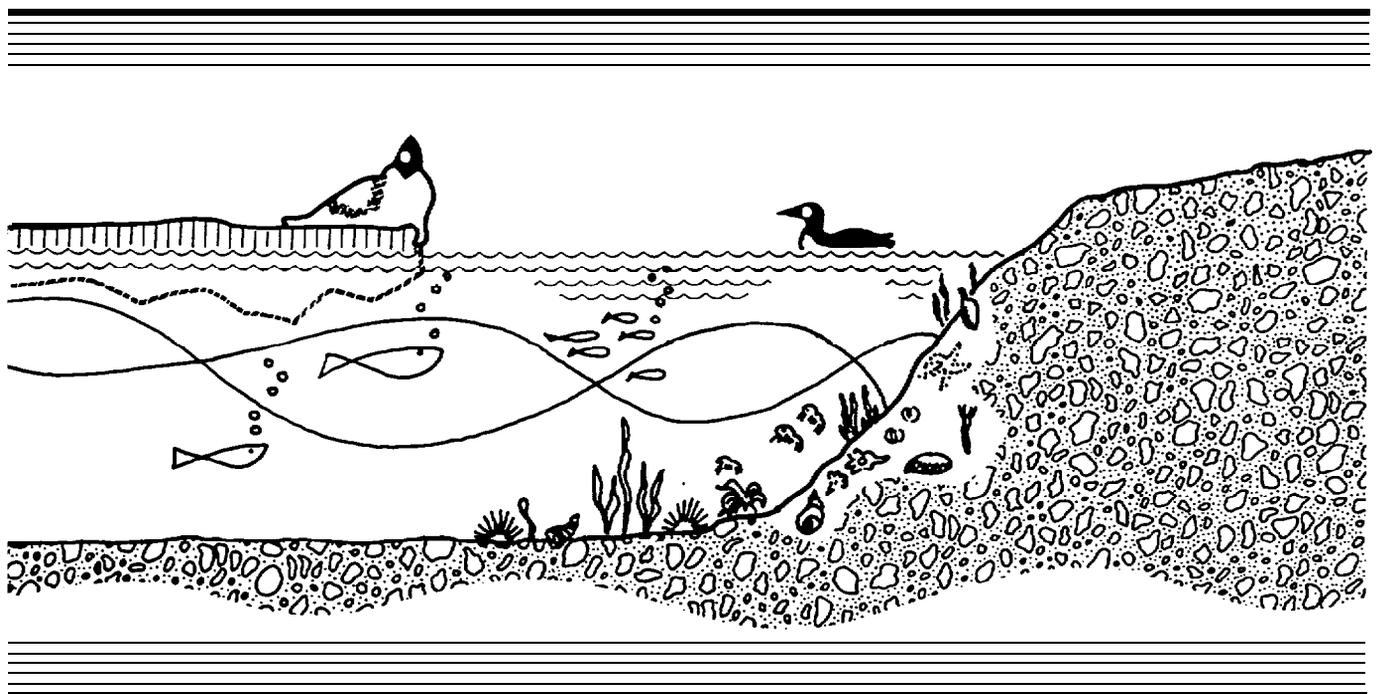


SPECIAL STUDIES



Baffin Island Oil Spill Project

WORKING REPORT SERIES

1981 STUDY RESULTS

FOREWORD

SPECIAL STUDIES 1981

This publication presents the results of studies which were carried out in 1981 at the BIOS Project site of Cape Hatt, **Baffin** Island, but which were ancillary to the original design and core programs of the Project. Although deemed to be worthwhile contributions to the Project and oil spill studies generally, the BIOS Management Committee did not directly fund these studies due to financial limitations.

Two reports are contained herein. The first presents the results of tissue analysis on two species of benthic fauna. This data is additional and complementary to the results in the BIOS Working Report 81-2: ANALYTICAL **BIOGEOCHEMISTRY**. The second report presents the results from the first year of a two-year study on under-ice **biota**.

Correct citations for this publication are as follows:

Engelhardt, **F.R.** and Norstrom, R. J., 1982, Petroleum hydrocarbons in two benthic invertebrates, the urchin Strongylocentrotus droebachiensis and the **polychaete** **Pectinaria granulosa**. IN: Special Studies - 1981 Study Results. (BIOS) **Baffin** Island Oil Spill Working Report 81-10.

Cross, W.E., 1982, In Situ studies of effects of oil and dispersed oil on primary productivity of ice algae and on under-ice amphipod communities. IN: Special Studies - 1981 Study Results. (BIOS) **Baffin** Island Oil Spill Working Report 81-10.

BAFFIN ISLAND OIL SPILL WORKING REPORT
1981 STUDY RESULTS
SPECIAL STUDIES

1. Petroleum Hydrocarbons in Two Benthic Invertebrates, the Urchin Strongylocentrotus Droebachiensis and the Polychaete Pectinaria Granulosa

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2. In Situ Studies of Effects of Oil and Dispersed Oil on Primary Productivity of Ice Algae and on Under-Ice Amphipod Communities

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BAFFIN ISLAND OIL SPILL PROJECT

Report on the 1981
Oil Spill Experiment

- Chemistry Component (Part B) -

PETROLEUM HYDROCARBONS IN TWO BENTHIC INVERTEBRATES,
THE URCHIN STRONGYLOCENTROTUS DROEBACHIENSIS AND THE
POLYCHAETE PECTINARIA GRANULOSA

Final Report

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September 1982

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SECTION ONE - INTRODUCTION

1.1 Spill Scenario

The design of the experimental oil spill protocol in the Baffin Island Oil Spill (BIOS) Project called for the discharge of chemically dispersed oil and untreated crude oil into various bays in Ragged Channel, Cape Hatt, N. W. T'. (Green et al. 1982) . A spill of 15m³ (75 drums) of slightly aged oil (7% air-stripped Lagomedio crude) was carried out in Bay 11 at 1540 on 19 August 1981. An estimated 37 drums of oil were collected from the surface one day after the spill. Bay 9 was treated at 1300 on 27 August 1981 with a sub-surface diffusion of 15m³ of aged Lagomedio crude. This oil was chemically dispersed using 10% Corexit 9527.

The concentration of oil in the water column was measured by Seakem Oceanography Ltd. using real-time fluorometry. Oil was found to be distributed in the two spill bays, as well as two other test bays, Bays 10 and 7. On the basis of 36 hours of monitoring, water column concentrations were found to be several hundred ppm-hrs. in Bay 9, tens of ppm-hrs in Bay 10, less than 1 ppm-hr in Bay 7, and several ppm-hrs in Bay 11. The details of the spill scenario and real-time water column data can be found in Green et al. (1982) . Further data on water column samples, as well as of sediment and shoreline substrates can be found in a report by Energy Resources Company Inc. - ERCO (Boehm et al. 1982) . Some of this physical information will be discussed later in reference to the findings of this study .

1.2 Chemistry Study Design

Part of the 1981 BIOS Project study was a detailed identification of petroleum hydrocarbon concentration and imposition in benthic biota. Seven species of in- and epifaunal invertebrates were collected at selected depths along defined transects in each of the four test bays. Five of the species were bivalves, Mya truncata, Serripes groenlandica, Macoma calcarea,

Astarte borealis and Nuculana minuta. Tissue hydrocarbon loads in these species were analyzed under contract by ERCO (see Boehm et al. 1982) . Two other species, the sea urchin Strongylocentrotus droebachiensis and the tube-dwelling polychaete Pectinaria granulosa, were analyzed by the National Wildlife Research Centre, Canadian Wildlife Service. This work was carried out in conjunction with Northern Environmental Protection, Indian and Northern Affairs, through the collegial association of the authors of this report. The assistance of M. Wong is gratefully acknowledged.

Analytical methodologies and instrumentation were standardized to a large degree, permitting ready comparison of data originating from both the CWS/DIAND and ERCO laboratories. An intercalibration exercise between the laboratories served as a further check on data consistency.

Hydrocarbon analysis was carried out also in 1980 on several species of plants and animals from the spill test area, including M. truncata and S. droebachiensis. The results of this work have been reported separately (Boehm 1981). In summary, both species showed evidence of hydrocarbons in body tissues, but this was predominantly of biogenic origin. Very low (ppb) and uniform levels of hydrocarbons of petroleum origin were also recorded, forming a tram level baseline.

SECTION 'IWO - MATERIALS AND METHODS

2.1 Sampling Protocol

A detailed description of the sampling protocol and methods for benthic biota is presented in a report by IGL Limited (Cross and Thomson 1982) . For purposes of this study, both urchins and polychaetes were collected in 1981 from each of the four test bays, on three occasions: before the oil discharges, one to four days after the discharge, and 14 to 20 days post-spill.

Both S. droebachiensis and P. granulosa were sampled from each of five sampling stations at the 7 m depth stratum of the tissue transect. Ten or more individuals were scheduled to be taken from each station. Additionally, a group of urchins was kept in screen enclosures (traps) at the 5 m depth, and sampled similarly.

After collection, all samples were wrapped in aluminum foil, frozen, and stored until dissection and analysis.

2.2 Analytical Methods

2.2.1 Tissue Processing

All individuals were dissected while still frozen in order to minimize loss of cellular fluids liberated during the freezing process, as well as volatile hydrocarbon losses. All dissecting implements and glassware were rinsed with pesticide grade acetone prior to contact with any sample. Only soft tissues were processed for analysis. In the case of S. droebachiensis, the test was scraped internally to free soft tissue adhesions, and discarded along with the mouthparts. Care was taken to avoid contamination of the sample by material on the external surface of the urchin test. The P. granulosa were removed from their tubes and the entire soft animal was processed.

Pooled samples of P. granulosa from 7 m stations were found to be too small individually to allow analysis (ea. lg or less) . All five samples from a depth stratum for each bay and one time point were pooled. Subsequent procedures were the same as for S. droebachiensis.

Prior to frozen storage of the homogenized pooled samples, an aliquot of each homogenate was dried overnight at 200°C for determination of water content and dry weight calculations.

2.2.2 Extraction and Cleanup

The method of alcoholic KOH saponification followed by extraction was adopted in favour of steam distillation because apparatus of a suitable scale for analysis of small samples using the latter procedure was not available. The main potential disadvantage of the saponification procedure is that removal of the large volumes of solvent employed in extraction and cleanup entails losses of the more volatile components. In order to minimize this problem, a procedure was developed which required only one evaporation step prior to screening by GC²FID (gas chromatography with flame ionization detectors) , and one more subsequent to addition of the aromatic internal standard (o-terphenyl) . A flow chart of the method is given in Fig. 2.1 and the procedure is given in detail below.

A single al.tins column cleanup step was found to be sufficient for analysis of n-alkanes, pristane and phytane by FID in spite of the relatively large quantities of biogenic compounds present. The latter, partly identified as squalenes (hexacosane isomers) and long-chain fatty acid alkyl esters by GC/mass spectrometry, occurred in the C20-28 region of the chromatogram, but were resolved from the n-alkanes. Similarly, there were no significant interferences from alkanes, olef ins or biogenic material in the analysis of aromatics by GC²/SIM (gas chromatography with single ion monitoring) so that separation into F1 and F2 fractions using silica gel chromatography was not required.

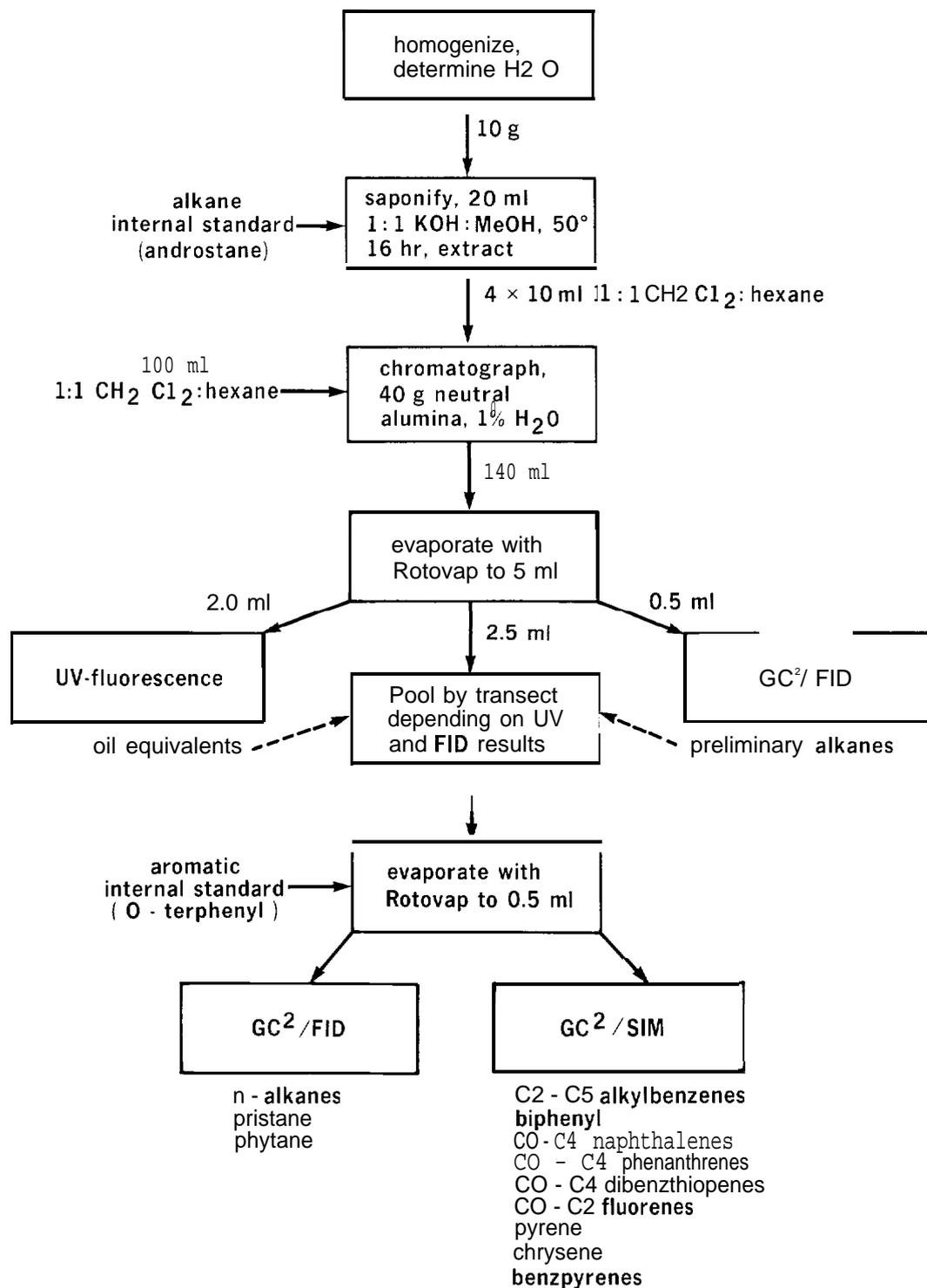


Figure 2.1 Extraction and cleanup schedule preparatory to GC²/FID and GC²/SIM analyses

Samples from each station were thawed and re-homogenized by shaking the vial vigorously. A 10 g sample (or sample plus water to make 10 g) was pipetted into a 50 ml centrifuge tube with teflon-lined screw cap, followed by the internal standard (0.2 ml, 10^{-4} g/ml androstane in hexane), and 20 ml of 1:1 methanol: 10N KOH. The sample was capped tightly, shaken, and digested in a 50°C waterbath shaker for 16 hr. After cooling in the refrigerator for $\frac{1}{2}$ hr., the tube was opened and 10 ml of 1:1 dichloromethane :hexane were added immediately. The sample was vortex fixed, centrifuged and the top layer transferred to a flask. This procedure was repeated a minimum of 4 times, or until the organic layer was colorless. The combined extracts were placed directly on the alumina clean-up column (40 g Fisher A950 neutral alumina, wet packed with 1:1 dichloromethane :hexane. The eluate was evaporated to ca. 2 ml with a rotary evaporator, transferred to a 15 ml centrifuge tube and made up to 5 ml with hexane. The sample was partitioned as follows: 0.5 ml for GC²/FID analysis, 2.0 ml for fluorescence analysis, and 2.5 ml for GC²/SIM analysis after pooling. All solvents and reagents used in processing were of ultra-pure grade.

2.2.3 UV/Fluorescence Analysis

The W/F analysis was carried out on clean extracted samples processed as described above. Fluorescence determination were carried out using a Turner Model 430 fluorometer at fixed wavelengths (excitation 300 nm, emission 350 nm, slit widths 15 nm).

The choice of wavelengths was predicated on needs for maximum absolute sensitivity, for realistic representation of total tissue hydrocarbon load, and for inter-convertibility of data originating from ERCO and the DIAND/CWS association. Spectral scans of aged (7% air-stripped) Lagomedio crude oil standards in hexane showed excitation peaks at 300 and 355 nm, although fluorescence was generally high between these two peaks. An emission maximum occurred at 405 nm, peaking from a gradual rise.

A better link with actual tissue hydrocarbon load was sought by verification of the emission fluorescence spectra of selected aromatic hydrocarbons, excited at 300 nm. The following relevant excitation peaks were found: benzene, 345 nm; naphthalene, 337 nm; phenanthrene, 364 nm; benzantracene, 411 nm; benzopyrene, 409 and 429 nm. These aromatic hydrocarbons were pure compounds dissolved as 5 rig/ml in dichloromethane and hexane, 1:1. A survey of the GC²/SIM data showed that tissue hydrocarbon compositions emphasized the two- and three-ringed aromatic structures which individually tended to have emission maxima closer to 350 nm than the 405 nm peak of the crude oil hydrocarbon mix-hue. This suggested an optimum choice for emission wavelengths in the mid-300's. Further, the decision had been made in the ERCO laboratory to use 325 and 350 nm as its quantitation settings.

Using a 300/350 nm combination, calibration was carried out using hexane as a blank and Legomedio crude oil in hexane as a standard. This was verified against the use of dichloromethane - hexane, 1:1 as a diluant which was not found to differ in its blank values. A calibration range of 0-15 rig/ml was used, a range in which the fluorescence readings were nearly linear and not limited by self-absorption. Calibration curves were determined, against which unknown and check samples could be read for concentration. Many of the tissue extracts required dilution with hexane to fit them into the calibration range. All solvents were of ultra pure grade. Final concentrations of tissue total hydrocarbons were expressed as rig/g dry weight on the basis of the crude oil standard.

2.2.4 GC²/FID Analysis

This analysis was performed with a Hewlett-Packard 5840 GC equipped with an autoinjector and 30 m fused silica column (DB-5, 0.25 mm i.d., 0.1 micron film thickness, J&W Scientific). Injections were performed automatically in the splitless mode, programming from 60°C to 300°C at 5°C/min. The injector was at 250°, and the detector at 300°. The carrier gas was helium at 20 psi head pressure. The recovery of androstane was calculated by

comparison to the area of external standards made directly from the spiking solution, and injected in the same series of runs.

The majority of samples did not contain significant quantities of n-alkanes. For those samples which contained alkanes, the procedure adopted was to calculate oil equivalents based on the sum of peak heights of the C16-26 n-alkanes. The standard used for this calculation was prepared from an aliquot of oil put through the entire analytical procedure (except saponification) . In addition, the distribution of n-alkanes relative to that in the oil was plotted (vide infra) for these samples. Pristane, which was a major biogenic hydrocarbon in *S. droebachiensis*, and phytane were determined using external standards. All results were corrected to androstane recovery.

2.2.5 GC²/SIM Analysis

This analysis was performed with a Hewlett-Packard 5985B GC/MS equipped with a 30 m fused silica column (SE-54, 0.25 mm i. d., J&W Scientific) . Injections were performed manually in the splitless mode using a temperature program and GC conditions identical to those for GC²/FID analysis . The column was inserted through the interface directly into the ion source. The source was at 200°C. and electron energy was 70 ev.

An early version of HP's 100 ion SIM software was used for analysis. The sensitivity was sufficient for analysis of the levels of aromatic hydrocarbons found in the samples. Table 21 lists the compounds determined, the molecular ion (used in all cases) , and the ion group in which the ion was employed. The latter is a procedure whereby the chromatogram was divided into 5 time segments, in each of which only 7 ions were scanned with a dwell time of 10 msec per ion. This procedure enhanced sensitivity, and the ability to detect and accurately quantitate narrow peaks.

Table 2.1 Summary of single ion monitoring (SIM) condition employed for analysis of aromatic hydrocarbons by GC² mass spectrometry

Compound or compound group	Abbreviation	Monitored ion	Present in ion group number
C2 alkyl benzenes	C2AB	106	1
C3 alkyl benzenes	C3AB	120	1
C4 alkyl benzenes	C4AB	134	1
C5 alkyl benzenes	C5AB	148	1
biphenyl	BP	154	2
naphthalene	N	128	1
C1 naphthalenes	C1N	142	1
C2 naphthalenes	C2N	156	2
C3 naphthalenes	C3N	170	2
C4 naphthalenes	C4N	184	2
fluorene	F	166	2
C1 fluorenes	C1F	180	3
C2 fluorenes	C2F	194	3
phenanthrene	Ph	178	3
C1 phenanthrenes	C1Ph	192	3
C2 phenanthrenes	C2Ph	206	4
C3 phenanthrenes	C2Ph	220	4, 5
C4 phenanthrenes	C4Ph	234	5
dibenzthiophene	BP	184	3
C1 dibenzthiophenes	C1BP	198	3
C2 dibenzthiophenes	C2BP	212	4
C3 dibenzthiophenes	C3BP	226	4
C4 dibenzthiophenes	C4BP	240	4, 5
pyrene	Py	202	4, 5
chrysene, benzanthracene	Chr	228	4, 5
benzopyrenes	BPy	252	5
o-terphenyl (intern.std.)	IS	230	2, 3

Samples with unusually high contamination as determined by UV fluorescence were analyzed individually. Otherwise, samples from a given Bay and time point were pooled by transect, the o-terphenyl internal standard (5 ug) added, and the volume (generally 12.5 ml) reduced to 0.5 ml in a small flask using a rotary evaporator. Each sample was re-analysed by GC²/FID at this point to obtain the final pristane, phytane and n-alkane results. The aromatic hydrocarbons were quantitated as o-terphenyl equivalents by measurement of areas for each ion in the correct retention time range for that compound class as determined from an authentic standard of the oil.

2.3 Methodology Validation and Quality Assurance 2.3.1 UV/F

The reproducibility of the fluorescence assay was tested by a series of duplicate analyses of tissue and oil extracts (n = 7). The error was found to average $3.2 \pm 0.9\%$. Recovery as expressed by the fluorescence data was assessed using four oil standards of 100 to 200 rig/ml. Recovery was found to be $70 \pm 4\%$, and the data presented in Section 3 (Results) are not corrected for extraction losses.

The suitability of the 300/350 nm wavelength settings was confirmed by spectral scans of extracted urchin samples. Excitation fluorescence peaks were found at 300 and 360 nm, and emission peaks broadly at 360 and 400 nm.

2.3.2 GC²/FID

The procedure for alkane analysis was tested by spiking oil to 10 ml of water at the 100 and 200 ug/ml level; to 10 g of clean, pre-spill S. droebachiensis homogenate at the 100 and 200 ug/ml levels in duplicate, and to 10 g P. granulosa homogenate at the 200 ug/ml level in duplicate. These samples were carried through the entire procedure and the recovery of n-alkanes was calculated by FID determination against an external standard of the same oil. The results are shown in Figure 2.2. The recovery above C16 was acceptable for quantitative analysis, i.e. greater than 80%, in all cases except the 100 ug/ml oil-in-water test sample. The loss of volatiles in this sample could have been due to the absence of biogenic "carrier" materials such as lipids. Biogenic hydrocarbons constituted significant interference at C23, C25 and C27 in the case of

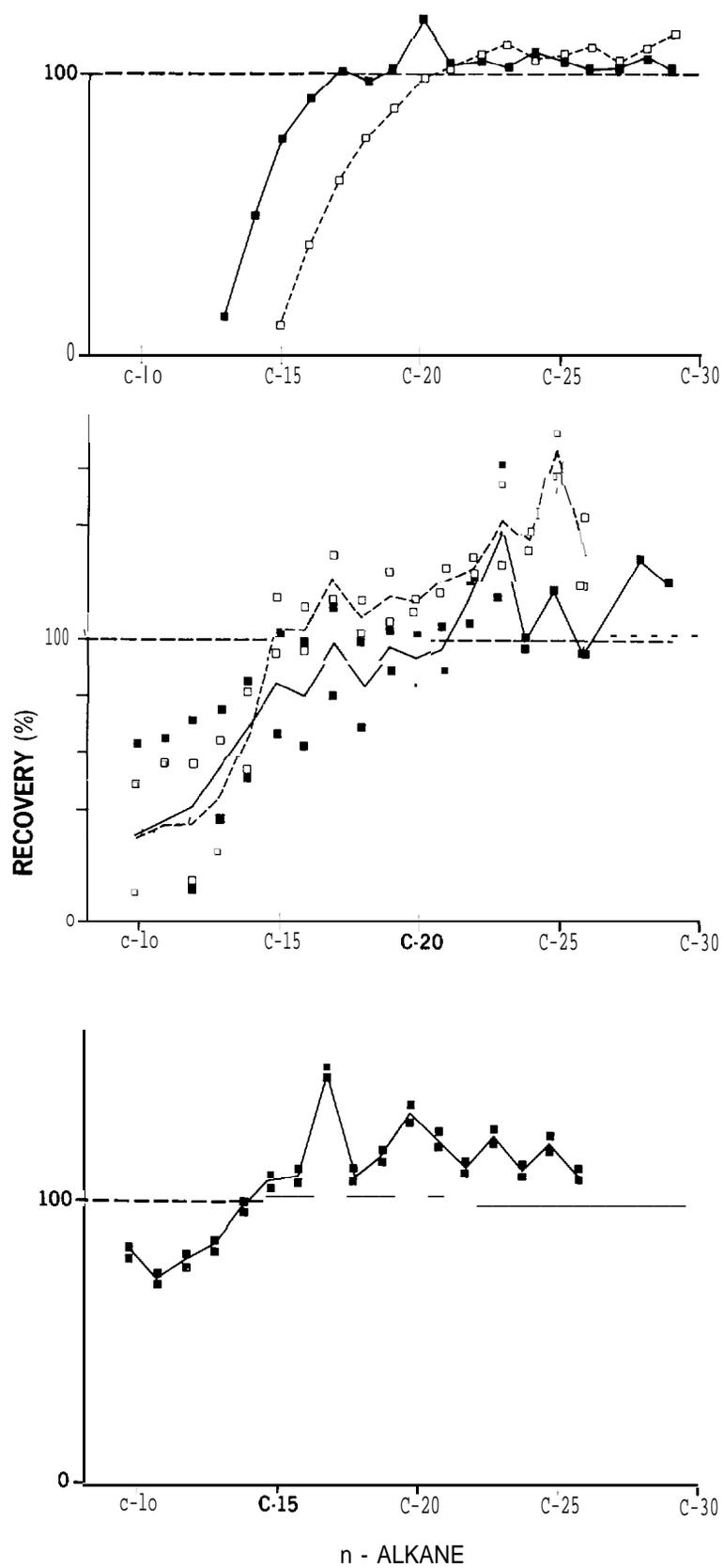


Figure 2.2 Recovery of n-alkanes by GC²/FID analysis

the urchins, and at C17, C20, C23 and C25 in the case of the annelids. Only seven samples had significant levels of alkanes in them, and of these, only two were as low as the 100-200 ug/g range. Interference from biogenic hydrocarbons was therefore not a significant source of error. Androstane recoveries were found to be $94 \pm 8\%$ ($n = 6$).

2.3.3 GC²/SIM

In order to determine recoveries of the aromatic compounds in the PAH fraction, a sample of oil was separated on silica gel into aliphatic and aromatic fractions. Pre-spill samples of urchins and polychaetes and water were spiked at the 50 or 100 ug/g oil equivalent level with the PAH fraction and carried through the whole procedure. Recovery was calculated by GC²/SIM determination against an external standard of the same PAH fraction of the oil. The o-terphenyl internal standard was added at the saponification step for this study only. The results are presented in Table 2.2. The o-terphenyl internal standard recovery was $101 \pm 4\%$ ($n = 7$). It is clear from the results that there is more of a problem in the determination of volatile compounds (e.g. , N and C/'N) and the relatively minor constituents {e. g. C3Ph, C2F) than there is for the major constituents , which was expected. If the major constituents are identified as C4AB, C1-C4N, DBT, C1-C3DBT, and C1-C2Ph, the overall recovery was 88%.

Because of potential errors introduced by the spiking procedure itself, the precision of the method was tested by analysis in quadruplicate of an urchin sample which had been screened and found to contain significant PAH contamination. The results of this analysis are presented in Table 2.3. The coefficient of variation for the major constituents of low volatility was less than 10%, which is a satisfactory precision for determination of compounds at levels less than 1 ppm.

2.4 Laboratory Intercalibration

In order to validate the comparability of data generated by CWS/DIAND and ERCO laboratory techniques it was decided to have each laboratory team analyze a number of samples of, to them, unknown concentration. Results would be compared after completion of the exercise.

Table 2.2 Recovery of the PAH fraction, spiked at 50 and 100 ug/g
in crude oil equivalents to pre-spill samples and blanks

SAMPLE TYPE	Spike ug Oil/ g net	IS	PERCENT RECOVERY ¹																							
			AB	BP	N	N ^{C1}	N ^{C2}	N ^{C3}	N ^{C4}	DBT	C1 DBT	C2 DBT	C3 DBT	Ph	C1- Ph	C2- Ph	C3- Ph	F	C1- F	C2- F						
<u>S. droeb.</u>	100	106	-	75	15	3.3	49	77	91	97	106	119	144	123	329	120	122	98	125	209						
· ·	100	99	56	10	6	8	6	9	0	9	5	9	4	-	8	4	91	94	99	107	110	98	91	86	93	144
" "	50	98	104	141	129	69	92	97	51	50	109	91	26	106	102	81	-	130	-	332						
· ·	50	105	97	324	89	95	89	86	61	54	2	8	-	6	2	6	3	7	9	-	-	-	-	-	-	-
<u>P. gran.</u>	100	96	116	110	206	83	69	83	132	102	106	133	135	114	142	71	1	3	6	-	-	-				
Blank	100	104	70	80	92	72	71	72	102	87	87	89	139	106	92	110	80	103	97	148						
Blank	50	96	42	85	75	67	71	72	76	84	81	87	-	111	94	102	136	-	-	-						

¹ IS = internal standard (o-terphenyl), AB = Alkyl benzenes, N = naphthalene, DBT = Dibenzthiophene
Ph = phenanthrene, F = fluorene

Table 2.3 Reproducibility of the fluorescence and GC²/SIM analysis procedures

STR, Bay 10 1st post spill Duplicate No.	oil Equivalent, (w fluoresc. ug/g dry weight	ng /g dry weight ¹																				Total Aroma- tic	Total PAH											
		C3 AB	C4 AB	C5 AB	BP	N	C1 N	C2 N	C3 N	C4 N	DBT	C1 DBT	C2 DBT	C3 DBT	C4 DBT	Ph	C1 Ph	C2 Ph	C3 Ph	C4 Ph	F			C1 F	C2 F	Chr								
P#1	86.6	400	463	23345	195557	883	1288	757	125	270	226	137	NA	35539220014820	5195	119	40	7069	5973															
P#2	92.7	263	364	23060	153522	919	1325	641	139	266	247	181	NA	385417	310	15029	63	111	25	NA	6860	6003												
P#3	95.8	607	766	34768	235	6981007	1503	675	134	245	210	162	34	341	380	263	156	20	6377	153	8	8332	6412											
P#4	94.2	322	553	26353	322	511	863	1192	618	134	238	235	162	43	324	392	261	148	NA	54	76	83	'6	6637	5509									
Mean	92.3	395	537	268	56	171	572	918	1327	673	333	255	227	161	39	351	39527835123	5890	108	-	7175	5974												
SD (±)	4.0	152	171	55	10	42	26	64	130	61	6	16	17	18	7	2	6	1	6	2	3	4	5	6	17	32	-	662	369					
N (%)	4	38	3	2	2	0	1	7	2	4	1	5	7	1	0	9	4	6	7	11	11	7	4	8	223	3	3	3	9	3	0	-	9	6

¹ AB = alkyl benzene DBT = dibenzthiophene
 BP = biphenyl m = phenanthrene
 N = naphthalene F = fluorene
 Chr = chrysene

The set of intercalibration samples were prepared by a chemist not associated with these analyses by spiking a clam (M. truncata) extract and an urchin (S. droebachiensis) extract with oil. In addition, a solution of Lagonedio crude oil in hexane at an unknown concentration was prepared. The biological samples were carried through the normal procedures in duplicate and oil equivalents calculated by comparison to an external standard of the same oil. The unknown oil sample was analysed directly.

SECTION THREE - RESULTS

3.1 Crude Oil Hydrocarbon Characteristics

Standards of the aged Lagomedio crude oil, as used in the oil spill study, were analyzed for hydrocarbon characteristics by UV/F, GC²/FID and GC²/SIM methods . The UV/F survey was carried out to optimize wavelength choice for tissue total hydrocarbon concentration and has been described in Section 2.2.

The chromatogram produced by GC²/FID analysis of Lagomedio crude shows a very typical and regular composition of n-alkanes from C10 to C32 (Figure 3.1) . Although the method as used without fractionation is less suitable for characterizing other hydrocarbons including aromatics, these are indicated as smaller peaks between the regular n-alkanes. A relatively large proportion of low to middle boiling fractions is evident from the chromatogram.

A fine definition of the quantitatively smaller aromatic component of Lagomedio crude was achieved by GC²/SIM analysis. Figure 3.2 shows a combined total ion chromatogram for the crude oil, a figure generated from the summed data obtained by SIM analysis. This combined chromatogram will serve as a handy reference for the other combined chromatograms depicting tissue hydrocarbons as determined by GC²/SIM. Single ion characteristics for the crude oil are detailed in Figure 3.2 to 3.4.

3.2 Tissue Hydrocarbons

3.2.1 Strongylocentrotus droebachiensis

3.2.1.1 UV/F

Figure 3.5 shows that the total oil burden as assessed by the UV/F method in urchins from all four bays increased within one to four days of the spill event from very low background or pre-oil levels. These increased by two weeks in Bay 9 and Bay 11 urchins to about 250 and 500 ug/g (oil equivalents per gram dry weight of soft tissue) . Bay 7 and Bay 10 samples showed little increase from the first post-spill sample.

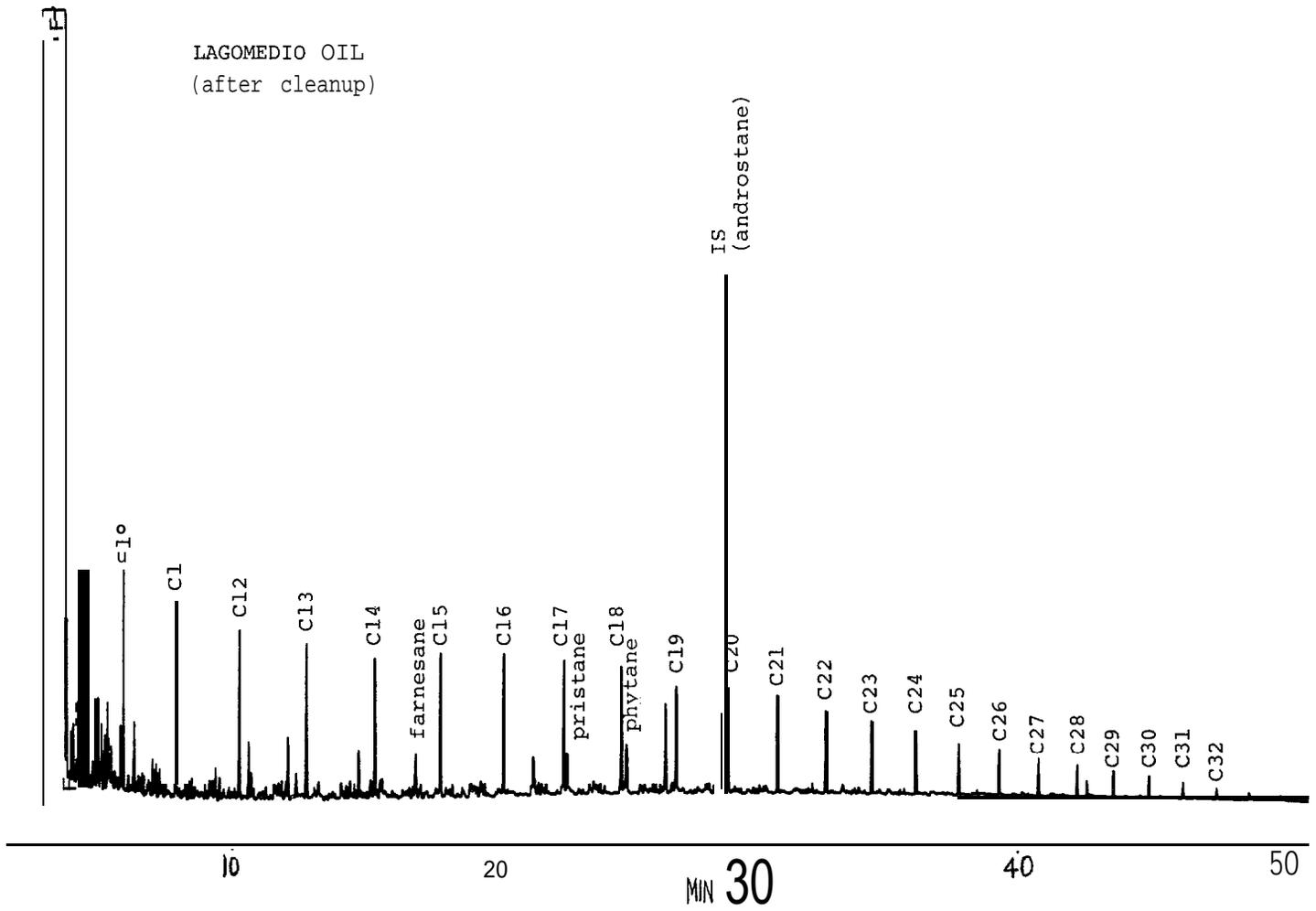
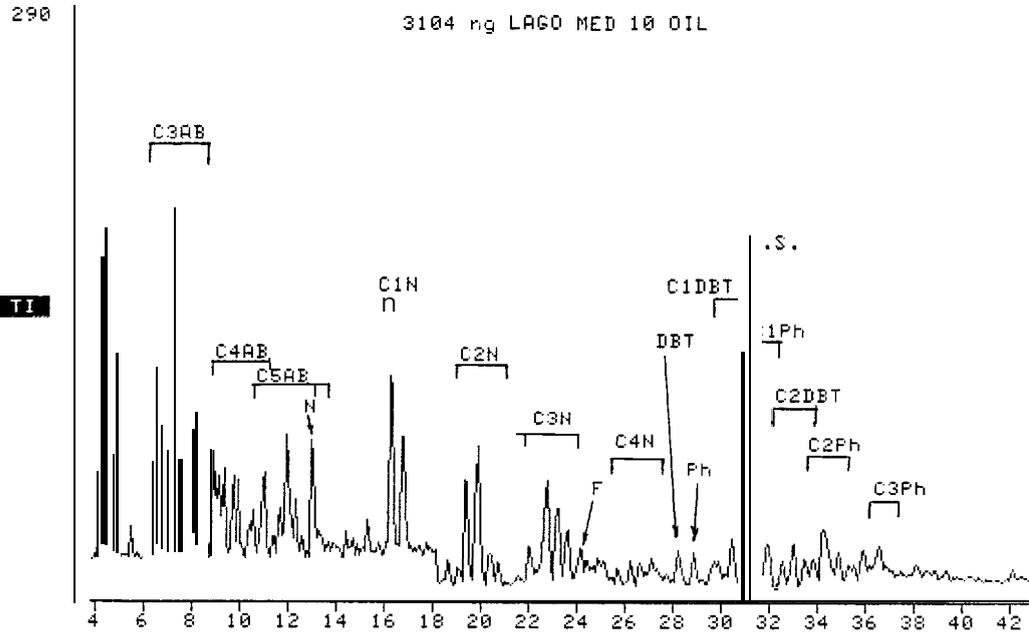


Figure 3.1 Hydrocarbon composition of Lagomedio crude oil as assessed by GC/FID

NAME OIL PAH FR.3MIC.L.FROM1ML,1034.6 MIC.G/1 ML
 MISC EI,SIM,2800V,MARCH2/82

FRN 11104



NAME OIL PAH FR.3MIC.L.FROM 1ML,1034.6 MIC.G/1 ML
 MISC EI,SIM,2800V,MARCH2/82

FRN 1

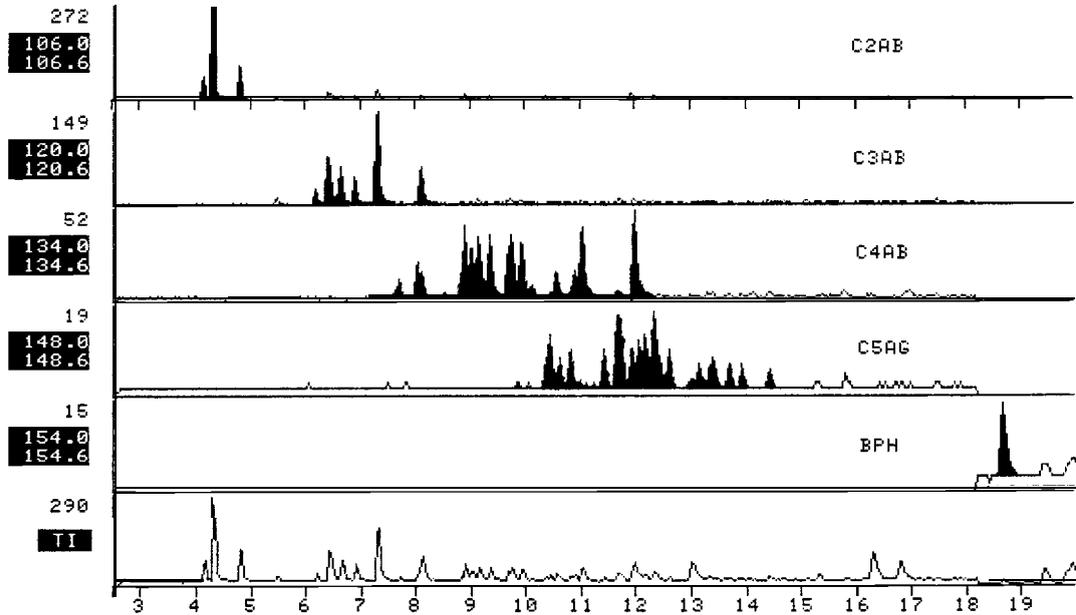


Figure 3.2 GC²/SIM chromatograms of Lagomedio crude oil, showing a total ion chromatogram (top) and benzene to biphenyl single ion chromatograms (bottom)

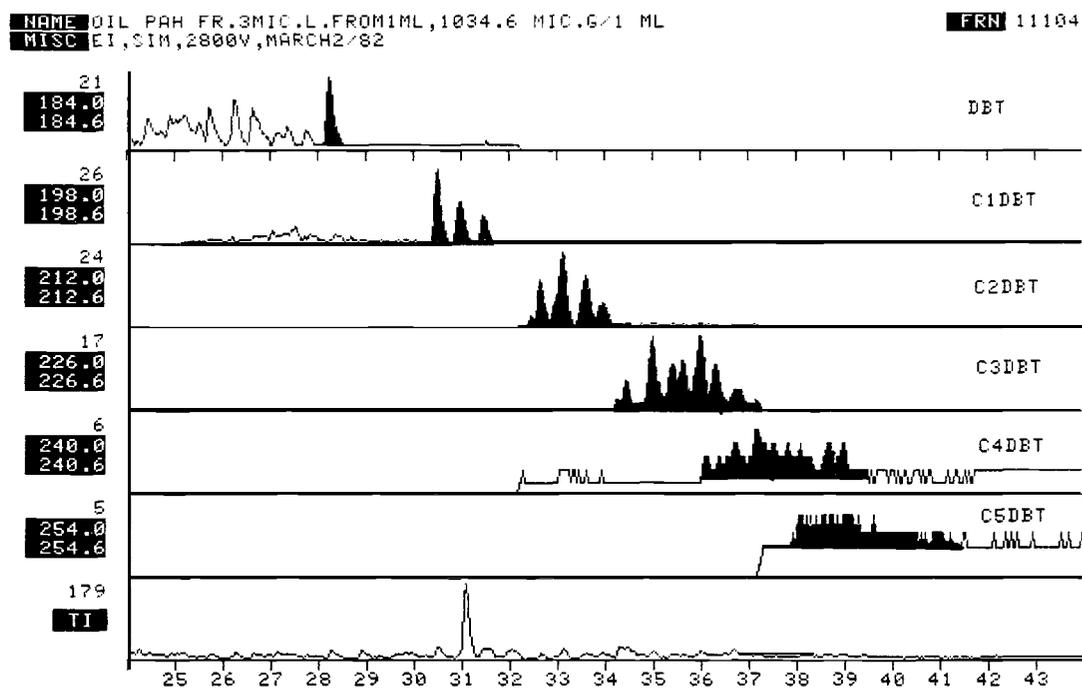
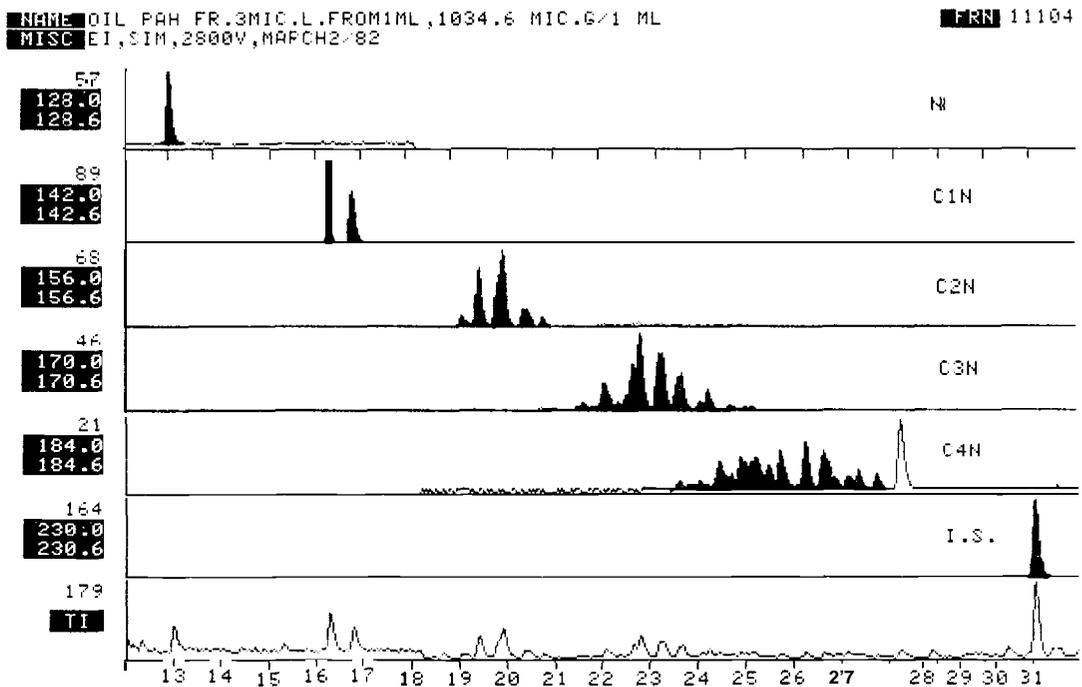


Figure 3.3 GC²/SIM chromatograms for Lagomedio crude oil, showing naphthalenes and dibenzthiophenes as single ions

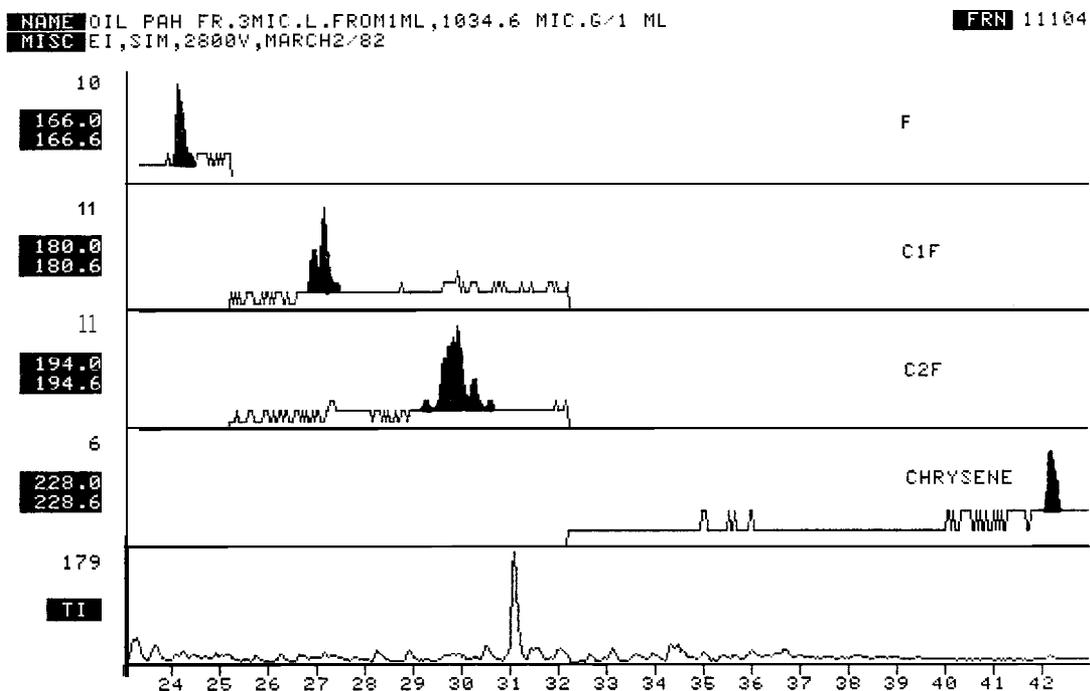
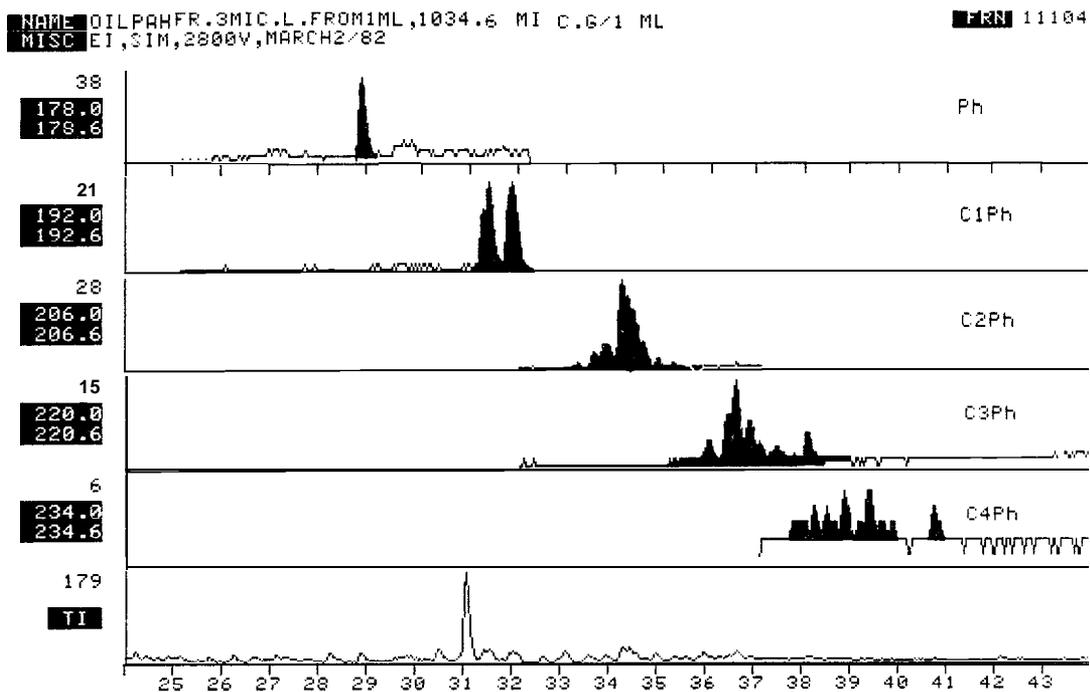


Figure 3.4 GC²/SIM chromatograms for Lagomedio crude oil, showing phenanthrenes, fluorenes, and chrysene as single ions

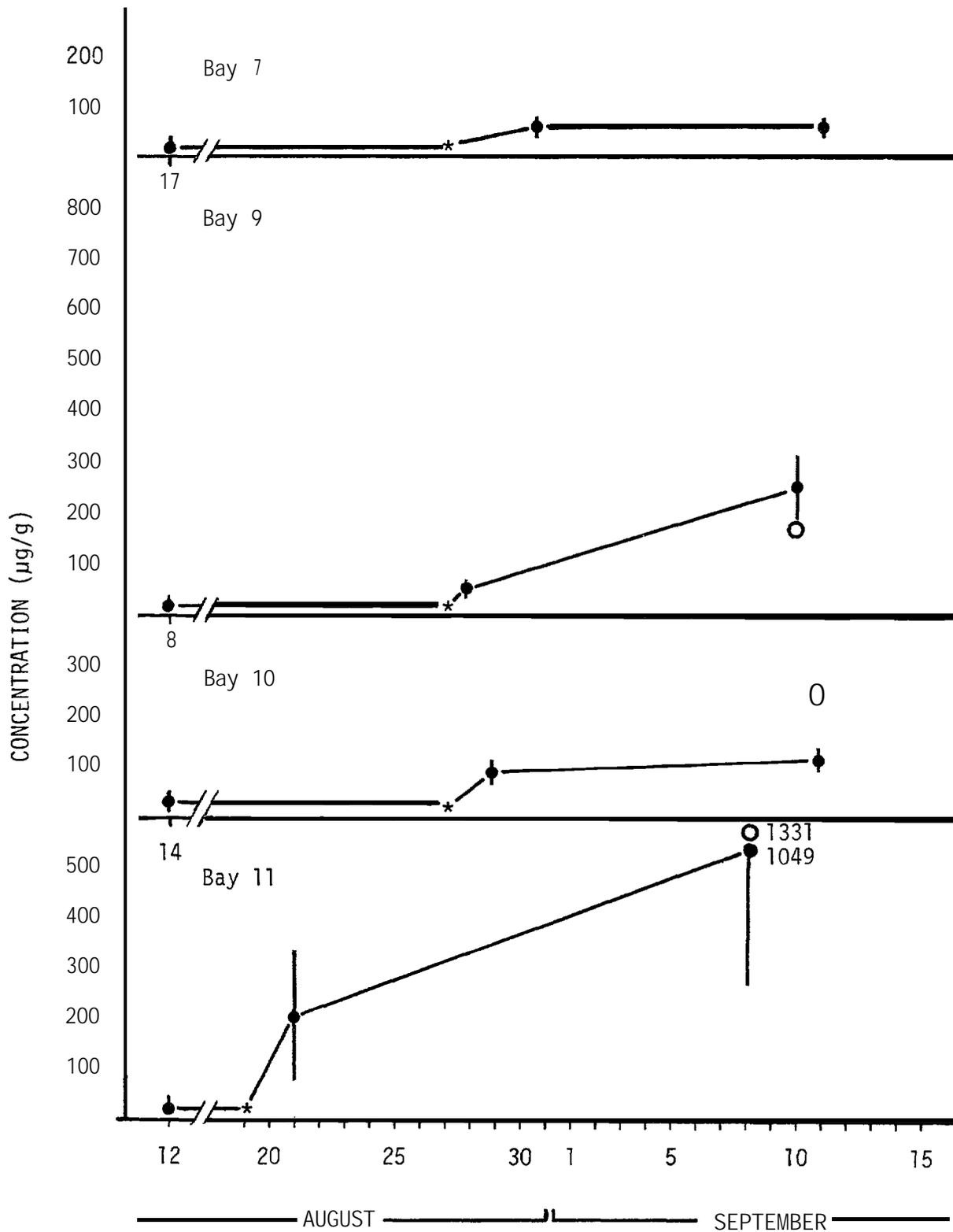


Figure 3.5 Trends in oil concentration in *Strongylocentrotus droebachiensis*, expressed as crude oil equivalents ($\mu\text{g/g}$ dry weight), by UV/F (*, day of spill; 0, 5m trap sample)

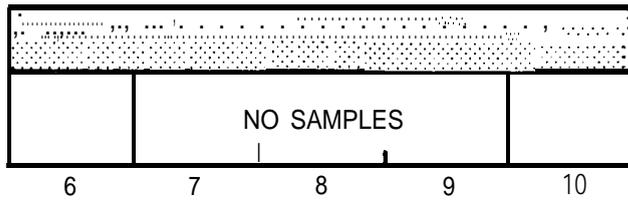
A more detailed examination of the individual sampling stations is presented in Figures 3.6 to 3.9. In pre-spill urchins, the station samples from the 7 m depth stratum varied by as much as a factor of four. An overall average of pre-spill tissue hydrocarbons (UV/F) from all four bays was 17.4 ug/g. The first post-spill samples were more consistent, except from Bay 11 where the values ranged from 20 to 596 ug/g among the 5 stations. Data from the second post-spill sample showed a similar relationship, with only Bay 11 being greatly variable, ranging from 174 to 2453 ug/g. Stations one and four were consistently higher in the post-spill samples of urchins from Bay 11. The urchin trap samples from 5m, examined at the second post-spill time period were the same as those from the 7m depth stratum in Bays 9 and 11, and somewhat higher in the case of Bay 10 (Figures 3.6, 3.7 and 3.9).

3.2.1.2 GC²/FID

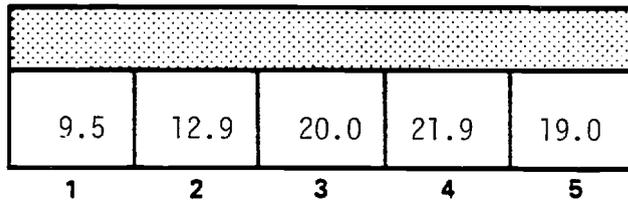
Very few of the urchin samples pooled for each 7m depth stratum contained quantifiable amounts of alkanes (>50 ppm oil equivalents) characteristic of Lagomedio crude oil (Figures 3.10 to 3.13). The urchin samples at Bay 11 (7m, Site No. 1 and 4) which were found to have high fluorescent and PAH content were also found to contain alkanes at both the first and second time points post-spill (Figures 3.14 and 3.15). The urchin trap samples at 5m from Bays 9, 10 and 11 also contained n-alkanes, the levels again being much higher in samples from Bay 11 (Figure 3.16). The results, calculated as outlined in the methods section, are presented in Table 3.1, and the abundance of the n-alkanes from C16 to C26 relative to C24 is shown in Figure 3.17.

Site No. 1 in Bay 11 was the most highly contaminated, with tissue levels in excess of 2,000 ug/g at the second time point post-spill. The distribution of the n-alkanes and the phytane/n-C18 ratio was also very similar to that in the oil at both time points. At Site No. 4 on the same transect, the distribution of n-alkanes below C23 was significantly different from that in the oil at both time points post-spill. This

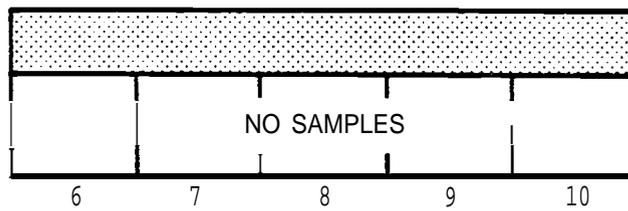
**BENTHIC
TRANSECT**
**TISSUE
PLOTS**



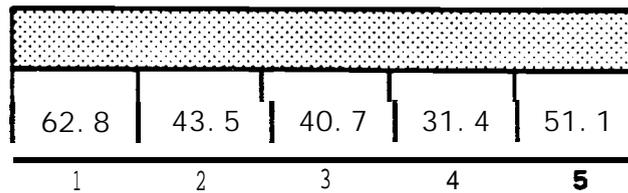
PRESPI LL
7-9 Aug.



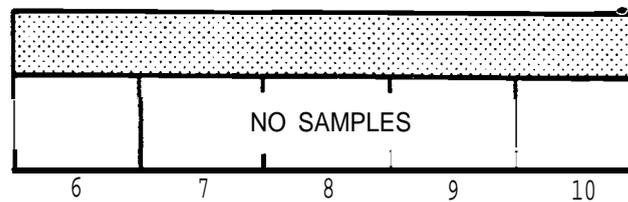
\bar{x} 16.5 ± 2.3



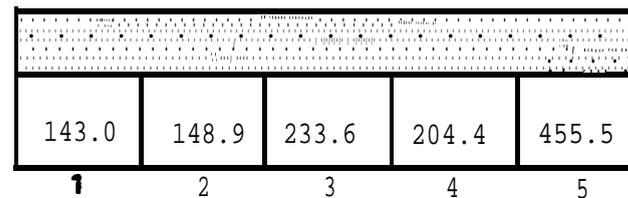
FIRST POSTSPI LL
28 Aug. {id}



\bar{x} 45.9 ± 5.3



SECOND POSTSPI LL
10 Sept. (14d)

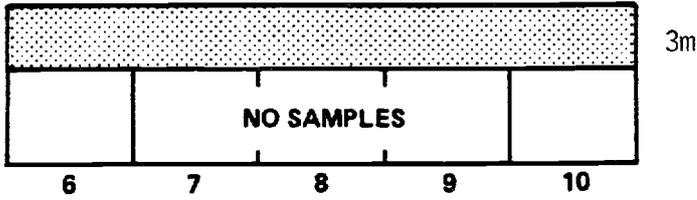


\bar{x} 237.1 ± 57.1

Figure 3.6 Concentrations of oil in Strongylocentrotus droebachiensis, Bay 9, by UV/F ($\mu\text{g/g}$ dry weight), \pm S.E.

**BENTHIC
TRANSECT**

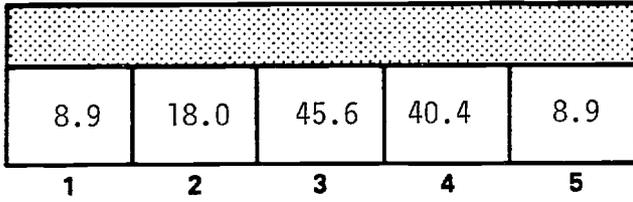
**TISSUE
PLOTS**



3m

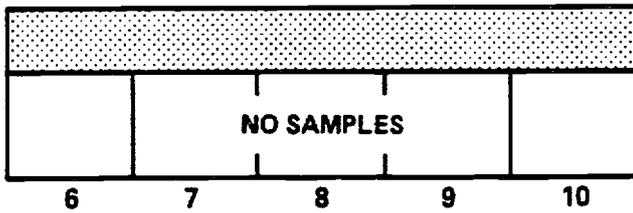
PRESPILL

14 Aug.



7m

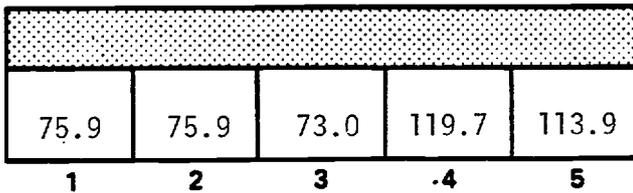
\bar{x} 24.9 ± 7.6



3m

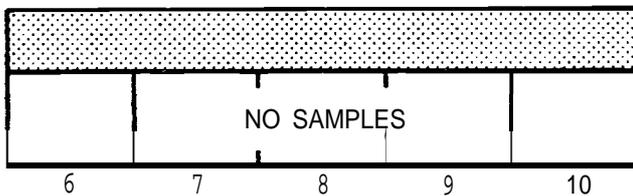
FIRST POSTSPILL

29 Aug. (2d)



7m

\bar{x} 91.7 ± 10.3



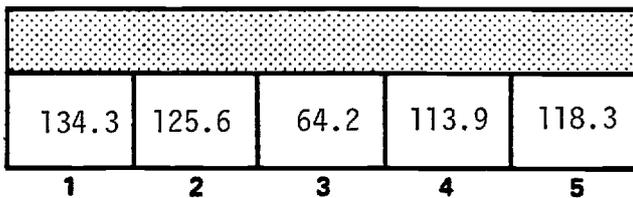
3m

SECOND POSTSPILL

11 Sept. (15d)



5m

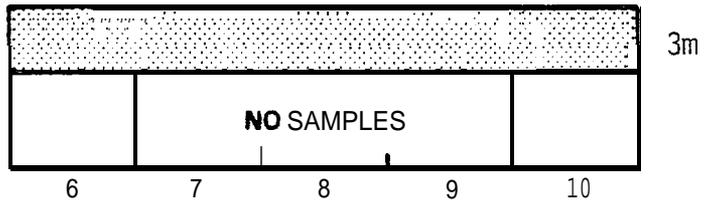


7m

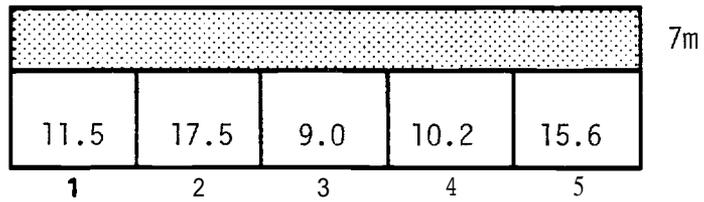
\bar{x} 111.2 ± 12.2

Figure 3.7 Concentrations of oil in *Strongylocentrotus droebachiensis*, Bay 10, by UV/F ($\mu\text{g/g}$ dry weight), \pm S.E.

**BENTHIC
TRANSECT
TISSUE
PLOTS**

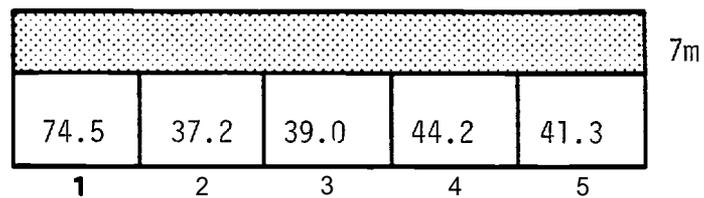
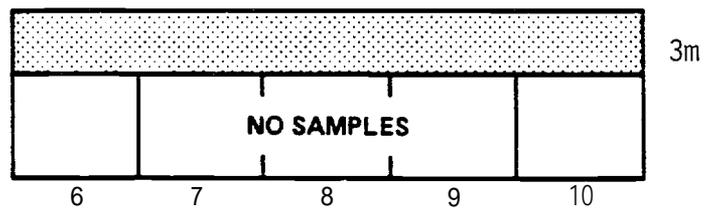


17 Aug.



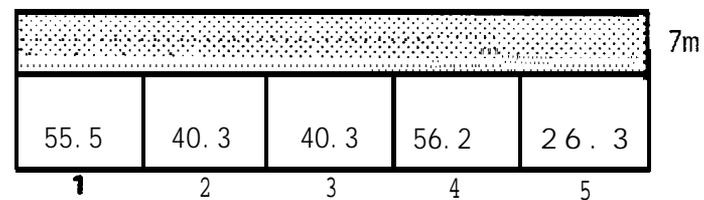
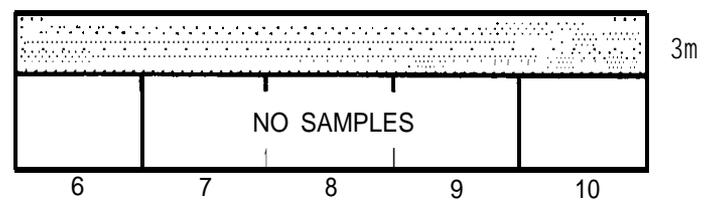
\bar{x} 12.8 ± 1.6

FIRST POSTSPILL
31 Aug. (4d)



\bar{x} 47.2 ± 6.9

SECOND POSTSPILL
11 Sept. (15d)

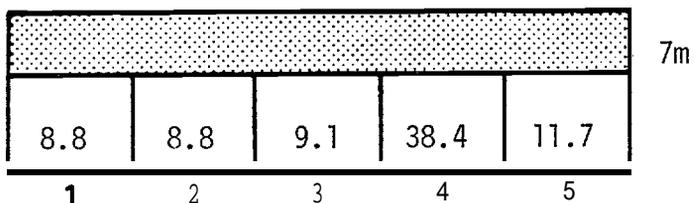
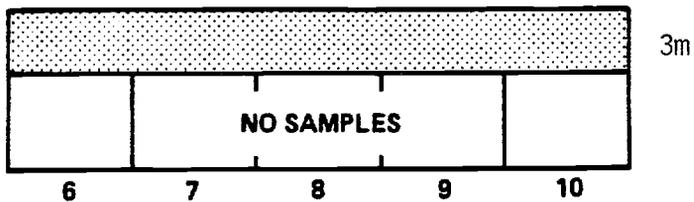


\bar{x} 43.7 ± 5.6

Figure 3.8 Concentrations of oil in Strongylocentrotus droebachiensis, Bay 7, by UV/F ($\mu\text{g/g}$ dry weight), \pm S.E.

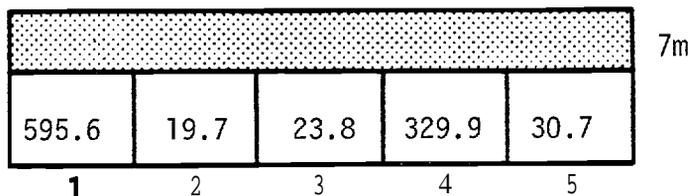
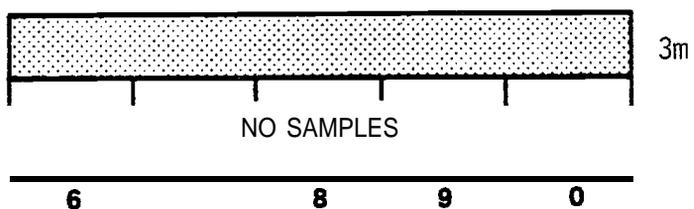
**BENTHIC
TRANSECT**

**TISSUE
PLOTS**



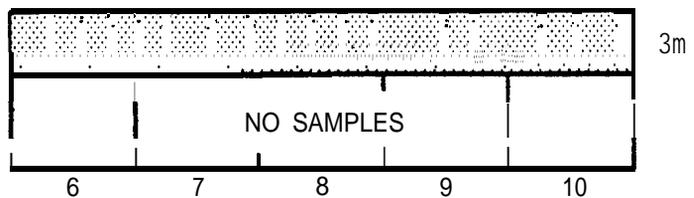
\bar{x} 15.3 \pm 5.8

FIRST POSTSPILL
21 Aug. (2d)

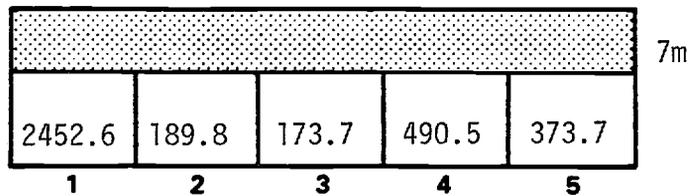


\bar{x} 199.9: 115.0

SECOND POSTSPILL
8 Sept. (20d)



5m



\bar{x} 1048.9 \pm 443.7

Figure 3.9 Concentrations of oil in *Strongylocentrotus droebachiensis*, Bay 11, by UV/F ($\mu\text{g/g}$ dry weight), \pm S.E.

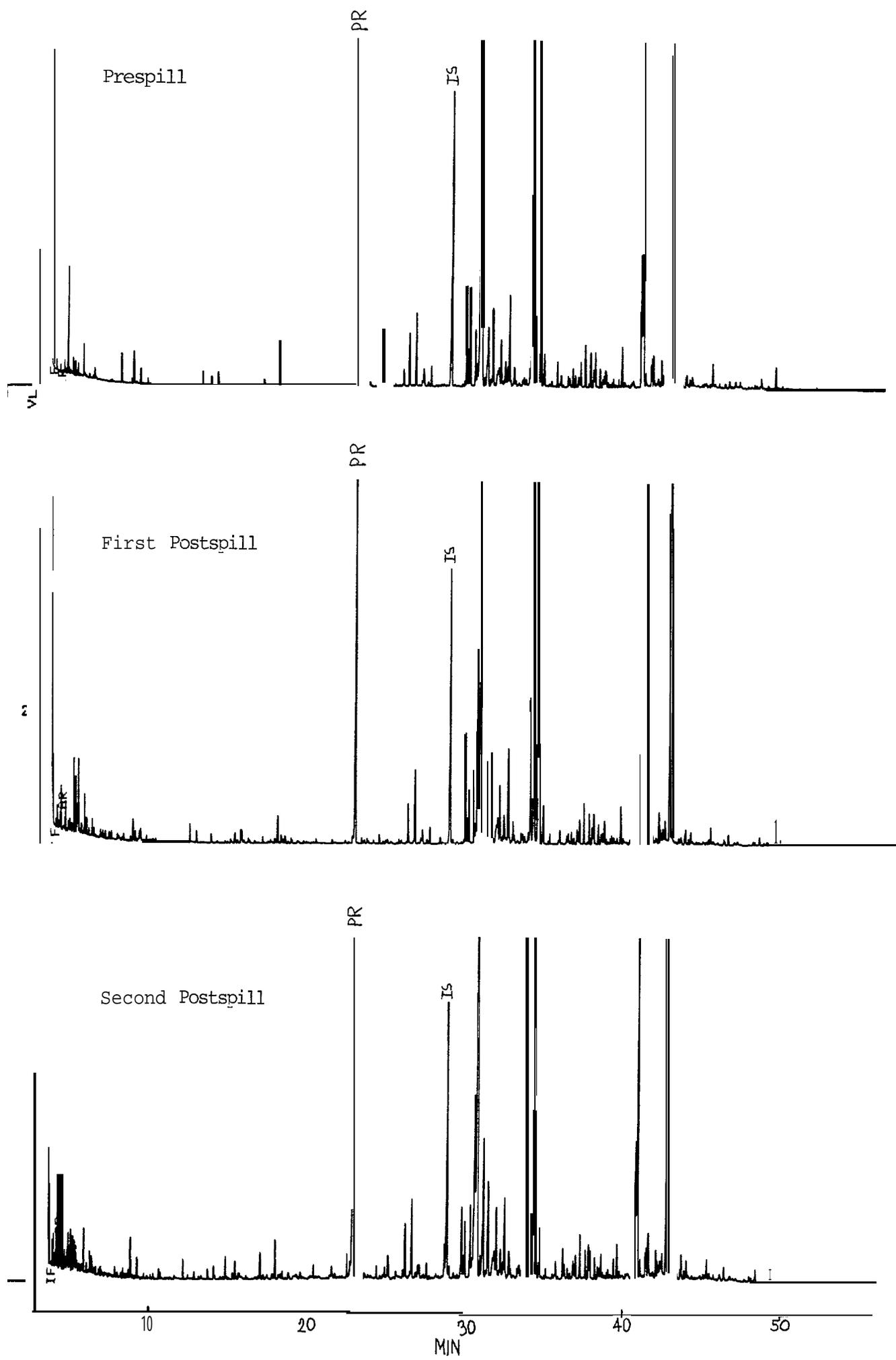


Figure 3.10 GC²/FID chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay B

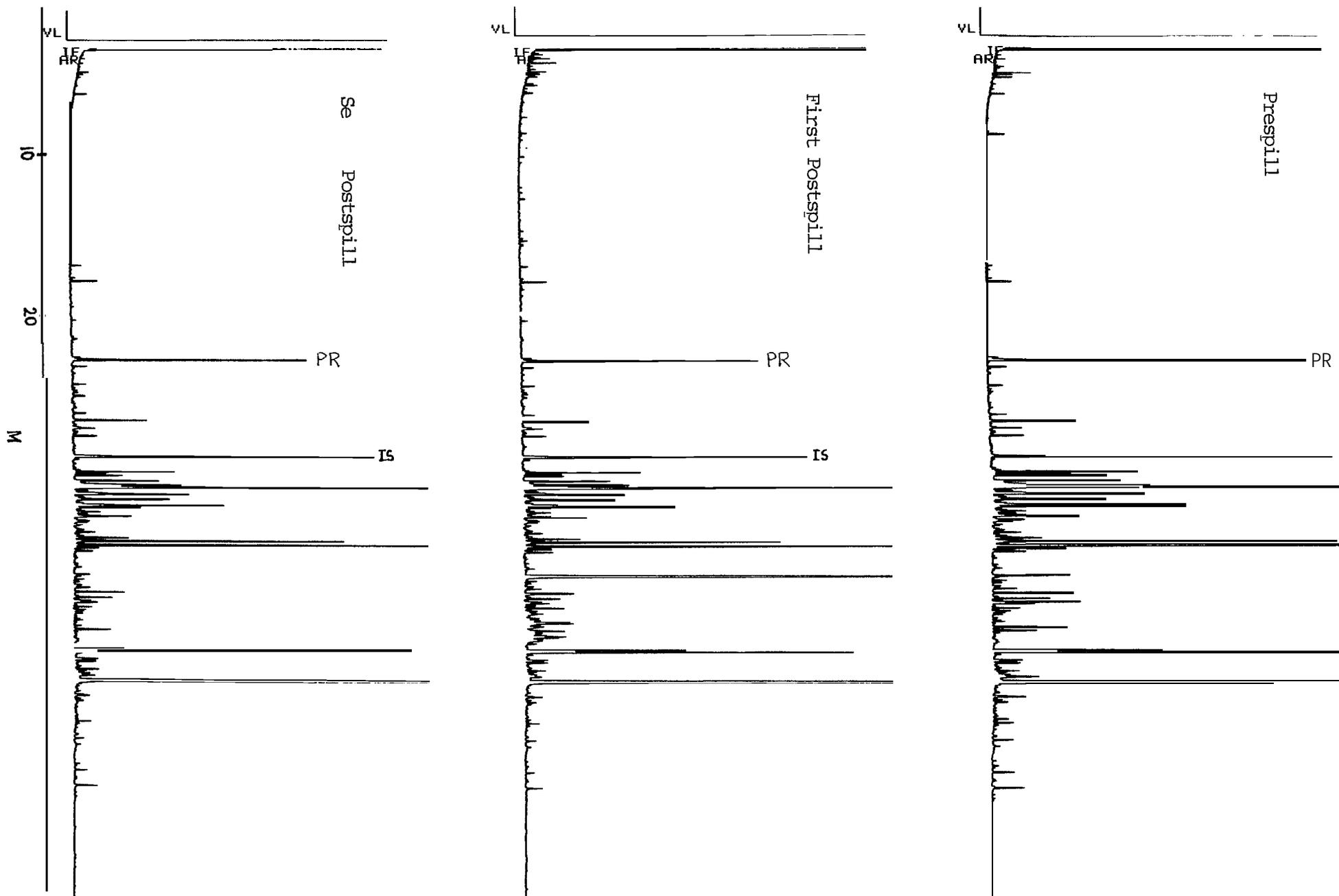


Figure 3.11 GC²/FID chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 10

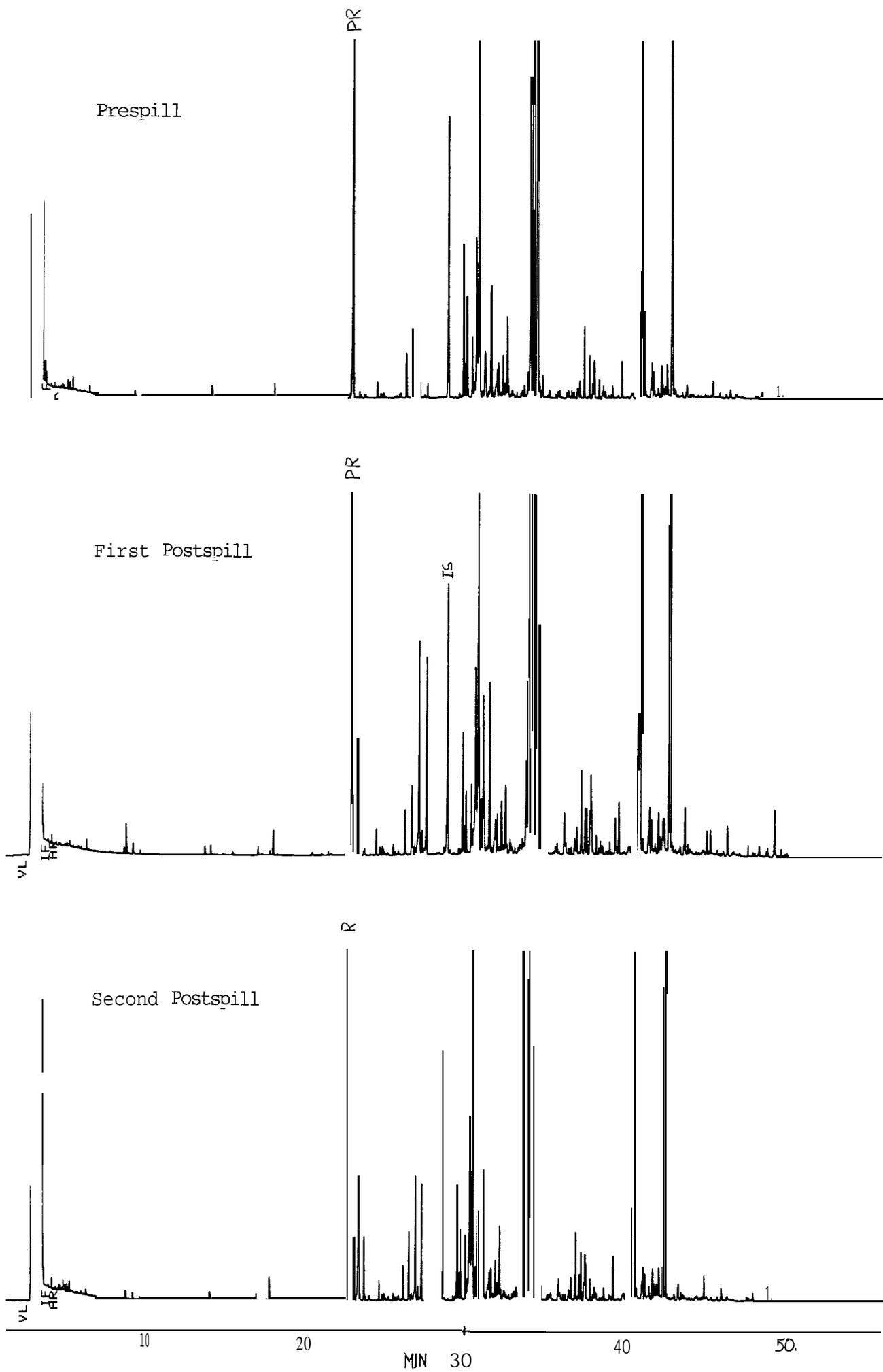


Figure 3.12 GC²/FID chromatograms of *Stronvlocentrotus droebachiensis* tissues from Bay 7

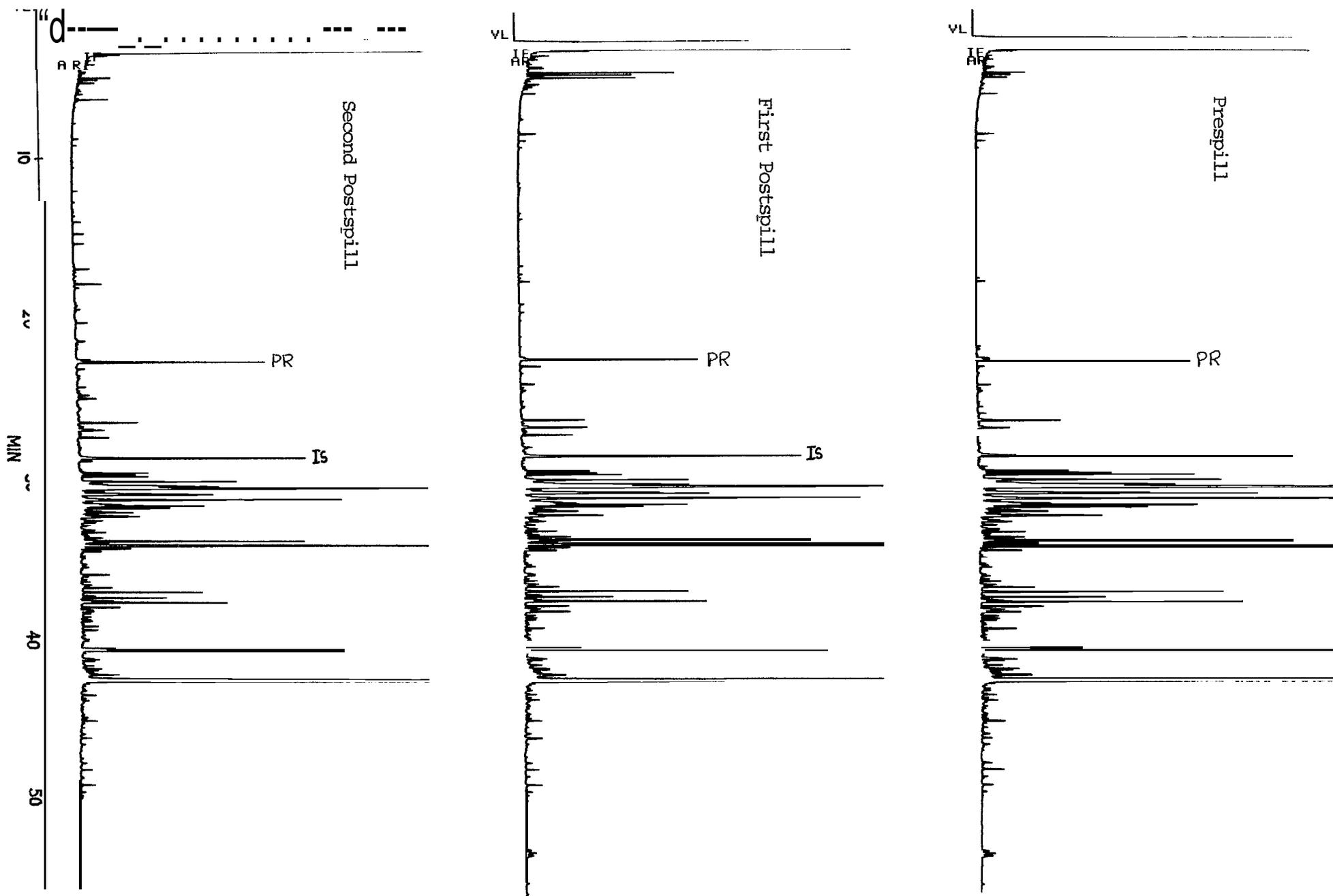


Figure 3.13 GC²/FID chromatograms of Strongylocentrotus droebachiensis tissues from Bay 11, sampling stations 2,3, and 5 combined

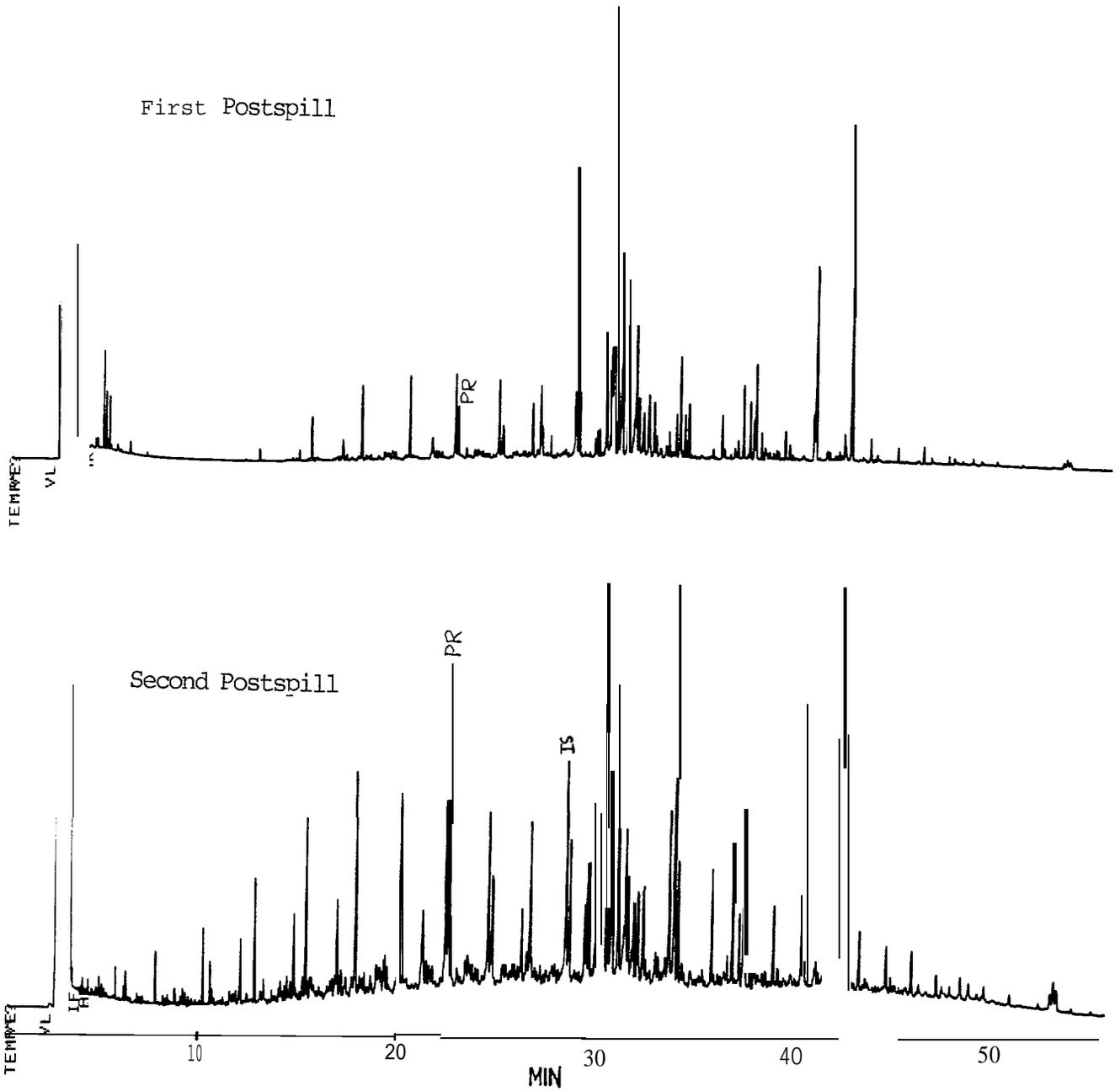


Figure 3.14 GC²/FID chromatograms of Strongylocentrotus droebachiensis tissues from Bay 11, sampling station 1

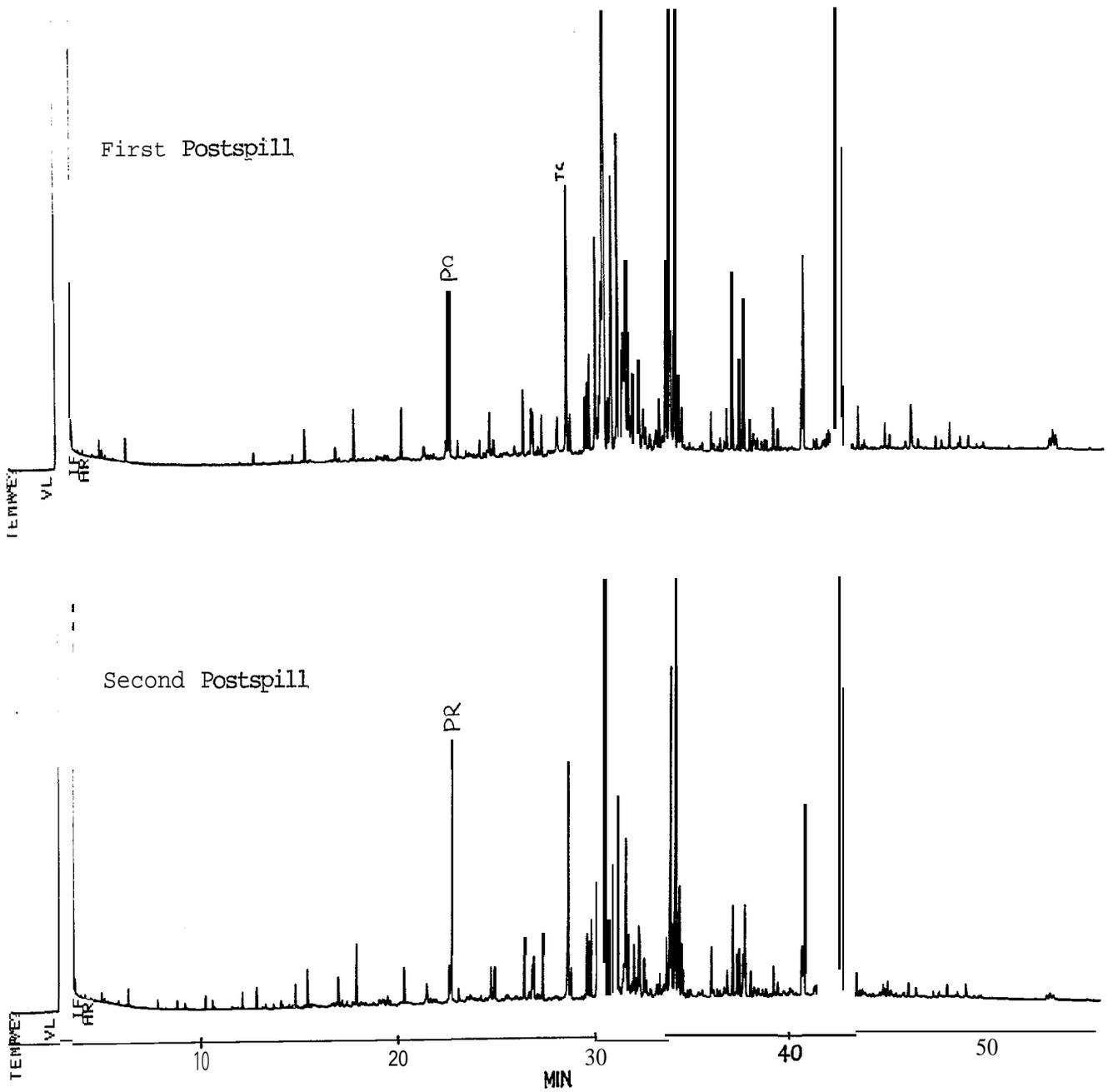


Figure 3.15 GC²/FID chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 11, sampling station 4

Table 3.1 n - Alkane levels in *Strongylocentrotus droebachiensis* tissue¹ (ug crude oil/g dry weight) based on sum of peak height of C16-C26 n-alkanes, compared to oil std. by FID

Site	Time Post-Spill	No. in Pool	n-alkane ug/g ²	Pristane/Phytane	Phytane/n-C ₁₈
Bay 11 7m, No. 1	1st	7	838	1.6	0.41
	2nd	10	2,349	3.1	0.63
Bay 11 7m, No. 4	1st	8	645	10.1	0.38
	2nd	10	519	7.4	1.00
Bay 11 Urchin trap	2nd	10	1,890	2.2	0.64
Bay 9 Urchin trap	2nd	9	243 ²	15.0	3.10
Bay 10 Urchin trap	2nd	9	121 ²	12.3	2.10
Crude Oil				0.9	0.40

¹ Percent water in soft tissue 86.5 ± 1.0

² Under 300 ug/g n-alkane levels quantitation is less reliable
Detection limit is 100 ug/g.

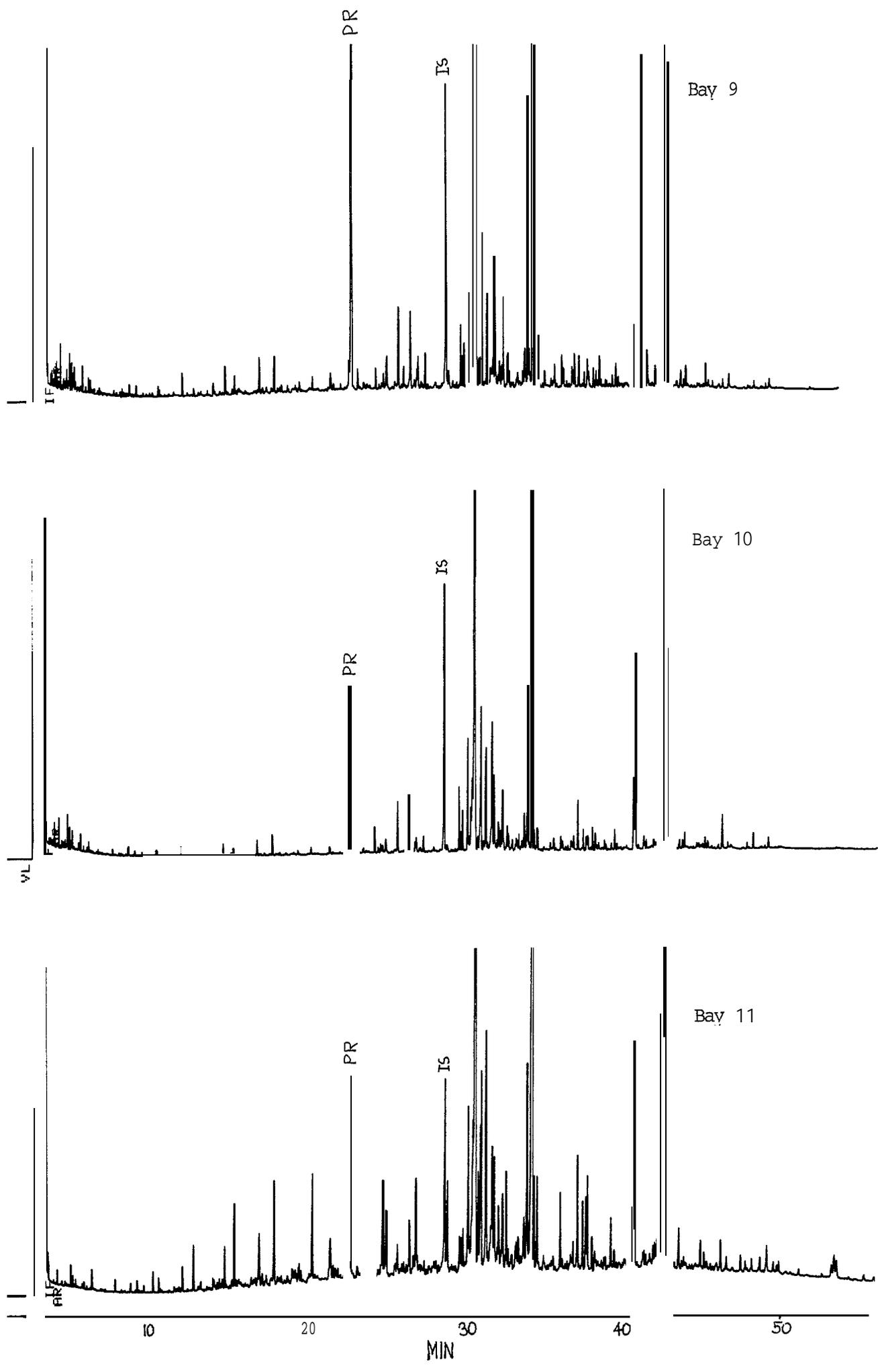


Figure 3.16 GC²/FID chromatograms of Stronovlocentrotus droebachiensis tissues from at 5 m depth in three bays

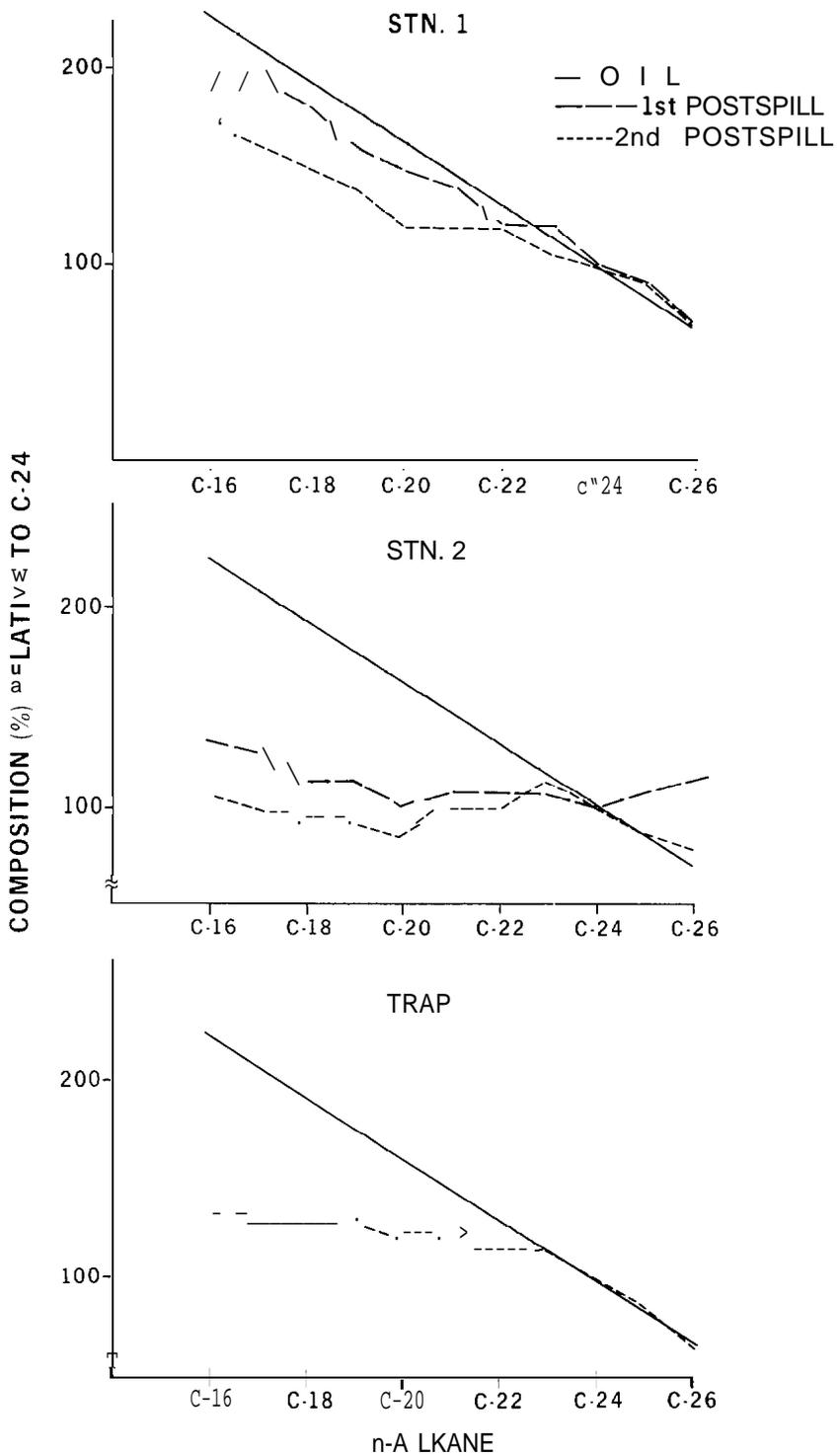


Figure 3.17 Relative n-alkane composition of Strongylocentrotus droebachiensis tissues from Bay 11

pattern existed also for the urchin trap sample from Bay 11 (Figure 3.18). The phytane/n-C₁₈ ratio was higher than that in the oil at the second time point post-spill, especially in the Bay 9 and 10 urchin trap samples. Pristane and phytane levels in urchin samples from all four bays, pooled by transect, are presented in Table 3.2.

3.2.1.3 GC²/SIM

The aromatic hydrocarbon profiles of urchin tissues from Bays 9, 10 and 11 indicate a proportional emphasis on the lower molecular weight hydrocarbons in the first post-spill samples (Figures 3.19 to 3.21). This is especially clear in the Bay 9 first post-spill urchin sample which showed naphthalene and alkyl benzene levels over 2,000 rig/g. The data are summarized in Figures 3.22 and 3.23. No such relative enhancements of the low molecular weight aromatics was noted for the first post-spill sample from Bay 11. While relatively low aromatic concentrations were found in the pooled samples from Stations 2, 3 and 5 (Figures 3.23 and 3.24), the two individual station samples from this surface-spill bay clearly showed a difference in early uptake patterns as compared to urchins from the dispersed oil bays (Figures 3.25 to 3.27).

Urchin tissues from the second post-spill samples exhibited an enhancement in the relative proportions of larger molecular weight aromatics, as well as a shift to a greater degree of methylation within an aromatic group (Figures 3.19 to 3.27). Levels over 2000 rig/g are recorded in urchin tissues from dispersed oil spill exposures; especially C₃- and C₄-naphthalene, C₂-phenanthrene, and C₂- and C₃- dibenzthiophene. The second post-spill samples for Bay 11 tissues similarly showed a relative predominance of larger molecular weight, polymethylated aromatics. Station 1 and 4 samples from Bay 11 illustrated this to the greatest degree, with individual selected PAH's from Station 1 showing concentration values over 3,000 ng/g (Figure 3.27).

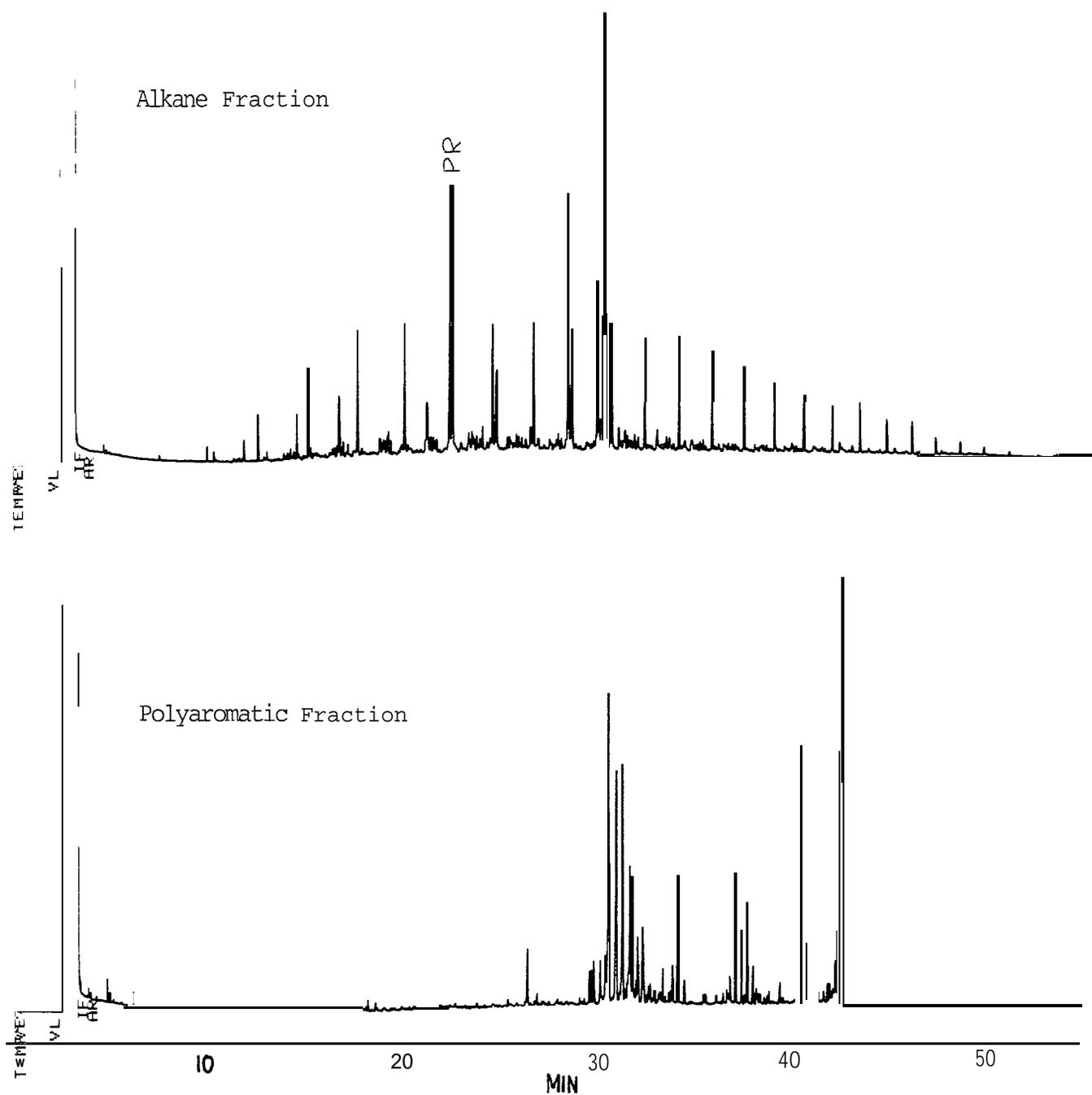


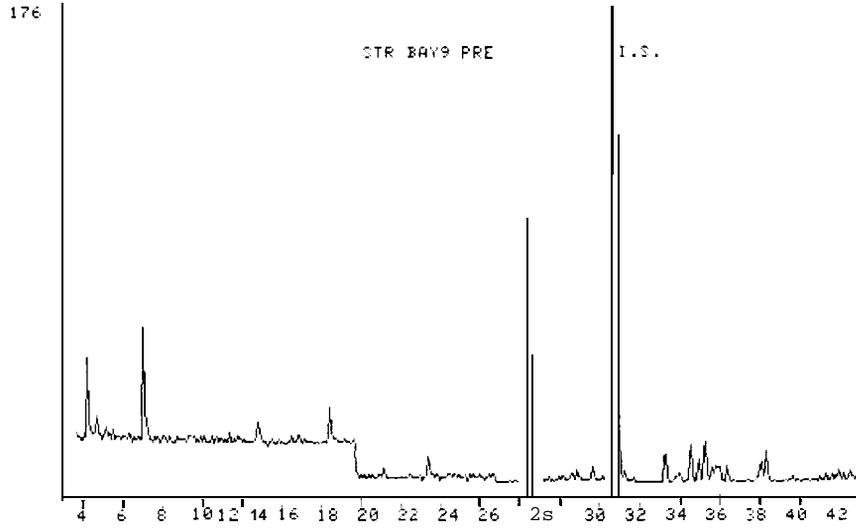
Figure 3.18 GC²/FID chromatograms (alkanes and polyaromatic hydrocarbons separated) of Strongylocentrotus droebachiensis tissues from the urchin trap at 5 m in Bay 11

Table 3.2 **Pristane and phytane levels (ug/g) dry weight)¹**
and ratios in Strongylocentrotus droebachiensis

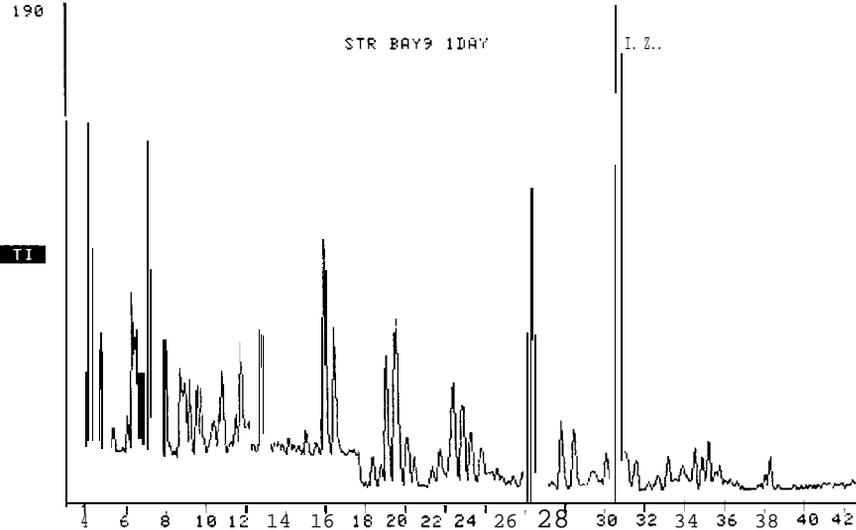
Site	Time Re. Spill	No. in Pool Stn . Individ.	Pristane	Phytane	Pristane-Phytane Ratio	
Bay 9-7m	pre	5	61	22.9	<0.20	> 115
	1st	4	50	24.1	<0.20	> 121
	2nd	5	55	28.7	1.36	21
Bay 10-7m	pre	5	44	14.4	<0.20	>70
	1st	5	38	11.8	<0.28	>42
	2nd	5	49	11.8	0.64	18
Bay 7-7m	pre	5	45	24.6	<0.20	> 123
	1st	5	48	19.4	<0.20	> 97
	2nd	5	49	22.3	0.41	54
Bay 11-7m	pre	4	31	9.6	<0.20	>48
	1st	3	22	9.4	<0.20	>47
	2nd	3	30	11.7	1.23	10

¹percent water in soft tissue, 86.5 ± 1.0

NAME BAY9,PRE,P,3MIC.L.FROM2.5ML,FEBR25/82 FRN 11303
MISC EI,SIM,2800V,T009,010,015,034,039



NAME BAY9,1DAY,P,3MIC.L.FROM2.0ML,FEBR26/82 FRN 11305
MISC EI,SIM,2800V,T327,332,337,347



NAME BAY9,2WKS,P,3MIC.L.FROM2.5ML,FEBR25/82 FRN 11304
MISC EI,SIM,2800V,T-635,640,645,650,656

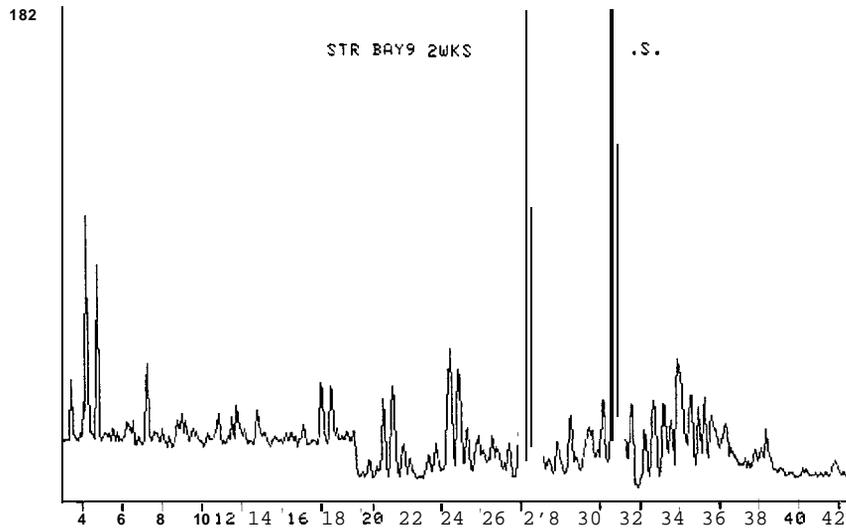
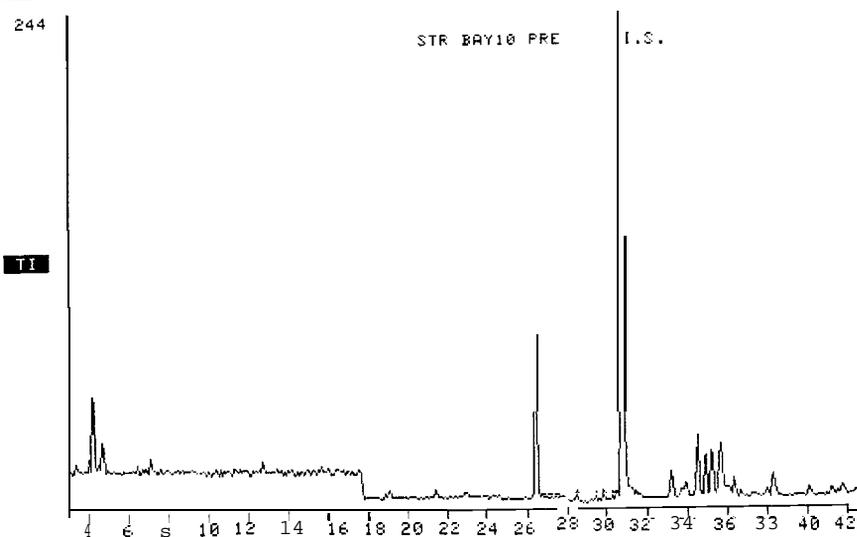


Figure 3.19 GC/MS total ion chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 9

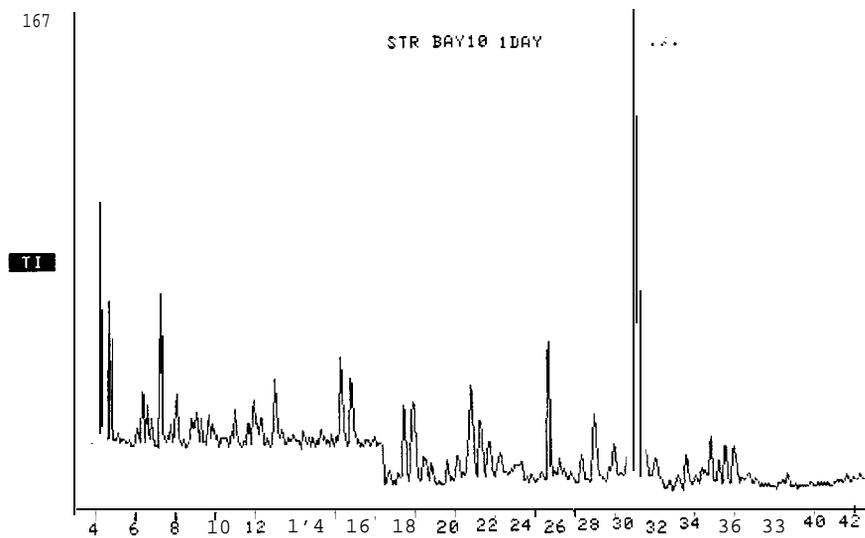
NAME BAY10,PRE,P,3MIC.L.FROM2.SML.FEBR25/82
MISC EI,SIM,2800V,T131,138,141,146,150*

FRN 11302



NAME STR,BAY10,1DAY,3MOC.L.,FR0, 0.5 ML
MISC EI,SIM,2800V,P #3,MARCH10/82

FRN 12107



NAME BAY10,2WKS,P,3MIC.L.FROM2.SML.FEBR25
MISC EI,SIM,2800V,T696,701,706,710,715.FEBR25

FRN 11301

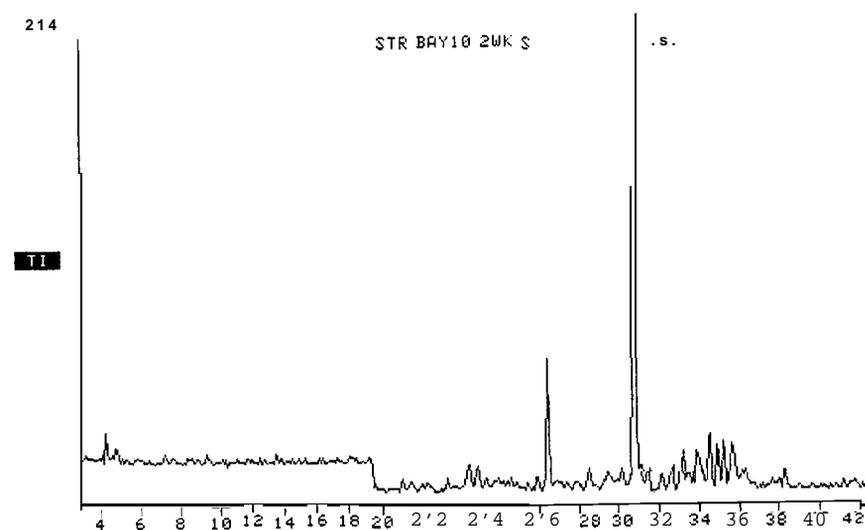
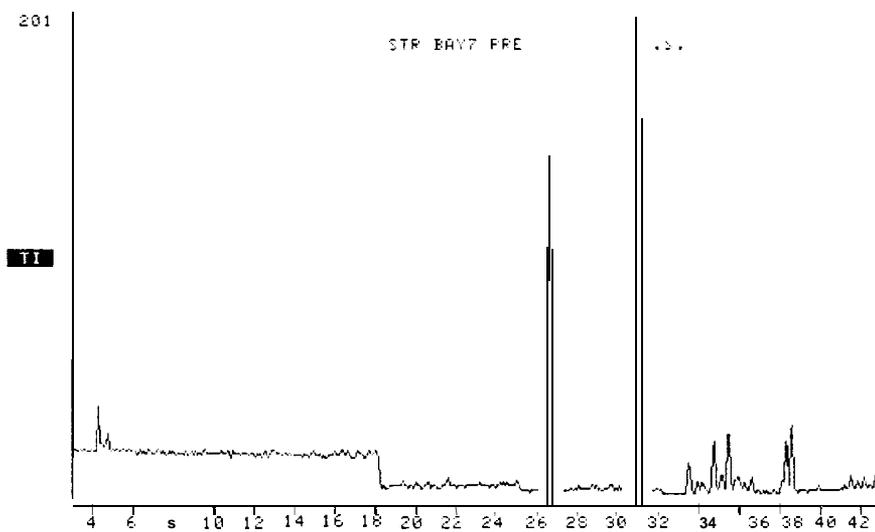


Figure 3.20 GC²/MS total ion chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 10

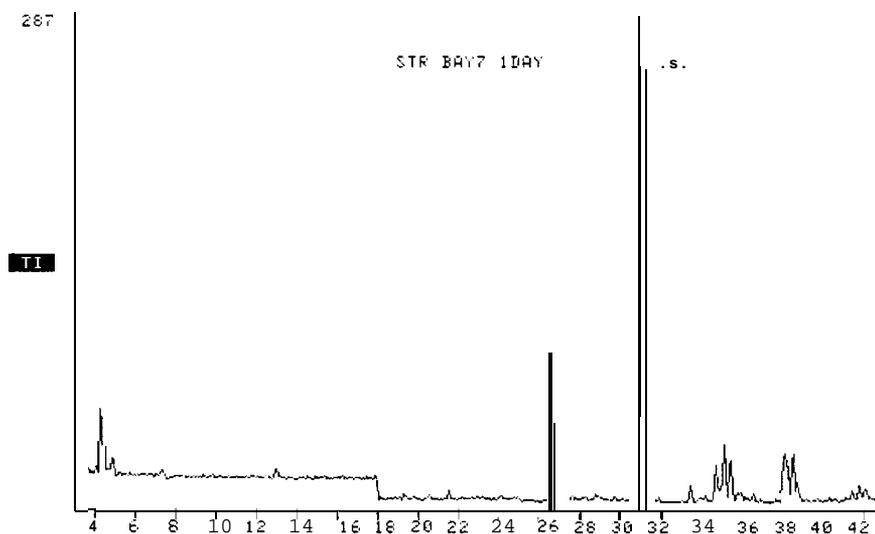
NAME BAY7,FRE,P,3MIC.L.FROM2.5ML
 MISC EI,SIM,2800V,T196,201,206,211,214

FRN 11103



NAME BAY7,1DAY,P,3MIC.L.FROM2.5ML,MARCH2/82
 MISC EI,SIM,2800V,T478,483,488,493,498

FRN 11101



NAME BAY7,2WKS,P,3MIC.L.FROM2.5ML,MARCH2/82
 MISC EI,SIM,2800V,T676,680,684,688,693

FRN 11102

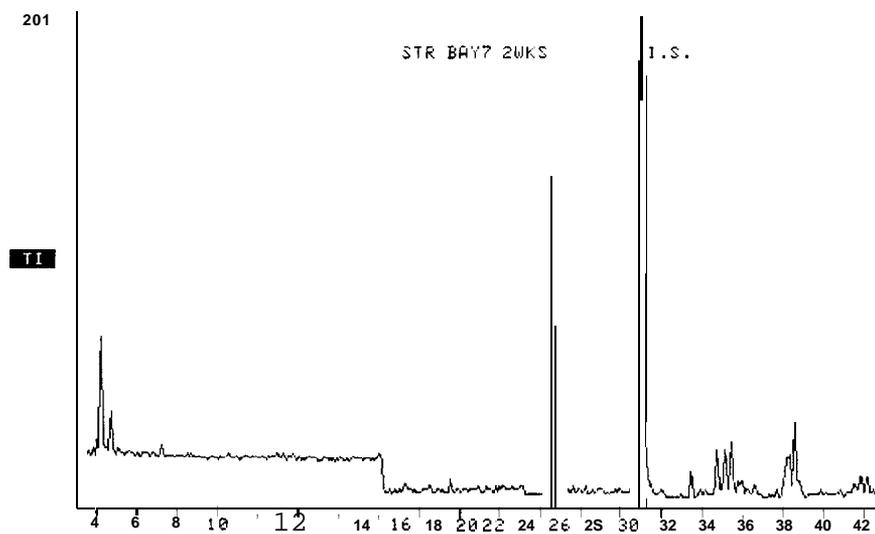


Figure 3.21 GC²/MS total ion chromatograms of *Strongylocentrotus droebachiensis* tissues from Bay 7

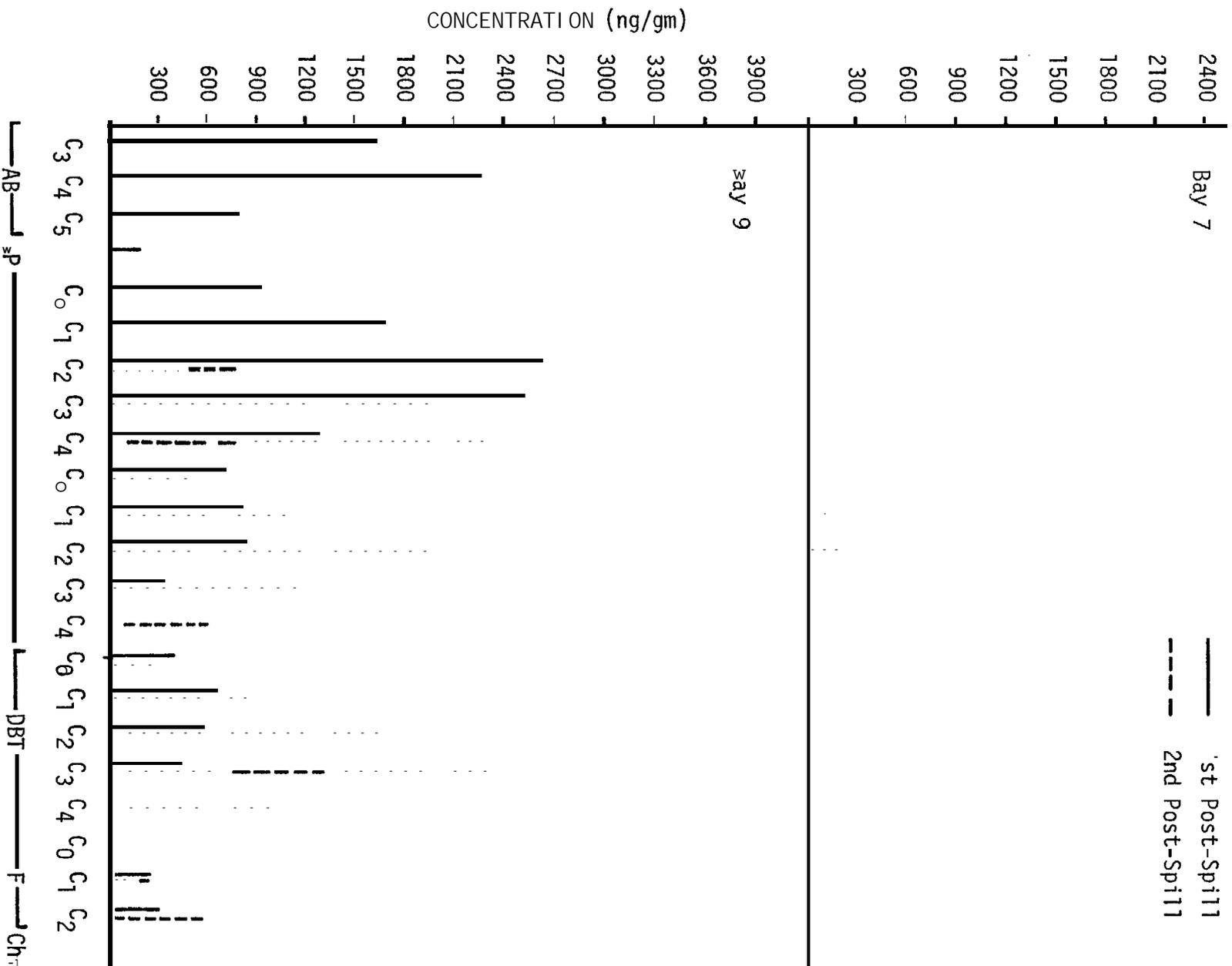
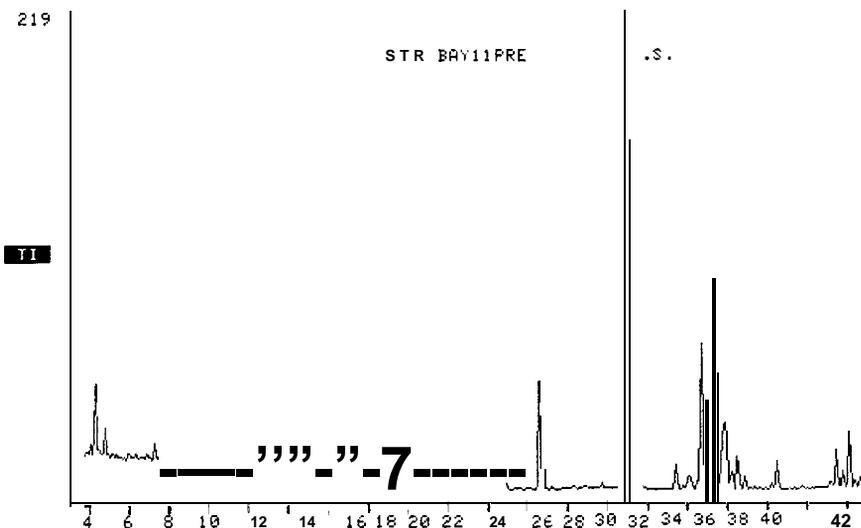


Figure 3.22 Aromatic hydrocarbon profiles in *Strongylocentrotus droebachiensis*

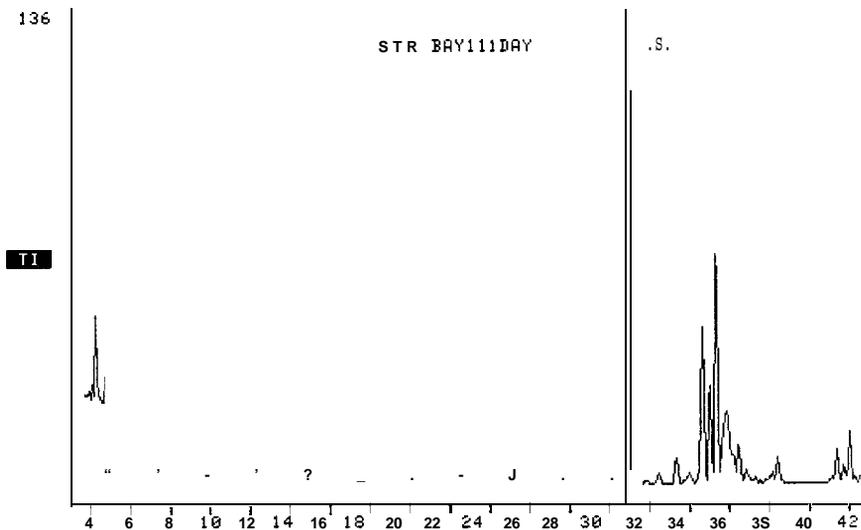
NAME BAY11,PRE,P,3MIC.L.FROM2.0ML
MISC EI,SIM,2800V,T80,85,90,100,MARCH1/82

FRN 11211



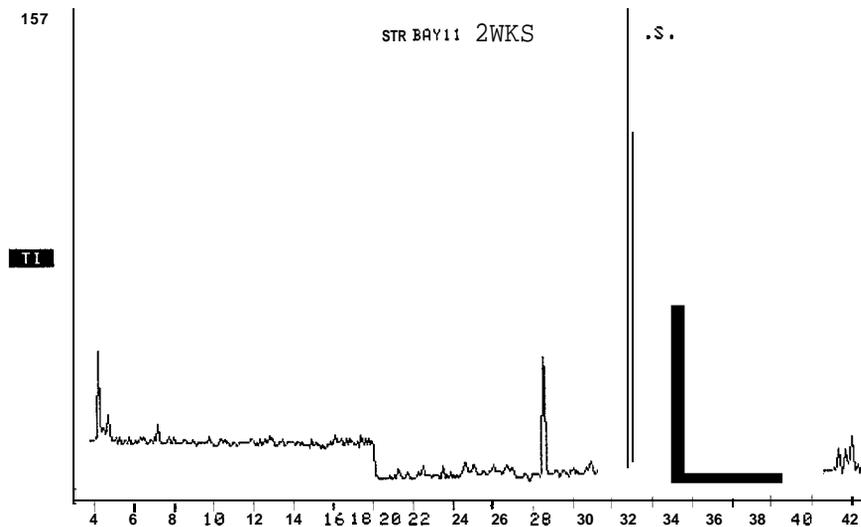
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MISC EI,SIM,2800V,T-226,231,241

FRN 11308



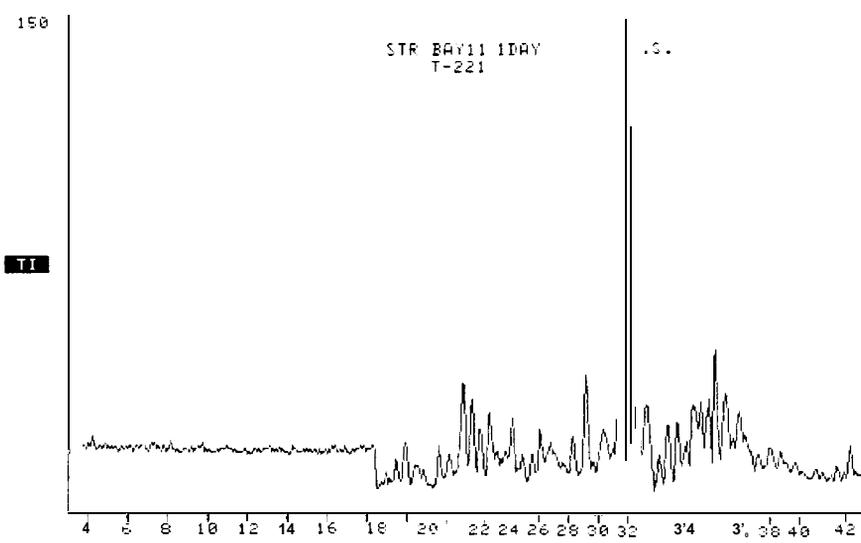
NAME BAY11,2WKS,P,3MIC.L.FROM1.5ML,MARCH1/82
MISC EI,SIM,2800V,T-210,623,627

FRN 11210



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chromatograms
GC /MS tota
Bay
amp
figure

NAME ITP, BAY11, 1DAY, T-221, 3MIC.L.FROM 0.5 ML FRN 12205
MISC EI, SIM, 2800V, MARCH15 '82



NAME ITP, BAY11, 2WKS, T-631, 3MIC.L.FROM 0.5 ML FRN 12207
MISC EI, SIM, 2800V, MARCH16 '82

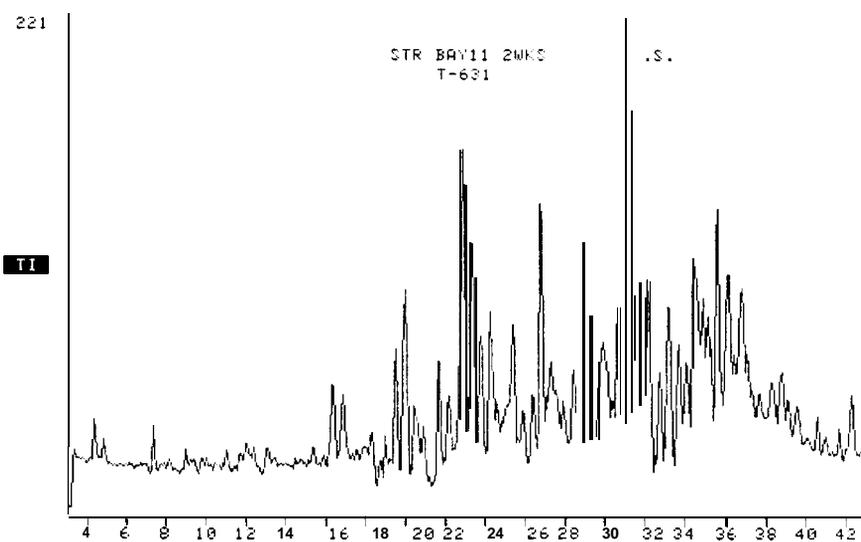
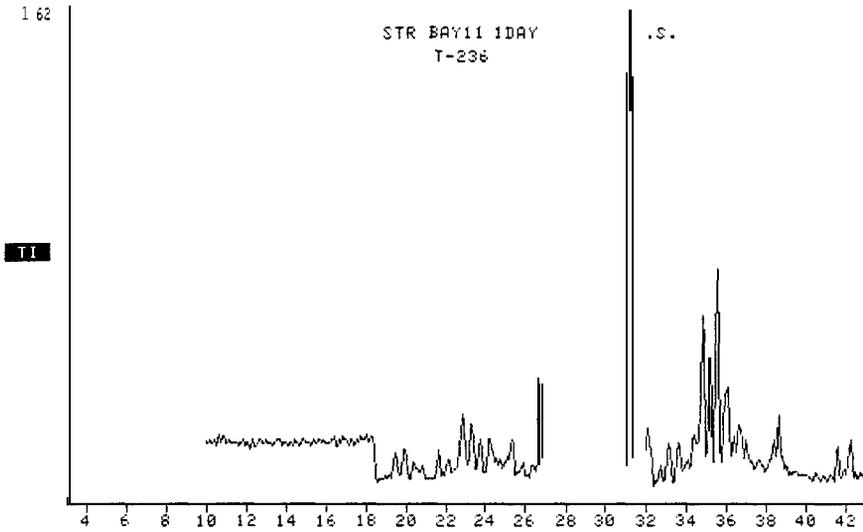


Figure 3.25 GC²/MS total ion chromatograms of Strongylocentrotus droebachiensis tissues from Bay 11, sampling station 1

NAME STR, BAY11, 1DAY, T-236, 3MIC.L. FROM 0.5 ML
FRN 12206
MISC EI, SIM, 2800V, MARCH15/82



NAME STR, BAY11, 2WKS, T-620, 3MIC.L. FROM 0.5 ML
FRN 12203
MISC EI, SIM, 2800V, MARCH17/82

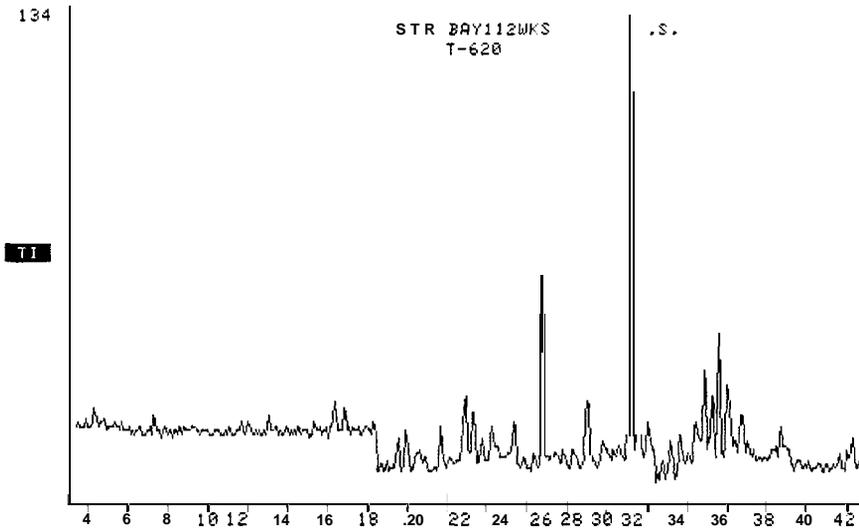


Figure 3.26 GC²/MS total ion chromatograms of Strongylocentrotus droebachiensis tissues from Bay 11, samplinf station 4

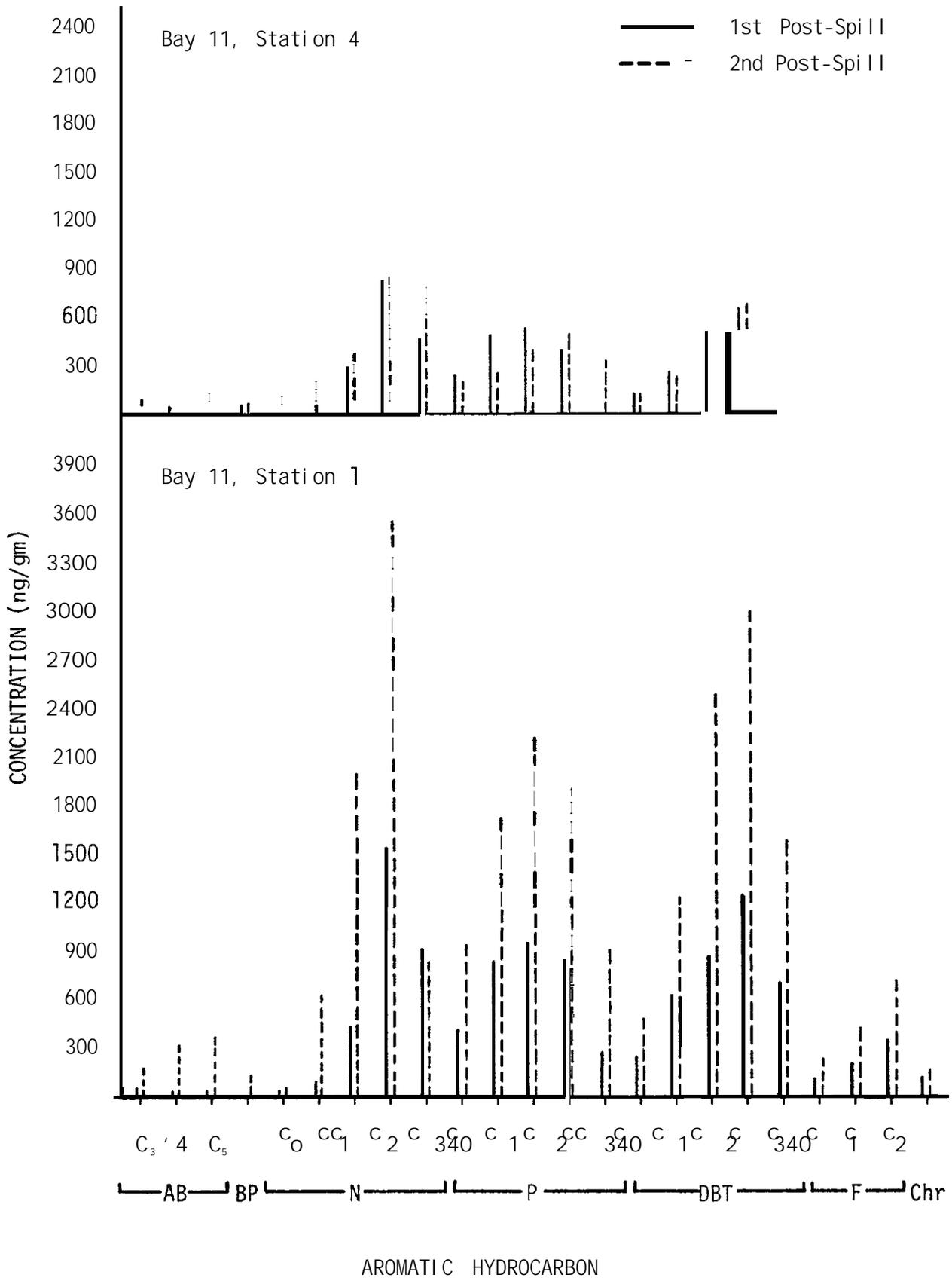


Figure 3.27 Aromatic profiles in Strongylocentrotus droebachiensis

The urchin trap samples from 5m showed PAH characteristics similar to the 7m samples (Figure 3.28). Polymethylated large molecular weight aromatics were enhanced in concentration. A comparison among trap samples from dispersed oil and surface spill conditions shows a marked similarity in relative composition (Figure 3.29) .

When total oil concentration in pooled urchin tissues is expressed using the GC²/SIM data, as related to aromatic levels in the crude oil (Figure 3.30), there are some difference from the concentration values determinedly UV/F (Figure 3.5). First post-spill samples in Bays 9, and 10 were markedly higher than when assessed on the basis of UV/F. Second post-spill samples for Bays 9 and 11 were lower on the basis of their aromatic composition, as was the first post-spill sample from Bay 11.

3.2.2 Pectinaria granulosa

3.2.2.1 UV/F

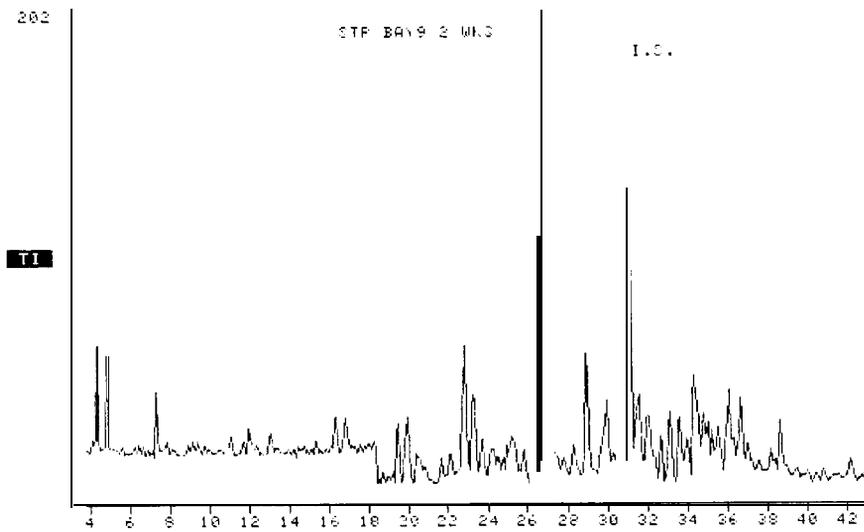
Tissue samples from polychaetes of Bays 9 and 10, pooled for the five stations of each 7m depth stratum, demonstrated an increase in total oil concentrations 1 to 2 days after oiling (Figures 3.31 to 3.33) . This increased by two weeks post-spill in Bay 9 polychaetes to nearly 300 ug/g, but remained relatively constant in tissues from Bay 10. No discernible increases were noted 2 to 4 days post-spill in Bay 7 and Bay 11 samples (Figures 3.31, 3.34 and 3.35) . Polychaetes from Bay 11 did show an increased body burden in the 2nd post-spill sample. Bay 7 tissues remained clean.

3.2.2.2 GC²/FID

The amounts of n-alkanes in polychaetes were found to be so low as to be almost unquantifiable. Figures 3.36 to 3.39 demonstrate qualitatively, however, that there was a minimal increase in n-alkanes in post-spill samples.

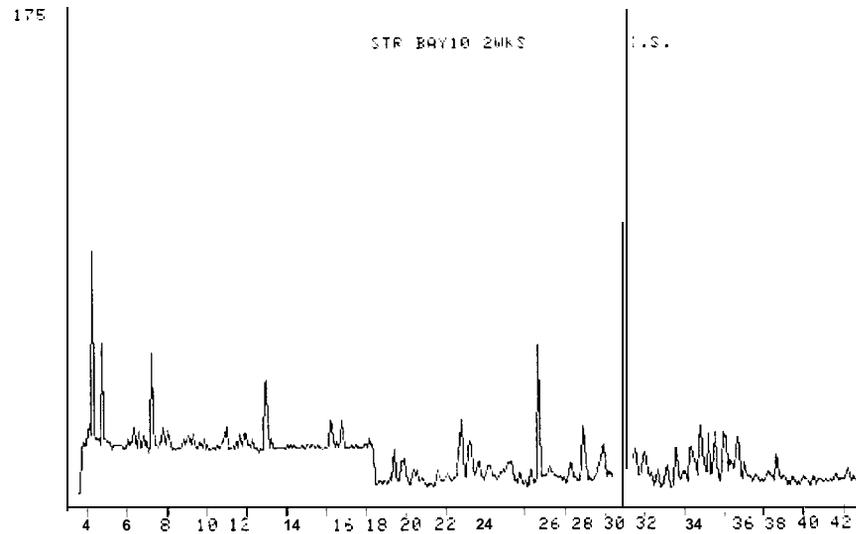
NAME STR_BAY9_2MKS_3MIC.L FROM 0.5 ML
MISC EI.SIM,2800V,T-824,MARCH8 82

FRN 12100



NAME STR_BAY10_2MKS_3MIC.L FROM 0.5 ML
MISC EI.SIM,2800V,T-823,MARCH8 82

FRN 12101



NAME STR_BAY11_2MKS_3MIC.L FROM 0.5 ML
MISC EI.SIM,2800V,T-822,MARCH8 82

FRN 12102

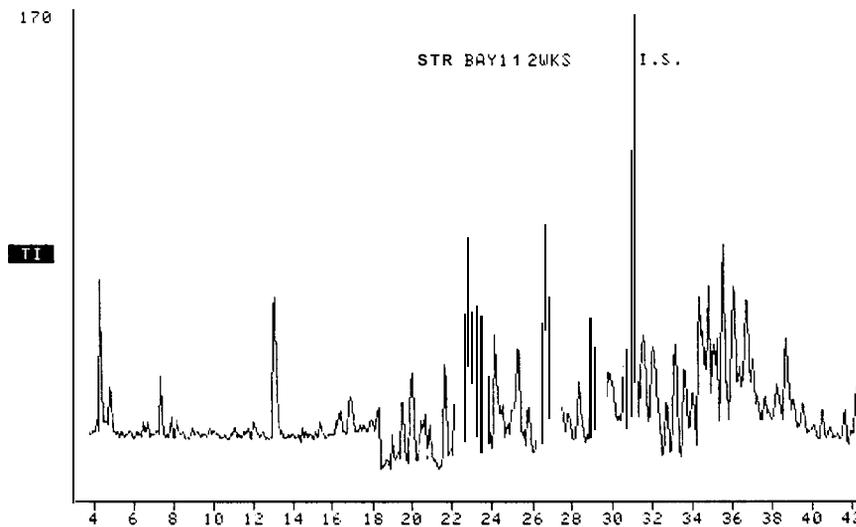


Figure 3.28 GC²/MS total ion chromatograms of *Strongylocentrotus droebachiensis* tissues from urchin traps at 5 m depth in three bays

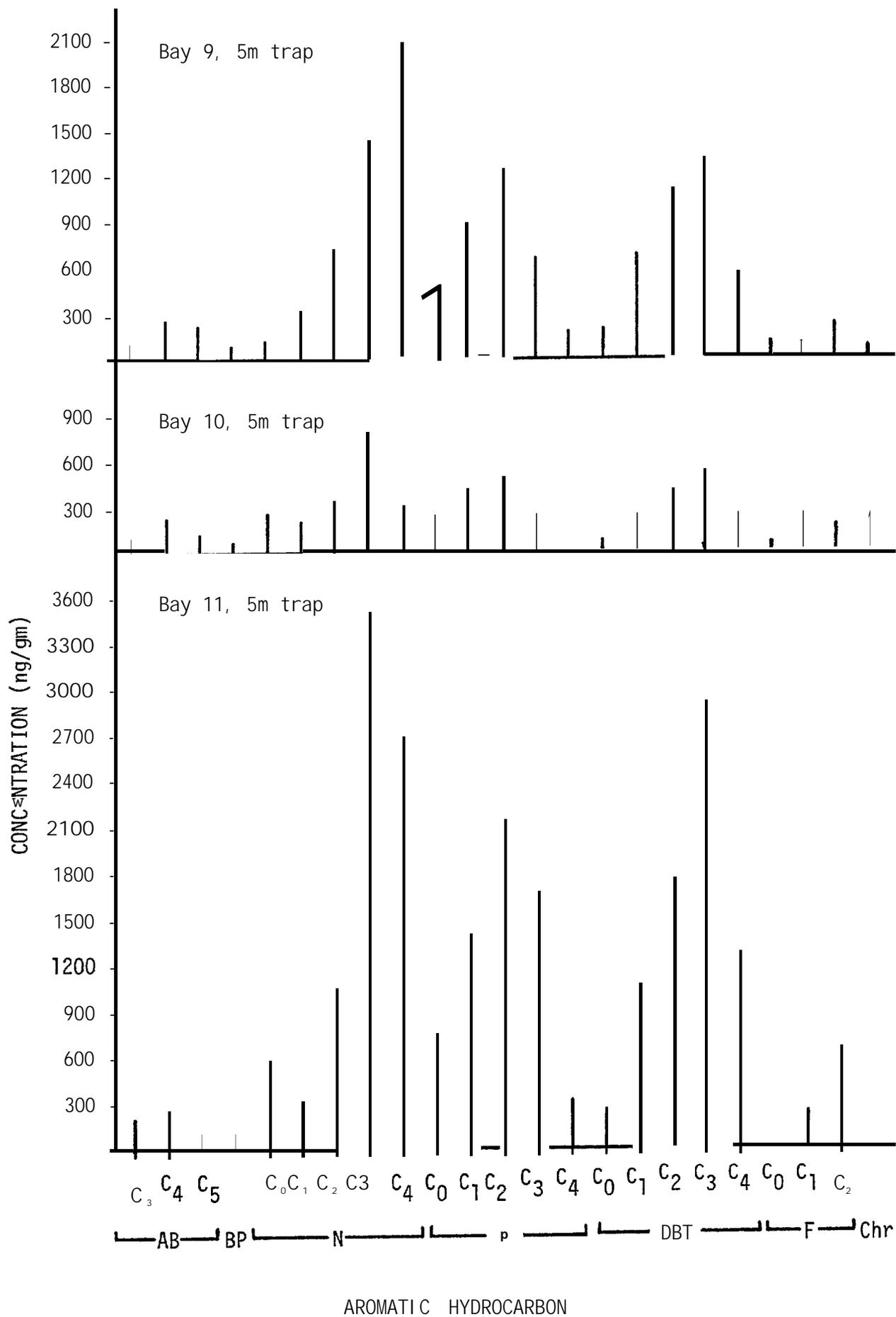


Figure 3.29 Aromatic profiles in *Strongylocentrotus droebachiensis*, 2nd post-spill sample

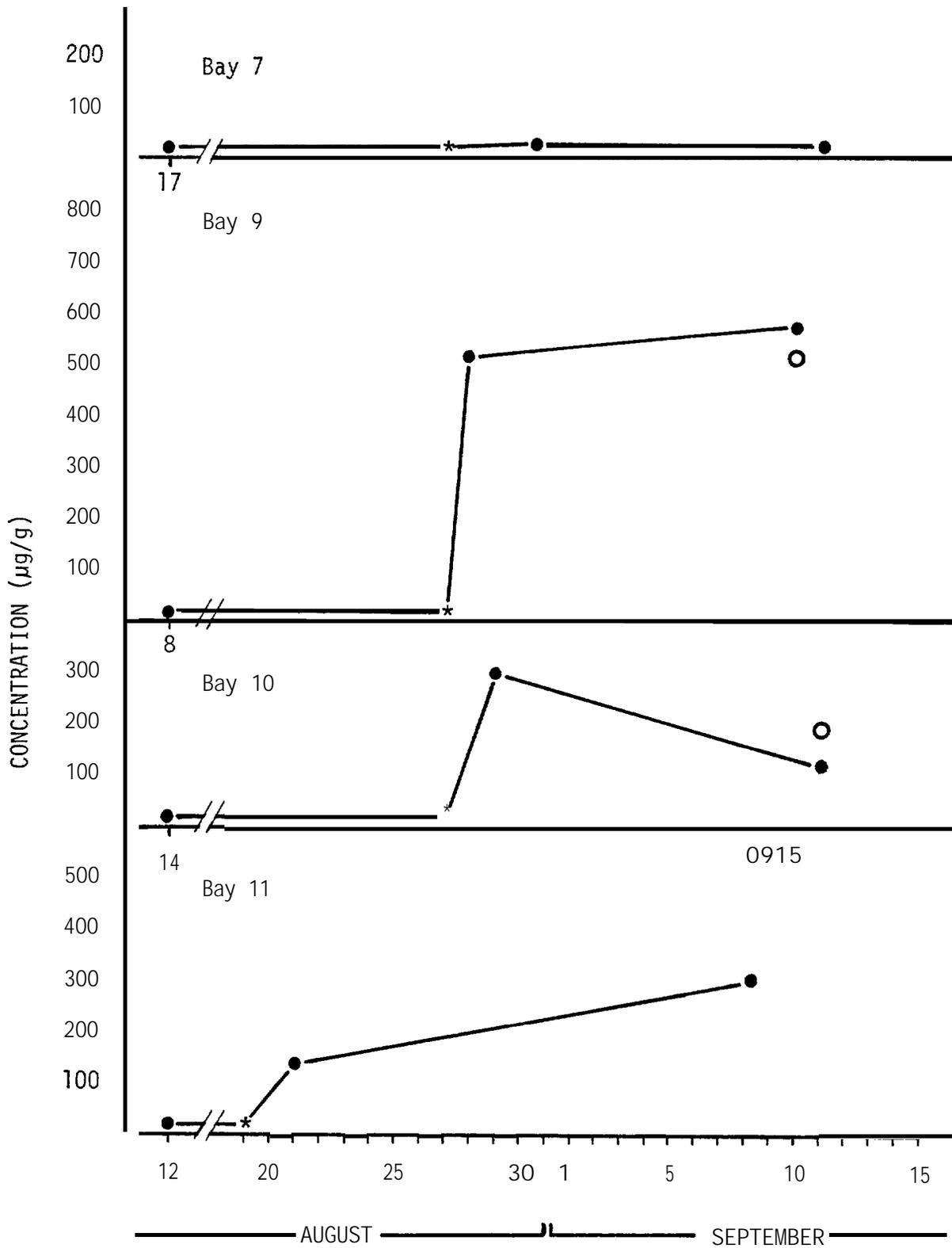


Figure 3.30 Trends in oil concentration in Strongylocentrotus droebachiensis, expressed as o-terphenyl equivalents (µg/g dry weight), (*, day of spill; ○, 5m trap sample)

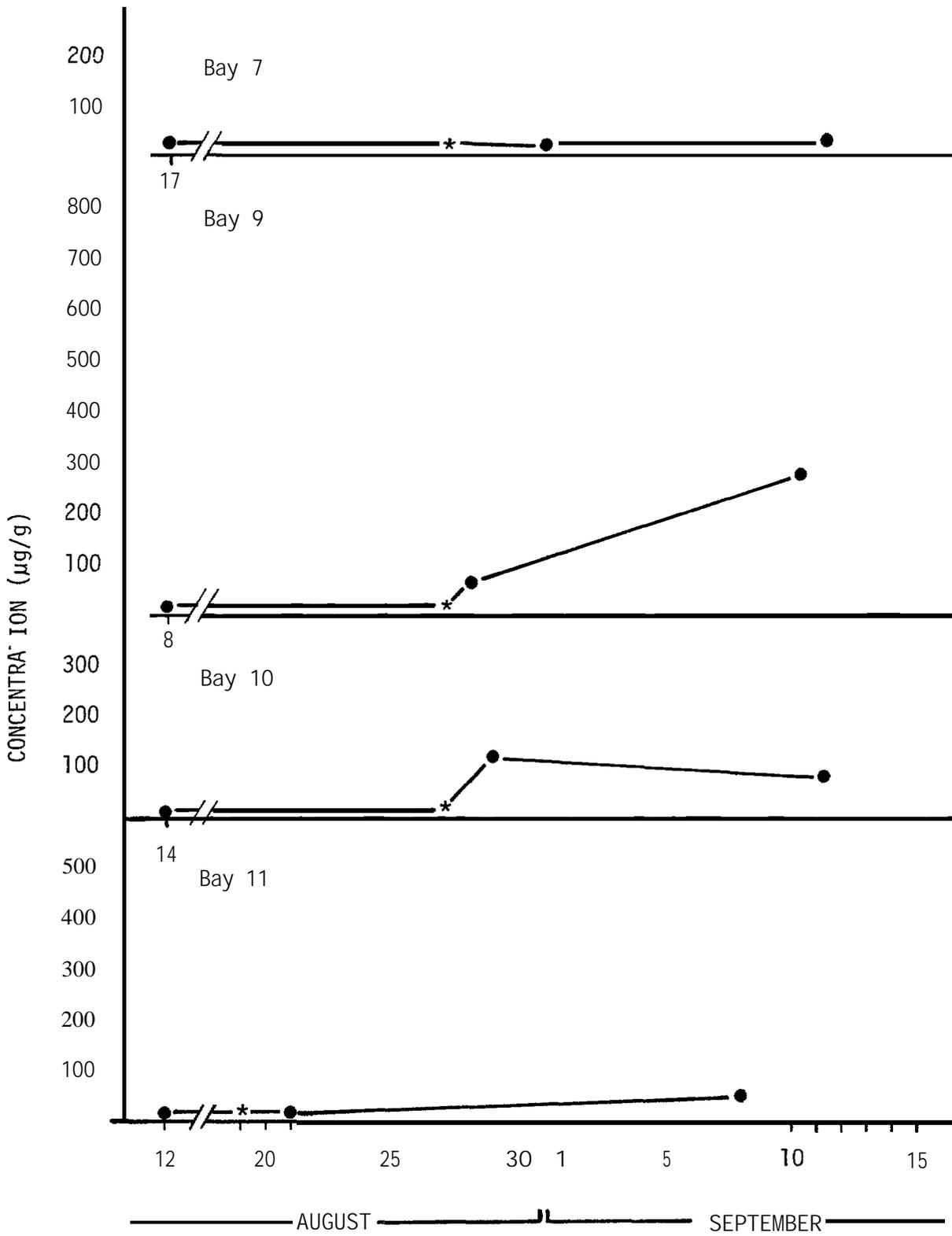
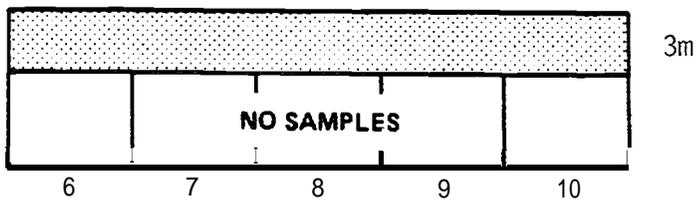
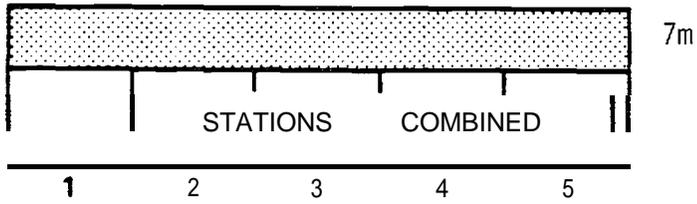


Figure 3.31 Trends in oil concentration in *Pectinaria granulosa*, expressed as oil equivalents (µg/g dry weight), by UV/F (*, day of spill)

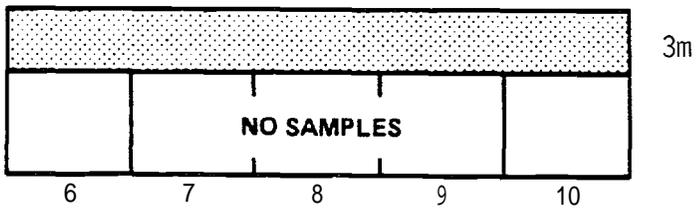
**BENTHIC
TRANSECT
TISSUE
PLOTS**



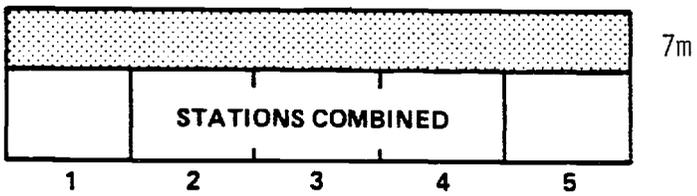
PRESPILL
7-9 Aug.



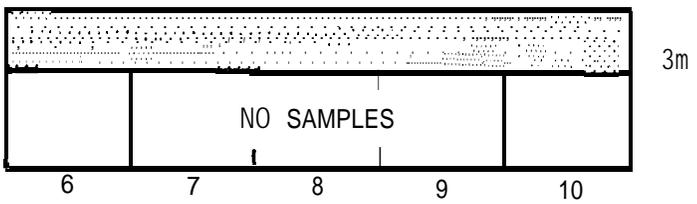
4.7



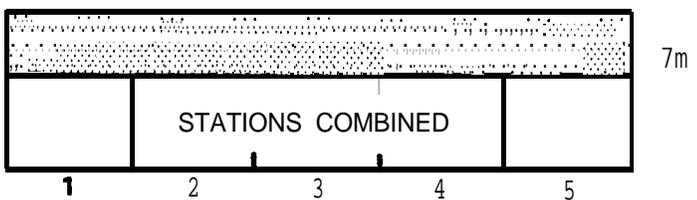
FIRST POSTSPILL
28 Aug. (1d)



64.5



SECOND POSTSPILL
10 Sept. (14d)



270.7

Figure 3.32 Concentrations of oil in Pectinaria granulosa from Bay 9, by UV/F (ug/g dry weight)

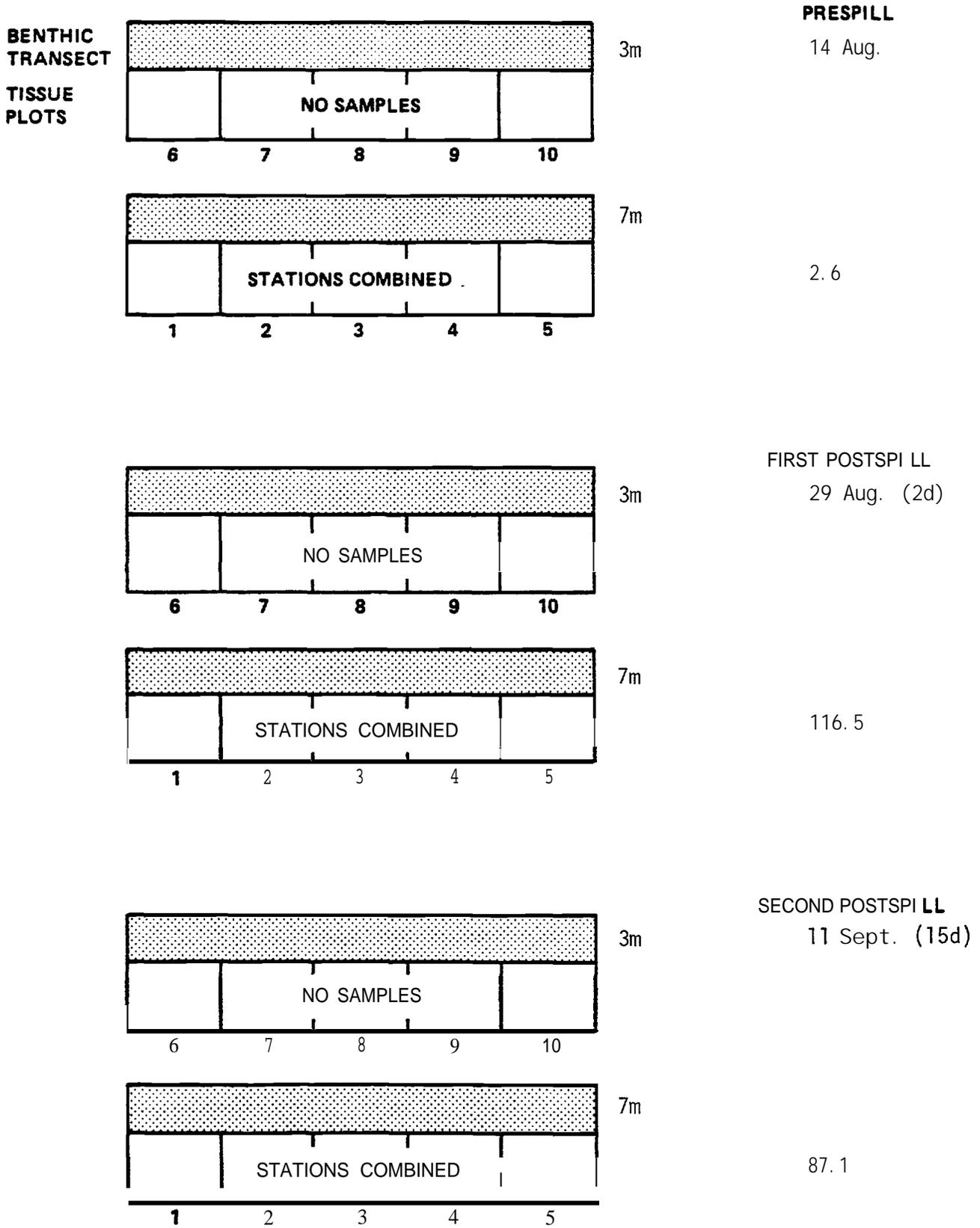
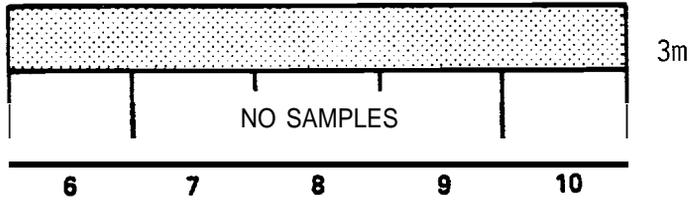


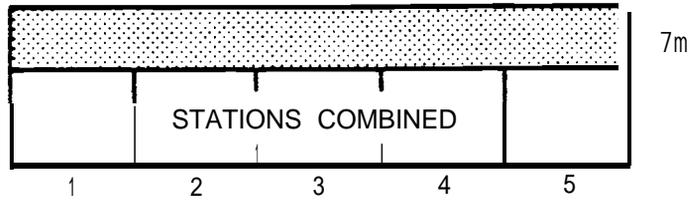
Figure 3.33 Concentrations of oil in *Pectinaria granulosa* from Bay 10, by UV/F (ug/g dry weight)

**BENTHIC
TRANSECT**
TISSUE
PLOTS

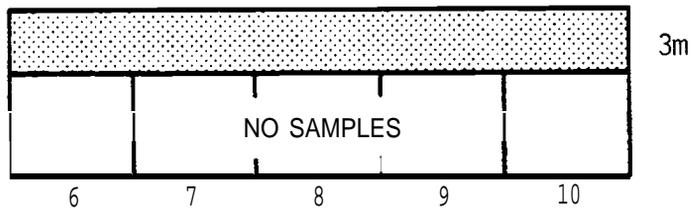


PRESPILL

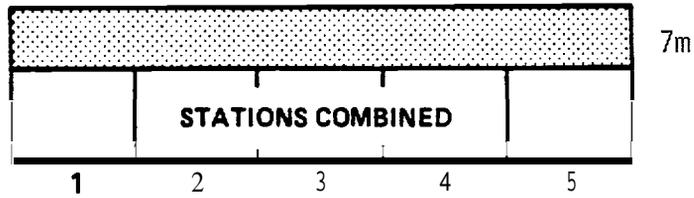
17 Aug.



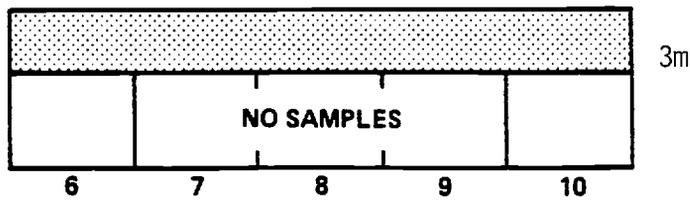
17.7



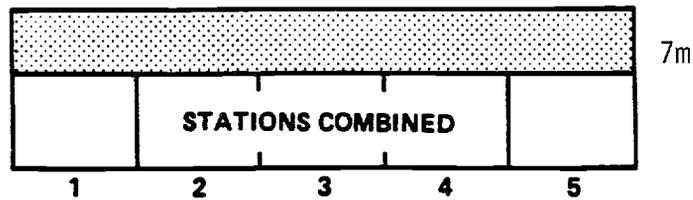
FIRST POSTSPILL
31 Aug. (4d)



12.6



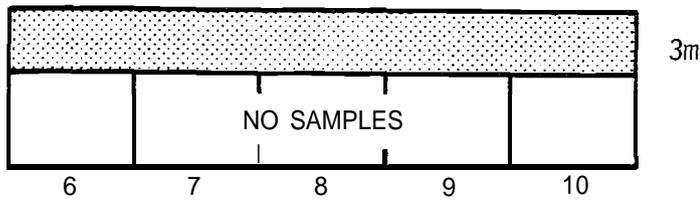
SECOND POSTSPILL
11 Sept. (15d)



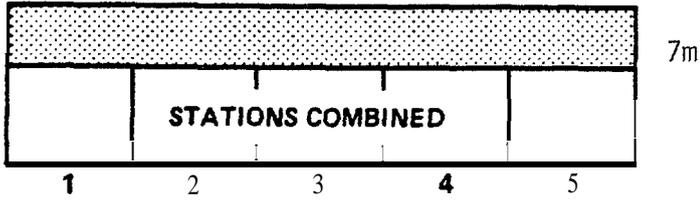
17.1

Figure 3.34 Concentrations of oil in Pectinaria granulosa from Bay 7, by UV/F (ug/g dry weight)

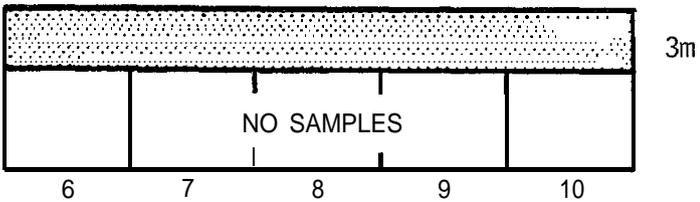
**BENTHIC
TRANSECT**
**TISSUE
PLOTS**



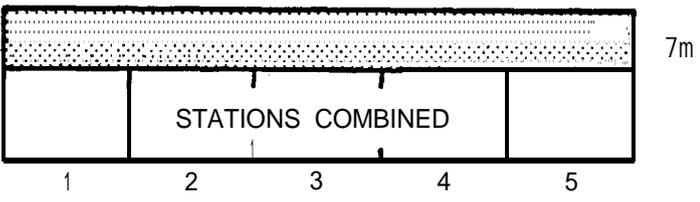
PRESPILL
12 Aug.



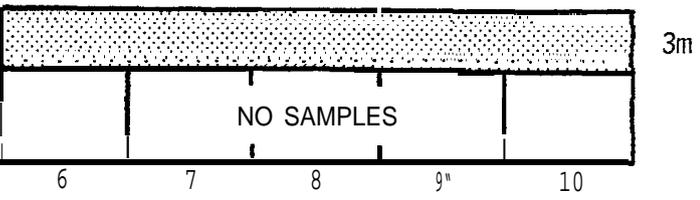
6.6



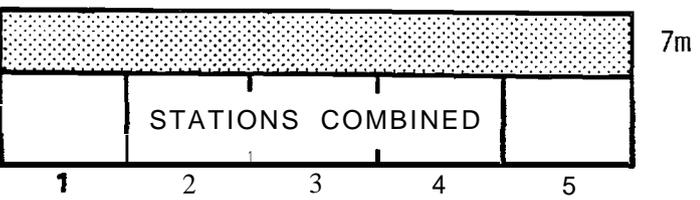
FIRST POSTSPILL
21 Aug. (2d)



6.8



SECOND POSTSPILL
8 Sept. (20d)



66.7

Figure 3.35 Concentrations of oil in Pectinaria granulosa from Bay 11, by UV/F (ug/g dry weight)

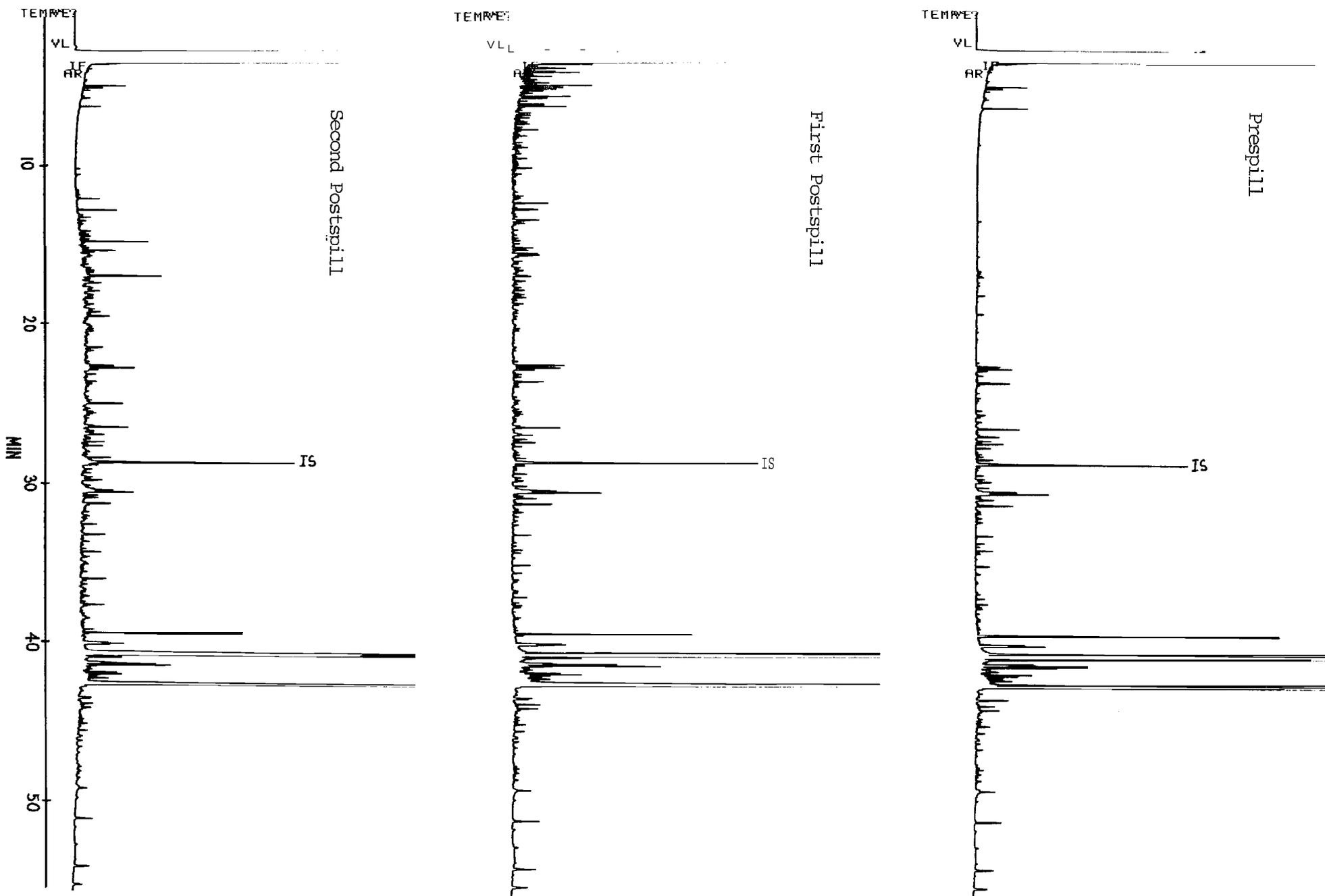


Figure 3.36 GC²/FID chromatograms of Pectinaria granulosa tissues from Bay 9

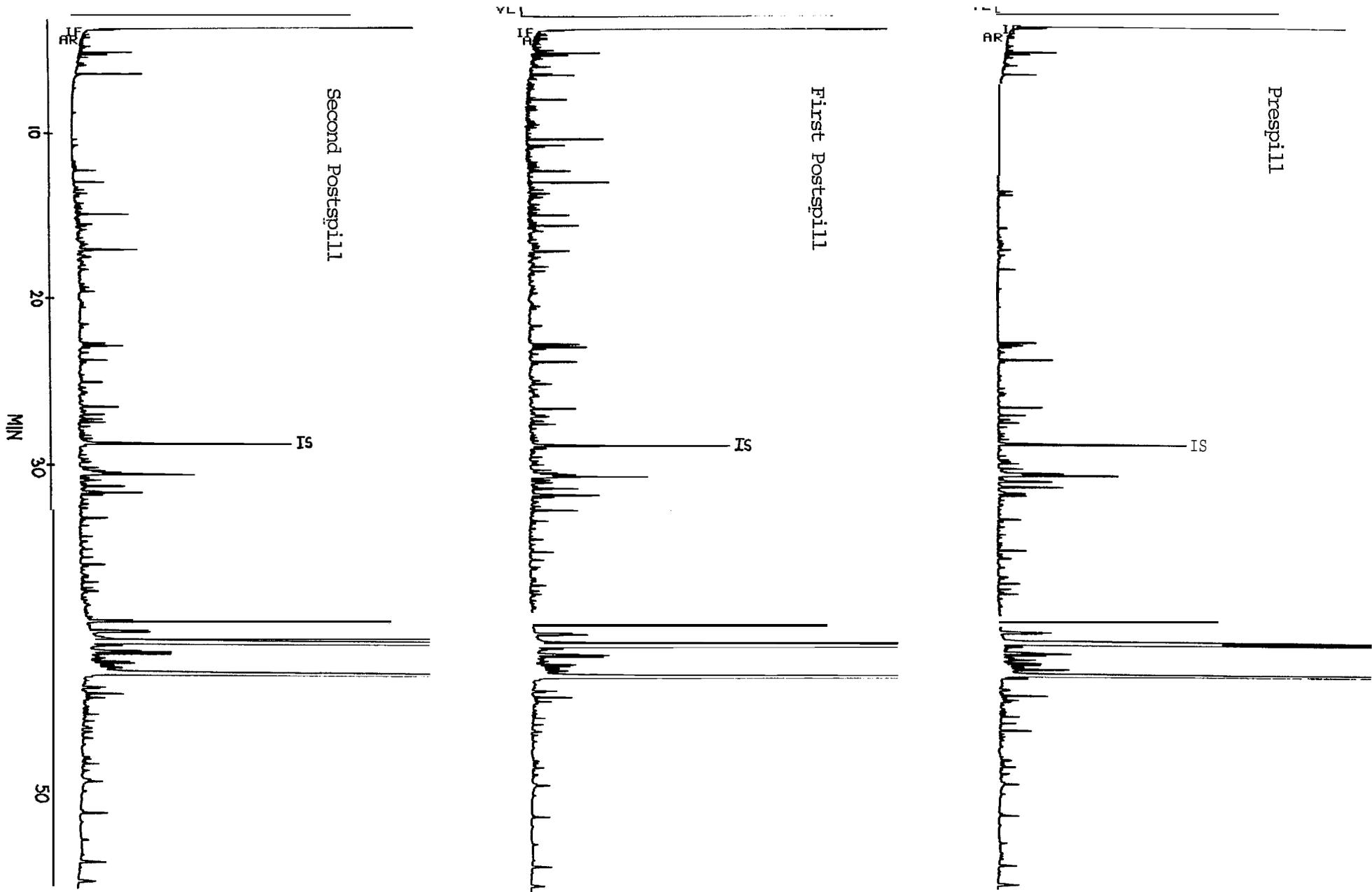


Figure 3.37 GC²/FID chromatograms of Pectinaria granulosa tissues from Bay 10

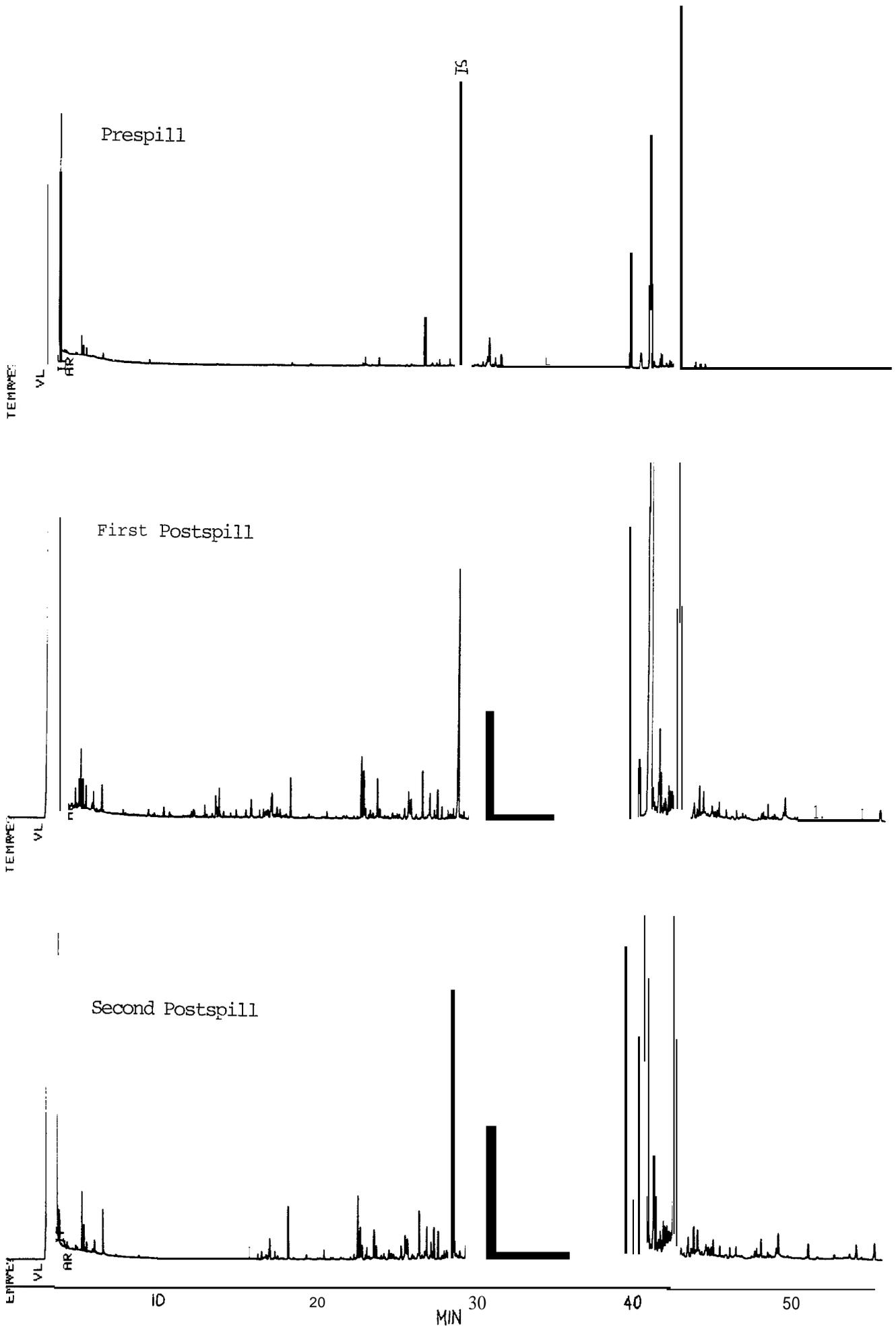


Figure 3.38 GC²/FID chromatograms of Pectinaria granulosa tissues from Bay 7

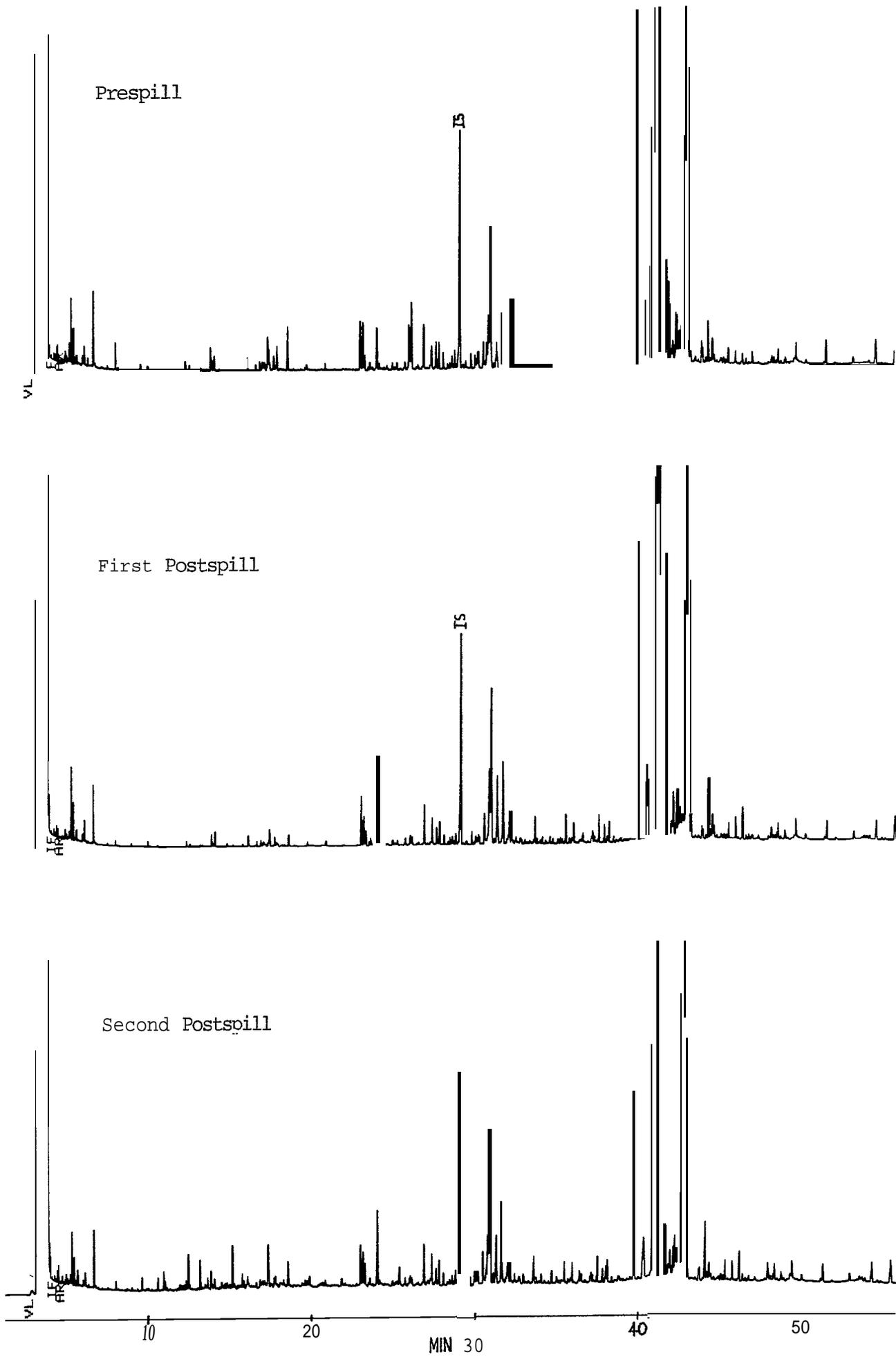


Figure 3.39 GC²/FID chromatograms of Pectinaria granulosa tissues from Bay 11

Levels of pristane were low (1-2 ug/g) , less than in urchins, and there was no discernible difference among bays (Table 3.3) . Generally, phytane levels increased from trace levels found in pre- and first post-spill tissue samples. A general effect was a decrease in the calculated pristane/phytane ratios.

Although there were not quantitated, it is interesting to note the behaviour of the iso-alkanes in the C12-C15 region of the chromatograms. By the second time period post-spill, iso-alkanes with retention indices 1273, 1377 and 1462 (farnesane) were distinct peaks in the chromatograms of the polychaetes from Bays 9, 10 and 11. Farnesane in particular was more abundant relative to phytane in these samples than it was in the oil. Small peaks in the C12-C15 region were also found for urchins (Figures 3.10 to 3.15) but there was no evidence for preferential accumulation of linear molecular weight iso-alkanes in this species as is suggested for the polychaetes.

3.2.2.3 GC²/SIM

Aromatic hydrocarbon profiles of polychaete tissues from Bay 9 showed a proportional emphasis on lower molecular weight aromatics in 1 day post-spill samples, shifting to an emphasis on larger molecular weight aromatics by 14 days post-spill (Figure 3.40) . The relationships are presented in detail in SIM assessments, as shown in Figures 3.41 to 3.45. This set of chromatograms for SIM data is qualitatively representative of all polychaete and urchin samples analyzed for aromatic composition by mass spectrometry.

Bay 9 polychaete tissues showed high levels of C3- and C4- alkyl benzenes and C1-, C2- and C3- naphthalenes approaching 2000 ng/g in the first post-spill samples (Figure 3.46) . Similarly high levels of C3- naphthalene and C2- phenanthrene were found in second post-spill tissues. The polymethylated phenanthrenes and dibenzthiophenes were markedly enhanced in the later sample.

Polychaete tissues from Bay 10 had an aromatic profile similar to that in Bay 9, but were of lower concentration (Figures 3.47 and 3.48) . Bay 7 tissues showed no conclusive trend for alterations in aromatic composition or concentration, in agreement with the UV/F data (Figures 3.49 and 3.46) .

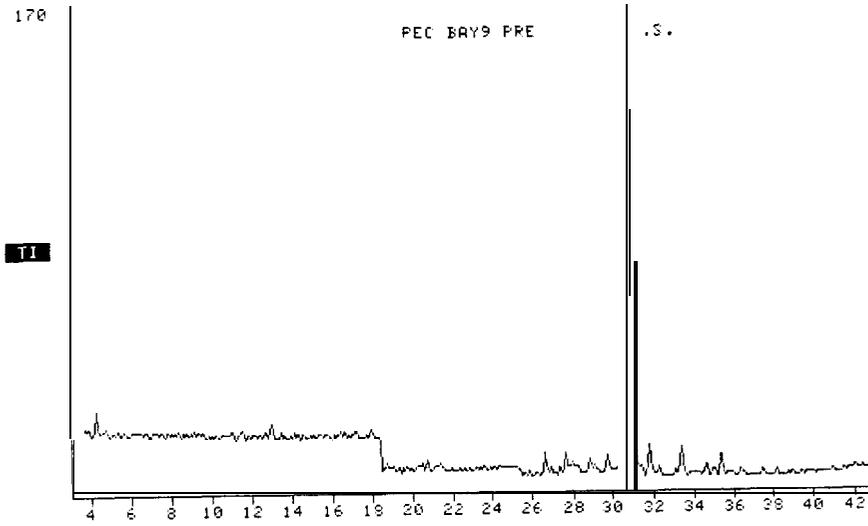
TABLE 3.3 Pristane and Phytane levels (ug/g dry weight) and ratios in Pectinaria granulosa

Site	Tire Re Spill	No. Individ. in Pool	Pristane	Phytane	Pristane- Phytane Ratio
Bay 9-7In	pre	50	1.24	<0.20	>6.2
	1st	48	1.54	<0.20	>7.7
	2nd	33	2.28	1.77	1.29
Bay 10-7m	pre	134	0.87	<0.20	>4.5
	1st	199	2.25	0.87	2.57
	2nd	37	1.61	0.85	1.89
Bay 7-7m	pre	9	1.36	<0.20	>6.8
	1st	90	1.80	<0.20	>9.0
	2nd	39	0.68	<0.20	>3.4
Bay 11-7m	pre	108	1.81	0.21	8.62
	1st	82	0.80	<0.2(3	>4.0
	2nd	66	1.36	0.74	1.84

¹Percent water in soft tissue, 73.0±4.9

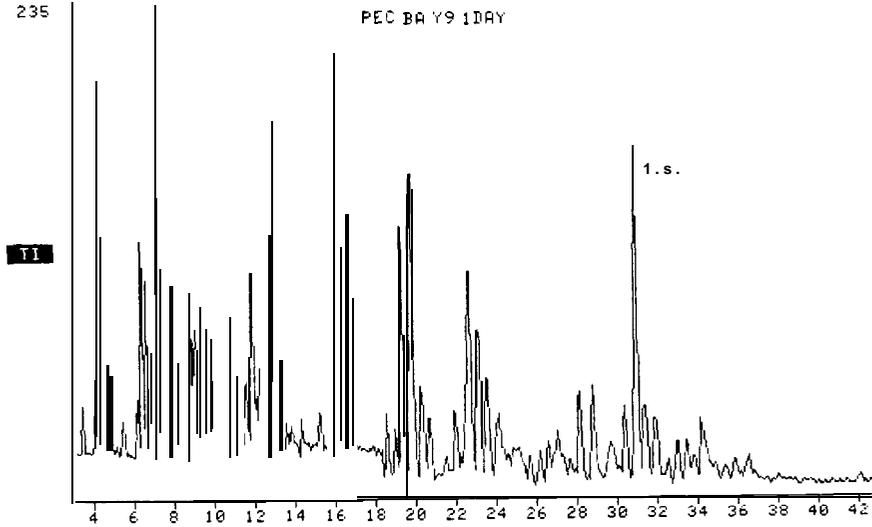
NAME PEC,BAY9,PRE,3MIC.L.FROM 0.5 ML
MISC EI,51M,3800V,T-025,MARCH3/82

FRN 11106



NAME PEC,BAY9,1DAY,3MIC.L.FROM 0.5 ML
MISC EI,51M,3800V,T-356,MARCH3/82

FRN 11106



NAME PEC,BAY9,2WKS,3MIC.L.FROM 0.5 ML
MISC EI,51M,2800V,T-755,7.86MC,MARCH3/82

FRN 11107

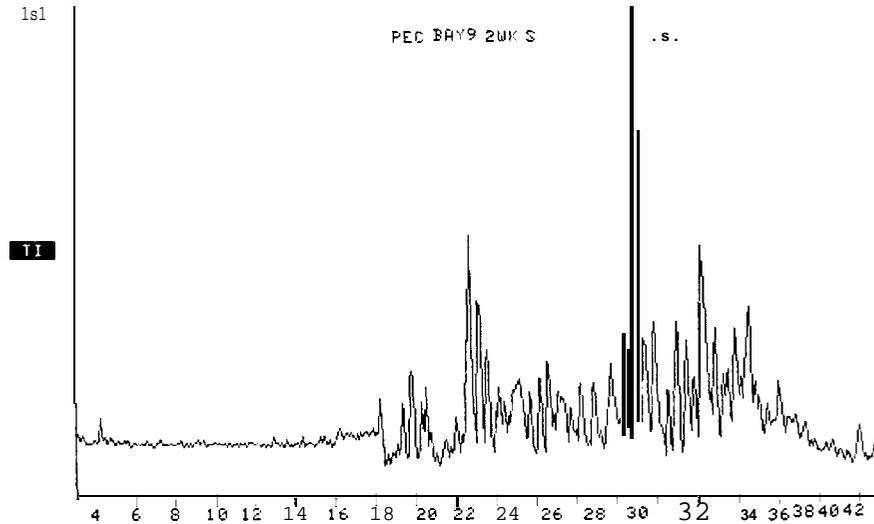
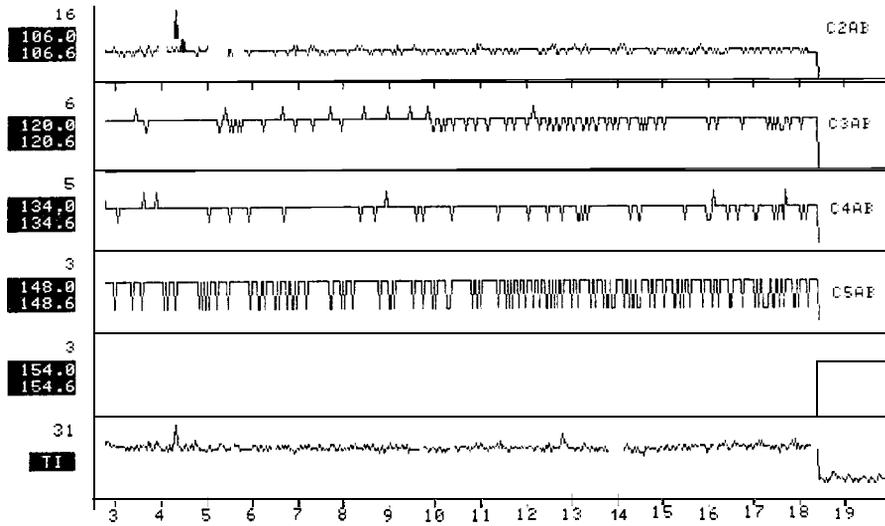
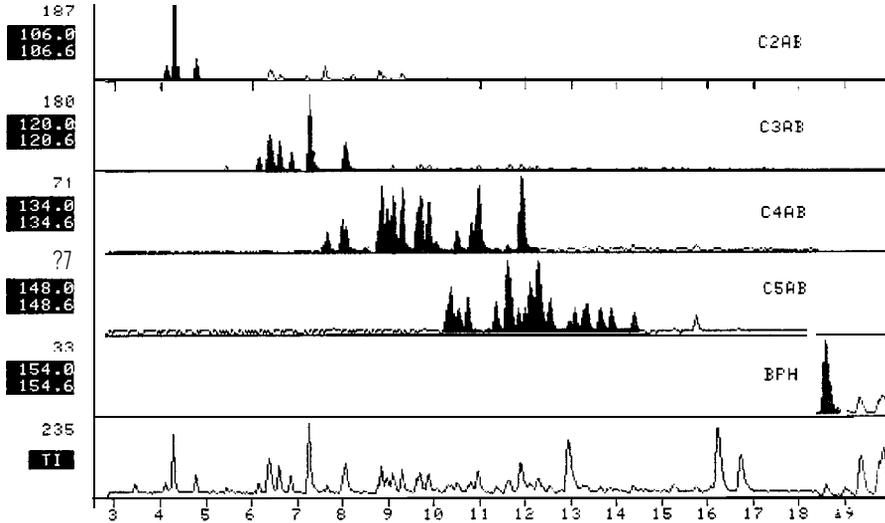


Figure 3.40 GC²/MS total ion chromatograms of Pectinaria granulosa tissues from Bay 9

NAME PEC, BAY9, PRE, 3MIC.L.FROM 0.5 ML Prespill FRN 11108
MISC EI, SIM, 2800V, T-025, MARCH3/82



NAME PEC, BAY9, 1DAY, 3MIC.L.FROM 0.5 ML First Postspill FRN 1:
MISC EI, SIM, 2800V, T-356, MARCH3/82



NAME PEC, BAY9, 2WKS, 3MIC.L.FROM 0.5 ML Second Postspill FRN 11107
MISC EI, SIM, 2800V, T-755, 7.8GMS, MARCH3-82

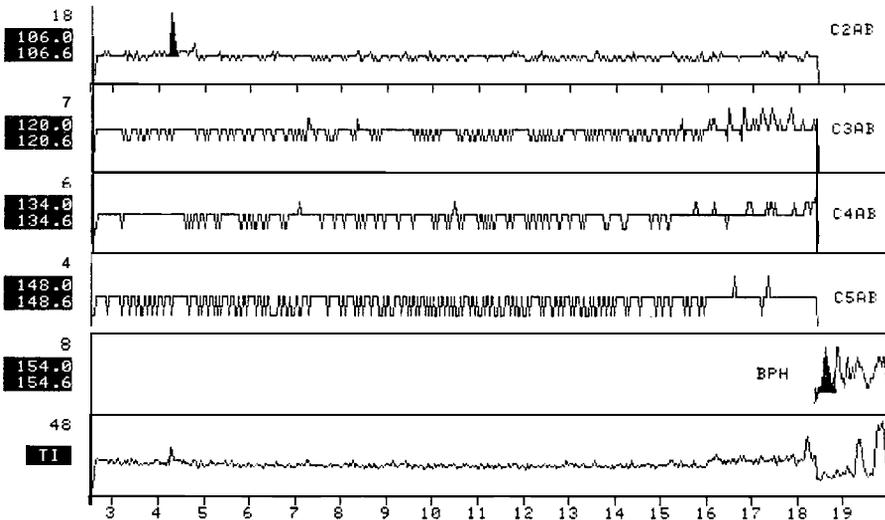


Figure 3.41 GC²/SIM chromatograms showing benzenes and biphenyl of *Pectinaria granulosa* tissues from Bay 9

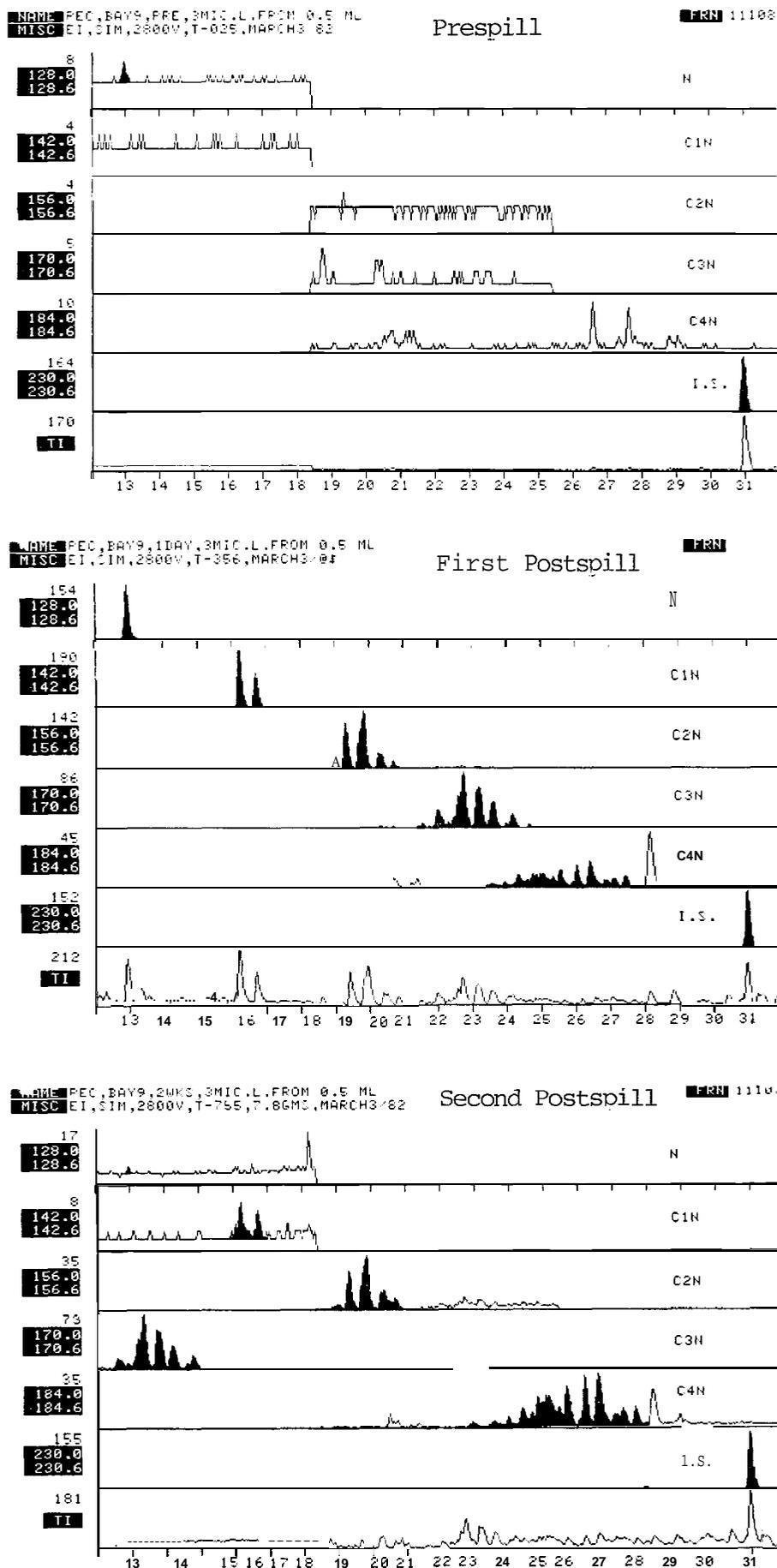


Figure 3.42 GC²/SIM chromatograms showing naphthalenes of *Pectinaria granulosa* tissues from Bay 9

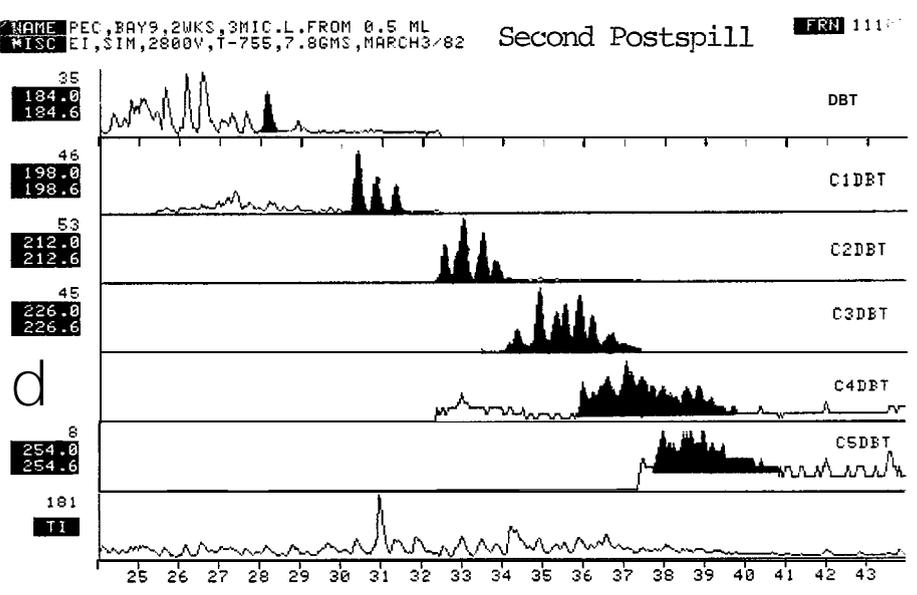
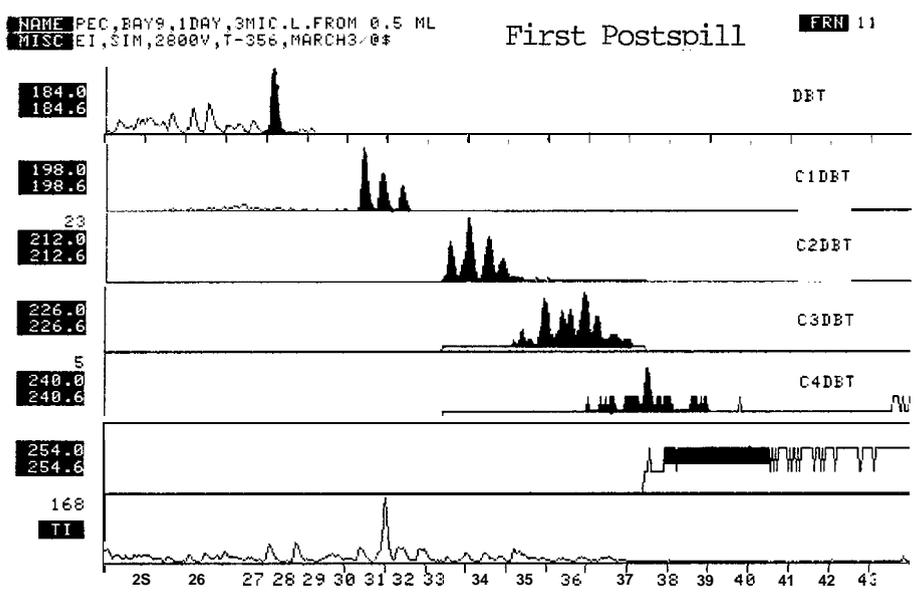
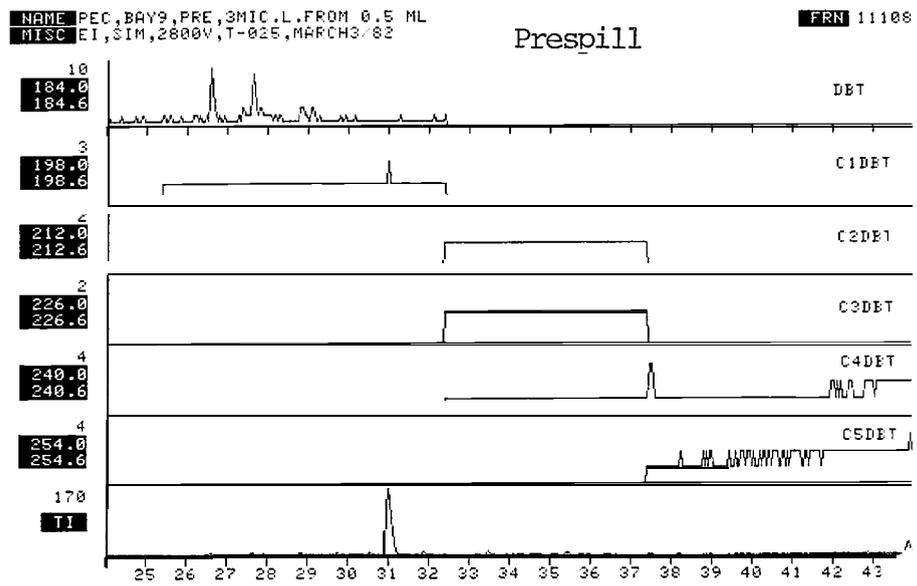


Figure 3.43 GC²/SIM chromatograms showing dibenzthiophenes of Pectinaria granulosa tissues from Bay 9

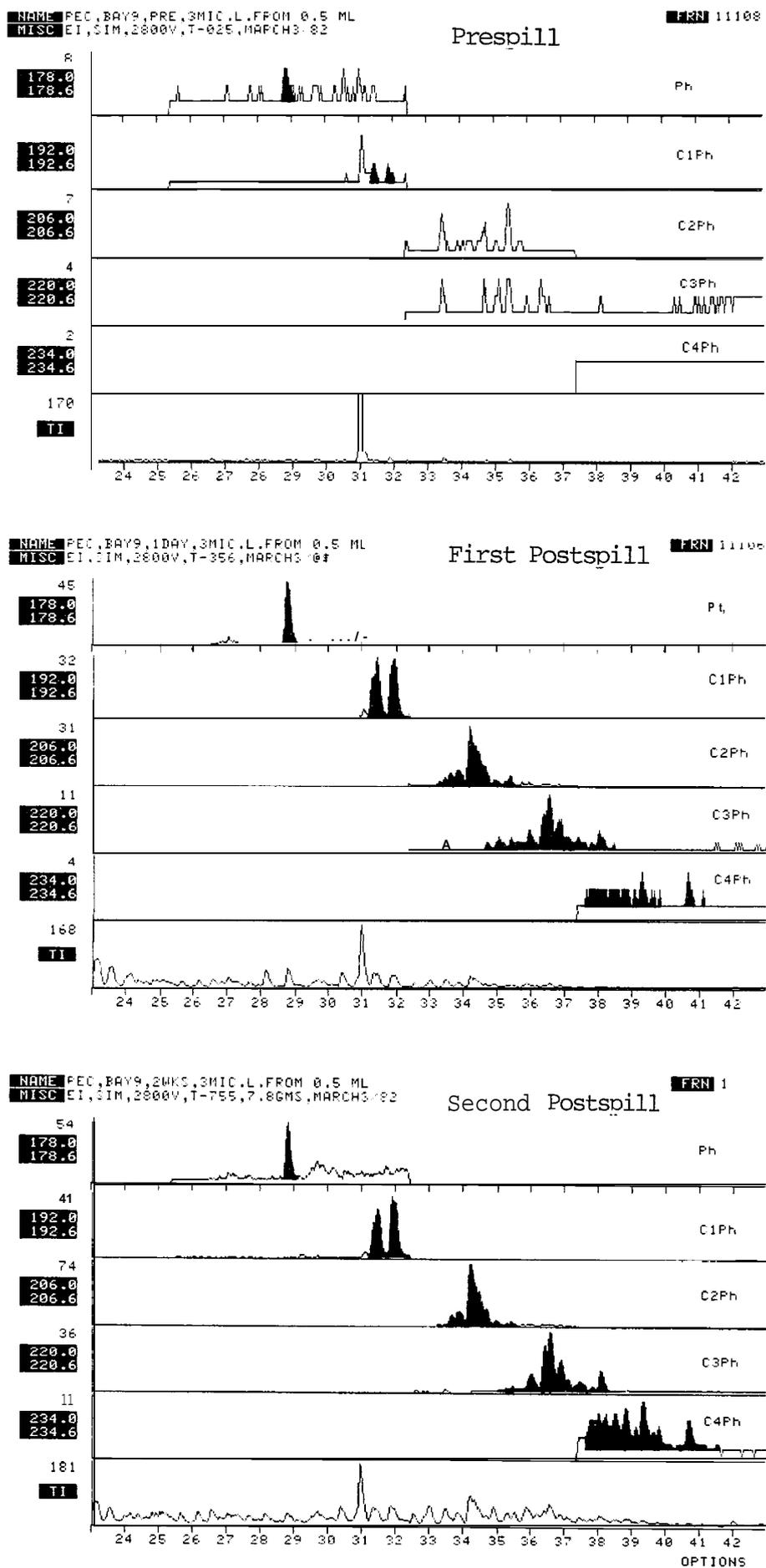
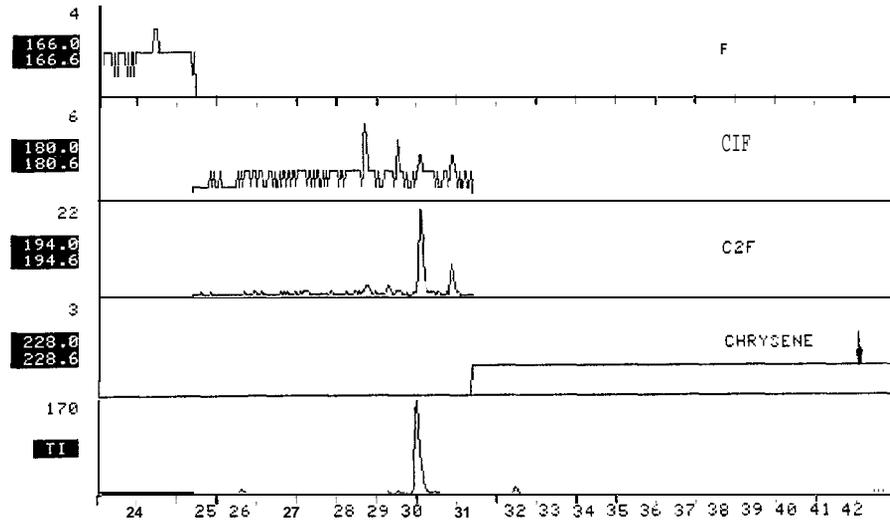


Figure 3.44 GC²/SIM chromatograms showing phenanthrenes of *Pectinaria granulosa* tissues from Bay 9

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MISC EI, SIM, 2800V, T-025, MARCH3/82

Prespill

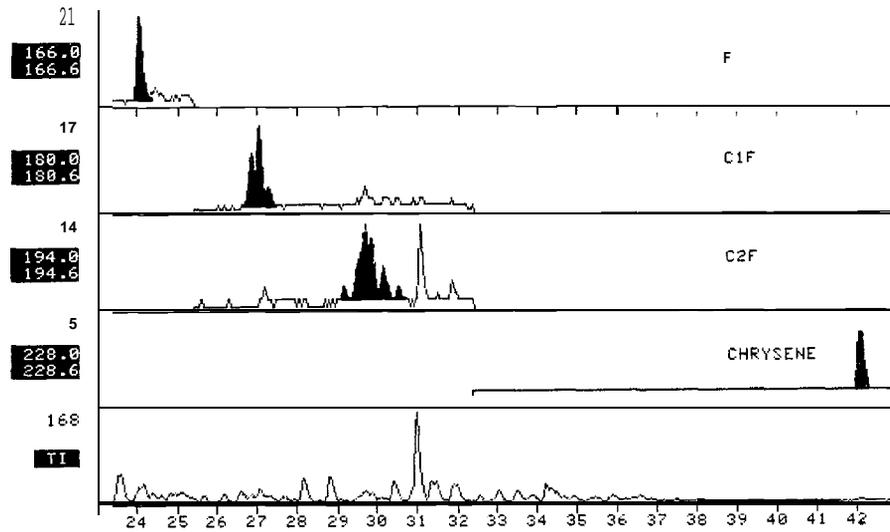
FRN 11108



PEC, BAY9, 1DAY, 3MIC.L.FROM 0.5 ML
EI, SIM, 2800V, T-356, MARCH3/82

First Postspill

FRN



NAME PEC, BAY9, 2WKS, 3MIC.L.FROM 0.5 ML
MISC EI, SIM, 2800V, T-755, 7.8 GMS, MARCH3/82

Second Postspill

FRN 11107

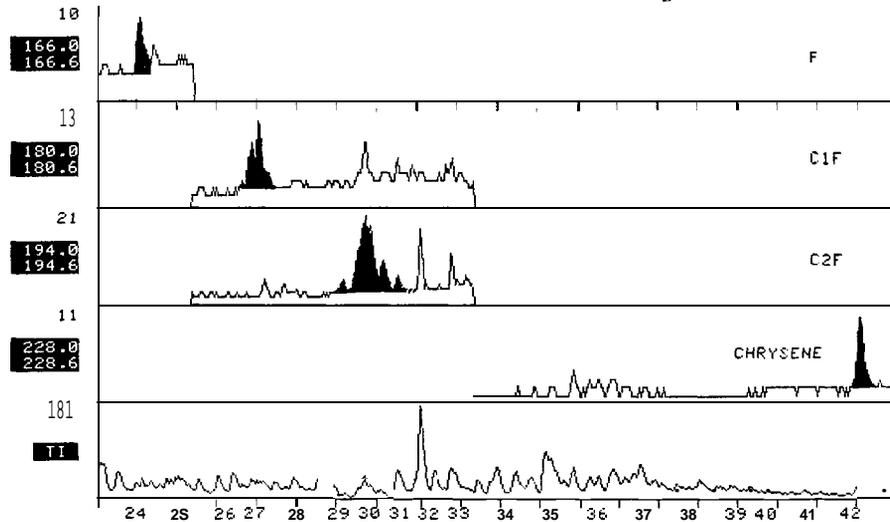


Figure 3.45 GC²/SIM chromatographs showing fluorenes and chrysene of *Pectinaria granulosa* tissues from Bay 9

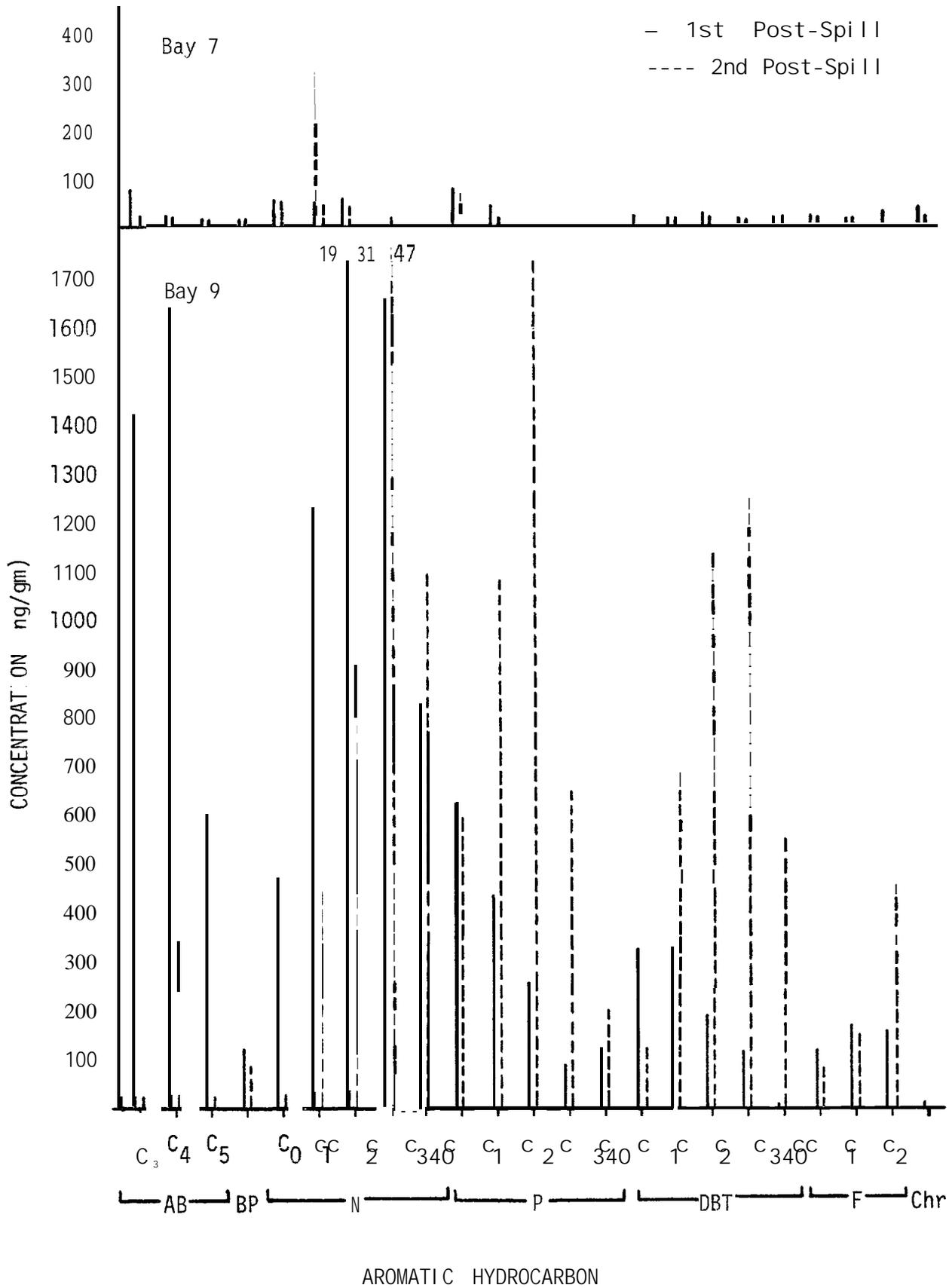
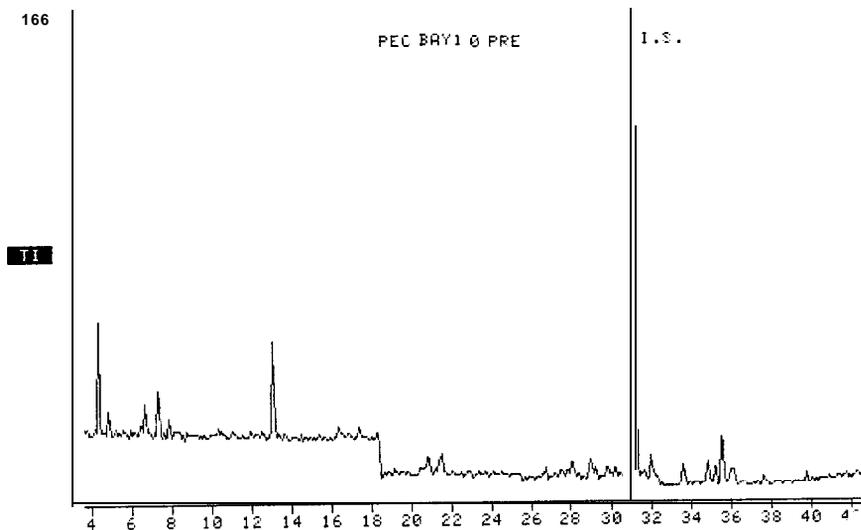


Figure 3.46 Aromatic profiles in Pectinaria granulosa

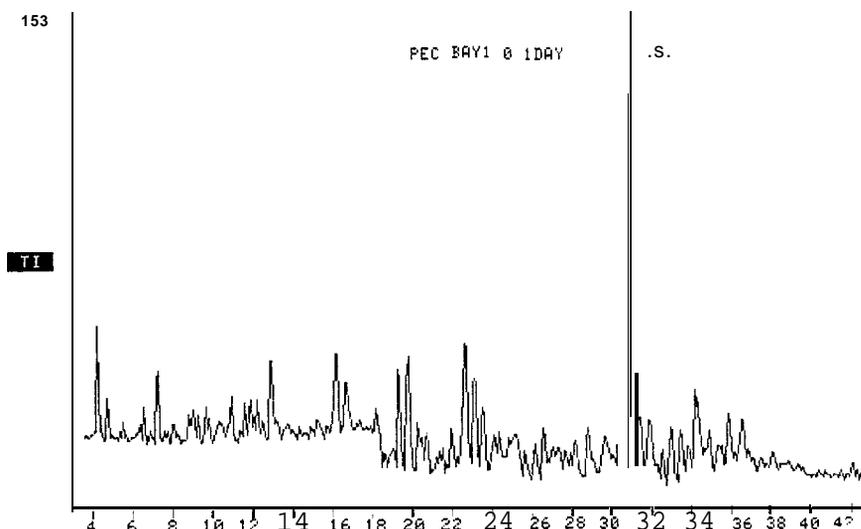
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MISC EI, SIM, 2800V, T-165, MARCH5/82

FRN 12006



NAME PEC, BAY10, 1DAY, 3MICL, FROM 0.5 ML
MISC EI, SIM, 2800V, T-519, MARCH3/82

FRN 11114



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MISC EI, SIM, 2800V, T-780, MARCH3/82

FRN 11002

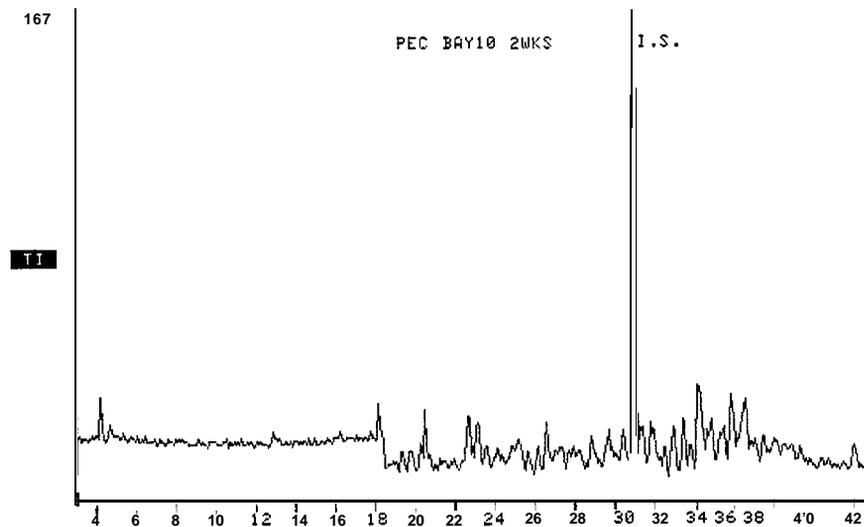
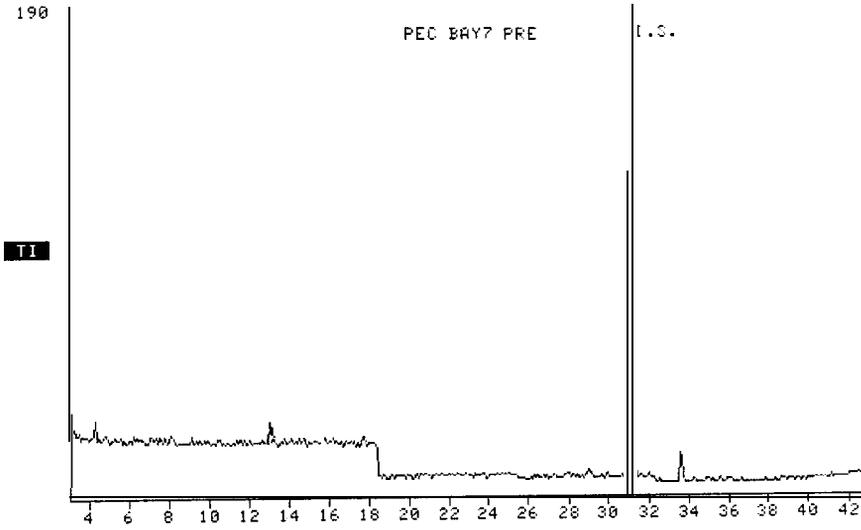


Figure 3.47 GC²/MS total ion chromatograms of Pectinaria granulosa tissues from Bay 10

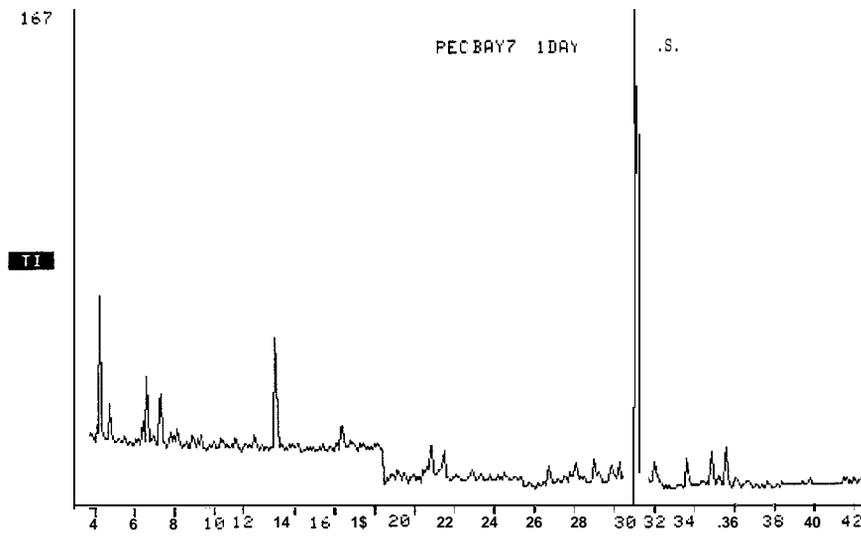
NAME PEC,BAY7,PRE,0.55G,3MICL.FROM 0.5ML
MISC EI,SIM,2800V,T-250,MARCH5/82

FRN 12005



NAME PEC,BAY7,1DAY,3MIC.L.FROM 0.5 ML
MISC EI,SIM,2800V,T-551,MARCH5/82

FRN 12003



NAME PEC,BAY7,2WKS,7.8G,3MIC.L.FROM 0.5ML
MISC EI,SIM,2800V,T-733,MARCH5/82

FRN 12004

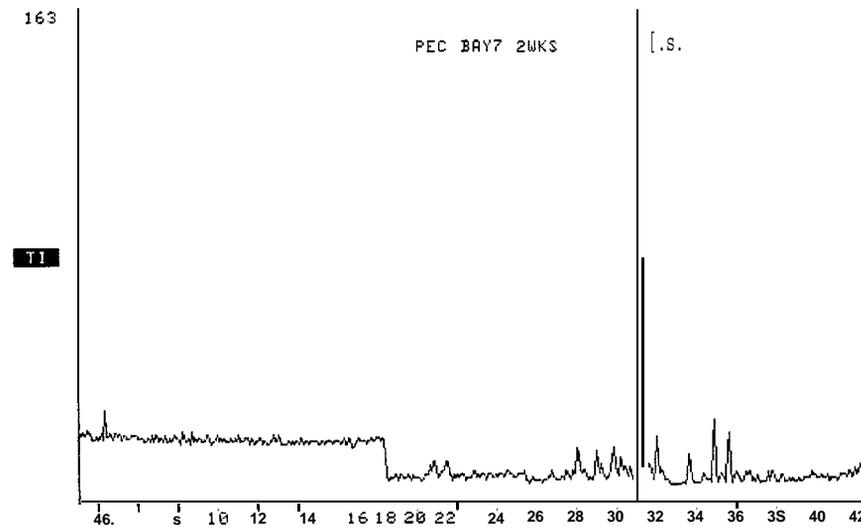


Figure 3.49 GC²/MS total ion chromatograms in Pectinaria granulosa tissues from Bay 7

Analysis of tissues from Bay 11 polychaetes demonstrated a relative emphasis on polymethylated and polynuclear aromatic hydrocarbons in the second post-spill sample (Figures 3.50 and 3.48).

A comparative calculation of total oil concentration using aromatic data was carried out for polychaete samples as well. Total tissue oil levels were greater when expressed on this basis (Figure 3.51) than on the basis of fluorescence (Figure 3.31). This was especially true for Bay 9 and Bay 10 post-spill samples, in particular Bay 9 first post-spill.

3.3 Laboratory InterCalibration

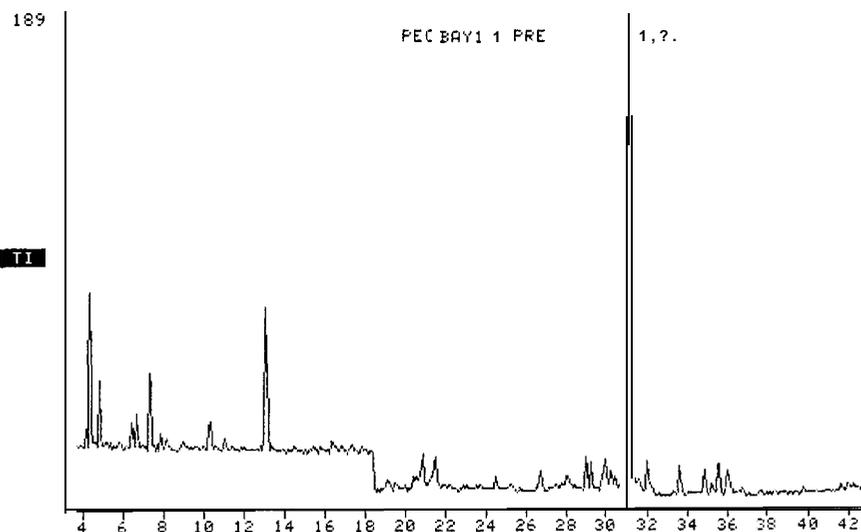
The results are shown in Tables 3.4 and 3.5. In all cases there was close agreement between oil equivalent concentrations derived from n-alkane (FID) and PAH (SIM) analysis. Average concentrations from GC² analysis of the oil spiked urchin and Mya tissues, as well as Lagomedio oil, were 23.4, 40.7 and 48.7 ug/ml respectively. This indicates reasonably good agreement with the presumed concentrations of total hydrocarbons in the unknown samples, taking into consideration the amount of the oil spike and background hydrocarbon concentrations in the tissue extracts.

Oil concentrations determined by UV/F agree in orders of magnitude with GC²-derived concentrations and with presumed background oil levels in the tissues prior to spiking. They are, however, less consistent in an absolute sense. Oil spill-derived urchin samples were under-estimated, similar to the situation described previously in Section 3.2.

The unknown Mya extract concentration was probably over-estimated by UV/F, although there was close accord in the oil-only sample concentration.

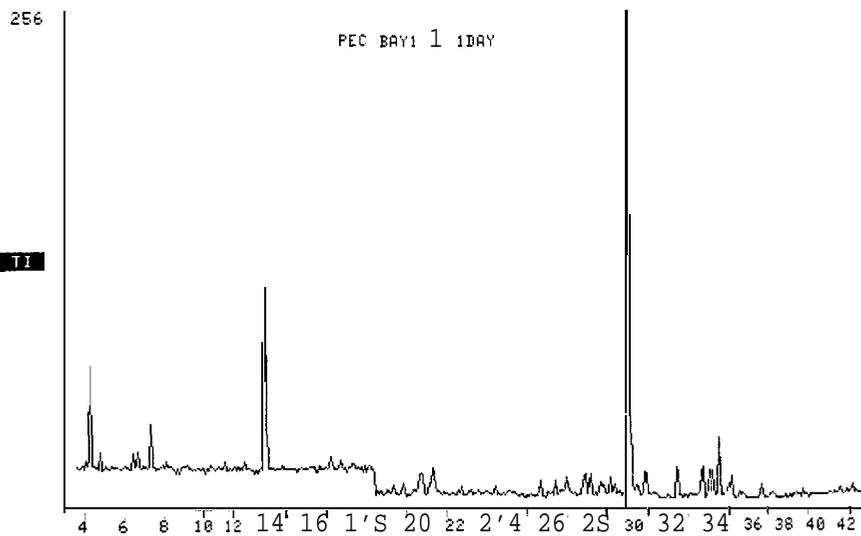
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FRN 12002



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MISC EI, SIM, 2800V, T-278, MARCH4 '82

FRN 12000



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MISC EI, SIM, 2800V, T-803, MARCH4 '82

FRN 12001

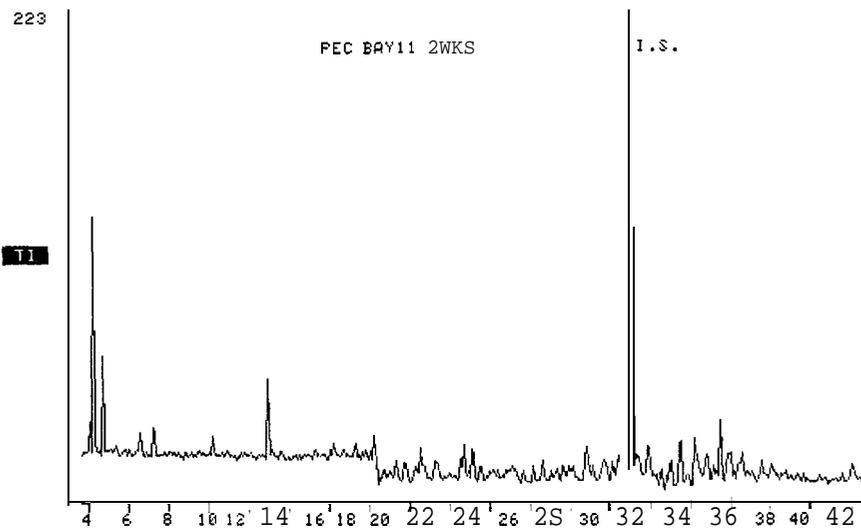


Figure 3.50 GC²/SIM total ion chromatograms in Pectinaria granulosa tissues from Bay 11

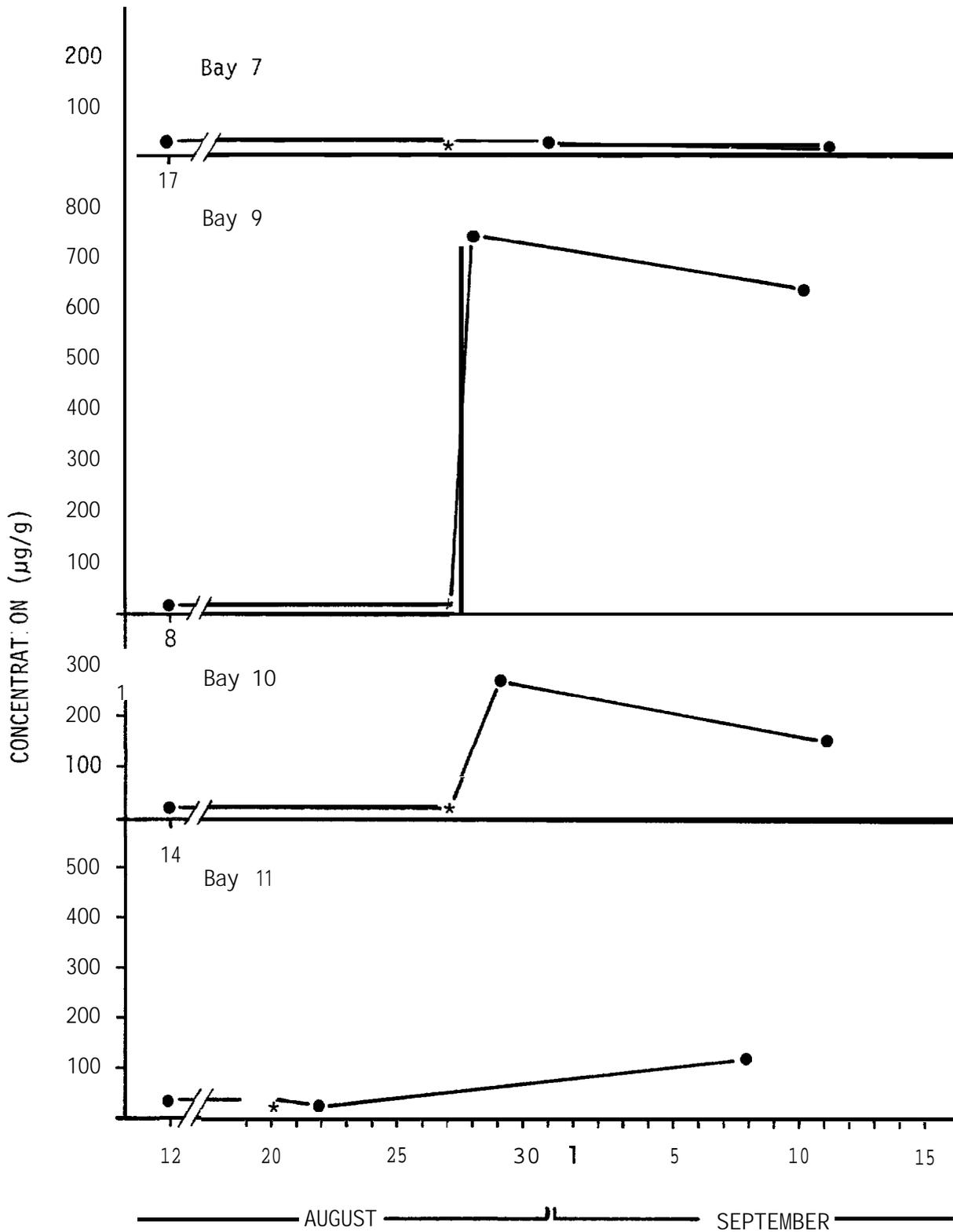


Figure 3.51 Trends in oil concentration in *Pectinaria granulosa*, expressed in o-terphenyl equivalents ($\mu\text{g/g}$ dry weight) (*, day of spill)

Table 3.4 Results of CWS/DIAND analysis of BIOS intercalibration samples, oil equivalent concentrations.

Unknown ¹ Sample	Amt. Oil Added (ug/ml)	Duplicate No.	Androstane Recovery (%)	Concentration of Oil in ug/ml ² calculated from		
				Alkanes ³	PAH ⁴	UV/F analysis ⁵
1	0	1			299	93.1
2	20.7	1	86.9	23.8	23.6 ± 2.5	38.8
		2	86.7	23.3	22.8 ± 2.8	39.0
3	29.2	1	98.4	44.8	38.8 ± 1.4	75.2
		2	107.3	42.7	36.6 ± 7.4	72.0
4	34.1	1	88.7	42.9	50.8 ± 3.9	31.7
		2	96.9	47.2	48.8 ± 2.8	33.7

¹Sample 1 - urchin homogenate, Bay 10 first post-spill, 0.2 g dry weight/ml.
 2 - urchin extract, pre-spill spiked, 0.60 cr. net weight/ml
 3 - Mya extract, approx. 0.35 g-net weight/ml
 4 - Lagomedio oil in solvent

²Except for sample 1, where concentration is ug/g dry weight

³Based on sum of peak heights of C18 - C22 n-alkanes compared to oil standard by FID

⁴Based on areas of individual PAH (C1-C2-naphthalenes, phenanthrenes and dibenzthiophenes) by SIM, mean ±5.0

⁵Excitation 300 nm, emission 350 nm

SECTION 4 - DISCUSSION

4.1 Dispersed Oil Exposures

Both S. droebachiensis and P. granulosa evidenced a rapid uptake of Lagomedio oil into soft tissues following release of the dispersion. Animals taken from Bay 9, the site of the dispersed oil release, had higher concentrations than those from Bay 10 which received the dispersed oil by current diffusion within about one-half day. Green et al. (1982) report that Bay 9 oil levels were of the order of 300 to 400 ppm-hours, measured over 36-hours, while Bay 10 levels were about 30 ppm-hrs.

Bay 7 received appreciably less oil over the 36 hour time period, resulting in an exposure level of 0.5 ppm-hrs. This lower level is reflected in the low tissue hydrocarbon levels found in urchins and particularly in polychaetes from Bay 7.

A distinguishing characteristic of the tissue hydrocarbon load accumulated initially from dispersed oil is the relatively high concentration of low molecular weight hydrocarbons. The shift to larger molecular weight and polymethylated aromatics about two weeks after the spill suggests an enhanced loss of the former component, although total oil quantities showed only small or no decrease in the second post-spill samples. A continued increase in tissue levels is probable in Bay 9 urchins and polychaetes. This suggests continued accumulation from the substrate or from food supplies since water levels of hydrocarbons become negligible within two days time after the spill.

It is interesting to note that pristane levels in urchins from Bays 7 and 9 were twice as high as those from Bays 10 and 11: 22 ± 3 and 25 ± 3 as compared to 13 ± 1 and 10 ± 1 ppm, respectively, from the means of the pre-, first and second post-spill determinations. This points out that there is probably a difference in feeding ecology for this species in these two areas. There was no discernable difference between Bays in the case of polychaetes. Generally, phytane levels increased in both species from trace levels found in pre- and first post-spill, to ppm levels in the second post-spill sample.

At the second time post-spill, the phytane/n-C18 alkane ratio was higher in animal tissues than it had been previously, and as compared to that in the Lagomedio oil. This was especially true in the Bay 9 and 10 urchin trap samples. It is probable that biodegradation occurred over the approximately two week post-oiling period. The origin of enhanced tissue isoalkanes may have been either from endogenous biodegradative processes, in particular gut flora, or from ingestion or absorption of biodegraded oil from the substrate or food material. It had been reported by divers carrying out observations after the oiling event that, by the second sampling period, urchins were feeding on incapacitated bivalves. The polychaetes, however, are considered to be surface deposit feeders.

A general conclusion which can be drawn from the dispersed oil spill test is that both S. droebachiensis and P. granulosa have a capacity to accumulate hydrocarbons to a high degree from either the water column or the sediment substrate when the oil is in particulate dispersed form. Later accumulation when the spilled oil has mainly disappeared from the water column may continue from the substrate and by ingestion of food material.

4.2 Oil Slick Exposures

In contrast to the generally similar hydrocarbon uptake picture shown by urchins and polychaetes when exposed to dispersed oil, the two species were found to differ in their response to the surface oil spill of Bay 11. Urchins showed a much greater degree of tissue accumulation under these conditions than did P. granulosa. It may be suggested that this difference is due to both the physiology and habitat of the species. The urchin would see an early continuous exposure to hydrocarbons dissolved in the water column because of its extensive water-vascular system. High levels in the second post-spill urchin sample may be attributed to either continuing absorption from very low background levels coupled with extensive retention of accumulated hydrocarbons, or as is more probable, a continued input from food sources. The sediment itself was of low contamination (Boehm et al. 1982) and had probably little effect. Low oil levels in the sediment of Bay 11, especially as compared to Bays 9 and 10, explain the low tissue

oil levels found in P. granulosa since this species is closely tied to the substrate for both habitat and food supply.

It is most interesting that urchins from Bay 11 had the highest tissue hydrocarbon levels of all four bays, although the concentrations in this bay were several orders of magnitude lower than that found at 5 or 7 m in Bay 9 (Green et al. 1982) . The oil levels are not likely due to extraneous contamination during sampling since the collection techniques guarded against such an occurrence. Further, the absence of low boiling hydrocarbons in the first post-spill sample set indicates that it was an uptake phenomenon which was described by the hydrocarbon data, not incidental contamination.

At present, there is no ready explanation for the marked differences in tissue oil levels noted between the five stations of Bay 11. There is some correlation with the sediment data which identified high hydrocarbon levels at the same sites (Boehm et al. 1982) . Presumably some physical factor such as current eddies or tide channels may have played a role in causing the station heterogeneity along the 7m depth stratum.

One may conclude that a surface oil spill which was not treated with chemical dispersants (although in this case much of the spilled oil was mechanically removed) leads to a high degree of accumulation of larger molecular weight aliphatics and aromatics in S. droebachiensis. This occurred to a greater degree than when the oil was clinically dispersed, even though in this case water and sediment oil levels were much higher. Given the general insistency in tissue hydrocarbons in urchins from a 5m trap as compared to freely mobile urchins from the same bay, one can conclude that there has been little movement of urchins among bays or into a given test bay from uncontaminated areas.

In contrast to the urchin, the polychaete P. granulosa exhibited less tissue uptake in the surface spill condition as compared to dispersed oil exposures. This finding is in agreement with low water and sediment hydrocarbon burdens.

4.3 Laboratory InterCalibration

Results from the CWS FID and SIM analyses agreed well one with the other and were also similar to the presumed hydrocarbon levels in the unknown samples. That the total oil concentrations calculated on the basis of alkanes and PAH were greater than the amount of oil added may have been due to background hydrocarbons in the tissues prior to spiking, or be a reflection of the apparent 50% enhancement in concentration of the oil-only check sample. The UV/F assessments agreed well with presumed real concentrations in the case of the oil sample, but gave enhanced values for the spiked urchins and Mya samples. This is in accord with the recognized limitations of the UV/F assay.. a method which may be more useful for screening purposes, yielding information on relative concentrations simply and rapidly.

The same check samples were assessed by ERCO (Boehm et al. 1982) . Based on the concentration data presented from fluorescence analysis, it is apparent that values derived by this method by the laboratory underestimate hydrocarbon concentrations, although the oil-only unknown corresponds closely to true values, and is similar to the CWS/DIAND analysis. Both the spiked urchin and spiked Mya samples by UV/F were found to be a factor of three lower than the data generated the CWS/DIAND UV/F method. Analysis by ERCO for the unspiked urchin sample gave a result two times greater than that of the CWS/DIAND assay. These inconsistent differences in W/F serve to point out the difficulties in comparing data between laboratories, even though methods were standardized as much as possible in this project. While an absolute transfer of data is thus not advisable, this comparison of UV/F data does point out the utility of the fluorescence method for screening.

SECTION FIVE - REFERENCES

- Boehm, P.D. 1981. Baffin Island Oil Spill Project Chemistry. 2. Hydrocarbon Chemistry, 1980 Study Results. BIOS Project Working Report Series, 1981. March 1981.
- Boehm, P.D., D.L. Fiest, and P. Hirtzer. 1982. Baffin Island Oil Spill Project Chemistry Component - Report on the 1981 Oil Spill Experiment. Vol. 2: Summary of Analytical Biogeochemistry. Final Report to the Environmental Protection Service, Department of the Environment Canada, May 1982.
- Cross, W.E., and D.H. Thomson. 1982. Effect of Oil and Dispersed Oil on Nearshore Macrobenthos at Cape Hatt, Northern Baffin Island. II. Results of 1980 and 1981 Pre- and Post-Spill Studies. BIOS Project Report for the Environmental Protection Service, Department of the Environment Canada, April 1982.
- Green, D.R., B.H. Humphrey, and B. Fowler. 1982. Report on the 1981 Oil Spill Experiments. Vol. 1: Summary of Field Work. BIOS Project Report for the Environmental Protection Service, Department of the Environment Canada, March 1982.

SAMPLE TYPE	Spike ug Oil/ g net wt.	PERCENT RECOVERY ¹																						
		IS	C4 AB	BP	N	C1 N	C2 N	C3 N	C4 N	DBT	C1 DBT	C2 DBT	C3 DBT	Ph	C1 Ph	C2 Ph	C3 Ph	F	C1 F	C2 F				
<u>S. drceb.</u>	100	106	-	75	15	11	49	77	91	97	106	119	144	123	129	120	122	98	125	209				
" "	100	99	56	106	86	90	95	94	-	84	91	94	99	107	110	98	91	86	93	144				
" "	50	98	104	141	129	89	92	97	51	50	109	91	26	106	102	81	-	130	-	111				
" "	50	105	97	124	89	95	89	86	61	54	2	8	-	-	6	2	6	3	7	9	-	-	-	-
<u>P. gran.</u>	100	96	116	110	206	83	89	83	112	102	106	111	135	114	142	71	1	3	6	-	-	-		
Blank	100	104	70	80	92	72	71	72	102	87	87	89	119	106	92	110	80	103	97	148				
Blank	50	96	42	85	75	67	71	72	76	84	81	87	-	111	94	102	1	3	6	-	-	-		

¹ IS = internal standard (o-terphenyl), AB = Alkyl benzenes, N = naphthalene, DBT = Dibenzthiophene
Ph = phenanthrene, F = fluorene

STR, Bay 10 1st post spill Duplicate No.	oil Equivalents, (UV fluoresc., ug/g dry weight	ng./g dry weight ¹																				Total Aroma- tic	Total PAH			
		C3 AB	C4	C5	BP	N	C1 N	C2 N	C3 N	C4 N	DBT	C1 DBT	C2 MT	C3 DBT	C4 MT	Ph	C1 Ph	C2 Ph	C3 Ph	C4 Ph	F			C1 F	C2 F	Chr
P#1	86.6	400	463	233	45	195	557	883	1288	757	125	270	216	137	NA	355	392	280	148	20	51	95	119	40	7069	5973
P#2	92.7	263	364	230	60	153	522	919	1325	641	139	266	247	181	NA	385	417	310	150	29	63	111	85	NA	6860	6003
P#3	95.8	607	766	347	68	215	698	1007	1503	675	134	245	210	162	34	341	380	263	156	20	63	77	153	8	8132	6412
P#4	94.2	312	553	263	53	122	511	863	1192	618	134	238	235	162	43	324	392	261	148	NA	54	76	83	NA	6637	5509
Mean	92.3	395	537	268	56	171	572	918	1327	673	133	255	227	161	39	351	395	278	151	23	58	90	108	-	7175	5974
SD (±)	4.0	152	171	55	10	42	86	64	130	61	6	16	17	18	7	2	6	1	6	2	3	4	5	-	662	369
CV (%)	4	38	32	20	17	2	4	1	5	7	1	0	9	4	6	7	4	8	2	23	U	19	30	-	9	6

¹
AB . alkyl benzene
BP . biphenyl
N . naphthalene

MT = dibenzthiophene
Ph = phenanthrene
F = fluorene
Chr = chrysene