncidence entre la distribution verticale des pigments et la valeur du rapport Nématodes/Copépodes : ce rapport présente ses plus fortes valeurs en hiver, période pendant laquelle les Copépodes, assez inféodés ici à la surface (au contraire des Nématodes), sont moins nombreux et où il y a aussi moins de pigments. L'instabilité de la couche superficielle semble donc être le facteur limitant de la biomasse primaire et secondaire à cette station et, de ce fait, une possible relation trophique est masquée.

Les Copépodes Harpacticoïdes : étude qualitative

Comme nous avons déjà eu l'occasion de le montrer (Bodin et Boucher, 1981), une étude qualitative est souvent plus révélatrice des perturbations d'un peuplement qu'une simple étude quantitative.

Variations temporelles des différents groupes écologiques

Après détermination, les espèces d'Harpacticoïdes ont été regroupées par affinités écologiques (Tableau 5) d'après nos observations personnelles et les données de la littérature, opération toujours délicate en raison des incertitudes qui pèsent sur l'écologie de certaines espèces. La comparaison de deux années consécutives : novembre 1978 à octobre 1979 et novembre 1979 à octobre 1980, met en évidence une certaine évolution des groupes écologiques au niveau de chaque station. Pour chacune des deux années et pour chaque groupe, deux variables ont été calculées : la somme des densités des espèces concernées (Σ densités) et la dominance générale moyenne (D.g.m.) (Bodin, 1977).

TABLEAU 5. Liste des espèces récoltées aux trois stations entre août 1979 et novembre 1980 (s = sabulicole, v = vasicole, p = phytophile, e = eurytope, m = mésopsammique).

		BROUENNOU (6/8/79 au 20/11/80)			CORN AR GAZEL (7/8/79 au 22/10/80)			KERSAINT (11/7/79 au 20/11/80)		
	Groupe écol.	D.g.m.	Fréquence %		D.g.m.	Fréquence %		D.g.m.	Fréquence %	
Canuella furcigera	v	+	6	R						
Canuella perplexa	s	12,0	100	C	80,3	100	C	0,4	35	F
Halectinosoma herdmani	s	+	12	R	+	7	R	+	12	R
Pseudobradya beduina	s				+	7	R			
Arenosetella sp.	m							0,9	18	R
Tachidius discipes	e	0,6	19	R	+	7	R	0,8	29	F
Microarthridion reductum	v	+	12	R						
Thompsonula hyaenae	s				1,1	20	R			
Harpacticus flexus	s	13,7	100	C	0,2	7	R	6,7	18	R
Tisbe sp.	p				+	13	R			
Parathalestris dovi	p	+	6	R	+	7	R			
Dactylopodia sp.	p							+	6	R
Parastenhelia spinosa bulbosa	p	0,1	6	R				+	6	R
Stenhelia (Del.) palustris bisp	v	0,5	25	F						
Robertsonia celtica	p	24,9	100	C				+	6	R
Bulbamphiascus imus	e	0,7	37	F						
Amphiascus varians	p				+	7	R			
Amphiascus longarticulatus	s				+	7	R			
Amphiascoïdes subdebilis	p	+	6	R						
Amphiascoïdes debilis s. str.	e	39,9	100	C						
Amphiascoïdes debilis limicolus	v	0,6	69	FF						
Schizopera sp.	p							+	6	R
Apodopsyllus arenicolus	m	+	6	R				10,9	100	C
Kliopsyllus constrictus s. str.	m							5,5	47	F
Intermedopsyllus intermedius	m							+	12	R
Paraleptastacus spinicauda	m	0,2	12	R	0,1	20	R	55,4	100	C
Mesochra pygmaea	e				+	7	R			
Enhydrosoma propinquum	v	+	19	R						
Rhizothrix minuta	s	+	6	R	2,2	80	C	1,9	71	FF
Huntemannia jadensis	v	0,3	37	F						
Heterolaophonte strömi s. str.	p	5,9	81	C	+	7	R			
Heterolaophonte littoralis	p							+	6	R
Paralaophonte brevirostris s. str.	p	+	6	R	+	7	R			
Paronychocamptus curticaudatus	s							+	6	R
Asellopsis hispida	s	+	6	R						
Asellopsis intermedia	s	0,4	44	F	15,8	100	C	16,9	88	C

A Brouennou, quatre groupes écologiques peuvent être distingués : les sabulicoles, les vasicoles, les phytophiles et les eurytopes. D'après les D.g.m., ces groupes se répartissent de la façon suivante :

	Période du 3/11/78 au 8/10/79		Période du 5/11/79 au 23/10/80	
	Σ densités	D.g.m.	Σ densités	D.g.m.
Sabulicoles	942	18,4	917	25,1
Vasicoles	477	9,2	62	1,6
Phytophiles	1 628	31,8	1 043	28,5
Eurytopes	1 525	29,9	1 620	44,4
Phytophiles + Eurytopes	3 153	61,7	2 663	72,9

La première année, les phytophiles dominent, avec près de 32 % des Harpacticoïdes ; viennent ensuite les eurytopes, puis les sabulicoles et, enfin, les vasicoles. La seconde année, les eurytopes deviennent largement prépondérants, avec plus de 44 %, et les phytophiles passent en seconde position. L'ensemble phytophiles + eurytopes progresse de plus de 11 %. Les sabulicoles et les vasicoles évoluent en sens inverse, c'est-à-dire que les sabulicoles progressent de près de 7 %, alors que les vasicoles sont réduits d'autant (Fig. 9).

A Corn ar Gazel, ces quatre mêmes groupes écologiques sont représentés la première année, alors que les vasicoles et les eurytopes disparaissent la seconde année. Mais, à cette station, les sabulicoles rassemblent toujours environ 99 % de la population :

	Période du 15/11/78 au 9/10/79		Période du 6/11/79 au 22/10/80	
	Σ densités	D.g.m.	Σ densités	D.g.m.
Sabulicoles	3 247	98,6	2 805	99,8
Vasicoles	4	0,1	-	-
Phytophiles	2	0,1	4	0,1
Eurytopes	27	0,8	-	-
Phytophiles + Eurytopes	29	0,9	4	0,1

Il n'est donc plus question de variations entre les groupes, mais il est intéressant de noter ici une variation à l'intérieur du groupe des sabulicoles. Celui-ci est composé essentiellement de deux espèces : *Asellopsis intermedia* et *Canuella perplexa*. La première année, *A. intermedia* est prépondérante, avec une D.g.m. de 71,5 % contre 18,2 % à *C. perplexa*. L'année suivante, c'est *C. perplexa* qui redevient largement dominante (comme c'était le cas en mars 1978) avec 88,7 % de la population, contre seulement 9 % à *A. intermedia* (Fig. 10).

A Kersaint, station de sable pratiquement pur, les espèces vasicoles sont évidemment absentes. Avec une médiane de près de 200 µm, ce sable est propice à l'installation des formes typiquement interstitielles ; il devient alors nécessaire de distinguer, parmi les Harpacticoïdes, un groupe d'espèces sabulicoles mésopsammiques et

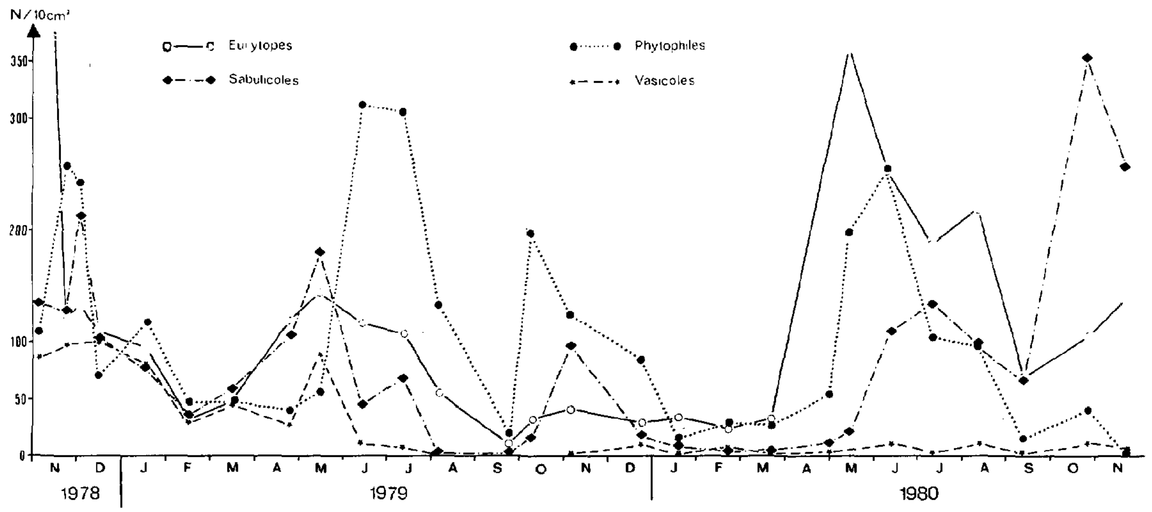

FIGURE 9. Brouennou : évolution temporelle de la densité des Harpacticoïdes regroupés par affinités écologiques.

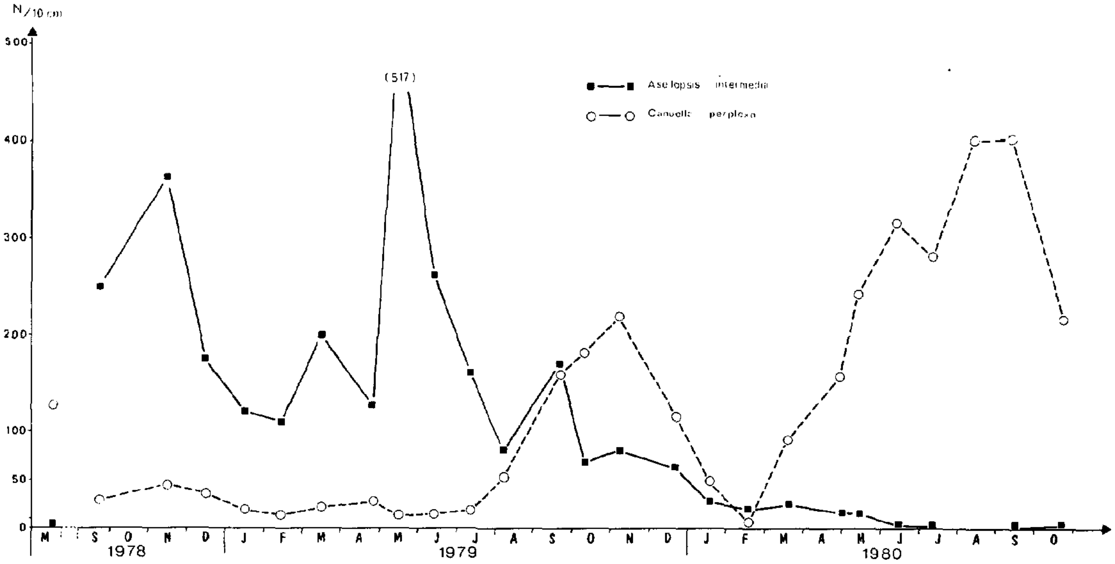

FIGURE 10. Corn ar Gazel : évolution temporelle de la densité des principales espèces d'Harpacticoïdes.

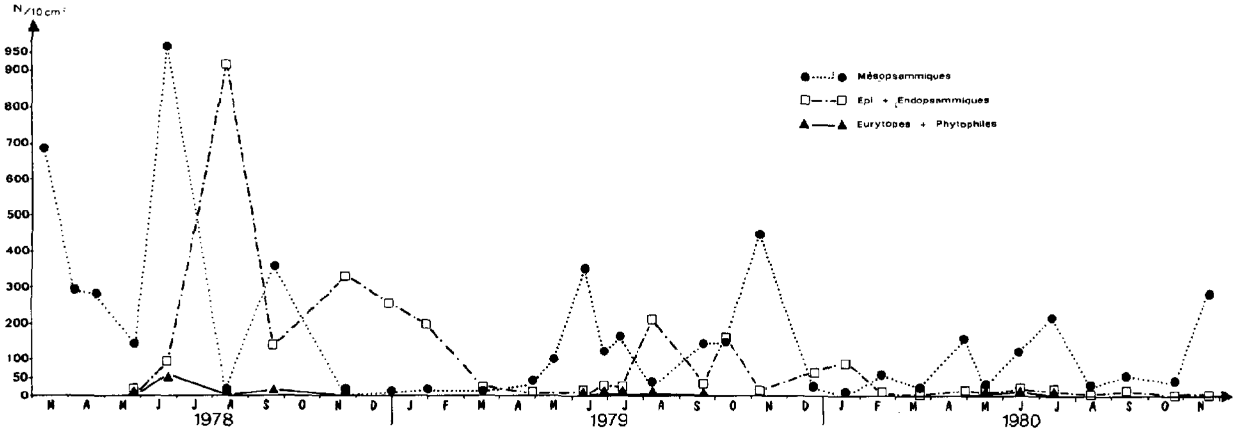

FIGURE 11. Kersaint : évolution temporelle de la densité des Harpacticoïdes regroupés par affinités écologiques.

un groupe de sabulicoles épi- et endopsammiques. Par ailleurs, comme elles sont peu nombreuses et peu abondantes, les espèces phytophiles et les espèces eurytopes sont regroupées dans un seul et même groupe :

	Période du 21/11/78 au 8/10/79		Période du 6/11/79 au 22/10/80	
	Σ densités	D.g.m.	Σ densités	D.g.m.
Sabulicoles mésopsammiques	1 120	45,9	1 181	81,2
Sabulicoles épi-endopsammiques	1 296	53,3	260	17,8
Phytophiles + Eurytopes	20	0,8	15	1,0

L'évolution de ces groupes est assez significative : durant la première année, les formes épi- et endopsammiques dominent avec plus de 53 % de la population, alors que, l'année suivante, les formes mésopsammiques reprennent largement la prédominance avec plus de 81 % (Fig. 11).

Diversité

D'une période à l'autre, on observe une chute importante de la richesse spécifique : 51 espèces avaient été recensées dans les trois stations jusqu'en juillet 1979, on n'en compte plus que 36 entre août 1979 et novembre 1980 (Tableau 5).

De plus, le nombre d'espèces dominantes (D.g.m. ⩾ 1 %) diminue aux trois stations : au total, on passe de 28 espèces dominantes durant la première période à 15 durant la seconde. Parallèlement, les espèces principales voient leur dominance générale moyenne augmenter. A Brouennou, la D.g.m. de *Amphiascoides debilis* s. str. passe de 23 à 40 %. A Corn ar Gazel, la D.g.m. de *A. intermedia* était de 63 % durant la première période étudiée, celle de *C. perplexa* est de 80 % durant la seconde période. A Kersaint, durant la première période, la D.g.m. de *A. intermedia* était de 26 %, celle de *Kliopsyllus constrictus* s. str. de 25 %, celle de *Paraleptastacus spinicauda* de 22 % ; durant la seconde période, la D.g.m. de *P. spinicauda* passe à plus de 55 %.

Enfin, le cas de *K. constrictus* est intéressant à considérer : cette espèce avait une position tout à fait prépondérante jusqu'en juin 1978 ; elle est restée fréquente par la suite, mais sa D.g.m. est tombée de 24,7 à 5,5 %. Cependant, on observe une recrudescence de cette forme mésopsammique en novembre 1980, où sa dominance partielle est de 48,2 %, ce qui nous rapproche de la situation initiale de mars 1978.

Discussion

Dans l'ensemble, on assiste donc à une progression des espèces sabulicoles et à une régression des vasicoles. A Corn ar Gazel et à Kersaint, l'évolution aboutit même à une situation proche de celle qui prévalait en mars 1978 ; la diminution de *A. intermedia* et l'augmentation du stock des mésopsammiques laissent supposer une dépollution du milieu, dépollution facilitée par un hydrodynamisme plus intense à ces stations. Mais, à Brouennou, la progression des eurytopes est encore plus nette que celle des sabulicoles, grâce à certaines espèces telles que *A. debilis* s. str. qui occupent encore largement le biotope.

Doit-on considérer ces espèces (*A. intermedia* et *A. debilis* comme des "opportunistes" au sens où l'entendent Bellan (1967) et Glémarec et Hily (1981) pour la macrofaune ? Il est sans doute encore trop tôt pour l'affirmer, car nous manquons d'états de références de ce type en méiofaune.

Du point de vue de la richesse spécifique, c'est la station de Kersaint qui a perdu le plus d'espèces (7) par rapport à la première période étudiée (en juin 1978, 18 espèces étaient présentes à Kersaint ; en juin 1980, il n'y en avait plus que 6) ; Brouennou en a perdu 5 et Corn ar Gazel en a gagné 2. Mais le phénomène le plus significatif, à notre avis, est la réduction du nombre des espèces dominantes de chaque station et la tendance à la concentration de la faune harpacticoïdienne sur quelques espèces particulièrement bien adaptées au biotope. A Corn ar Gazel, cette tendance est poussée à l'extrême, c'est-à-dire qu'on a un peuplement presque monospécifique, correspondant à un biotope très sélectif d'où les espèces qui avaient envahi le milieu à la suite de la pollution disparaissent peu à peu.

CONCLUSION

Le microphytobenthos est très vite apparu, sur ces deux plages, peu sensible à l'action directe de la pollution (dosages de pigments et observations microscopiques *in vivo* réalisés en avril 1978) mais, partie intégrante de l'écosystème, il réagit au déséquilibre provoqué dans celui-ci. Il est un révélateur des caractères édaphiques du biotope et représente un maillon du réseau trophique benthique sous sa forme active (chlorophylle *a*) ou détritique (phéophytine). Son étude apporte des éléments dans la distinction entre des fluctuations naturelles provoquées par l'hydrodynamisme et une réaction à la pollution des peuplements animaux interstitiels, ce qui expliquerait la différence constatée entre les deux années.

Le méiobenthos, en tant que niveau trophique essentiellement lié au substrat, est particulièrement sensible aux fluctuations des paramètres écologiques, comme l'ont montré de nombreux auteurs (Gray, 1971 ; Arlt, 1975 ; Giere, 1979, Frithsen et Elmgren, 1979 ; Coull et Bell, 1979 ; Renaud-Mornant et Gourbault, 1980 ; Boucher *et al.*, 1981). Le méiobenthos est particulièrement précieux dans le cas des biotopes pauvres en macrofaune (Kersaint).

Du point de vue quantitatif, on peut constater qu'il n'y a pas eu d'"hécatombe" dans la méiofaune, comme ce fût le cas en d'autres circonstances (Wormald, 1976). Mais on observe des perturbations au niveau des cycles saisonniers. A Kersaint, par exemple, il semble que la présence d'hydrocarbures ait provoqué une régression des peuplements (en particulier des Copépodes Harpacticoïdes) jusqu'en mai 1978, ce qui a eu pour effet de retarder de deux mois le pic de printemps. Ce décalage n'est plus que de un mois l'année suivante et il est complètement résorbé en 1980. L'élévation temporaire des densités observée au bout de quelques mois peut être une autre conséquence de la pollution liée à une eutrophisation inhabituelle du milieu provoquée par un éventuel apport de matières organiques. Dans les milieux de mode battu, l'hydrodynamisme a agit rapidement pour, au bout de 12 à 16 mois, opérer un retour à l'oligotrophie habituelle de ces milieux. D'une certaine manière, on retrouve ici le schéma des mécanismes régulateurs des écosystèmes étudiés en baie de Morlaix par Boucher *et al.* (1981). Mais, en l'absence d'états de références antérieurs à la marée noire pour ces stations, nous resterons prudents dans l'interprétation des chutes de densités observées à Corn ar Gazel et Kersaint depuis juillet 1979, où intervient probablement aussi la réapparition des prédateurs de la macrofaune.

Beaucoup plus révélatrices sont les perturbations au niveau qualitatif observées chez les Copépodes Harpacticoïdes. Après l'afflux d'espèces qui a fait suite à la marée noire (juin 1978 à Kersaint), une diminution de la richesse spécifique jointe à une certaine évolution des groupes écologiques montrent qu'un processus de retour à l'état initial est sur le point d'aboutir, en novembre 1980, sur les plages de mode battu. Par contre, une plage de mode abrité telle que Brouennou semble à un stade de dépollution moins avancé, à moins que ce ne soit là son état normal... Encore une fois, l'absence de références ne nous permet pas de nous prononcer avec certitude.

En tout état de cause, l'évolution de la méiofaune en milieu pollué par les hydrocarbures est donc liée essentiellement à l'oxygénation du sédiment et, par conséquent, à l'intensité de l'hydrodynamisme.

Une "veille écologique" devrait permettre de mieux apprécier l'impact de cette marée noire, de préciser les délais de retour à l'état d'équilibre initial au sein des biotopes pollués et, d'une manière générale, de mieux comprendre les perturbations des écosystèmes susceptibles d'être pollués dans le futur. La valeur d'une approche par une étude des écosystèmes dans leur ensemble n'est plus à démontrer pour l'étude des effets des pollutions sur l'environnement (Linden *et al.*, 1979).

Il est souhaitable que les études entreprises, tant au niveau de la macrofaune que de la méiofaune et du microphytobenthos, puissent être poursuivies encore plusieurs années avec, en parallèle, un suivi des paramètres physico-chimiques et sédimentologiques.

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LONG-TERM IMPACT OF THE AMOCO CADIZ CRUDE OIL SPILL ON OYSTERS *Crassostrea gigas* AND PLAICE *Pleuronectes platessa* FROM ABER BENOIT AND ABER WRAC'H, BRITTANY, FRANCE

I. OYSTER HISTOPATHOLOGY

II. PETROLEUM CONTAMINATION AND BIOCHEMICAL INDICES OF STRESS IN OYSTERS AND PLAICE

by

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INTRODUCTION

On the evening of 16 March 1978, the Liberian-registered supertanker *Amoco Cadiz* (233,680 tons deadweight) ran aground and subsequently broke up on Men Goulven rock, Roches de Portsall, approximately 2 km off Portsall on the Breton coast of France. Over a period of several days the complete cargo of the supertanker, which consisted of 120,000 metric tons of light Iranian crude oil, 100,000 tons of light Arabian crude oil and 4,000 tons of bunker fuel was spilled into the coastal waters. By mid April the oil had spread to and contaminated in varying degrees 375 km of the north and west coasts of Brittany (Hess, 1978; Spooner, 1978; Southward, 1978). At the time, it was the largest oil spill in maritime history. There have been two larger spills since then. Two estuaries in the heavily impacted area, l'Aber Benoit 6 km east of the spill and l'Aber Wrac'h 9 km east of the spill, face west and became heavily contaminated with spilled oil.

Aber Benoit and Aber Wrac'h are biologically rich and before the spill supported large oyster mariculture operations and other commercial fisheries. It was therefore of considerable economic and hygenic importance to accurately assess the progress of the long-term recovery of the estuarine biota from the impact of the oil spill.

Several factors relating to this spill, including the large volume of oil spilled, the prevailing winds and currents which drove much of the oil ashore, adverse weather conditions and large tidal prisms which resulted in the incorporation of large amounts of oil into bottom sediments, and the extreme biological richness of the impacted area, all conspired to create a "worst case" scenario for marine oil pollution. Therefore, the *Amoco Cadiz* spill offered a unique opportunity to study in detail the long-term impact and timecourse of biological recovery from a catastrophic pollution incident.

While we already know that the immediate biological effects of the spill were very serious in some areas (Cross et al., 1978; Chasse, 1978; Chasse and Morvan, 1978), there was very little information upon which to base estimates of the rate at which the impacted area would be returned to pre-spill biological productivity. We have used several biochemical parameters and histopathological examination in an ongoing biological survey to assess the health and rate of recovery of marine animals from the two heavily polluted estuaries.

The primary objective of this research program was to assess the degree of chronic sublethal pollutant stress experienced by representative species of benthic fauna from Aber Benoit and Aber Wrac'h. Two indices of stress were used. These are histopathology and biochemical composition. We expected the fauna of these severely impacted estuaries to exhibit an elevated incidence of various histopathological lesions directly or indirectly related to oil pollution stress. As the estuaries recovered from the spill the incidence of these lesions was expected to diminish. Similarly, the concentrations of certain diagnostic biochemical components of the severely stressed fauna were expected to deviate significantly from normal. These diagnostic biochemical indices were expected to return to normal as the estuaries recovered and the resident fauna became less severely stressed. The results of this investigation provide valuable information for assessing the biological recovery of these severely polluted estuaries. They also provide a means of diagnosing pollutant stress in other polluted environments.

I. Histopathology of Oysters *Crassostrea gigas*

Marine animals readily accumulate petroleum hydrocarbons in their tissues from dispersion or solution in sea water and to a lesser extent from petroleum-contaminated sediments and food (see recent reviews by Neff et al., 1976 a,b; Lee, 1977; Varanasi and Malins, 1977; Neff, 1979; Neff and Anderson, 1981). The accumulated hydrocarbons and in particular the more toxic aromatic hydrocarbons interact with cellular membranes and interfere with membrane-mediated biological processes (Roubal and Collier, 1975). Two types of histopathological lesions may result from chronic contamination of marine animals with oil.

The first type is due to the direct toxic effects of petroleum hydrocarbons and associated heavy metals on cells. These compounds may produce a variety of histopathological lesions in the affected organ systems. There are several reports that exposure to sublethal concentrations of oil in laboratory or field studies resulted in epithelial sloughing and discharge of mucus glands in the gills of teleost fish (Blanton and Robinson, 1973; Gardner, 1975; Hawkes, 1977; McKeown and March, 1978). McCain et al. (1978) reported severe hepatocellular lipid vacuolization in English sole *Parophrys ventulus* following exposure for four months to experimentally oiled (Alaskan North Slope crude oil) sediments. Rainbow trout fed Prudhoe Bay crude oil-contaminated food showed several histopathological changes in the liver (Hawkes, 1977). These included glycogen depletion, proliferation of the endoplasmic reticulum and focal necrosis with connective tissue infiltration in necrotic regions. We have described a wide variety of histopathological lesions to embryos and fry of the killifish *Fundulus heteroclitus* exposed chronically during embryonic development to the water-soluble fraction of No. 2 fuel oil (Ernst et al., 1977). In a recent laboratory study of the effects of water soluble fractions of crude oil on marine fish, one of us (Eurell and Haensly, 1981) observed a variety of histopathologic changes in liver and gill tissues.

Little research has been published on the histopathological effects of petroleum in benthic marine invertebrates. However lesions similar to those described in fish can be expected in the analogous organs of marine invertebrates.

Although crude oil contains known carcinogens such as benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene, petroleum-induced cancer has not been unequivocally demonstrated in any marine species (Neff, 1979). However, there are several reports of increased incidence of cancer-like lesions in natural populations of marine invertebrates and fish from hydrocarbon polluted sites (See recent symposium volumes edited by Dawe et al., 1976 and Kraybill et al., 1977).

The second type of histopathological lesion resulting from chronic exposure to sublethal concentrations of oil is caused by elevated susceptibility of contaminated animals to bacterial, virus or parasite infection. This increased susceptibility may result from damage to protective epithelia in the affected animals or to deleterious effects of the pollutant hydrocarbons on the immune system of the animal (Hodgins et al., 1977; Sinderman, 1979). Marine animals which have been subjected to chronic sublethal oil pollution stress can be expected to exhibit an elevated incidence of disease in comparison to non-contaminated animals.

MATERIALS AND METHODS

Oysters *Crassostrea gigas* were collected during five sampling trips to France. Dates of these trips were December 1978, April 1979, July-August 1979, February 1980, and June-July 1980. In Aber Benoit, oysters were obtained from commercial oyster parc owners in St. Pabu and Prat Ar Coum. Oysters from Aber Wrac'h were obtained from a commercial operation near Paluden. Aber Benoit oysters were not available in August 1979. Reference oysters were obtained from several places. None were completely uncontaminated with oil. On the first two trips, December 1978 and April 1979, the oyster parc operator at St. Pabu had oysters from the Rade de Brest (supposedly uncontaminated) which he was holding for later sale. We used these as reference oysters. Subsequent hydrocarbon analysis revealed that these oysters were as heavily contaminated with petroleum as Aber Benoit oysters. They had probably become contaminated during brief holding in the contaminated water of the Aber, as Michel and Grizel (1979) subsequently showed in transplant experiments. On the third trip, August 1979, reference oysters were obtained from the CNEXO mariculture field station at Ile Tudy. On the fourth and fifth trips, February 1980 and June 1980, reference oysters were obtained from a commercial oyster parc owner on the Rade de Brest at Plougastel. As soon as possible after collection, the oysters were shucked and the soft tissues fixed whole in freshly prepared Helly's fixative. The visceral mass was incised to insure rapid penetration of the fixative. After fixation the oysters were washed, dissected into several organs or body regions, dehydrated in ethyl alcohol and embedded in paraffin embedding medium. Organ systems processed for histopathological examination included: visceral mass (includes digestive tract, digestive gland, kidney and gonad), gill, and mantle. Sections were cut a 6 µm with a rotary microtome and stained with hematoxylin-eosin. All tissue blocks and prepared microslides of oyster tissues were labeled, inventoried and archived.

Tissue sections were evaluated qualitatively. The qualitative procedures included a description of the average and limits of normal for the histological status of each tissue. All histopathological lesions were described in full. The incidence of different types of lesions in each tissue was recorded. The incidence of different types of lesions in each tissue was recorded. These data for the three populations (2 oil-contaminated stations and one control station) were compared. Seasonal and temporal differences in the incidence of pathological lesions were also recorded. A photographic record of normal tissue histology and of all types of histopathological lesions was made and archived.

RESULTS

Tissues from 134 specimens of *Crassostrea gigas* from four sites were examined for histopathologies over five sampling trips. From the specimens collected, tissue samples of 131 adductor muscles, 127 stomach/intestines, 129 digestive glands, 130 gonads, 134 gills, and 130 mantles were examined for a total of 781 tissues out of a possible 804.

A total of nine types of pathologies were found with an incidence of 241 occurrences (Table 1). Five-hundred and ninety of the 781 (75.6%) tissues examined were free of pathologies; or, 191 of the 781 (24.3%) tissues examined bore one or more pathologies. Of the 241 pathologies found, 77 (32.0%) were various types of symbioses, while 164 (68.0%) cases apparently were not correlated with symbioses. Table 2 summarizes the distribution of pathologies among the tissues examined. Adductor muscle had the lowest incidence (3.8%). Digestive gland tissue had the highest incidence (23.9%) followed by gill (22.0%), mantle (21.4%), gut (17%), and gonad (11.9%). The number of tissues with pathologies was nearly evenly distributed among the collecting sites. Oysters from reference stations had a higher incidence of lesions, particularly in gonad and gill, than oysters from oil-polluted sites. Thirty percent of the oyster tissues from both Aber Wrac'h and Aber Benoit bore one or more pathologies. Forty percent of the tissues from Rade de Brest and Ile Tudy combined contained one or more pathologies.

Overall, mantle bore the lowest number of pathology types (3) while digestive gland contained the most types of pathologies (9) followed by gut (7), gill (6), gonad (5), and muscle (4).

Pathologies and their distributions among organs and sites are described below.

1. Muscle. - Muscle tissues were examined from 131 *C. gigas*. Samples for microscopic examination were dissected from the adductor muscle and both fast and catch muscles were examined when possible. Generally, two tissue samples were taken from each muscle and oriented to give both longitudinal and cross sections.

Histopathologies occurred in 4.6% (6 of 131) of the muscle samples examined. There were a total of 9 incidences of the three pathologies described below. Muscle from reference stations contained the widest variety of pathologies. No pathologies were found in muscles from Aber Benoit.

Table 1. Types of pathologies, total incidence of each, affected organ and collecting site of occurrence

Pathology	Incidence	Organ*	Site+
Amoebae	3	DG,MA	C
Ciliates	21	GU,DG,GI	W,B,C
Sporozoans	29	MU,GU,DG,GO,GI,MA	W,B,C
Copepods	23	GU,DG,GI	B,C
Nematodes	1	DG	W
Degeneration	10	MU,GU,DG,GO	W,C
Necrosis	9	GU,DG,GO	W,B,C
General leucocytosis	93	MU,GU,DG,GO,GI,MA	W,B,C
Focal leucocytosis	52	MU,GU,DG,GO,GI,MA	W,B,C
Total	241		

* MU - Muscle
GU - Gut
DG - Digestive gland
GO - Gonad
GI - Gill
MA - Mantle

\+ C - Control (Rade de Brest and Ile Tudy)
W - Aber Wrac'h
B - Aber Benoit

Table 2. Distribution of pathologies in tissues of oysters *Crassostrea gigas* from two oil contaminated estuaries and from reference stations, with sampling times combined.

	Organ						
Station	Muscle	Gut	Digestive Gland	Gonad	Gill	Mantle	Total
Reference	5	17	20	15	21	18	96
Aber Benoit	0	18	19	6	14	17	74
Aber Wrac'h	5	6	18	7	18	17	71
Total	10	41	57	28	53	52	241

Abnormally high numbers of eosinophilic leucocytes (general leucocytosis) were apparent in 1.5% (2 of 131) of the adductor muscle samples exmained. Leucocytes were generally spread throughout the muscle rather than being in focal aggregations.

Aggregated eosinophilic leucocytes were present in 1 of 131 adductor muscles examined. For the purposes of this report, this aggregation was classified as a focal leucocytosis although there was no central core or tight concentric arrangement of leucocytes as reported from *Crassostrea virginica* (Armstrong et al., 1980). This may be an inflammatory response to what appears to be a foreign body, possibly a nematode, at the edge of the aggregation.

Five (3.8%) of the muscles examined contained areas of degenerated muscle bundles. This condition was characterized by a breakdown or liquefaction of the cellular integrity. Degenerated areas contained amorphous, light staining debris and fibers. No pyknotic nuclei were present in surrounding whole muscle fibers and no inflammation (increased number of leucocytes) was apparent.

Unidentified sporozoans in the plasmodial stage were found in 1 of the 131 muscles examined.

2. Digestive Gland. - The digestive glands of 129 *C. gigas* were examined. Generally, two samples were taken from each specimen at different levels (anterior and posterior) of the digestive gland.

Histopathologies were noted in 44.2% (57 of 129) of the digestive gland samples examined. There were a total of 64 incidences of the 9 types of pathologies described below. Twenty-seven of these or 42.2% apparently were not attributable to symbioses, while 37 (57.8%) were a type of symbiont or were clearly attributable to symbioses (i.e. inflammation). The distribution of these histopathologies among sampling sites is summarized in Table 3. Digestive gland samples from Aber Benoit contained more pathologies than samples from the other two sites. All digestive gland samples from the December, 1978 collection at Aber Benoit bore one or more pathologies. Samples from other sites over the five collections had no more than 62% incidence of pathologies.

Abnormally high numbers of eosinophilic leucocytes were dispersed throughout the leydig tissue between diverticula in 5 (3.7%) of the 129 samples examined. In some, leucocytes were also invading the diverticular epithelium. These cases could have been inflammatory responses to parasites such as copepods which were not included in the sectioned material. That is, the sections could be at the edge of an inflammatory response as described below.

Table 3. Distribution of histopathologies of *C. gigas* digestive gland among sampling sites. Numbers in parentheses represent the number of specimens examined.

		Site		
Pathology	Aber Wrac'h (45)	Aber Benoit (42)	Rade de Brest & Ile Tudy (42)	Total (129)
Leucocytosis (general)	1	2	2	5
Leucocytosis (focal)	8	7	1	16
Degeneration	1	-	-	1
Focal Necrosis	1	4	-	5
Amoebae	-	-	1	1
Ciliates	4	6	6	16
Sporozoa	2	7	7	16
Nematode	1	-	-	1
Copepoda	-	3	-	3
Total	18	29	17	64

Aggregates of eosinophilic leucocytes, were present in 12.4% (16 of 129) of the digestive glands examined. Almost all cases were in specimens from Aber Wrac'h or Aber Benoit (Table 3). For the purpose of this study, these were termed focal leucocytoses. They differed from a general leucocytosis in that the leucocytes were in a dense clump, sometimes focal, rather than being dispersed throughout the tissues. General leucocytosis may possibly, in some cases, be a part of a focal inflammation viewed some distance from the focal foreign body or parasite. Some cases of focal leucocytosis appeared to be confined to the leydig tissue surrounding the diverticulae and were not totally "focal". In most cases, however, the condition involved mass invasion of the lumina by leucocytes and/or phagocytes with large numbers of leucocytes and/or phagocytes massed in the surrounding leydig tissue. Decomposed portions of copepods were present in the lumina of two specimens and no doubt were responsible for the mass inflammation. Copepods were not apparent in the leucocytic inflammations in the digestive glands of the other specimens. These inflammations, or focal leucocytoses, may also have been responses to copepods as they were identical in all aspects except for the observed presence of copepods in the section.

In one case, well-formed focal aggregates were present in the leydig tissue adjacent to the digestive gland. In one, the leucocytes were confined to a well-formed "pocket", while in another the leucocytes were also spread from the "pocket" to adjacent leydig tissue. A massive pocket of leucocytes was present in one of the digestive glands examined. Leucocytes were confined to the large "pocket". Adjacent leydig cells were compressed.

A degeneration of two or three diverticula was observed in one digestive gland and was associated with a copepod parasite. This involved a breakdown of the diverticular epithelia and basal membranes with leucocytic inflammation.

Five (3.9%) of the samples examined bore small necrotic areas on one to four diverticula. These areas were characterized by a breakdown of cellular integrity accompanied by light staining cellular debris and a limited number of leucocytes. Necrosis appeared to be minor.

Amoebae were present in the digestive gland of one *C. gigas*.

Digestive glands of 16 (12.4%) of the *C. gigas* examined contained ciliates. Ciliates were evenly distributed among Aber Wrac'h, Aber Benoit and Rade de Brest oysters and were sometimes quite numerous in the diverticular lumina. Ciliates were oblong, with a somewhat pointed antenai, and longitudinal spiral rows of short, stout cilia. They did not appear to damage the diverticula.

Sporozoa were present in the diverticular epithelium of 16 (12.4%) of the specimens examined. All but two of the cases were from Aber Benoit or Rade de Brest and Ile Tudy (Table 3). Sporozoans were spherical and stained very intensely. They were often surrounded by a clear (lysed ?) zone.

The digestive gland of one specimen bore a nematode which elicited an inflammatory response, an aggregation of eosinophilic leucocytes.

Remains of copepods were noted in the diverticula of 3 *C. gigas* from Aber Benoit. They were accompanied by heavy leucocytic inflammation and were being phagocytized as evidenced by the presence of leucocytes in the copepods.

3. Gut. - Samples consisting of stomach, intestine and often esophageal and rectal tissues were examined from 127 *C. gigas*. Generally, two tissue samples were taken from each specimen (anterior and posterior portions of the visceral mass).

Histopathologies were noted in 32.2% (41 of 127) of the gut samples examined. There were 62 cases of the 6 pathology types discussed below. Thirty-five percent (22) involved a symbiont while 40 cases (65%) apparently were not symbiotic in nature, although there may be some question about this. Specimens from the combined reference stations bore more than two and a half times the pathologies as Aber Wrac'h specimens. Oysters from Aber Benoit contained slightly fewer pathologies than those from the reference stations and twice the number of pathologies as specimens from Aber Wrac'h.

Abnormally high numbers of eosinophilic leucocytes (general leucocytosis) were noted in the intestinal epithelium, and sometimes surrounding leydig tissue, of 15% (19 of 127) of the *C. gigas* examined. Almost all cases were in oysters from Rade de Brest. This condition was difficult to judge. Oyster intestinal epithelium normally has some leucocytes between columnar cells. However, the large number of leucocytes in the intestinal epithelium of these 14 specimens appeared abnormally high. the number was considered abnormally high if the basal portion of columnar cells was completely, or almost completely, obscured by leucocytes. However, there still is some doubt about whether this is a "pathology" or a normal condition. Except for the large number of leucocytes, the intestinal tissues appeared very healthy.

Focal aggregates of leucocytes were present in the intestinal epithelium, or adjacent to it, in 14.2% (8 of 127) of the gut tissues examined. One case involved a large, loose aggregation of leucocytes in the leydig tissue beneath the basement membrane. Another involved small clumps of leucocytes between the columnar epithelial cells. Like the general leucocytosis, this condition was difficult to judge. Leucocytes are normally present in the epithelium, but more or less scattered about. These clumps could be normal phagocytosis, although no foreign matter was ever observed in such clumps. The intestinal epithelium containing the above clumps appeared otherwise very healthy.

Focal necrotic areas were present in the gut epithelium of 3 (2.3%) oysters examined. In two incidences, the gastric shield was involved. This condition was characterized by a breakdown of the structure of the gastric shield and/or epithelium, a concentration of debris at the affected area, and leucocytic inflammation of the gastric shield and/or epithelium.

Ciliates were present in the gut lumen of one *C. gigas*. These ciliates were the same type as described above in the digestive gland.

The plasmodial stage of an unidentified sporozoan was noted in the epithelium of a single *C. gigas*. The plasmodium was amoeboid in appearance with several nuclei. The gut otherwise appeared in very good condition.

Copepods were present in the stomach of 15.7% (20 of 127) of the specimens examined. No oysters from Aber Wrac'h bore copepods. None of the copepods observed were being phagocytized as was the case in the digestive gland. Up to three copepods were observed in some sections.

4. Gonad. - Gonadal tissues of 130 *C. gigas* were examined. Generally, two tissue samples were taken from each specimen (anterior and psoterior visceral mass).

Gonadal tissues from *C. gigas* were a very difficult tissue type to assess for non-symbiotic pathologies. Possible histopathologies were noted in 38% (9 of 130) of the gonadal tissues examined. There were 50 incidences of the three non-symbiotic pathology (?) types and one symbiotic pathology discussed below. Only six of the 50 conditions were of an apparent symbiotic nature.

Half (36 of 71) of the female gonadal tissues examined exhibited moderate to heavy aggregations, both focal and general, of eosinophilic leucocytes. This presented a perplexing problem in determining if this represented an inflammatory response to a stressful condition and therefore a pathology due to such stress, or if it was a normal condition in the reproductive cycle of *C. gigas*. This condition was present in only one *Crassostrea virginica* from South Louisiana oil platforms (Armstrong et al., 1980) but was observed in other bivalve species (10% of specimens examined in association with degeneration or necrosis of the gonad). Eight female *C. gigas* from the Pacific Northwest (Sequim, Washington) were examined for comparison. All eight appeared to be in a post-spawn condition and all had heavy aggregation of leucocytes in the gonadal tissues.

The spawning cycle of the *C. gigas* from France could not be definitely determined. Undifferentiated (could not determine if it was male or female), undeveloped, developing (immature), ripe (mature) and spawned stages were present in samples from all five of the collecting periods (December 1978, April 1979, August 1979, February 1980, and June 1980). The majority of the specimens from December 1978, however, appeared to be of the spawned stage at Aber Wrac'h, ripe at Aber Benoit, and undifferentiated at Rade de Brest. In April 1979, the majority of the specimens appeared to be in the developing stage at all three sites. The majority of the specimens taken during August 1979 and June 1980 appeared to be of the ripe stage at all three sites, although there were some spawned-appearing specimens from Aber Wrac'h in August 1979. The February, 1980 collection yielded more undifferentiated and developing specimens. This does somewhat indicate an early winter spawn, but as already stated, all reproductive stages were present in samples from all five collection periods.

In the *C. gigas* from France, 13.8% (18 of 130) of the gonads examined contained large numbers of leucocytes dispersed throughout the tissues. All 18 incidences were in female gonads (18 of 76 or 23.8%). This condition was present in undeveloped, developing (immature), ripe and post spawn ovaries. In some cases it could not be determined if the ovary was in a developing stage or a post-spawn stage because of the large numbers of leucocytes present. In some, the gonad appeared fully spawned (entire gonad examined contained only a few ova and ovacytes, follicles largely empty), while in others part of the ovary was packed with ova (ripe) and the other part contained few ova and ovacytes (spawned) and many leucocytes. In gonads with large numbers of leucocytes, all or almost all ova appeared normal (not degenerating or lysing). The 29 normal ovaries (no aggregations of leucocytes present) included the undeveloped, developing (immature), ripe and spawned stages.

Twenty-two of the 130 (16.6%) gonads examined contained compact clumps of leucocytes ranging from foci in the follicular wall to large clumps in the ovary to foci in the leydig tissue of the testes. Nineteen (86.4%) of the cases were in female gonads. Reproductive stages varied from undeveloped to spawned. Three of the cases were found in testes.

In the general and focal leucocytoses discussed above, the difference between the two was in the extent (small area, tight clump vs. general dispersion over several follicles) of inflammation, but this was sometimes difficult to ascertain and the two may blend together.

Necrotic appearing areas were noted in 3.1% (4 of 130) of the gonads examined. These areas were characterized by cellular debris, degenerating ova, and leucocytosis.

Sporozoa were present in 4.6% (6 of 130) of the specimens examined. Sporozoans were spherical, densely staining, and were embedded in the gonadal tissue. The specimens appeared to be surrounded by a small lysed "halo" area.

5. Gill. - Gills from 134 *C. gigas* were examined for pathologies. Generally, three pieces of gill (consisting of both lamellae) were dissected from one side and oriented (when possible) to give both longitudinal and transverse sections.

Histopathologies were noted in 31.3% (42 of 134) of the gills examined. There were a total of 49 cases of the six pathology types described below. Forty-three (87.8%) were apparently not symbiotic or related to a symbiotic condition.

Abnormally high numbers of eosinophilic leucocytes were present in 31.3% (42 of 134) of the gills examined. In most incidences, the leucocytes were dispersed throughout several plica, but four cases appeared to be more focally organized in one or two plica.

Amoebae were noted in the gills of one *C. gigas*. The infection appeared to be light as only two amoebae were found. The amoebae were circular in outline with a hyaline cytoplasm. The nucleus occupied approximately one-third of the cell. A small, spherical inclusion body was adjacent to the nucleus.

The gills of four (3%) specimens examined harbored ciliates in their water tubules. Ciliates were somewhat crescent-shaped with tufts of stout cilia extending downward from the two tips. The arms of the crescent were sometimes turned inward so that the tips of the cilia were touching, giving a partially hollow, circular shape to the ciliate. Two lateral nuclei were present. The ciliates apparently provoked an inflammatory response as most were surrounded by eosinophilic leucocytes in the water tubules, or the surrounding tissues contained abnormally high numbers of lucocytes.

One specimen contained the plasmodial stage of a sporozoan. Multinucleate plasmodia were subspherical to ovate. Numerous plasmodia were dispersed throughout the gill, but most heavily in the leydig tissue of the interlamellar area.

A single copepod was found on a gill filament of one *C. gigas*.

No necrotic areas were observed on the gills examined and the outer columnar epithelium of the specimens examined appeared healthy. The number of mucous glands in sections of randomly selected plica and terminal grooves were counted in an effort to determine if specimens from Aber Wrac'h and Aber Benoit contained more active glands than those from Rade de Brest and Ile Tudy. The results were inconclusive. The number of mucus cells per unit area of gill was not statistically significantly different among the three populations.

6. Mantle. - Sections of mantle from 130 specimens were examined. Two or three pieces of mantle were dissected from specimens and oriented to give a transverse section across the tri-lobed edge.

Histopathologies were noted in the mantle of 31.5% (41 of 130) of the specimens examined. There were a total of 47 of the three pathology types described below. The distribution of the pathologies among sampling sites was nearly equal.

Abnormally high numbers of eosinophilic leucocytes were noted in 35.4% (46 of 130) of the specimens examined. Thirty-eight of the incidences involved large numbers of leucocytes dispersed beneath the epithelium or in the leydig tissue. Eight cases, however, involved leucocytes which were more aggregated in clusters.

Sporozoans were found in the mantle of a single specimen.

No necrotic areas were found on any of the mantles examined. All epithelial cells appeared healthy. In an effort to determine if the mantle epithelium of oysters from Aber Wrac'h and Aber Benoit contained significantly more mucous cells than specimens from Rade de Brest and Ile Tudy, the number of mucous cells in a high power field were counted at a level even with the circumpallial nerve and an area three fields higher. Specimens from Aber Wrac'h contained slightly more (average of 27.5 to 34 for the five collections) mucous cells than those from Aber

Benoit (average of 20 to 32) and Rade de Brest and Ile Tudy (average of 22 to 31). The differences were not statistically significant.

CONCLUSIONS

In general, oysters *Crassostrea gigas* from all five collections and all four sampling stations appeared to be extremely healthy as determined by histopathological examination. Incidence of parasitic infestation was very low, especially when compared to incidence of parasitism in *C. virginica* from the northwest Gulf of Mexico. The low incidence of parasitism in *C. gigas* from Brittany may be due to the fact that they are a recently-introduced mariculture species in the area. There probably has not been enough time for their parasites to catch up with them. According to Henri Grizell (personal communication), parasitism and disease are increasing in these oysters.

The most prevalent pathologic lesion in *C. gigas* from Brittany was leucocytosis. In mollsucs, this condition is usually a response to chemical or physical irritation. It is an inflammatory defensive response. However, size and distribution of leucocyte populations varies greatly in different mollusc species under different environmental conditions. *C. gigas* generally seems to have more leucocytes than the closely-related *C. virginica*. Thus, the extent to which observed leucocytoses in *C. gigas* were normal or pathologic is uncertain. In any event, incidence of leucocytosis was similar in oysters from oil-contaminated Aber Benoit and Aber Wrac'h and from reference stations in the Rade de Brest and at Ile Tudy.

Necrosis was observed several times but no definitive cases of hyperplasia, neoplasia or other precancerous conditions was noted in any of the four oyster populations.

There were no consistent temporal trends in incidence of pathology in the oysters from oiled and reference stations. Oysters collected in December 1978, nine months after the spill, had an incidence of pathological conditions similar to that in oysters collected in June 1980, twenty-seven months after the spill. One difference that may have obscured other effects was size. By June 1980, oysters which had been in the Abers at the time of the spill had grown to very large size. During the first year after the spill, there was little evidence of growth in oysters from the two Abers. During the second year, growth appeared normal or even accelerated.

There was also some indication, based on observations of gonadal condition, that oysters from the Abers had an altered reproductive cycle compared to reference oysters, possibly including near complete reproductive suppression for one year after the spill. Sample sizes and frequencies were not great enough to demonstrate this convincingly.

II. Petroleum Contamination and Biochemical Indices of Stress in Oysters and Plaice

The most obvious immediate biological effect of the *Amoco Cadiz* spill was a very large kill of benthic estuarine and coastal marine organisms (Cross et al., 1978). The rate of recovery of these benthic communities would depend on the rate and success of reproduction by the surviving animals in the affected area and on the success of recruitment from adjacent unpolluted areas. The resident benthic fauna in the oil-impacted area which survived the spill were undoubtedly severely stressed. Because of the heavy contamination of the estuarine sediments with oil it is highly probable that the surviving resident benthic fauna would continue for some time to be stressed and potential immigrants to the estuaries would be subjected to stress as they settled there.

Considerable research has been conducted in recent years on sublethal physiological stress responses of marine animals to oil and other types of pollution (Neff et al., 1976a; Anderson, 1977; Johnson, 1977; Patten, 1977; Neff, 1979; Thomas et al., 1980; Neff and Anderson, 1981). A variety of sublethal physiological and biochemical responses to pollutant stress have been described. In an ecological perspective, the net effect of chronic pollutant stress on marine organisms is to shunt limited energy resources away from growth and reproductive processes to maintenance and homeostatic functions. The result is decreased growth, fecundity and reproductive success in the stressed population. A variety of biochemical parameters are altered in stressed animals and reflect the stress-induced changes in energy balance and partitioning. These biochemical parameters can be used as an index of pollutant stress in marine animals.

Biochemical indices of pollutant stress chosen for use in this investigation include hemolymph glucose concentration and adductor muscle-free amino acids in oysters; and blood glucose and cholesterol, liver glycogen and ascorbic acid, and muscle-free amino acids in plaice. We have discussed elsewhere the rationale for using these parameters as indices of pollutant stress (Thomas et al., 1980, 1981 a,b).

When exposed to petroleum, marine molluscs and teleost fish readily accumulate hydrocarbons in their tissues (Neff et al., 1976b; Varanasi and Malins, 1977; Neff and Anderson, 1981). Molluscs tend to release accumulated hydrocarbons relatively slowly when concentrations in the ambient medium are reduced. However, under similar conditions, teleost fish release hydrocarbons very rapidly. Differences in hydrocarbon release rate by molluscs and fish can be attributed to differences in ability to convert hydrocarbons to polar more readily excreted metabolites by the cytochrome P-450 mixed function oxygenase system and related pollutant-metabolizing enzyme systems (Varanasi and Malins, 1977; Neff, 1979). In the present investigation, aliphatic and aromatic hydrocarbons were analyzed in oysters and plaice from oiled and reference stations to assess patterns of hydrocarbon accumulation and release and to allow for correlations between levels of hydrocarbon contamination of animals and histopathological/biochemical responses.

MATERIALS AND METHODS

Oysters *Crassostrea gigas* were collected for biochemical analysis on the first three sampling trips. Sampling sites were as described earlier in the section on oyster histopathology. Oysters were shucked and a sample of hemolymph was collected immediately from the heart or the adductor muscle and stored frozen until analyzed. Adductor muscle was also sampled and stored at -60°C until analyzed.

Plaice *Pleuronectes platessa* were collected by otter trawl from oil-contaminated Aber Benoit and Aber Wrac'h. Reference stations for plaice samples were as follows: December 1978, Baie de Douarnenez; April 1979, Loc Tudy; August 1979, February 1980, June 1980, Ile Tudy. Fish from the Baie de Douarnenez and Loc Tudy were collected by otter or beam trawl. Fish from Ile Tudy were captured by net at the sluice gate of the CNEXO mariculture pond and held in large circular holding tanks with flowing seawater until sampled.

Samples were taken as soon as possible after capture and while the fish were still alive. Tissue samples included blood, muscle and liver. Blood samples were centrifuged to remove red blood cells. Serum, muscle, and liver were frozen immediately in liquid nitrogen and kept frozen at -60° until analyzed.

Samples from 5-10 animals from each station and each trip were analyzed biochemically. Blood glucose and liver glycogen were measured with a Yellow Springs Instruments automatic glucose analyzer, Model 23A.

This method, based on the glucose oxidase enzymatic reaction, is highly specific for glucose and required only 25 μl of serum. Replicate determinations of each serum sample were performed. Total and esterified cholesterol in serum was determined by a cholesterol oxidase assay system which is both highly snesitive and specific.

For tissue-free amino acid analysis, muscle tissue was thawed, weighed and homogenized in distilled water using a 2/1 ratio of distilled water/wet weight. Homogenates were deproteinized with 12.5% trichloroacetic acid and then centrifuged. The supernates were frozen, thawed, and centrifuged again to remove additional TCA precipitates. The supernates were then evaporated to dryness on a rotary evaporator and the residue dissolved in 0.2 M Citrate buffer adjusted to pH 2.2. The extracts were analyzed with a Beckman automatic amino acid analyzer. The amino acid composition of the extract and the concentration of individual amino acids in it were determined. Taurine/glycine molar ratios were computed. Variations in amino acid compositions and concentrations among fish and oysters from different sampling stations were analyzed statistically.

Plaice liver was analyzed for ascorbic acid. Tissue samples were thawed, weighed and homogenized in 3% metaphosphoric acid-8% acetic acid solution. After centrifugation, the supernates were analyzed immediately by the α,α-diperidyl technique of Zannoni et al. (1974).

Oysters and plaice samples for hydrocarbon analysis were taken at the same times and places as samples for biochemical/histopathological analysis. Ten to twelve whole oysters were pooled for each sample. They were shucked and tissues were rinsed in distilled water, blotted dry, wrapped in hexane-cleaned aluminum foil and frozen at -60°C until analysis. For the April 1979 sample, whole fish were used. For subsequent samples, pooled samples of liver and muscle from 5-10 fish were used. Fish tissue samples were handled like oyster samples.

Hydrocarbon analyses were performed by Dr. Paul Boehm, ERCO, Cambridge, Massachusetts using capillary gas chromatography/mass spectrometry.

RESULTS AND DISCUSSION

Petroleum Hydrocarbons

Concentrations of total aliphatic and aromatic hydrocarbons in tissues of oysters *Crassostrea gigas* from Aber Benoit and Aber Wrac'h, heavily contaminated with *Amoco Cadiz* oil, and from supposedly clean reference stations are summarized in Table 4. Reference oysters for the first two collections were Rade de Brest oysters which had been held for a short period of time in concrete holding tanks on the shore of Aber Benoit at St. Pabu. These reference oysters were heavily contaminated with *Amoco Cadiz* oil as were the authentic Aber Benoit and Aber Wrac'h oysters. "Hydrocarbon status" of samples was determined by comparing GC peak profiles of f_1 and f_2 hydrocarbon fractions of tissue extracts to GC profiles of authentic weathered *Amoco Cadiz* oil. Apparently, sufficient oil was still leaching from the sediments of the Aber 13 months after the spill to allow rapid and heavy contamination of oysters exposed to waters of the bay. Michel and Grizel (1979) reported similar rapid hydrocarbon contamination of oysters transplanted to stations in Aber Benoit and Aber Wrac'h. Subsequent reference oyster samples were obtained from sites which had not received *Amoco Cadiz* oil. They contained low levels of petroleum hydrocarbons not of *Amoco Cadiz* origin. Concentrations of total aliphatic and aromatic hydrocarbons in oysters from Aber Benoit and Aber Wrac'h did not vary substantially over the time-course of this investigation (up to 27 months after the spill). The persistence of petroleum hydrocarbons in tissues of oysters probably represents, in part, a continuous recontamination with hydrocarbons leaching gradually into the water from the heavily contaminated sediments of the Abers. Oysters from the Baie of Morlaix, east of Aber Wrac'h and less heavily contaminated with *Amoco Cadiz* oil than the Abers, collected 17 months after the spill, contained about half the aromatic hydrocarbons of Aber Wrac'h oysters. It is interesting to note that Aber Benoit oysters collected in December 1978 and April 1979 had a distinctly oily taste. Oysters sampled in August 1979 and later did not taste oily. Apparently, 200 ppm aromatics is not readily detected by taste, whereas 500 ppm is.

More detailed analysis of the aliphatic fraction of the oyster samples revealed some interesting trends (Tables 5-7). In all but one case (Aber Wrac'h, April 1979), the aliphatic fraction of Aber Benoit and Aber Wrac'h oysters was dominated by the low boiling aliphatics, C_{10} - C_{20}, including n-alkanes, branched and isoprenoid compounds. This is quite unlike weathered *Amoco Cadiz* oil or oil in the Aber

Table 4 . Concentrations of total aliphatic and aromatic hydrocarbons (measured gravimetrically) in oysters *Crassostrea gigas* from reference stations and from two estuaries contaminated with *Amoco Cadiz* oil. Status determined according to pattern and identity of GC peaks.

Date/Sample	Hydrocarbon Fraction (μg/g dry tissue) Aliphatics	Aromatics	Status[a]
December 1978 (9)[b]			
Rade de Brest (reference)	47.8	208.0	AC oil
Aber Benoit	136.7	552.2	AC oil
Aber Wrac'h	115.4	540.0	AC oil
April 1979 (13)			
Rade de Brest (reference)	153.9	1001.0	AC oil
Aber Benoit	114.9	690.0	AC oil
Aber Wrac'h	225.8	986.1	AC oil
August 1979 (17)			
Ile Tudy (reference)	39.3	51.9	Other oil
Baie de Morlaix	134.6	206.4	Other oil
Aber Wrac'h	101.0	485.4	AC oil
February 1980 (23)			
Rade de Brest (reference)	62	87	Other oil
Aber Benoit	154	275	AC oil
Aber Wrac'h	217	599	AC oil
June 1980 (27)			
Rade de Brest (reference)	33	60	Other oil
Aber Benoit	238	283	AC oil/Other oil
Aber Wrac'h	132	430	AC oil

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[a] AC oil - *Amoco Cadiz* oil; other oil - definitely petroleum, but cannot be identified as *Amoco Cadiz oil.*

[b] month after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 5. Concentration of aliphatic hydrocarbons in tissues of oysters *Crassostrea gigas* from Aber Benoit, Brittany France collected at different times after the *Amoco Cadiz* oil spill. Values are in ng/g dry weight (parts per billion).

Compound	Sample Date Dec 1978 (9)[a]	 Apr 1979 (13)	 Aug 1979 (17)	 Feb 1980 (23)	 Jun 1980 (27)
n-C_{10}	ND	32	NS	ND	ND
n-C_{11}	316	328	NS	162	ND
n-C_{12}	43	47	ND	44	ND
n-C_{13}	99	31	NS	7	ND
n-C_{14}	394	18	NS	16	20
Farnesane	1,343	776	NS	66	92
n-C_{15}	22	84	NS	68	10
n-C_{16}	44	46	NS	62	62
n-C_{17}	184	61	NS	144	22
Pristane	376	232	NS	40	22
n-C_{18}	ND	ND	NS	51	ND
Phytane	613	375	NS	39	122
n-C_{19}	47	68	NS	17	42
n-C_{20}	77	57	ND	7	
n-C_{21}	ND	ND	NS	18	49
n-C_{22}	ND	ND	NS	10	53
n-C_{23}	24	ND	NS	20	57
n-C_{24}	43	ND	NS	21	53
n-C_{25}	55	ND	NS	21	17
n-C_{26}	51	ND	NS	9	24
n-C_{27}	53	ND	NS	37	52
n-C_{28}	37	ND	NS	12	11
n-C_{29}	72	ND	NS	18	66
n-C_{30}	ND	ND	NS	74	343
n-C_{31}	ND	ND	NS	13	27
n-C_{32}	ND	ND	NS	12	134
n-C_{33}	ND	ND	NS	ND	49
n-C_{34}	ND	ND	NS	ND	12
Total Resolved Aliphatics	3,893	2,155	NS	981	1,346

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978

ND, not detected

NS, no sample available.

Table 6. Concentration of aliphatic hydrocarbons in tissues of oysters *Crassostrea gigas* from Aber Wrac'h, Brittany France collected at different times after the *Amoco Cadiz* oil spill. Values are in ng/g dry weight (parts per billion).

Compound	Sampling Date Dec 1978 (9)[a]	 Apr 1979 (13)	 Aug 1979 (17)	 Feb 1980 (23)	 Jun 1980 (27)
$n\text{-}C_{10}$	46	ND	110	47	84
$n\text{-}C_{11}$	383	55	759	170	255
$n\text{-}C_{12}$	86	365	ND	126	63
$n\text{-}C_{13}$	39	70	32	ND	15
$n\text{-}C_{14}$	36	650	12	519	35
Farnesane	222	617	953	370	148
$n\text{-}C_{15}$	13	ND	172	56	13
$n\text{-}C_{16}$	53	ND	098	198	31
$n\text{-}C_{17}$	37	177	577	218	ND
Pristane	319	52	39	44	78
$n\text{-}C_{18}$	ND	ND	ND	27	ND
Phytane	571	242	141	194	64
$n\text{-}C_{19}$	187	ND	ND	12	ND
$n\text{-}C_{20}$	74	ND	ND	12	ND
$n\text{-}C_{21}$	ND	ND	ND	ND	ND
$n\text{-}C_{22}$	ND	ND	ND	ND	ND
$n\text{-}C_{23}$	ND	30	ND	141	ND
$n\text{-}C_{24}$	15	135	ND	ND	ND
$n\text{-}C_{25}$	15	222	ND	19	12
$n\text{-}C_{26}$	14	289	ND	27	ND
$n\text{-}C_{27}$	11	280	ND	19	ND
$n\text{-}C_{28}$	ND	241	ND	23	ND
$n\text{-}C_{29}$	ND	219	ND	18	ND
$n\text{-}C_{30}$	ND	132	ND	16	ND
$n\text{-}C_{31}$	ND	ND	ND	ND	ND
$n\text{-}C_{32}$	ND	ND	ND	ND	ND
Total Resolved Aliphatics	2,121	3,776	2,893	2,256	798

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978

ND, not detected.

Table 7. Concentration of aliphatic hydrocarbons in tissues of oysters *Crassostrea gigas* from reference stations on the Brittany coast of France collected at different times after the *Amoco Cadiz* oil spill. Values are in ng/g dry weight (parts per billion).

Compound	Dec 1978[a] (9)[d]	Apr 1979[a] (13)	Aug 1979[b] (17)	Feb 1980[c] (23)	Jun 1980[c] (27)
	Sampling Date				
n-C_{10}	53	ND	ND	86	226
n-C_{11}	360	441	ND	339	486
n-C_{12}	15	49	ND	86	112
n-C_{13}	17	111	ND	18	9
n-C_{14}	52	29	ND	33	18
Farnesane	11	1,346	4	45	35
n-C_{15}	172	123	29	122	66
n-C_{16}	139	55	17	69	41
n-C_{17}	252	228	61	81	46
Pristane	18	331	ND	ND	ND
n-C_{18}	39	ND	ND	58	45
Phytane	117	529	17	ND	13
n-C_{19}	40	35	ND	22	28
n-C_{20}	43	73	ND	20	24
n-C_{21}	42	20	ND	16	22
n-C_{22}	40	47	ND	15	23
n-C_{23}	42	85	21	16	36
n-C_{24}	49	135	29	15	45
n-C_{25}	50	173	33	16	47
n-C_{26}	56	204	44	15	55
n-C_{27}	65	207	54	20	60
n-C_{28}	64	167	36	16	48
n-C_{29}	66	165	64	29	47
n-C_{30}	22	100	54	50	42
n-C_{31}	ND	71	59	13	22
n-C_{32}	ND	ND	ND	ND	8
Total Resolved Aliphatics	1,824	4,724	522	1,200	1,604

[a], from Rade de Brest, but maintained in Aber Benoit before sampling

[b], from oyster mariculture ponds of CNEXO at Ile Tudy

[c], from a commercial oyster parc in the Rade de Brest

[d], months after the *Amoco Cadiz* oil spill, 16 March 1978

ND, not dectected.

sediments which is dominated by higher boiling saturated hydrocarbons. This phenomenon is unexplained and could represent selective accumulation and/or retention of lighter aliphatics or more rapid metabolism and excretion of heavier aliphatics. The most likely explanation is that oysters were being contaminated with hydrocarbons leaching from bottom sediments into the water column. Lighter aliphatics, because of their slightly higher aqueous solubility than heavy aliphatics, are desorbed more readily from sediments and therefore are more available for uptake by the oysters. Aliphatic hydrocarbon fractions of reference oysters were more uniform (Table 7). Relative abundances of C_{10} to C_{32} aliphatics were similar.

There were no consistent differences in characteristics of the aliphatic hydrocarbon fraction between reference oysters and oysters from oil-polluted Aber Benoit and Aber Wrac'h (Table 8). With one exception (April 1979), alkane/isoprenoid ratios were higher in oysters from reference stations than in those from oil-polluted stations. Pristane/phytane ratios were quite variable and without pattern. All but two carbon preference indices were near one indicating a petroleum origin for the high molecular weight aliphatic fraction.

Composition of the aromatic fraction of oysters, as determined by gas chromatography/mass spectrometry, revealed a great deal about the origin of the hydrocarbon contamination of the oysters (Tables 9-11). High concentrations of alkyl naphthalenes through alkyl dibenzothiophenes are characteristic of samples contaminated with crude oil. *Amoco Cadiz* oil was particularly rich in alkyl phenanthrenes and alkyl dibenzothiophenes. These were the most abundant aromatics/heterocyclics in oyster samples from oil-contaminated Aber Benoit and Aber Wrac'h. Aromatic hydrocarbon assemblages of crude oil origin are dominated by alkylated species, whereas aromatic assemblages of pyrogenic origin are dominated by the unalkylated parent compound (Neff, 1979). Thus we can conclude that oysters from Aber Benoit and Aber Wrac'h at all five sampling times, and reference oysters from the December 1978 and April 1979 collections were heavily contaminated with crude oil, resembling the *Amoco Cadiz* oil. The other three reference samples contained some oil, but it did not resemble *Amoco Cadiz* oil. In oysters from the two Abers, there was a general trend for the concentration of aromatics/heterocyclics in the alkyl naphthalenes to alkyl dibenzothiophenes series to decrease slowly with time. The February 1980 samples contained higher concentrations of alkyl phenanthrenes and alkyl dibenzothiophenes than expected. It is possible that winter storms in December and January resuspended oil-contaminated sediments causing recontamination of resident oysters.

Table 8 . Characteristics of the aliphatic hydrocarbon fraction of oysters *Crassostrea gigas*. from reference stations and from two estuaries contaminated with *Amoco Cadiz* oil

Date/Sample	Pristane/Phytane	Alkanes/Isoprinoids	Carbon Preference Index (C_{26}-C_{30})
December 1980(9)			
Rade de Brest (reference)	0.15	2.34	1.27
Aber Benoit	0.61	0.18	2.0
Aber Wrac'h	0.56	0.07	1.57
April 1979(13)			
Rade de Brest (reference)	0.63	0.12	1.16
Aber Benoit	0.62	0.13	ND
Aber Wrac'h	0.21	1.00	1.10
August 1979(17)			
Ile Tudy (reference)	ND	5.15	1.39
Baie de Morlaix (reference)	ND	ND	0.93
Aber Benoit	NS	NS	NS
Aber Wrac'h	0.28	0.48	ND
February 1980(23)			
Rade de Brest (reference)	ND	4.37	1.00
Aber Benoit	ND	1.30	1.02
Aber Wrac'h	0.23	0.81	ND
June 1980(27)			
Rade de Brest (reference)	ND	2.64	1.10
Aber Benoit	0.18	0.28	0.61
Aber Wrac'h	1.68	0.19	0.93

Table 9. Concentration of aromatic hydrocarbons in tissues of oysters *Crassostrea gigas* from Aber Benoit, Brittany, France at different times after the *Amoco Cadiz* oil spill. Values are in ng/g tissue (parts per billion).

Compound	Sampling Date Dec 1978 (9)[a]	Apr 1979 (13)	Aug 1979 (17)	Feb 1980 (23)	Jun 1980 (27)
Alkyl naphthalenes	NA	1,243	NS	ND	300
Alkyl fluorenes	NA	2,230	NS	891	850
Phenanthrene	NA	590	NS	43	64
Alkyl phenanthrenes	NA	17,345	NS	6,088	3,014
Dibenzothiophene	NA	123	NS	ND	ND
Alkyl dibenzothiophenes	NA	15,380	NS	8,860	5,420
Fluoranthene	NA	665	NS	150	84
Pyrene	NA	600	NS	150	87
Benz[a]anthracene	NA	263	NS	200	ND
Chrysene	NA	490	NS	600	180
Benzofluoranthenes	NA	570	NS	670	100
Benzopyrenes	NA	339	NS	413	80
Perylene	NA	80	NS	ND	ND
Total Resolved Aromatics	NA	39,918	NS	18,065	10,179

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978.

NA, sample not analyzed by GC/MS

NS, no sample available

ND, not detected

Table 10. Concentration of aromatic hydrocarbons in tissues of oysters *Crassostrea gigas* from Aber Wrac'h, Brittany, France at different times after the *Amoco Cadiz* oil spill. Values are in ng/g tissue (parts per billion).

Compound	Sampling Date				
	Dec 1978 (9)[a]	Apr 1979 (13)	Aug 1979 (17)	Feb 1980 (23)	Jun 1980 (27)
Alkyl naphthalenes	781	594	150	ND	10
Alkyl fluorenes	2,203	1,453	980	560	ND
Phenanthrene	89	69	ND	ND	170
Alkyl phenanthrenes	12,114	14,989	5,030	10,089	4,550
Dibenzothiophene	24	ND	ND	ND	ND
Alkyl dibenzothiophenes	21,748	11,521	9,900	15,820	5,530
Fluoranthene	258	58	50	150	70
Pyrene	291	105	65	190	90
Benz[a]anthracene	557	330	ND	1,100	63
Chrysene			300		230
Benzofluoranthenes	ND	237	260	410	190
Benzopyrenes	ND	161	50	170	140
Perylene	ND	ND	ND	ND	50
Total Resolved Aromatics	38,065	29,517	16,785	28,489	11,093

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978;

ND, Not detected.

Table 11. Concentration of aromatic hydrocarbons in tissues of oysters *Crassostrea gigas* from "reference" stations on the Brittany coast of France at different sampling times after the *Amoco Cadiz* oil spill. Values are in ng/g tissue (parts per billion).

Compound	Sampling Date Dec 1978[a] (9)[d]	 Apr 1979[a] (13)	 Aug 1979[b] (17)	 Feb 1980[c] (23)	 Jun 1980[c] (27)
Alkyl naphthalenes	327	467	ND	ND	180
Alkyl fluorenes	689	1,562	ND	ND	180
Phenanthrene	129	85	5	180	350
Alkyl phenanthrenes	4,375	10,679	285	527	630
Dibenzothiophene	30	56	ND	ND	20
Alkyl dibenzothiophenes	3,668	10,590	283	975	675
Fluoranthene	220	171	130	180	200
Pyrene	170	98	130	180	90
Benz[a]anthracene	252	290	ND	140	58
Chrysene			410	350	170
Benzofluoranthenes	88	48	350	410	160
Benzopyrenes	64	65	140	182	83
Perylenes	ND	ND	ND	ND	ND
Total Resolved Aromatics	10,012	24,111	1,733	3,124	2,796

[a], from Rade de Brest, but maintained in Aber Benoit before sampling

[b], from oyster mariculture ponds of CNEXO at Ile Tudy

[c], from commercial oyster parc in the Rade de Brest

[d], months after the *Amoco Cadiz* oil spill, 16 March 1978

ND, not detected.

Higher molecular weight aromatics, fluoranthene through perylene, although present in small amounts in crude oil, are more characteristic of pyrogenic hydrocarbon assemblages (Neff, 1979). Concentrations of these aromatics were similar in reference and Aber oysters and there was no consistent pattern of temporal change. These hydrocarbons probably have a similar origin in all three populations, namely from particulate organic matter derived from smoke of wood and fossil fuel combustion. Several of these aromatics, including benz[a]anthracene, benzofluoranthenes, and benzopyrenes, are known carcinogens. Their presence in tissues of oysters at relatively high concentration could be cause for concern.

Whole fish and muscle samples of plaice *Pleuronectes platessa* contained low concentrations of aliphatic and aromatic hydrocarbons (Table 12). Most of the muscle samples contained aliphatic hydrocarbon distributions characteristic of oil (Tables 13-15). Nearly tenfold higher concentrations of aliphatics were found in liver samples than in muscle samples of reference plaice and plaice from the oil-polluted Abers. In the August 1979 samples, some of this was identified as petroleum. In later samples, no petroleum-derived hydrocarbons were detected in liver samples. The aromatic fraction showed a distribution pattern similar to that of the aliphatic fraction. Liver aromatic fractions were dominated by biogenic squalene. Liver samples also contained high concentrations of what appeared to be naphthenic (cyclic alkanes) hydrocarbons.

Aliphatic fractions from all liver samples were dominated by hydrocarbons in the C_{21} - C_{32} molecular weight range. In the three liver samples from Aber Benoit, two of the three samples from Aber Wrac'h, and one reference sample, dominant aliphatics were C_{27} and C_{29}. In the remaining two samples, dominant aliphatics were C_{25} and C_{29}. With few exceptions light aliphatics, C_{10} - C_{20}, were present at low or nondetectable concentrations in the plaice livers.

Plaice muscle contained 1-10% of the concentration of aliphatics that liver did. Alkane distribution patterns in muscles varied considerably. In most cases alkanes above C_{24} were dominant. Concentrations of aliphatic hydrocarbons in muscle and liver were higher in summer (August 1979 and June 1980) than in winter (February 1980), suggesting a seasonal cycle of tissue hydrocarbon concentration. This seasonal pattern was not correlated with seasonal changes in total lipid content of plaice tissues (Table 18). As in the oysters, there was no consistent difference between reference plaice and plaice from oil-contaminated Aber Benoit and Aber Wrac'h with respect to pristane/phytane ratio, alkane/isoprenoid ratio, or carbon preference index (Table 16).

Table 12. Concentrations of total aliphatic and aromatic hydrocarbons (measured gravimetrically) in tissues of plaice *Pleuronectes platessa* from reference stations and from two estuaries contaminated with *Amoco Cadiz* oil. Status determined according to pattern and identify of GC peaks.

Date/Sample	Hydrocarbon Fraction (µg/g dry tissue) Aliphatics	 Aromatics	Status[a]
April 1979 (13)[b]			
Whole Fish			
Loc Tudy (reference)	2.9	91	Biogenic
Aber Benoit	38.0	24	Biogenic
Aber Wrac'h	7.1	83	Small U.C.M.
August 1979 (17)			
Muscle			
Aber Benoit	7.7	9.0	Other oil/biogenic
Aber Wrac'h	19.9	12.7	Other oil/biogenic
Liver			
Aber Benoit	801.9	235.6	Other oil/biogenic
Aber Wrac'h	1034.0	317.9	Other oil/biogenic
February 1980 (23)			
Muscle			
Ile Tudy (reference)	23	19	Other oil/biogenic
Aber Benoit	83	22	Other oil/biogenic
Aber Wrac'h	66	12	Other oil/biogenic
Liver			
Ile Tudy (reference)	736	548	Biogenic
Aber Benoit	1510	352	Biogenic
Aber Wrac'h	831	355	Biogenic
June 1980 (27)			
Muscle			
Ile Tudy (reference)	16	23	Biogenic
Aber Benoit	38	17	Other oil/biogenic
Aber Wrac'h	146	41	Other oil
Liver			
Ile Tudy (reference)	1210	723	Biogenic
Aber Benoit	1810	682	Biogenic
Aber Wrac'h	1130	511	Biogenic

[a] biogenic - probably of biological origin; small U.C.M. - small unresolved complex mixture, typical of weathered oil; other oil - definitely petroleum but cannot be identified as *Amoco Cadiz* oil.

[b] months after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 13. Concentration of aliphatic hydrocarbons in tissues of plaice *Pleuronectes platessa* from Aber Benoit, Brittany France, collected at different times after the *Amoco Cadiz* oil spill. Values are in ng/g dry weight (parts per billion).

Compound	Date/Sample						
	Apr 1979(13)	Aug 1979(17)		Feb 1980(23)		Jun 1980(27)	
	Whole Fish	Muscle	Liver	Muscle	Liver	Muscle	Liver
n-C_{10}	ND	ND	ND	ND	ND	ND	ND
n-C_{11}	3	15	ND	6	531	ND	208
n-C_{12}	ND	ND	ND	ND	324	ND	ND
n-C_{13}	ND	ND	ND	ND	ND	4	ND
n-C_{14}	ND	ND	ND	ND	ND	8	ND
Farnesane	ND	ND	ND	ND	ND	ND	ND
n-C_{15}	3	6	ND	13	ND	8	ND
n-C_{16}	3	12	ND	14	132	8	ND
n-C_{17}	9	44	ND	31	336	13	ND
Pristane	18	12	ND	16	ND	5	2,530
n-C_{18}	4	47	ND	21	299	13	ND
Phytane	31	20	ND	29	ND	5	ND
n-C_{19}	3	24	ND	15	331	11	ND
n-C_{20}	6	13	ND	29	459	22	ND
n-C_{21}	4	9	555	39	425	50	1,130
n-C_{22}	3	10	1,927	44	321	120	3,470
n-C_{23}	3	13	3,695	51	206	221	6,460
n-C_{24}	2	15	5,109	61	152	324	9,030
n-C_{25}	4	21	6,476	78	452	436	10,500
n-C_{26}	7	23	8,315	92	2,530	486	13,700
n-C_{27}	15	31	14,363	95	6,660	503	19,600
n-C_{28}	8	28	10,552	84	4,100	421	15,200
n-C_{29}	22	42	14,282	87	6,770	359	23,500
n-C_{30}	ND	43	4,573	57	750	299	9,040
n-C_{31}	10	43	4,425	99	1,430	197	9,150
n-C_{32}	ND	36	1,414	20	ND	117	3,280
n-C_{33}	ND	ND	ND	ND	ND	86	ND
n-C_{34}	ND	ND	ND	ND	ND	51	ND
Total Resolved Aliphatics	158	507	76,586	991	26,208	3,767	126,798

[a] months after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 14. Concentration of aliphatic hydrocarbons in tissue of plaice *Pleuronectes platessa* from Aber Wrac'h, Brittany France collected at different times after the *Amoco Cadiz* oil spill. Values are in ng/g dry weight (parts per billion).

	Date/Sample						
	Apr 1979(13)[a]	Aug 1979(17)		Feb 1980(23)		Jun 1980(27)	
Compound	Whole Fish	Muscle	Liver	Muscle	Liver	Muscle	Liver
$n^{-}C_{10}$	ND	16	ND	ND	562	16	152
$n^{-}C_{11}$	ND	105	ND	ND	943	46	ND
$n^{-}C_{12}$	ND	ND	ND	ND	155	16	ND
$n^{-}C_{13}$	ND	ND	ND	ND	ND	ND	ND
$n^{-}C_{14}$	ND	ND	ND	ND	ND	ND	ND
Farnesane	ND	ND	ND	ND	ND	ND	ND
$n^{-}C_{15}$	ND	6	ND	6	ND	ND	ND
$n^{-}C_{16}$	ND	8	ND	11	ND	9	ND
$n^{-}C_{17}$	8	16	ND	27	ND	28	151
Pristane	10	ND	ND	5	370	12	ND
$n^{-}C_{18}$	3	7	ND	19	ND	22	ND
Phytane	15	ND	ND	8	ND	17	ND
$n^{-}C_{19}$	ND	ND	ND	9	ND	6	ND
$n^{-}C_{20}$	ND	ND	ND	ND	ND	23	ND
$n^{-}C_{21}$	3	ND	ND	8	164	45	1,040
$n^{-}C_{22}$	3	ND	ND	7	ND	91	3,410
$n^{-}C_{23}$	2	9	ND	7	46	162	6,460
$n^{-}C_{24}$	2	14	207	6	ND	223	9,150
$n^{-}C_{25}$	3	23	4,722	6	56	335	10,600
$n^{-}C_{26}$	4	34	1,467	7	379	328	16,300
$n^{-}C_{27}$	7	64	3,781	7	1,100	280	20,100
$n^{-}C_{28}$	4	76	3,883	7	857	255	16,600
$n^{-}C_{29}$	6	147	6,625	8	2,480	266	21,700
$n^{-}C_{30}$	ND	189	3,032	5	599	254	10,800
$n^{-}C_{31}$	ND	237	1,747	4	912	80	10,900
$n^{-}C_{32}$	ND	241	ND	ND	134	100	3,130
$n^{-}C_{33}$	ND	237	ND	ND	ND	18	2,050
$n^{-}C_{34}$	ND	176	ND	ND	ND	17	586
Total Resolved Aliphatics	70	1,605	25,464	157	6,914	2,649	133,129

[a] months after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 15. Concentration of aliphatic hydrocarbons in tissues of plaice *Pleuronectes platessa* from reference stations on the Brittany coast of France at different times after the *Amoco Cadiz* oil spill. Values are in ng/g dry weight (parts per billion).

Compound	Date/Sample Apr 1979(13)[a] Whole Fish	Feb 1980(23) Muscle	Feb 1980(23) Liver	Jun 1980 (27) Muscle	Jun 1980 (27) Liver
$n\text{-}C_{10}$	ND	17	99	37	231
$n\text{-}C_{11}$	ND	44	275	75	618
$n\text{-}C_{12}$	ND	11	53	17	130
$n\text{-}C_{13}$	ND	1	ND	ND	ND
$n\text{-}C_{14}$	ND	3	ND	1	ND
Farnesane	ND	4	ND	ND	ND
$n\text{-}C_{15}$	ND	11	ND	7	ND
$n\text{-}C_{16}$	2	16	ND	8	ND
$n\text{-}C_{17}$	5	32	ND	16	ND
Pristane	3	10	ND	3	ND
$n\text{-}C_{18}$	4	21	ND	18	ND
Phytane	2	18	ND	6	ND
$n\text{-}C_{19}$	3	8	ND	7	ND
$n\text{-}C_{20}$	3	7	80	9	187
$n\text{-}C_{21}$	2	8	302	17	1,740
$n\text{-}C_{22}$	2	7	283	41	5,510
$n\text{-}C_{23}$	2	9	412	76	1,060
$n\text{-}C_{24}$	1	10	571	110	14,500
$n\text{-}C_{25}$	2	7	771	141	23,600
$n\text{-}C_{26}$	2	12	1,150	158	20,000
$n\text{-}C_{27}$	3	12	2,380	158	21,400
$n\text{-}C_{28}$	2	10	2,060	134	16,200
$n\text{-}C_{29}$	6	9	5,270	112	24,200
$n\text{-}C_{30}$	ND	5	1,320	82	10,500
$n\text{-}C_{31}$	1	5	3,730	58	12,500
$n\text{-}C_{32}$	ND	ND	749	34	482
$n\text{-}C_{33}$	ND	ND	ND	ND	402
$n\text{-}C_{34}$	ND	ND	ND	ND	1,160
Total Resolved Aliphatics	45	297	19,505	1,325	154,420

[a] months after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 16. Characteristics of the aliphatic hydrocarbon fraction of plaice *Pleuronectes platessa* from reference stations and from two estuaries contaminated with *Amoco Cadiz* oil.

Date/Sample	Pristane/Phytane	Alkanes/Isoprenoids	Carbon Preference Index (C_{26} - C_{30})
April 1979(13)[a]			
Whole Fish			
Loc Tudy (reference	1.40	2.34	3.72
Aber Benoit	0.59	0.39	3.11
Aber Wrac'h	0.66	0.45	2.41
August 1979(17)			
Muscle			
Aber Benoit	0.61	3.38	1.19
Aber Wrac'h	ND	6.14	1.12
Liver			
Aber Benoit	ND	ND	1.64
Aber Wrac'h	ND	ND	1.70
February 1980(23)			
Muscle			
Ile Tudy (reference)	0.58	2.37	1.13
Aber Benoit	0.55	1.77	1.11
Aber Wrac'h	0.64	4.54	1.27
Liver			
Ile Tudy (reference)	ND	ND	2.32
Aber Benoit	ND	ND	2.34
Aber Wrac'h	ND	ND	2.65
June 1980(27)			
Muscle			
Ile Tudy (reference)	0.56	5.22	1.07
Aber Benoit	1.00	5.00	1.06
Aber Wrac'h	0.68	2.07	1.00
Liver			
Ile Tudy (reference)	ND	ND	1.45
Aber Benoit	ND	ND	1.62
Aber Wrac'h	ND	ND	1.38

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978.

The hydrocarbon data demonstrate convincingly the dramatic differences in patterns of petroleum hydrocarbon contamination of oysters and plaice from the same oil-contaminated Abers. Oysters contained high concentrations of alkanes, dominated by low molecular weight compounds, while in plaice, the dominant alkanes in liver samples were the higher molecular weight compounds. Oysters contained abundant petrogenic and pyrogenic aromatic hydrocarbons spanning a wide molecular weight range. Plaice on the other hand contained little true aromatic hydrocarbon. These differences undoubtedly reflect the markedly different capabilities of bivalve molluscs and teleost fish to metabolize and actively excrete petroleum hydrocarbons. Most teleosts studied to date have a highly active and inducible cytochrome P-450 mixed function oxygenase system capable of converting aromatics and some aliphatics to polar and more easily excreted matabolites (Neff, 1979). This enzyme system is absent altogether or present at very low activity in bivalve mollusc tissues.

Biochemical Indices of Stress

Total lipid concentration in tissues of oysters and plaice, determined in connection with hydrocarbon analyses, showed no consistent patterns in relation to station or season (Tables 17-18). In June 1980, but not at other sampling times, oysters from the two oil-contaminated Abers contained 2-3 times as much lipid as oysters from the reference station. It is quite possible that this is related to differences between reference and Aber oysters in state of reproductive ripeness, and not directly to oil-induced effects.

Hemolymph glucose concentrations in oysters were low, highly variable, and showed no relationship to station (Table 19). No statistically significant differences were noted in values for reference and Aber oysters. There was a trend at all stations toward increasing hemolymph glucose concentration between December 1978 and August 1979.

Some patterns did emerge in serum glucose concentrations of plaice (Table 20). In December 1978, April 1979 and August 1979, with one exception, serum glucose concentrations of plaice from oil-contaminated Aber Benoit and Aber Wrac'h were lower than values for reference plaice. Two of these differences were statistically significant. The collecting technique (otter trawl) is highly stressful, and maximal hyperglycemic stress response occurs rapidly in fish (Thomas et al., 1980). The data suggest, not that Aber plaice were less stressed than reference plaice, but that they had become refractory--perhaps due to chronic stress--to capture-induced hyperglycemia. Inability to respond biochemically to stress has been demonstrated in plaice held in the

Table 17. Concentration of total lipids (determined gravimetrically) in whole oysters *Crassostrea gigas* from reference stations and from estuaries contaminated by *Amoco Cadiz* oil. Values are in µg/g dry tissue.

Station	August 1979	February 1980	June 1980
Reference	9,775	6,650	5,580
Aber Benoit	NS	9,150	15,900
Aber Wrac'h	6,151	4,860	11,200
Baie de Morlaix	6,188	NS	NS

NS, no sample available.

Table 18. Concentration of total lipids (determined gravimetrically) in tissues of plaice (*Pleuronectes platessa*) from reference stations and from two estuaries contaminated by *Amoco Cadiz* oil. Values are in µg/g dry tissue.

Station	Tissue	August 1979	February 1980	June 1980
Reference	Muscle	NS	2,310	1,870
	Liver	NS	11,300	8,770
Aber Benoit	Muscle	1,921	2,170	1,740
	Liver	16,729	12,700	11,200
Aber Wrac'h	Muscle	3,278	1,750	1,810
	Liver	14,725	20,300	4,380

NS, no sample available.

Table 19. Hemolymph glucose concentration in oysters *Crassostrea gigas* from reference stations and from oil-polluted Aber Benoit and Aber Wrac'h. Values and standard deviations are in mg glucose/100 ml hemolymph. n = 8 replicates.

Station	Sampling Date Dec 1978(9)[a]	 April 1979(13)	 Aug 1979(17)
Reference	5.12 ± 3.1	13.05 ± 2.9	23.53 ± 4.0
Aber Benoit	3.00 ± 1.8	12.57 ± 2.4	NS
Aber Wrac'h	4.80 ± 2.6	11.25 ± 3.8	23.87 ± 2.8

NS, no sample analyzed

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 20. Serum glucose concentration in plaice *Pleuronectes platessa* from reference stations and from oil-polluted Aber Benoit and Aber Wrac'h. n = 10 replicates. Values and standard deviations are in mg glucose/100 ml serum.

Station	Sampling Date Dec 1978(9)[a]	 Apr 1979(13)	 Aug 1979(17)	 Feb 1980(23)	 Jun 1980(27)
Reference	158.1 ± 11.6	149.6 ± 23.5	160.4 ± 36.9	27.1 ± 19.2	37.2 ± 12.8
Aber Benoit	118.3 ± 32.9	57.0 ± 32.9*	93.7 ± 27.3*	85.9 ± 33.2*	147.3 ± 46.0*
Aber Wrac'h	NS	125.6 ± 19.5	168.4 ± 31.3	135.0 ± 28.7*	135.0 ± 55.4*

*, significantly different from reference at $\alpha = 0.05$

NS, no sample analyzed

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978.

laboratory (Wardle, 1972). In the last two samples, February 1980 and June 1980, reference plaice were sampled very rapidly after capture and their blood glucose values represent the normal unstressed values. Plaice from the Abers were stressed by capture and showed a hyperglycemic response, suggesting some recovery of physiological function with time. These data show some of the difficulties in using blood glucose concentration as an index of stress in fish. If blood samples cannot be taken immediately after the fish are captured, capture-induced responses may obscure any due to pollution.

Liver glycogen concentrations in fish from the last two collections were highly variable (Table 21). Because of extremely large standard deviations, no patterns could be discerned.

Total cholesterol and high density lipoprotein (HDL) cholesterol concentrations in the blood of plaice were measured in sampels from the last two collecting trips (Table 22). The general trend was for total cholesterol to be elevated and HDL cholesterol concentration to be depressed in fish from the two oil-contaminated Abers. Several of these differences were statistically significant. As a result, HDL cholesterol as percent of total cholesterol was lower in plaice from Aber Benoit and Aber Wrac'h than in plaice from the reference station at Ile Tudy.

Concentration of liver-free ascorbic acid was measured in plaice from all five sampling trips (Table 23). In all but the February 1980 sample, liver ascorbate concentrations in plaice from oil-contaminated Aber Benoit and Aber Wrac'h were substantially lower than concentrations in livers of plaice from reference stations. In four cases, the difference was statistically significant. In the February collection, the pattern was reversed. Reference fish contained hepatic ascorbate concentrations significantly lower than concentrations in livers of fish from the two Abers. At this time, all the reference fish were gravid females ready to spawn. Onlya few of the fish from the Abers were in this condition. It is highly likely that the extreme depletion of liver ascorbate reserves in the reference fish is the result of ascorbate mobilization for gonadal maturation and ovogenesis. These gravid reference fish also had relatively low hepatic glycogen reserves (Table 21).

Adductor muscle-free amino acid profiles and concentrations were measured in oysters from the first three collecting trips (Tables 24-26). Total free amino acid concentrations were always lower in adductor muscles of oysters from oil-contaminated Aber Benoit and Aber Wrac'h than in adductors of oysters from reference stations. This difference cannot be attributed to differences in seawater salinity between Aber and reference stations, since all stations had salinities in the 30-34 o/oo

Table 21. Concentrations of glycogen in the liver of plaice *Pleuronectes platessa* from a reference station at Ile Tudy and from oil-polluted Aber Benoit and Aber Wrac'h. Values are in mg glycogen/g wet weight.

Station	February 1980(23)[a]	June 1980(27)
Reference	4.81 ± 9.05	8.45 ± 7.03
Aber Benoit	0.48 ± 0.39	6.68 ± 7.56
Aber Wrac'h	14.37 ± 17.42	12.35 ± 12.0

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 22. Concentration of serum total cholesterol and HDL cholesterol in plaice *Pleuronectes platessa* from a reference station at Ile Tudy and from oil-polluted Aber Benoit and Aber Wrac'h. Values are in mg cholesterol/100 ml serum.

Station	Sampling Date					
	February 1980(23)[a]			June 1980(27)		
	Total Chol.	HDL Chol.	HDL Chol. % Total	Total Chol.	HDL Chol.	HDL Chol. % Total
Reference	196.9 ± 68.1	170.2 ± 57.8	86.5 ± 4.0	260.9 ± 81.2	229.5 ± 44.1	89.0 ± 11.8
Aber Benoit	244.3 ± 63.2	159.3 ± 39.4	66.2 ± 12.4	406.1 ± 101.3*	189.6 ± 23.3*	48.4 ± 9.4
Aber Wrac'h	271.7 ± 75.0*	107.3 ± 43.5*	70.7 ± 13.8	269.9 ± 53.0	165.5 ± 36.7*	62.3 ± 11.4

*, significantly different from reference at $\alpha = 0.05$. n = 10.

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 23. Concentration of ascorbic acid in the liver of plaice *Pleuronectes platessa* from reference stations and from oil-polluted Aber Benoit and Aber Wrac'h. Values are in mg ascorbate/g wet weight.

	Sampling Date				
Station	Dec 1978(9)[a]	Apr 1979(13)	Aug 1979(17)	Feb 1980(23)	Jun 1980(27)
Reference	136.6 ± 22.5	137.4 ± 16.2	131.2 ± 15.2	5.4 ± 2.3	69.7 ± 21.2
Aber Benoit	108.7 ± 20.9	80.6 ± 29.8*	88.3 ± 37.1	17.3 ± 3.5*	44.7 ± 14.8*
Aber Wrac'h	NS	93.1 ± 28.5*	64.3 ± 32.5*	25.1 ± 6.0*	50.1 ± 8.7*

*, significantly different from reference at $\alpha = 0.05$. n = 8-10.

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 24. Concentrations of free amino acids in the adductor muscle of oysters *Crassostrea gigas* from a reference station in the Rade de Brest and from oil-polluted stations in Aber Benoit and Aber Wrac'h. n = 5 unless stated otherwise.

December 1978 (nine months after spill)

Amino Acid	FAA Concentration (μM/g wet weight and standard deviation) Rade de Brest	 l'Aber Benoit	 l'Aber Wrac'h
LYS	0.65 ± 0.06	1.02 ± 0.16*	0.68 ± 0.11
HIS	0.25 ± 0.04	0.53 ± 0.09*	0.25 ± 0.03
ARG	6.71 ± 0.45	6.68 ± 0.29	5.67 ± 1.05
TAU	62.90 ± 8.61	68.70 ± 7.99	63.91 ± 3.41
ASP	3.57 ± 0.55	0.78 ± 0.06*	0.41 ± 0.12*
THR	--	--	2.83^{++}
SER	2.85 ± 1.96	3.93 ± 0.52	3.05 ± 0.16
GLU	8.81 ± 1.12	7.59 ± 1.09	5.14 ± 0.09*
PRO	28.41 ± 6.39	40.82 ± 9.97*	24.01 ± 3.01
GLY	67.69 ± 3.44	30.56 ± 7.82*	26.54 ± 2.88*
ALA	18.98 ± 1.52	13.50 ± 2.90	10.15 ± 0.49
CYS	--	--	--
VAL	0.33^{++}	--	0.19 ± 0.06^{+}
MET	0.11 ± 0.01^{+}	0.31 ± 0.21	0.18 ± 0.09^{+}
ILE	0.15 ± 0.03^{+}	0.12 ± 0.06^{+}	0.10 ± 0.03^{+}
LEU	0.20 ± 0.03^{+}	0.41 ± 0.21^{+}	0.19 ± 0.01^{+}
TYR	0.12^{++}	0.33 ± 0.18^{+}	--
PHE	--	0.22^{++}	--
NH_3	2.55 ± 0.45	2.74 ± 0.33	2.33 ± 0.19
Total FAA	201.73 ± 24.21	175.50 ± 31.52	143.30 ± 11.54

-- , not detected

+, detected in two samples

++, detected in one sample

*, significantly different from reference at α = 0.05

Table 25. Concentration of free amino acids in the adductor muscle of oysters *Crassostrea gigas* from a reference station in the Rade de Brest and from oil-polluted stations in Aber Benoit and Aber Wrac'h. n = 5 unless otherwise stated.

	April 1979 (thirteen months after spill)		
	FAA Concentration (μM/g wet weight and standard deviation)		
Amino Acid	Rade de Brest	Aber Benoit	Aber Wrac'h
LYS	0.91 ± 0.15	0.79 ± 0.15	1.04 ± 0.46
HIS	0.29 ± 0.14	0.24 ± 0.07	0.33 ± 0.10
ARG	5.06 ± 0.84	4.31 ± 0.65	4.64 ± 0.72
TAU	57.41 ± 9.77	64.39 ± 6.19	65.58 ± 3.87
ASP	1.46 ± 0.58	1.31 ± 0.58	0.92 ± 0.39
THR	--	--	--
SER	2.40 ± 0.82	3.59 ± 0.92	2.76 ± 0.46
GLU	9.89 ± 2.08	10.27 ± 1.21	10.37 ± 1.11
PRO	26.15 ± 9.53	20.76 ± 9.29	6.76 ± 5.28*
GLY	28.02 ± 4.38	25.62 ± 20.46	26.09 ± 4.38
ALA	11.59 ± 3.74	10.33 ± 2.87	10.94 ± 0.96
CYS	--	0.170^{++}	--
VAL	--	0.26 ± 0.03^{+}	--
MET	0.10 ± 0.02^{+}	0.21 ± 0.05^{+}	0.11 ± 0.06^{+}
ILE	0.15 ± 0.06^{+}	0.13 ± 0.01^{+}	0.11 ± 0.03^{+}
LEU	0.27 ± 0.11^{+}	0.26 ± 0.02^{+}	0.21 ± 0.06^{+}
TYR	0.163^{++}	0.134^{++}	--
PHE	--	--	--
NH_3	2.44 ± 0.79	1.82 ± 0.62	1.87 ± 0.44
Total FAA	146.94	143.60	129.86

--, not detected

$^{+}$, detected in two samples

$^{++}$, detected in one sample

*, significantly different from reference at $\alpha = 0.05$.

Table 26. Concentration of free amino acids in the adductor muscle of oysters *Crassostrea gigas* from a reference station at Ile Tudy and from an oil-polluted station in Aber Wrac'h. n = 5 unless otherwise stated.

August 1979 (sixteen months after spill)

Amino Acid	FAA Concentration (μM/g wet weight and standard deviation) Ile Tudy	 Aber Wrac'h
LYS	0.43 ± 0.20	0.21 ± 0.27
HIS	0.35 ± 0.17	0.28 ± 0.13
ARG	3.82 ± 0.27	6.67 ± 1.83
TAU	62.09 ± 6.54	63.32 ± 5.58
ASP	2.83 ± 0.92	0.97 ± 0.49*
THR	--	--
SER	1.59 ± 0.71	2.73 ± 1.23
GLU	8.07 ± 4.59	7.66 ± 2.13
PRO	33.46 ± 25.72	28.57 ± 9.74
GLY	41.11 ± 16.13	26.11 ± 11.52
ALA	11.92 ± 5.31	14.09 ± 3.72
CYS	--	--
VAL	--	--
MET	0.29 ± 0.02+	0.23 ± 0.13+
ILE	0.03 ± 0.03+	0.23 ± 0.30+
LEU	0.11 ± 0.07+	0.19 ± 0.003+
TYR	--	--
PHE	--	--
NH_3	2.44 ± 1.13	2.43 ± 0.56
Total FAA	166.12	151.77

--, not detected

+, detected in two samples

*, significantly different from reference at α = 0.05.

range. Dominant tissue-free amino acids in all samples were taurine (TAU), glycine (GLY), proline (PRO) and alanine (ALA). In all samples from all collections and stations, taurine concentration was maintained nearly constant (range of means, 57.4 - 68.7 μM/g wet weight). There was a trend for glycine and aspartic acid concentrations to be lower in adductors of oysters from the two oil-contaminated Abers than in adductors of reference oysters. The result was that free taurine:-glycine molar ratios (a recommended index of pollutant stress) were significantly higher in adductor muscles of oysters from Aber Benoit and Aber Wrac'h than in adductors of reference oysters in all but one instance (Table 27). Jefferies (1972) has suggested that taurine:-glycine ratios higher than about 2.0 in mollusc tissues may be a good index of stress. As indicated above, the high taurine:glycine ratios are attributed almost exclusively to a decrease in free glycine concentration. This, in turn, may be attributed to poorer nutritional status or altered patterns of amino acid metabolism in oil-stressed oysters.

Similar patterns were observed in free amino acid profiles and concentrations in skeletal muscle of plaice (Table 28-32). Total free amino acid concentrations were much lower in plaice muscle than in oyster muscle, reflecting the well-developed capability of plaice to regulate body fluid concentration hypoosmotic to the ambient seawater medium. As in oyster muscle, taurine, glycine and alanine were the dominant free amino acids in plaice muscle. Concentrations of several free amino acids were statistically significantly different in muscle of plaice from Aber Benoit and/or Aber Wrac'h than in muscle of reference plaice. However, there was no consistent pattern of change. Free glycine concentration was lower in muscle of plaice from the Abers than in muscle of plaice from reference stations in December 1978 and August 1979. In February and June 1980, free taurine concentration in muscle of Aber Wrac'h plaice was lower than in muscle of reference fish. In February 1980, it was higher. Despite these as yet unexplained variations, in seven out of nine cases where comparative data were available, mean free taurine;glycine molar ratios in muscle of plaice from Aber Benoit and Aber Wrac'h were statistically significantly different from ratios in muscle of reference fish (Table 33). Because of seasonal variations in free taurine:glycine ratios in muscle tissue of oysters and plaice, it is important when using this parameter as an index of stress to compare values for pollutant-impacted and reference animals collected at the same time from nearby locations.

Several biochemical parameters were evaluated as potential indices of pollutant stress in oysters and plaice from oil-contaminated Aber Benoit and Aber Wrac'h. Values of some of these parameters were statistically significantly different in populations from the

Table 27 . Mean free taurine:glycine molar ratios in adductor muscle of oysters *Crassostrea gigas* from reference stations (Rade de Brest or Ile Tudy) and from oil-contaminated estuaries (Aber Benoit and Aber Wrac'h). Seven replicate samples from each station were analyzed.

	Sampling Date		
Station	Dec 1978(9)[a]	April 1979(13)	July 1979(16)
Reference	0.93	2.05	1.51
Aber Benoit	2.25*	2.51	NS
Aber Wrac'h	2.41*	2.51*	2.42*

*, significantly different from reference sample at $\alpha = 0.05$.

NS, no sample analyzed.

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978.

Table 28 Concentration of free amino acids in skeletal muscle plaice *Pleuronectes platessa* from a reference station in Baie de Douarnenez and from oil-polluted Aber Benoit. n = 5 unless stated otherwise.

	December 1978 (nine months after spill)	
	FAA Concentration (μM/g wet weight and standard deviation)	
Amino Acid	Baie de Douarnenez	Aber Benoit
LYS	0.28 ± 0.10	1.47 ± 0.76*
HIS	0.40 ± 0.04	0.53 ± 0.14
ARG	0.37 ± 0.09	0.26 ± 0.08
TAU	11.33 ± 2.68	11.23 ± 3.06
ASP	--	--
THR	0.84 ± 0.19	0.66 ± 3.06
SER	0.92 ± 0.68	0.71 ± 0.18
GLU	0.35 ± 0.13	0.15 ± 0.06^{+}
PRO	0.27 ± 0.11	0.48 ± 0.18
GLY	11.86 ± 4.81	5.58 ± 1.48*
ALA	2.91 ± 0.99	1.34 ± 0.09*
CYS	--	--
VAL	0.12 ± 0.01^{+}	0.23 ± 0.17^{+}
MET	0.07 ± 0.01^{+}	0.07 ± 0.02
ILE	0.06 ± 0.01^{+}	0.11 ± 0.07
LEU	0.11 ± 0.01^{+}	0.13 ± 0.08
TYR	--	--
PHE	--	--
NH_3	6.36 ± 0.39	6.29 ± 0.34
Total FAA	29.89 ± 9.86	22.95 ± 6.58

--, Not detected

+, two samples

*, significantly different from reference at $\alpha = 0.05$.

Table 29 . Concentration of free amino acids in skeletal muscle of plaice *Pleuronectes platessa* from a reference station at Loc Tudy and from oil-polluted Aber Benoit and Aber Wrac'h. n = 5 unless otherwise stated.

	April 1979 (thirteen months after spill)		
	FAA Concentration (μM/g wet weight and standard deviation)		
Amino Acid	Loc Tudy	Aber Benoit	Aber Wrac'h
LYS	0.47 ± 0.23	0.88 ± 0.21*	0.18 ± 0.11
HIS	0.92 ± 0.31	0.99 ± 0.21	0.56 ± 0.35
ARG	--	0.21 ± 0.12[+]	--
TAU	14.67 ± 3.19	11.41 ± 1.38	8.94 ± 2.43*
ASP	0.13 ± 0.06	0.05 ± 0.03	0.06 ± 0.02
THR	0.89 ± 0.31	1.21 ± 0.58	0.59 ± 0.27
SER	0.77 ± 0.19	0.97 ± 0.09	0.66 ± 0.16
GLU	0.29 ± 0.14	0.29 ± 0.07	0.30 ± 0.05
PRO	0.25 ± 0.03	0.87 ± 0.24*	0.56 ± 0.31
GLY	7.38 ± 0.47	9.81 ± 1.50	8.86 ± 3.01
ALA	1.77 ± 0.28	1.28 ± 0.18	1.15 ± 0.24*
CYS	--	0.12[++]	--
VAL	--	0.11 ± 0.01[+]	0.12 ± 0.01[+]
MET	0.49 ± 0.01[+]	0.06 ± 0.02[+]	0.06 ± 0.02[+]
ILE	0.09 ± 0.03[+]	0.04 ± 0.03[+]	0.07 ± 0.01[+]
LEU	0.08 ± 0.03[+]	0.11 ± 0.02[+]	0.06 ± 0.05[+]
TYR	--	--	--
PHE	--	--	--
NH_3	4.69 ± 0.74	5.07 ± 0.61	5.30 ± 1.24
Total FAA	28.21	28.41	22.18

--, not detected

+, detected in two samples

++, detected in one sample

*, significantly different from reference at $\alpha = 0.05$

Table 30. Concentration of free amino acids in skeletal muscle of plaice *Pleuronectes platessa* from a reference station at Ile Tudy and from oil-polluted Aber Benoit and Aber Wrac'h. n = 5 unless otherwise stated.

	August 1979 (seventeen months after spill)		
	FAA Concentration (µM/g wet weight and standard deviation)		
Amino Acid	Ile Tudy	Aber Benoit	Aber Wrac'h
LYS	0.96 ± 0.65	0.64 ± 0.33	1.40 ± 0.70
HIS	0.97 ± 0.66	0.66 ± 0.12	1.50 ± 0.21
ARG	0.17 ± 0.02^{+}	--	0.21 ± 0.01^{+}
TAU	9.28 ± 1.22	10.08 ± 1.32	8.01 ± 1.16
ASP	0.03 ± 0.01^{+}	0.05 ± 0.03^{+}	0.06 ± 0.05
THR	0.36 ± 0.15	0.48 ± 0.07	0.65 ± 0.24
SER	0.27 ± 0.22	0.41 ± 0.22	0.47 ± 0.25
GLU	0.10 ± 0.01	0.18 ± 0.05^{+}	0.17 ± 0.11
PRO	0.50 ± 0.03^{+}	0.74 ± 0.20^{+}	1.78 ± 1.41
GLY	9.96 ± 3.40	6.57 ± 3.14	3.70 ± 1.56*
ALA	1.67 ± 0.81	0.86 ± 0.26	1.27 ± 0.29
CYS	--	0.18^{+}	--
VAL	--	--	0.16 ± 0.15^{+}
MET	0.03 ± 0.04^{+}	0.39 ± 0.53^{+}	0.17 ± 0.09^{+}
ILE	0.41 ± 0.03^{+}	0.07 ± 0.03^{+}	0.11 ± 0.09^{+}
LEU	0.04 ± 0.001^{+}	0.10 ± 0.08^{+}	0.16 ± 0.11^{+}
TYR	--	--	--
PHE	--	--	--
NH$_3$	5.65 ± 0.58	5.38 ± 2.19	6.34 ± 1.27
Total FAA	24.40	21.39	20.29

--, Not detected

$^{+}$; detected in two samples

$^{++}$, detected in one sample

*, significantly different from reference at $\alpha = 0.05$.

Table 31. Concentration of free amino acids in skeletal muscle of plaice *Pleuronectes platessa* from a reference station at Ile Tudy and from oil-polluted Aber Benoit and Aber Wrac'h. n = 8 to 10 unless otherwise stated.

	February 1980 (23 months after spill)		
	FAA Concentration (µM/g wet weight and standard deviation)		
Amino Acid	Ile Tudy	Aber Benoit	Aber Wrac'h
LYS	0.57 ± 0.40	0.95 ± 0.58	0.55 ± 0.42
HIS	--	0.50 ± 0.27(7)[1]	0.70 ± 0.39
ARG	--	--	--
TAU	7.55 ± 2.90	12.57 ± 3.38*	12.36 ± 2.33*
ASP	0.20 ± 0.15(8)	--	--
THR	0.33 ± 0.11	0.63 ± 0.20*	0.78 ± 0.42*
SER	0.70 ± 0.52	0.93 ± 0.50	0.79 ± 0.36
GLU	0.57 ± 0.20	0.24 ± 0.13*	0.27 ± 0.10*
PRO	--	--	1.51 ± 0.72(3)
GLY	7.42 ± 2.64	15.49 ± 7.96*	18.61 ± 5.32*
ALA	3.70 ± 0.74	1.63 ± 0.59*	1.53 ± 0.40*
CYS	--	--	--
VAL	--	--	--
MET	0.32 ± 0.08(5)	--	0.03(1)
ILE	0.21 ± 0.14(7)	--	--
LEU	0.29 ± 0.17(7)	--	0.16(1)
TYR	--	--	--
PHE	--	--	--
NH_3	NA	NA	NA
Total FAA	20.86	32.94	38.80

--, not detected

NA, not analyzed

*, significantly different from reference at α = 0.05

[1], number of samples in which amino acid was detected.

Table 32. Concentration of free amino acids in skeletal muscle of plaice *Pleuronectes platessa* from a reference station at Ile Tudy and from oil-polluted Aber Benoit and Aber Wrac'h. n = 8 to 10 unless otherwise stated.

June 1980 (twenty-seven months after spill)			
	FAA Concentration (µM/g wet weight and standard deviation)		
Amino Acid	Ile Tudy	Aber Benoit	Aber Wrac'h
LYS	0.34 ± 0.30	0.63 ± 0.52	1.05 ± 0.32*
HIS	0.29 ± 0.13	1.39 ± 0.63*	1.87 ± 0.46*
ARG	--	--	--
TAU	16.92 ± 3.96	12.37 ± 3.63	11.81 ± 2.30*
ASP	0.13 ± 0.14(7)[1]	0.08 ± 0.03	0.06 ± 0.02
THR	0.33 ± 0.17	0.87 ± 0.37	0.77 ± 0.35
SER	0.80 ± 0.32	0.59 ± 0.38	0.63 ± 0.37
GLU	0.26 ± 0.19	0.25 ± 0.08	0.19 ± 0.06
PRO	0.24 ± 0.28	1.73 ± 2.34(9)	2.30 ± 2.33(9)
GLY	2.50 ± 2.08	14.65 ± 6.95*	8.77 ± 4.52*
ALA	2.26 ± 0.60	1.28 ± 0.43*	1.07 ± 0.32*
CYS	--	--	--
VAL	0.34 ± 0.37(3)	0.15 ± 0.02(6)	0.16 ± 0.02(6)
MET	0.15 ± 0.08	0.12 ± 0.02(9)	0.14 ± 0.06(9)
ILE	0.12 ± 0.18	0.08 ± 0.04	0.09 ± 0.02(8)
LEU	0.16 ± 0.25	0.16 ± 0.05	0.16 ± 0.03(7)
TYR	--	--	--
PHE	--	--	--
NH_3	NA	NA	NA
Total FAA	24.84	35.37	29.07

--, not detected

NA, not analyzed

*, significantly different from reference at $\alpha = 0.05$

[1], number of samples in which amino acid was detected.

Table 33. Mean free taurine: glycine molar ratios in skeletal muscle of plaice *Pleuronectes platessa* from reference stations (Baie de Douarnenez, Loc Tudy, or Ile Tudy) and from oil-contaminated estuaries (Aber Benoit and Aber Wrac'h). Seven or ten replicate samples from each station were analyzed.

	Sampling Date				
Station	Dec 1978(9)[a]	Apr 1979(13)	Aug 1979(17)	Feb 1980(23)	Jun 1980(27)
Reference	0.96	1.99	0.93	0.66	1.35
Aber Benoit	2.10*	1.16*	1.53*	0.81	0.84
Aber Wrac'h	NS	1.01*	2.16*	1.02*	6.77*

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*, significantly different from reference sample at $\alpha = 0.05$.

NS, no sample analyzed.

[a], months after the *Amoco Cadiz* oil spill, 16 March 1978.

oil-polluted Abers and from nearby reference stations. These may be useful indices of pollutant stress. They include blood cholesterol/HDL cholesterol, liver ascorbic acid, and skeletal muscle-free amino acid ratios in fish; and adductor muscle-free amino acid ratios in oysters. Blood glucose also has potential as an index of stress in fish, if the fish can be captured and blood samples taken very quickly. Alternatively, useful information can be obtained if degree and duration of capture-induced stress can be standardized for reference and experimental fish. In such a case, the index of chronic pollutant stress is hypoglycemia, reflecting a loss or diminuation of the capacity of the hypophyseal-interrenal system to respond to stress.

Several of the alterations in biochemical parameters in oil-polluted fish and oysters are indicative or symptomatic of poor nutritional status (e.g., depressed muscle glycine, depletion of liver glycogen and ascorbate, etc.). This may be related to histopathological lesions, reported by Haensly and Neff in this publication in the gut and liver of plaice from the oil-contamianted Abers.

One difficulty in using biochemical and histopathological parameters as indices of pollutant stress is that one is not always certain that animals from the impacted and reference sites are from the same population and therefore can be compared biochemically and histopathologically. The only way to establish convincingly that differences observed in indicator parameters in reference and impacted populations are due solely or primarily to the pollution incident under investigation, is to have comparative data collected before the pollution incident. This usually is not available. The oysters used in this investigation are a recently introduced species *Crassostrea gigas* and are from a common breeding stock throughout Brittany. Genetic differences between reference oysters and oysters from the Abers are therefore extremely unlikely. However, the extent to which plaice from the west and south coast of Brittany (reference sites) mix and interbreed with plaice from the northwest coast (site of the Abers) is not known. Some intermixing undoubtedly occurs. It seems likely, therefore, that many of the differences we have reported between oysters and plaice from the Abers and those from reference stations are attributable directly or indirectly to impacts of the *Amoco Cadiz* oil spill.

There has been substantial improvement in condition of oysters and plaice in the Abers during the timecourse of this investigation (up to 27 months after the spill). Recovery is still not complete, however.

A most interesting observation from our investigations is that oysters, which were heavily contamianted by the oil spill and remained so for the duration of the investigation, showed little evidence of histopathological or biochemical damage, whereas plaice from the Abers, although not heavily contaminated with oil, showed evidence of serious and progressive histopathological and biochemical damage. This may be due to differences in the sensitivity of molluscs and fish to petrolum. However, an alternative hypothesis is that the metabolites of petroleum hydrocarbons, particularly of the polycyclic aromatic hydrocarbons, are much more toxic than the unmetabolized parent compounds and cause much of the damage in a chronic pollution situation. It is well-established that the phenolic, epoxide and diol metabolites of polycyclic aromatic hydrocarbons are much more elctrophilic and biologically reactive than the unoxygenated parent compounds (Neff, 1979). Since oysters have little or no capability to oxygenate polycyclic aromatic hydrocarbons to reactive metabolites, they are quite tolerant to oil. Fish on the other hand have a highly active mixed-function oxygenase system and so rapidly convert polycyclic aromatics to reactive metabolites which cause tissue damage. This hypothesis warrents further investigation.

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RETABLISSEMENT NATUREL D'UNE VEGETATION DE MARAIS MARITIMES ALTEREE PAR LES HYDROCARBURES DE L'AMOCO-CADIZ:MODALITES ET TENDANCES

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RESUME

Le rétablissement de la végétation des marais de l'Ile Grande partiellement détruite par les hydrocarbures est significativement engagé et ce depuis 1980. Les modalités et la chronologie du rétablissement sont fonction de la dominance relative, en chaque point, de deux processus : regénération *in situ* d'individus pérennes, germination de graines et semences produites sur place ou dans le voisinage. La colonisation est surtout le fait d'espèces annuelles, alors que la germination des espèces pérennes est très peu fréquente, sauf dans les zones abritées à substrat meuble et propre. Elle est toutefois ralentie ou nulle dans les secteurs exposés aux effets directs de la marée et/ou piétinés intensément lors du nettoiement de 1978. Celà justifie les efforts de restauration volontaire, au moyen de plantations, tentés dans de tels sites et dont un des intérêts est d'accélérer les phénomènes de dépôt des sédiments et des semences.

D'autre part, des espèces initialement "résistantes" présentent actuellement une sensibilité marquée à la pollution endogée toujours active qui se traduit par le déclin et, à terme, par la disparition sur de larges espaces de populations entières (*cf. Juncus maritimus* Lam.).

Ainsi, ces processus, agissant simultanément ou successivement conduisent-ils à des séquences de rétablissement variées, à des stades transitoires (?) marqués par une redistribution spatiale des espèces qui s'écarte notablement de la distribution antérieure.

ABSTRACT

Recovery of Ile Grande salt-marsh vegetation, partially destroyed by hydrocarbons has been significantly started up since 1980. Ways and timing of recovery are due to the relative dominance, in each point, of two processes viz. *in situ* regeneration of perennial individuals, germination of seeds producted near or on the site. Colonisation is mainly due to annual species while germination of perennials is a rare event, except in shades places with loose and clean substrate. However, it is impeded either in tide exposed points or in formerly heavily trampled places.

So, efforts of volontary restoration are justified in such locations ; planting acts besides by the speeding up of the aggregation of sediments and seeds close to the transplants.

In an other hand, some species, initially "resistant" show a marked sensibility to underground actual pollution and consequently, large populations may decline or even die (cf. Juncus maritimus Lam.).

Finally, these processes, acting in simultaneous or successive manners, will lead to varied recovery sequences, to transitory (?) stages characterized by a spatial species redistribution which may be quite different from the original pattern.

MOTS-CLES : Rétablissement, regénération, restauration, successions secondaire et primaire, pollution par les hydrocarbures, nettoiement, végétation de marais maritimes.

KEY WORDS : Recovery, regeneration, restoration, secondary and primary succession, hydrocarbons pollution, cleaning up, salt marsh vegetation.

INTRODUCTION

Le rétablissement d'un couvert végétal perturbé est un processus complexe qui recouvre des réalités et présente des modalités très diverses, d'autant que les causes perturbantes n'ont pas eu le même impact suivant les lieux et suivant les espèces composant le tapis végétal (Baker, 1979 ; Levasseur _et al._, 1981).

Les marais maritimes constituent un ensemble hétérogène, qui quoique fondamentalement organisé en habitats étagés, aux conditions mésologiques variées, supporte une végétation qualitativement ou dans les espaces intrazonaux, quantitativement variée. Cependant, étant donné qu'il s'agit d'environnements physiquement déterminés, surtout dans les parties moyennes et basses des marais, la diversité spécifique est faible, ce qui signifie qu'une perturbation peut avoir un effet drastique sur des communautés végétales et ceci d'autant plus qu'elles seront paucispécifiques et/ou particulièrement sensibles, de par leurs composantes, à une cause perturbatrice particulière.

Ayant dans un travail antérieur (Levasseur _et al._, _l. c._) détaillé cet aspect des choses, nous ne présenterons, dans cette communication, que quelques données relatives au rétablissement de la végétation au cours des trois années écoulées, et ceci aussi bien dans les espaces non modifiés par l'homme que dans ceux qui ont été transformés du fait des opérations de nettoiement.

Pour ce faire, nous utiliserons les documents suivants, quoique non exhaustifs des différents cas de figures rencontrés :
- cartographie chronologique d'un marais choisi pour sa diversité intrinsèque initiale, mais aussi pour la diversité des perburbations l'ayant affecté depuis mars 1978 ;
- transects permanents, régulièrement relevés depuis 1979 et destinés, au plan des populations végétales, à illustrer à la fois la chronologie des reprises, le développement végétatif ultérieur, les réorganisations spatiales interclones et la colonisation directe par les individus nouveaux.

LE RETABLISSEMENT : DEFINITION ET PROCESSUS GENERAUX

Définition

Il y a lieu de distinguer entre :

1 - le rétablissement dans un marais donné du couvert végétal, qui se traduit par une cicatrisation se déroulant non nécessairement linéairement dans le temps et non synchroniquement dans l'espace et dont la durée probable, pour être menée à son terme, est fonction de nombreux paramètres, essentiellement :

- le degré de destructuration et/ou de destruction initiales ;
- les nouvelles conditions écologiques (p. ex. la permanence, dans et sur le sol, et sous différentes formes, de quantités importantes de pétrole est un nouveau facteur de l'environnement).

Cette cicatrisation (i. e. gains en recouvrement) est indépendante des voies suivies et des moyens mis en oeuvre.

Elle peut quelquefois avoir pour conséquence la constitution d'un peuplement végétal qualitativement et/ou structuralement différent du peuplement d'origine; mais la dimension temps manque pour évaluer le degré de permanence de l'état atteint au moment du constat car, en cette matière, tous les états sont conditionnels et contractuels !

2 - le rétablissement d'une communauté végétale particulière, en qualité et en structure, dans un lieu donné. Cet état, nécessitant des références antérieures précises est beaucoup plus difficile à évaluer que le premier cité, immédiatement appréhendable car il s'agit du degré de recouvrement par la végétation, au temps t, d'un espace donné.

Cependant, le rétablissement après perturbation d'une végétation peut être estimé, lorsqu'il est compris comme étant la réparation naturelle des dommages subis, au moyen de constats établis à intervalles réguliers.

Processus généraux.

Le rétablissement est à la fois un processus et un résultat, lorsque l'on considère qu'il est mené à terme. De ce point de vue il est largement engagé en de nombreux sites et même localement achevé. Mais sous ce phénomène, en dépit des expressions spatiales et des chronologies si diverses actuellement, se retrouvent les mêmes mécanismes. Ceux-ci sont fondamentalement au nombre de deux auxquels il faudrait ajouter les actions volontaires de restauration par plantations :

Successions secondaire d'une part, primaire d'autre part. En fait, la distinction dans un lieu donné de ces deux processus est loin d'être nette car fréquemment ils agissent synchroniquement et non séquentiellement et de plus ils inter-et rétroagissent quelquefois continuement.

La réparation naturelle des destructions peut se faire soit dans un premier temps à partir de la régénération in situ d'éléments vivaces ayant survécu, que ceux-ci soient situés à l'intérieur de la zone atteinte ou périphériquement, soit, dans un second temps, par des implantations nouvelles à partir de migrules provenant d'individus ou de clones situés en dehors de la zone intéressée, ou d'individus ou de portions de clones autochtones ayant pu poursuivre ou retrouver un cycle phénologique normal ou ayant retrouvé cette capacité après un délai plus ou moins long de survivance en vie ralentie. Dans ce cas, il s'agit alors d'une colonisation interstitielle et/ou séquentielle puisque commandée spatialement par l'ordre de réapparition, la localisation, le nombre et la nature des individus vivaces ayant survécu, mais aussi par les conditions écologiques règnant dans le lieu.

Ainsi, un processus de régénération qui à notre sens se rapporte d'abord aux espèces pérennes implique la poursuite normale du cycle végétatif d'individus épargnés et la reprise de développement épigé d'individus survivants du fait de dispositions morphologiques particulières ou de conditions d'habitats plus favorables.

Nous distinguerons alors les phases suivantes :

1 - Après une période plus ou moins longue de vie ralentie, reprise du cycle phénologique normal

2 - Extension végétative éventuellement centrifuge consécutive ou concomittante de la première phase et/ou formation de graines et semences viables

3 - Poursuite du processus si les conditions mésologiques restent adéquates et si les conditions d'intéractions coenotiques (concurrence) le permettent.

Le second processus de colonisation (*i. e.* succession primaire *s. l.*) implique :

1 - La formation de graines et semences dans, en périphérie ou à l'extérieur de la zone concernée

2 - La non-exportation pour celles produites sur place ou inversement l'accessibilité des lieux pour les autres

3 - Des conditions de germination et de développement favorables

4 - Le maintien de ces conditions auxquelles vont s'ajouter les conditions coenotiques, les unes et les autres variant avec le temps, dans l'espace, du fait du processus fondamental de rétroaction.

La colonisation peut aussi être le fait de fragments végétatifs détachés de pieds-mères, dans le lieu ou y ayant accès par le jeu des courants. (*cf.* dispersion actuelle, qui utilise ces deux modalités, de *Spartina cf. anglica*, dans le marais 2, mais aussi, à l'Ouest du pont, dans le marais 4).

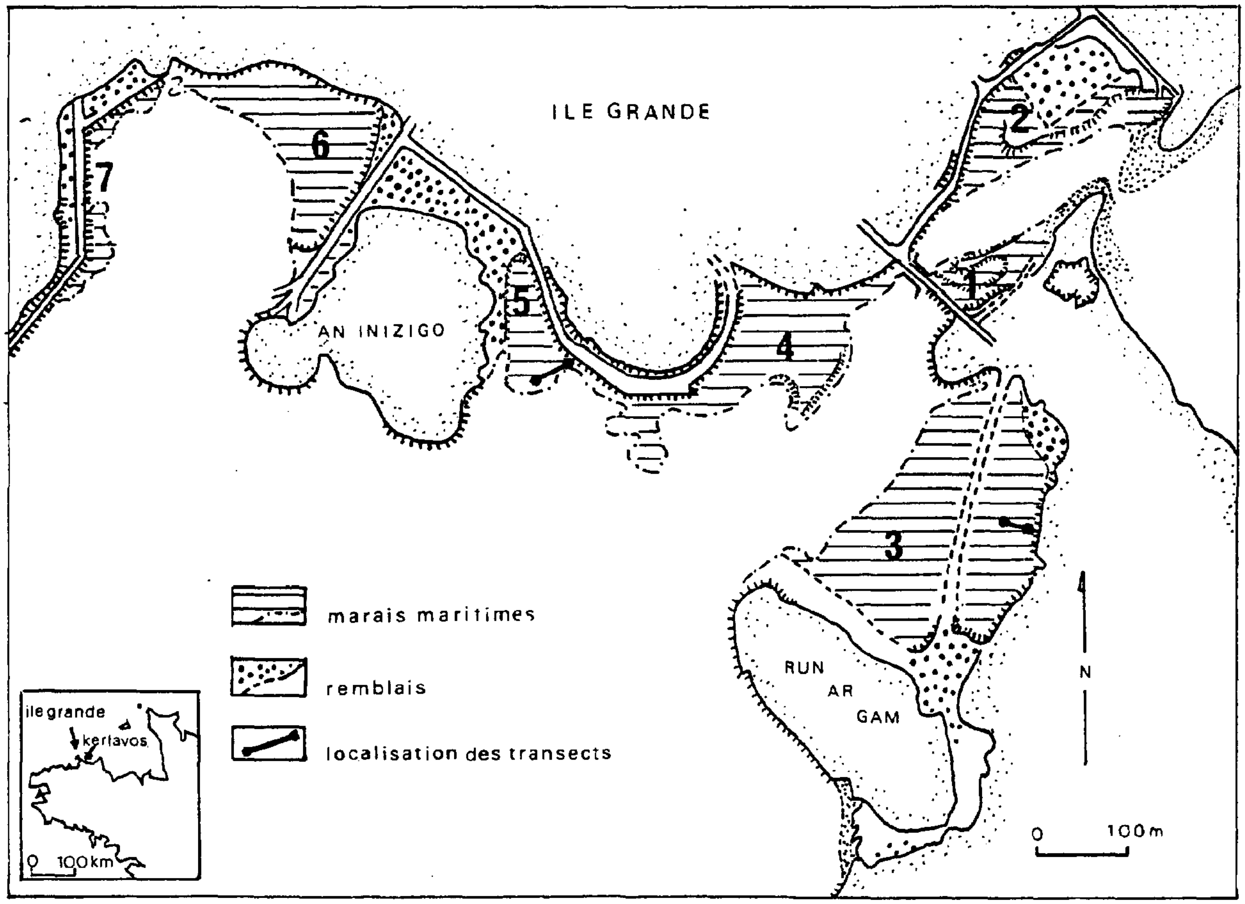


Figure 1. Carte de localisation des marais de l'Ile Grande.

INVENTAIRE SOMMAIRE DES SITUATIONS HERITEES

A la fin de 1978, les cinq situations suivantes ont été distinguées, sur la base du recouvrement ou non par les hydrocarbures, de l'intensité du piétinement ou du passage répété d'engins lors du nettoiement intervenu en 1978, des opérations connexes de ce nettoiement telles le décapage au bulldozer de la couche superficielle du sol ou l'établissement de remblais à l'emplacement des fosses de stockage du pétrole.

Groupe A : 1) zones non touchées ou touchées seulement marginalement par l'épandage d'hydrocarbures, mais qui ont pu être secondairement piétinées.

Groupe B : zones ayant été soumises à l'impact direct du pétrole.
2) zone pétrolée mais non nettoyée intensivement (i. e. sans piétinement important ayant entraîné une compaction durable des couches supérieures du sol).
3) zone pétrolée et nettoyée intensivement.

Groupe C : zones profondément modifiées par rapport à leur statut antérieur :
4) zones remblayées (mort-terrains, sédiments meubles)
5) zones étrépées au bulldozer.

Caractéristiques générales de ces zones.

Situation 1) Secteurs non atteints ou peu atteints par les hydrocarbures du fait de leur situation topographique ou des dispositions prises immédiatement après la catastrophe (cf. marais 1 et 2, à l'Est du pont).

Localisation :
Parties internes des marais ou dunes bordières

Processus en cours :
Succession secondaire de cicatrisation dans les zones piétinées.

Situation 2) Territoires pollués mais non ou peu piétinés. Dépôt initial de pétrole sur les parties aériennes des plantes et sur le sol formant ensuite sur celui-ci un revêtement cohérent qui se desquame localement avec le temps, dans les sites exposés (modalité 1). Dépôt intrasédimentaire de pétrole ; celui-ci encore actuellement sous forme semi-liquide dans les chenaux, dans la partie haute de la slikke, mais aussi en haut-schorre, dans les zones saturées en eaux douces par les sources venant du domaine terrestre et qui constituent des marécages supralittoraux saumâtres comme dans le marais 6 (modalité 2).

Processus en cours :
Très variables. Successions secondaires ayant débuté dès l'automne 1978 et qui sont caractérisées essentiellement par une régénération in situ d'espèces pérennes épargnées et survivantes. Lorsque la destruction du tapis végétal a été plus complète, il y a possibilité de colonisation directe par des éléments allochtones si les conditions s'y prêtent. Il y a ainsi possibilité d'une succession primaire interstitielle.

Situation 3) Territoires fortement piétinés et/ou soumis à des passages d'engins, notamment d'engins chenillés qui détruisent les organes endogés de pérennance.

Destruction quasi-totale de la végétation ; survivance d'un très faible pourcentage(quelquefois inférieur à 5 %) d'individus de type géophyte à rhizome et hémicryptophyte à souche.

Compaction secondaire forte avec pour conséquences le tassement du sol et la pénétration forcée du pétrole dans ses premiers centimètres. Peu de changements en trois ans de cet état, sauf en mode exposé où des délitations et desquamations se produisent, sauf encore dans les zones proximales soumises à sédimentation.

Localisation :

Cette situation se rencontre surtout dans les parties des marais les plus proches des chenaux et criques, là où était effectué le pompage du pétrole. Les communautés les plus fréquemment destructurées ou détruites sont les suivantes :

peuplement à Spartina maritima,
" à Salicornia perennis,
" à Halimione portulacoides,
" à Puccinellia maritima et Triglochin maritima,
p.p. " à Juncus maritimus du schorre moyen (végétation à Limonium vulgare et Plantago maritima.(*)

Processus en cours :

Le rétablissement de la végétation, par des voies naturelles y est très lent sinon nul, actuellement encore ; succession secondaire possible à partir des éléments épargnés, mais ceux-ci sont en quantité insuffisante pour permettre une cicatrisation rapide de ces lieux. En fait la reprise de la végétation y est quasi-nulle. Le rétablissement d'un couvert végétal ne peut être que la conséquence d'une succession primaire en quelque sorte obligatoire dans le lieu, mais qui pour de nombreux sites n'est encore que potentielle, ou bien encore d'opérations de restauration par plantations ad hoc d'espèces vivaces.

Situation 4) Remblais

Les parties de marais remblayées sont localisées à l'emplacement de fosses, ce qui explique les tassements ultérieurs observés depuis 1980, et la réintégration de certains de ces espaces dans le domaine maritime s. l. (marais 2 p. ex.)

Processus en cours :

Succession primaire obligatoire. D'ailleurs très rapidement initiée et qui, en 1981, en est déjà au stade développement végétatif horizontal d'espèces vivaces- surtout Puccinellia maritima. Il faut noter que dans ces lieux la germination d'espèces vivaces a été observée et que la colonisation s'est faite à partir de graines et semences ayant eu accès au site et ayant pu germer sur place, alors que ces germinations n'ont pas été observées dans les autres sites des marais à sédiments non meubles.

(*)Nomenclature d'après Abbayes H.des et al.(1970)

Situation 5) Territoires étrépés.

Solution la plus extrême de nettoiement puisque la roche-mère, ici limons quaternaires décalcifiés, est mise à nu. A part quelques exceptions très locales, à la fois le contingent de graines produites avant 1978, et l'ensemble de la masse végétale épi - et endogée a été détruite sur de vastes espaces comme par exemple dans l'estuaire de Kerlavos, situé à quelques kilomètres à l'Est de l'Ile Grande. Ailleurs (marais 1, 3, 4, 5, 6, 7) c'est essentiellement la partie proximale des marais qui a été amputée de la sorte.

Processus en cours :

Initiation d'une nouvelle pédogénèse.

Le rétablissement d'un couvert végétal ne peut provenir que d'une succession primaire obligatoire effectivement engagée après une période de latence de 1 an *via* l'installation de populations thérophytiques essentiellement constituées de différentes espèces annuelles du genre *Salicornia*.

CARTOGRAPHIE CHRONOLOGIQUE D'UN MARAIS PRIS COMME EXEMPLE : le MARAIS NORD D'AN INIZIGO.

Les caractères particuliers de ce marais dont la localisation est précisée sur la figure 1, sous le numéro 6, et qui nous l'ont fait choisir comme exemple réside dans la variété des habitats contigüs, particulièrement l'existence de prairies saumâtres supra-littorales dans sa partie N, dominées soit par des roselières à *Scirpus tabernaemontani* et *Scirpus maritimus* soit par des prairies à *Juncus maritimus*, espèce quasi-exclusive sur de grands espaces.

Morphologiquement, ce marais se relève périphériquement, le réseau hydrographique convergeant en son centre se résoud en deux chenaux majeurs orientés Est-Ouest. Sa partie Ouest a été partiellement étrépée tandis que sa partie Sud a fortement été piétinée, le sous-ensemble Nord étant de ce point de vue, peu touché.

La figure 2 renseigne schématiquement sur la distribution spatiale des agressions qu'il a subi en 1978 : absence de pollution, pollution sans nettoyage, pollution puis nettoyage, étrépage proximal à l'Ouest. La partie Nord-Est a été remblayée à différents moments depuis 1978.

Ainsi, les cinq situations précitées se retrouvent ici, mais les trois premières dominent.

Les figures 3, 4, 5 se rapportent à des constats établis en Août 1979, 1980 et 1981. Il ne s'agit pas à proprement parler de cartes de végétation, puisque l'aspect qualitatif n'est pas le but de ces représentations. Celui-ci en effet, est de fixer, à un moment donné, l'état de la végétation d'un double point de vue :

- progrès de la cicatrisation,
- niveau de reconstitution des communautés végétales.

Plus précisément le codage correspond :

- Pour le premier groupe (A) à des peuplements recouvrant la quasi-totalité du sol, au moment de la cartographie ; le caractère différentiel intergroupe étant celui de la diversité spécifique à ce moment là,

- Pour le second groupe (B), il s'agit de peuplements en cours de régénération ou soumis à succession primaire. La destructuration, la dénudation a pu y avoir été très forte et le recouvrement (hormis en niveau 6 pour les espèces annuelles) est toujours inférieur à 50 % de la surface.

Code des figures 3, 4 et 5.

Groupe A : recouvrement des espèces pérennes pouvant atteindre 100 %

■ Niveau 1 : Végétation plurispécifique, non touchée par les hydrocarbures ou végétation ayant recouvré, à la fois sa diversité originelle, mais aussi sa structure antérieure. Dans ce cas on parlera de rétablissement achevé.

☰ Niveau 2 : Végétation paucispécifique (n inf. à 3 espèces).

▥ Niveau 3 : Végétation monospécifique. Ce résultat peut être atteint de deux façons ; la régénération est le fait d'une seule espèce ou le fait du rétablissement complet d'un clone (cf. roselières).

Groupe B : recouvrement des espèces pérennes inférieur à 50 % ; diversité spécifique indifférente :

▨ Niveau 4 : Recouvrement compris entre 50 et 25 %

▦ Niveau 5 : Recouvrement compris entre 25 et 5 %

░ Niveau 6 : Recouvrement inférieur à 5 % pour les espèces pérennes, mais pouvant dépasser 80 % en ce qui concerne les thérophytes.
Dans ce dernier cas, il s'agit d'un recouvrement saisonnier.

☐ Niveau 7 : Recouvrement nul aussi bien pour les espèces pérennes que pour les espèces annuelles.

Il est ainsi possible, en un lieu donné, de suivre les changements de statuts, et de comparer d'un site à l'autre, l'amplitude de ces changements et leur vitesse relative, et ceci pour une même gamme d'habitats (cf. ci-après).

La figure 6, quant à elle, est plus synthétique puisqu'elle indique les écarts des états constatés en Août 1981 par rapport à ceux d'Août 1979.

Code de la figure 6 :

□ Pas de changement

░ Passage du niveau 7 au niveau 6 (*i. e.* acquisition d'un couvert phanérogamique saisonnier à thérophytes).

▥ Changement d'état correspondant à 1 niveau

▥ Changement d'état correspondant à 2 niveaux

■ Changement d'état correspondant à 3 niveaux et ce, jusqu'à la catégorie 4 incluse.

▤ Passage d'un niveau inférieur à 3 à un niveau 3, 2 ou 1

▦ Passage du niveau 3 au niveau 2, de celui-ci au niveau 1

▩ Passage du niveau 3 au niveau 1

cas particuliers :

⊞ Evolution régressive, mais qui peut correspondre à des gains spatiaux d'une espèce sociale, au détriment d'une végétation non concurrentielle.

▨ Secteurs plantés (zone de restauration de l'équipe "américaine" Pr. Seneca-Raleigh).

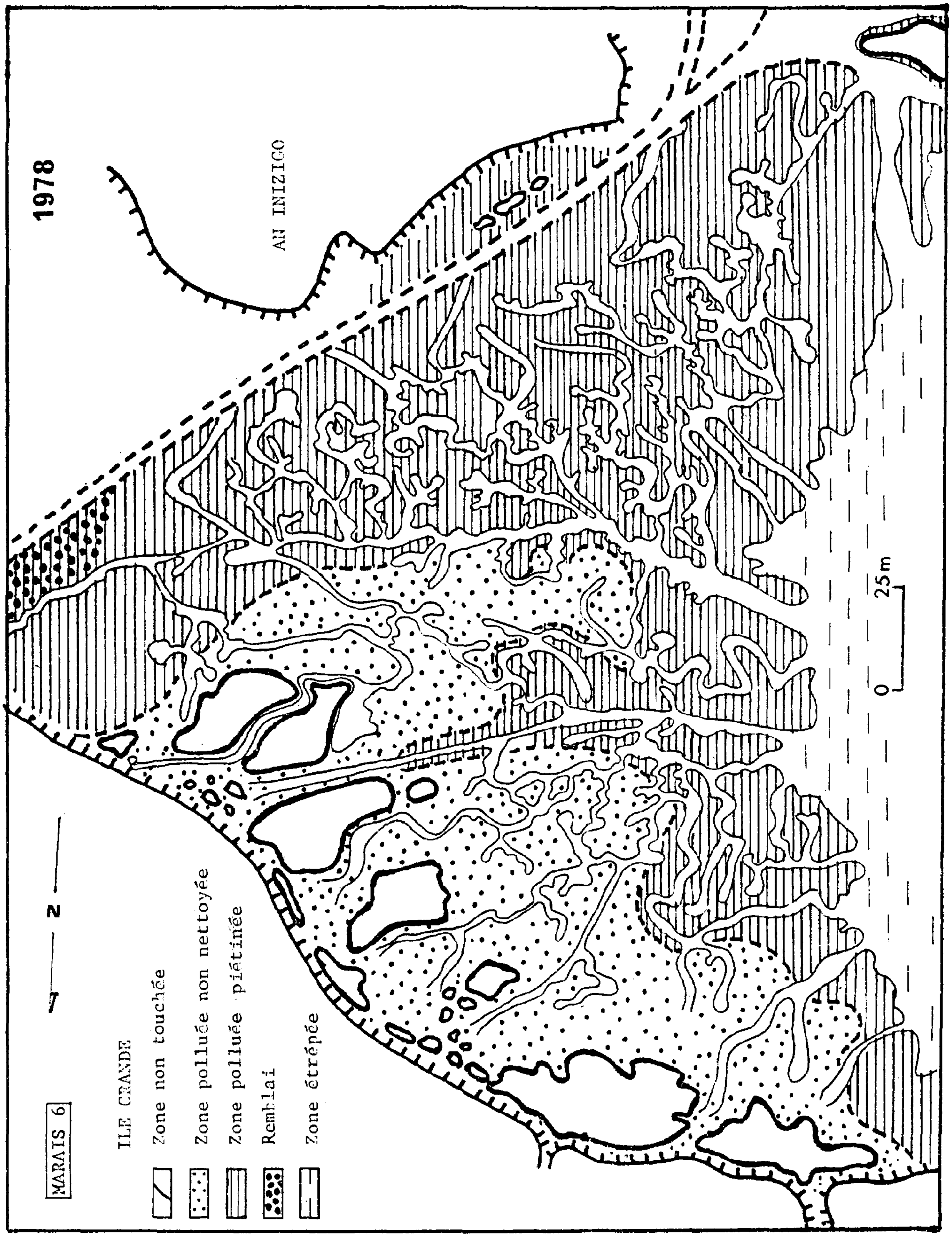


Figure 2. Marais 6 -Extension de la pollution et modalités de nettoiement.

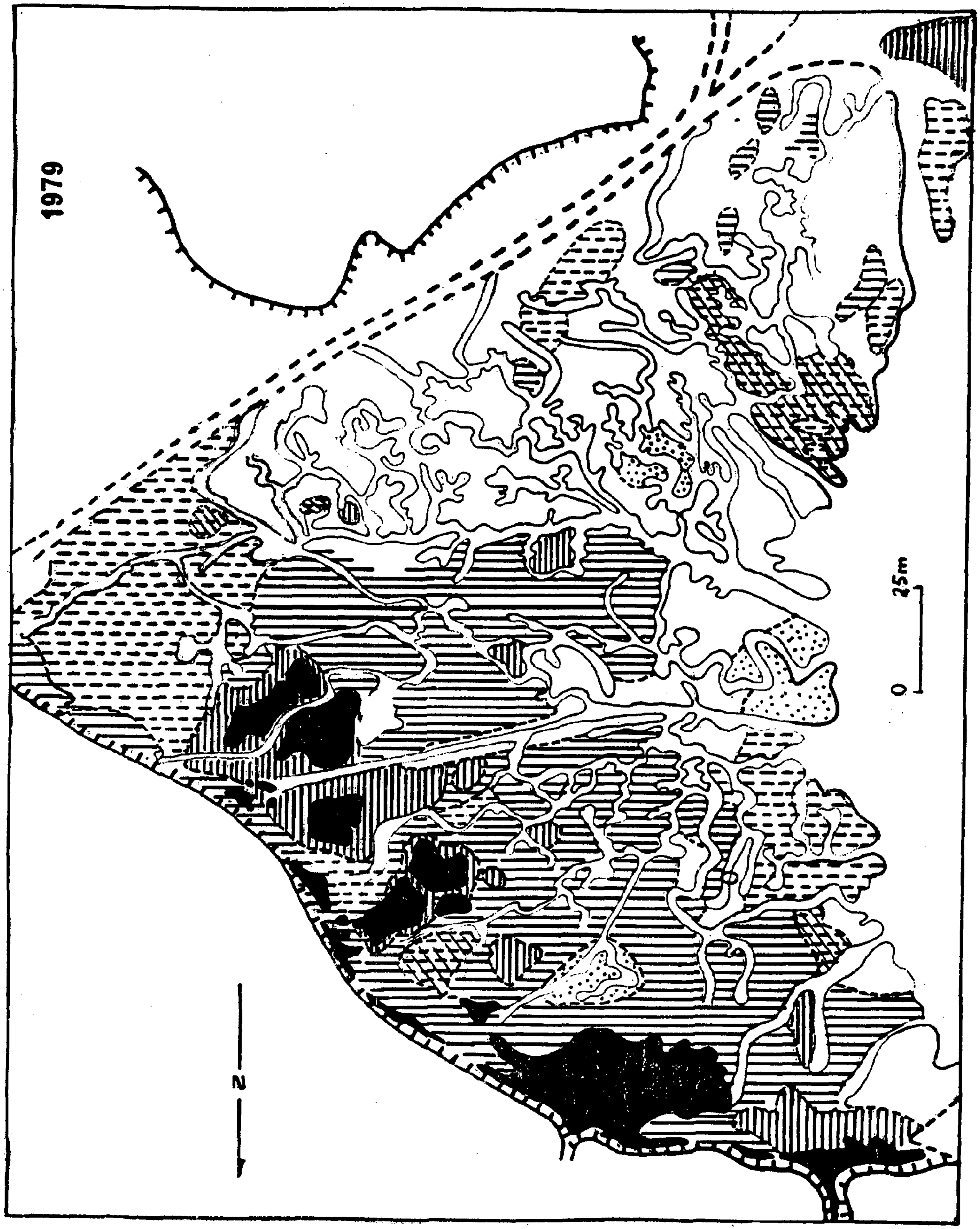


Figure 3. Marais 6-Etat en 1979 (légende dans le texte).

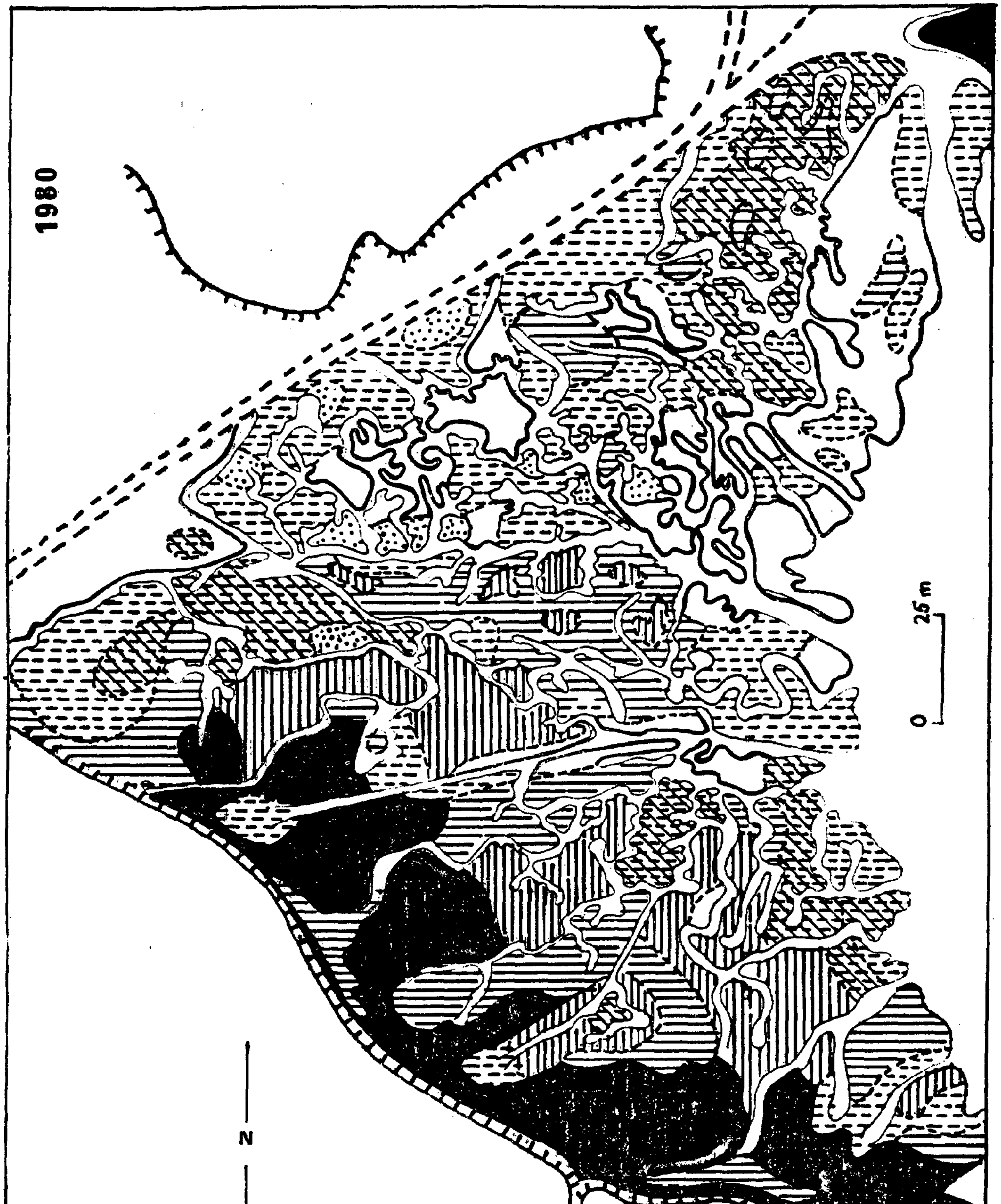


Figure 4. Marais 6-Etat en 1980 (légende dans le texte).

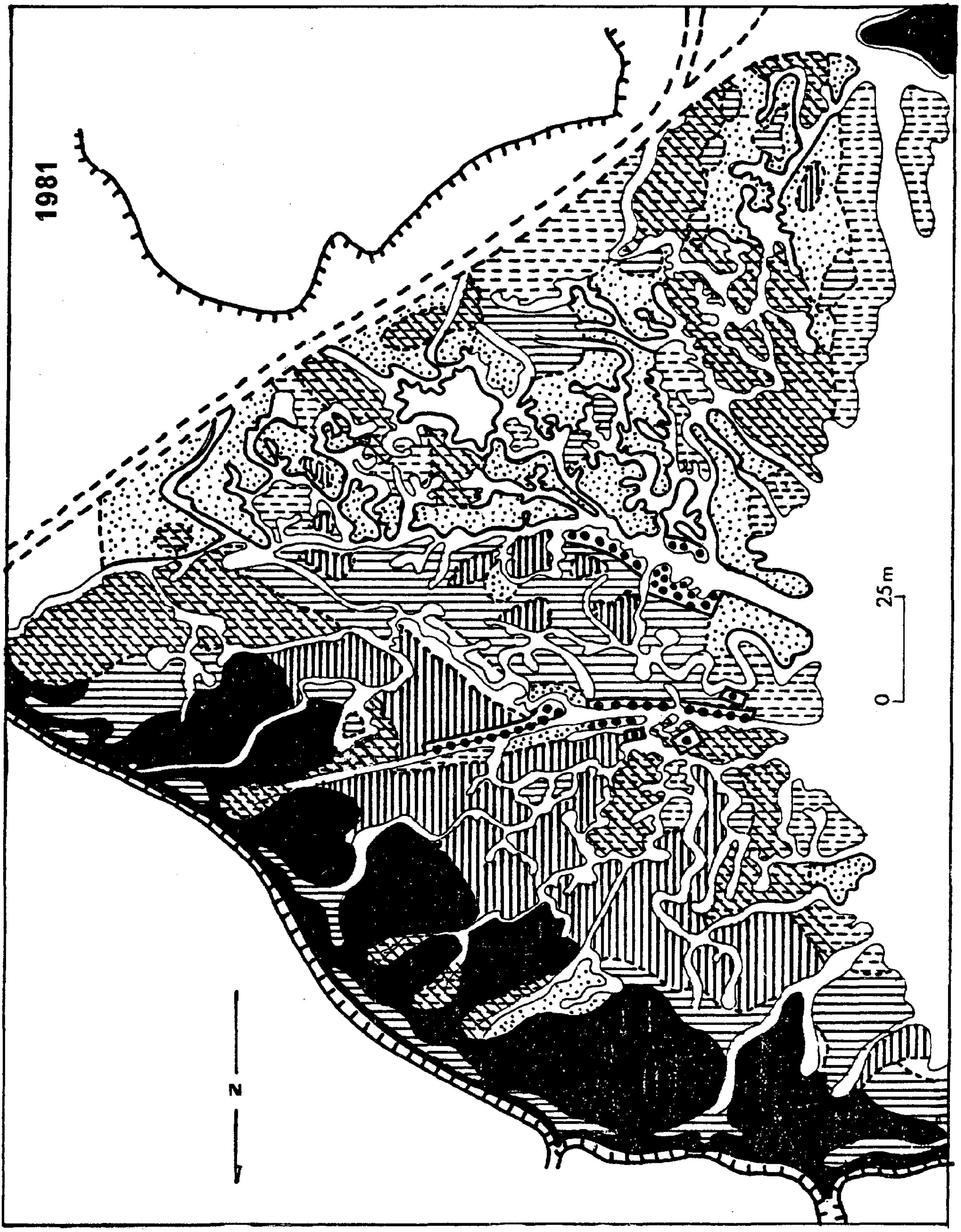


Figure 5. Marais 6-Etat en 1981 (légende dans le texte).

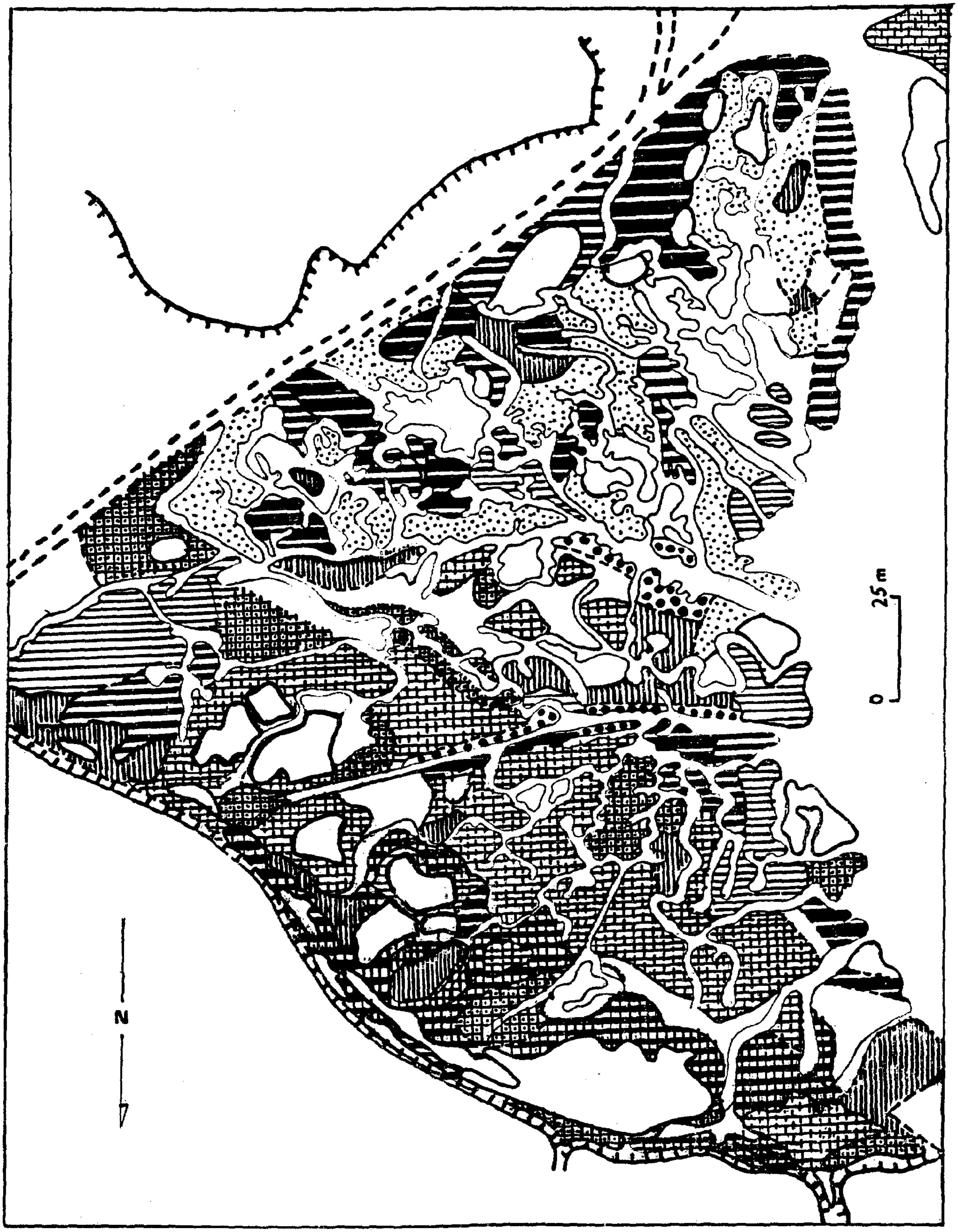


Figure 6. Marais 6- Distribution spatiale des écarts de niveaux observés entre 1979 et 1981.(légende dans le texte).

Commentaires

Mis à part un petit nombre de points dépourvus de toute végétation, y compris thérophytique, tous les autres ont vu leur couvert végétal, même fragmentaire, évoluer avec le temps. Ces transformations sont, dans la plupart des cas et selon nos conventions, progressives - i. e. tendent vers une cicatrisation des espaces dénudés. Celle-ci peut être accompagnée par une augmentation de la diversité spécifique, lorsqu'il y a eu destruction sélective d'une partie du contingent spécifique de départ, mais non dénudation extensive. Cependant des tendances régressives s'observent plus localement.

Il existe une certaine opposition entre les parties Nord et Sud du marais (gauche et droite sur les figures 2 à 6), de part et d'autre des deux chenaux majeurs médians. L'état de la végétation, en secteur Sud, ne dépasse pas le stade peuplement thérophytique, ce qui correspond, 43 mois après le dépôt de pétrole, à la destruction effective presque totale des espèces pérennes dans ces zones bordières.

On constate néanmoins (cf. figure 6) que la cicatrisation à partir de régénérations autochtones est comparativement assez rapide le long de la route bordant An Inizigo au Nord. En trois ans, l'amplitude du changement est de l'ordre de deux ou trois niveaux. Une cause possible de cette régénération plus rapide, qui intéresse surtout les hémicryptophytes à souche et les chamaephytes ligneuses mais aussi les hémicryptophytes stolonifères telles Puccinellia maritima, est à rechercher dans le ruissellement permanent ou la percolation latérale de sources provenant de l'île. C'est effectivement en tête des chenaux et sur les marges que ce processus est le plus rapide.

Lorsque l'on compare les figures 2 et 6, on remarque que ce sont les zones piétinées qui présentent le plus faible taux de reprises, exprimé par le progrès, en recouvrement, des espèces vivaces. Il faut noter toutefois que la végétation initiale, dans la haute slikke, le bas-schorre et le schorre moyen était pauci - ou monospécifique. Le piétinement, ajouté à l'effet immédiat du pétrole sur des plantes à appareil végétatif essentiellement épigé a des effets drastiques et surtout durables. Ainsi ces deux facteurs, l'un initial, l'autre consécutif, mais encore opérant actuellement, constituent-ils, en première hypothèse, la raison majeure du retard dans le rétablissement d'une végétation, du fait de leurs effets multiples, directs ou indirects.

L'exposition aux effets dynamiques de la marée joue également, aussi bien sur le plan de la reprise que sur celui de l'installation d'individus nouveaux dans les espaces très dénudés. L'exposition favorise, étant donné l'absence de couverture végétale, l'érosion des berges, ce qui conduit à des effondrements locaux qui, à terme, peuvent entraîner des modifications dans les drainages, par occultation des chenaux les plus étroits ou les moins profonds. Mais cette exposition, comme nous l'avons dit plus haut, a aussi pour conséquence une destruction accélérée de la couche cohérente de pétrole déposée sur les sédiments, même lorsque celle-ci a été tassée. Des souches survivantes ainsi libérées ont pu alors reprendre leur développement, par formation de nouvelles pousses aériennes. à partir de bourgeons adventifs.

Par contre, en ce qui concerne la colonisation de ces espaces, l'exposition aux effets directs de la mer joue comme un facteur contraire. Il n'en est pour preuve que l'installation réussie, dans des situations mésologiques homologues, des espèces annuelles, en l'absence de ce facteur (i. e. dans les zones abritées, en position plus interne).

La partie Nord du marais se différencie des autres pour deux raisons :

- sa physiographie,
- la nature de sa couverture végétale.

La topographie et l'existence de nombreuses sources qui ont assuré un auto-nettoyage précoce du pétrole déposé dans ces secteurs distaux font que le degré de destruction et de destructuration du couvert végétal y a été comparativement plus faible. Structuralement en effet, les géophytes à rhizomes dominent et les parties aériennes, qui de toutes façons, ont une durée de vie limitée, ont joué le rôle de piège à pétrole, sans que les organes endogés de pérennance n'aient été immédiatement atteints. Seule, la sous-strate des peuplements, composée d'hémicryptophytes stolonifères, a été endommagée, mais la reconstitution de ce tapis interstitiel est en cours et même localement, dans les zones de stagnation des eaux continentales, presque achevée.

Il ressort de ces observations que dans un même marais les conditions et les modalités de rétablissement de la végétation sont très variées. Si la régénération, à proprement parler, est engagée, à des degrés divers et pour des raisons et avec des moyens divers, l'installation de nouveaux individus, par colonisation directe ne s'observe pas, hormis le cas des espèces annuelles dans les lieux protégés. Aussi peut-on dire que le rétablissement est assuré par le développement végétatif des seuls individus ou clones survivants, sans qu'il y ait ajout quantitatif ultérieur significatif.

Les évolutions ultérieures sont cependant dans la majorité des cas conditionnelles et dépendantes de la résistance des populations régénérées à la pollution rémanente diffuse ou directe, de là les délais variables observés dans l'initiation du processus ! (cf. figure 6).

ANALYSE DIACHRONIQUE DE QUELQUES TRANSECTS PERMANENTS.

Parmi les transects établis en 1979 et régulièrement suivis depuis, nous en avons sélectionné trois, deux dans les marais de l'Ile Grande, le dernier dans l'estuaire de Kerlavos. La localisation des deux premiers est indiquée sur la figure 1.

- A Kerlavos, il s'agit d'un schorre moyen à *Armeria maritima* et *Plantago maritima*, étrépé par décapage des dix premiers centimètres du sol. Le substrat plan est constitué de limon. Le mode d'exposition est abrité. En utilisant le code des figures 3 à 5, le niveau de départ, en 1979 est 7.

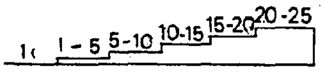
- Marais 5-Est d'An Inizigo. Transect d'orientation ENE-WSW, établi dans une zone exposée en partie aux actions dynamiques de la marée et qui fut à la fois très polluée, très piétinée et soumise également au passage d'engins. Deux petits chenaux recoupent ce transect qui se relève légèrement vers l'Est. Selon les sections de celui-ci, les niveaux de départ sont de 4 ou 5.

- Marais 3. Ce transect, situé en mode très abrité, est orienté NW-SE. Son point de départ haut est situé sur une levée artificielle non touchée par le pétrole, mais très piétinée. Il se termine dans une zone marécageuse occupée par une prairie à Juncus maritimus, encore actuellement très polluée, mais non piétinée. Les niveaux de départ vont de 1 à 4.

Ces transects illustrent ainsi les différents cas de figures rencontrés, que ce soit sur le plan de la végétation, des habitats, des destructions.

Légendes des figures 7A - 7B, 8A-8B , 8C, 9A - 9B.

Figures 7A, 8A, 9A. Les transects sont constitués de carrés contigüs de 0,50 x 0,50 m subdivisés chacun en 25 cases de 0,10 x 0,10 m. Les présences spécifiques sont relevées dans chacune des cases. L'échelle des hauteurs, dans le diagramme est établie comme ci-après :



Présence de l'espèce dans n cases

Afin de faciliter la comparaison des distributions linéaires et des recouvrements sur la ligne, les données relatives à chaque année, pour une espèce donnée, sont rapprochées.

L'ordre de présentation des espèces, dans les diagrammes est conventionnel. Il s'appuie sur les types suivants :

- plante à organe de résistance endogé. [géophyte à rhizome
  hémicryptophyte à souche
- plante sans organe de résistance endogé [chamaephyte ligneuse
  hémicryptophyte stolonifère
- thérophytes (y compris espèces biannuelles)

Figures 7B, 8B, 9B : sont indiqués:
- le nombre de cases par carré occupées par la végétation, toutes espèces confondues.
- le nombre d'espèces présentes dans chaque carré.

Figure 8C : Pour ce qui est du transect 5, nous avons présenté différemment les données, par la distinction des espèces annuelles et pérennes et leur importance, exprimée comme la somme des cases occupées par chacune des espèces. Ces sommes sont ensuite cumulées, de là les dépassements possibles dans le cas de cooccurence ou même de début de stratification. La somme des recouvrements individuels peut ainsi être supérieure aux 100 % d'un carré élémentaire.

Code espèces:Arm.mar.=Armeria maritima;Ast.trip.=Aster tripolium;Cochl.angl.=Cochlearia anglica;Halim.port.=Halimione portulacoides;Juncus mar.=Juncus maritimus;Limon.vulg.=Limonium vulgare;Paraph.strig.=parapholis strigosa;Plant.mar.=Plantago maritima;Pucc.mar.=Puccinellia maritima;Salic.div.sp. (Th)=Salicornia div.sp.(annuelles);Salic.perenn.=Salicornia perennis;Sperg. mar.=Spergularia marina;Suaeda mar.=Suaeda maritima;Trigl.mar.=Triglochin maritima.

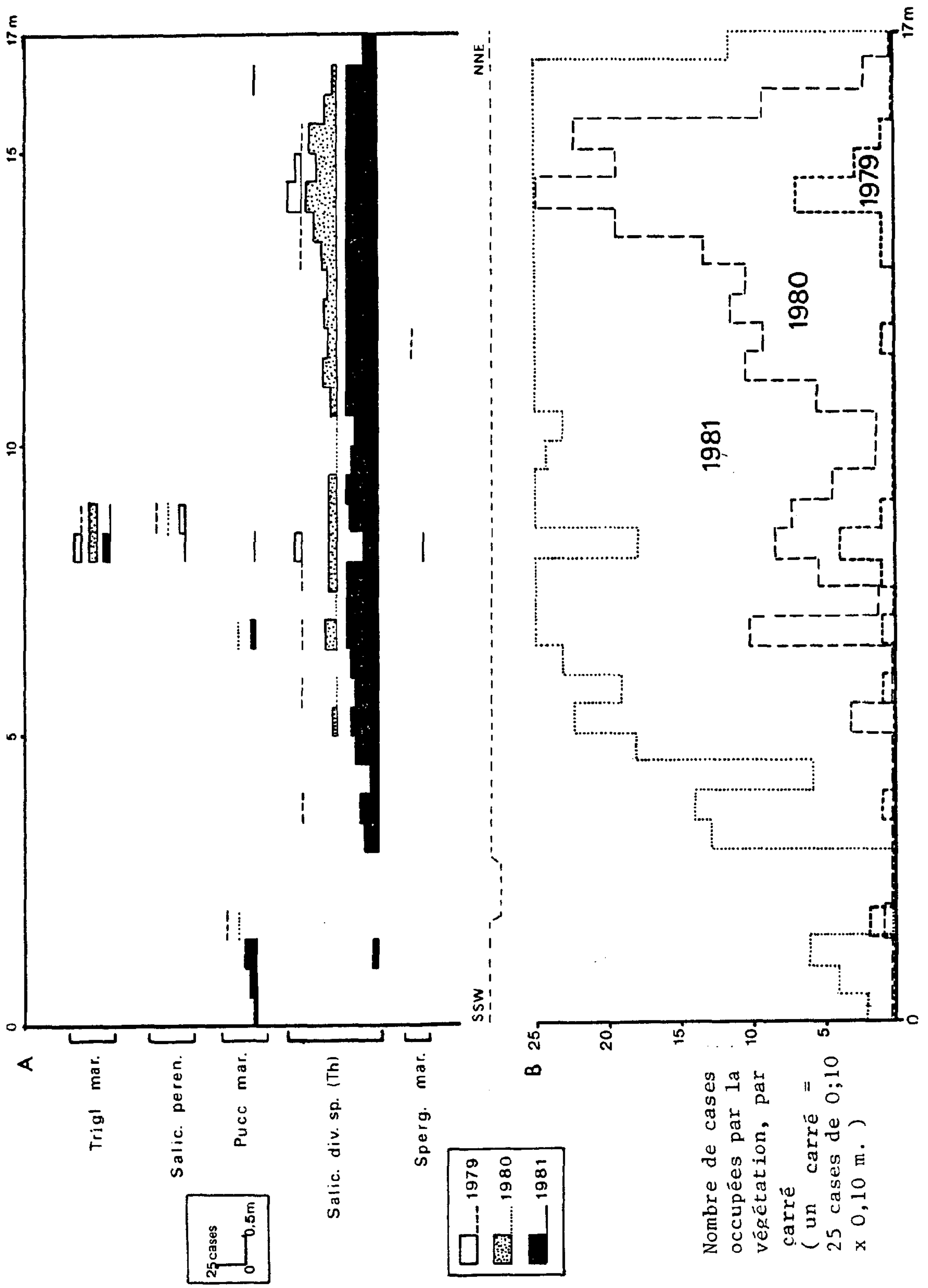


Figure 7. Transect permanent -Estuaire de Kerlavos.
A-Distribution spatiale des espèces
B-Variation longitudinale du recouvrement et de la diversité.

TRANSECT MARAIS 5

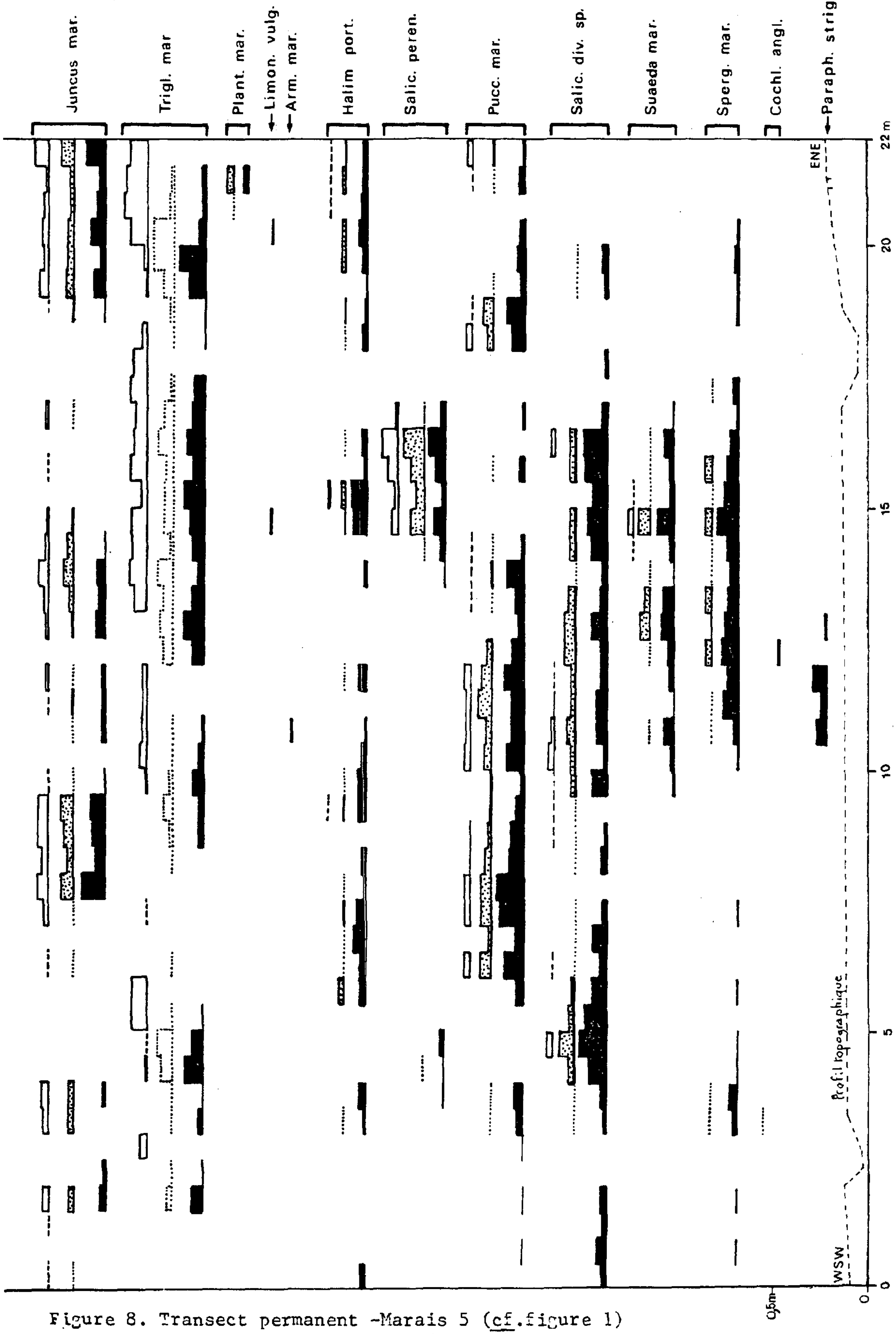


Figure 8. Transect permanent -Marais 5 (cf.figure 1)
A-Distribution spatiale des espèces.

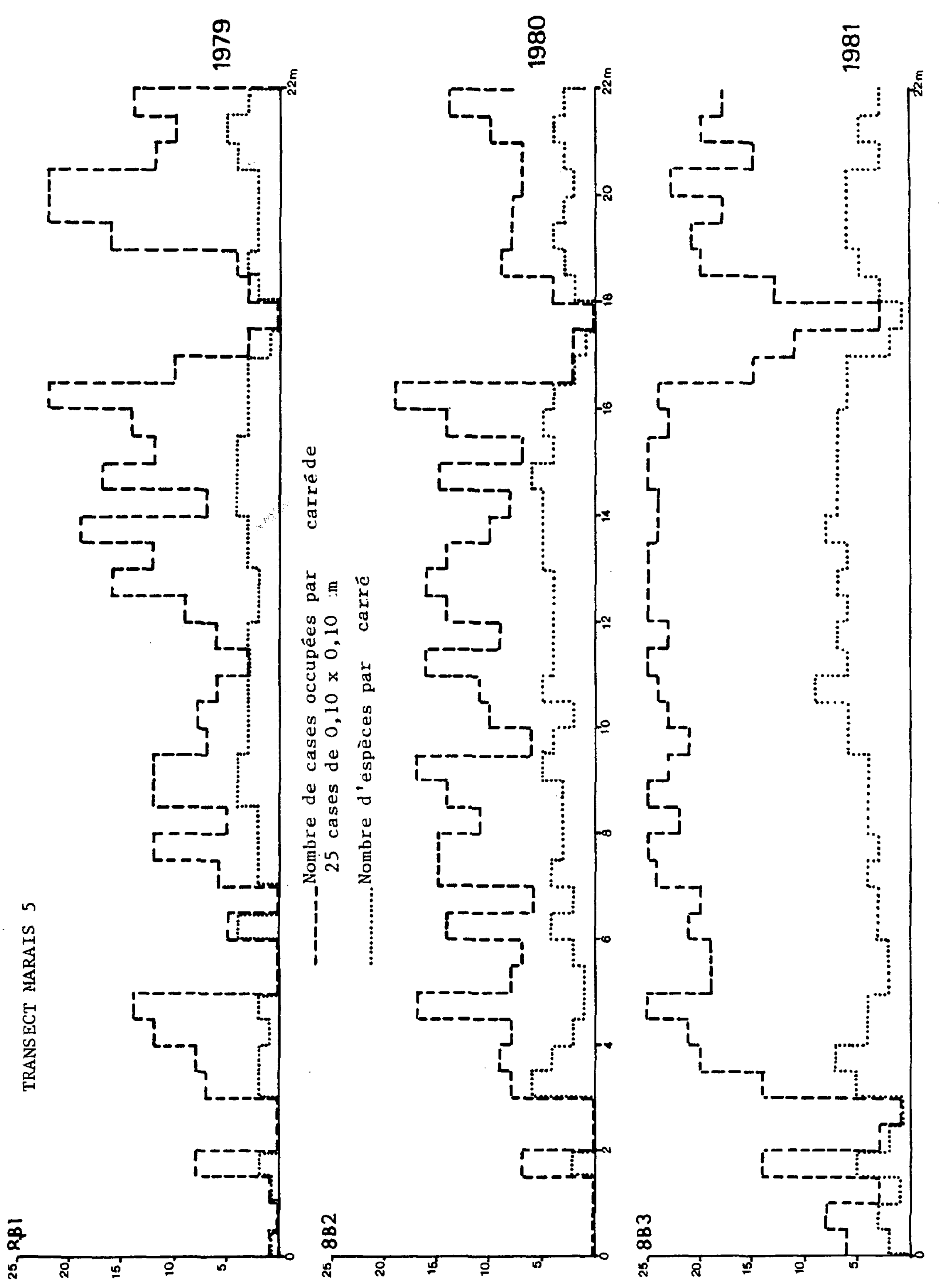


Figure 8. Transect permanent-Marais 5 -B-Variation longitudinale du recouvrement et de la diversité

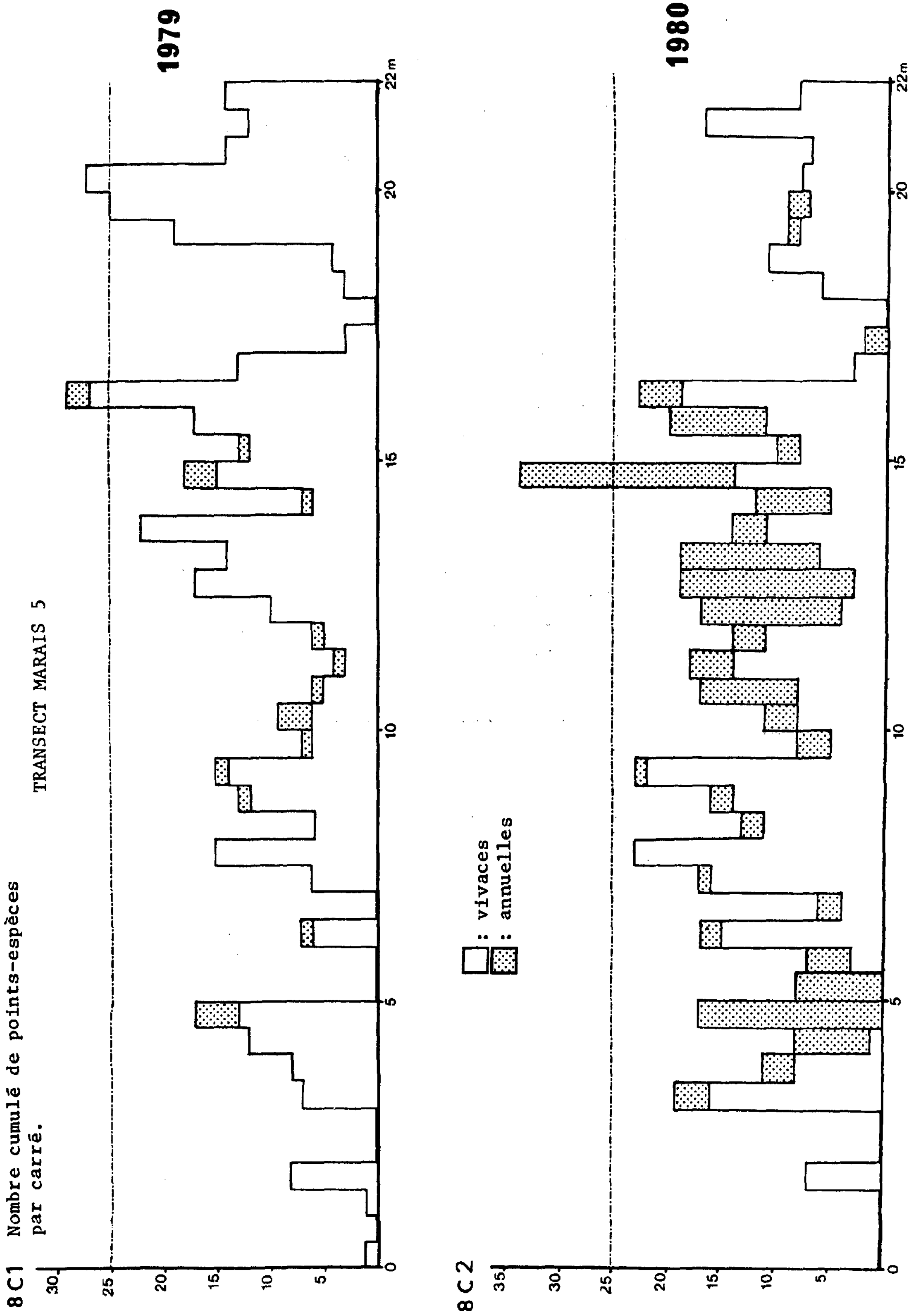


Figure 8. Transect permanent-Marais 5-C-Variation longitudinale du recouvrement des plantes annuelles et vivaces (cf.texte).

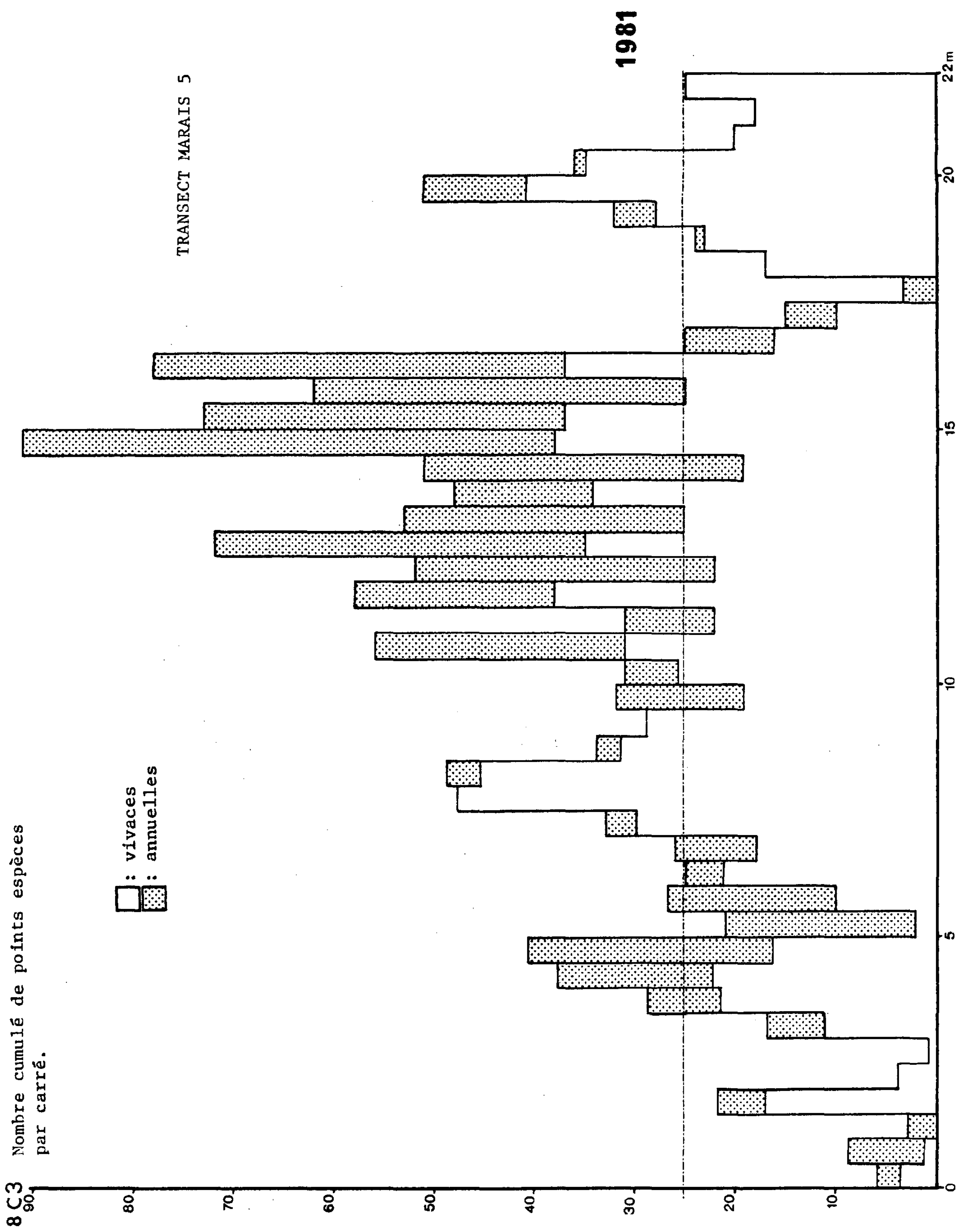


Figure 8. Transect permanent-Marais 5- C -Variation longitudinale du recouvrement des plantes annuelles et vivaces (cf.texte)

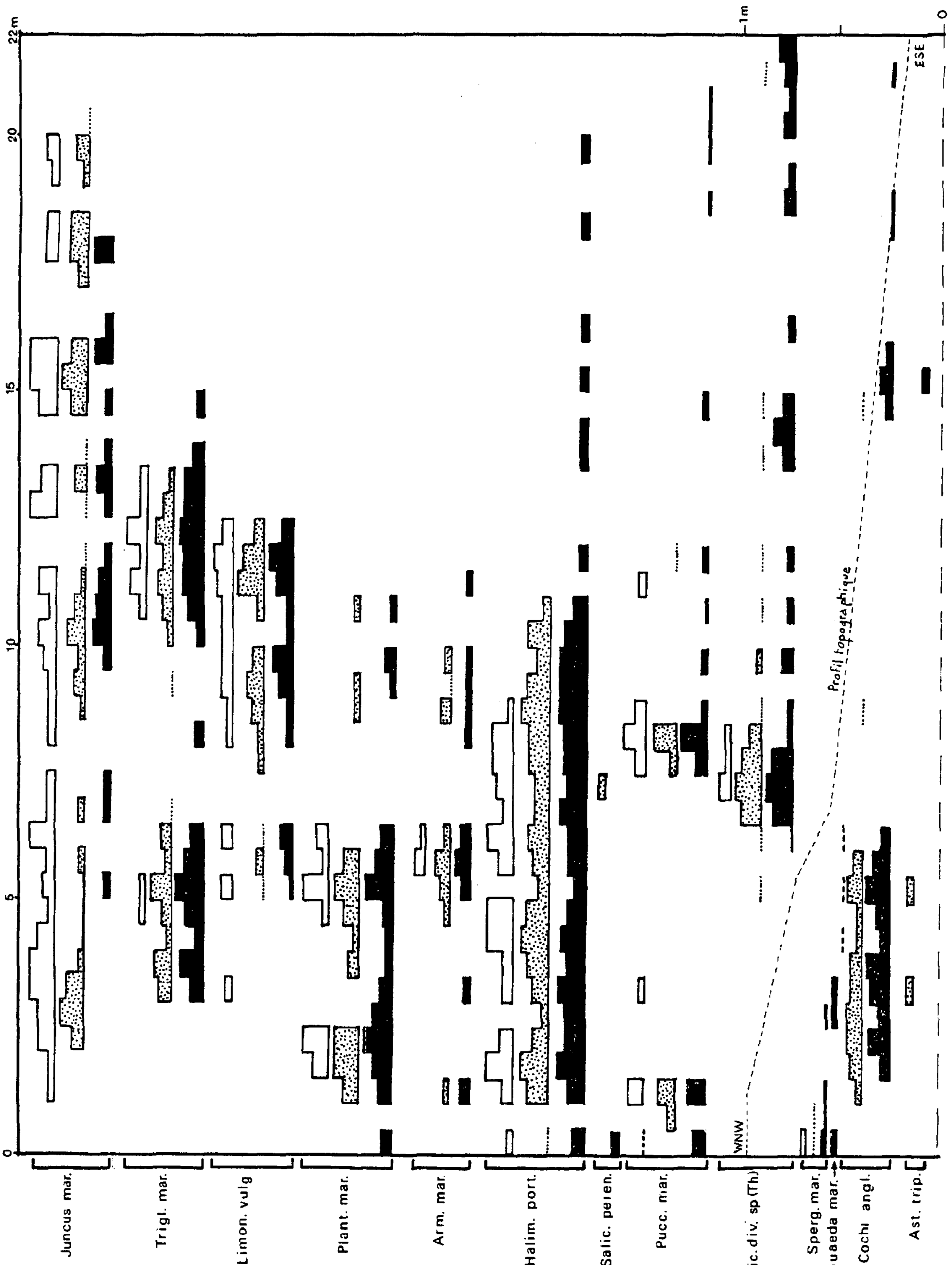


Figure 9. Transect permanent-Marais 3 (cf. figure 1)
A-Distribution spatiale des espèces

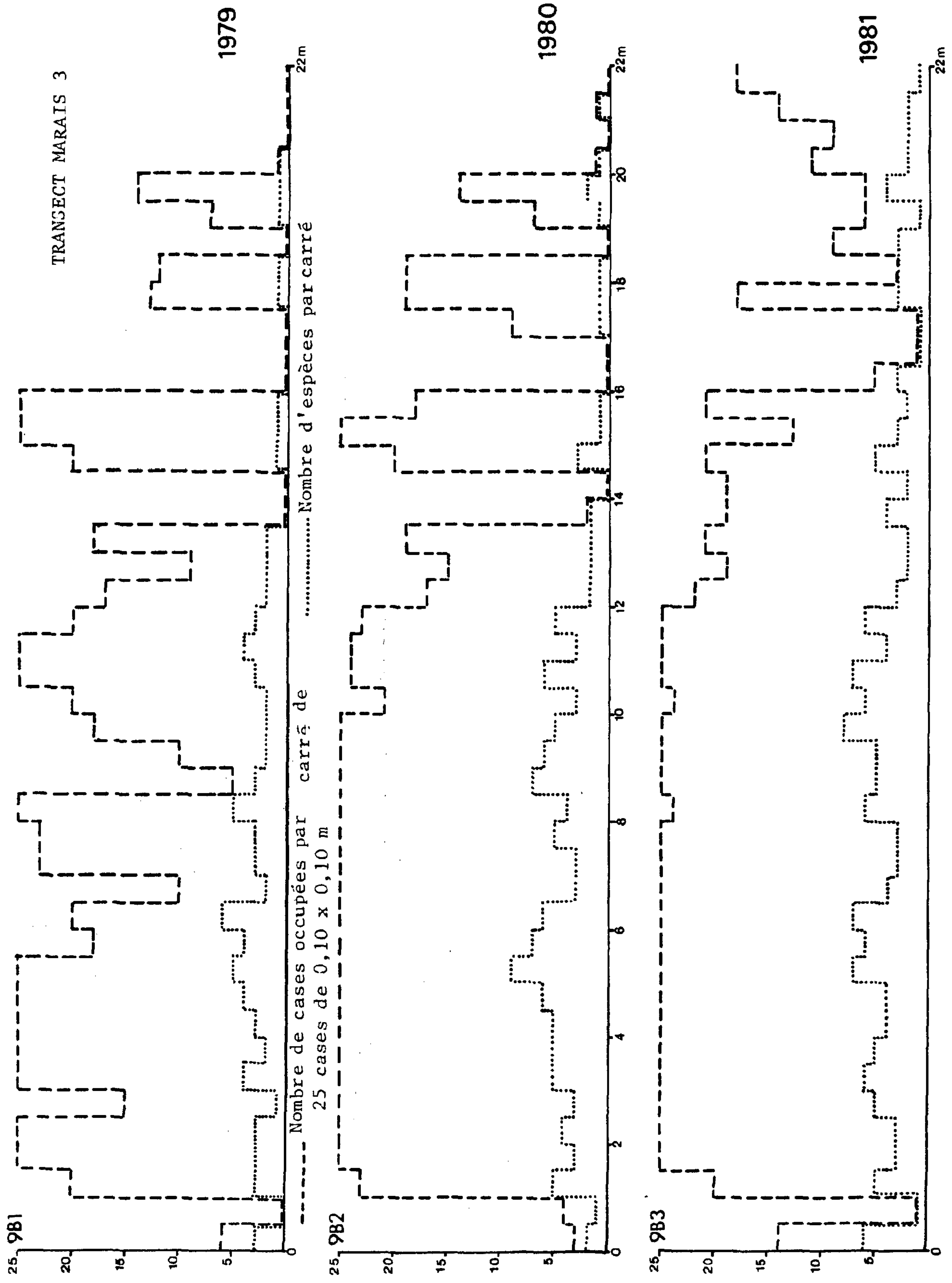


Figure 9. Transect permanent-Marais 3
B-Variation longitudinale du recouvrement et de la diversité

Commentaires

- Estuaire de Kerlavos (figures 7A, 7B)

Les espèces vivaces ont un recouvrement presque nul en 1979, tandis que s'installent quelques pieds de Salicornia ramosissima et Salicornia gr. herbacea (non distinguées sur les transects !).

Le fait remarquable est la rapidité avec laquelle, en moins de trois ans, les thérophytes ont saturé lespace disponible, ce peuplement saisonnier ayant un recouvrement qui peut atteindre 100 %. Il s'agit d'une colonisation primaire active (stade I - espèces "opportunistes" dites à stratégie "r"). Il faut noter l'importance des centres de dispersion initiaux (cf.Brereton, 1971). Les pieds-mères établis en 1979 ont produit des graines qui n'ont pas été exportées étant donné le niveau topographique des lieux et leurs positions très internes dans l'estuaire. Il y a ainsi capitalisation locale des graines qui germent pratiquement sur place. La présence saisonnière d'un revêtement algal microphytique exerce une influence aussi bien dans le piégeage des graines déposées sur le sol que sur leur germination, au printemps suivant (protection thermique, hydrique, photique, mais aussi protection contre les agents dynamiques).

- Marais 5 (E-An Inizigo) (figures 8A, 8B et 8C)

Augmentation, entre 1979 et 1981 de la richesse spécifique dans certaines sections du transect, notamment celles correspondant au schorre moyen. Cette augmentation vient de reprises relativement tardives d'hémicryptophytes à souche et rosette telles Plantago maritima, Limonium vulgare et Armeria maritima dont l'importance numérique reste néanmoins très faible. De nouvelles thérophytes apparaissent en 1981 : Cochlearia anglica et Parapholis strigosa.

En ce qui concerne les plantes pérennes, et si l'on met à part Juncus maritimus, il y a gain dans le recouvrement de chaque population. Le phénomène intéressant est celui du décalage progressif des optimums, d'une année sur l'autre, ce qui peut traduire deux faits :

. l'importance de la compétition interspécifique, du fait de la mise en contiguité de clones régénérés, et qui se sont ensuite développés latéralement d'une façon centrifuge.

. la biologie de la régénération qui favorise un développement plus actif des parties du clone les moins endommagées. Si cette partie est en situation marginale, par rapport au clone ancien, il peut y avoir double mouvement, normalement vers l'extérieur, mais aussi vers l'intérieur (développement centripète) assurant ainsi une reconquête des positions antérieures.

Un clone épargné, à développement plagiotropique dominant, se cicatrise lui-même tout en gagnant des espaces, à sa périphérie, qu'il n'occupait pas précédemment du fait de l'occupation des lieux par d'autres espèces ou par des clones de la même espèce (on peut rattacher ce phénomène à celui du "die-back" observé chez les espèces à extension végétative).

C'est ainsi que ce processus peut conduire à des redistributions spatiales de dominance, après perturbation, telles celles signalées par Baker (1973).

De ce point de vue, des espèces stolonifères ou radicantes comme Puccinellia maritima et Halimione portulacoides ont un développement spatial qui s'accélère avec le temps et qui est rapide compte-tenu du nombre de pieds survivants au départ. Comme dans la section du transect où ces deux espèces se développent, le terrain est plan, donc la pente, via les durées et fréquences de submersion n'est pas un facteur limitant, l'aire actuelle de ces plantes tend à rejoindre leur aire potentielle, en l'absence de concurrence.

L'occupation de l'espace par les annuelles montre la même tendance qu'à Kerlavos. Ilya saturation des espaces interstitiels. Le même processus de capitalisation à partir des pieds-mères s'observe encore ici. De plus, apparaît un phénomène de nucléation (Yarranton et Morrisson, 1974). En effet, toute végétation dans un espace intertidal soumis à submersion est un obstacle pour les particules sédimentaires-et les semences-véhiculées par l'eau. Dans un second temps, ces semences qui peuvent provenir de plantes pérennes également, germent dans le lieu où elles se sont déposées à la faveur des et dans les sédiments meubles déposés à la base de l'obstacle. On observe de la sorte, dans les secteurs où ce phénomène se produit, à la fois une accélération autoentretenue de la sédimentation, et, d'une façon concomittante, une accélération de la colonisation accompagnée d'une augmentation de la diversité spécifique locale. Cette phase d'augmentation transitoire de la diversité est bien connue: transitoire car elle est suivie (cf. marais 2, remblai artificiel, non étudié dans cette communication) par une légère décroissance de la richesse spécifique, conséquence du comportement "impérialiste" de certaines espèces "couvre-sol".

L'observation de la figure 8B montre, à un autre niveau le phénomène déjà décrit à Kerlavos de saturation du plan -toutes espèces confondues- également l'augmentation de la diversité dans certaines sections, en même temps que la variation horizontale de celle-ci, entre 1979 et 1981, traduisant l'hétérogénéité, à grande échelle d'un tel espace. L'explication est plus à rechercher du côté de la compétition interspécifique que de celui de contraintes mésologiques (types biomorphologiques compatibles ou incompatibles, cf. discussion in Levasseur et al., l.c.).

La figure 8C montre la part prise, dès 1980, par les thérophytes, part croissant très brutalement en 1981. Mais l'accélération la plus forte se tient précisément là où une végétation vivace déjà installée fait protection alors que dans la partie gauche du transect, plus exposée, la régénération d'espèces vivaces est moins active ou la destruction initiale de celles-ci plus complète aussi les annuelles sont-elles numériquement moins abondantes.

- Marais 3 dit de Notenno (figures 9A, et 9B)

Ce transect est d'une interprétation plus délicate que les précédents étant donné l'hétérogénéité des situations présentes et les tendances en quelque sorte inverses qui s'y développent depuis 1980.

Ce qui frappe à première vue dans la figure 9A est le déséquilibre entre les parties gauche et droite du diagramme pour ce qui est de la diversité spécifique en chaque point. Deux raisons peuvent être évoquées :

1 - appel à la notion de composition floristique initiale (*cf.* Egler, 1954), fonction, dans ce cas particulier, de gradients mésologiques le long de cette toposéquence induisant en particulier une richesse spécifique maximale dans la partie moyenne du gradient topographique, mais avec cooccurence des limites basses.

2 - l'opposition peut aussi être interprétée comme le résultat des perturbations différenciées qui se sont exercées et qui continuent de s'exercer dans ces lieux, notamment dans les parties déprimées du transect et qui marquent l'influence effective de l'épandage du pétrole, et ce jusqu'à la limite supérieure de dépôt. On notera à ce propos que la cicatrisation a été rapidement effective au dessus de cette limite et ceci dès 1980, alors que le recouvrement végétal reste discontinu en dessous (*cf.* figure 9B).

Le fait à retenir reste cependant le remplacement de la population primitive à *Juncus maritimus*, seule espèce rescapée en 1978 par une espèce de type biomorphologique différent, *Halimione portulacoides* -chamaephyte ligneuse à tiges radicantes- qui étend son aire, dans les parties basses, depuis 1980. Dans l'espace disponible libéré, au moins au niveau et au dessus du sol, le même phénomène de colonisation en nappe par les annuelles s'observe, de la part de *Salicornia* du groupe *herbacea*, tandis que les autres thérophytes -y compris des formes annuelles d'*Aster tripolium*- s'installent, à leur niveau bionomique habituel sur les parties moyennes et hautes du transect.

L'évolution des performances de *Juncus maritimus*, géophyte à rhizome est intéressante car très représentative de la tendance générale au déclin qui affecte, en divers lieux, et ce depuis 1980, des populations entières de cette espèce. Celle-ci, rappelons-le, est une des plantes qui occupait et qui occupe encore la plus grande partie des marais 3, 4, 5 et 6 et qui a été considérée par nous en 1979 comme une plante *résistante*. Comme son rôle physionomique, structural et coenotique est considérable, toutes modifications dans sa distribution et son abondance spatiale pourront avoir, à plus long terme, des incidences certaines.

Les données quantitatives suivantes (figures 10 et 11) illustrent de telles tendances régressives. La figure 10 présente l'évolution saisonnière du rapport biomasse épigée sur nécromasse aérienne exprimées en poids sec. Les données de base sont le résultat de fauches effectuées au ras du sol, dans trois quadrats de 0,50 x 0,50 m, pris au hasard à trois niveaux topographiques, les récoltes étant ensuite séchées 48 heures à 65°C. On notera l'inversion du rapport à partir de la fin de l'année 1980 et à terme, ceci conduira à la disparition de l'espèce dans ce lieu.

D'une façon concomittante, les capacités de reproduction sont altérées et se traduisent, selon les cas, par une réduction du nombre de tiges fertiles(*), par des malformations de l'inflorescence et, pour ces dernières, par une diminution du nombre de rameaux florifères, par l'absence d'étamines ou la non-formation de capsules et de graines ou seulement par la production d'un petit nombre de graines avortées. Dans le même temps, les pièces du périanthe peuvent revêtir un aspect bractiforme (*cf.* figure 12).

De plus, l'observation de coupes transversales de rhizomes montre une nécrose des parenchymes; ceci, ajouté à un dessèchement des apex végétatifs, pose le problème de la nutrition hydrique et minérale de

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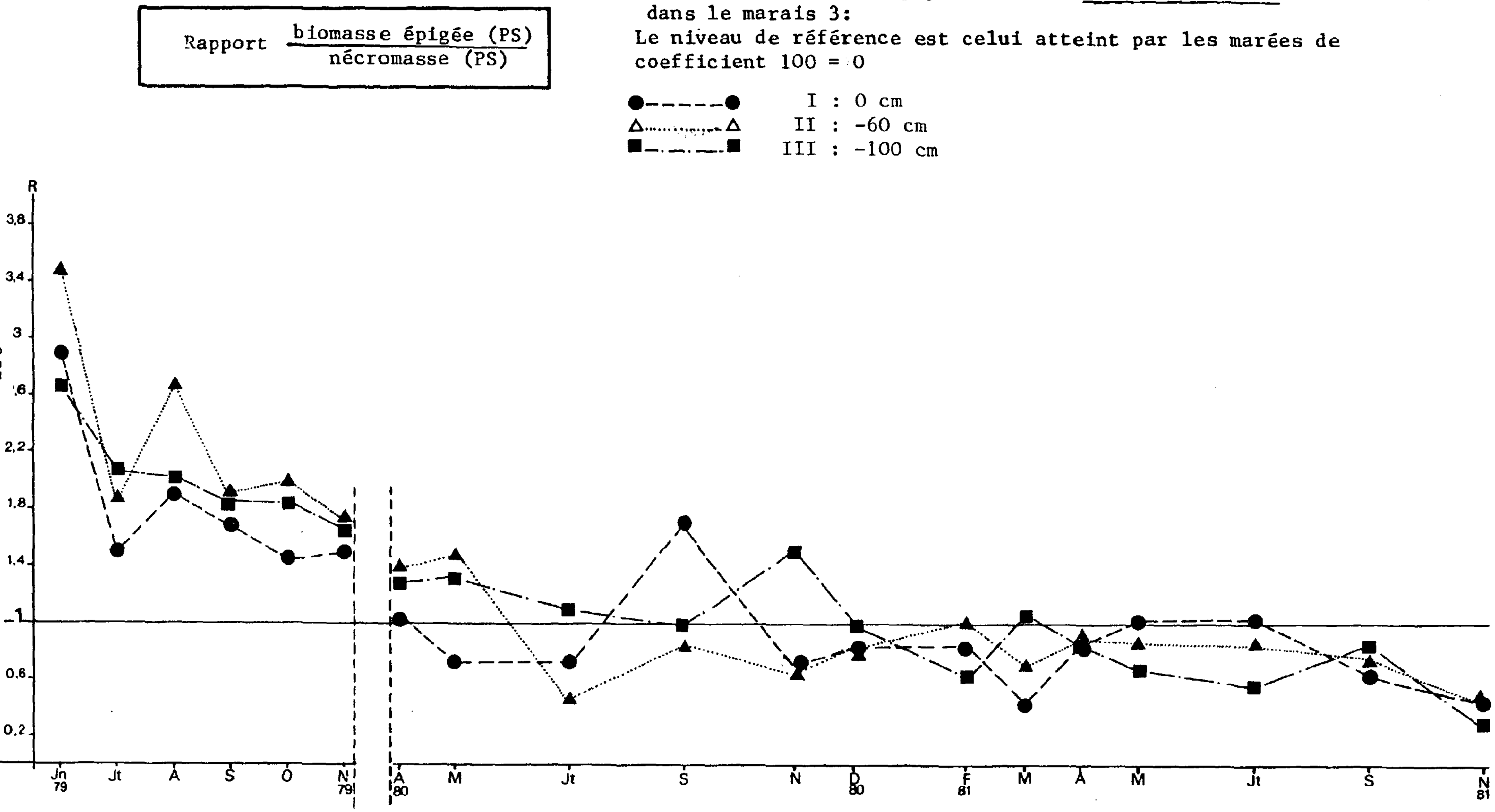
(*) *cf.* figure 11.



Altitude relative des populations de Juncus maritimus échantillonnées dans le marais 3:
Le niveau de référence est celui atteint par les marées de coefficient 100 = 0

Figure 10. Evolution saisonnière du rapport biomasse épigée sur nécromasse aérienne.

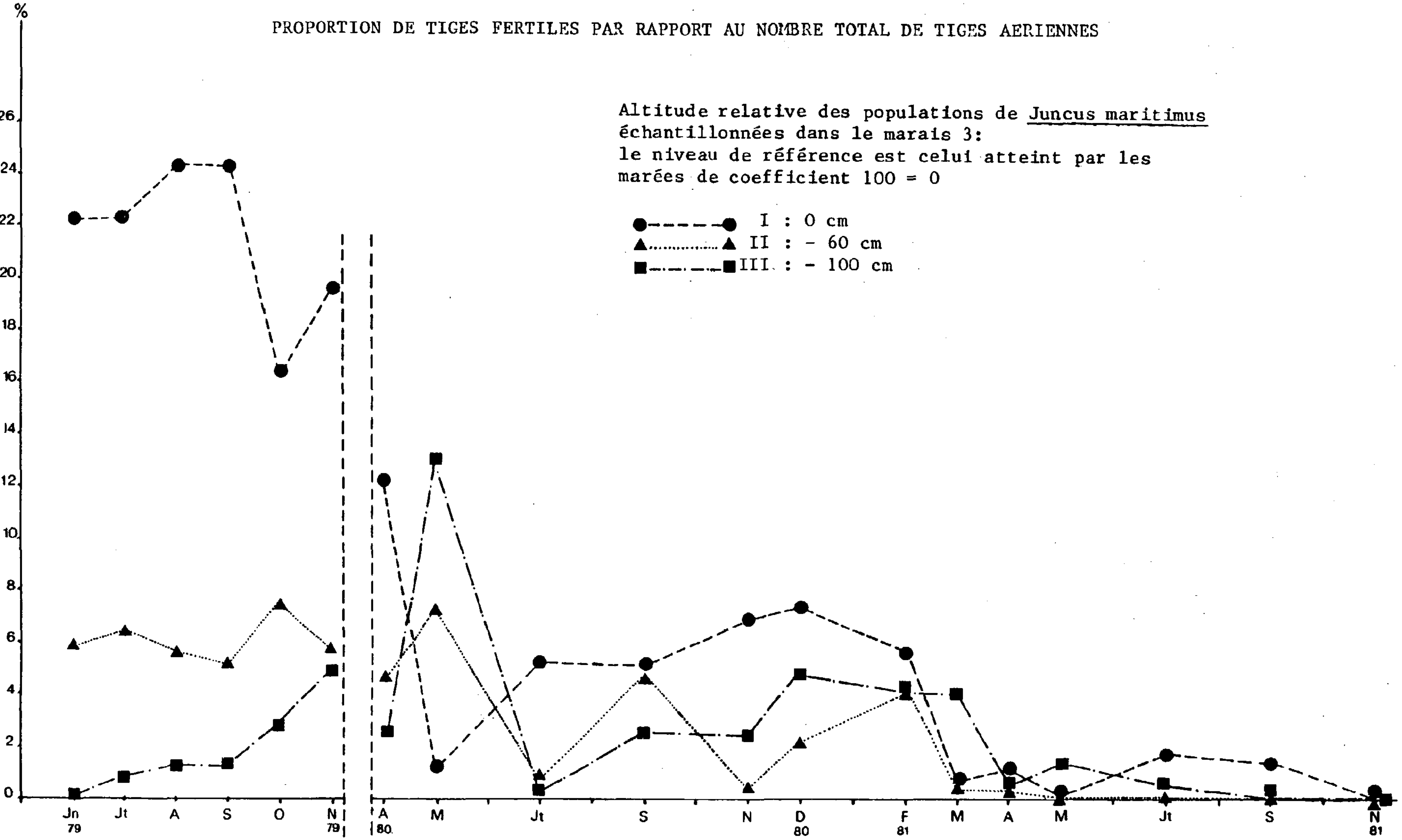


Figure 11. Evolution saisonnière du nombre de tiges fertiles par rapport au nombre de tiges aériennes ($s=0,250$ m^2).

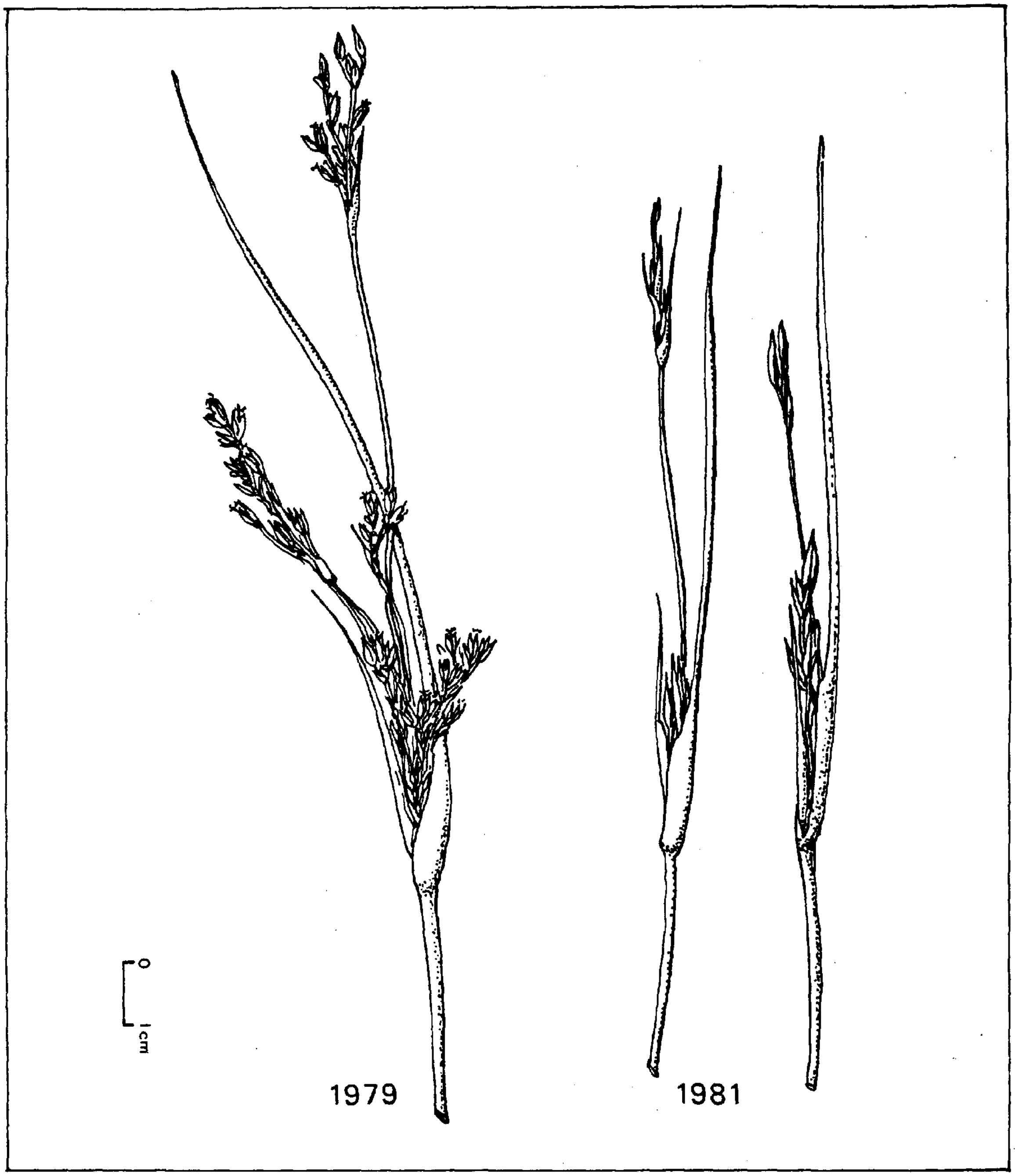


Figure 12. Morphologie comparée d'inflorescences de Juncus maritimus Lam. provenant d'un même clone situé au S du marais 4 (cf.figure 1) prélevées en Août 1979 et Août 1981.

ces organes, donc des effets à long terme du pétrole, toujours présent, sur la physiologie et sur certains métabolismes de la plante. Apparemment, il n'y a pas renouvellement des réserves dans le rhizome : celles subsistant après 1978 ont maintenant été consommées. Il faut rappeler à ce propos les conclusions de Baker (l.c.) relatives à la sensibilité de cette espèce à des pollutions chroniques par les hydrocarbures.

L'observation simultanée des deux courbes montre la réalité d'une tendance exprimée de deux façons différentes et qui ne touche pas seulement l'appareil reproducteur ! Rappelons cependant qu'une "allocation d'énergie" plus forte en faveur de l'appareil végétatif est considérée comme classique par Ranwell (1972) chez les plantes des marais maritimes. La véritable question relative à la nature et aux délais de rétablissement de la végétation, dans les marais de l'Ile Grande, est là. Il est vraisemblable que ce rétablissement aura lieu, naturellement mais aussi avec l'aide des plantations volontaires; mais, sur de grands espaces encore occupés par le Jonc, il aura lieu sans lui, ce qui pourra modifier passablement le paysage végétal, mais aussi les stratégies de restauration, celle-ci ne pouvant alors être uniquement focalisée sur les seuls secteurs actuellement dénudés.

CONCLUSIONS

Les quelques exemples présentés montrent qu'au delà de la variété constatée, un processus de rétablissement du couvert végétal est effectif en de nombreux lieux; les gains en recouvrement, par rapport à la situation de 1978, représentent environ 35 %. Il est vraisemblable que localement on assistera à une accélération du phénomène puisque par nucléations en chaîne, le nombre de points d'agglutination des sédiments et des semences va croître d'une façon non linéaire.

Il reste encore des sites où la destruction de la végétation a été totale. Pour différentes raisons ils demeurent stériles, en ce sens que des germinations ne peuvent s'y effectuer. Aussi un rétablissement naturel y est-il peu probable au moins dans un avenir proche. Il semble alors que des restaurations au moyen de plantations soient la seule voie réaliste possible, comme en témoignent les succès enregistrés, à la suite des deux années d'expérimentations menées dans ces secteurs par le Dr. Seneca et son équipe. En effet, l'introduction de boutures, selon les cas, avec ou sans sol, leur reprise et leur développement ultérieur, montre a contrario que c'est la phase "germination" qui est inhibée et donc qu'en la court-circuitant, on accélère la cicatrisation. De la même manière, comme ces boutures font obstacle, d'autres espèces peuvent alors s'implanter naturellement, mais dans un second temps, dans ces lieux, rétablissant une diversité spécifique qui n'existait pas au départ lors de l'expérience. Notons qu'un phénomène similaire peut être initié par les nappes de thérophytes qui marquent fréquemment la première phase de la recolonisation.

Si l'on compare l'état actuel de la végétation avec l'état primitif, on constate que le rétablissement de celle-ci passe en de nombreux lieux par une redistribution spatiale des espèces, au profit d'un petit nombre d'entre elles. Cette redistribution, qui peut quelquefois aller jusqu'à la monopolisation (transitoire ?) d'un espace peut avoir deux causes :

- les plantes survivantes -initialement "résistantes" ne possèdent pas des capacités d'extension végétative suffisantes pour cicatriser les espaces interstitiels dénudés, alors que d'autres espèces,"sensibles" celles-là aux premières perturbations présentent ces qualités,de part leur organisation et leur éthologie; de là l'écart actuellement observé entre la composition floristique initiale du site et la composition du moment.

- des espèces tout-à-fait résistantes, telles *Juncus maritimus* et dans une moindre mesure, *Triglochin maritima*, deviennent sensibles à la pollution chronique qui affecte maintenant ces marais. Leurs populations, en déclin, sont envahies périphériquement par des espèces autrefois cantonnées, du fait de la saturation de l'espace par les premières ,en dehors des clones les plus denses. Ce sont d'ailleurs les mêmes espèces qui jouent ce rôle dans les deux cas, à savoir *Puccinellia maritima* et *Halimione portulacoides*,toutes deux capables, par stolons ou tiges radicantes,de couvrir le sol, même lorsque celui-ci est encombré, à un niveau endogé,par des souches ou des rhizomes qui se maintiennent longtemps après la mort de la plante.

La résistance d'une plante est donc une notion très relative, elle est en quelque sorte individuelle et thématique mais l'organisation future d'un couvert végétal après perturbation doit autant aux plantes dites résilientes qu'à des espèces "résistantes" en nombre insuffisant ou devenant sensibles à d'autres causes que celles qui avaient autorisé la résistance de départ.

La soi-disant robustesse d'un tel écosystème tient plus à ses capacités de cicatrisation *via* des colonisations périphériques ou implantations directes, lorsqu'elles sont possibles, qu'à la résistance aléatoire, à plus long terme, d'autres espèces. Mais encore y a-t-il une nuance fondamentale entre la réaction vis-à-vis d'une perturbation exceptionnelle, mais finie dans le temps et une perturbation qui devient chronique et qui n'a pas été intégrée dans le passé par exemple au moyen d'une sélection particulière d'espèces. C'est peut-être ce qui est en train de se dessiner actuellement.

Encore faudrait-il affiner le concept d'écosystème littoral. Il vaut mieux en effet parler de plusieurs écosystèmes superposés ou inclus dont les caractères qualitatifs, structuraux et dynamiques sont différents au travers des types biomorphologiques représentés, de leur abondance relative, de leur distribution spatiale. Une même perturbation s'exercera alors d'une façon sélective et différenciée sur les éléments composant une végétation locale, delà les délais et les modalités différentes du rétablissement consécutif. Celui-ci pourra même ne pas être possible : une Spartinaie altérée ne pourra être reconstituée que par la Spartine elle-même.

Plus fondamentalement, la nature, l'abondance relative, la distribution spatiale des espèces présentes ou apparaissant pendant la succession pourront être soumis à variation, changeant dans un premier temps la composition mais aussi la structure des peuplements en cours de rétablissement, ceci à l'intérieur de certaines limites imposées par l'environnement mésologique. Ces écarts et ces divergences, par rapport à l'état ancien, ne constituent pas des phénomènes "anormaux" et/ ou éventuellement inquiétants. Ils représentent seulement la matérialisation instantanée du processus fondamental qui conduit à une saturation par la végétation de l'espace disponible, lorsque l'opportunité s'y prête, comme c'est le cas en ce moment.

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RESTORATION OF MARSH VEGETATION IMPACTED BY THE AMOCO CADIZ OIL SPILL AND SUBSEQUENT CLEANUP OPERATIONS AT ILE GRANDE, FRANCE

Ernest D. Seneca and Stephen W. Broome[1]

INTRODUCTION

General

Tidal salt marshes functon to stabilize estuarine shorelines, to exchange nutrients with sediments and the surrounding waters, to provide energy as detrital material to the estuarine food web, and to serve as nursery grounds for many commercially important fish and shellfish. Because competing land uses have resulted in a decrease in areal extent of these valuable resources in the past, there have been concerted efforts recently to preserve the remaining marshlands and to reestablish marshes at selected sites. Techniques and procedures have been developed to: (1) reestablish marsh in areas where Man has destroyed natural marsh, (2) reestablish marsh along shorelines where storms have damaged or destroyed natural marsh, (3) establish marsh along canal banks and shorelines to stabilize the substrate and retard erosion, and (4) establish marsh on dredged material (Woodhouse et al., 1974; Garbisch et al., 1975; Seneca et al., 1976).

Our research efforts in marsh establishment along the southeastern coast of the United States led us to respond to an invitation from the joint scientific commission of the National Oceanic and Atmospheric Administration (NOAA)/Centre National pour l'Exploration des Oceans to study the effects of the Amoco Cadiz oil spill. We developed a proposal for restoring marsh at the Ile Grande site adapting techniques and procedures developed for *Spartina alterniflora* Loisel. in North Carolina (Woodhouse et al., 1974; Seneca et al., 1976) to restoration of a part of the Ile Grande marsh using vegetation indigenous to that region. This interim report contains results from 2 years' marsh rehabilitation research at Ile Grande and a nearby estuary at Kerlavos.

Literature Review

The effects of oil pollution on salt marsh vegetation have been studied and reported by European researchers. Based on observations of Welsh salt marshes affected by oil spills from the Chryssi P. Goulandris in January 1967 and the Torrey Canyon in March 1967, Cowell (1969) rated susceptibility of marsh plants to crude oil and concluded that salt marshes dominated by *Spartina townsendii* H. and J. Groves and *Puccinellia maritima* (Huds.) Parl. were most subject to damage. Stebbings (1970) studied the effects of oil from the Torrey Canyon spill on salt marshes in Brittany and found that these marshes were

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able to withstand 2 to 10 cm of oil with only slight, short-term, floral composition changes. Apparently, most of the toxic fractions had been lost from the Torrey Canyon oil, since it had been weathered at sea for 2 to 18 days. Stebbings noted that oil appeared to form an impervious layer on the substrate preventing gaseous interchange between soil and air, causing reducing conditions in the mud, and ultimately chlorotic symptoms in plants. Stands of Agropyron pungens (Pers.) R. and S., Festuca rubra L., Juncus maritimus Lam., and Scirpus maritimus L. were extremely vigorous and seemed to derive some nutritional benefit from the breakdown products of this Torrey Canyon oil. Cowell and Baker (1969) noted that populations of annuals such as Suaeda maritima (L.) Dum. and Salicornia spp. near Pembroke, Southwest Wales, were reduced initially but were recovering a year after oiling from the Chryssi P. Goulandris. Halimione portulacoides (L.) Aell. was the plant most badly damaged. In June 1968 the plant species with the greatest coverage in the upper, middle, and lower marsh (Festuca rubra, Puccinellia maritima, and Spartina townsendii, respectively) had recovered completely (Cowell and Baker, 1969). Baker (1971a-i) reported on several aspects of the effects of oil pollution on salt marsh and concluded that single oil spillages do not cause long-term damage to marsh vegetation (Baker, 1971a).

These studies indicate that marsh vegetation is resilient and often can recover from single oil spills. Baker (1971e) suggests that it is best to let an oiled marsh recover naturally. However, persistent oil pollution has killed Spartina marsh at Southampton Water (Ranwell, 1968). Such sites may develop extremely anaerobic conditions in the mud so that higher plants can no longer grow on them. Cowell (1969) states that repeated contamination is likely to have increasingly serious effects if anaerobic conditions are created due to bacterial use of oxygen in the biological oxidation of the oil. We found no account of marsh recovery after removal of the upper layer of marsh substrate and vegetation.

Study Sites

The Ile Grande site is a relatively protected estuary with a mean tide range of ca. 6 m, a spring tide range of ca. 8 m, and a mean tide level of ca. 5 m. Our first visit to Ile Grande was in December 1978. Our NOAA liason representative, Douglas Wolfe, indicated that the marsh west of the bridge at Ile Grande was to be our primary study site (Fig. 1). There were extensive stands of Juncus maritimus on both sides of the estuary with lesser stands composed of a mixture of species including Puccinellia maritima, Triglochin maritima L., Limonium vulgare Mill., Spartina maritima (Curtis) Fern., and Halimione portulacoides. There were vast areas with no vegetation cover, the result of cleanup operations by the French military to rid the marsh of Amoco Cadiz oil. In many areas only the aboveground marsh vegetation and associated oil had been removed and in other areas the entire marsh surface including the root mat had been removed to a depth of over 30 cm. The intertidal creek banks were almost completely lacking in vegetation cover. A limited number of substrate samples from the disturbed sites were taken which subsequently indicated a

FIGURE 1. Marsh west of the bridge at Ile Grande. Area without vegetation is due to removal of oil and vegetation during Amoco Cadiz cleanup operations.

material which was sandy loam in texture and low in nitrogen and phosphorus.

Marsh vegetation adjacent to the disturbed sites indicated that prior to the oil spill the natural marsh was composed primarily of Juncus maritimus, Puccinellia maritima, Triglochin maritima, Limonium vulgare, with lesser amounts of Spartina maritima. Halimione portulacoides was dominant along the creek banks. We noted considerable variation in the relative dominance of these species and others within marshes in the vicinity. Spartina anglica C. E. Hubbard was present only at a single site at Ile Grande as a small clump less than 3 m in diameter. This species is abundant in the Bay of Mt. St. Michel some 125 km to the east of Ile Grande.

Juncus stands generally occupied the highest elevations of the marsh relative to the other species mentioned. Subsequent observations indicated that the Juncus marsh is flooded for about 3 days each spring tide cycle. Above the level of Juncus there was in some areas a narrow fringe of Festuca rubra and Agropyron pungens with associated species. Many salt marsh ecologists consider this vegetation to be a part of the marsh. This higher zone of vegetation which extends up to ca. 1 m above the Juncus marsh is flooded relatively infrequently on extremely high storm tides and spring tides. It lies above the marsh impacted by Amoco Cadiz oil and cleanup operations. Our marsh rehabilitation efforts were confined to elevations from 0.8 m below to 0.3 m above that of the Juncus marsh.

Because we wanted to compare the marsh at Ile Grande with other marshes in the vicinity, we also visited the marsh in the estuary at Kerlavos ca. 5 km from Ile Grande (Fig. 2). This marsh contained less Juncus, no Spartina, and was dominated by Puccinellia maritima, Armeria maritima (Mill.) Willd., and Triglochin maritima along with Plantago maritima L., Cochleria officinalis L., Halimione portulacoides and Aster tripolium L. There was evidence of marsh removal by cleanup operations in the Kerlavos marsh also, but it appeared that the marsh was much less heavily impacted than that at Ile Grande. We chose to use this marsh area as a supplemental study site.

FIGURE 2. Marsh in estuary at Kerlavos. Areas without vegetation represent sites of marsh removal during Amoco Cadiz oil cleanup operations.

PROCEDURE

Substrate

Substrate samples were taken at the transplant source sites for each species and also at the experimental planting sites each year. These samples were analyzed for elemental concentrations, pH, organic matter, and volume weight by the North Carolina Department of Agriculture Soil Testing Division using their routine methods.

1979 Plantings

Based on our preliminary plantings made in December 1978 and the nutrient analysis of initial substrate samples, we established 9 experimental plantings in May 1979, using primarily Puccinellia maritima (Fig. 3), to a lesser extent Juncus maritimus (Fig. 4), and to a lesser extent still because transplants were not locally abundant, Spartina maritima (Fig. 5). These experimental plantings were designed to determine transplant response to conventional ammonium sulfate + concentrated superphosphate and slow release (Mag Amp and Osmocote) fertilizer materials at different rates over a wide range of tidal elevations. All transplants were taken from the natural marshes at Ile Grande and Kerlavos. Digging of transplants was confined to small areas along narrow drainageways (Fig. 6) and protected sites so as to impact the marsh as little as possible. Half of the 2900 May transplants were plugs (10 to 15 cm deep cores from 5 to 7 cm in diameter composed of root material with attached substrate) and half were sprigs (root material only) (Figs. 7, 8, 9). Holes for the transplants were made with a 6.5-cm diameter soil auger (Fig. 10). Transplants were spaced 0.5 m apart and the appropriate amount of fertilizer material was placed into the transplant hole prior to insertion of the transplant (Fig. 11). Planting was conducted just prior to the spring tide cycle so that transplants would be flooded shortly after planting.

FIGURE 3. Puccinellia maritima.

FIGURE 4. *Juncus maritimus*.

FIGURE 5. *Spartina maritima*.

FIGURE 6. Digging Puccinellia along a narrow drainageway.

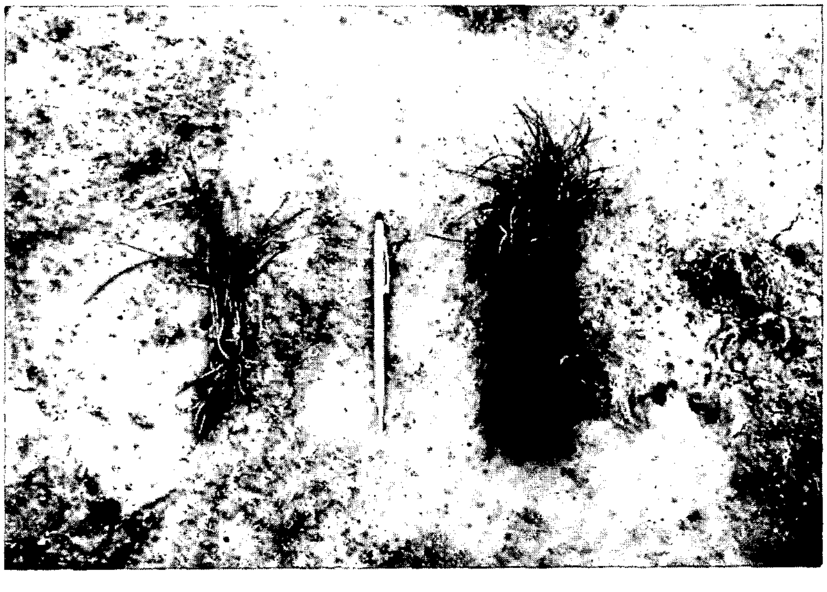

FIGURE 7. Transplants of Puccinellia: sprig on left, plug on right.

FIGURE 8. Plug type transplants of *Juncus*.

FIGURE 9. Plug type transplants of *Spartina*.

FIGURE 10. A 6.5-cm diameter soil auger used to make holes for transplants in experimental plantings.

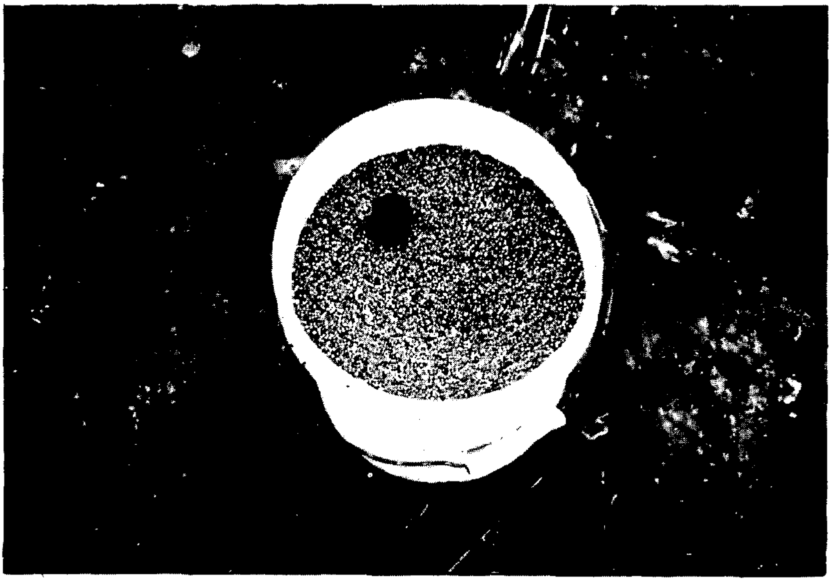

FIGURE 11. Osmocote (a slow release fertilizer material) + concentrated superphosphate. Black cup measures a dose of fertilizer (2.8 g N + 1.2 g P) per transplant. Holes for transplants are spaced 0.5 m apart.

FIGURE 12. *Triglochin maritima*.

FIGURE 13. Plug type transplants of *Triglochin*.

In September, we made 9 additional plantings of Juncus maritimus, Puccinellia maritima, and Spartina maritima and established initial plantings of another species, Triglochin maritima (Figs. 12, 13). Although not recognized as such on our initial visits to the site, the latter species appears to be a common pioneer species on disturbed sites alone or with Puccinellia maritima. Both Puccinellia and Triglochin appear to invade by seed.

Height, number of stems, cover (a measure of spread) and aboveground dry weight per transplant were determined in September 1979, 4 months after planting. Cover determinations were made by measuring the average maximal diameter and the average minimal diameter of the transplant and using these dimensions in the formula for the area of an ellipse. Percent survival by transplant type and species was also assessed at this time and at each subsequent visit. Because our major objective was to establish vegetation, destructive sampling for biomass determinations was held to a minimum of three samples per treatment per location. A photographic record of all plantings was initiated.

1980 Plantings

Based on results of our 1979 plantings, we established 14 additional plantings at higher elevations in May 1980 utilizing plugs of Puccinellia, Juncus, Spartina, and Triglochin and sprigs of Halimione (Figs. 14, 15). Remains of stems and intact root systems of Halimione indicated that this species was the dominant along the creek banks prior to the Amoco Cadiz oil (Fig. 16). Consequently, we began preliminary tests of reestablishing this species along the creek banks (Fig. 17) and included it in an experiment to determine the feasibility of nursery production for transplants. Like the earlier plantings, these 1980 plantings were designed to determine transplant response to fertilizer materials at different rates over a range of substrate and exposure conditions. Cover was determined for selected plantings. All experimental plantings were surveyed to determine relative elevations, i.e. relative to the natural marshes (Fig. 18).

In September we made 8 additional plantings using primarily Puccinellia and Halimione with some Spartina. Based on results from our earlier plantings, further planting of Triglochin seemed impractical. As in September 1979, all earlier plantings were assessed for survival, height and cover with sampling for dry weight determinations limited to only two plantings. Photographic surveillance was considered even more important because we did not conduct intensive destructive sampling.

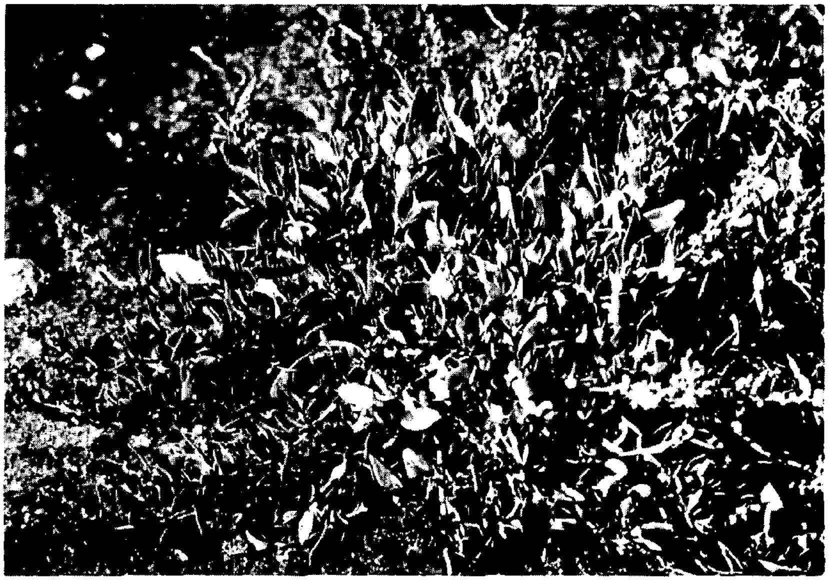

FIGURE 14. *Halimione portulacoides.*

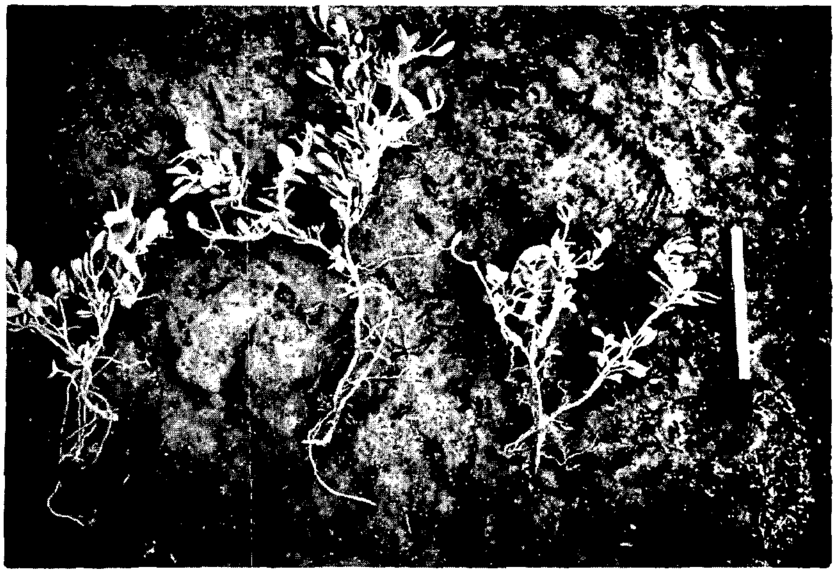

FIGURE 15. Sprig type transplants of *Halimione.*

FIGURE 16. Creek bank without vegetation as a result of removal of oil and vegetation in cleanup operations. Old root systems of Halimione are visible on lower portion of banks. Note lack of natural marsh plant invasion of these sites at time of photo, May 1980.

FIGURE 17. Making transplant holes along creek banks, May 1981. Experimental plantings made in May 1980 are visible in background on creek bank.

FIGURE 18. Surveying to determine the elevation of our plantings in relation to that of the natural marsh at Ile Grande, May 1981. Note transplants on creek banks between surveyors and on right creek bank toward the village from the white stake.

1981 Plantings

Based on results of all earlier plantings, we established 21 additional experimental plantings in May 1981 utilizing about 4900 transplants at Ile Grande. Most of the planting effort was concentrated on establishing cover on the bare creek banks which were at this time beginning to erode due to decay of the binding root mat and undercutting by tidal waters (Fig. 19). Two rows of Halimione transplants (sprigs) were planted on the edge of the creek banks with two rows of Puccinellia transplants (plugs) adjacent to and toward the marsh along several intertidal creeks (Fig. 20). Many other areas, still bare of vegetation 2 years after the catastrophe, were planted to increase the probability of revegetation (Figs. 21, 22, 23). All transplants were spaced 0.5 m apart. Cover was determined for selected plantings. All experimental plantings were surveyed to determine relative elevations, and the photographic record was continued.

FIGURE 19. Eroding creek bank at Ile Grande, May 1981.

FIGURE 20. Creek banks that had no vegetation over 2 years after the catastrophe. Each bank was planted with two rows of Halimione sprigs in May 1981.

FIGURE 21. Site at Ile Grande without vegetation prior to planting in May 1981.

FIGURE 22. Making holes for transplants and applying fertilizer in preparation for planting at same site as shown in Figure 21, May 1981.

FIGURE 23. Same site as that in Figures 21 and 22 just after planting Puccinellia in interior and Halimione on the perimeter of area, May 1981.

Nursery Plantings

It was obvious from our initial visit to Ile Grande that transplant sources could become exhausted as we began scaling up the planting operation. With rehabilitation of larger areas as a goal, we explored the possibility of establishing nursery areas for two of the most promising species, Puccinellia and Halimione. The Puccinellia nursery area was established at Kerlavos in May 1979 in conjunction with a type of transplant and fertilizer materials experiment. The nursery area for Halimione was incorporated into a fertilizer materials experiment with three other species at Ile Grande in May 1980. Both areas were refertilized with Mag Amp + Osmocote to determine the effect of fertilizer in addition to that applied at planting (Fig. 24). A limited number of transplants were taken from each nursery area in May 1981 and compared with transplants of the same species taken from the natural marsh in experimental plantings at Ile Grande.

Another approach to the problem of transplant propagation was undertaken in a joint venture with Monsieur Levasseur in 1981. He took Puccinellia plants from a natural marsh, transplanted them into small plastic pots, and grew them in his garden in Rennes for several weeks in the spring (Fig. 25). These transplants were planted in an experimental plot at Ile Grande in May 1981 to compare their growth response with transplants taken from the natural marsh at the time of planting (Fig. 26).

FIGURE 24. Puccinellia transplants in September 1981 being refertilized with Mag Amp + Osmocote 16 months after planting at Kerlavos.

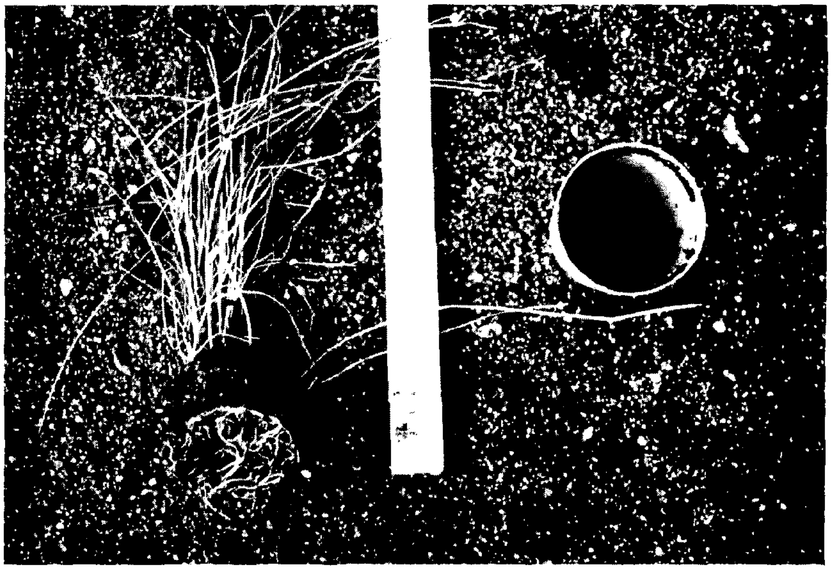

FIGURE 25. Puccinellia transplant grown by Monsieur Levasseur in his garden for several weeks prior to planting at Ile Grande, May 1981.

FIGURE 26. Experimental planting established with transplants shown in Figure 25, May 1981.

Data Analysis

Data were analyzed using the Statistical Analyses System (SAS) programs for analysis of variance and least significant difference (LSD) (Barr et al., 1976). All statistically significant differences were determined at the .05 level. Variability was generally high and not all data could be analyzed statistically. We feel that in field experiments of the type conducted on disturbed marsh sites that overall observations, photographs, and at times fragmentary data have to be interpreted and used as best they can even when statistical significance cannot be documented. Consequently, because of these conditions and the fact that data of this type are not readily available, we have included data in this report that we consider important even though the variability is high.

RESULTS AND DISCUSSION

Substrate

Results of analyses of substrate samples from Ile Grande indicated important differences among sites which affect plant growth. Samples from relatively undisturbed areas of marsh from which transplants were taken and from the root mat of marsh killed by oil but with no surface manipulation, had relatively high ammonium, phosphorus and organic matter concentrations and low volume weight and pH values compared to substrate below the undisturbed root mat and that exposed by the cleanup operations (Table 1). The low ammonium and phosphorus concentrations of the subsurface material definitely were limiting to plant growth. Data to be presented later in the report indicate that fertilizer materials were necessary for significant plant growth in these disturbed substrates.

TABLE 1. Values for five substrate variables for seven sites (source of transplant sites for four species, two strata of marsh without vegetation but with surface not removed, upper stratum of a creek bank without vegetation but with surface not removed, and a site from which the marsh surface was removed) at Ile Grande.

Site	Ammonium (mg/dm^3)	Phosphorus (mg/dm^3)	Organic matter (%)	pH	Volume weight (g/cc)
Puccinellia site	88	12	8.9	3.4	0.7
Triglochin site	67	30	8.3	5.4	0.7
Juncus site	56	19	6.5	6.0	1.0
Spartina site	117	15	5.8	3.3	0.8
Marsh without vegetation[a]					
upper 10 cm (root mat)	50	32	4.8	5.9	0.7
below root mat	14	7	0.6	7.1	1.4
Creek bank (upper 10 cm)[a]	40	17	3.5	4.7	1.0
Site with marsh removed[b]	5	2	0.0	7.0	1.2

[a] Marsh vegetation removed as a result of cleanup operations but marsh surface not removed; examples of site in Figures 1 and 16.

[b] Marsh surface including root mat was removed during cleanup operations.

Elevation

All elevations are given in relation to the average elevation of the natural marsh at the particular site, Ile Grande or Kerlavos. At Ile Grande the average elevation of the *Juncus* marsh on the southwest side of the bridge was the reference datum. This average elevation is tied to a white mark on the rock wall of the bridge for which Mademoiselle Odile Guerin, who has worked on the Ile Grande project, has elevation tied to a national datum. The average elevation is also tied to the concrete foundation of the bridge itself. At Kerlavos the average elevation of the marsh is tied to a bench mark at an electric station tower ca. 0.5 km from the study site. Based on relating water levels at the two sites, the *Juncus* marsh at Ile Grande is about 0.1 m above the elevation of the natural marsh at Kerlavos.

At Ile Grande we planted *Juncus*, *Puccinellia*, *Spartina* and *Triglochin* over a range of elevation from 0.8 m below to 0.3 m above the average elevation of the natural *Juncus* marsh. *Juncus* transplants did not survive at elevations below that of the natural *Juncus* marsh and best survival occurred at 0.3 m above that of the natural *Juncus* marsh. *Puccinellia* transplants did not survive at elevations of 0.7 m below that of the natural *Juncus* marsh and survival was less than 10% at elevations of 0.5 m below that of the natural *Juncus* marsh. The best growth and survival of *Puccinellia* transplants was achieved in the range of elevation between 0.1 m below and 0.3 m above that of the natural *Juncus* marsh. *Spartina* transplants survived at the lowest elevations of all species tested. Although *Spartina* transplants survived at elevations of 0.8 m below that of the natural *Juncus* marsh, growth was best at 0.3 m below that of the natural *Juncus* marsh. Survival and growth of *Triglochin* was generally poor but its elevation response was similar to that of *Puccinellia* with no survival at elevations of 0.7 m below that of the natural *Juncus* marsh.

At Kerlavos experimental plantings of *Puccinellia* and *Triglochin* were established over a range of elevation from 0.5 m below to 0.1 m below that of the natural marsh. The best survival and growth of these *Puccinellia* transplants occurred at 0.2 m below that of the natural marsh. Transplants at 0.4 m below the elevation of the natural marsh did very poorly. *Triglochin* transplants responded in a similar manner.

Halimione was planted at elevations from 0.1 m below to 0.3 m above that of the natural marsh at Ile Grande. Survival and growth of these transplants were best at about 0.3 m above the elevation of the natural marsh, but survival was good throughout the range of elevations planted. At Kerlavos, *Halimione* was planted from 0.4 m below to 0.2 m below the elevation of the natural marsh. Survival and growth was best in the upper half of this elevation range.

Plantings in General

About 9,700 transplants have been planted at Ile grande and about 1,800 others at Kerlavos over the period May 1979 through May 1981 (Table 2). Although half of these transplants were those of

TABLE 2. Number of transplants planted at Ile Grande and Kerlavos for five species for five dates from May 1979 to May 1981.

Species	Number of transplants[a] by year by month by site[b]									
	1979				1980				1981	
	May		Sep		May		Sep		May	
	IG	K	IG	K	IG	K	IG	K	IG	K
Halimione[c]	0	0	0	0	332	108	220	0	2756	0
Juncus	518[d]	0	173	0	360	0	0	0	0	0
Puccinellia	1298[d]	718[d]	180	80	448	645	179	40	2186	0
Spartina	258[d]	0	62	0	105	0	85	0	0	0
Triglochin	0	0	117	40	447	105	0	0	0	0
Total	2074[d]	718[d]	532	120	1692	858	484	40	4942	0

[a] All transplants were plugs except as otherwise noted.

[b] IG = Ile Grande, K = Kerlavos.

[c] All transplants were sprigs.

[d] Half were sprigs and half were plugs in May 1979.

Puccinellia, four other species were also studied intensively. We tested two different types of transplants of four species, spring versus fall planting for four species, conventional and slow release fertilizer materials over a wide range of substrate and elevation conditions for five species, and developed nursery areas for two species. These comparisons and tests resulted in the establishment of 61 separate experiments and plantings over about 0.3 ha (Figs. 27, 28, 29, 30). The smallest experiment contained only 27 transplants while the largest contained over 1,000 transplants. The results from selected plantings are contained in the sections of this report that follow.

Although quantitative measures (survival, cover and dry weight) are important in assessing transplant response to fertilizer materials and local site conditions, qualitative measures of plant response such as sequential photographs can also be revealing and supplement data. One of our best documented experimental plantings is at Kerlavos where we compared sprigs and plugs of *Puccinellia* in several fertilizer treatments. The planting was established in May 1979 and after realizing the initial objectives, we refertilized the area to develop a nursery for *Puccinellia* transplants. The pictorial sequence shows the site prior to planting (Fig. 31), immediately after planting and initial fertilization (Fig. 32), 1 year after planting (Fig. 33), and 2 years after planting, 8 months after refertilization (Fig. 34).

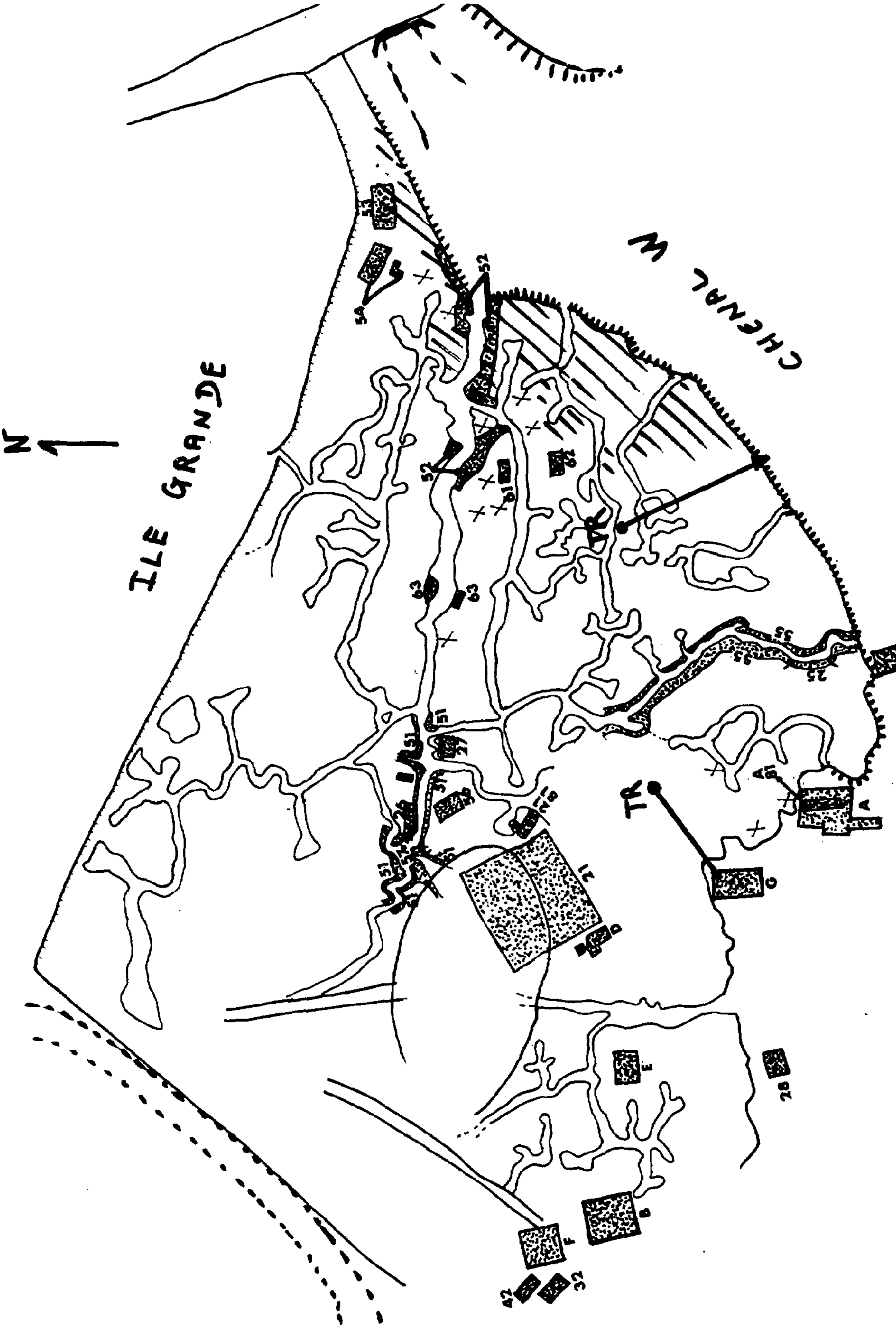

FIGURE 27. Map of study area on northwest side of the bridge at Ile Grande showing location of experimental plantings (stippled areas). Base map by Monsieur Levasseur.

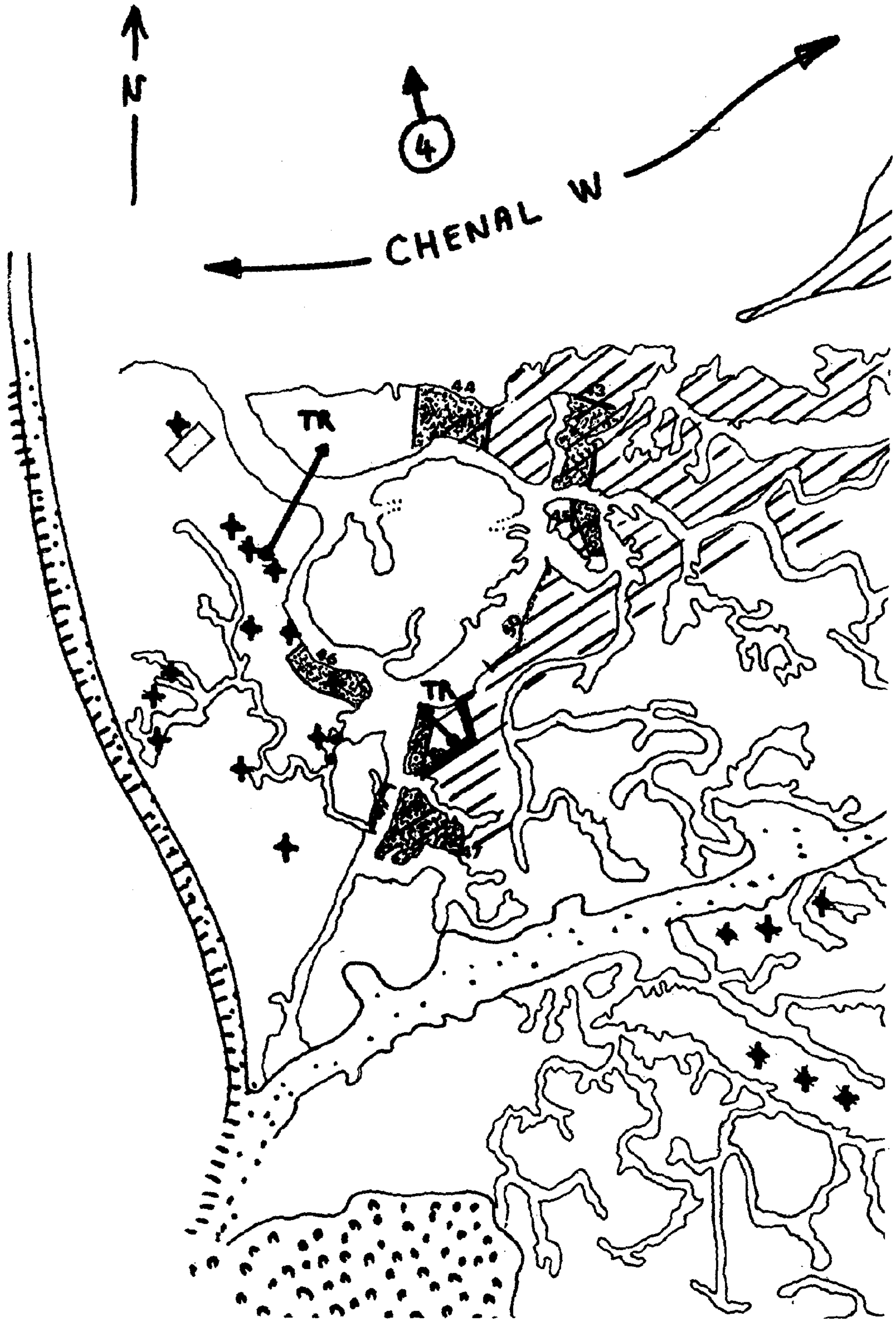

FIGURE 28. Map of study area on southeast side of bridge at Ile Grande showing location of experimental plantings (stippled areas). Base map by Monsieur Levasseur.

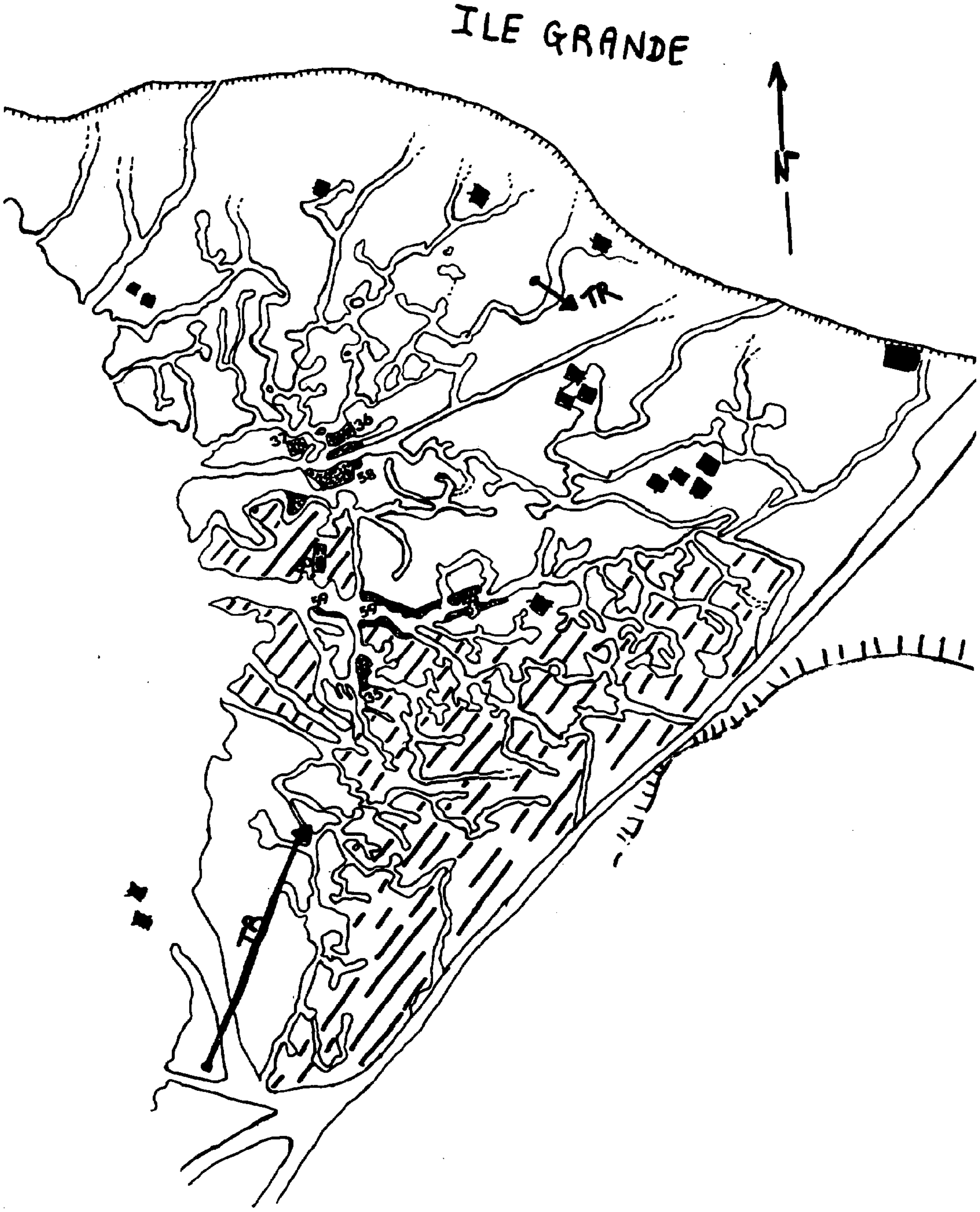

FIGURE 29. Map of study area on northwest side of estuary at Ile Grande beyond area in Figure 28 from bridge showing location of experimental plantings (stippled areas). Area is just beyond road from village to several houses on edge of estuary. Base map by Monsieur Levasseur.

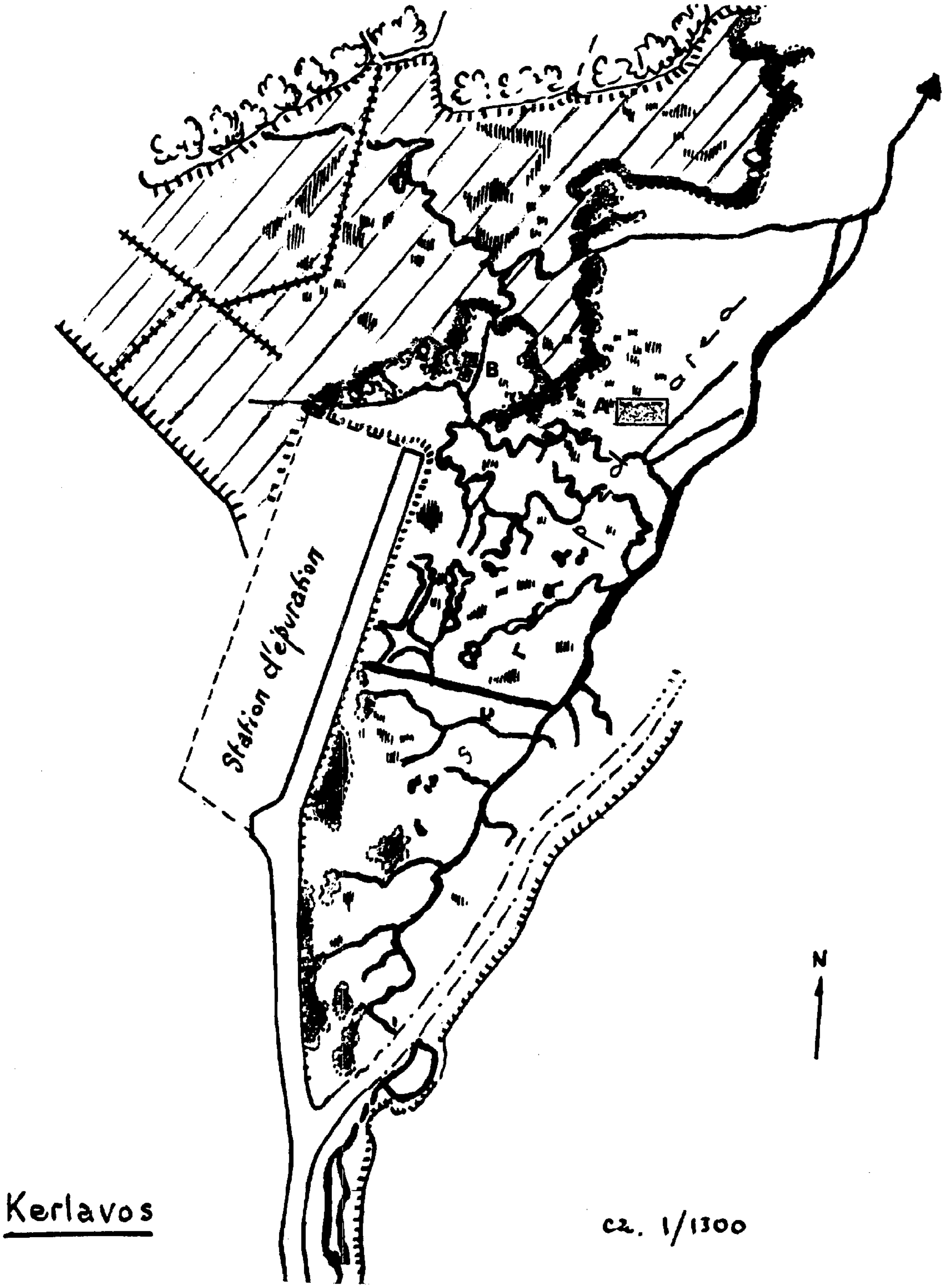

FIGURE 30. Map of study area in estuary at Kerlavos showing location of experimental plantings (stippled areas). Base map by Monsieur Levasseur.

FIGURE 31. Site for experimental planting at Kerlavos prior to planting in May 1979.

FIGURE 32. Same site as in Figure 31 just after planting with *Puccinellia* sprigs and plugs on 0.5-m spacing in May 1979.

FIGURE 33. Same site as in Figure 32 in May 1980, 1 year after planting. There are six rows of sprigs and six rows of plugs which alternate with each other beginning with a row of sprigs on the extreme left. The tallest plants in the center are plugs in the Mag Amp + Osmocote fertilizer treatment.

FIGURE 34. Same site as in Figure 32 in May 1981, 2 years after planting. The planting is now a Puccinellia nursery area.

Survival

In September, 4 months after our initial planting, the survival of plugs was significantly greater than that of sprigs for all three species (Table 3). Survival averaged over transplant type was about 65% for both Puccinellia and Spartina but only about 50% for Juncus. Reevaluation of these May 1979 transplants 1 year after planting indicated that significant mortality of both transplant types occurred over winter (Table 3). Greater overwinter mortality occurred in plug than in sprig transplants for both Puccinellia and Juncus. These results suggest that whatever factors were causing mortality in the sprigs were still affecting the plugs and that it was simply taking longer to cause mortality in the larger transplant type. Overall survival was less than 50% for both transplant types for all species, but still significantly higher for plugs than for sprigs. These relatively low survival percentages included plantings in unfavorable (low elevation, exposed, poorly drained) locations, since we were trying to determine response over a wide range of conditions. In the more favorable sites, at about the elevation of the natural Juncus marsh, survival of plug transplants was consistently above 70% for Puccinellia. On those planting sites where 10% or more of the transplants survived through the second year, plugs continued to survive better than sprigs for Puccinellia and Juncus (Table 4). Of all the 1979 transplants, only those of Puccinellia on the better sites yielded survival values of greater than 60%.

TABLE 3. Percent survival at 4 and 12 months for two types of transplants[a] for three species for the combined plantings made at Ile Grande and Kerlavos in May 1979.

Species	Survival (%) by time[b] by type			
	4 months		12 months	
	Sprig	Plug	Sprig	Plug
Juncus	22	80	14	37
Puccinellia	48	84	31	47
Spartina	56	80	10	26
Averaged over species	44	84	29	37

[a] There were 230, 979, and 100 transplants of each type for Juncus, Puccinellia and Spartina, respectively.

[b] Survival of plugs was significantly greater than that of sprigs within and over all three species at each of the two sampling periods based on chi-square analysis. The reduced survival of both transplant types over species between the two sampling periods was also significant based on chi-square.

TABLE 4. Survival of two types of transplants of three species 1 and 2 years after planting (May 1979) averaged over all locations on more favorable sites.

Species	Survival (%)[a]			
	May 1980		May 1981	
	Sprig	Plug	Sprig	Plug
Puccinellia	64	87	63	82
Juncus	4	39	2	23
Spartina	10	26	4	10

[a] There were 379, 120 and 50 transplants planted of each type for Puccinellia, Juncus and Spartina, respectively.

Except for Puccinellia transplants, these survival data from May 1979 plantings are not impressive. We were in the process of learning where to plant with regard to elevation, the best type of transplant to use, the appropriate species, whether spring was better than fall planting, and how to satisfy the nutrient requirements of the transplants with fertilizer materials. Survival data for the May 1980 plantings indicate a significant increase in survival over those of the earlier plantings (Table 5). These results indicate that we were making progress and that except for Juncus, survival values greater than 50% were achieved for all species tested. The seemingly erroneous survival value for Halimione 4 months after planting is due to aboveground material appearing dead but the underground material being alive and giving rise to new aboveground growth the following spring. A decrease in survival overwinter of greater than 14% was only noted for Juncus which experienced a 41% decrease. Spring appears to be a better season to transplant than fall but the data are incomplete at this time.

Type of Transplant

The best comparison between sprig and plug transplants of Puccinellia from our experiments is the data from Kerlavos where cover and survival were higher for plug transplants except for the conventional ammonium sulfate + concentrated superphosphate treatment where the cover of sprigs was higher than that of plugs (Table 6, Fig. 33). The reduced survival for both types of transplants and especially sprigs in the ammonium sulfate + concentrated superphosphate (2.8 g N + 4.1 g per transplant) treatment was due to a small depression without exterior drainage which occupied a portion of this treatment and in which transplants did not survive (Fig. 33). Increased salinity due to evaporation or waterlogged substrate conditions due to prolonged ponding could have contributed to the reduced survival of this treatment. Although this drainage condition was restricted to a small

TABLE 5. Survival of sprig type Halimione transplants and plug type transplants of four other species at two sampling dates averaged over all locations; planted May 1980.

Species	Survival (%)[a]	
	Sep 1980	May 1981
Halimione	45	52
Juncus	80	39
Puccinellia	95	83
Spartina	96	89
Triglochin	82	68

[a] There were 440 Halimione, 360 Juncus, 823 Puccinellia, 105 Spartina, and 822 Triglochin transplants planted.

TABLE 6. Cover in September of 1979 and 1980 and survival in September 1980 for sprig and plug type Puccinellia transplants for six fertilizer treatments at Kerlavos; planted May 1979.

Treatment	Amount (g) per transplant		Cover (cm^2)[a]				Survival (%)[b]	
			Sep 1979		Sep 1980		Sep 1980	
	N	P	Sprig	Plug	Sprig	Plug	Sprig	Plug
Control	0	0	34	91	46	174	65	95
Ammonium sulfate[c]	2.8	1.2	249	128	338	292	78	95
Mag Amp + Osmocote 3	2.8	4.1	77	260	234	533	50	83
Ammonium sulfate[c]	2.8	4.1	154	188	177	275	40	70
Ammonium sulfate	2.8	0	79	108	135	264	75	95
Concd superphosphate	0	1.2	62	107	145	206	75	78
Avg. over treatment			109	147	152	291	64	86

[a] Cover was ca. 5 cm^2 for sprigs and ca. 25 cm^2 for plugs at planting. Standard error of difference between equally replicated transplant type means and among fertilizer treatment means: 40 for September 1979, 57 for September 1980, n=10.

[b] There were 40 transplants of each transplant type per fertilizer treatment at planting.

[c] Source of P was concentrated superphosphate.

area (12 m^2), and was an exception to the relatively uniform topography of the experimental site, it served to emphasize the importance of adequate drainage for plantings of Puccinellia.

When cover is averaged over treatment for the period from September 1979 to September 1980, the cover value for plugs increased two fold whereas that for sprigs increased by about 40%. Average survival over this same period of time was 22% higher for plugs than for sprigs. Sixteen months after planting, cover for plugs was significantly higher in the Mag Amp + Osmocote 3 (estimated to last for 3 months) treatment and for sprigs it was significantly higher in the ammonium sulfate + concentrated superphosphate treatment (2.8 g N + 1.2 g P). The controls achieved only 14 and 33% of the cover of the best treatments for sprigs and plugs, respectively, over this 16-month period.

Response to Fertilization

Kerlavos

Analysis of variance of cover and dry weight data of plug type transplants of Puccinellia on a disturbed site at Kerlavos indicated a significant response to fertilizer materials (Tables 7, 8). One year after planting the cover of plugs in all three fertilizer treatments containing both nitrogen and phosphorus was significantly greater than that of plants in those treatments which provided only nitrogen or phosphorus or neither (Table 7). These results emphasize the requirement for fertilizer materials on those disturbed sites which substrate samples indicated contained amounts of nitrogen and phosphorus which were too low for good initial growth of transplants.

The dry weight of aboveground plant samples from the Mag Amp + Osmocote 3 slow release fertilizer treatment was significantly greater than that of plants from any other treatment at 4 and 16 months after planting (Table 8). Although cover in the Mag Amp + Osmocote 3 treatment was not significantly different from that in the two conventional ammonium sulfate + concentrated superphosphate treatments 1 year after planting, by 16 months after planting, cover of plants in this Mag Amp + Osmocote 3 treatment was significantly greater than that of those in any other treatment. Cover of plants in this treatment was about twice that of transplants in the second best treatment. The center row of transplants (plugs) in Figure 33 is the Mag Amp + Osmocote 3 treatment. These data indicate the advantage of a slow release over a conventional fertilizer material on this disturbed site for a relatively long period (16 months). We excavated the belowground portion of several healthy transplants in the Mag Amp + Osmocote 3 treatment and noted that the fertilizer material still present below the transplant could be identified after 4 months (Fig. 35).

Cover in the control plants remained significantly below that of plants in those three treatments which provided both nitrogen and phosphorus 16 months after planting. Cover in these control plants was

TABLE 7. Cover of plug-type <u>Puccinellia</u> transplants on three sampling dates for six fertilizer treatments at Kerlavos; planted May 1979.

Treatment	Amount (g) per transplant		Cover (cm^2)[a,b]		
	N	P	May 1980	Sep 1980[c]	May 1981
Control	0	0	66	174	388
Ammonium sulfate[d]	2.8	1.2	122	292	638
Mag Amp + Osmocote 3	2.8	4.1	209	533	819
Ammonium sulfate[d]	2.8	4.1	187	275	687
Ammonium sulfate	2.8	0	73	264	481
Concd superphosphate	0	1.2	106	206	501
Avg. over treatment			127	291	586

[a] Cover of a plug at planting was ca. 25 cm^2.

[b] Standard error of difference among equally replicated fertilizer treatment means: 48 for May 1980, 49 for September 1980, 107 for May 1981; n=10. Comparison of initial fertilizer treatments for May 1981 may not be entirely appropriate because of the refertilization.

[c] Refertilized with Mag Amp + Osmocote 3 (2.8 g N + 4.1 g P per transplant) in September 1980 after data collection.

[d] Source of P was concentrated superphosphate.

TABLE 8. Aboveground dry weight of plug type *Puccinellia* transplants in September 1979 and 1980 for six fertilizer treatments at Kerlavos; planted May 1979.

	Amount (g) per transplant		Aboveground dry wt (g)[a]	
Treatment	N	P	Sep 1979	Sep 1980
Control	0	0	1.9	10.7
Ammonium sulfate[b]	2.8	1.2	3.8	23.3
Mag Amp + Osmocote 3	2.8	4.1	10.1	52.5
Ammonium sulfate[b]	2.8	4.1	4.5	22.1
Ammonium sulfate	2.8	0	3.3	14.6
Concd superphosphate	0	1.2	3.1	15.5
$s_{\bar{d}}$[c]			3.0	6.2

[a] Aboveground dry weight at planting was less than 1 g.

[b] Source of P was concentrated superphosphate.

[c] Standard error of difference among equally replicated fertilizer treatment means, n=3.

FIGURE 35. Excavated *Puccinellia* transplant 4 months after planting showing new roots (white) and slow release fertilizer material still in place.

significantly below that of those same three fertilizer treatments 2 years after planting even though these initial control plants were fertilized with Mag Amp + Osmocote 3 in September 1980. Although comparison of the original fertilizer treatments is confounded and may not be entirely appropriate in May 1981 since all transplants were refertilized in September 1980, it is interesting to note that the same three fertilizer treatments with both nitrogen and phosphorus continued to have cover values which were significantly higher than those of plants in any other treatment. Cover of transplants in the best fertilizer treatment achieved an average radial spread of about 10 cm annually (Fig. 36). At this rate of spread, these Puccinellia plants would achieve complete substrate cover in about 3 years after planting (Fig. 37).

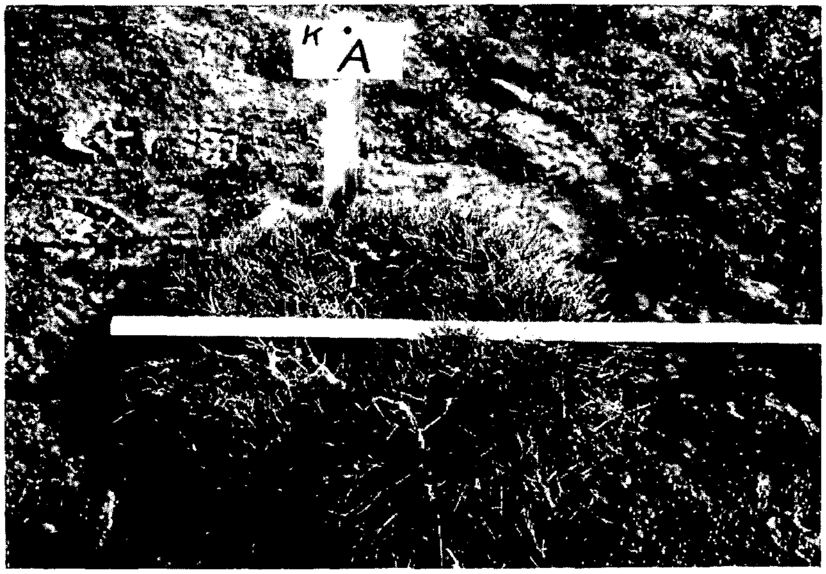

FIGURE 36. A 2-year old Puccinellia transplant with an average diameter of ca. 60 cm and cover of ca. 2,800 cm^2 or 112 times that of the transplant at planting.

FIGURE 37. Several 2-year old Puccinellia transplants that were planted 0.5 m apart. The substrate should be completely covered by these plants by May 1982.

Ile Grande

Analysis of variance of cover data of Halimione and Puccinellia transplants 1 year after planting on a disturbed site at Ile Grande indicated a significant response to fertilizer materials (Table 9). Best growth as measured by cover was achieved by both species in the Mag Amp + Osmocote 3 treatment (Table 9, Figs. 38, 39). Cover of Halimione transplants in this treatment was significantly higher than that of transplants in any other treatment except for the Osmocote 8-9 (estimated to last 8 to 9 months) + concentrated superphosphate treatment. The cover data for Puccinellia transplants indicate the advantage of slow release over conventional fertilizer materials at this particular site. Apparently leaching of the conventional fertilizer materials was a problem because of the coarse sandy substrate in this planting. Significantly greater cover of Puccinellia was produced by the slow release fertilizer treatments than by ammonium sulfate + concentrated superphosphate except where the rate of ammonium sulfate was doubled (5.6 g N per transplant).

Differences among the cover values of the Triglochin transplants are meaningless except to document the poor growth by this species under all experimental treatments. The response by Triglochin in the particular experiment is representative of its response at several experimental sites and is the reason for our decision to delete it from

TABLE 9. Cover and survival of transplants of three species in May 1981 for nine fertilizer treatments at Ile Grande, planted May 1980.

	Amount (g) per transplant		Cover (cm^2)[a] by species[c]			Survival (%)[b] by species[c]		
Treatment	N	P	H	P	T	H	P	T
Control	0	0	326	319	26	100	100	87
Ammonium sulfate	2.8	0	108	305	14	60	100	73
Ammonium sulfate[d]	2.8	1.2	128	302	25	67	100	93
Ammonium sulfate[d]	5.6	1.2	100	460	12	100	100	93
Mag Amp + Osmocote 3	2.8	4.1	535	725	23	100	100	60
Osmocote 3	2.8	1.2	277	556	14	67	100	93
Osmocote 8-9	2.8	0.4	228	449	31	73	100	73
Osmocote 8-9	5.6	0.8	272	578	9	73	100	60
Osmocote 8-9[e]	2.8	1.2	366	611	21	60	100	93
$s_{\bar{d}}$[f]			103	93	7			

[a] Cover of a plug transplant for *Puccinellia* and *Triglochin* was ca. 25 cm^2 at planting; n=14 for *Puccinellia*, n=8 for *Triglochin*. Sprigs of *Halimione* had quite variable cover at planting, but generally less than 50 cm^2; n=8.

[b] There were 15 transplants per treatment for each species.

[c] H = *Halimione*, P = *Puccinellia*, T = *Triglochin*.

[d] Source of P was concentrated superphosphate.

[e] Source of additional P was concentrated superphosphate.

[f] Standard error of difference among equally replicated treatment means.

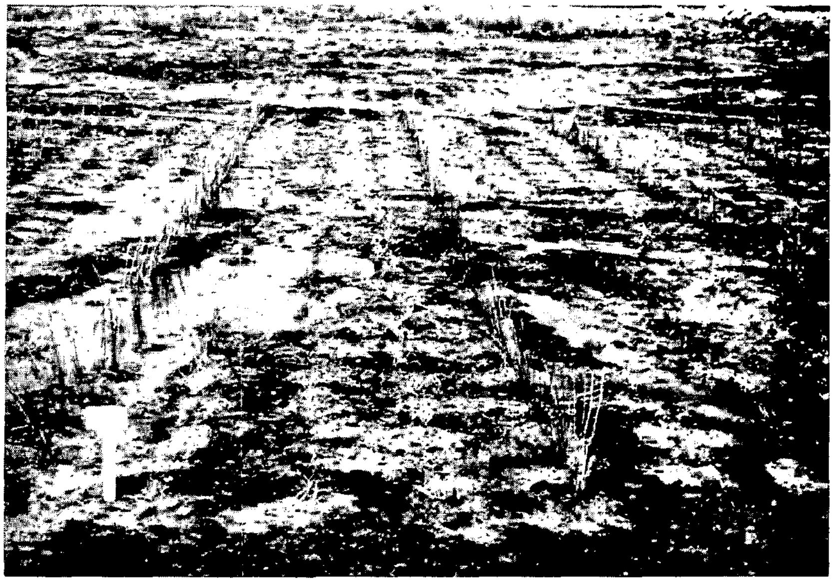

FIGURE 38. Experimental planting at Ile Grande to determine response to fertilizer by four species, from right of stake: Puccinellia, Triglochin, Halimione, and Juncus just after planting in May 1980.

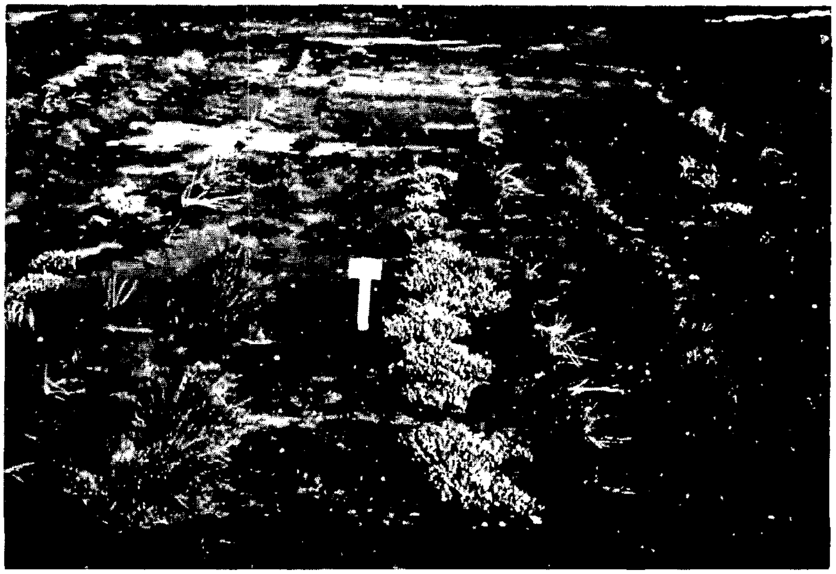

FIGURE 39. Same experimental planting as shown in Figure 39 in May 1981, 1 year after planting. First seven plants in foreground were refertilized with same fertilizer materials as in initial treatments (Table 9) in September 1980, 4 months after planting.

further consideration as a desirable species in our rehabilitation efforts.

The relatively high survival of all three species indicates a marked improvement in our selection and handling of transplants as well as the selection of a favorable planting site. These survival percentages were based on 135 transplants per species in this particular experiment. The high survival of Puccinellia transplants coupled with the relatively high cover values as compared to those of the other two species indicates that our emphasis on this species is justified.

Analysis of variance of cover data indicates that the Osmocote 8-9 slow release fertilizer material maintained the original cover of Spartina transplants at planting with very little growth through the first year (Table 10). Transplants in the control and ammonium sulfate + concentrated superphosphate treatments decreased in cover over the first year. Although growth was not good, survival of these and other 1980 Spartina transplants was consistently above 80% (Table 5, Figs. 40, 41). Growth of this species has been very slow in all our experimental plots but because it can occupy lower elevations than most of the other species, we plan to continue to experiment with it on a limited scale.

TABLE 10. Cover and survival of plug type Spartina transplants in May 1981 for three fertilizer treatments at Ile Grande; planted May 1980.

Treatment	Amount (g) per transplant N	P	Cover[a] (cm^2)	Survival[b] (%)
Control	0	0	11	93
Ammonium sulfate[c]	2.8	1.2	15	100
Osmocote 8-9[d]	2.8	1.2	29	80

[a] Cover of a plug transplant at planting was ca. 25 cm^2. Standard error of difference among equally replicated treatment means = 6.1, n=6.

[b] There were 15 transplants per treatment planted.

[c] Source of P was concentrated superphosphate.

[d] Source of additional P was concentrated superphosphate.

FIGURE 40. Experimental plantings of Spartina at Ile Grande to determine transplant response to fertilizer materials just after planting in May 1980.

FIGURE 41. Same experimental planting as shown in Figure 40 in May 1981, 1 year after planting.

Response to Refertilization

Analysis of variance indicated that refertilized Puccinellia transplants produced significantly more cover than those not refertilized (Table 11). Without refertilization transplant cover increased by 2.1 times from September 1980 to May 1981 whereas with one refertilization in September 1980 cover increased 2.9 times by May 1981. Those plants refertilized tiwce (May and September 1980) increased their cover 3.8 times by May 1981 or by 1.9 times between May and September 1980 and by 1.9 times between September 1980 and May 1981. One refertilization increased cover 1.4 times that of the plants which were not refertilized over an 8-month period and two refertilizations increased cover 1.8 times that of the unrefertilized plants over a period of 1 year.

TABLE 11. Cover of Puccinellia transplants at two sampling dates for three fertilizer treatments[a] at Kerlavos, planted May 1979.

Treatment	Cover (cm^2)[b] Sep 1980	 May 1981
Not fertilized	222	460
Refertilized Sep 1980[c]	221	645
Refertilized May 1980[d] and again Sep 1980[c]	428	833

[a] All treatments were fertilized in May 1979 at planting.

[b] Standard error of difference among equally replicated treatment means = 115, n=11.

[c] Refertilized with Mag Amp + Osmocote 3 (2.8 g N, 4.1 g P per transplant).

[d] Refertilized with Osmocote 8-9 + P (2.8 g N + 1.2 g P per transplant).

Response to Fresh Oil

In the spring of 1980 oil from the Tanio reached the estuary at Kerlavos. Although it was observed on several of our 1979 transplants, we could not document any adverse effects. We decided to take advantage of the opportunity to plant in some of the fresh oil deposits along a creek bank. The marsh surface of the planting site had been removed in a cleanup of Amoco Cadiz oil earlier. The fresh Tanio oil was a superficial layer on the substrate which did not appear to penetrate into the substrate. In May 1980 transplants of Halimione and

Puccinellia were planted at one of these freshly oiled sites (Fig. 42). Cover and survival data determined 1 year later indicate no noticeable effect of the oil on either species (Table 12, Fig. 43) as compared to these data from transplants at unoiled sites which were planted the same year (Table 9). As in many other experiments, cover data indicated a significant transplant response to fertilizer materials with best growth realized in the Osmocote slow release treatment.

TABLE 12. Cover and survival of Halimione (sprigs) and Puccinellia (plugs) transplants in May 1981 at a site oiled by the Tanio in the spring 1980; planted May 1980.

			May 1981			
	Amount (g) per transplant		Halimione		Puccinellia	
Treatment	N	P	Cover (cm^2)[a]	Survival (%)	Cover (cm^2)[a]	Survival (%)
Control	0	0	86	67	381	100
Ammonium sulfate[b]	2.8	1.2	258	100	493	67
Osmocote 8-9[c]	2.8	1.2	631	67	550	100

[a] Cover of Halimione sprigs was quite variable at planting, but was generally less than 50 cm^2; that of a Puccinellia plug was ca. 25 cm^2. Standard error of difference among equally replicated treatment means; 100 for Halimione, 160 for Puccinellia, n=3.

[b] Source of P was concentrated superphosphate.

[c] Source of additional P was concentrated superphosphate.

Creek Bank Plantings

Creek banks with no vegetation cover are one of our top priority planting sites. Preliminary plantings of Halimione made in May 1980 (Fig. 44) achieved over 90% survival and good growth by the following May (Fig. 45). Puccinellia plantings have also achieved good survival and growth over this period of time (Figs. 46, 47). About half of the over 4,900 May 1981 transplants were planted along creek banks (Fig. 20). A similar proportion of the overall planting effort is planned for creek bank sites in May 1982.

FIGURE 42. Planting Puccinellia and Halimione in fresh oil from the Tanio in the estuary at Kerlavos in May 1980.

FIGURE 43. Same experimental planting as shown in Figure 42 in May 1981, 1 year after planting: Puccinellia on left, Halimione on right.

FIGURE 44. Preliminary planting of *Halimione* sprigs along a creek bank at Ile Grande in May 1980.

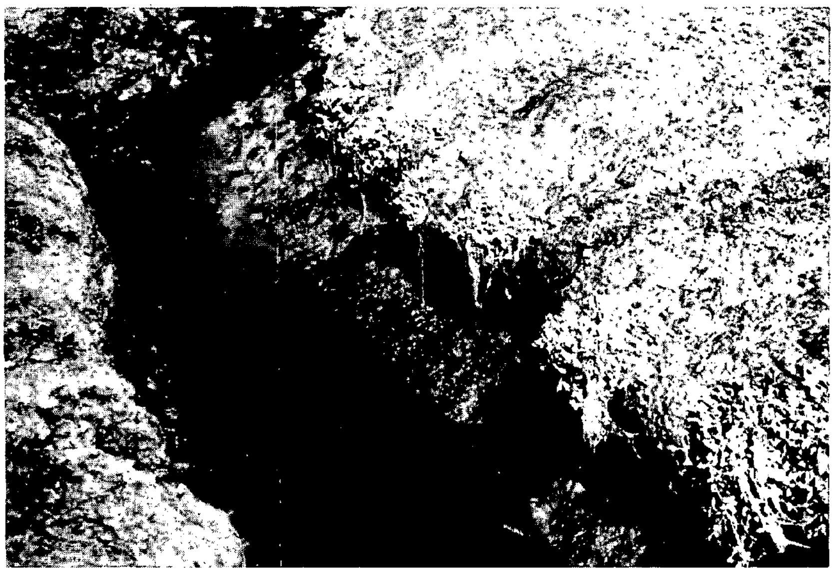

FIGURE 45. Same site as shown in Figure 44 in May 1981, 1 year after planting.

FIGURE 46. Experimental planting of Puccinellia along a creek bank at Ile Grande in May 1980 just after planting.

FIGURE 47. Same site as shown in Figure 46 in May 1981, 1 year after planting.

Transplant Time Requirement

It is difficult to determine the time involved in the transplanting operations when experimental plantings are being established. In May 1981 we kept records of the time required for four persons to dig and transplant sprigs of *Halimione* and plugs of *Puccinellia*. *Halimione* plants were dug, separated into sprigs and put into plastic bags for transport to the planting site at the rate of about 180 per person hour. *Puccinellia* plants were dug, cut into plugs and put into a container for transport to the planting site at the rate of about 75 per person hour. These rates indicate that *Halimione* sprigs can be obtained about 2.4 times faster than *Puccinellia* plugs.

The planting operation includes opening the transplant hole with a soil auger, inserting the appropriate amount of fertilizer, and inserting the transplant and firming the substrate around it. Both types of transplants can be planted at the rate of about 40 per person hour.

These time requirements for digging and planting make no allowance for travel, supplies, and equipment, which must also be considered in the total cost of a planting operation. Based on our digging and planting time requirements only, the time required to plant 1 ha of *Halimione* on a 0.5 m spacing (40,000 transplants) would be about 1,220 person hours (220 person hours to dig sprigs + 1,000 person hours to plant). The time required to plant 1 ha of *Puccinellia* on a 0.5 m spacing would be about 1,530 person hours (530 person hours to dig + 1,000 person hours to plant). These cost estimates indicate that it would take four persons working 8 hour days about 38 days to plant 1 ha of *Halimione* on a 0.5 m spacing and about 48 days to do the same using *Puccinellia*.

Recovery of Transplant Source Sites

From the beginning of our restoration efforts we were aware of the potential for impact to the natural marsh in digging transplants for the plantings. Consequently, we confined our digging of plants in the natural marsh to areas adjacent to narrow drainageways (Fig. 6) or to small areas (0.25 m^2) in the marsh. All *Puccinellia* transplant source sites were replanted and those areas that were dug in 1979 and 1980 were almost completely revegetated by May 1981 (Figs. 48, 49). In a further attempt to lessen the pressure for obtaining transplants from the natural marsh, we have initiated nursery areas for *Halimione* and *Puccinellia*. These combined actions will help keep impact to the natural marsh to a minimum and serve as a model for others who may engage in similar activities in the future.

Nursery Plantings

The *Puccinellia* nursery area at Kerlavos was established in May 1979 and now contains about 300 plants that can be dug and separated into transplants. Although the plants vary in size, the average cover

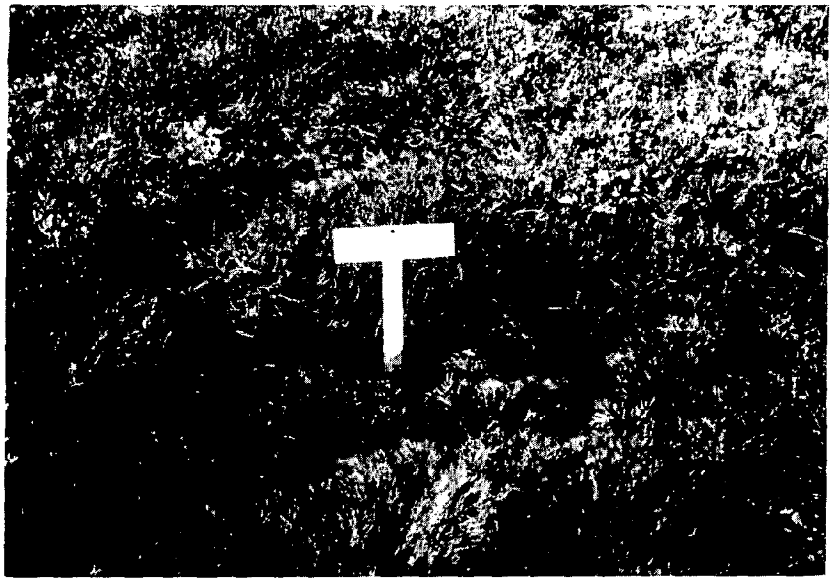

FIGURE 48. Site where Puccinellia transplants were dug in May 1980. The area was replanted and was becoming rapidly revegetated in September 1980, 4 months after digging.

FIGURE 49. Same site as shown in Figure 48 in May 1981, 1 year after digging. Vegetation cover is almost complete.

is about 540 cm^2 or over 20 times that of a plug type transplant. To determine the actual number of plugs that could be obtained from a sample of plants, we dug 11 plants from the row nearest the estuary in May 1981 (Fig. 50). One of the largest plants yielded 50 plugs (Figs. 51, 52, 53), but the average number of plugs per plant dug was 20, which agreed well with what we predicted based on the average cover. Since the nursery area contains about 300 plants, we can predict that it could have yielded a minimum of 6,000 plug type transplants in May 1981. It seems reasonable to assume that cover will increase from 50 to 100% by our next major planting effort in May 1982. This assumption translates into a conservative estimate of about 10,000 plug type transplants or enough to plant 0.25 ha on a 0.5 m spacing.

The Halimione nursery area at Ile Grande was established in May 1980 and added to in May 1981. It contains about 200 plants that can be dug and separated into transplants (Fig. 54). In May 1981 we dug a sample of seven plants to determine the average number of sprig type Halimione transplants that could be obtained per plant dug. We obtained an average of five sprigs from each plant dug (Fig. 55). Based on 200 plants in the nursery area, we estimate that there were about 1,000 Halimione transplants available in May 1981. We estimate that the increase in cover by May 1982 will result in about 1,500 to 2,000 Halimione sprigs available for digging at that time which would plant about 0.05 ha on a 0.5 m spacing.

FIGURE 50. Row of 2-year old Puccinellia transplants nearest the estuary in the nursery area at Kerlavos, May 1981.

FIGURE 51. Sample Puccinellia plant that was dug for transplants from the same row of plants shown in Figure 50, May 1981.

FIGURE 52. Cutting the same plant shown in Figure 51 into plug type transplants.

FIGURE 53. Plant shown in Figure 51 yielded 50 plug type transplants.

FIGURE 54. A 1-year old Halimione transplant in the nursery area at Ile Grande.

FIGURE 55. Halimione sprigs being dug from the nursery area at Ile Grande.

Invasion of Plantings by Other Plants

Observations at Ile Grande and Kerlavos indicate that other marsh plants invade our experimental plantings more rapidly than they colonize areas that still lack vegetation cover as a result of cleanup operations. In one of our May 1979 experimental plantings of Puccinellia at Kerlavos (Figs. 56, 57, 58), 97% of the transplants in the 60 m^2 area had been invaded by at least one other species by May 1981 (Fig. 59). Of these transplants which had been invaded, 66% were invaded by two or more other species. The most abundant invader was an annual species of Salicornia which was present in 94% of the transplants sampled. Other invading genera in the order of their percentage of presence per transplant sampled were Cochleria (49%), Halimione (24%), Spergularia (10%), and Armeria (1%).

FIGURE 56. Experimental planting of *Puccinellia* at Kerlavos in May 1979 just after planting.

FIGURE 57. Same experimental planting as shown in Figure 56 in May 1980, 1 year after planting.

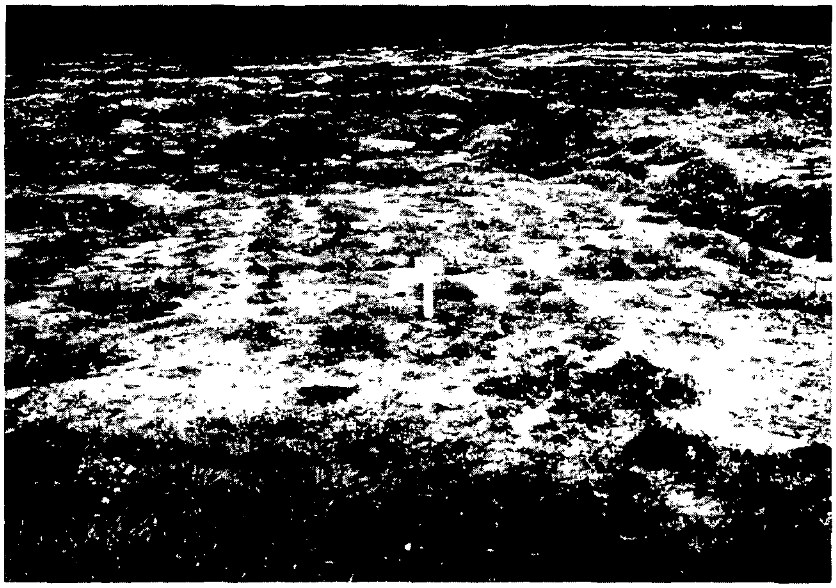

FIGURE 58. Same experimental planting as shown in Figure 56 in May 1981, 2 years after planting.

FIGURE 59. A 2-year old Puccinellia transplant from the experimental planting shown in Figure 56 with two invading marsh plants: Cochlearia (white flowers) and Salicornia (left center near cluster of white flowers).

ACKNOWLEDGEMENTS

Cooperation with our French colleagues (Madames Le Campion-Alsumard, Plante-Cuny, and Vacelet from Marseille and Monsieur Levasseur and Mademoiselle Jory from Rennes) has been invaluable. In fact, our work would have just about been impossible without their help. They have gone out of their way to help us while we were in France, such as arranging a meeting with the Mayor of Pleumeur-Bodou, making observations on our experimental plots during the interim of our visits, and providing laboratory facilities for processing samples. Presently we are cooperating with Monsieur Levasseur on nursery production of transplants and monitoring of our plantings until November 1982. He has also agreed to serve as the major professor for a graduate student from the United States who is a candidate for a Fulbright Scholarship. Among his tasks, this student would follow our plantings and document the invasion of these plantings by other marsh plants subsequent to our active involvement in the project. In summary, the association with our French colleagues has been very beneficial from our standpoint and we cannot overemphasize the vital part they have played in making our research proceed smoothly.

We thank Amoco Oil for providing the funds for our research through NOAA Contract No. NA79RAC00018. We thank NOAA personnel especially Drs. W.N. Hess and D.A. Wolfe for providing us the opportunity to conduct this very timely and environmentally beneficial research and for their cooperation throughout the period of the project.

Last, special thanks for technical assistance in field work and in data analysis go to Messrs. C.L. Campbell and L.L. Hobbs who made the overall research effort a success.

SUMMARY

Experimental plantings of *Halimione portulacoides*, *Juncus maritimus*, *Puccinellia maritima*, *Spartina maritima*, and *Triglochin maritima* have been made at Ile Grande and Kerlavos, France in an attempt to rehabilitate salt marsh that was impacted by the Amoco Cadiz oil spill and subsequent cleanup operations. Over 61 experimental plantings including over 11,000 transplants have been established to test two types of transplants, conventional and slow release fertilizer materials over a wide range of substrate and elevation conditions and to develop nursery areas. *Spartina* transplants survived at lower elevations than those of any other species tested, but the best growth of transplants of all species tested occurred within ± 0.3 m of the elevation of the natural marsh in the vicinity. Survival and growth data indicate that transplants of *Puccinellia* with a core of root and substrate material intact (plugs) were superior to those transplants with roots only (sprigs).

Although there was considerable variation in response to fertilizer materials and rates, both nitrogen and phosphorus were required for good transplant growth on the disturbed sites tested. Slow release fertilizer materials produced better growth over a wide range of substrate types than did the conventional, more soluble fertilizer materials. Higher survival and better growth were obtained with Halimione and Puccinellia transplants than with those of the other three species tested. Aboveground growth of the best experimental plantings of Puccinellia spread radially at the rate of about 10 cm annually. At this rate of spread, these experimental plantings would achieve complete substrate cover in about 3 years after planting. Refertilization at various periods after planting produced a significant increase in cover.

Halimione sprigs were dug at the rate of about 180 per person hour and plugs of Puccinellia at the rate of about 75 per person hour. Transplants of both species were planted and fertilized at the rate of about 40 per person hour. Sites in the natural marsh from which Puccinellia transplants were dug, were replanted and became almost completely revegetated within 1 year. Nursery areas were established for both Halimione and Puccinellia and estimates indicated that in May 1981 they contained about 6,000 transplants of Puccinellia and 1,000 of Halimione. Preliminary data indicate that other marsh plants invade our plantings more rapidly than they invade unplanted disturbed sites.

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ETUDES MICROBIOLOGIQUES ET MICROPHYTIQUES DANS LES SEDIMENTS DES MARAIS MARITIMES DE L'ILE GRANDE A LA SUITE DE LA POLLUTION PAR L'AMOCO CADIZ

par

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PRESENTATION SOMMAIRE DES BIOTOPES ET STATIONS ETUDIES

Trois des biotopes caractéristiques des marais maritimes - schorres, chenaux de schorre, et haute-slikke - ont été retenus pour cette étude dans deux sites, différant très nettement quant à l'importance de la pollution par le pétrole de l'Amoco Cadiz (Fig. 1A - site Sud, Sud-Ouest très pollué ; site Est, Nord-Est peu pollué).

Le bloc diagramme (Fig. 1B) montre la différence de niveau altitudinal entre les 3 biotopes, entraînant évidemment des différences de durée d'immersion.

Les Schorres

Les schorres de l'Ile Grande sont des prés-salés à *Juncus maritimus* et *Halimione portulacoides* qui présentent une surface plus ou moins tabulaire et un réseau de drainage variable (Fig. 1A, schorre D1 mieux drainé que le schorre A1).

Les deux stations de référence pour le "biotope schorre" sont B1 et C1 (site Est, Nord-Est, Fig. 1A) faiblement atteintes par la pollution du fait de la mise en place d'un barrage de protection sous le pont reliant l'Ile Grande à la terre.

Les schorres très pollués, A1 et D1, sont donc situés dans la partie Sud, Sud-Ouest. Cette partie du marais servit pendant un certain temps de zone de stockage d'hydrocarbures issus du nettoyage des plages et rochers.

Les Chenaux

Ces schorres sont drainés par des chenaux presque constamment immergés, dont le sédiment est une vase fluide (station de référence C2 ; station très polluée A2 - Fig. 1A et B).

Le Biotope Haute-Slikke

Le biotope de vase sableuse intertidale ou haute-slikke, de part et d'autre du chenal central, a été étudié en A3 pour le site pollué et en B3 pour le site peu pollué. Cette dernière station fut remplacée à partir de mars 1980 par E3 (même type de biotope) lorsque les travaux de surcreusement du chenal central éliminèrent la station B.

FIGURE 1

(OPPOSITE)

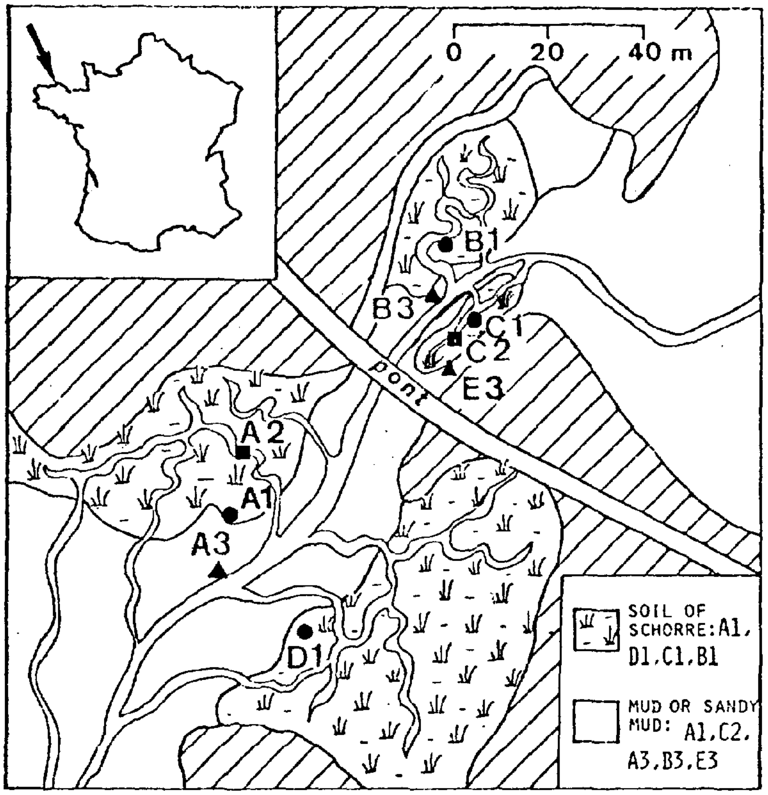
A - Schéma de localisation des stations

Stations de référence = C_1, B_1, schorres
(Est, Nord-Est du pont) C_2, chenal
B_3, E_3, haute-slikke

Stations trés polluées = A_1, D_1, schorres
(Sud, Sud-Ouest du pont) A_2, chenal
A_3, haute-slikke

B - Bloc diagramme représentant les 3 types de biotopes et le niveau des hautes-mers.

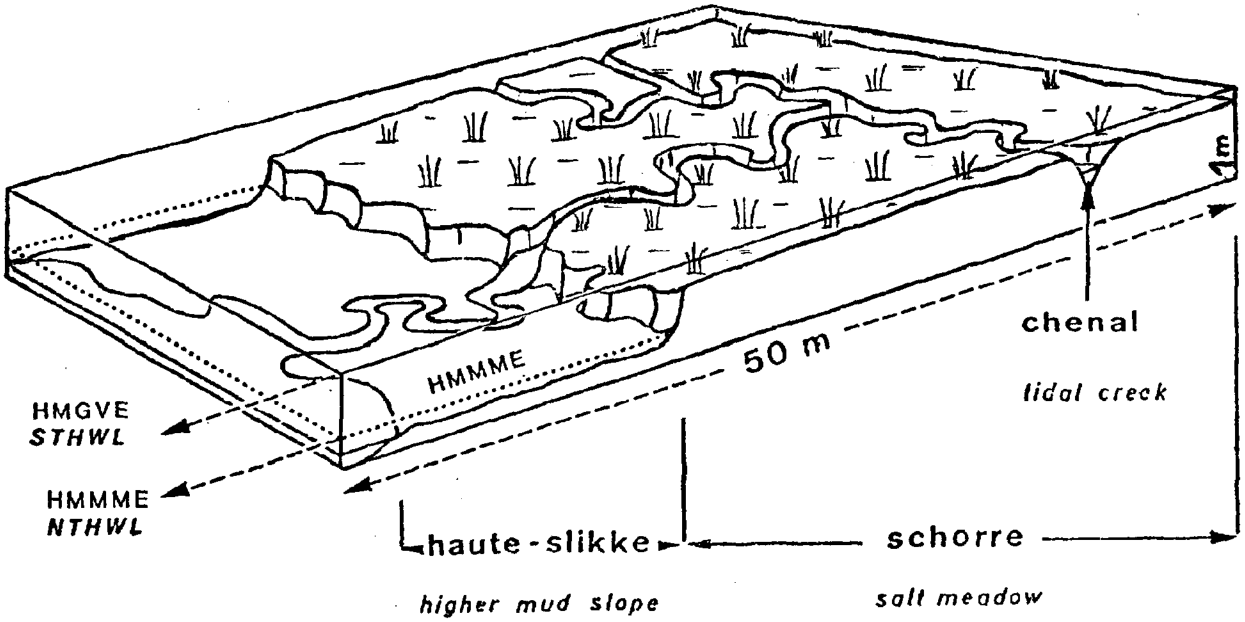
A



SAMPLING SITES

REFERENCE STATIONS = C1, B1 SCHORRES (SALT MEADOWS)
C2 TIDAL CREEK
B3, E3 SLIKKE (MUD SLOPE)
POLLUTED STATIONS = A1, D1 SCHORRES (SALT MEADOWS)
A2 TIDAL CREEK
A3 SLIKKE (MUD SLOPE)

B



ETUDES REALISEES DANS CES BIOTOPES ET METHODES UTILISEES

Neuf missions d'échantillonnage pour l'étude des hydrocarbures et des peuplements bactériens et microphytiques ont été réalisées entre décembre 1978 et novembre 1980 (une dernière mission est programmée en novembre 1981, ce qui donnera un "suivi" de 4 ans).

Divers paramètres physiques et chimiques (températures, salinités, p^H, Eh) ont été mesurés dans l'eau et les sédiments.

Les Hydrocarbures

Les hydrocarbures (HC) ont été dosés et leur composition analysée dans des fractions de carottes à différents niveaux (Tab. 1, 2, 3).

Les concentrations en hydrocarbures totaux (HCT) exprimées en $g.kg^{-1}$ de sédiment sec sont définies comme étant la fraction FA après les traitements suivants appliqués aux échantillons de sédiments :

- extraction de la matière organique au toluène-méthanol sur échantillon humide (Farrington et Tripp, 1975).
- élimination du soufre (pesée avant saponification ⟶ poids AV-SP).
- saponification à la potasse (pesée après saponification ⟶ poids AP-SP).
- fractionnement du produit de la saponification par la méthode dite au "Sep-pak"(micro-colonne de silice à compression radiale) (Giusti et al., 1979).
- élution par trois solvants successifs :

1° hexane	fraction FA = HCT
2° chloroforme	fraction FB = fraction polaire
3° méthanol	fraction FC

L'extrait à l'hexane (FA = HCT) est ensuite analysé en chromatographie liquide haute pression (HPLC reverse-phase) puis en chromatographie gazeuse capillaire (CPG) et infra-rouge lorsque la concentration est suffisante. Une analyse plus fine par spectrométrie de masse couplée avec la CPG est effectuée si nécessaire.

Etude Bactérienne

Pour l'étude bactérienne, l'échantillonnage des sédiments était effectué par carottages.

Les fractions étudiées étaient :

dans les schorres (carotte de 60 cm environ)

- couche de surface (su)
- rhizosphère (rh)
- couches plus profondes (couche argileuse : ca ; couche sableuse : cs) ;

dans les chenaux (carottes de 30 à 40 cm)
- couche de surface (su)
- zone réduite (zr)
- couches plus profondes (ca ou cs) ;

dans les slikkes (carottes de 10 à 40 cm)
- mêmes couches que les chenaux.

L'étude bactérienne de sous-échantillons comprenait :

- dénombrement de la microflore hétérotrophe par MPN sur eau de mer peptonée à 5 $g.l^{-1}$;
- estimation de l'activité bactérienne sur les mêmes ensemencements ; établissement d'une courbe d'activité qui dépend de la présence de souches à croissance rapide ;
- dénombrement des germes capables de dégrader les hydrocarbures par MPN sur milieu minéral contenant du pétrole "Arabian light" comme source de carbone ;
- enzymologie : mise en évidence des différentes hydrolases présentes et estimation comparative de leur activité par la méthode APIZYM.

A la surface des carottes, l'activité enzymatique est due à l'ensemble du peuplement "bactéries + microphytes". Dans les couches plus profondes, seule intervient l'activité bactérienne.

Les peuplements microphytiques

Les peuplements microphytiques étaient étudiés sur des carottes plus courtes (3 premiers centimètres d'épaisseur).

Aspect Quantitatif

L'aspect quantitatif du peuplement est essentiellement appréhendé par l'estimation d'un indice chlorophyllien de biomasse que nous abrègerons en ICB : extraction à l'acétone et mesures (avant et après acidification des extraits) des concentrations en chlorophylle a (Chla ou ICB) et en produits de dégradation de la chlorophylle (phéopigments = Phéo.), méthode de Lorenzen (1967) modifiée par Plante-Cuny (1974) pour les sédiments. Résultats exprimés en $\mu g.g^{-1}$ de sédiment sec.
Rapport Chla/Chla + Phéo : indice de vitalité des peuplements s'il y a prépondérance de la chlorophylle (rapport $>$ 0,5).

Aspect Qualitatif

L'aspect qualitatif du peuplement consiste en une étude écotaxinomique des principales espèces de diatomées et cyanophycées présentes.

Note

Il est évident que dans la présente synthèse tous les résultats obtenus dans les études microbiologiques et microphytiques n'ont pu être pris en compte. Ils feront l'objet de rapports séparés.

(Voir aussi Vacelet et al., et Plante-Cuny et al., 1981).

SYNTHESE DES PRINCIPAUX RESULTATS

Les résultats concernant l'analyse fine des hydrocarbures et de leur éventuelle dégradation font l'objet d'un rapport séparé [x].

Des résultats succints seront donnés ici seulement pour servir à l'interprétation des phénomènes biologiques.

Différents Degrés de Pollution au Début des Observations

Il faut noter ici l'absence de "point zéro" concernant l'état des marais de l'Ile Grande avant l'échouage de l'Amoco Cadiz en mars 1978. Aucune étude préalable n'existait sur ce site et il nous a été impossible de trouver dans une région avoisinante un marais maritime de même type, indemne, pouvant servir de référence.

De sorte que, les stations choisies présentent en fait différents degrés de pollution en décembre 1978, au début de nos observations (HCT en surface, exprimés en g. kg^{-1} de sédiment sec).

Les valeurs ci-dessous, et notamment celles de A1 et D1, sont très élevées en comparaison des valeurs données par Marchand (1981) pour des sédiments profonds (15 à 100 mètres) à la suite du naufrage du "Bohlen". Rappelons que le site de l'île Grande était un lieu de stockage des hydrocarbures après nettoyage d'autres sites.

- schorres encore couverts de mazout en décembre 1978 :
  A1 : 32,97 ; D1 : 94,68
- schorre avec traces de pollution :
  C1 : 4,17
- schorre apparemment indemne :
  B1 : 1,9
- chenaux drainant les schorres :
  très pollué, A2 : 7,69 •
  apparemment indemne, C2 : 3,26
- haute-slikke bordant le chenal central :
  visiblement très polluée, A3 : 5,56 •
  apparemment indemne, B3 : 0,50

Les concentrations évaluées en A2 et A3 (•) ont été mesurées sur des échantillons provenant de l'extrême pellicule superficielle du sédiment (moins de 1 cm d'épaisseur) déjà colonisée par des microphytes.

Evolution des Concentrations en Hydrocarbures et des Peuplements Bactériens et Microphytiques à Partir de Décembre 1978

Dans chacun des 3 biotopes - schorres, chenaux et slikkes - seront faites des comparaisons entre les stations très polluées à différents

[x] Etudes réalisées par Henri Dou, Gérard Giusti et Gilbert Mille. Laboratoire de Chimie Organique A et LA 126 CNRS - Faculté des Sciences de Saint Jérôme - 13397 Marseille cedex 13.

degrés d'une part, et entre les stations très polluées et les stations peu polluées d'autre part.

On expose dans chaque cas : - l'évolution des concentrations en HCT ($g.kg^{-1}$), et de leur éventuelle dégradation ; - l'évolution de l'activité bactérienne, du nombre de germes dégradant les HC, de l'activité enzymatique ; - l'évolution quantitative et qualitative des peuplements microphytiques.

Evolution dans les schorres

Schorres très pollués, D1 et A1.

Schorre D1.

En surface, la station D1 (Tab. 1) présente, presque deux ans après l'échouage, la même concentration en HCT (94,51) qu'en décembre 1978. Il existe dans ce site des zones encore plus polluées (prélèvement intentionnel dans une tache d'hydrocarbures en mai 1980 : 230,60).

Il semble y avoir, en ces points, une accumulation en surface par drainage du schorre environnant, lui-même encore visiblement très pollué en 1980.

Le rapport AV/AP (Tab. 2), indicateur présumé d'une biodégradation, augmente légèrement en 1979 ainsi que l'activité bactérienne : un début de dégradation aurait eu lieu entre 1978 et 1979. On note parallèlement une augmentation du nombre de germes dégradant les HC jusqu'en novembre 1979 (10^4 à 10^7 $germes.ml^{-1}$ de sédiment) suivi d'une régression en 1980 (Fig. 2).

L'activité enzymatique de l'ensemble de la microflore a été importante en décembre 1978, s'est prolongée jusqu'en 1979 ce qui suggère la possibilité d'un effet favorisant des HC. Cette activité régresse en 1980 indiquant peut-être un appauvrissement de la microflore bactérienne (régression de chymotrypsine, trypsine et hydrolases des glucides) (Fig. 5).

Les microphytes (Fig. 8A,D1)totalement éliminés et encore absents en novembre 1978, ont recolonisé peu à peu la surface du sol et ont présenté un maximum non négligeable en juillet 1979 (50 μg $Chla.g^{-1}$). Ensuite, la prépondérance au printemps 80 des phéopigments indique un état peu florissant de la population. Une reprise est amorcée en novembre 1980 (40 μg $Chla.g^{-1}$). Il est possible que cette population végétale comportant un fort pourcentage de cyanophycées réputées riches en hydrocarbures naturels en C^{17} (Han et Calvin, 1969 ; Saliot, 1981) contribue à l'augmentation observée du rapport C^{17}/Pr (Tab. 3) ce qui rend difficiles les interprétations quant à l'état de dégradation des HC.

D1 : Dans la rhizosphère (cf. schémas des carottes, Tab. 1), on note dans le temps une diminution (de 8,13 à 0,23) de la quantité d'HCT et une augmentation du rapport AV/AP (1,20 à 2,06) pouvant indiquer une forte biodégradation. En effet, la concentration en germes dégradant les HC croît de 10^2 à 10^6 $germes.ml^{-1}$ jusqu'en 1980 (Fig. 2). Quant à l'activité enzymatique, elle est fluctuante et plus faible en général en 1980 (Fig. 5).

D1 : Dans les couches plus profondes (30 cm), les concentrations en HCT, faibles au départ (0,48) diminuent en 1979 (0,10). Le nombre des germes dégradant les HC a cru jusqu'en avril 1980 puis a diminué (Fig.2). L'activité enzymatique régresse depuis décembre 1978. Tous les groupes d'hydrolases sont concernés (Fig. 5).

Schorre A1.

En surface, contrairement au schorre précédent, où persistent de fortes concentrations en HCT, les valeurs passent de 32,97 à 18,84 de 1978 à 1979, mais la biodégradation paraît peu importante (AV/AP $\simeq$ 1). En 1980, la concentration tombe à 14,98. Les résultats concernant la rhizosphère en 1979 tendraient à prouver qu'il y a eu percolation. L'analyse montre en 1980, l'absence d'alcanes linéaires et la persistance des HC saturés ramifiés et aromatiques. On observe également que la densité des germes dégradants les HC augmente jusqu'en novembre 1979 et régresse en 1980 (Fig. 2), soit faute de substrat (alcanes linéaires), soit par suite d'un phénomène climatique général (voir B1 et C1).

L'activité enzymatique en A1 en surface, est comparable à celle de D1 (accroissement en 1979, régression en 1980) (Fig. 5).

Les microphytes ont été éliminés sur le sol A1 par l'arrivée du mazout et étaient encore absents en décembre 1978, comme en D1 (Fig. 8A).

Par contre, une très légère colonisation seulement était amorcée en 1979, suivie d'une diminution en 1980 (quelques μg Chla.g^{-1} seulement). Les pigments dégradés sont dominants en toutes saisons, traduisant le peu de vitalité de la population (Chla/Chla + Phéo. : 0,2 en moyenne).

A1 : Dans la rhizosphère du schorre A1, il y a augmentation de 0,47 à 3,68 des concentrations en HCT indiquant probablement une percolation en 1979, accompagnée d'une dégradation importante (AV/AP : 1,47 et 1,26, Tab. 2). Ensuite, la pollution diminue en 1980. La microflore dégradant les HC était en densité maximale en 1979, puis a régressé fortement en 1980, davantage qu'en surface (Fig. 2).

L'activité enzymatique est en augmentation depuis 1978, la pollution étant proportionnellement moins forte que dans le schorre D1 (Fig. 5).

Conclusion sur les schorres très pollués.

Ces deux biotopes, très pollués au départ, ont réagi différemment puisque le plus pollué (D1) n'est pas le moins recolonisé par les microphytes. Il faut sans doute y voir l'influence bénéfique d'une plus forte humectation : D1, mieux drainé donc plus souvent inondé, est plus rapidement recolonisé du fait de l'apport de particules argileuses favorisant la fixation des microphytes.

Dans les rhizosphères, la dégradation paraît se faire convenablement, même en cas de percolation.

C'est donc à la surface des schorres les plus élevés (ou les plus souvent exondés) que se restaure le plus lentement le peuplement microbiologique.

Schorres peu pollués, C1 et B1.

Schorre C1.

En surface, sur le schorre C1, moins pollué (4,17 en 1978) les concentrations en HCT ont rapidement diminué (0,54 en 1980). Les germes dégradant les HC sont restés en nombre stable (10^4 à 10^5 germes.ml^{-1} de sédiment (Fig. 2)). L'activité enzymatique a été constamment élevée. La dégradation semble donc s'effectuer normalement (Fig. 5).

Dans la rhizosphère, il y a forte diminution des HCT avec le temps et forte dégradation (AV/AP = 1,25). Les germes dégradant les HC se sont maintenus en nombre stable (10 à 10^3 germes.ml^{-1}). L'activité enzymatique a décru en 1980.

Dans les couches les plus profondes, les concentrations en HCT sont très faibles en 1980 (0,05) et l'activité enzymatique constante.

Schorre B1.

En surface, le deuxième schorre de référence, peu pollué (1,90 en décembre 1978) n'a pratiquement pas montré de variations jusqu'en 1979 (1,75). Le nombre de germes dégradant les HC et les indices de biodégradation (1,34) tendent à prouver qu'il y aurait eu forte dégradation (Fig. 2).

Les HCT dosés en 1979 n'ayant pas les caractères d'HC dégradés ($C^{17}/Pr = 4,43$), nous émettons les hypothèses que de faibles apports chroniques d'HC se produisent en cette station, ou plutôt que les microphytes, très abondants sur ce site, seraient responsables d'apports d'HC biogènes (Prédominance d'imparité $>$ 1).

Contrairement aux faits observés sur les schorres très pollués, les microphytes n'ont pas été ici éliminés au départ et ne paraissent pas affectés par une faible pollution, tout au moins en 1978 et 1979. Une densité maximale du peuplement est observée en juillet 1979 avec 130 µg Chla.g^{-1} (Fig. 8A) et un rapport Chla/Chla + Phéo. de 0,75 indice de bon fonctionnement de la population.

Tout au long de l'année, et contrairement aux schorres pollués, cet indice est toujours supérieur à 0,5. Le peuplement de cyanophycées et diatomées est toute l'année bien diversifié et les espèces caractéristiques des marais maritimes y sont présentes.

En 1980, cependant, un ICB moyen de 50 µg Chla.g^{-1} est plus faible que celui des années précédentes, mais comparable à celui du schorre C1. On ne peut, dans ces deux stations, exclure l'influence néfaste éventuelle d'un facteur climatique en 1980.

Dans la rhizosphère B1, l'activité bactérienne et l'activité enzymatique ont été importantes et les concentrations en HCT, faibles au départ, ont diminué encore.

Conclusions sur le biotope "schorre".

Dans les stations très polluées au départ :

1° Une dépollution se produit peu à peu, sauf si de nouveaux apports par drainage (?) maintiennent des taux de concentrations élevés.

2° La dégradation des HC est toujours plus faible, en surface et dans la rhizosphère, que dans les schorres peu pollués. Cette dégradation est, dans les sites peu humectés, pratiquement stoppée.

3° Les concentrations en germes dégradant les HC sont toujours supérieures de 1 à 2 ordres de grandeur à celles des schorres peu pollués.

4° Par contre, l'activité enzymatique est 2 à 3 fois plus faible que dans les schorres peu pollués.

5° Après avoir totalement disparu, les microphytes recolonisent très lentement les schorres "asphaltés", un peu plus rapidement les schorres pollués mais plus humectés. Par contraste, les schorres peu pollués sont très florissants (ICB 10 à 20 fois supérieur).

Evolution dans les Chenaux

Chenal très pollué, A2.

En surface, les concentrations en HCT ont diminué de 10,78 à 2,57. Les indices adéquats prouvent qu'une forte dégradation a eu lieu. Les chromatogrammes des HC restant en 1980 ne présentent plus les pics des alcanes linéaires. La dégradation rapide paraît terminée et les autres fractions resteront probablement en l'état. Les germes dégradant les HC ont augmenté en nombre jusqu'en 1979 (10^6) puis ont décru en 1980 (10^4 germes.ml^{-1}, Fig. 3), ce qui corrobore l'hypothèse précédente de la non poursuite de la dégradation mais n'exclut pas l'hypothèse du facteur climatique.

L'activité enzymatique est plus faible que dans la station non polluée (Fig. 6).

Les microphytes ont recolonisé très rapidement la surface de la vase et ont atteint en novembre 1980, un maximum de 588 μg Chla.g^{-1}, maximum absolu dans toutes les stations étudiées (Fig. 8B).

En 1978, cette microflore était paucispécifique (*Phormidium* et *Amphiprora alata*). En 1980, la diversité spécifique d'une vase normale équivalente (Carter, 1933) est retrouvée. Pourtant un raclage effectué en mai 1980 montre encore une concentration d'HC de 14,20 g.kg^{-1}. Les chromatogrammes montrent l'absence totale d'alcanes linéaires (Tab. 3).

Dans la zone réduite (Tab. 1 : zr), les concentrations en HCT ayant augmenté jusqu'en 1980, on peut évoquer la possibilité d'une percolation. A ce même niveau, en 1980, tous les alcanes linéaires sont dégradés. La concentration mesurée concerne donc des HC plus résistants. On observe en outre que le nombre de germes dégradant les HC décroît fortement (10 germes.ml^{-1}). L'activité enzymatique décroît également.

Chenal peu pollué, C2.

En surface, les concentrations en HCT sont passées de 3,26 à 0,70. La biodégradation est importante (AV/AP élevé). Le nombre de germes dégradant les HC est stable (10^3 à 10^4 germes.ml^{-1}).

L'activité enzymatique dans cette station "propre" est aussi importante que dans les schorres peu pollués. Elle est en nette augmentation en 1980, ce qui est peut-être à relier aux travaux d'aménagement effectués à cet endroit (voir ci-dessous).

Les microphytes ont manifesté un maximum de développement en été 1979 suivi d'une décroissance. Les variations saisonnières paraissent donc très différentes dans les deux stations de type "chenaux", mais le chenal où se trouve la station C2 a été obturé momentanément, en mai 80, par des travaux de surcreusement du chenal central. La composition des peuplements de microphytes a été nettement perturbée et s'est appauvrie en espèces et en individus.

La zone réduite et la couche sableuse sous-jacente sont fortement dépolluées (0,09 et 0,05). Le nombre de germes dégradant les HC est stable. Dans la zone réduite, une diminution de l'activité enzymatique se poursuit depuis décembre 1978, indice possible de la restauration d'un état d'origine (disparition des lipases, augmentation des activités estérases, aminopeptidases, chymotripsines et glucidases).

Conclusions sur le biotope "chenal de schorre".

1° Dans le cas de pollution forte, la biodégradation, après avoir été active, s'est ralentie ou arrêtée. Les HC encore présents ont peu de chances d'être dégradés rapidement. Dans le cas de pollution faible, la dégradation a été presque complète.

2° Parallèlement, le nombre de germes dégradant les HC a augmenté puis diminué dans le cas de forte pollution. Il est stable ailleurs.

3° L'activité enzymatique est moins importante, en surface, en cas de pollution. Elle est toujours plus faible en zone réduite.

4° Les microphytes se développent en surface de façon luxuriante dans les chenaux pollués. Les concentrations en Chla sont en 1980 nettement supérieures à celles du chenal non pollué.

Evolution dans les slikkes

Slikke très polluée, A3.

En surface, à l'endroit précis où nous avons situé la station A3, on peut dire que les concentrations en HCT sont passées dans la pellicule superficielle (ps = quelques millimètres d'épaisseur) de 5,56 à 0,27 $g.kg^{-1}$ entre décembre 1978 et mai 1980 (Tab. 1).

Dans la couche sous-jacente (su : 2 à 3 cm d'épaisseur) les concentrations sont passées de 24,95 à 0,60 entre 1979 et 1980. Il y a donc eu dépollution en surface. Dans le même temps, les indices de biodégradation ont augmenté jusqu'en 1979 (Tab. 2) et le nombre de germes dégradant les HC également (Fig. 4).

Dans la couche sableuse, par contre, les concentrations en HC ont augmenté de 0,65 à 2,40 en 1979, puis diminué en 1980 (0,50). Les indices de biodégradation sont faibles. L'activité enzymatique est réduite (Fig. 7). Il semble y avoir eu percolation, surtout en novembre 1979.

Devant les difficultés d'interprétation des résultats dans un tel biotope (problèmes d'échantillonnages), des prélèvements par raclages ont été faits en mai 1980. Ils ont permis d'effectuer des dosages à partir d'un matériel abondant et de différencier, d'une part la pellicule superficielle constituée essentiellement de particules argileuses compactées par les microphytes, et, d'autre part, la couche sableuse visiblement encore très polluée.

Les résultats sont éloquents : 0,27 dans le premier cas, 15 g.kg^{-1} dans le second. Ce sont des hydrocarbures d'origine "Arabian light" dont les alcanes linéaires certes sont dégradés, mais dont les autres constituants sont toujours présents (Tab. 1,3).

Notre première hypothèse du "piégeage" du pétrole sous la matte végétale se trouve confirmée.

En effet, les filaments de cyanophycées et les diatomées compactant les particules argileuses avaient très rapidement colonisé ces vases imprégnées d'HC puisqu'en décembre 1978, on observait un ICB de 140 µg Chla. g^{-1} (Fig. 8C), et très peu de phéopigments (rapport Chla/Chla + Phéo. de 0,96 , le plus élevé de cette étude) indiquant une population jeune. Ce peuplement se révélait paucispécifique (*Phormidium*, deux espèces de *Nitzschia*, *Amphipleura*, *Rhopalodia*) mais se diversifiait très rapidement. Les deux cycles annuels de 1979 et 1980 présentent tous deux un maximum en automne ou en hiver, tout comme la vase du chenal pollué, avec des ICB presque aussi élevés et toujours très peu de phéopigments.

En 1980, le peuplement est très riche et très diversifié (présence de nombreuses espèces caractéristiques de ces milieux).

La matte algale recouvre donc un sédiment dans lequel une dépollution a eu lieu, tout au moins dans la partie superficielle, mais dont la couche sableuse est toujours imprégnée d'HC dont la dégradation paraît très ralentie. Cette matte semble toujours jouer un rôle de frein à une dépollution mécanique par le jeu des marées.

Slikkes peu polluées, B3 et E3.

La station B3 nous a paru devoir être remplacée, en mars 1980, par une nouvelle station de référence (E3), la slikke centrale ayant été perturbée par l'obturation momentanée du pont, après l'échouage du Tanio (mars 1980) puis par des travaux d'aménagement.

Les concentrations en HCT dans ces stations sont faibles. La biodégradation a été importante. Le nombre de germes dégradant les HC est stable (Fig. 4). L'activité enzymatique de surface est en augmentation en 1980 (Fig. 7).

Le peuplement microphytique est florissant, particulièrement en E3, ou un indice C^{17}/Pr de 4,47 en mai 1980 pourrait traduire, comme dans les schorres non pollués B1 et C1, la présence d'un hydrocarbure biogène particulièrement abondant chez certaines cyanophycées (Fig. 8C).

Contrairement à la slikke polluée, les couches sous-jacentes ici ne renferment pas d'hydrocarbures.

Conclusions sur le biotope "slikke".

Ces conclusions sont très voisines de celles qui concernent les chenaux :

1° En cas de pollution grave, la biodégradation, active d'abord, s'est ralentie ou même arrêtée.

2° Le nombre de germes dégradant les HC a diminué depuis 1978. Dans les stations peu polluées il est stable.

3° L'activité enzymatique paraît toujours freinée en surface en cas de pollution forte.

4° Les microphytes se sont développés rapidement sur les sédiments pollués, piégeant des particules argileuses et constituant une "matte algale" plus compacte que dans les chenaux, pellicule qui freine la dépollution de ces sédiments.

CONCLUSIONS

Nous avons essayé au cours de cette étude de nous attacher à comprendre les interrelations qui pouvaient exister entre l'état de dégradation des hydrocarbures et l'évolution des peuplements bactériens et microphytiques des marais maritimes. La complexité de ces milieux et les problèmes d'échantillonnage qui en découlent rendent parfois difficile la compréhension du fonctionnement d'un tel écosystème perturbé.

En ce qui concerne l'ensemble des biotopes.

1° Il apparaît tout d'abord que les marais maritimes de l'Ile Grande restent très pollués malgré une biodégradation importante.

2° Les hydrocarbures présents actuellement à la surface des sédiments ou dans des couches plus profondes (percolation ou "piégeage") sont à un stade tel (disparition totale des alcanes linéaires) que la dégradation ne paraît pas se poursuivre actuellement. Ce ralentissement peut avoir plusieurs causes : persistance des seules fractions les plus résistantes des HC, toxicité, pour le peuplement bactérien, de certains produits de dégradation, facteur climatique défavorable à l'activité bactérienne.

3° L'évolution de ces milieux s'est avérée différente suivant le degré initial de pollution :

- dans les stations très polluées, les concentrations en germes dégradant les hydrocarbures ont été très élevées. Puis leur nombre a décru en 1980. L'activité enzymatique a été moins élevée en surface et dans les rhizosphères.

- dans les stations peu polluées, l'impact sur les peuplements microphytiques et bactériens a été peu perceptible, et la dégradation a été plus poussée, mettant en évidence l'existence probable d'un seuil de concentration en HC en-dessous duquel la "restauration" est possible.

En ce qui concerne chaque biotope en particulier, le déversement massif du pétrole "Arabian light" n'a pas eu les mêmes conséquences dans les sols du pré-salé, le plus souvent exondé (schorres) que dans les sédiments fins, le plus souvent immergés (chenaux et slikkes). L'aspect microbiologique et l'aspect mécanique sont à prendre en compte dans les différences de dépollution.

Les vases intertidales

Elles ont été probablement plus vite nettoyées par le jeu des marées que la surface des schorres. Mais, par ailleurs, elles ont été très rapidement recolonisées du fait de l'apport de particules argileuses favorisant l'installation d'un peuplement paucispécifique de cyanophycées et de diatomées qui, par la suite, s'est diversifié. Le mazout restant s'est trouvé ainsi plus ou moins piégé sous cette "croûte" algale et pourrait s'y maintenir longtemps.

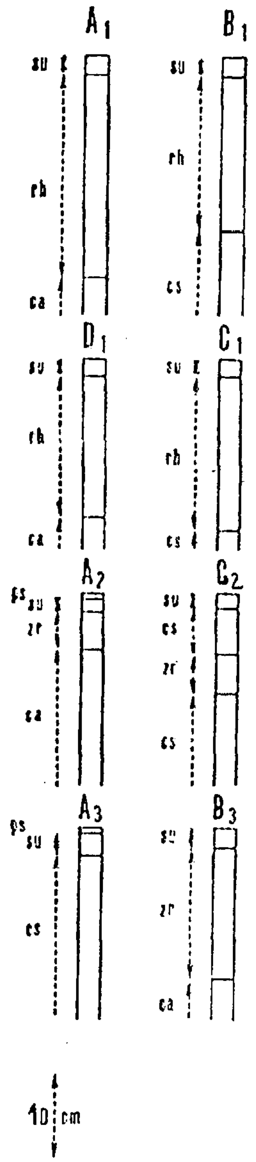
Les schorres

Les schorres, par contre, ont été moins rapidement dépollués par les marées, certains présentant même des zones d'accumulation. Après avoir totalement disparu, les microphytes recolonisent très lentement les sols, d'autant plus lentement qu'ils sont moins souvent immergés. La colonisation est actuellement environ 10 fois inférieure à la normale sur le sol des schorres à *Juncus*, observation qui concorde avec les résultats obtenus par Levasseur et Seneca sur la flore macrophytique (voir contributions de ces auteurs).

TABLEAU 1

EVOLUTION DES CONCENTRATIONS EN HYDROCARBURES TOTAUX DANS LES SEDIMENTS DES MARAIS MARITIMES DE L'ILE GRANDE.

Biotopes et Stations	Différents niveaux dans la carotte	HC totaux $g.kg^{-1}$ de sédiment sec			
		IG_1 12/78	IG_2 3/79	IG_6 11/79	IG_8 5/80
Schorre					
	su	32,97		18,84	14,98
A_1	rh	0,47		3,68	0,03
	ca				0,04
	su	94,68		94,51	230,60*
D_1	rh	8,13		0,23	17,78*
	ca	0,48		0,10	0,18*
B_1	su	1,90		1,75	
	rh	0,17		0,10	
	su	4,17		0,43	0,54
C_1	rh	0,26		0,03	0,15
	cs (14-32 cm)				0,05
Chenaux					
	ps	7,69			14,20
	su		10,78	6,59	2,57
A_2	zr	0,48	0,22	0,29	1,14
	ca	0,10		0,03	0,08
	- 28-36 cm				0,08
	su	3,26		1,41	0,70
C_2	zr	0,77		0,09	
	cs	0,23		0,05	
Haute Slikke					
	ps	5,56			0,27-154**
A_3	su		24,95	3,45	0,60
	cs		0,65	2,40	0,50
	su				0,89
E_3	zr				0,08
	ca				0,09
	su			0,52	0,56
B_3	zr			0,19	0,22
	ca				0,16



ps : pellicule superficielle, prélèvement par raclage
su : partie superficielle, quelques centimètres
rh : rhizosphère
cs : couche sableuse
zr : zone réduite
ca : couche argileuse

* Cette carotte n'a pas été prélevée au hasard mais dans une tache d'hydrocarbures afin d'en étudier l'état de dégradation.

** Deux raclages ont été effectués dans le chenal central.

TABLEAU 2

HYDROCARBURES DANS LES SEDIMENTS DES MARAIS MARITIMES DE L'ILE GRANDE
(POLLUTION PAR L'AMOCO CADIZ)

ANALYSE CHIMIQUE

stations		Poids humide des échantillons g.		AV-SP g.kg^{-1}		AP-SP g.kg^{-1}		AV/AP		FB g. kg^{-1}		Hydrocarbures totaux (FA) g. kg^{-1}	
		IG_1 12/78	IG_6 11/79	IG_1 12/78	IG_6 11/79	IG_1 12/78	IG_6 11/79	IG_1 12/78	IG_6 11/79	IG_1 12/78	IG_6 11/79	IG_1 12/78	IG_6 11/79
Schorre													
A1	su	13,40	25,10	73,60	43,48	67,60	39,58	1,09	1,10	35,70	16,93	32,97	18,84
	rh	68,70	99,50	2,00	8,92	1,37	7,10	1,47	1,26	0,90	2,99	0,47	3,68
D1	su	12,55	14,60	173,90	243,45	162,50	210,50	1,07	1,15	67,90	102,12	94,68	94,51
	rh	59,15	90,10	19,78	1,98	16,50	0,96	1,20	2,06	8,40	0,46	8,13	0,23
	ca	24,25	74,80	1,89	0,24	1,48	0,19	1,26	1,26	1,00	0,06	0,48	0,10
B1	su	13,30	10,85	8,00	10,29	5,15	8,98	1,34	1,14	4,00	5,56	1,90	1,75
	rh	53,50	97,80	2,12	1,99	1,30	0,89	1,63	2,24	1,06	0,58	0,17	0,10
C1	su	15,35	19,85	13,74	2,43	12,27	1,93	1,12	1,26	8,10	1,19	4,17	0,43
	rh	58,80	132,50	2,41	0,15	1,59	0,12	1,52	1,25	1,33	0,05	0,26	0,03
Chenaux de schorre													
A_2	ps	51,20	6,10	18,52	21,49	16,70	12,41	1,11	1,73	9,00	4,25	7,69 *	6,59
	zr	83,50	120,60	1,78	0,95	1,42	0,60	1,25	1,58	0,91	0,23	0,48	0,29
	ca	23,10	139,20	0,63	0,17	0,31	0,07	2,03	2,43	0,21	0,02	0,10	0,03
C_2	su	10,50	15,90	14,07	6,32	8,27	4,28	1,70	1,48	5,00	2,17	3,26	1,41
	zr	39,80	41,20	3,07	1,89	2,26	0,94	1,36	2,01	1,50	0,66	0,77	0,09
	cs	18,65	76,90	0,60	0,92	0,45	0,40	1,33	2,3	0,22	0,25	0,23	0,05
Haute-slikke													
A_3	ps	37,80	1,05	11,48	16,91	11,44	12,91	1,00	1,31	5,88	8	5,56*	3,45
	cs		106,50		7,43		6,66		1,12		3,54		2,40
B_3	ps		7,50		6,18		2,66		2,32		1,34		0,52
	cs		112,30		3,43		1,76		1,95		1,14		0,19
Arabian light				100 g		94 g		1,07		32%		68%	

AV-SP : avant saponification
AP-SP : après saponification
poids en g par kg de sédiment sec
AV/AP : rapport
FB : fraction B séparée sur Sep-Pak, élution $HC\ Cl_3$
FA : fraction A, élution hexane : hydrocarbures totaux
ps : pellicule superficielle

su : partie superficielle : quelques centimètres.
rh : rhizosphère
zr : zone réduite
ca : couche argileuse
cs : couche sableuse

* Ces concentrations ont été évaluées sur des échantillons de sédiment raclés à la spatule sur le terrain (0,5 cm d'épaisseur environ).

TABLEAU 3

HYDROCARBURES DANS LES SEDIMENTS DES MARAIS MARITIMES DE L'ILE GRANDE (POLLUTION PAR L'AMOCO CADIZ)

ANALYSE CHROMATOGRAPHIQUE DE LA FRACTION SATUREE

Stations		C_{17} / Pr			C_{18} / Ph			Pr / Ph			Prédominance d'imparité		
		IG_1 12/78	IG_6 11/79	IG_8 5/80	IG_1 12/78	IG_6 11/79	IG_8 5/80	IG_1 12/78	IG_6 11/79	IG_8 5/80	IG_1 12/78	IG_6 11/79	IG_8 5/80
Schorre													
A_1	su	1	0,5	XX	0,77	0,58	XX	0,54	0,67	XX	≃1	XX	XX
	rh	0,48	0,5	12	0,41	0,21	4,5	0,61	0,35	0,25	≃1	XX	1,30
D_1	su	0,60	0,2	1,88	0,47	0,11	1,21	0,60	0,54	0,67	≃1	XX	0,91
	rh	1,24	1,5	2,14	0,93	1,92	1,40	0,75	0,66	0,64	≃1	1,40	1
	ca	3,30	2	3	0,78	1,92	2,38	0,17	0,83	0,61	XX	XX	1,12
B_1	su	XX	4,43		XX	2,62		XX	0,54		XX	1,57	
	rh	0,21	1,31		1,75	4		7,80	3,2		2,37	XX	
C_1	su		1,42	6		6,20	4,64		3,80	0,55		1,11	1,27
	rh		2,80	6,82		6,67	7,23		1,60	0,85		1,05	1,84
Chenaux de schorre													
A_2	ps		0,35	XX		0,13	XX		0,56	XX		XX	XX
	zr		0,50			0,26			0,58			1,25	
	ca		1,00	2,83		3,50	2,89		2,20	0,67		1,03	1,17
C_2	su	0,13	0,19	XX	0,39	0,44	XX	3,50	23,33	XX	0,99	1,84	XX
	zr	0,13	1,25		0,26	2,5		2,28	4		1,06	1,48	
	cs	2,33	3,33		5,30	3,5		2	0,75		0,89	1,30	
Slikke													
A_3	su		4,10	XX		6,00	XX		1,25	XX		1,03	XX
	cs		0,09	XX		<0,06			<0,85	XX		XX	XX
B_3	ps		0,53	XX		0,53	1,42		0,94			1,04	1,77
	cs		0,29			0,14			4,72			1,60	
E_3	su			14,47			1,18			0,5			1,19
	ca			XX			6,5			XX			2,36
Arabian light		10,30			4,70			0,48			0,88		

$$\text{Prédominance d'imparité} = \frac{2\,(C_{23} + C_{25} + C_{27})}{C_{22} + 2C_{24} + 2C_{26} + C_{28}}$$

XX les chromatogrammes ne montrent plus la présence d'alcanes linéaires.

su = partie superficielle : quelques centimètres

rh = rhizosphère

cs = couche sableuse

zr = zone réduite

Pr = pristane

ca = couche argileuse

Ph = phytane

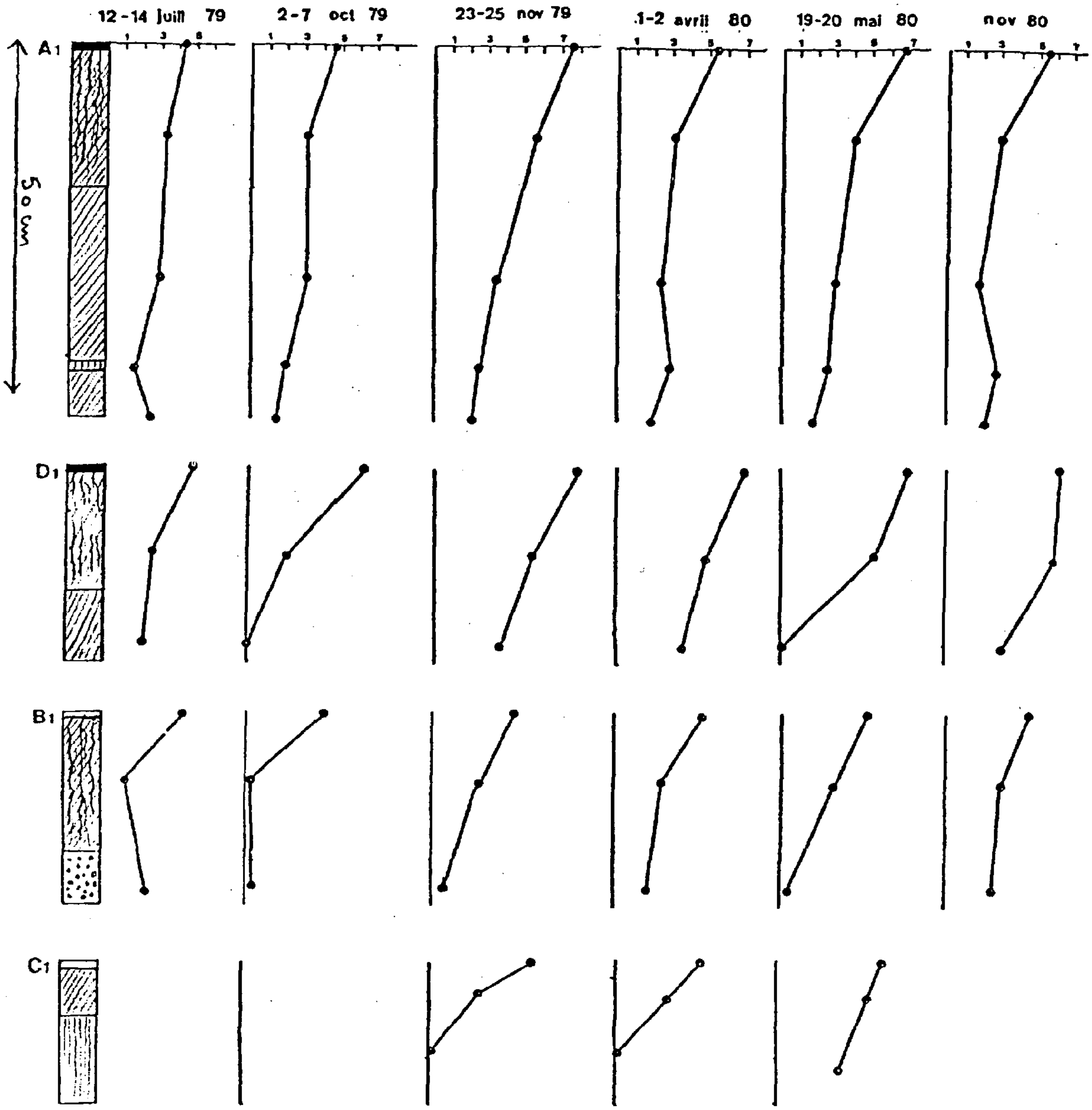


FIGURE 2. Nombre de germes (log) dégradant les hydrocarbures à différentes profondeurs dans les schorres très pollués (A1 et D1) et moins pollués (B1 et C1) des marais maritimes de l'Ile Grande.

▬	couche d'hydrocarbure	▨	argile
▭	couche à microphytes	▥	couche réduite
▤	rhizosphère	⛆	sable

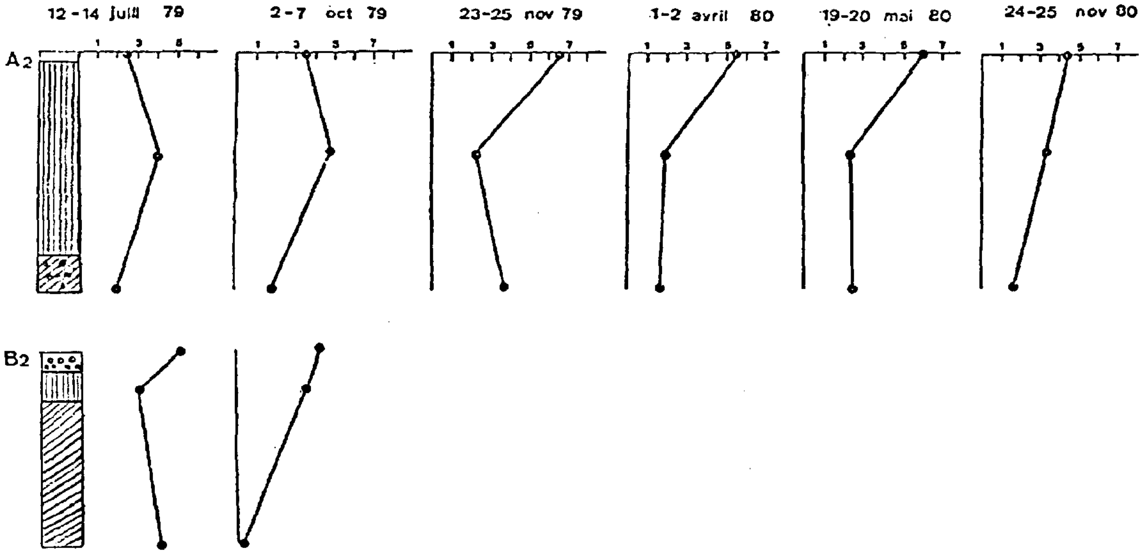


FIGURE 3. Nombre de germes (log) dégradant les hydrocarbures à différentes profondeurs dans les chenaux très pollués (A2) et moins pollués (B2) - même légende que figure 2 -.

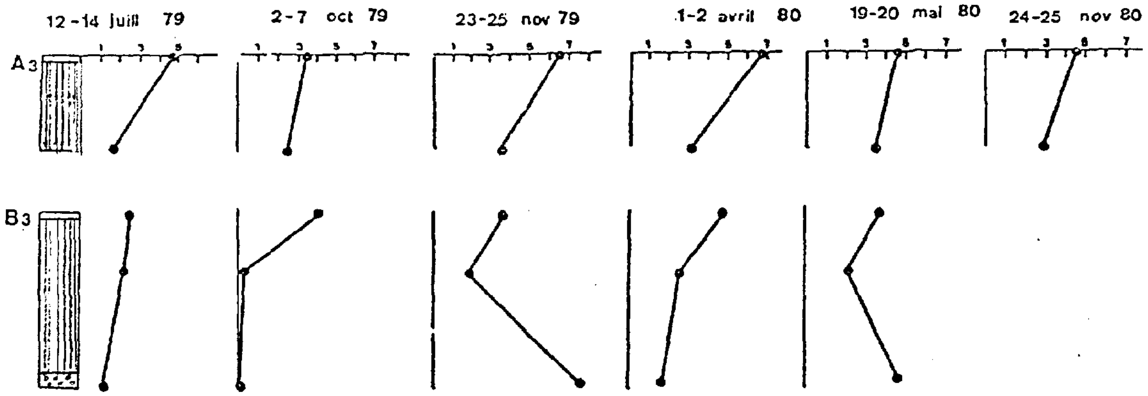


FIGURE 4. Nombre de germes (log) dégradant les hydrocarbures à différentes profondeurs dans les slikkes très polluées (A3) et moins polluées (B3) - même légende que figure 2) -.

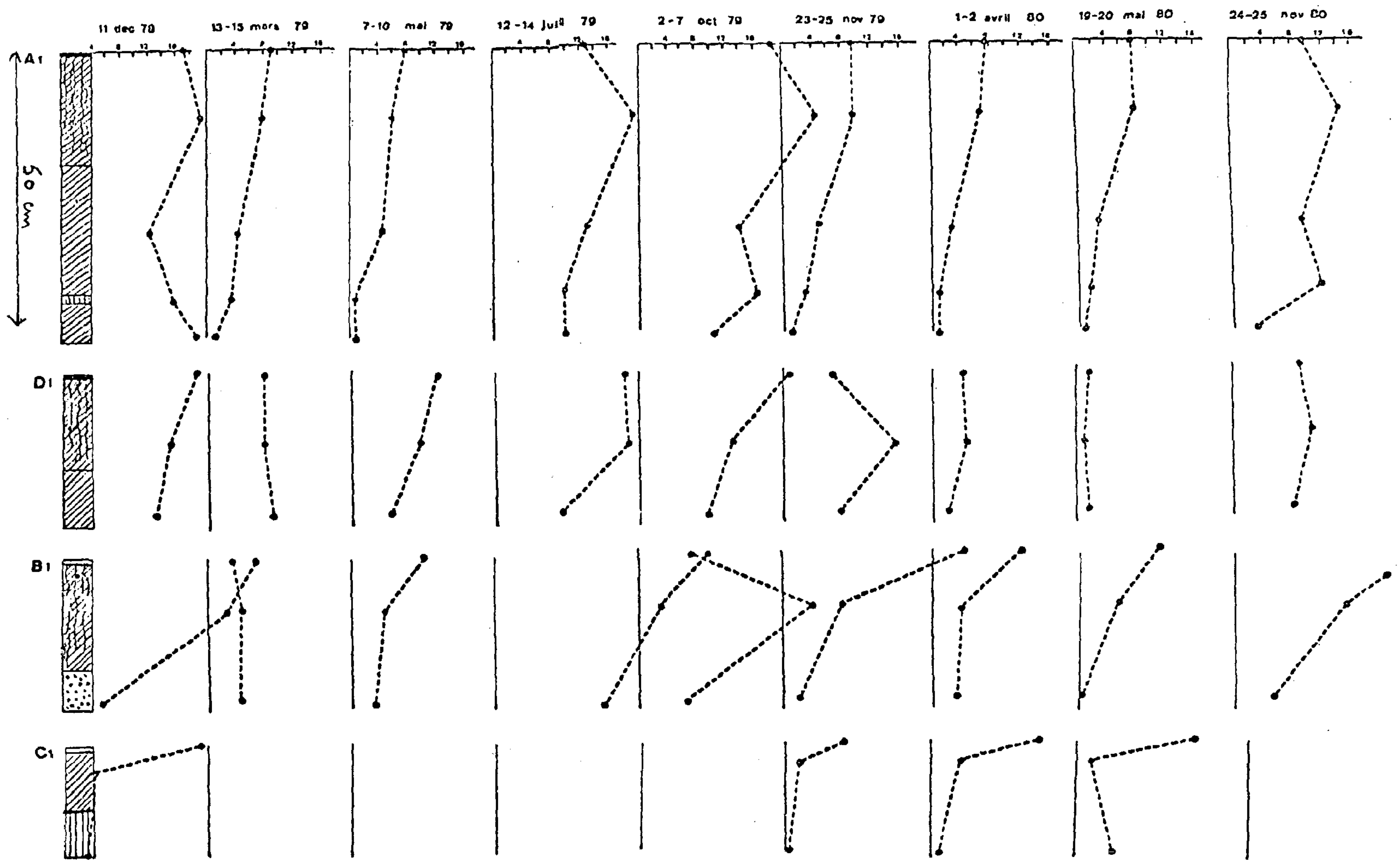


FIGURE 5. Activité enzymatique (cf. méthode) de l'ensemble des peuplements bactériens et microphytiques dans les schorres très pollués (A1 et D1) et moins pollués (B1 et C1).

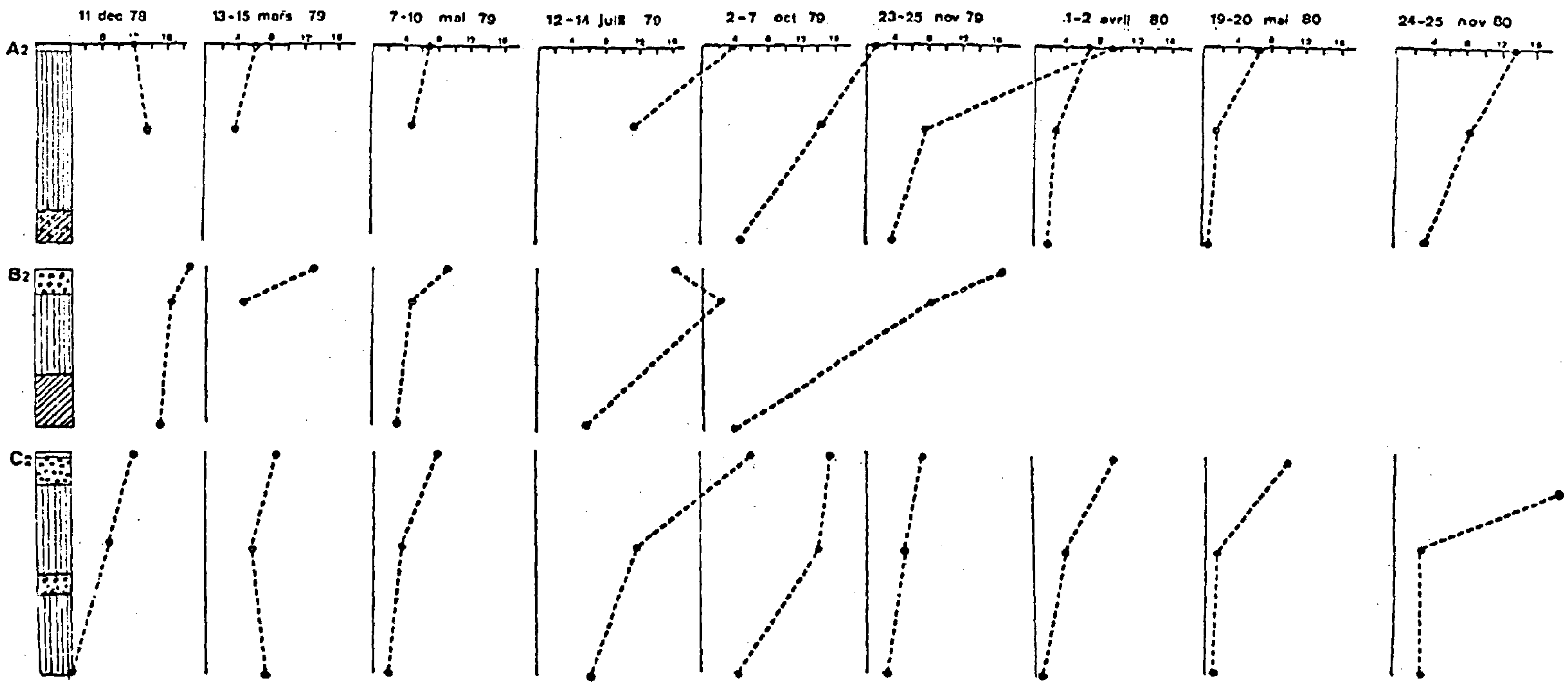


FIGURE 6. Activité enzymatique dans les chenaux très pollués (A2) et moins pollués (B2 et C2).

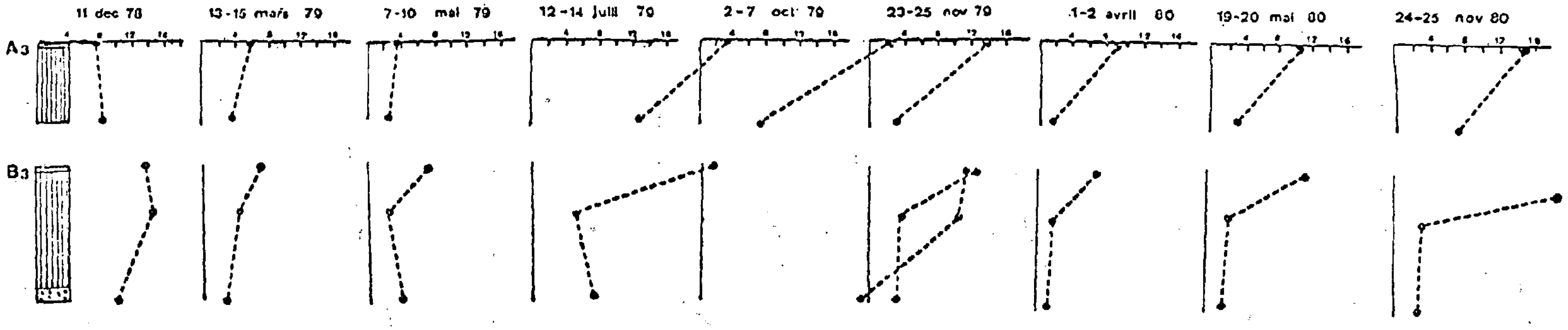


FIGURE 7. Activité enzymatique dans les slikkes très polluées (A3) et moins polluées (B3).

FIGURE 8

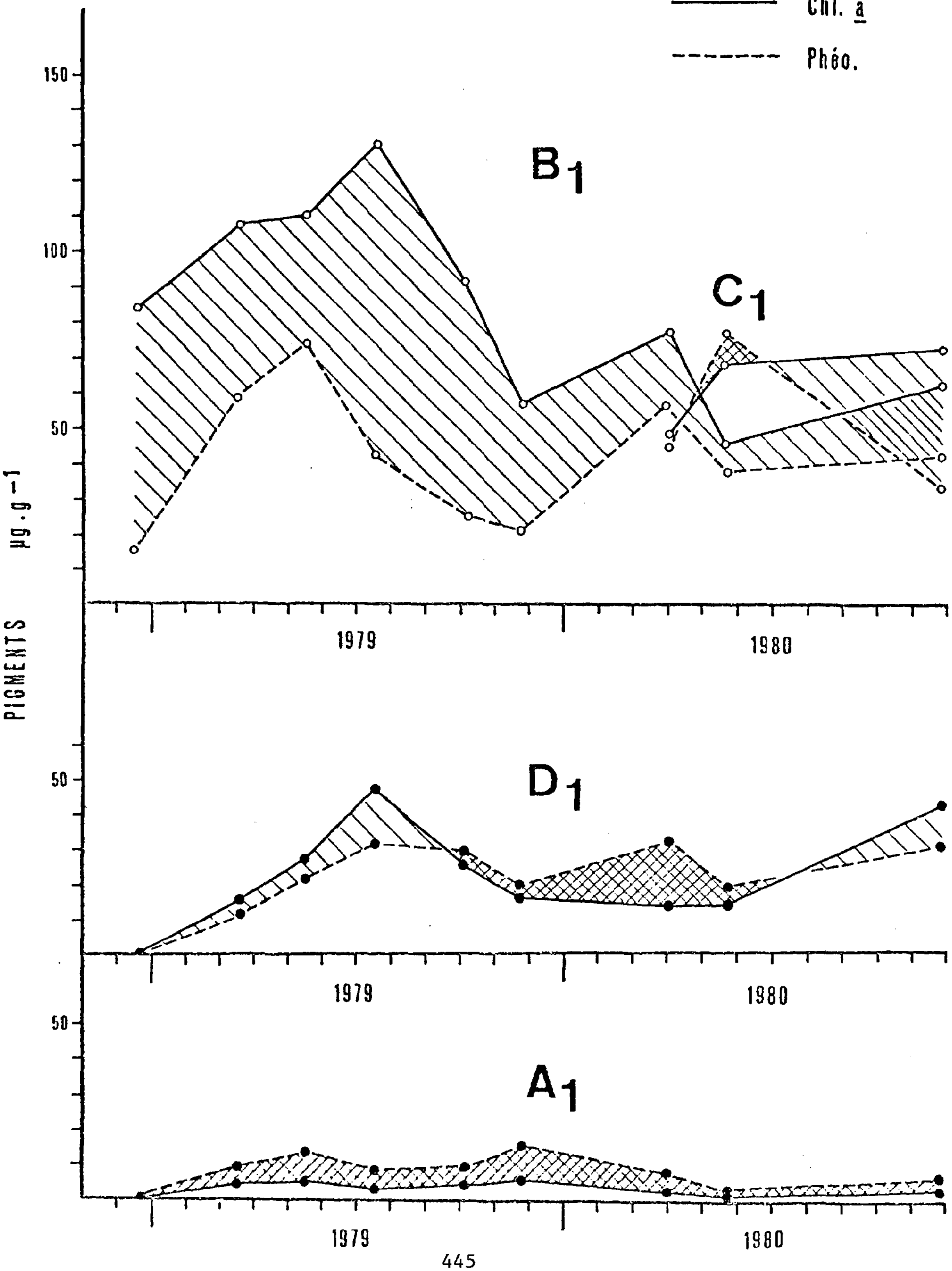
Evolution temporelle des concentrations en chlorophylle *a* (trait plein) et en phéopigments (pointillés) à la surface des sédiments (en $\mu g\text{-}g^{-1}$ de sédiment sec).

A - Stations de schorres B_1, C_1 peu pollués
D_1, A_1 trés pollués

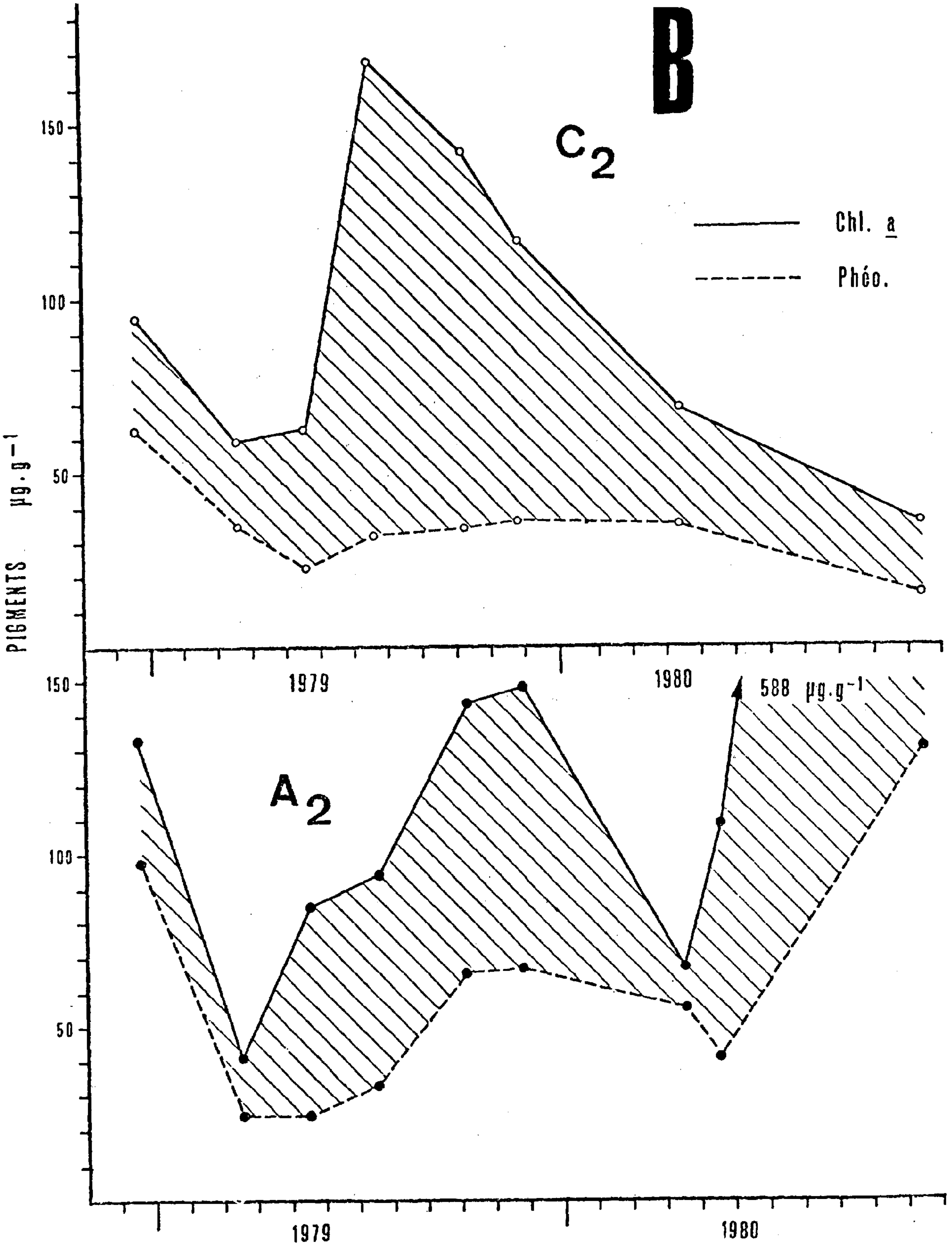
B - Stations de chenaux C_2 peu pollué
A_2 trés pollué

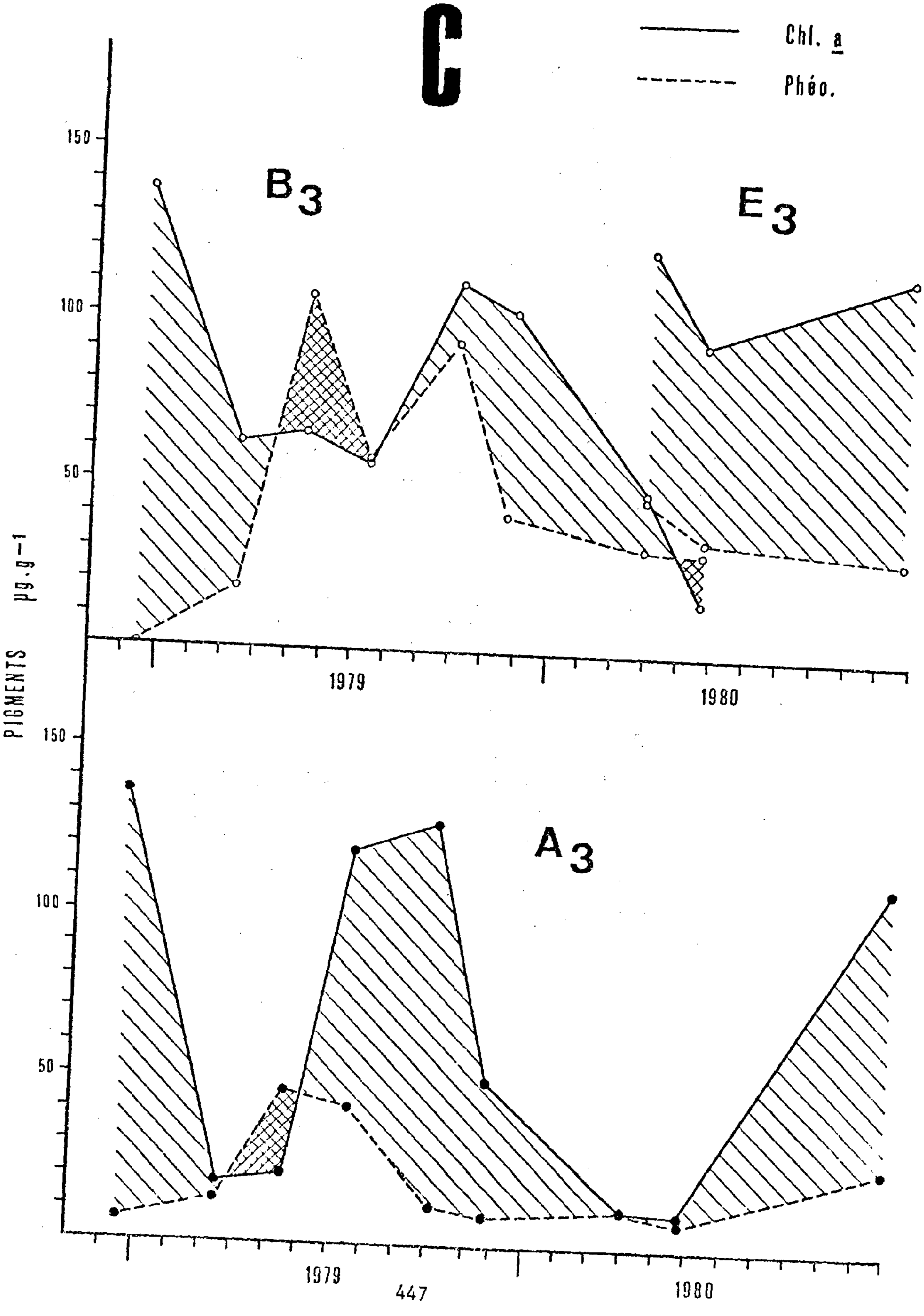
C - Stations de slikkes B_3, E_3, peu polluées
A_3 trés polluée

A



Chl. a
Phéo.
150
100
50
B1
C1
µg.g−1
PIGMENTS
1979
1980
50
D1
1979
1980
50
A1
1979
1980


B
C2
Chl. a
Phéo.
150
100
50
PIGMENTS µg.g-1
1979
1980
A2
588 µg.g-1
150
100
50
1979
1980


C
Chl. a
Phéo.
150
100
50
B3
E3
µg.g-1
PIGMENTS
1979
1980
150
100
50
A3
1979
1980

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Résumé

La baie de Lannion, largement ouverte face à la progression des nappes de pétrole de l'*AMOCO CADIZ*, fut particulièrement souillée : 60 millions de cadavres échoués furent dénombrés sur les deux plages du fond de la baie de St-Efflam et Locquémeau.

Des études antérieures sur l'estran de St-Efflam, représentatif des nombreuses plages de sable fin de Bretagne, ont servi de référence à ce travail.

L'impact du pétrole est très variable sur les diverses espèces d'une même station. La partie Est de la plage, plus contaminée, montre une plus forte mortalité. Le haut et le bas de l'estran sont plus affectés que la partie intermédiaire. Deux processus semblent intervenir :

- les nappes d'échouages en haut,
- le pétrole dissout ou en émulsion dans la masse d'eau en bas et dans tout l'infratidal.

A la mortalité immédiate s'ajoutent des mortalités et des effets pathologiques à long terme. Certaines espèces continuent à regresser même en 1981 et les recrutements souvent inexistant en 1978 s'amorcent en 1980, pour certains timidement encore en 1981. L'Est de la plage reste fortement touché bien que des signes de recouvrance certains apparaissent sur le reste de la plage mais les gros peuplements du bas de la plage à *Solen, Ensis, Echinocardium, Lutraria, Mactra corrallina* ne sont pas réapparus.

INTRODUCTION

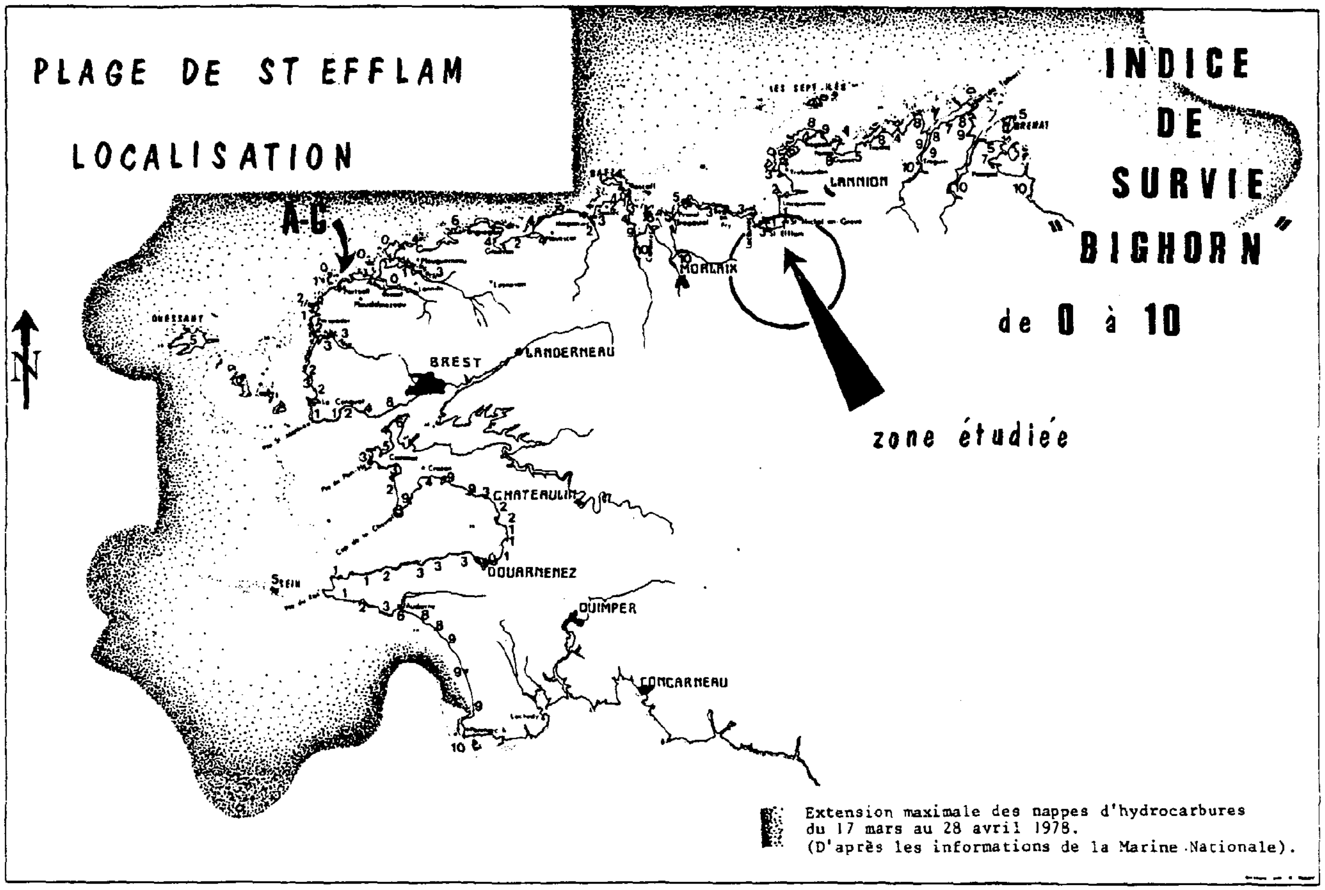
Les nappes d'émulsion pétrolière de l'*AMOCO CADIZ* poussées à la surface de la Manche, le long de la côte, par les vents d'Ouest, ont été freinées en se heurtant sur les saillants successifs du rivage. Quatre zones principales d'obstacles se sont dressées face à leur progression vers l'Est. Ce sont les roches et les îlots des abers et de la presqu'île Ste-Marguerite, d'abord ; le champ de roches de l'île de Batz, de Santec, de Roscoff, ensuite ; puis les roches de Primel et du Guersit ; enfin, celles des rebords Est de la baie de Lannion avec l'île Grande. En chacun de ces lieux, et surtout dans les criques, les estuaires et les baies de sable fin les plus proches qui les précèdent, le pétrole s'est abondamment accumulé en provoquant de lourdes mortalités donnant lieu à d'importants échouages de cadavres de poissons, d'oiseaux et de coquillages.

La baie de Lannion, déjà fortement souillée, en 1967, par le pétrole du TORREY CANYON, a été atteinte par les nappes de l'*AMOCO CADIZ* dès le 5e jour après le naufrage. Sur les deux grandes plages de sable fin du fond de la baie nous avons recensé 60 millions d'individus morts, dont la moitié sur la Grande Grève (St-Efflam). Le cinquième seulement des cadavres était accumulé dans le spectaculaire et nauséabond cordon d'échouage du niveau des hautes mers ; la fraction la plus importante, bien que plus discrète, était éparpillée en nuages à la surface de l'ensemble des plages. Par des transects de plages, réalisés avec des gabarits métalliques d'1/4 de m^2, par 5 équipes d'étudiants opérant durant 3 jours, nous avons obtenu le décompte suivant pour les principales espèces :

ESPECES	Nbre d'individus (cadavres)	Poids en matière organique sèche(t)	Poids brut (t)
Echinocardium cordatum	20.10^6	4	260
Cardium edule	16.10^6	5	70
Mactra corallina	14.10^6	4	50
Pharus legumen	5.10^6	10	100
Ensis siliqua	1.10^6	2,5	25
Lutraria lutraria	$0,1.10^6$	0,8	10
Donax vittatus	1.10^6	0,04	0,6
Tellina fabula	$0,03.10^6$	0,001	0,01
Tellina tenuis	$0,02.10^6$	0,001	0,01
SOMME	$57,15.10^6$	25,366	515,62

A cette mortalité initiale, brutale, s'est ajoutée une importante mortalité ultérieure plus discrète. L'observation des phénomènes dans leur ensemble géographique ne nous a pas permis de cerner cette mortalité durant les premiers mois. Deux campagnes trimestrielles, avec seulement 10 stations régulièrement suivies à St-Efflam, ont pu être effectuées. Ce n'est qu'en janvier 1979, dans le cadre d'un contrat avec la NOOA, que nous avons pu mettre en place un réseau d'observations mensuelles mais qui ne couvrait l'ensemble de la plage qu'en 6 mois.

Les peuplements des plages de la Grande Grève (St-Michel et St-Efflam) et de Locquemeau ont été étudiés qualitativement depuis un siècle par les chercheurs et les étudiants de la station biologique de Roscoff. Des états de références quantitatifs, établis sous forme de cartes d'isobiomasses, de 1965 à 1968 (C. Chassé, 1972), donnaient, pour la plage de St-Efflam, des points de comparaison utiles à la fois pour le suivi des principales espèces et pour l'impact des hydrocarbures déjà évalué lors du naufrage du TORREY CANYON, en 1967.


PLAGE DE ST EFFLAM
LOCALISATION
INDICE
DE
SURVIE
"BIGHORN"
de 0 à 10
zone étudiée
A-C
N
OUESSANT
LES SEPT-ILES
LANNION
MORLAIX
BREST
LANDERNEAU
CHATEAULIN
DOUARNENEZ
QUIMPER
CONCARNEAU
S^tEIN
Extension maximale des nappes d'hydrocarbures
du 17 mars au 28 avril 1978.
(D'après les informations de la Marine Nationale).

L'ensemble de la nouvelle cartographie des peuplements de la plage de St-Efflam a été réalisé avec une centaine de stations quantitatives dont 65 ont été étudiées d'une manière plus approfondie. Chaque station a fait l'objet de 3 prélèvements de sédiment effectués à la benne à main de 1/16 de m^2 sur 20 cm de profondeur. La faune est recueillie sur place par tamisage sous l'eau sur maille de 1 mm, elle est déterminée, comptée et pesée. Des cartes d'isobiomasses des principales espèces ont été dressées, comparables aux cartes antérieures réalisées avant et après le naufrage du TORREY CANYON.

Dix stations caractéristiques des principaux peuplements, situées au bas et au centre de la plage, et suivies durant toutes les opérations, ont permis d'établir l'évolution des espèces dans le temps.

LE MILIEU

Sur la côte Nord de Bretagne, s'ouvrant sur la baie de Lannion, face aux vents dominants de secteur Nord-Ouest, à quelque 20 km à l'Est de la baie de Morlaix, la « Lieue de Grève » est une vaste plage de 5 km^2 tapissée de sable fin de 100 à 130 μ, émergeant presque entièrement aux grandes basses mers. Elle est profondément encaissée entre des collines élevées, large de 2 km au niveau des basses mers, elle atteint 4 km au niveau des hautes mers, d'où son nom de « Lieue de Grève ». Il y a 1,6 km en moyenne entre ces deux niveaux. Une butte énorme, Roc'al haz, haute de 99 m, s'avance légèrement en compartimentant faiblement le fond de la baie, séparant les localités de St-Efflam, à l'Ouest, de St-Michel-en-Grève, à l'Est. Six ruisseaux issus des coteaux élevés qui bordent le fond de la baie coulent en convergeant, à basse mer, vers l'entrée de la baie entre les pointes de Plestin, à l'Ouest, et de Beg-an-Fourn, à l'Est. Les trois ruissellements de l'anse orientale sont les plus importants. Par leur action de dessalure ils sont responsables de l'appauvrissement considérable des peuplements des niveaux moyens de cette anse, liée, par ailleurs, à l'exposition maximale aux houles des vents dominants du secteur Nord-Ouest. L'anse occidentale ne reçoit que des apports d'eau douce très limités (une légère salure estivale apparaît). Elle est relativement abritée des vents de Nord-Ouest par la pointe de Plestin, et partiellement protégée des vents importants de Nord-Est par les pointes de Beg-an-Fourn, Locquémeau, et la côte Est de la baie de Lannion. Aussi, le sédiment y est-il légèrement plus fin, moins perméable et moins oxygéné ; c'est la zone la plus richement peuplée. Elle présente un petit massif de roches métamorphiques noires, dures et tourmentées : le « Rocher Rouge », qui, à 1 km du fond de l'anse, couvre 1 ha et s'élève depuis les basses mers moyennes jusqu'au niveau des pleines mers de vives-eaux. Il offre un abri permettant le développement de sédiments légèrement envasés en arrière. Un maigre herbier de *Zostera nana* s'étend au niveau des basses mers de mortes-eaux ; il ne modifie que très peu le sédiment, la biomasse des *Zostera* y étant faible (250 à 600 g frais au m^2, moyenne 350). Notons son léger déplacement vers l'Ouest, depuis 1968. Le phénomène de sursalure estivale qui s'y produit, dû à l'évaporation du film d'eau qui n'a pas le temps de s'écouler durant la basse mer, est insuffisant pour modifier les peuplements, à moins que l'on puisse lui attribuer la présence très clairsemée de quelques *Nereis diversicolor*.

Un aspect important particulier à cette grève est l'accumulation croissante, d'année en année, d'algues non fixées, d'ectocarpales brunes libres au niveau des basses mers, mais surtout d'*Ulva lactuca* verte au début du printemps et à l'automne, au-dessus du niveau de la mi-marée. Ces algues couvrent à basse mer, d'un revêtement parfois continu, de très larges étendues de sable ; elles sont animées d'un balancement pendulaire au gré des marées et des vents. Ces « marées vertes » proviennent de l'eutrophisation croissante de l'impluvium des bassins de drainage des ruisseaux par les nitrates, les phosphates, la potasse, les pesticides et les lisiers d'origine agricole. Elles jouent un rôle trophique important notamment par la libération des spores et gamètes et par le support qu'elles constituent pour une riche faune d'Harpacticoïdes, de Foraminifères, de Ciliés et d'Amphipodes (*Dexamine spinosa*). Elles sont partiellement consommées par les Talitres des hauts niveaux. La destruction des Amphipodes par le pétrole explique peut-être partiellement la particulière abondance des « marées vertes » de 1978 et 1979.

EFFETS DE LA MAREE NOIRE SUR LES PEUPLEMENTS SEDIMENTAIRES

Sur les cartes suivantes sont reportés 4 états successifs de distribution pour chaque espèce : 1964-1968, 1979, 1980 puis 1981.

Sur les cartes A, on lit les zones de forte densité des principales espèces. Nous comparons 4 périodes, les espèces sont représentées par leurs initiales :

Ba : Bathyporeia pilosa, sarsi et guillamsonniana
(notées respectivement P, S)
Ne : Nerine cirratulus
Ur : Urothoe brevicornis
Ar : Arenicola marina
Ow : Owenia fusiformis
Tt : Tellina tenuis
Tf : Tellina fabula
Do : Donax vittatus
Oph : Acrocnida brachiata

X { *Ensis ensis et Ensis siliqua*
Mactra corallina
Pharus legumen
Lutraria lutraria }

Les espèces étudiées réagissent de manière différente comme en témoigne la courbe de l'évolution des biomasses et le tableau Notons qu'il s'agit là de l'évolution moyenne de 10 stations. Des déplacements des zones de peuplements visibles sur les cartes n'ont pas pu être pris en compte dans le tableau.

Les cartes B à O présentent les courbes d'isovaleurs en cal/m^2 des biomasses des principales espèces de la macrofaune endogée. Les facteurs de conversion sont :

1 cal = 1 g de matière organique fraîche = 0,2 g de matière organique sèche.

Les populations de quelques espèces n'ont apparemment pas été touchées , elles paraissent même s'étendre : par exemple, les deux polychetes errantes *Nephtys hombergii* (Cartes B) et *Sigalion mathildae* (Carte C) ce dernier ayant tendance à coloniser maintenant le haut de la plage.

Le crabe *Platyonichus latipes* (cartes D) dont les noyaux se décalent vers l'Ouest de la grêve où ils s'étendent en densité, et le bivalve *Tellina tenuis* (carte E), qui après une très forte progression jusqu'en 1980, voit ses effectifs amorcer une diminution sur les noyaux Est et Ouest mais avec le maintien du noyau central fixe.

D'autres espèces par contre ont beaucoup régressé après la catastrophe.

En opposition à *Tellina tenuis*, *Tellina fabula* (cartes F) qui occupait en 1968 tout le niveau de basse mer, continue à regresser dans les très bas niveaux où elle disparaît actuellement à l'est.

L'ophiure *Acrocnida brachiata* (cartes G) est de moins en moins présente. On assiste à une diminution du nombre des noyaux plus étalés vers l'Ouest.

Les polychetes sédentaires *Arenicola marina* (cartes H) et *Owenia fusiformis* (carte I) ont regressé après la marée noire, mais à partir de 1980 ; les deux espèces se développent à nouveau sur le coté Est de l'Estran, bien que pour *Owenia* on constate une légère diminution en 1981.

Le bivalve *Donax vittatus* prépondérant en 1968, a probablement bien diminué avant 1978 puis à complétement disparu après l' "AMOCOCADIZ". Après une timide réapparition en 1980, le noyau central prend de l'importance , s'étend vers le bas de la plage et le noyau de l'Est se consolide (carte J).

En 1979 on trouve les Amphipodes *Bathyporeia* (carte K) et *Urothoe* (Carte L) en petite quantité juste au Nord-Ouest de la plage, ils ont beaucoup regressé après la marée noire en 1980, ils n'ont pas récupéré en biomasse mais se sont étendus.

En 1981 une légère regression pour *Bathyporeia* pourrait être attribué à une mortalité estivale tandis qu'*Urothoe* se déplace vers le centre et non l'Ouest de la plage.

Les cartes M représentent des espèces non cartographiées auparavant il semblerait qu'elles aient pris de l'importance depuis 1968 : mais à partir de 1980, *Magelona papillicornis et Glycera convoluta* voient leurs effectifs diminuer fortement surtout à l'Est de la plage.

En 1980, on a pu cartographier *Cardium edule et Nerine cirratulus* qui avaient disparu après la marée noire (carte N). Ils sont actuellement en régression.

Spiophanes bombyx apparu en 1980 s'étend vers le centre et le bas de la plage (cartes O)

Par contre les trois autres espèces apparues en 1980 *Paradoneis armata, Eteone flava et Capitomastus* (cartes O) ont beaucoup diminué en 1981. Notons que *Capitomastus minimus* est significatif d'un milieu pollué (LE MOAL Y.et QUILLIEN-MONOT,1979)

CONCLUSIONS

1- PERTES DE BIOMASSE DIFFEREES

La contamination des organismes n'a pas toujours été immédiatement mortelle. Dans les sédiments restés longtemps contaminés, certaines espèces qui avaient bien survécues au printemps de la marée noire ont vu leurs populations s'effondrer en 6 mois, voire un an plus tard. Le tableau suivant, portant sur 10 stations de sable fin du bas de la plage de St Efflam en baie de Lannion, milieu bien représentatif par sa nature et par son degré de contamination, montre que le taux de survie ultime pour les espèces qui avaient bien survécues est nettement plus faible que celui enregistré à la fin du printemps 1978 pris comme référence, soit après la marée noire.

ESPECES	PRINTEMPS 1978	ETE 1978	1ER SEMESTRE 1979	2ème SEMESTRE 1980	1ER SEMESTRE 1981
TELLINA FABULA	1	0,20	0,20	0,035	0,09
TELLINA TENUIS	1	0,65	1,39	1	0,88
OWENIA FUSIFORMIS	1	0,75	0,32	0,34	0,36
ARENICOLA MARINA	1	0,72	1,67	3,30	3,11
NEPHTYS HOMBERGII	1	2	0,33	0,42	0,68
BIOMASSE TOTALE	1	0,77	1,18	1,02	0,95

Le facteur multiplicatif est proche des valeurs ultérieures les plus faibles des dates variées rencontrées dans ce tableau soit : 0,09 ; 0,65 ; 0,32 ; 0,72 ; 0,33.

On peut estimer que les espèces qui avaient bien survécues initialement accusent une mortalité additionnelle raisonnable proche de : (0,o9 + 0,65 + 0,32 + 0,72 + 0,33) /5 soit 0,4
La mortalité totale ultime étant 1,4 fois plus élevée que celle calculée pour la fin du printemps 1978.

2- *DIVERSITE DES COMPORTEMENTS SPECIFIQUES*

Le comportement relatif des diverses espèces est très variable et assez imprévisible.

En ce qui concerne les effets immédiats pour une même station certaines espèces résistent parfaitement (*Tellina tenuis, Owenia fusiformis*) d'autres sont presque intégralement détruites (*Donax vittatus, Cardium edule, Bathyporeia, Echinocardium cordatum, Pharus legumen, Ensis ensis, Ensis siliqua, Mactra corallina, Lutraria lutraria*. Seules les trois premières espèces de cette liste sont timidement reparues aujourd'hui.

A plus long terme, les comportements sont aussi disparates :

Tellina tenuis non affecté a prospéré jusqu'en 1980 et amorce une diminution de même pour *Nephtys hombergii.*

Tellina fabula, Owenia fusiformis, Arenicola marina peu affectés initialement ont considérablement regréssés en 1979, quelquefois très tardivement bien que certains signes d'un rétablissement certain apparaissent (*Arenicola marina*) en 1981.

Urothoe et Bathyporeia qui avaient initialement disparus sont réapparus mais seulement dans la partie la plus occidentale et sans encore atteindre les densités initiales.

Depuis 1980 on assiste à la réapparition de certaines espèces qui avaient complétement disparu après la marée noire, *Donax vittatus* se consolide alors que *Nerine cirratulus, Ensis ensis et Cardium edule* ont du mal à se réimplanter.

Des espèces nouvelles pour la localité apparues en 1980 dont certaines seraient significatives d'une pollution résiduelle, commencent à diminuer en 1981.

On doit donc considérer que les peuplements présentent encore en fin 1981 surtout dans la partie Est de la plage un déséquilibre écologique profond alors qu'à l'Ouest des signes d'une recouvrance avancée apparaissent.

3- EVOLUTION GLOBALE

Il semble s'amorcer une dérive qualitative générale des peuplements de sables fins bien calibrés très typiques à *Donax vittatus*, *Tellina fabula*, *Echinocardium cordatum* et grands *Solenidae* vers des peuplements plus banalisés de sables fins plus eutrophisés qu'envasés à *Arenicola marina*.

La marée noire n'est sans doute pas seule en cause ("marées vertes") mais elle a accéléré cette évolution régressive des peuplements originels.

Les parties hautes et surtout basses de l'estran, plus que les niveaux médians, sont les plus touchées. Ceci coïncide avec une plus forte accumulation des échouages des nappes dans le haut de la plage et dans la moitié Est, suivie d'une persistance des hydrocarbures dans l'épaisseur du sédiment. Ceci est conforme à ce qui a été observé sur tout le littoral en matière d'accumulation d'hydrocarbures sous l'influence des vents d'Ouest dominants.

La forte régression constatée dans le bas de la plage, confirmée par la nature essentiellement infratidale de la grande masse des cadavres retrouvés dans les échouages, souligne un autre fait majeur en dehors des hauts de plage directement atteints par les nappes, l'essentiel de la mortalité est à imputer au pétrole dispersé ou dissout au sein de la masse d'eau.

Au niveau biomasse totale, on constate une chute importante en 1978 et 1979 par rapport à 1964-1968. Des affaiblissements des noyaux de peuplements de la partie Est par rapport à ceux de la partie Ouest, et du bas par rapport au haut, sont très notables. Cette distribution se maintient en 1980 puis 1981 en s'appauvrissant encore. Notons que la progression des *Arenicoles* se fait au dépend d'espèces de petite taille plus productives. Il en résulte donc une baisse générale de la fertilité de l'ensemble de la baie par rapport à 1968 de l'ordre de près des deux tiers.

Le rétablissement encore très incomplet des peuplements demanderait des études ultérieures.

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ESPECES / DATE (Nb m² / cal/m²)	1964 – 1968	AMOCO CADIZ	1978 AVRIL	1978 JUIN	1978 SEPT.	1979 1^e sem.	1980 1^e sem.	1980 SEPT.	1980 NOV.	1981 FEV.	1981 AVRIL	1981 AOUT
TELLINA FABULA	157/13	↘	29/5	9/1	5/1	11/1	2/1	8/0,05	16/0,3	20/0,3	33/0,6	18/0,4
TELLINA TENUIS	500/45	↗	624/104	554/63	448/74	810/145	711/128	660/119	560/89	580/84	630/98	590/93
DONAX VITTATUS	160/57	↘↗	1/0	2/1	0/1	0/0	10/1	11/1,7	70/1,8	40/2,4	37/2,5	42/3
OWENIA FUSIFORMIS	576/39	↘	440/31	294/22	340/25	98/10	143/9	144/11,4	106/10,2	136/10,5	184/13	120/10
ARENICOLA MARINA	5/9	↗	2/9	1/9	1/4	3/15	7/29	9/31	8/28	8/24	10/32	7/28
NEPHTHYS HOMBERGII	6/2	↗	12/6	23/15	19/9	3/2	1/1	26/2	31/3	37/3,5	51/4,6	41/4,2
BATHYPOREIA	17/0,7	↘↗	0/0	0/0	1/0	0/0	0/0	24/0,2	24/0,2	58/0,6	32/0,3	26/0,2
UROTHOE	3/0,2	↘↗	3/0,2	0/0	0/0	1/0	0/0	30/0,5	27/0,5	28/0,4	29/0,4	30/0,5
CAPITOMASTUS MINUTUS		↗						10/0,01	21/0,03	80/0,16	46/0,07	18/0,02
TOTAL Cal./m²	*165,9*	∼	*146,2*	*111*	*114*	*173*	*162*	*165,8*	*133,03*	*125,86*	*151,57*	*139,32*

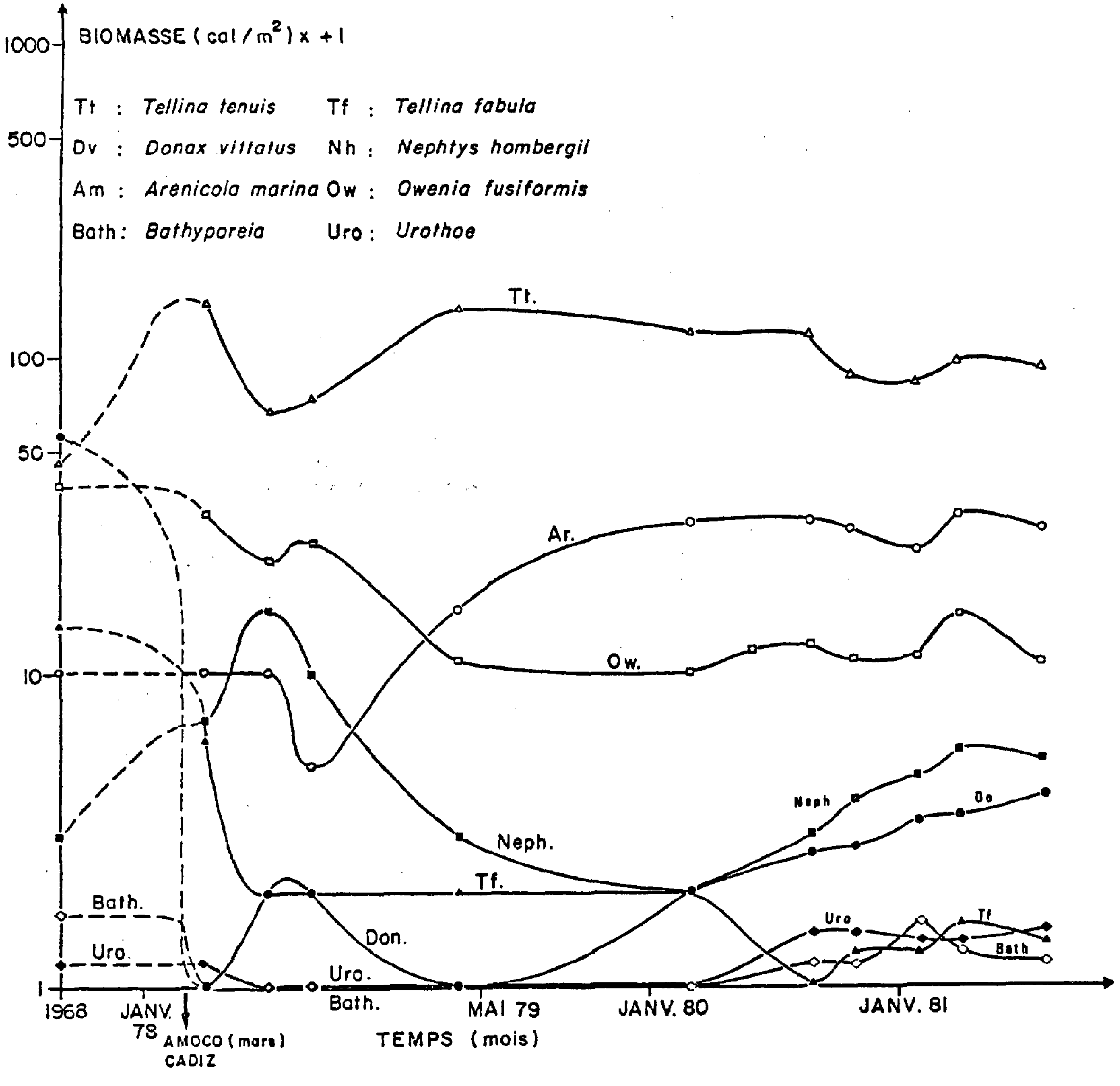
Variation dans le temps du nombre d'individus au m² et de la biomasse en cal./m² pour les principales espèces de la plage de St-EFFIAM, suite à l'échouage de l'AMOCO-CADIZ.

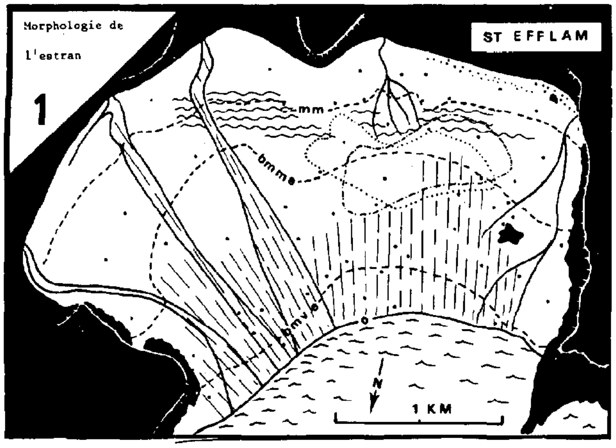
(Le nombre d'individus au m² et la biomasse sont des chiffres établis d'après une moyenne de 10 stations du bas et centre de plage, ce qui représente une surface de 2 m² au total)

COURBE DE L'EVOLUTION DES BIOMASSES

Moyenne de 10 stations de bas de plage.

Les nombres d'individus au m^2 et de la biomasse en cal/m^2 pour tous les especes examines (44 especes differentes) sont disponibles sur demande de l'auteur.





EMPLACEMENT DES STATIONS DE PRELEVEMENTS.

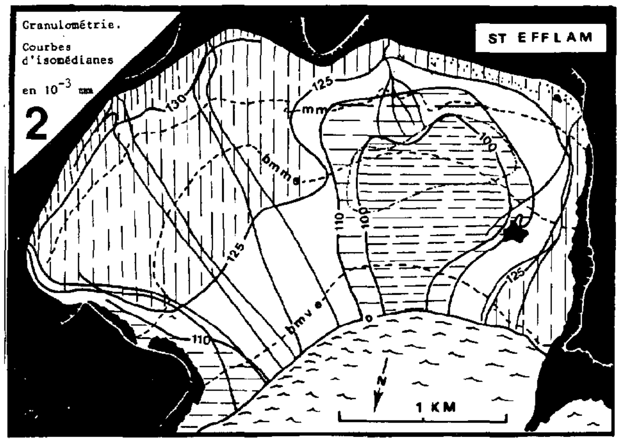
1 RUISSEAUX.

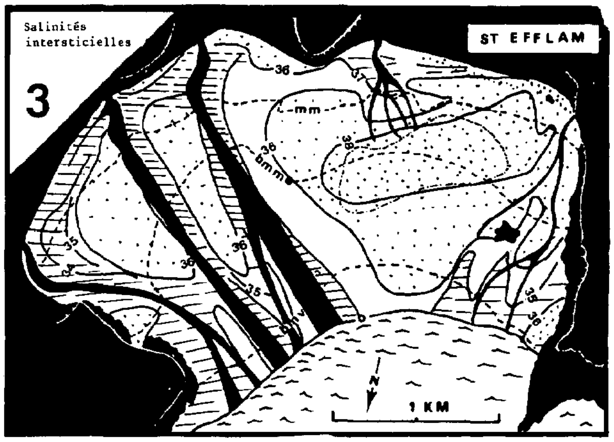
2 NAPPES DE RUISSELLEMENT.

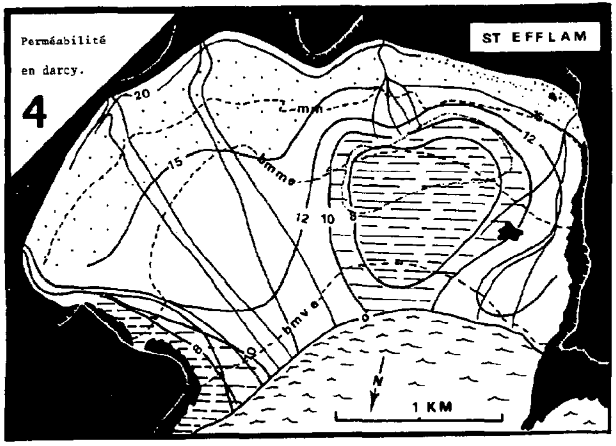
3 GRANDS RIPPLE-MARKS.

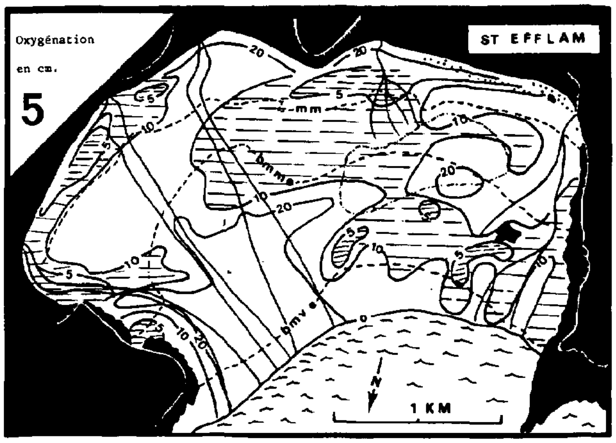
4 HERBIER DE *ZOSTERA NANA* 1979

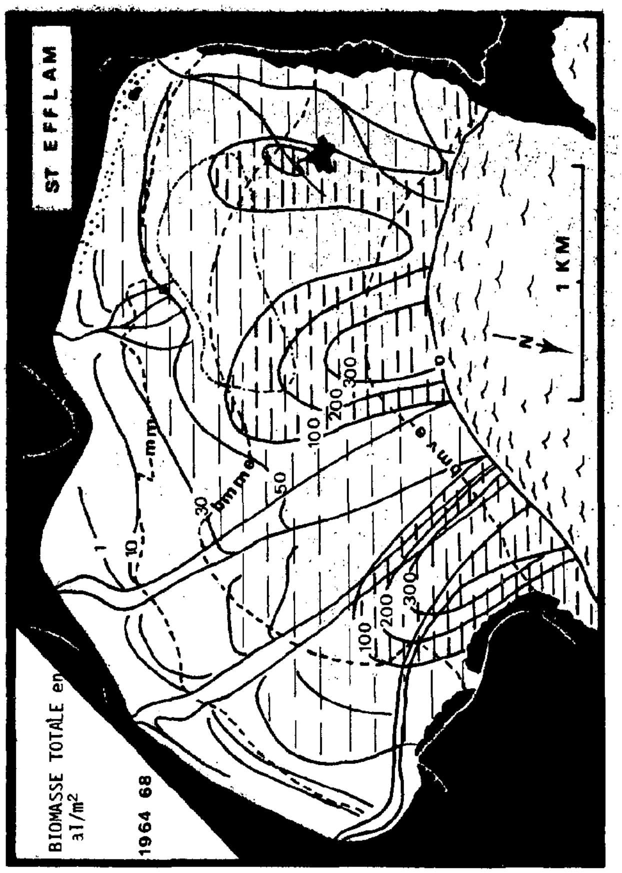
5 HERBIER DE *ZOSTERA NANA* 1964-68







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BIOMASSE TOTALE en al/m²
1964 68
1 KM
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bmve
mm
0
1
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50
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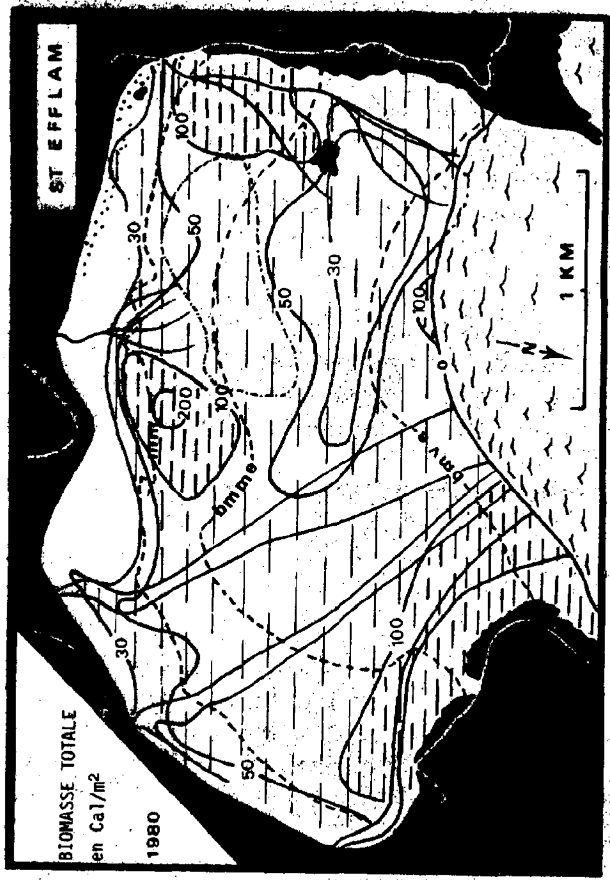
200
300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en Cal/m²
1980
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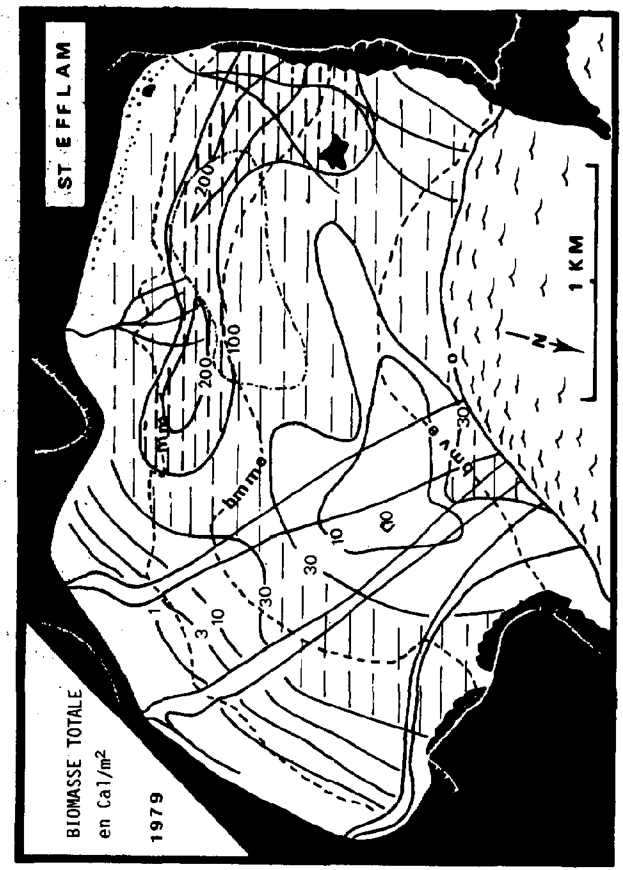
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en Cal/m²
1979
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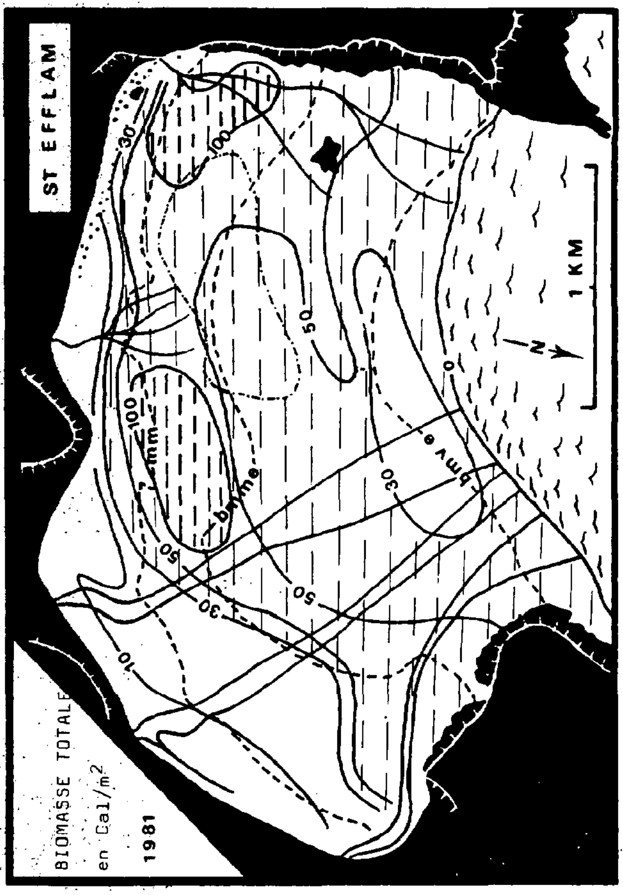
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en Cal/m²
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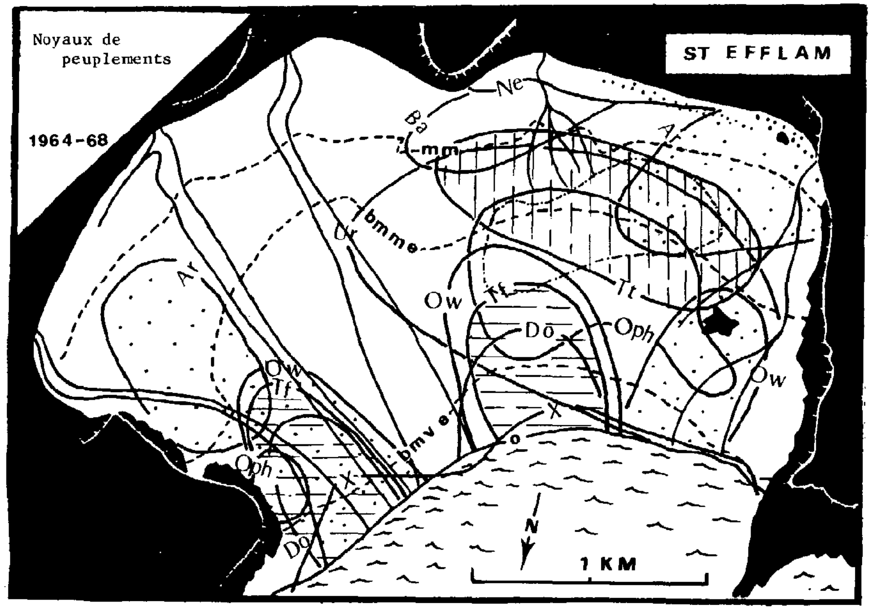
Noyaux de peuplements
1964-68
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Ba
mm
Ar
bmme
Ur
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Tf
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Dо
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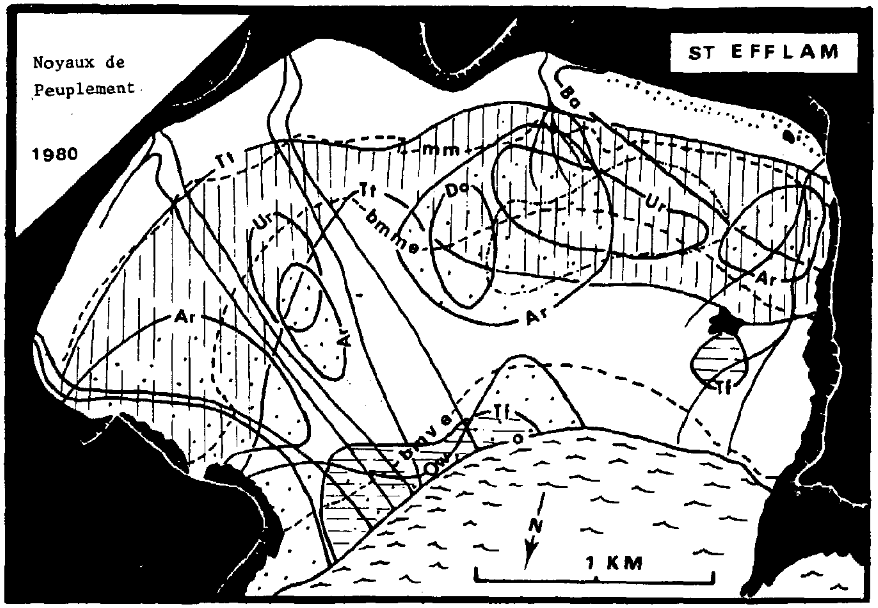
Tf
Oph
X
bmve
o
X
Do
N
1 KM

A


Noyaux de
Peuplement
1980
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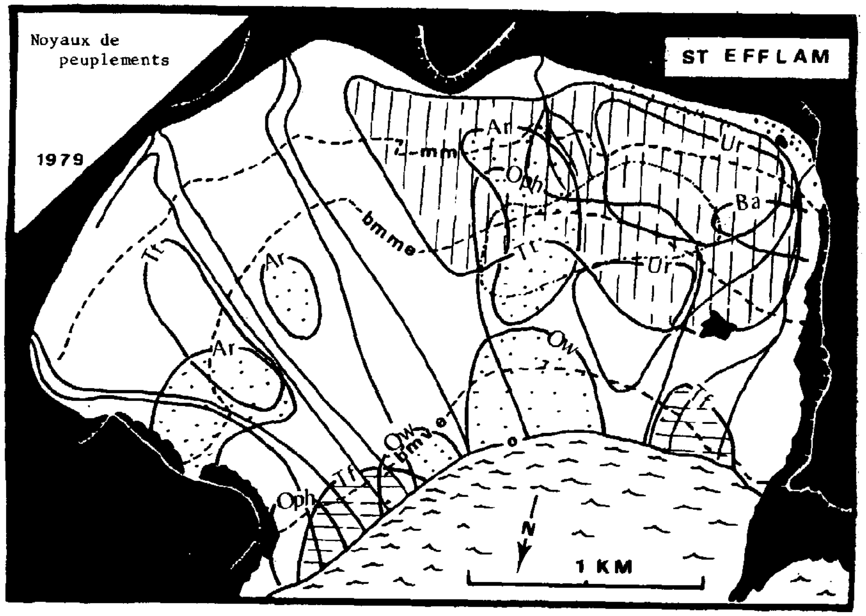
Tf
Ow
o
N
1 KM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o
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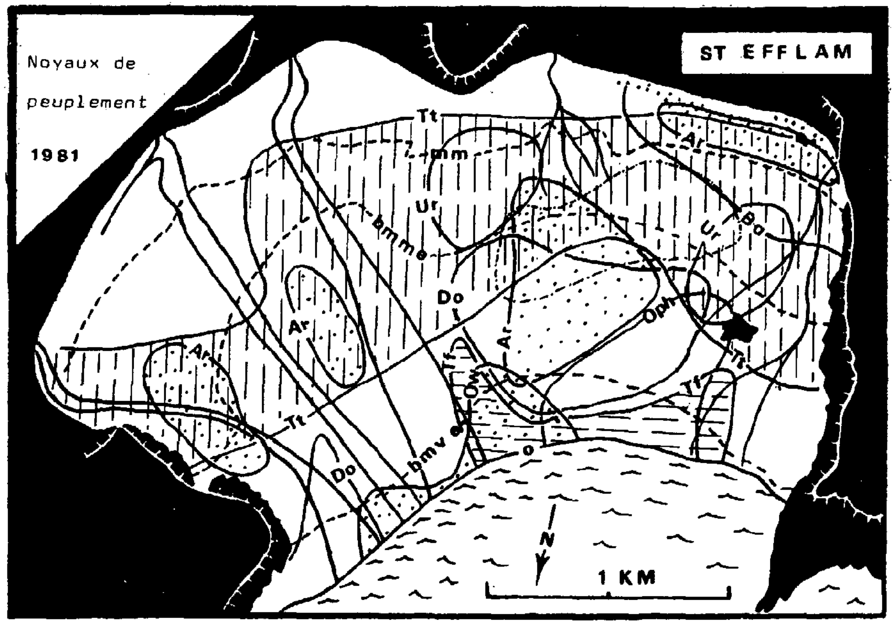
1 KM

A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peuplement
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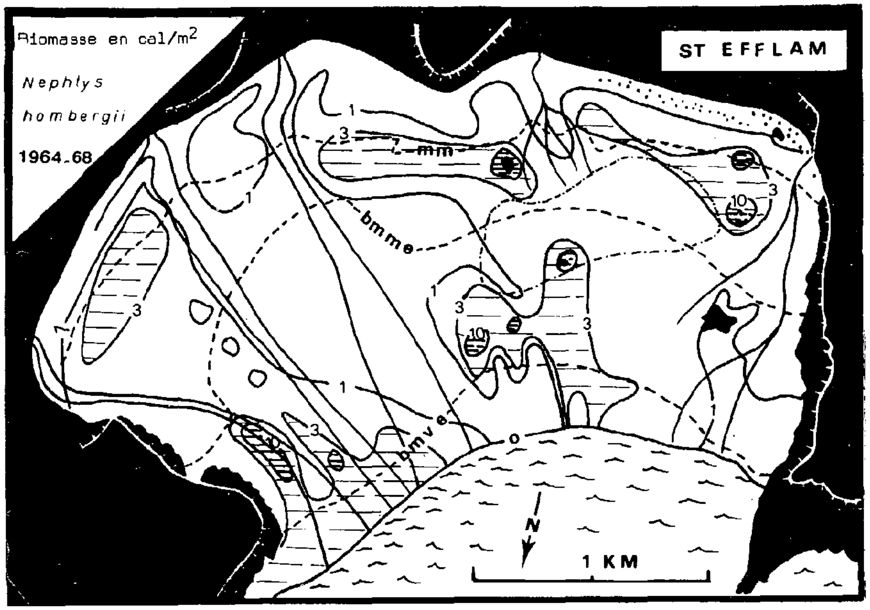
1 KM


Biomasse en cal/m2
Nephtys
hombergii
1964-68
ST EFFLAM
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3
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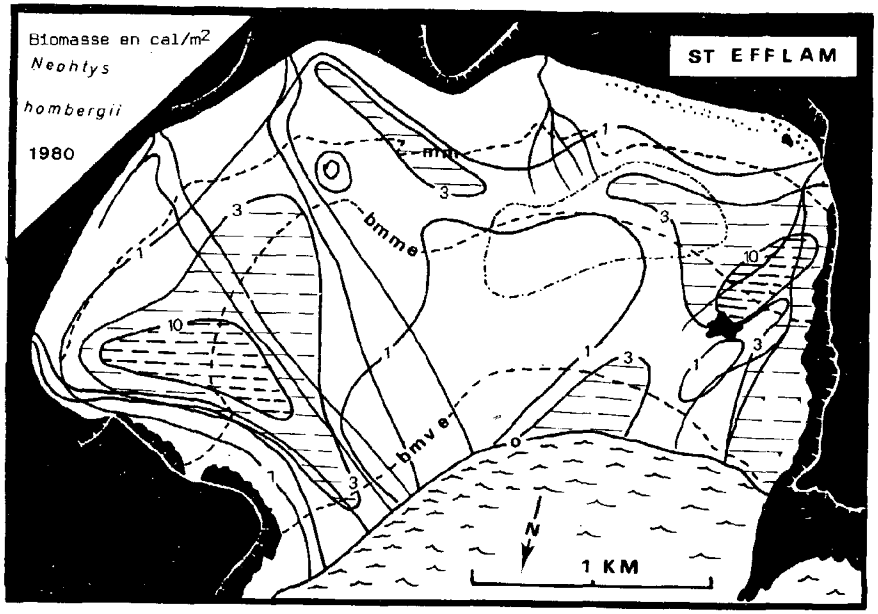
2 mm
bmme
bmve
0
N
1 KM

B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hombergii
1980
ST EFFLAM
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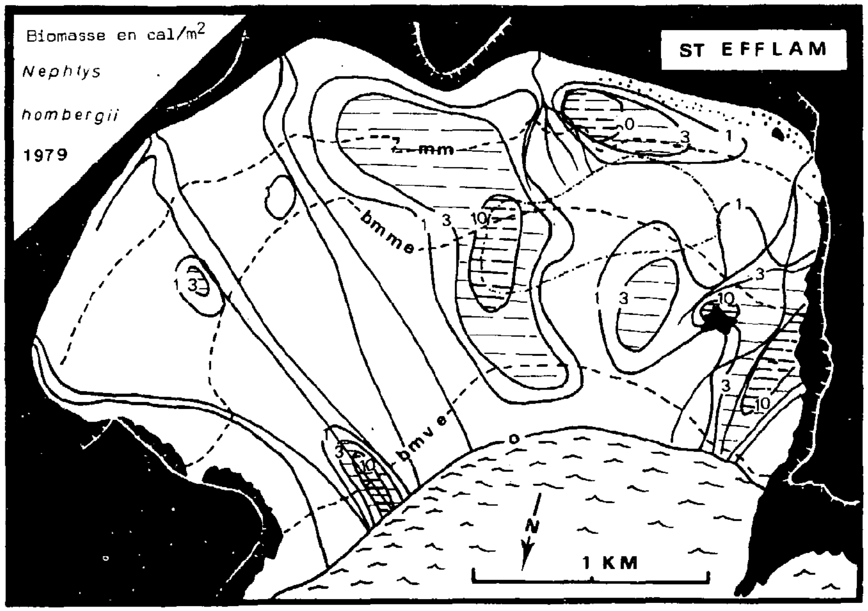
1 KM


Biomasse en cal/m2
Nephtys
hombergii
1979
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2 mm
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bmve
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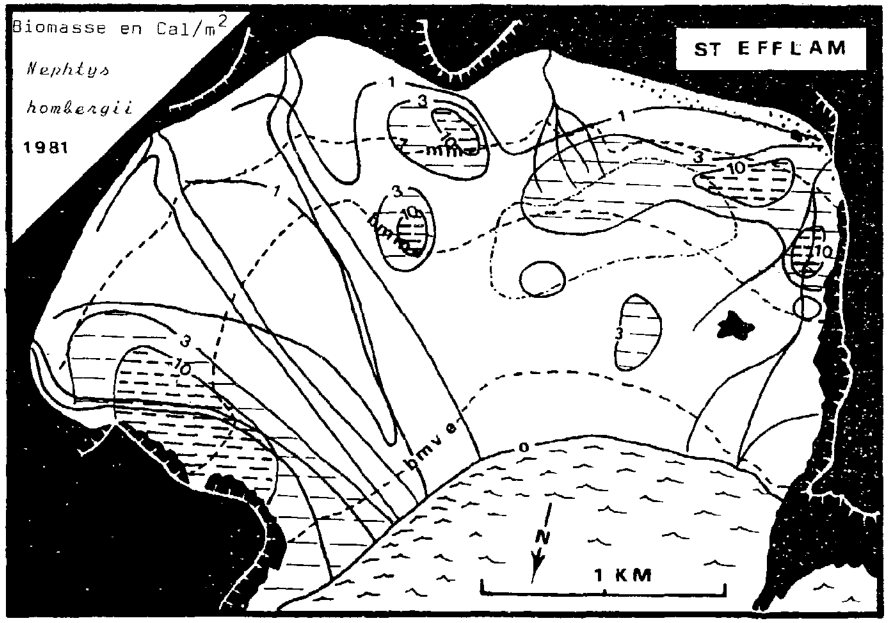
1 KM

B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hombergii
1981
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2 mm
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bmve
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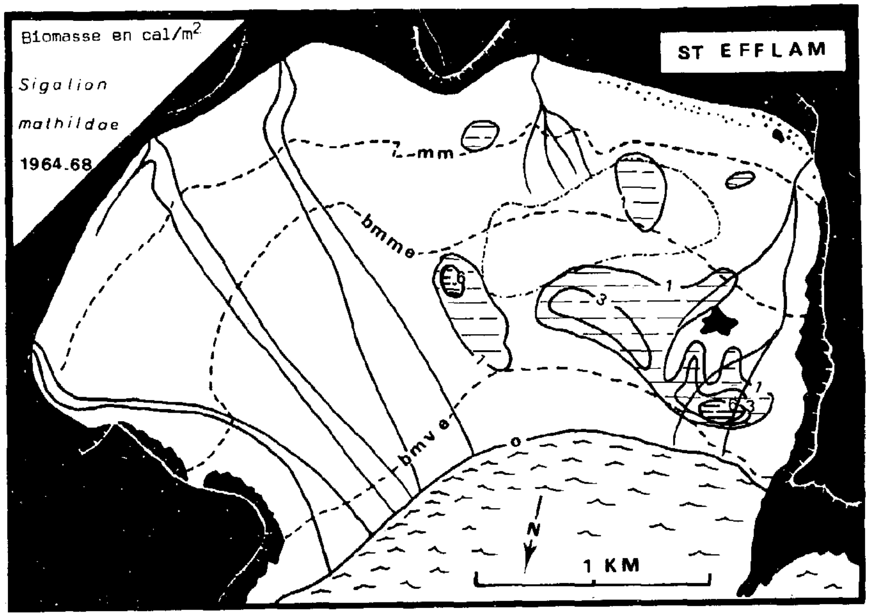
1 KM


Biomasse en cal/m^2
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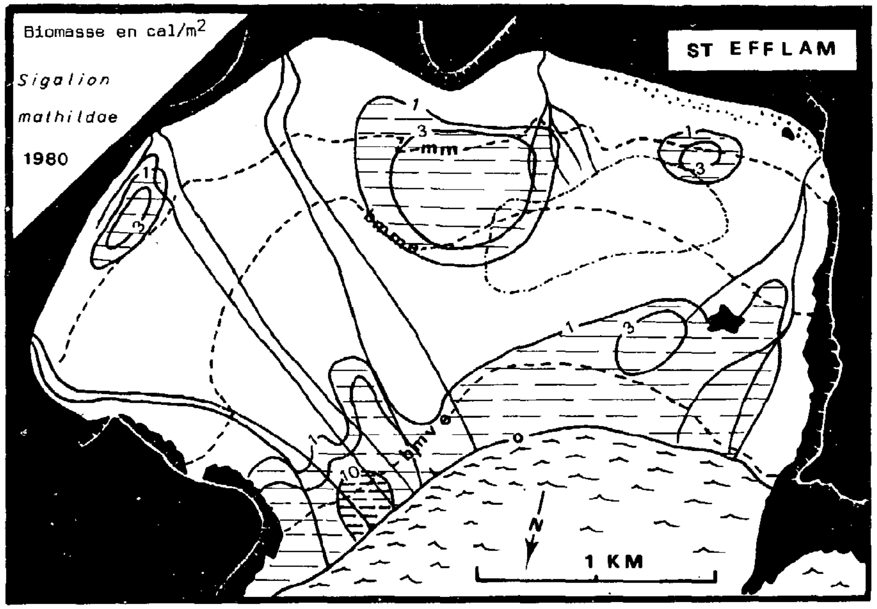
mathildae
1964.68
ST EFFLAM
7 mm
bmme
bmve
1
3
0
N
1 KM

C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Sigalion
mathildae
1980
ST EFFLAM
7 mm
bmme
bmve
1
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0
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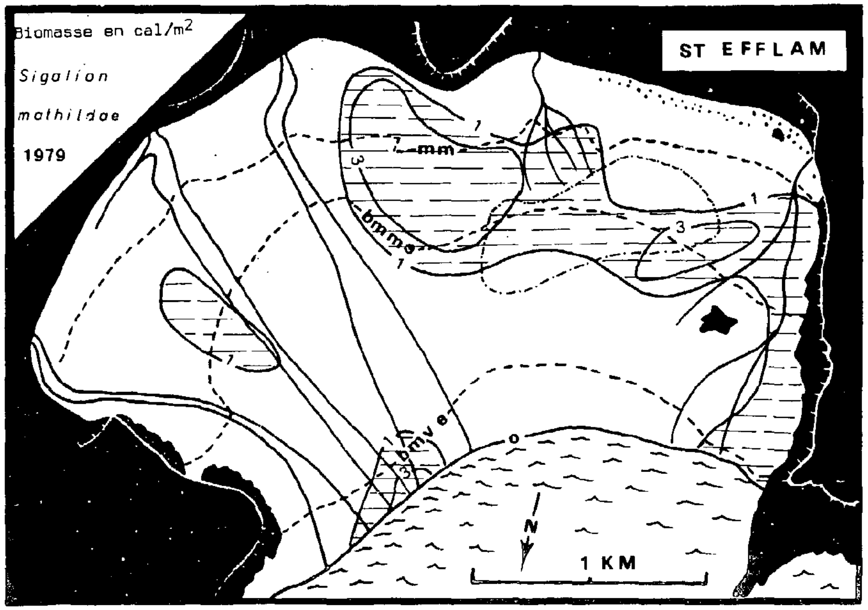
1 KM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Sigalion
mathildae
1979
ST EFFLAM
7 mm
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bmve
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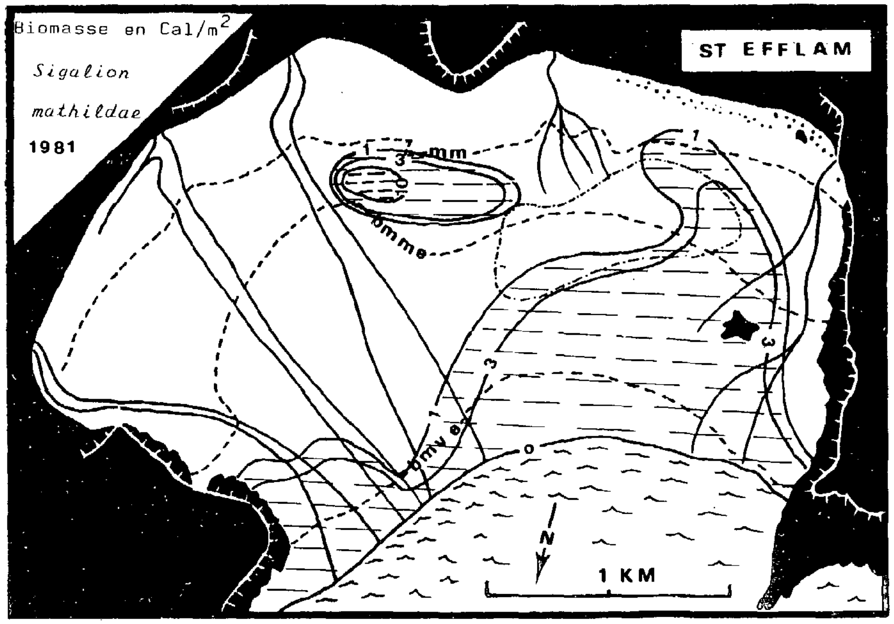
N
1 KM

C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Sigalion
mathildae
1981
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bmme
bmve
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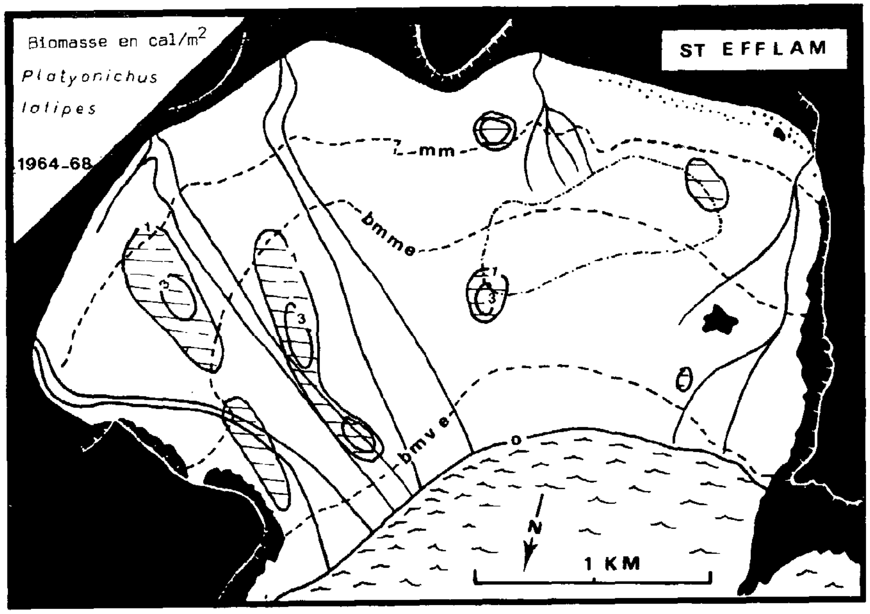
1 KM


Biomasse en cal/m²
Platyonichus
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1964-68
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3
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mm
bmme
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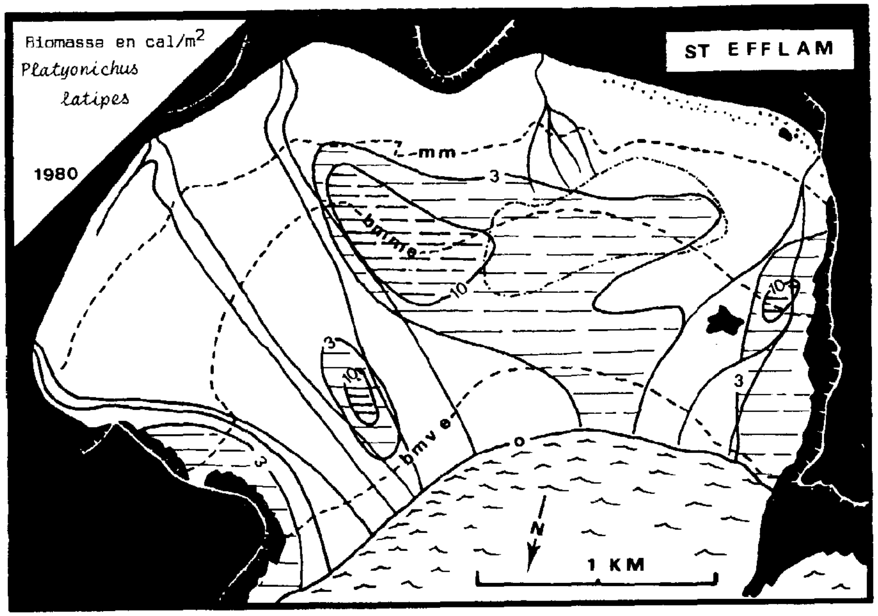
0
N
1 KM

D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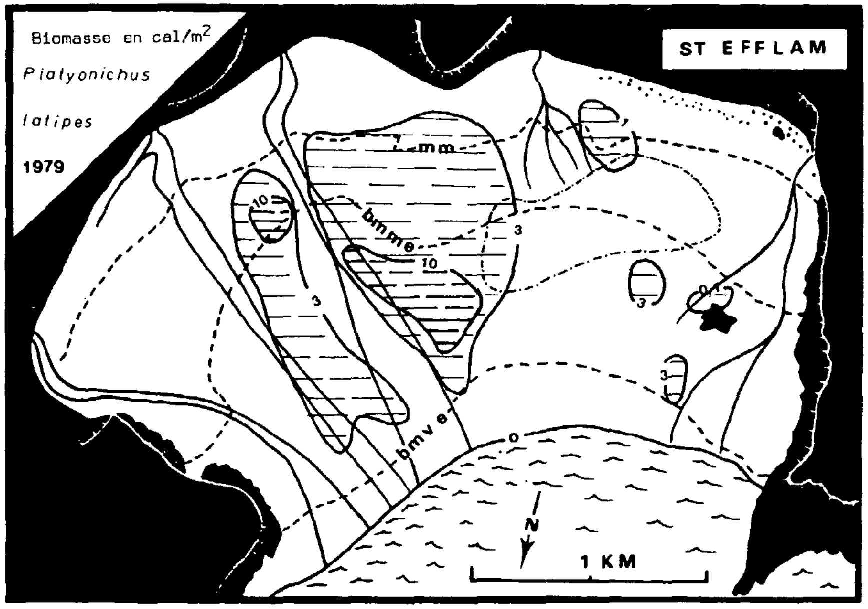
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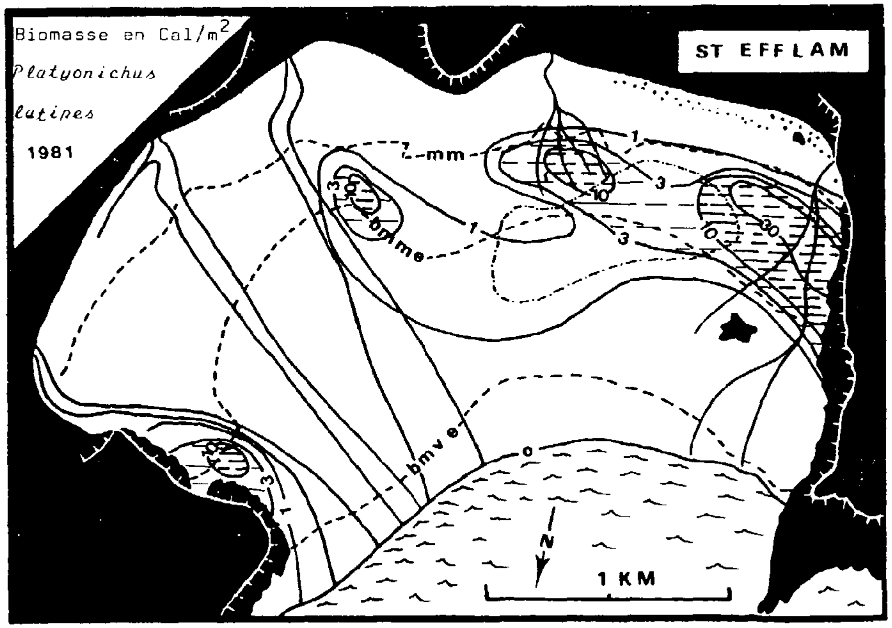
D


Biomasse en Cal/m²
Platyonichus
latipes
1981
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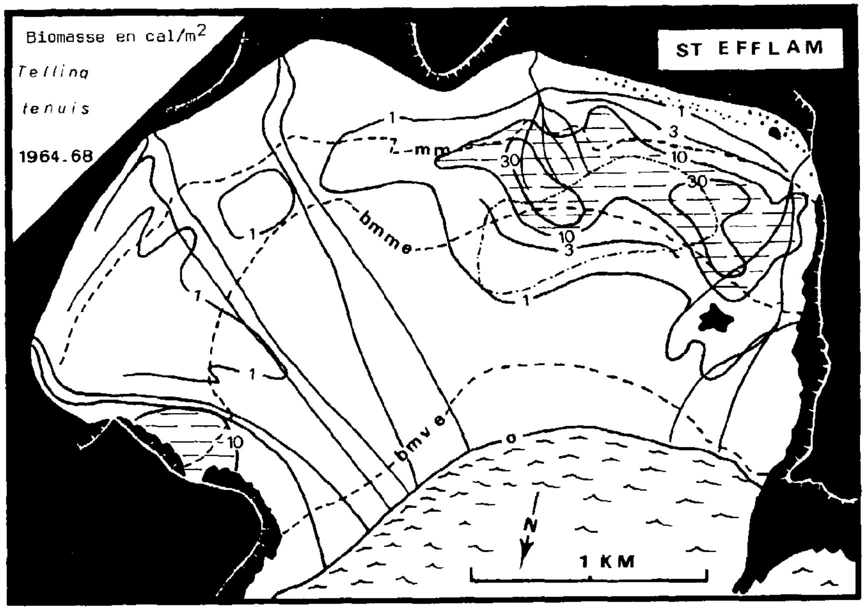
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1964.68
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bmme
bmve
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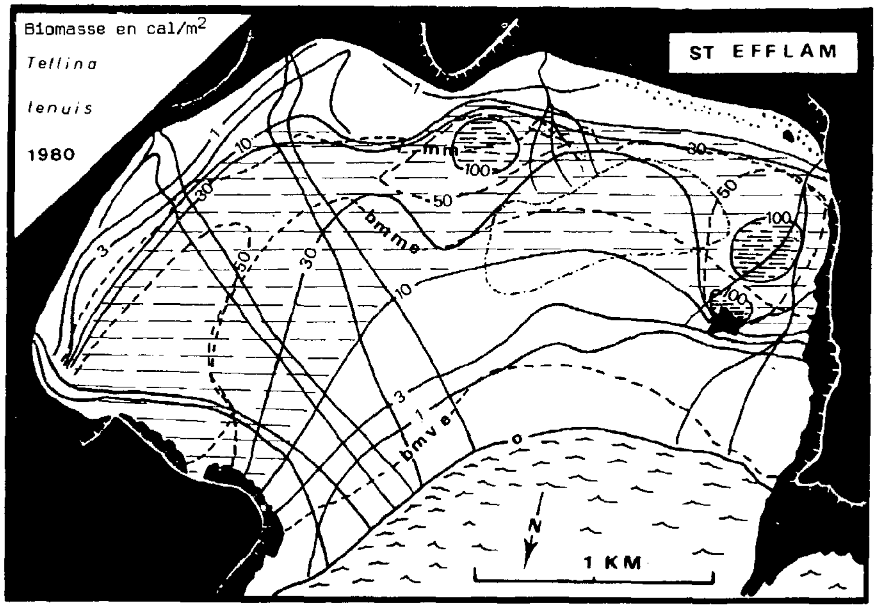
1 KM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tenuis
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bmve
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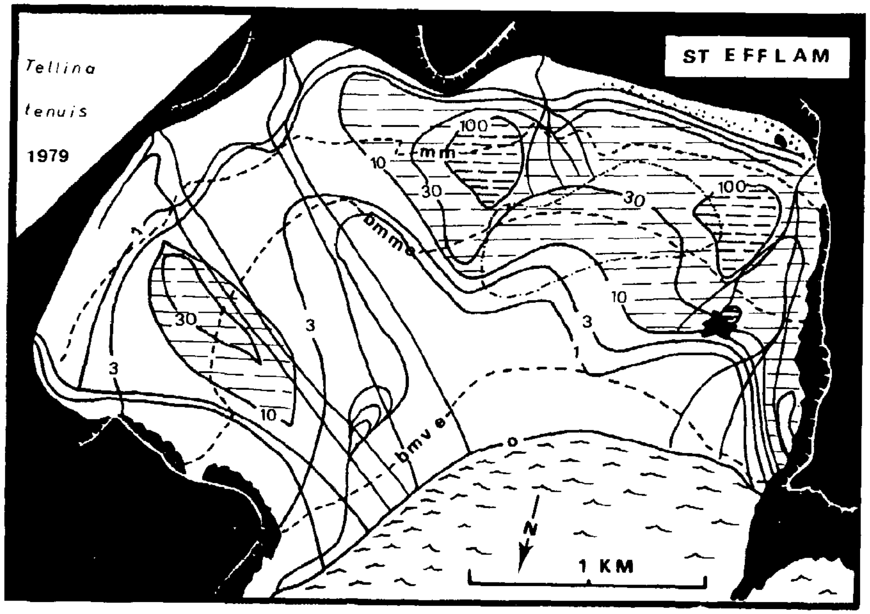
1 KM

E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1979
ST EFFLAM
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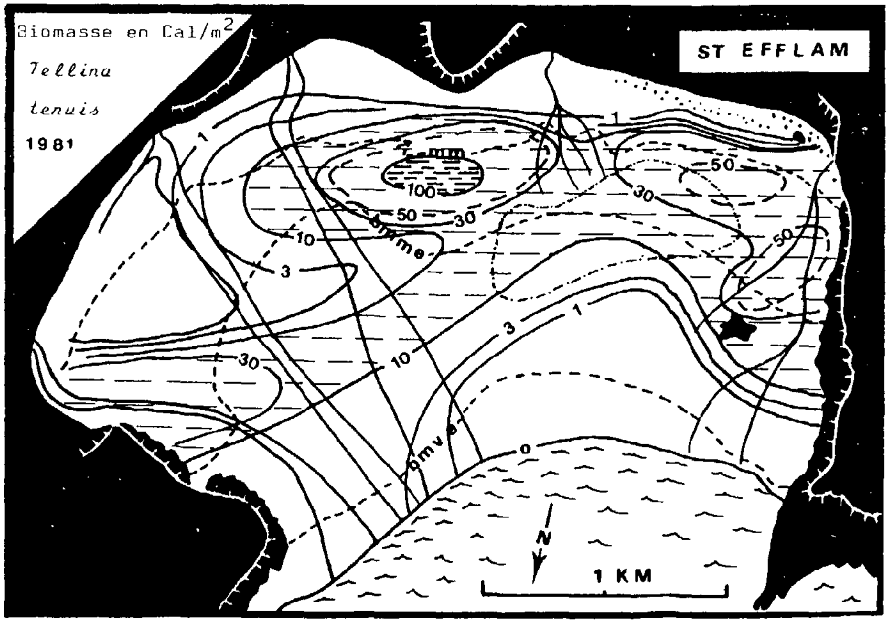
N
1 KM


Biomasse en Cal/m2
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tenuis
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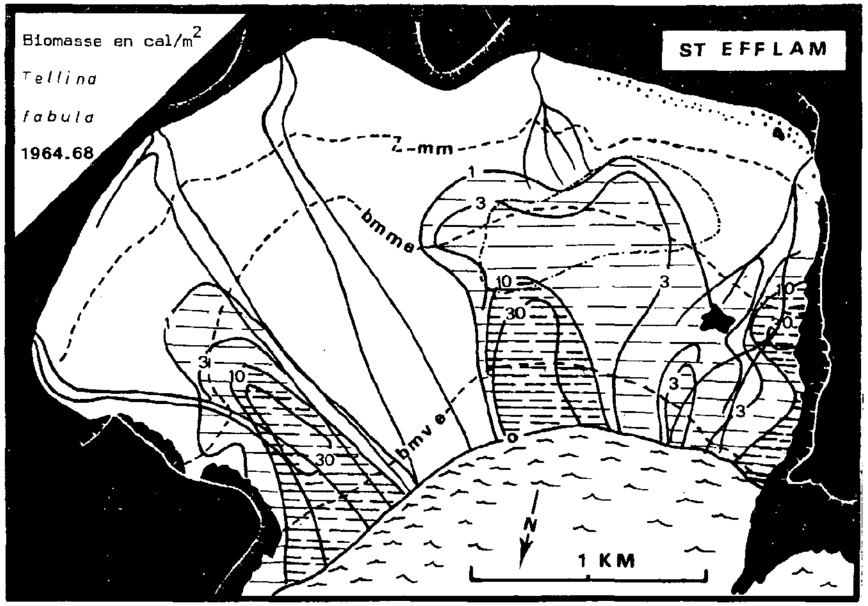
1 KM

E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1964.68
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Z-mm
bmme
bmve
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3
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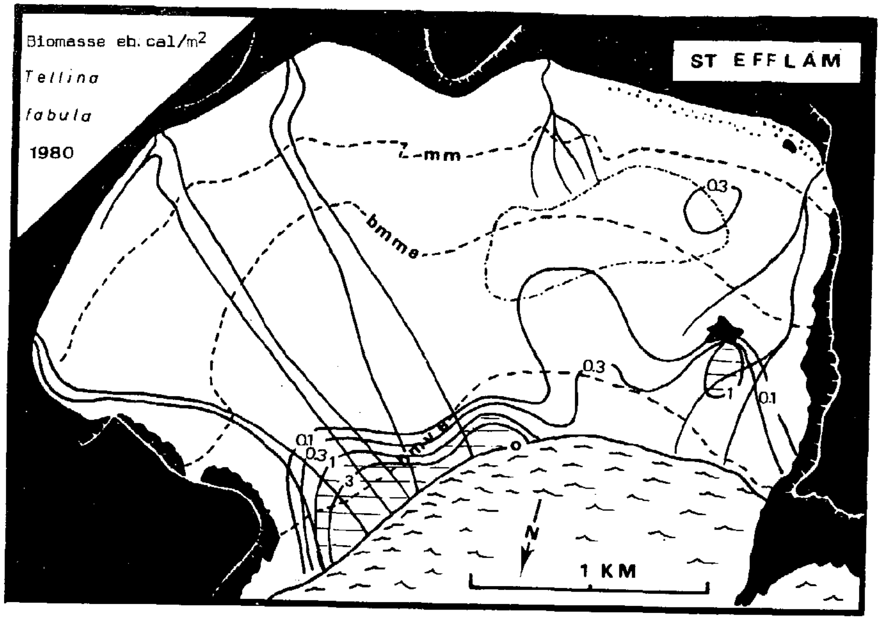
N
1 KM

F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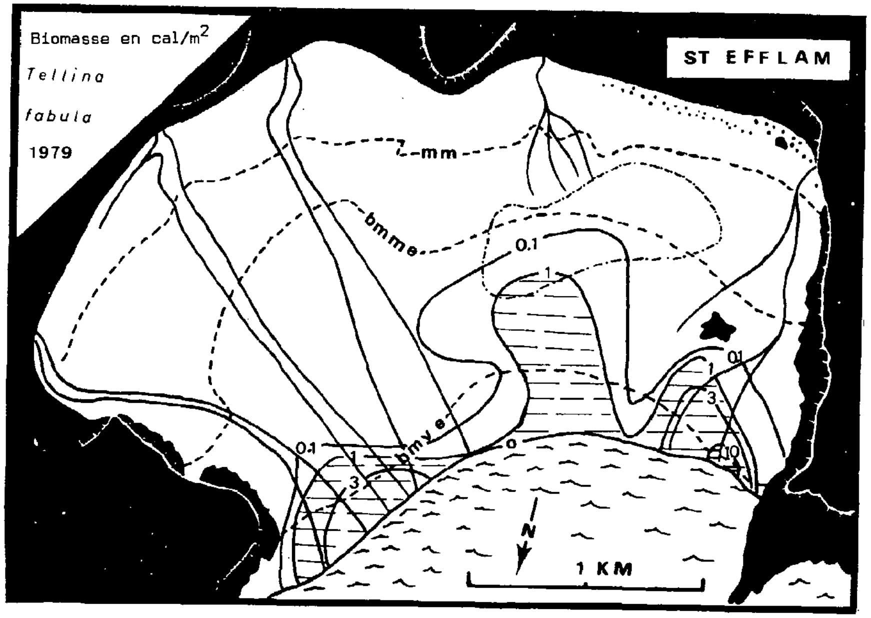
Biomasse en cal/m^2
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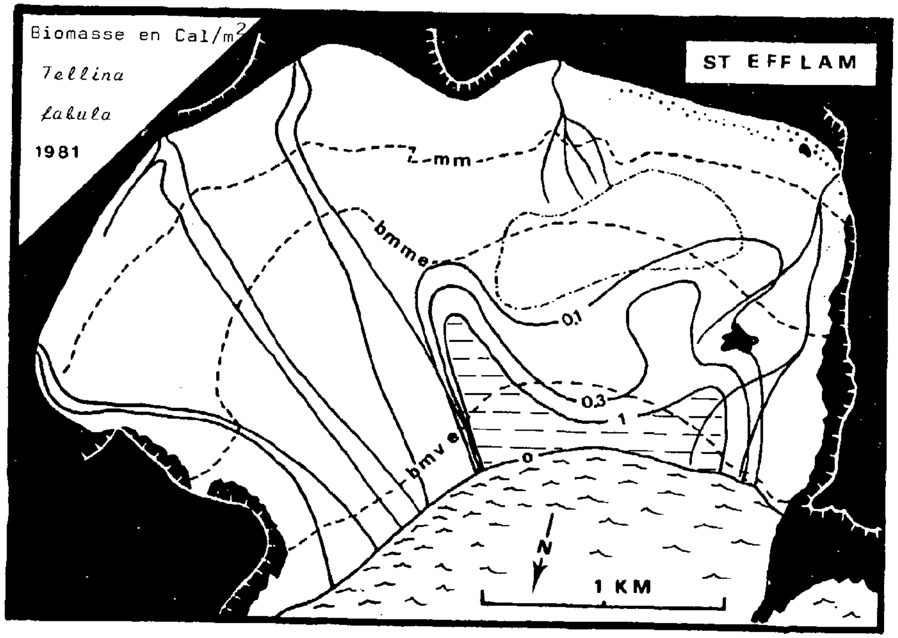
Biomasse en Cal/m^2
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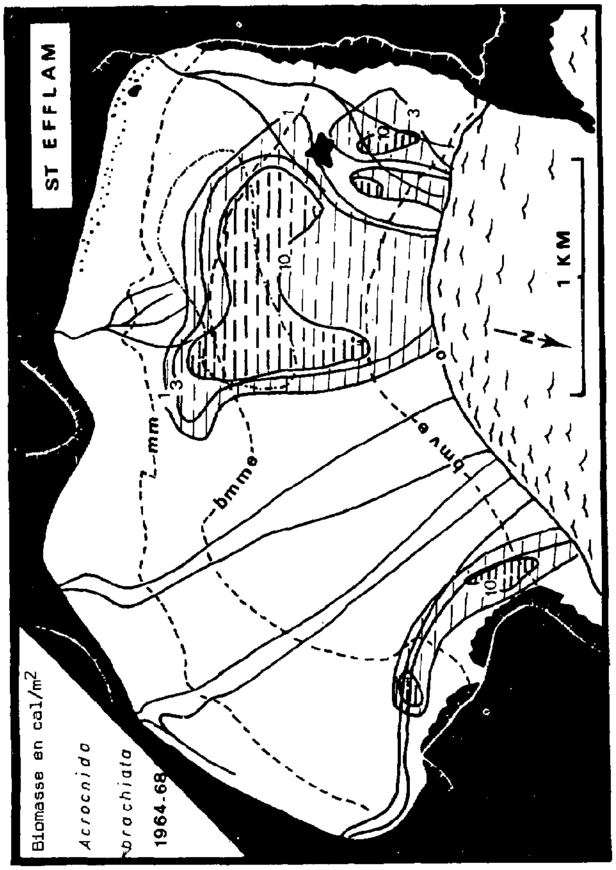
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1964-68
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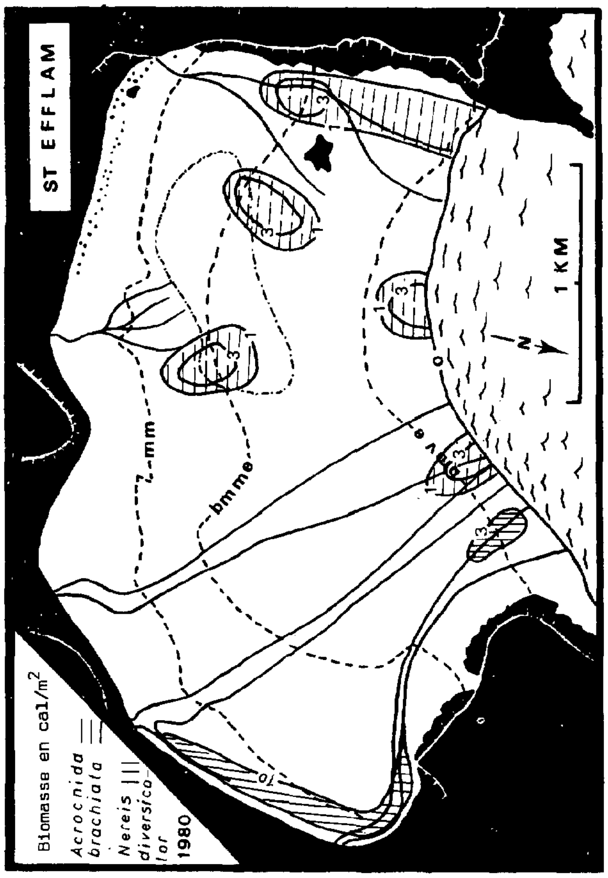
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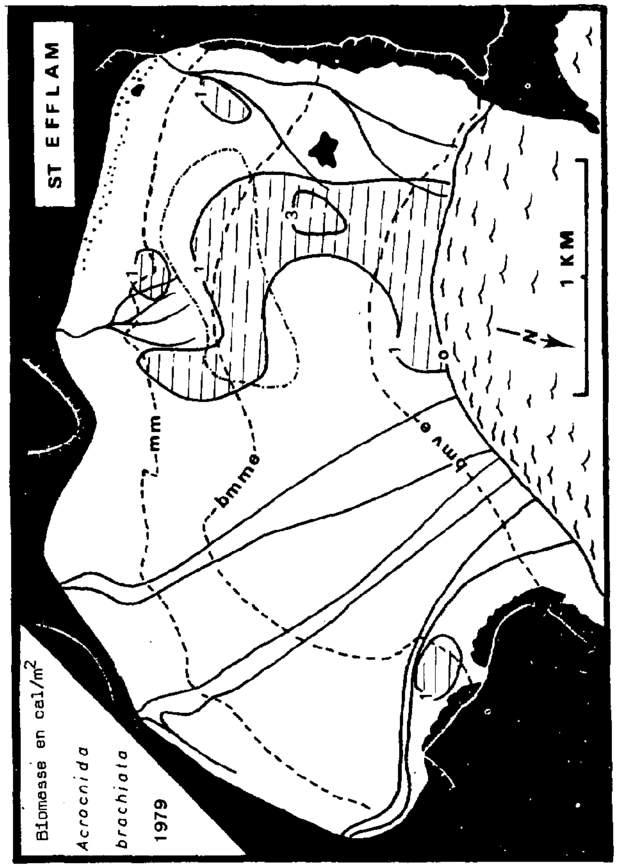
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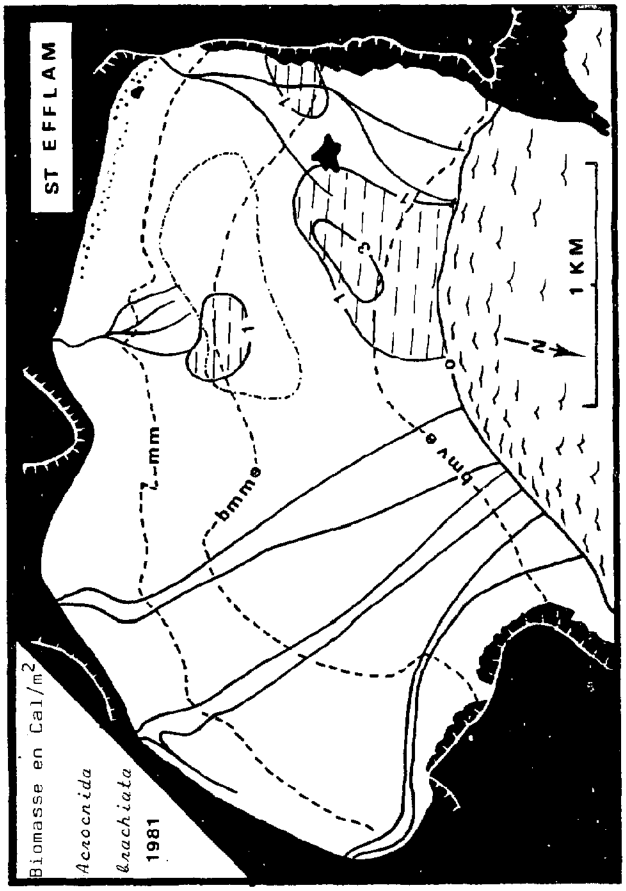
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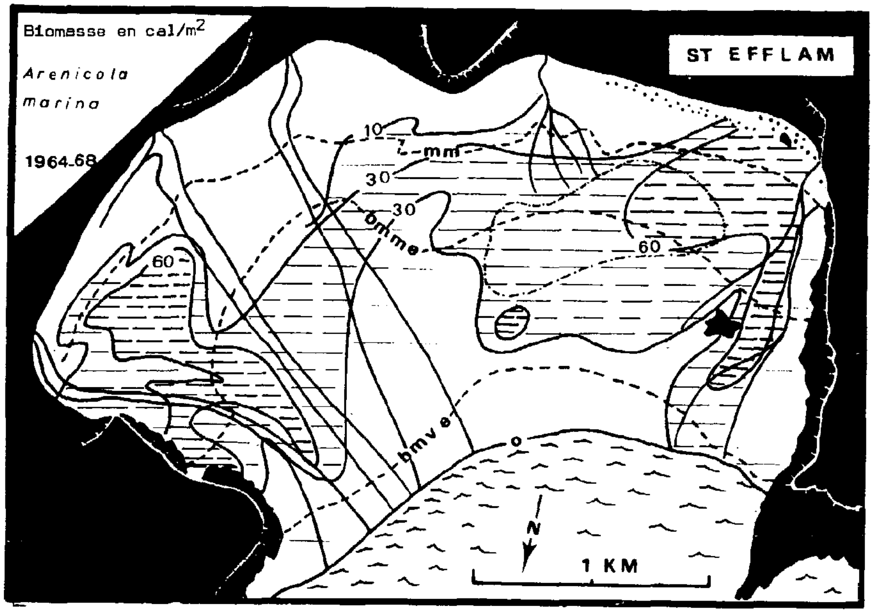
Biomasse en cal/m²
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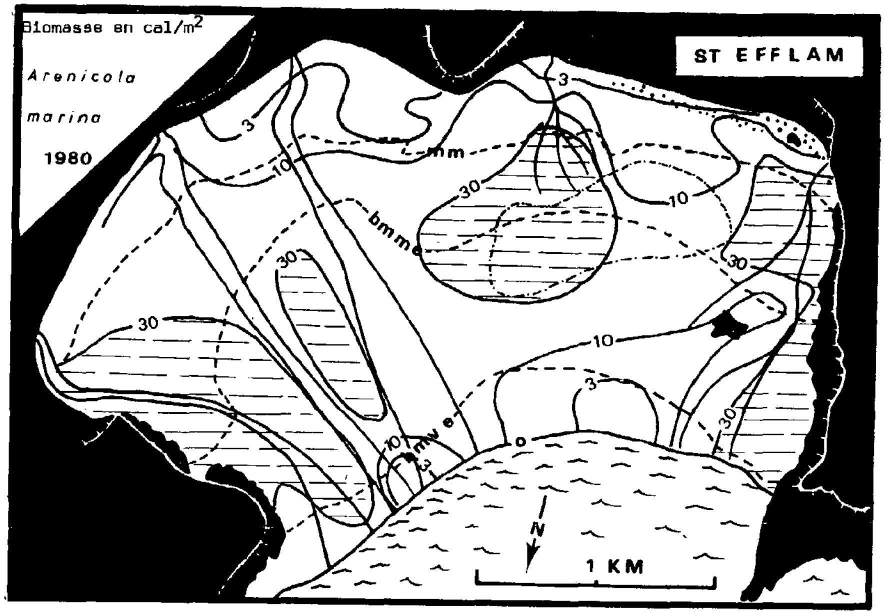
N
1 KM

H


Biomasse en cal/m²
Arenicola
marina
1980
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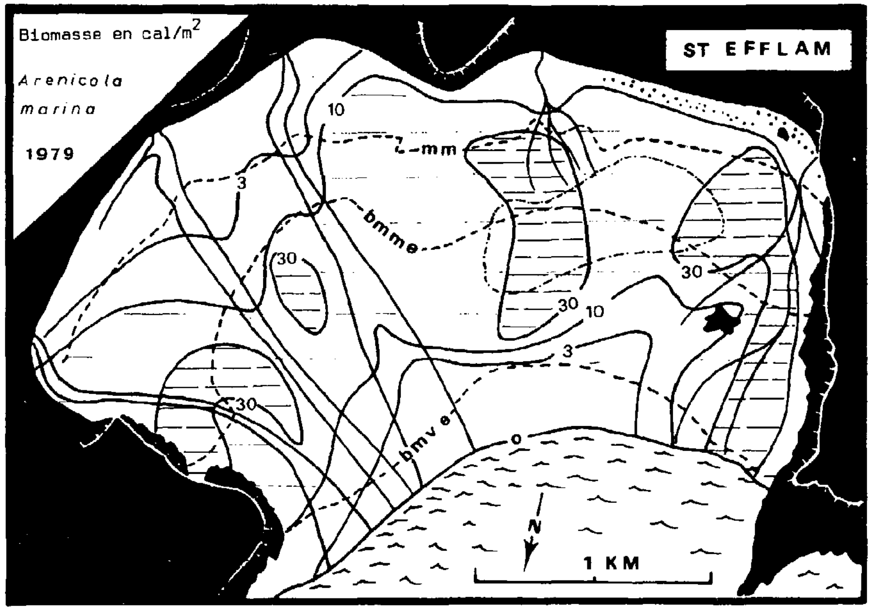
Biomasse en cal/m²
Arenicola
marina
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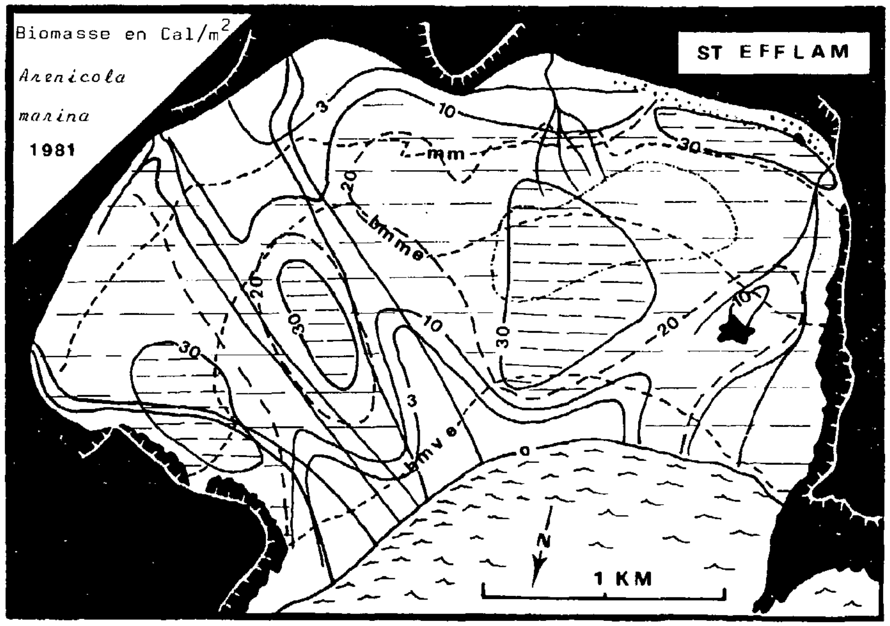
H


Biomasse en Cal/m²
Arenicola
marina
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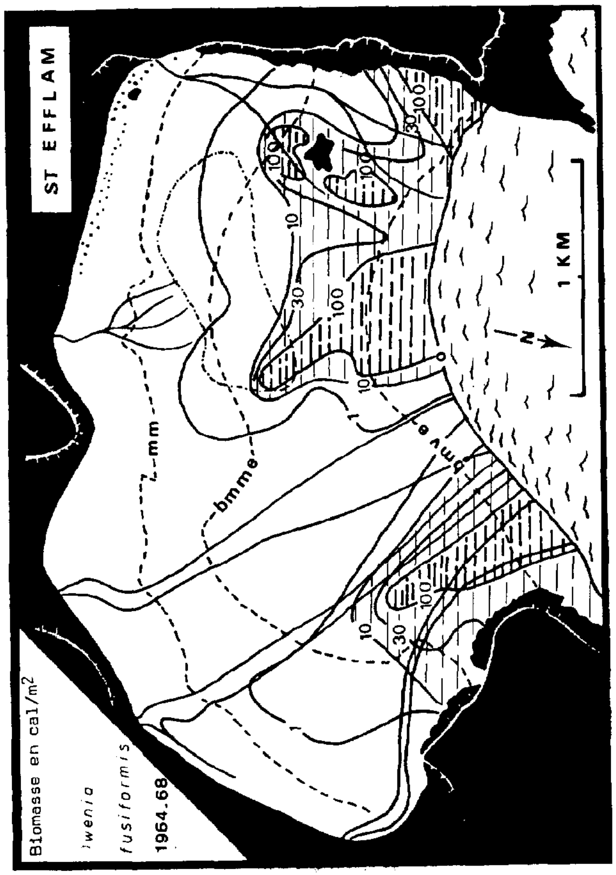
Biomasse en cal/m^2
Owenia
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1964.68
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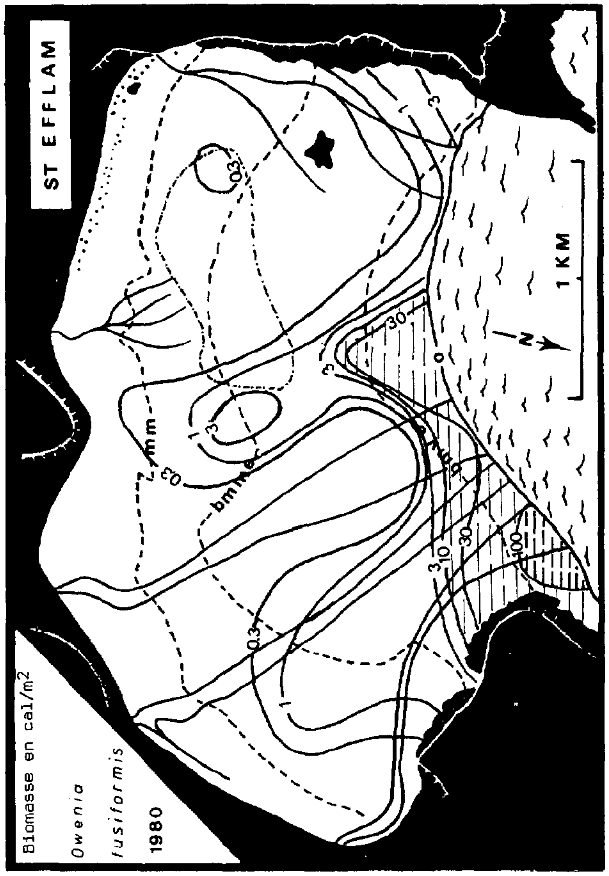
Biomasse en cal/m^2
Owenia
fusiformis
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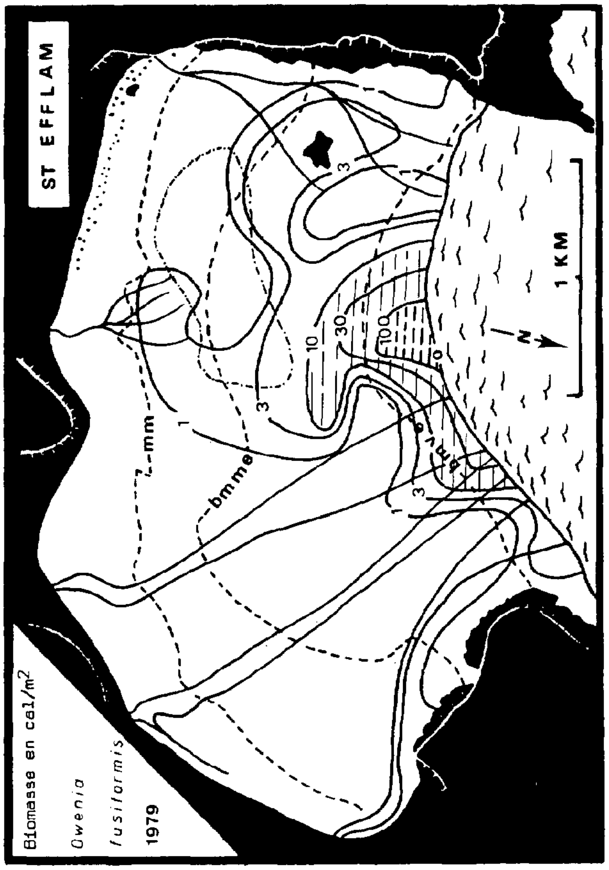
Biomasse en cal/m^2
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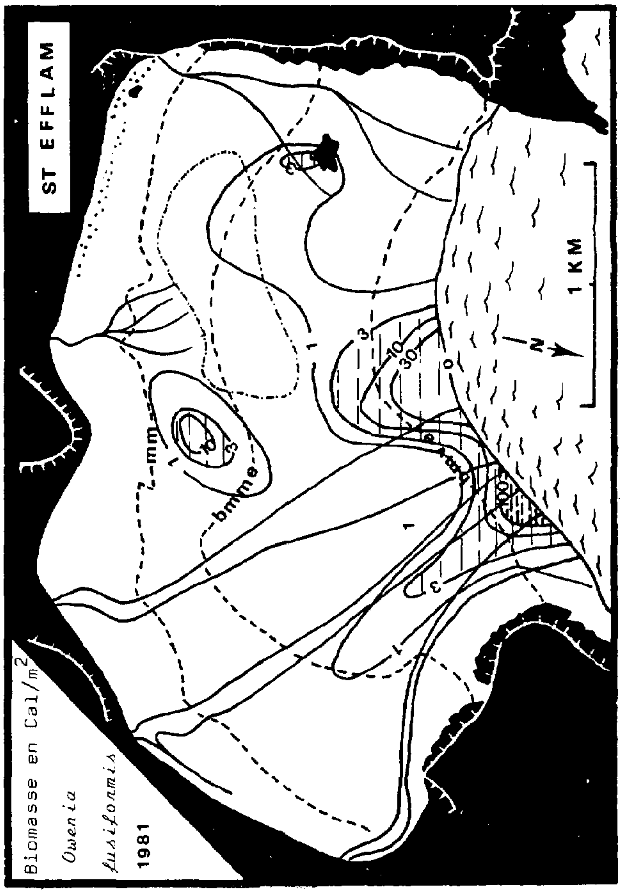
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Owenia
fusiformis
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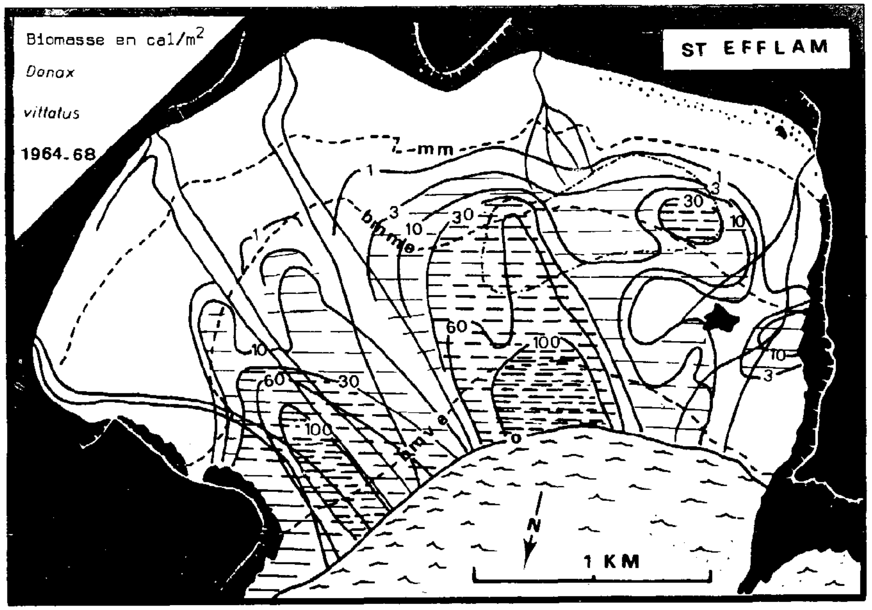
Biomasse en cal/m2
Donax
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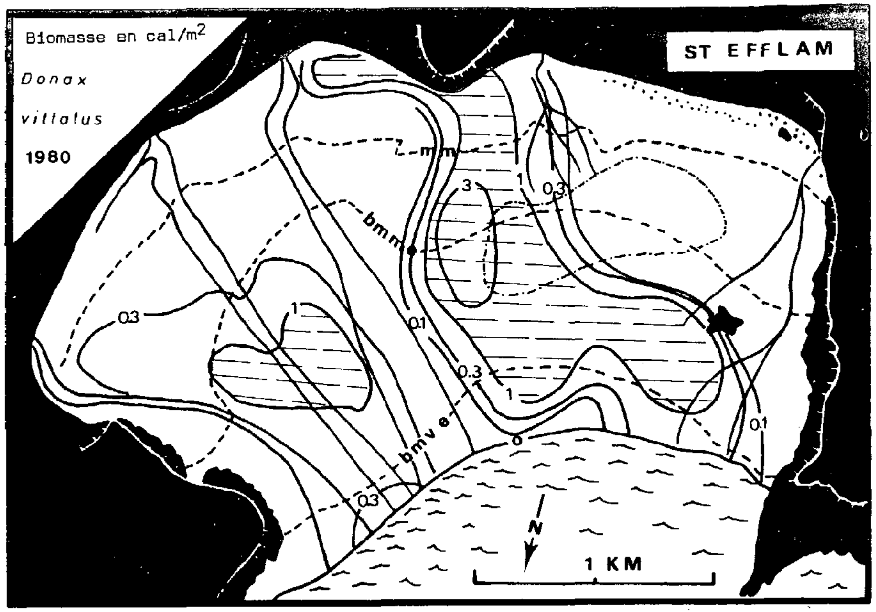
1 KM

J


Biomasse en cal/m2
Donax
vittatus
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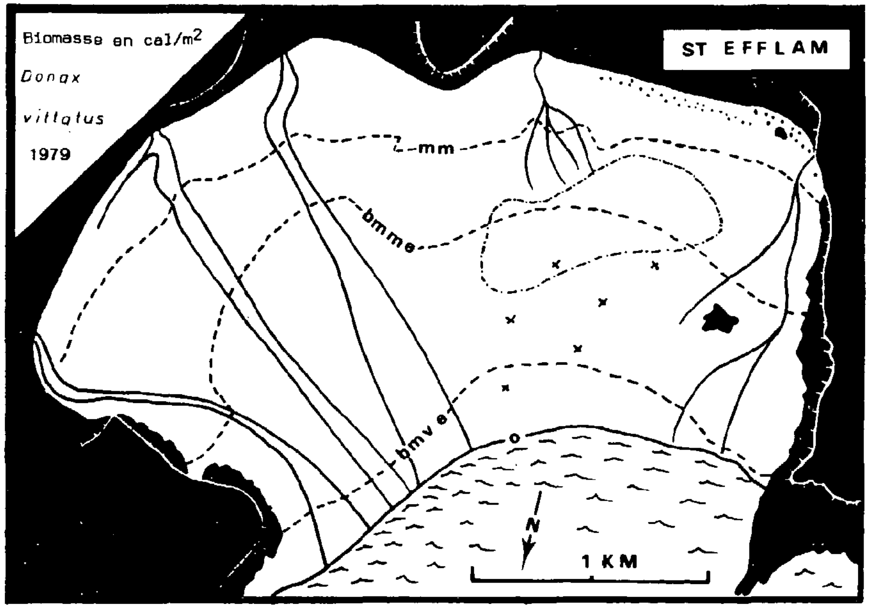
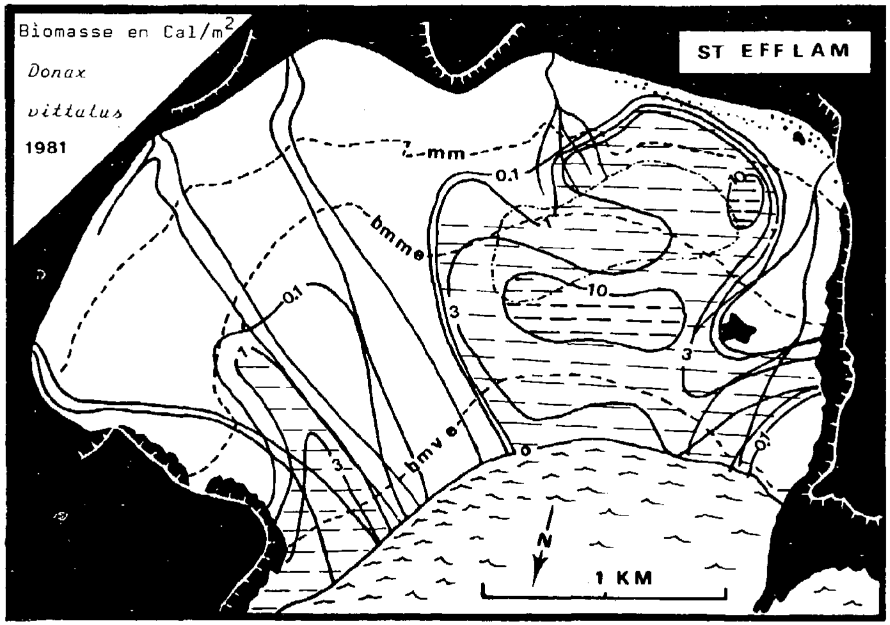
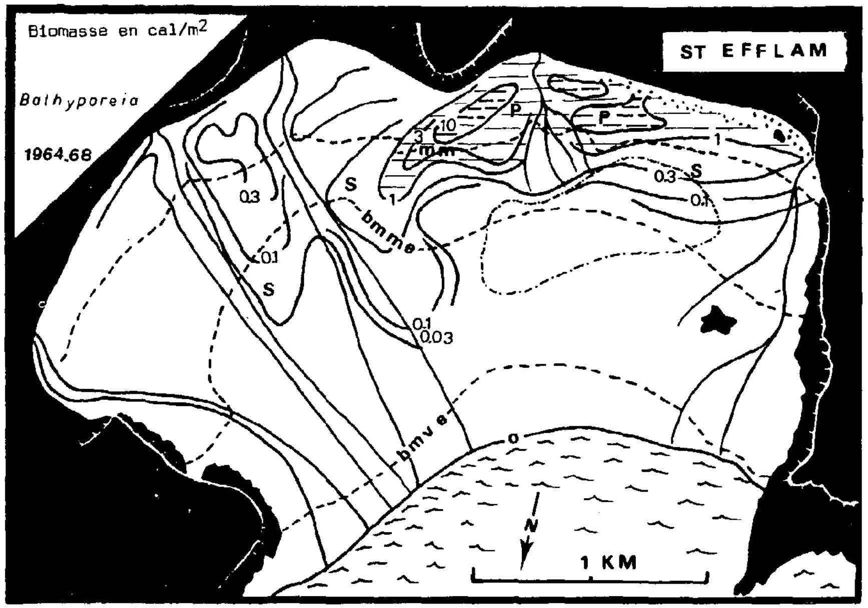
3
0
N
1 KM

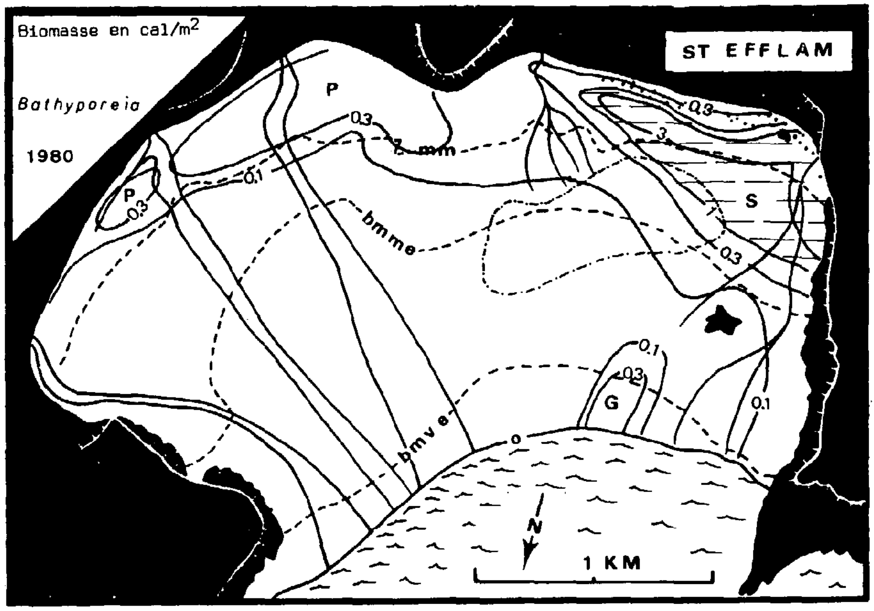

Biomasse en cal/m2
Donax
vittatus
1979
ST EFFLAM
Z-mm
bmme
bmve
0
N
1 KM

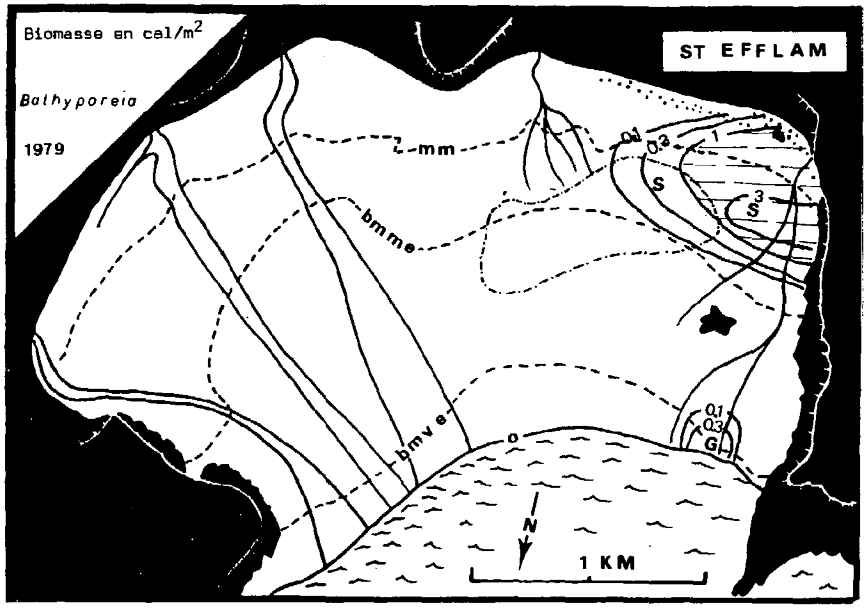
J


Biomasse en Cal/m2
Donax
vittatus
1981
ST EFFLAM
Z-mm
bmme
bmve
0.1
1
3
10
0
N
1 KM

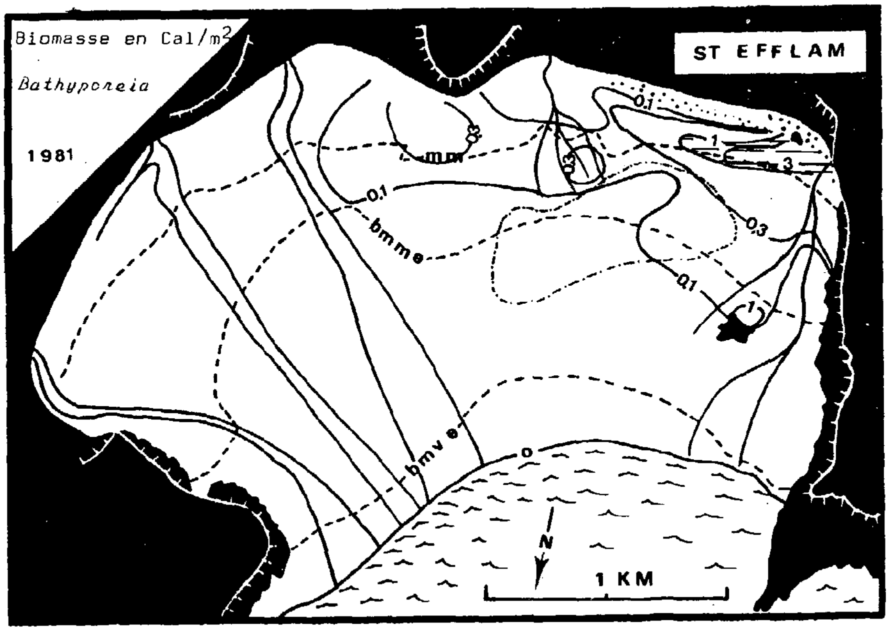

Biomasse en cal/m²
Bathyporeia
1964.68
ST EFFLAM
P
P
10
3
mm
1
S
0.3
S
0.1
0.3
0.1
S
bmme
0.1
0.03
bmve
o
N
1 KM

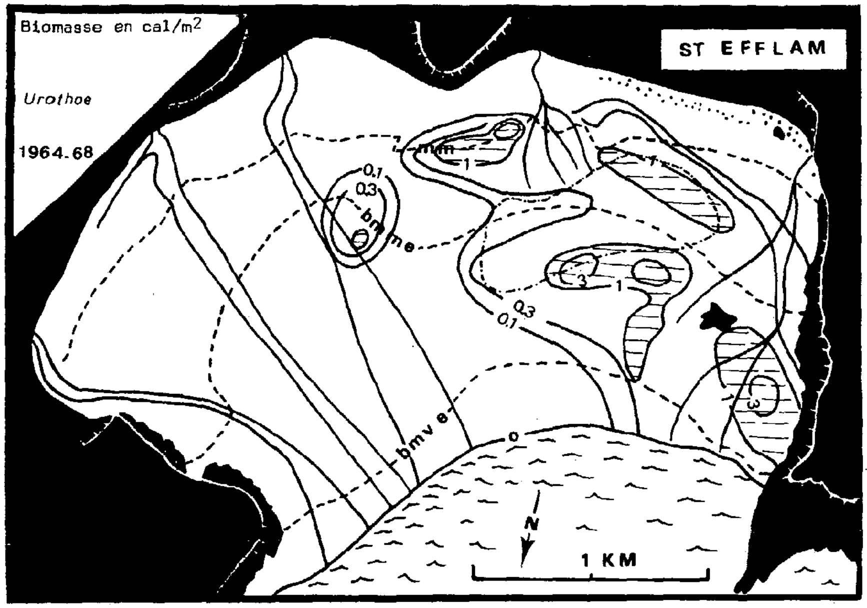
K

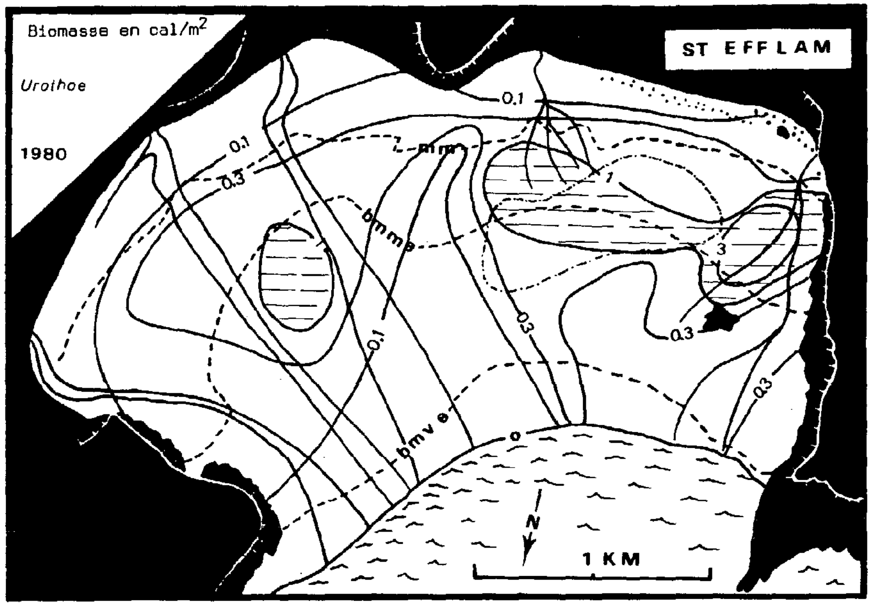

Biomasse en cal/m²
Bathyporeia
1980
ST EFFLAM
P
0.3
mm
0.1
P
0.3
0.3
3
S
1
0.3
bmme
0.1
0.3
G
0.1
bmve
o
N
1 KM

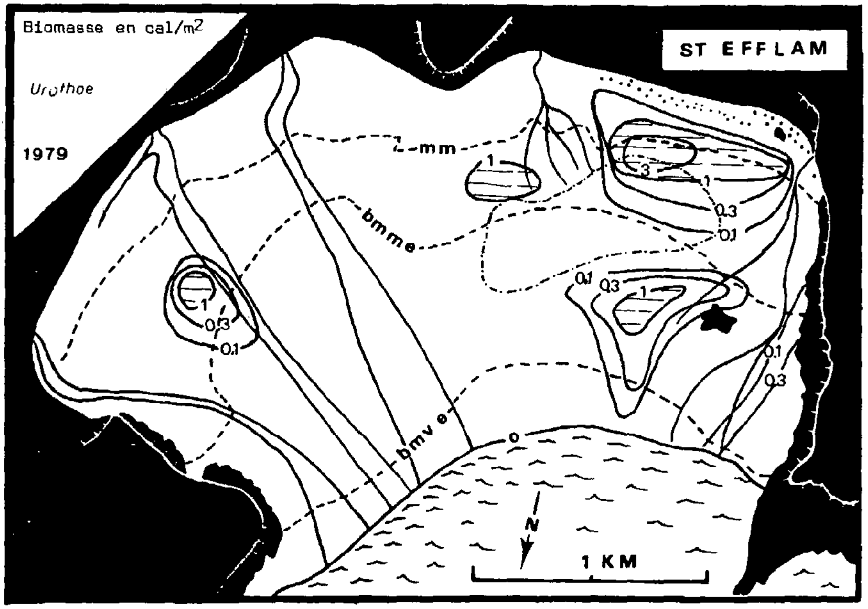

Biomasse en cal/m²
Bathyporeia
1979
ST EFFLAM
mm
0.1
0.3
1
S
3
S
bmme
0.1
0.3
G
bmve
o
N
1 KM

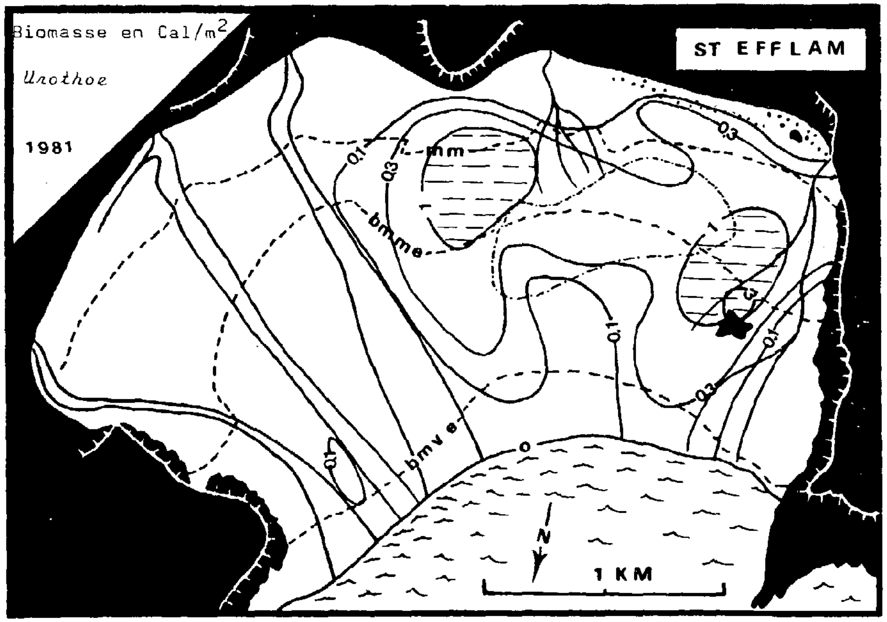
K

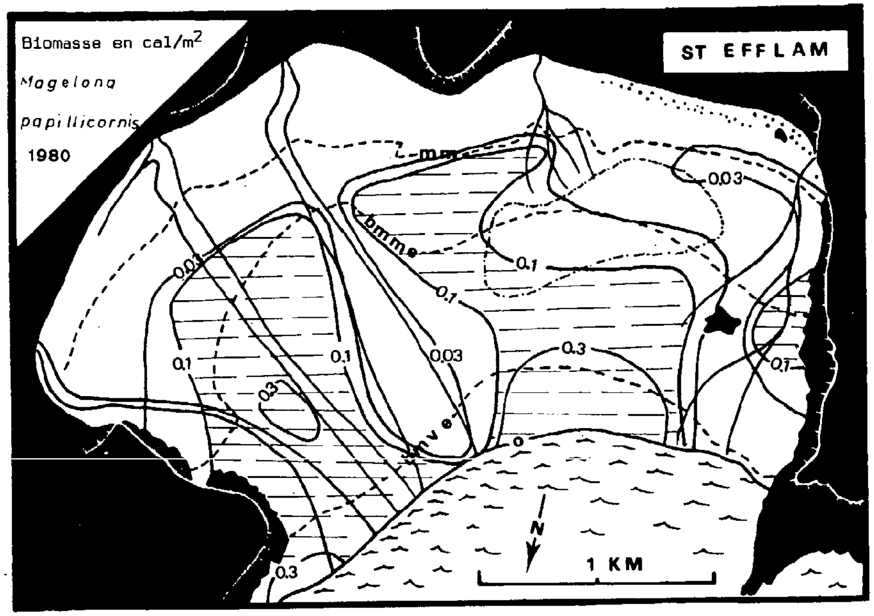

Biomasse en Cal/m²
Bathyporeia
1981
ST EFFLAM
0.1
1
3
0.3
0.3
mm
0.1
bmme
0.3
0.1
1
bmve
o
N
1 KM


Biomasse en cal/m2
Urothoe
1964.68
ST EFFLAM
Z-mm
bmme
bmve
0.1
0.3
1
3
0
N
1 KM

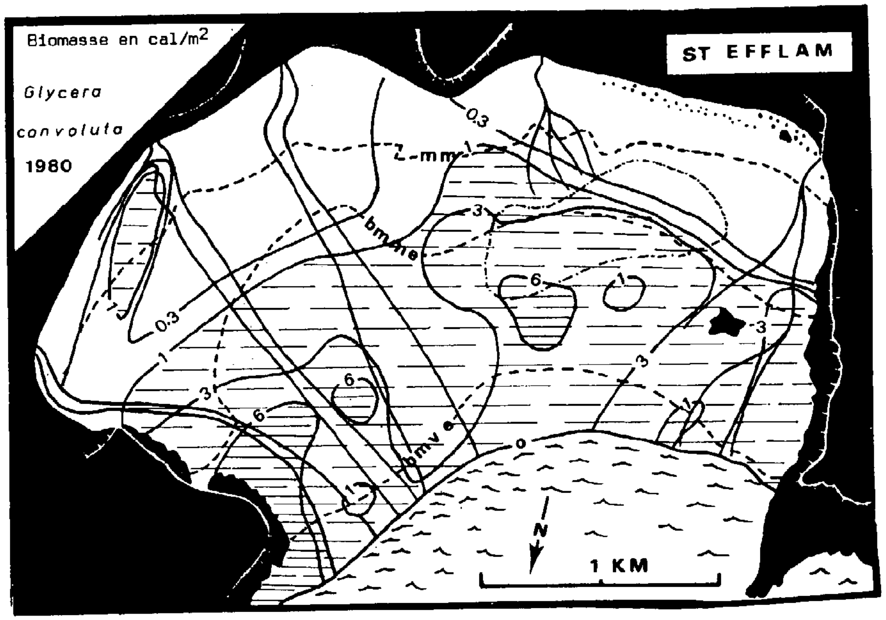

Biomasse en cal/m2
Urothoe
1980
ST EFFLAM
Z-mm
bmme
bmve
0.1
0.3
1
3
0
N
1 KM

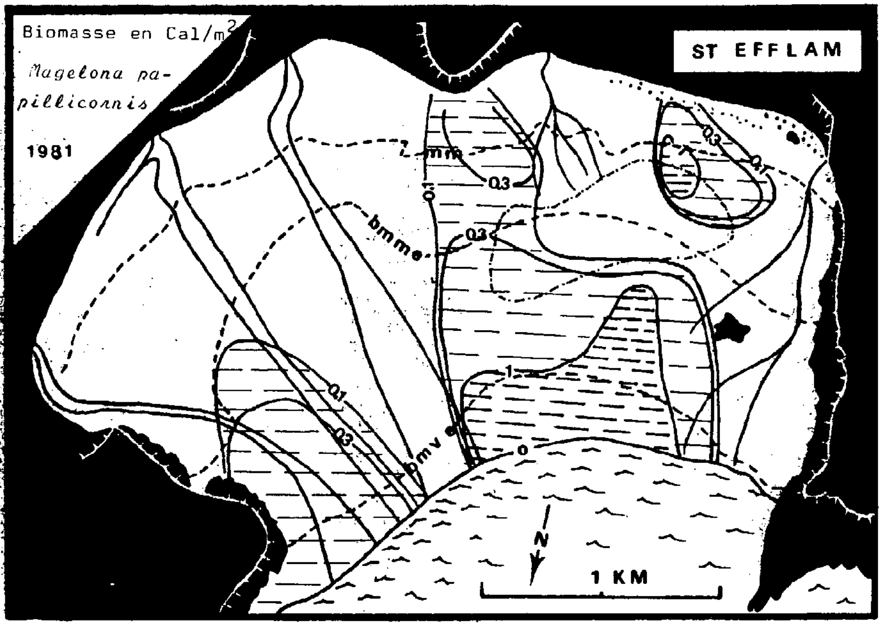

Biomasse en cal/m2
Urothoe
1979
ST EFFLAM
Z-mm
bmme
bmve
0.1
0.3
1
3
0
N
1 KM


Biomasse en Cal/m2
Urothoe
1981
ST EFFLAM
Z-mm
bmme
bmve
0.1
0.3
1
0
N
1 KM

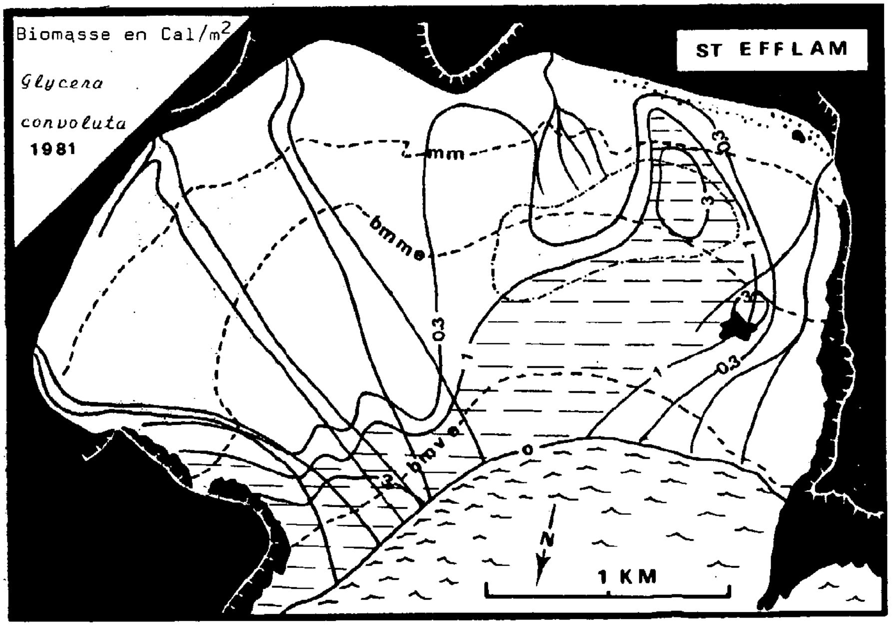

Biomasse en cal/m²
Magelona
papillicornis
1980
ST EFFLAM
Z-mm
bmme
bmve
0.03
0.1
0.3
0
N
1 KM

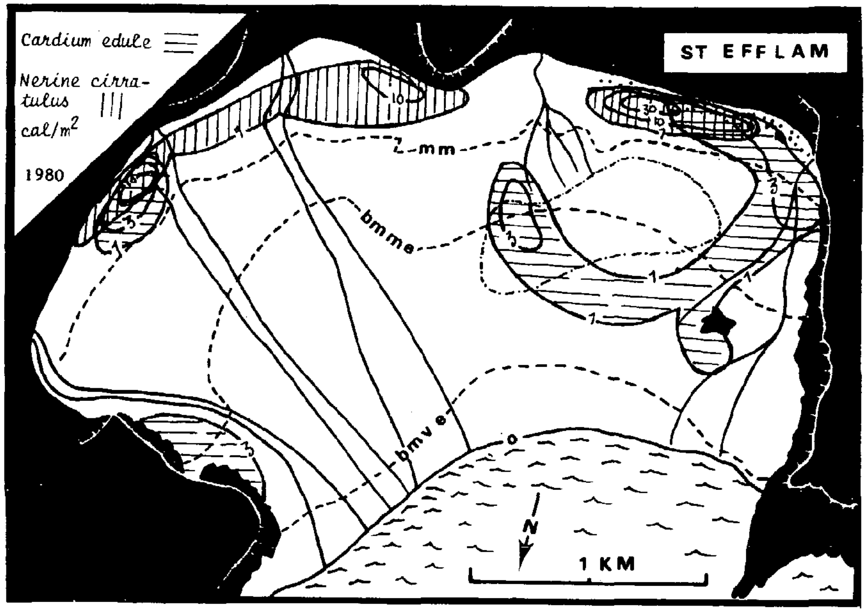
M

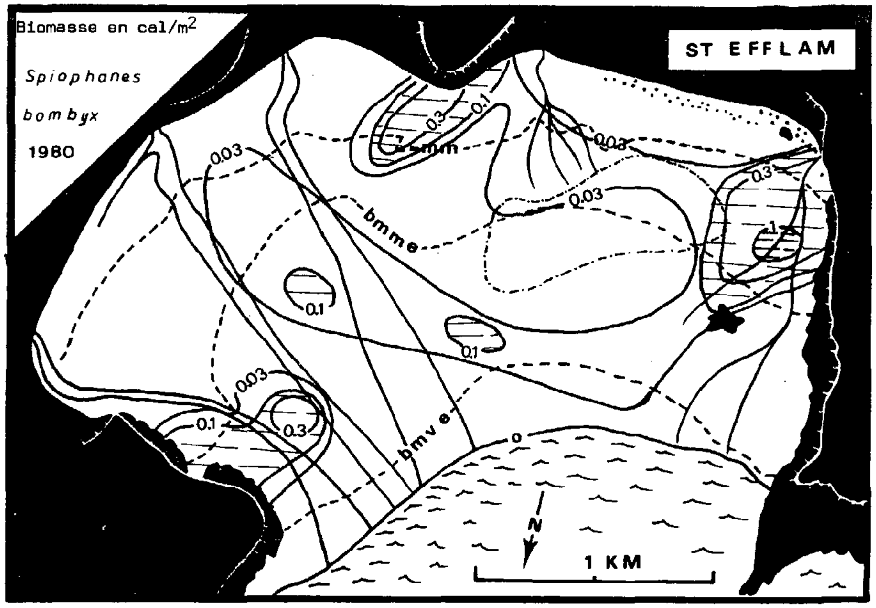

Biomasse en cal/m²
Glycera
convoluta
1980
ST EFFLAM
Z-mm
bmme
bmve
0.3
1
3
6
0
N
1 KM


Biomasse en Cal/m²
Magelona pa-
pillicornis
1981
ST EFFLAM
Z-mm
bmme
bmve
0.1
0.3
1
0
N
1 KM

M

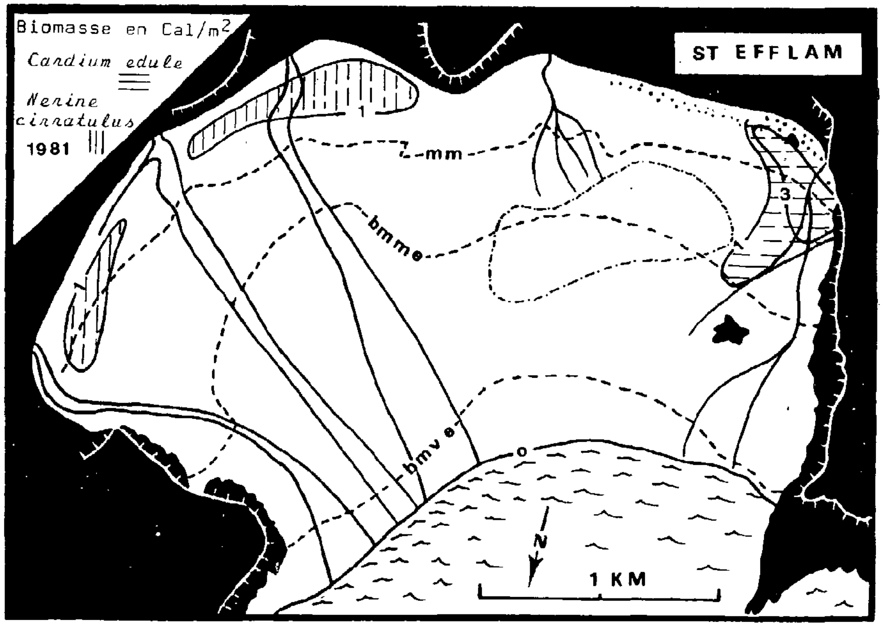

Biomasse en Cal/m²
Glycera
convoluta
1981
ST EFFLAM
Z-mm
bmme
bmve
0.3
1
3
0
N
1 KM

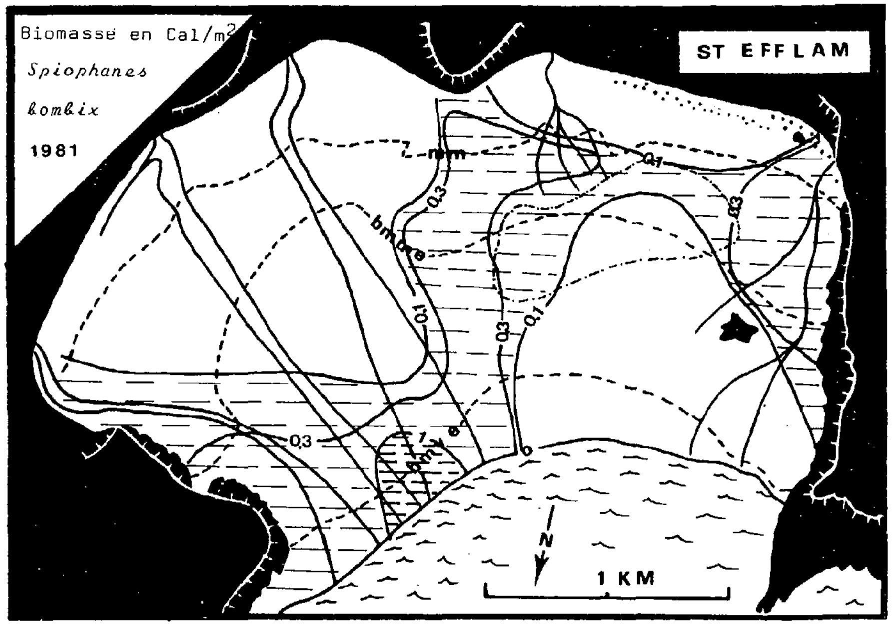

Cardium edule ☰
Nerine cirratulus |||
cal/m²
1980
ST EFFLAM
10
30
10
3
1
3
1
Z-mm
bmme
bmve
0
N
1 KM

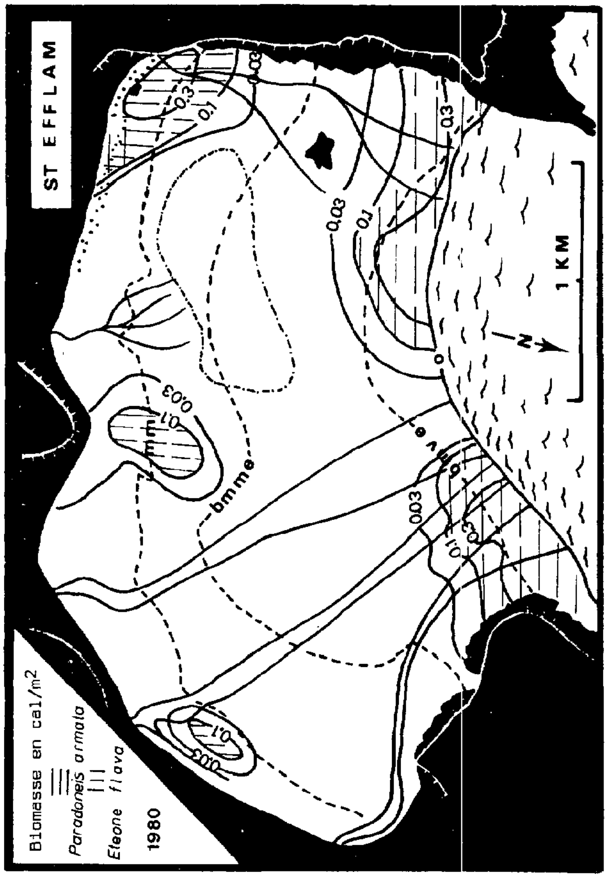

Biomasse en cal/m²
Spiophanes bombyx
1980
ST EFFLAM
0.3
0.1
1
0.03
0.03
0.03
0.3
1
0.1
0.1
0.03
0.1
0.3
Z-mm
bmme
bmve
0
N
1 KM

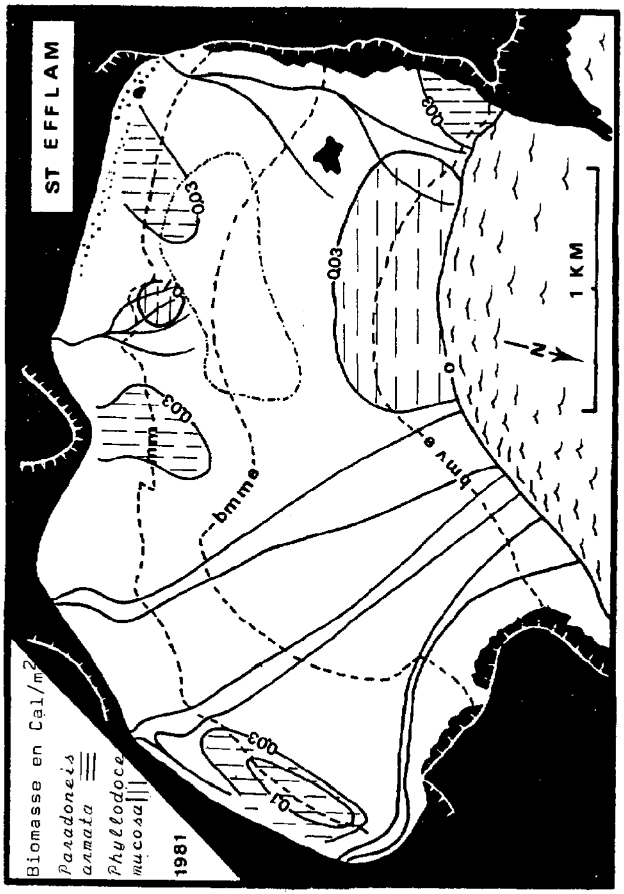
N

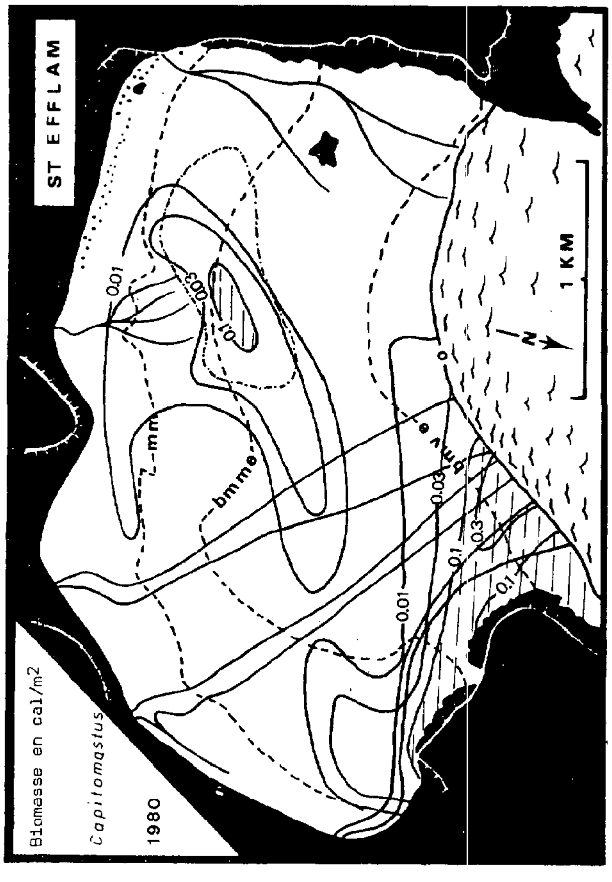
O

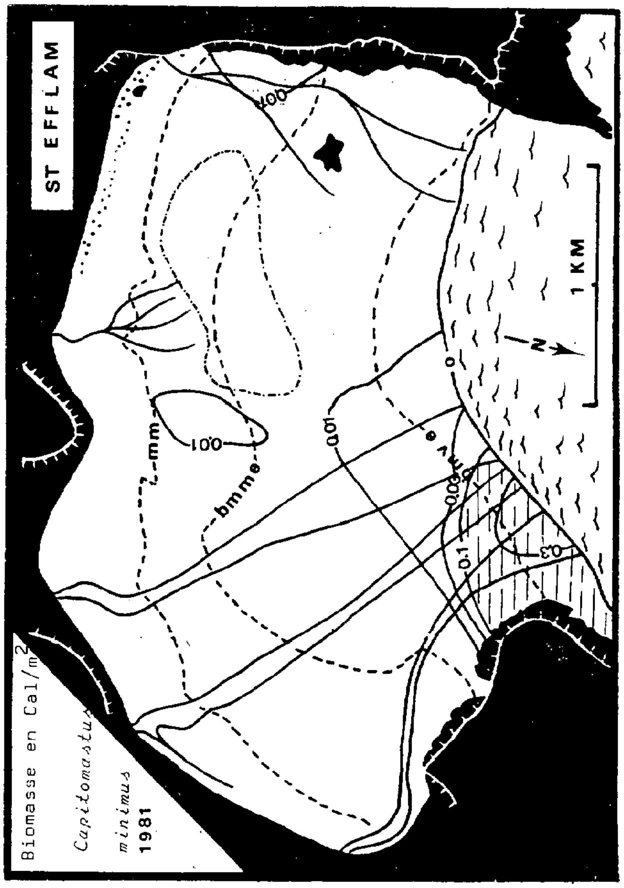

Biomasse en Cal/m²
Cardium edule ☰
Nerine cirratulus
1981 |||
ST EFFLAM
1
1
3
Z-mm
bmme
bmve
0
N
1 KM


Biomasse en Cal/m²
Spiophanes bombix
1981
ST EFFLAM
0.1
0.3
0.3
0.1
0.3
0.1
0.3
1
Z-mm
bmme
bmve
0
N
1 KM









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