BNWL-SA-6559

CONF-7710169--1

RECRUITMENT OF BENTHIC ANIMALS AS A FUNCTION OF PETROLEUM HYDROCARBON CONCENTRATIONS IN THE SEDIMENT

by

J. W. Anderson, R. G. Riley* and R. M. Bean*

Battelle
Pacific Northwest Division
Marine Research Laboratory
Route 5, Box 1000
Sequim, Washington 98382

and

*Environmental Chemistry Section
P. O. Box 999
Richland, Washington 99352

DISCLAIMER

RECRUITMENT OF BENTHIC ANIMALS AS A FUNCTION OF PETROLEUM HYDROCARBON CONCENTRATIONS IN THE SEDIMENT

by

J. W. Anderson, R. G. Riley and R. M. Bean

Abstract

Three separate field installations, consisting of clean and oiled sediment in fiberglass trays, were placed in the intertidal zone of Sequim Bay, Washington to determine rates of hydrocarbon depuration and recruitment of benthic organisms. Detailed chemical analysis, using glass capillary gas chromatography, and GC/MS, were conducted such that individual components and hydrocarbon classes associated with the sediment after varying periods of field depuration could be quantitated. Depuration rates of hydrocarbon types in sediment receiving oil on the surface decreased in the order of saturates, methylnaphthalenes and methylphenanthrenes. The time to 50% depuration (half-time) for these coarse sediments was approximately 100 days, while sediment mixed with oil had only decreased by about 20 to 30% by 100 days. Levels of the aromatics (naphthalenes and phenanthrenes) in the two systems followed the general pattern, but very little loss was exhibited when oil was mixed with sediment.

No substantial inhibition of benthic organism recruitment was produced by either type of sediment contamination. There was a tendency for suppression of populations of mature and juvenile bivalves (*Mysella tumida*) at the last sampling interval for all three installations. Future sampling of these populations and further analyses of all benthic organisms may provide a better evaluation of effects of specific hydrocarbon components in sediments on benthic recruitment. These results are discussed in light of oil spill studies and other field experiments.

RECRUITMENT OF BENTHIC ANIMALS AS A FUNCTION OF PETROLEUM HYDROCARBON CONCENTRATIONS IN THE SEDIMENT

by

J. W. Anderson, R. G. Riley and R. M. Bean

Introduction

As a result of oil production from the Alaskan Outer Continental Shelf, the Pacific Northwest is one of the areas of the United States most likely to receive increased inputs of petroleum hydrocarbons (PHC) from transport, port operations, and refining. There is the possibility that exploration on the coasts of Oregon and Washington will indicate the potential for drilling, and, thus, production-related inputs are possible. Present and future activities associated with this energy source necessitate research on the potential impacts of oil in the Pacific Northwest marine environment. While the results of recent studies on the fate and effects of PHC in the marine environment have strengthened our understanding of some aspects of oil pollution, we are not yet to the point at which an accurate prediction of the effects of acute or chronic oil inputs can be made.

In order to more clearly define the potential for petroleum to exert significant long-term environmental effects in marine sediments, studies on the fate of crude oil in sediments are being conducted. These studies are interdisciplinary, involving aspects of physical organic chemistry and marine biology. The principal objectives of these studies are to determine the rates and mechanisms

of degradation of petroleum in the marine environment, to determine which component types are persistent, and to correlate such information, where possible, with observed effects on the marine biota.

To meet the analytical objectives, considerable effort has been directed toward development of analytical methods for the separation, identification, and quantitation of classes of compounds and individual compounds which comprise saturate and aromatic fractions of Prudhoe Bay Crude oil (PBC). Analytical methodologies developed on PBC have been appropriately modified for analysis of petroleum compounds in sediments, tissues, and laboratory exposure systems.

In this paper, we wish to report on methods which are being used to meet some of the above needs. These techniques are being used to separate, identify, and quantitate the components of PBC and to quantitate these components in sediment samples contaminated with PBC as a part of laboratory and field depuration studies. The methods used in these studies employ the techniques of liquid-column chromatography, capillary-column chromatography, gas chromatography-mass spectrometry(GC-MS), high performance liquid chromatography (HPLC), and infrared spectroscopy (IR).

The experimental approach utilized was designed to provide correlations between the rates of oil depuration and benthic organism recruitment, in the natural environment. This type of field experiment has been recommended in past workshops (Beynon and Cowell, 1974; EXXON, 1974), but to our knowledge has not yet been investigated. With present restrictions on the spillage of oil in

the territorial boundaries of the United States, it appears that this is the only type of field experiment that can be conducted. As in other types of experimentation, both chemical and biological analytical methods were developed as the investigation progressed. The research is continuing such that alterations in the biota and chemical characteristics of the sediment will be followed for a number of years to provide realistic information on the fate and effects of petroleum hydrocarbons in marine sediments.

Materials and Methods

Analytical Chemistry

Analysis of PBC. The separation of PBC into saturate and aromatic compound fractions was adapted from a silica gel chromatography technique described by Warner (1976) and modified by MacLeod *et al.* (1976). Two fractions (saturate and aromatic) were collected. Each fraction with its appropriate internal standard was concentrated and analyzed by gas chromatography on a 30-meter SE-30 glass capillary column (J & W Scientific) programmed from an initial temperature of 70° to 250° at a rate of 4°/min. Compounds in the saturate and aromatic fractions were identified by peak enhancement analysis with known standards. A Hewlett Packard 5982A quadrapole mass spectrometer operating in the argon chemical ionization mode and employing single ion monitoring was used to determine approximate retention time ranges for specific compound types within the aromatic fraction.

Analysis of total oil in sediments by IR. Concentrations of total oil in sediments were determined on carbon tetrachloride extracts of sediments with a Beckman Acculab 6 spectrophotometer by comparison of the amount of absorbance obtained at 2930 cm^{-1} to a calibration curve prepared with Prudhoe Bay Crude oil (API, 1958). Recoveries of total oil from carbon tetrachloride extracts of oil-amended sediments were found to be greater than 90%.

Separation and quantitation of compounds in contaminated sediment. Triplicate 20-gram samples of sediment were extracted for 24 hours in glass bottles containing Teflon-lined caps, 20 grams of anhydrous sodium sulfate, and 50 ml of hexane (Burdick & Jackson). Appropriate amounts based on total oil analyses of each sample were concentrated to 1 ml and separated into saturate and aromatic fractions using a silica gel method adapted from that of Warner (1976). Saturate and aromatic fractions were analyzed by capillary column chromatography as previously described, employing 2, 6, 10-trimethyldodecane and hexamethylbenzene as internal standards for the saturate and aromatic fractions, respectively. Peak areas and internal standard calibrations were determined with an Autolab Systems IV Computer integrator.

Recovery studies. One milliliter of two concentrations of oil in hexane was added to separate 20 gram samples of sediment. The concentrations of oil chosen approximated the extremes of the oil concentration range observed in the sediment field depuration studies. Each sample was extracted, concentrated on the basis of total oil

concentration and analyzed as previously described. Percent recoveries of individual compounds and compound types isolated from the oil were calculated on the basis of the known amount of oil added to the sediment and the concentrations of individual compounds present in PBC.

Biological

Experimental System. The methods and materials utilized in these studies are essentially those described for oil-on-sediment and oil-in-sediment exposures conducted with sipunculids (*Phascolosoma*) (Anderson *et al.*, 1977). Either fiberglass or polyvinylchloride sediment trays (36 x 46 x 81 cm), containing 3 equal compartments and fiberglass mesh (1.6 mm) openings in the bottom, were used to hold clean and contaminated sediment in the intertidal zone. Sediment was collected from the same area that was used for placement of experimental trays. Sieving of natural substrate was necessary to reduce large particle size variability, ranging from fine detritus to large cobbles. The first two installations (I on April 17, and II on May 3, 1976) consisted of 6 trays each, four of which received oil while 2 served as controls. The sediment used in these installations was sieved thru a 12 mm mesh screen in the field and transferred in buckets to a freezer at the laboratory. After three cycles of freezing and thawing, the substrate was placed in the trays and transferred to large aquaria receiving flowing seawater (one liter per min.). The aquaria were equipped with a system to provide tidal flushes such that water would drain completely from the sediment (through the mesh bottom) and fill with

aerated water after a "low tide" period of 1 hour. When the water level was approximately 1 cm above the sediment surface, a 4% (v/v) volume of oil was poured over the water forming a uniform layer. "Low tide" was then initiated, and the oil impacted the surface and remained there for about 2 hours, until the next "high tide." At the high water level, excess oil (not adsorbed to sediment) was skimmed off by the outflow tube. After 3 more tides (48 hours total), the 4 exposed trays and 2 controls were transferred to the zero tide level on the sand spit east of the laboratory. Exactly the same procedure was utilized in the preparation of installation II in May, 1976.

To simulate a spill where finer sediment is thoroughly mixed with oil, installation III (October, 1976) was prepared in a somewhat different manner. Sediment collected from the same local was sieved a second time to eliminate particles greater than 2 mm. As described in the oil-in-sediment procedure of Anderson *et al.* (1977), this substrate was mixed thoroughly in a fiberglass-lined cement mixer with a volume of oil approximating 0.1% of the sediment volume. The oil was mixed in a blender with 500 ml of seawater before addition to the tumbling moist sediment, and a 500 ml rinse was added a few moments later. Using a C^{14}-labelled-2 methylnaphthalene spike in the oil, periods of mixing between 15 min. and 1 hour have been shown to be sufficient (Anderson *et al.* 1977). These trays were also flushed for 48 hours in the laboratory tidal flux system to remove excess oil. They were placed in the field near the first two installations on October 28, 1976.

Sampling and Analyses. The schedule of sampling is shown in Table 1. Using the initiation of installation I as an example, on April 17, 1976, after a 48 hr flushing period in the laboratory, 3 cores were taken from section 1 of Trays 1, 3 and 5, and frozen for later analyses. Since organisms had been killed previously by freezing and thawing, no samples were taken for biota analyses at initiation of installations (April 17, May 3, and October 28, 1976). The cores were sections of standard PVC pipe measuring about 13.2 cm in length, with an inside diameter of 3.8 cm. The volume of sediment extracted by a core varied from 81 to 109 ml, but within a given installation, the means and standard deviations were 93 ± 14 (I and II) or 97 ± 12 (III). These cores represented about 5% of a section, which was approximately 2 liters in sediment volume. The differences between installations were the result of small sediment height variations. Both in the laboratory and in the field, core samples were easily removed by inserting all replicates (usually 5) and placing a neopreme stopper over one at a time before slowly extracting the tube. The contents of 3 or more cores were allowed to extrude into widemouth jars and within 2 hours, 5% Formalin was added to each. After about one week, the fixative was carefully decanted, and a 70% ethanol solution containing Rose Bengal stain was added to aid in distinguishing between organisms and detritus particles. Other replicate cores for chemical analyses were, within 1 hour, placed in a low temperature (-70°C) freezer until transport to Richland on dry ice and subsequent analyses.

Early field collections on June 21, August 24, and November 23, 1976, utilized somewhat different techniques. Two core samples were

Table 1. Sampling schedule for field installations. Trays (T) 4 and 5 contained control sediment in all installations. There were 3 sections per tray and one was completely emptied at each time interval. Parenthetical numbers represent the number of replicate cores sampled.

Install-ation	Condition	Dates of Sampling Trays and Sections																			
		1976												1977							
		A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A			
I	Coarse sediment (12 mm sieve) oil on top, flushed 48 h in lab.	4/17 T.1,3,5 Sec. 1 (3)		6/21 T.2,4,6 Sec. 1 (3)*		8/24 T.1,3,5 Sec. 3 (3)*			11/23 T.2,4 Sec. 3 (3)**			2/15 T.1,3,5 Sec. 2 (5)			(Term.) 5/6 T.2,4,6 Sec. 2 (5)						
II	(As I above)		5/3 T.1,3 Sec. 1 (3)			8/24 T.2,4,6 Sec. 1 (3)*			11/23 T.1,3,5 Sec. 3 (3)**			2/15 T.2,4,6 Sec. 3 (5)			5/6 T.1,3,5 Sec. 2 (5)				8/14 T.2,4,6 Sec. 2 (5)		
III	Fine sediment (2 mm sieve), oil mixed in., flushed 48 h in lab.							10/28 T.1,3,5 Sec. 1 (3)				2/15 T.2,4,6 Sec. 1 (5)			5/6 T.1,3,5 Sec. 3 (5)				8/14 T.2,4,6 Sec. 3 (5)		

* After extraction of 2 cores for chemical analyses all the remaining sediment in the section was sorted for organisms.

** As above, except the remaining sediment was mixed and 4 subsample cores were taken in the laboratory.

taken as already described and frozen for chemical analyses, but for the June and August collections, all the remaining sediment in the section was sorted for organisms. This process was clearly too labor intensive, so subsample cores were taken from the November collection, which were comparable to those taken in the field at all later dates.

Sorting of organisms from small portions (about 5 ml) of the sediment was conducted under a dissecting microscope at 15X. Animals were placed in small vials with 70% ethanol and held for later identification and counting. While a large number of species were identified, the more prominent species which were present at nearly every interval and in each sample were selected for comparisons.

Results

Chemical

Analysis of PBC. Compounds identified in the saturate fraction of PBC by gas capillary column chromatography included the straight chain hydrocarbons from C_{11} to C_{26}, pristane and phytane. Pristane and phytane were baseline resolved from heptadecane and octadecane (Figure 1). Identification and quantitation of compounds in the aromatic fraction proved to be more difficult because of the lack in the availability of commercial standards and because many were present in PBC at much lower concentrations relative to the saturate compounds. Identifications that were made are listed on Figure 2. Many compounds in the aromatic fraction were categorized by GC/MS according to compound types: i.e., monomethyl- dimethyl- and

trimethylnaphthalenes, monomethyl-, C_2- and C_3-phenanthrenes, etc. The GC/MS information has thus enabled us to estimate the concentration of hydrocarbon types by calculating the total concentrations on the basis of those compounds which reside within a given retention time range.

Sediment Analyses. In order to determine the efficiency of extraction of hydrocarbons from the oiled sediment, recovery studies with hexane as the solvent were conducted. Average recoveries of saturate hydrocarbons from sediment for both concentration levels ranged from 83% to 85%. Similar results were obtained for aromatic compounds and compound types. High average recoveries were obtained for naphthalene (100%), methylnaphthalenes (88% - 99%), phenanthrene (80% - 96%) and methylphenanthrenes (84% - 90%).

The above method was applied to field studies of the depuration of the components of PBC from a coarse intertidal sediment. The concentrations of compounds in the saturate and aromatic fractions from sediment taken from the first day of the first installation are listed in Table 2. Docosane, naphthalene and phenanthrene were selected as representative saturate and aromatic components of oil, and their rate of depuration in the sediment was determined over a period of seven months. The results are shown in Figure 3. Only 2.1%, 1.0% and 2.6% of the original docosane, naphthalene and phenanthrene, respectively remained in the surface portion of the sediment after seven months of exposure in the beach environment. Similar trends were found for three representative compound classes (Figure 4). Only 1.8%, 1.1% and 4.0% of the saturates, methylnaphthalenes, and methylphenanthrenes remained after the same period

Table 2. Concentrations of saturate and aromatic hydrocarbons (parts per million, PPM) in sediment from the day of installation of field experiment I (4-17-76).

Saturates	Concentration (PPM)	Aromatics	Concentration (PPM)
C_{11}	13.34 ± 1.21	Naphthalene	7.20 ± 0.77
C_{12}	16.11 ± 1.53		
C_{13}	17.79 ± 1.82	2-MN	14.31 ± 3.10
C_{14}	17.44 ± 2.39	1-MN	10.51 ± 2.90
C_{15}	19.39 ± 2.94	Total	24.82 ± 5.32
C_{16}	18.84 ± 3.00		
C_{17}	17.03 ± 2.92	1-ethyl + 2-ethyl	
Pristane	10.74 ± 1.95	Naphthalene	6.93 ± 0.35
C_{18}	16.20 ± 3.22		
Phytane	8.05 ± 1.43	2,6 + 2,7-DMN	9.52 ± 0.75
C_{19}	18.79 ± 3.00	1,3 + 1,6-DMN	9.93 ± 0.94
C_{20}	16.40 ± 2.88	1,7-DMN	7.81 ± 0.64
C_{21}	17.56 ± 3.09	1,4 + 2,3 + 1,5 DMN	9.14 ± 0.72
C_{22}	16.02 ± 3.37	1,2-DMN	1.60 ± 0.45
C_{23}	15.18 ± 2.65	Total	42.68 ± 2.74
C_{24}	13.80 ± 2.84		
C_{25}	11.71 ± 2.47	TMN-1	3.80 ± 0.64
C_{26}	10.21 ± 1.97	TMN-2	3.65 ± 0.55
		TMN-3	5.69 ± 1.11
Total saturates		TMN-4	4.26 ± 0.76
measured	274.61 ± 42.32	2,3,6-TMN	2.08 ± 0.24
		TMN-5	5.99 ± 0.77
		Total	28.44 ± 6.10
		Phenanthrene	3.05 ± 0.85
		MP-1	2.74 ± 0.56
		MP-2	2.34 ± 0.40
		MP-3	3.94 ± 1.71
		MP-4	3.49 ± 1.67
		Total	12.53 ± 4.31
		C_2-phenanthrene-1	7.30 ± 1.56
		C_2-phenanthrene-2	2.36 ± 0.68
		C_2-phenanthrene-3	2.56 ± 0.44
		C_2-phenanthrene-4	2.47 ± 0.87
		Total	14.70 ± 3.52
		Total aromatics measured	140.35 ± 23.96

MN = Methylnaphthalene
DMN = Dimethylnaphthalene
TMN = Trimethylnapthalene
MP = Methylphenanthrene

of time. These results were in excellent agreement with the observed depuration according to total oil analysis obtained on the same samples (Figure 4).

The rates of depuration of these compounds and compound types in the second installation were very similar to the rates observed in the first field installation. Initial oil concentrations were approximately the same. After a 7-month time period, only trace amounts of docosane remained, and naphthalene and phenanthrene were undetectable in the sediments (Figure 5). Similar results were observed for the compound classes and total oil concentration (Figure 6).

The results of the analyses of installations one and two are in marked contrast to the depuration rates observed in the third installation. Initial concentrations of total oil in the sediment were approximately 10% of the concentrations observed in installations one and two. This is reflected in the determined concentrations of individual compounds and compound types (Figures 7 and 8). After 9 months, 46.9%, 17.6% and 50.0% of the original docosane, naphthalene and phenanthrene, respectively, remained in the sediment (Figure 7). Greater persistence of saturates (40.1%), methylnaphthalenes (46.5%) and methylphenanthrenes (49.6%) as compound types was also observed (Figure 8). Total oil analysis indicated 73% of the IR detectable oil remained after 9 months of exposure (Figure 8).

The ratios of nC_{17}/pristane and nC_{18}/phytane were calculated from analysis of the saturate fraction from sediment extracts of field installations I and III. The results are shown in Table 3.

Table 3. Ratios of nC_{17}/pristane and nC_{18}/phytane derived from analysis of sediment extracts from two field installations.

Date	Days of Depuration	Installation	nC_{17}/pristane	nC_{18}/phytane
4/17/76	0	I	1.59 ± 0.35	2.01 ± 0.54
6/21/76	65	I	1.60 ± 0.39	2.01 ± 0.41
8/24/76	130	I	1.61 ± 1.80	2.04 ± 2.32
11/23/76	221	I	0.22 ± 0.05	0.32 ± 0.06
10/28/76	0	III	1.59 ± 0.03	2.21 ± 1.08
2/15/77	110	III	1.68 ± 0.18	2.12 ± 0.31
5/06/77	190	III	1.46 ± 0.12	2.26 ± 0.32
8/14/77	290	III	0.81 ± 0.10	0.98 ± 0.16

In both installations, the ratios remained constant through the period of 4 to 6 months. After this period of time, considerable changes occurred in the ratios. In the first installation, the nC_{17}/pristane and nC_{18}/phytane ratios were lower by factors of 7.3 and 6.4, respectively. The changes observed in the third installation were significant but less than those observed in the first installation. The nC_{17}/pristane and nC_{18}/phytane were lower by factors of 1.8 and 2.3 respectively.

Biological

From examination of the list of species identified from field collections (Table 4), it is clear that a good diversity of organisms were recruited. As might be expected, the dominant phyla were Annelida, Mollusca and Anthropoda. Since a reasonable portion of the crustaceans may have been transient occupants of the particular sediment tray, these more epibenthic forms were not chosen as good examples of recruited animals. Gastropods were also eliminated from more extensive comparisons between clean and oiled sediment for the same reason. On examination of the numbers of individuals found in each tray at all or nearly every collection interval, only three polychaetes and two bivalves were shown to be present in significant numbers. We do not feel that selection of 5 species from the total represents a bias toward oil resistant species, since these were the only species that were consistently found in control sediments, with one or more individual per core (in most cases).

An overview of the distribution of the five benthic species is shown in Figure 9. Installation I contains the largest compilation

Table 4. Animals Found in Recruitment Trays.

Phylum Annelida

Class Polychaeta

Order - Errantia:
- *Anataides groenlandica*
- *Dorvillea moniloceras*
- *Dorvillea* sp.
- *Eulalia nigrimaculata*
- *Harmothoe* sp.
- *Hemipodus borealis*
- *Lepidonotus caelorus*
- *Lumbrineris* sp.
- *Nothria elegans*
- *Ophiodromus pugettensis*
- *Pholoe minuta*
- *Platynereis bicanaliculata*
- *Typosyllis fasciata*

Order - Sedentaria:
- *Armandia bioculata*
- *Axiothella rubrocincta*
- *Boccardia* sp.
- *Cirractulus cirratus*
- *Owenia collaris*

Phylum Mollusca

Class - Bivalvia
- *Clinocardium nuttallii*
- *Macoma* sp.
- *Mysella tumida*
- *Mytilus edulis*
- *Protothaca staminea*
- *Psephidia lordi*

Class - Gastropoda
- *Acmea* sp.
- *Alabina* sp.
- *Fartulum* sp.
- *Lacuna* sp.
- *Margarites* sp.
- *Nucella lamellosa*

Phylum Anthropoda

Class Crustacea

Order - Decapoda
- *Cancer productus*
- *Hemigrapsus oregonensis*
- *Pagurus beringanus*
- *Pagurus hirsutiusculus*
- *Pagurus* sp.
- *Pandalus* sp.
- *Pinnixa* sp.
- *Telmessus cheirogonus*
- *Upogebia pugettensis*
- *Pugettia gracilis*

Subclass - Cirripedia
- *Balanus* sp.

Subclass - Malacostraca

Order - Amphipoda
- *Corophium* sp.
- Unknown amphipod

Order - Isopoda
- *Exosphaeroma amplicauda*
- *Gnorimosphaeroma oregonensis*

Order - Tanaidacea
- *Leptocheila* sp.

Order - Cumacea
- *Cumella* sp.

of data. The trays had been in the field for 385 days by the time the last set of sections was sampled, thus, terminating this installation. At the first sampling date (August 24, 1976; 130 days), when the greatest effect from oil in sediment would be expected, larger numbers of individuals were present in the contaminated substrate than in clean sediment. The absence of standard deviations or the large variations (when shown) are due to the fact that the entire sections (about 2 liters of sediment) were sorted. The numbers of individuals were then converted to numbers present in cores on a volume/volume basis. The only replication was, therefore, the result of sampling 2 exposed trays, while only one control tray was analyzed. When replication was possible by use of field collected core samples, variations were still quite large, as shown by the remaining time intervals in installation I. Only in one case was abundance of an organism in clean sediment significantly higher than that in exposed substrate, and the difference was very small (*Mysella tumida* on May 6, 1977). On the other hand, in August and November of 1976, the polychaete, *Armandia bioculata*, was more abundant in oiled sediment than in clean.

For the bivalves, *Mysella* and *Psephidia*, it was possible to discriminate between juvenile and adult organisms. The numbers of mature bivalves increased with time in installation I and at the final interval (385 days), there was a significantly higher number of mature *Mysella* in the control sediment (5 ± 1 compared to 0.8 ± 1.1). This, however, was the only instance of greater abundance of mature bivalves in the clean sediment of installation I.

The conditions of exposure for installation II were exactly the same as I, except that the date of initiation was approximately 3 weeks later (May 3, 1976). As can be seen from Figure 9, only the bivalves from this installation have been identified and counted at the writing of this paper. The results of these analyses are much the same as those observed in installation I. There are very small differences between the abundance of these bivalves at the first two intervals, but *Psephidia* appears to be more abundant in the clean sediment on November 15, 1977. Unfortunately, not all replicates have been sorted and identified so the statistical significance of this difference can not be evaluated at this time. However, it would appear from data collected on the May sampling date (368 days), that *Psephidia* in oiled substrate were not eliminated in November. At this last interval, there are no significant differences between mature or juvenile bivalves in the clean and oiled sediment of installation II.

Installation III was prepared in a manner which was significantly different than the first two installations. Sediment was first screened through a 12 mm sieve and then a finer mesh, which allowed only particles less than 2 mm to pass through. This relatively homogeneous substrate was then thoroughly mixed with oil, before being placed in the sediment trays. The significantly different chemical characteristics of this installation have already been discussed, and they provide evidence of the marked difference between these sediments and those of the earlier installations. It was noted during field collections that these more homogeneous

particles tended to be much more compact thus making coring more difficult. However, the cores remained intact on extraction and transfer to jars for preservation. It was rather remarkable that this finer, more compact substrate possessed populations of the five species which were apparently equivalent to those in the other two installations. After only 110 days in the field, reasonably good recruitment had occurred in both clean and oiled sediment trays. From this February collection the only significant differences between abundance in clean and oiled substrate were exhibited by *Psephidia* and *Ophiodromus*. There were more of the small bivalves (*Psephidia*) in the clean sediment, and a small portion of the population was mature. On the other hand, the polychaete, *Ophiodromus*, was more abundant in the oiled substrate at this time interval. In May, (80 days later) there were no significant differences between the populations of any of the 5 species in clean and oiled substrate, but both bivalve species tended to be somewhat suppressed in the oil-contaminated sediment. The proportions of mature bivalves in oiled and clean substrate were very similar.

To attempt to correlate season with changes in the abundance of species, the mean values for numbers of individuals were plotted against sampling dates for all three installations. There was no apparent pattern of abundance for four of the five species in regards to season or the nature of the substrate (clean vs. oiled). The only trend that was observed is shown in Figure 10, where the mean number of *Mysella* individuals per core are plotted against the calendar. An increase in the numbers of individuals, in all installations and both clean and oiled sediment, is observed

for the period between February 15 and May 6, 1977. This period obviously correlates with increases in day length, air temperature and water temperature (7 - 10°C). Populations would be expected to increase during this time, and it is interesting to note that the means of all exposed collections are lower than the corresponding control populations. This pattern is even exhibited by *Mysella* in the third installations which had been in the field a significantly shorter period of time (110 in February and 190 in May). As noted above, the only significant difference between *Mysella* populations in clean and oiled substrate was that of installation I, and, unfortunately, those were the last sections available for sampling. Later intervals will be examined by collections from installations II and III to see if the pattern of *Mysella* suppression in oiled substrate continues. Samples were taken from II and III during August, 1977, but data are not yet available.

Discussion

Chemical

The capillary gas-chromatographic method has been applied to three installations of a field study to evaluate the persistence of PBC in coarse beach sediment. Using this method, considerable differences were observed in the depuration of PBC from the sediment depending on whether the oil was layered on the surface of the sediment, simulating a spill during calm conditions (installation I and II), or thoroughly mixed into the sediment (installation III) prior to field exposure, as might be the case during strong storms

(West Falmouth Spill, 1969). When PBC was layered on the surface of the sediment (installation I), 4% or less of the individual hydrocarbons or hydrocarbon types remained in the sediment after a period of 7 months (Figures 3 and 4). In contrast to this, individual hydrocarbons and hydrocarbon types were more persistent in the sediment thoroughly mixed with oil. After 9 months of exposure, 46.9%, 17.9% and 50.0% of the original docosane, naphthalene and phenanthrene remained in the sediment of installation III. Similar percent losses of other hydrocarbon types were observed (Figure 7 and 8) when oil was mixed with sediment.

The differences in the rates of depuration of individual hydrocarbons and hydrocarbon types from the two different oil-sediment field installations can be attributed to many physical, chemical and biological mechanisms. For the sediment that contained a surface layer of oil, tidal and wave action, surface volatility, photochemical and biodegradative processes are more than likely major contributors to the rapid depuration of oil from installations I and II of the field experiment. In the case of PBC that is thoroughly mixed into the sediment, the slower depuration rates observed can be attributed, to some extent, to a reduction in the activity of all of the above processes. Changes in C_{17}/pristane and C_{18}/phytane ratios have been cited as a measure of biodegradation of oil in the environment (Blumer and Sass, 1972) and we observed changes in these ratios for installations I and III (Table 2). It is suggested that the contribution of the biodegradative process to the depuration of saturate compounds from oil layered sediment was

minimal, because greater than 96% of the oil in the sediment was lost before the biodegradative process apparently began. The greater biodegradative activity for saturate compounds in the layered oil-sediment system can be attributed to the more aerobic environmental conditions expected to be present near the sediment surface. It is interesting to note that increased rates of depuration are observed after six months for both docosane and total saturates in the mixed oil-sediment system (Figures 7 and 8). The fact that this occurs at the same time that we observe changes in the pristane-phytane ratios suggest that part of this increase in the depuration rates is due to the biodegradative process.

There are quite interesting relationships between the rates of depuration of various components in the two different types of installations (I and II vs. III). In the surface-oiled system, both individual compounds (docosane, naphthalene and phenanthrene) and classes of components decrease in rate of depuration from saturates to phenanthrenes. However, when oil is mixed into sediment, the pattern is somewhat confused, as very slow depuration of all classes occurs. From Figure 7, it appears that naphthalene decreased substantially during the first 100 days, while docosane and phenanthrene concentrations remained relatively stable. When the classes of compounds are considered (Figure 8), few changes were exhibited until 190 days, when only the saturates showed a decrease.

Biological

A fact, which we are sure is not surprising to biologists, is that the results of biota analyses are not as definitive as chemical determinations. The variability between core samples in a given

sediment tray and between collection dates precludes absolute evaluation of effects on recruitment. While a large number of species was represented at least once in a sample, only five were present in sufficient numbers and frequency for comparisons to be made between populations in clean and oiled substrate. Wide fluctuations in the seasonal abundance of several species is apparently the factor which eliminates most organisms from consideration. Control sediments have never shown any indication of "carry-over" contamination, so the absence of most species in clean sediment at one or more collection dates is not the result of oil in the environment

When comparing the abundance of the two bivalves and three polychaete species, few significant differences between populations in clean and oiled substrate were observed. Perhaps the major finding was that populations of the bivalve *Mysella tumida* were depressed at the final collection date (May, 1977). Abundance of this species in control sediments of all three installations increased markedly between February and May of 1977, but the populations in corresponding sediments lagged behind (Figure 10). The May values for both total animals and mature individuals were significantly different in installation I, but only a trend was indicated in the other two installations. It was interesting that the seasonal effect (spring increase) on abundance of *Mysella* was consistent for all three installations even though the system of oil application and the duration of depuration for installation III was quite different. From chemical data, the levels of total hydrocarbons in the oiled sediments of installations I and II were 100 ppm or less, while those of installations III still contained 490 ppm on May 6, 1977. It is most interesting that this latter

concentration did not produce a greater suppression of the population of *Mysella*, as well as other species during the spring season.

When comparing this investigation to others on the fate and effects of petroleum hydrocarbons in the marine environment, one must either consider chemical studies or biological studies, as others have generally not utilized a "team" approach in field experiments or spill studies. Very often field studies have been conducted after a spill and baseline data are not available for comparison of pre- and post-spill conditions. As pointed out by Michael (1977), it is often difficult to obtain funding for long-term studies, which are needed to evaluate effects on populations and communities. There are, however, a few relatively recent studies which present data that may be compared to those of this investigation.

A very large field study has been described by McAuliffe *et al.*, (1975) which concerned the effects of a 65,000 barrel oil spill, 11 miles east of the Mississippi River Delta on the continental shelf. They reported that oil in sediment was generally restricted to a 10 mile radius from the platform and that linear regression coefficient did not show significant correlation between any of the biological parameters measured (no. of species, no. of individuals, diversity index and crustacean/polychaete ratio) and the amounts of either C_{12}-C_{23} or C_{12}+ hydrocarbons. It was estimated that 0.4 to 0.5% of the total oil discharged was present on the sediments within 5 miles radius of the platform. A value of about 130 ppm total hydrocarbons (measured gravimetrically) represented the contaminated area and could be compared to the IR values for the exposed sediments in this study which were initially as high as 770 ppm. Using total organic carbon as a measure of the extent

of oil contamination, Wormald (1976), studied the devastation and later recovery of meiofauna in beach sediments of Picnic Bay, Hong Kong, which received a spill of Heavy Marine Diesel oil. He showed that nematodes and harpacticoid copepods were almost completely killed within 4 days after the spill and populations remained very low for 8 months. Factors which were apparently controlling the rate of recovery (recruitment) were total oil levels, percent of aromatics, and position on the tide level. The latter factor seemed to be important because of physical/chemical and perhaps microbiological activities which in turn affected the retention time of oil in the sediment. Nematode populations recovered before those of harpacticoids. About 11 months were required to reduce hydrocarbon concentrations in sediment from about 1% to background, and after 15 months the meiofauna was present at "normal" population levels.

Another investigation of the effects of spilled oil was recently reported by Krebs and Burns (1977), in which they described the gradual recovery of fiddler crab, *Uca pugnax*, populations corresponding to concentrations of weathered No. 2 Fuel Oil in sediments. Recovery is still not complete 7 years after the spill, and concentrations in the mud greater than 1000 ppm were toxic to adults, while those of 100 to 200 ppm were toxic to juveniles. Abnormalities related to sublethal chronic exposures were also found, and it appears that this species may not have the capabilities to metabolize hydrocarbons exhibited by other crustaceans (see review by Lee, 1977). About 7 years after a Bunker C fuel oil spill (Arrow, February, 1970) Vandermeulen and Kiezer (1977) reported on the persistence of components of this oil in the beach sediments of

Chedabucto Bay and the contamination of molluscs. They found that bivalve contamination was likely the result of the absence of metabolic pathways to depurate the hydrocarbons. A striking difference between their study of sediment oil components and our data is evident. Hydrocarbons leaching from tar on the beach to sediments apparently are being selectively retained in the beach sand. As n-alkanes are almost completely absent from the sand, which has considerable contamination from aromatics and cyclo-alkanes, they propose that selective biodegradation is responsible. Our data do not indicate a preferential degradation of n-alkanes, and a relatively slow biodegradation component. It may be that during the 7 years of oil leaching high populations of oil degrading micro-organisms have been established in their study site, and we see an indication of this process after about 7 months.

In addition to oil spill studies, a few field experiments have been conducted which provide information pertaining to this investigation. Bieri and Stamoudis (1977) reported on the results of a No. 2 Fuel oil spill experiment in a small area of a marsh. While data were generated regarding levels of hydrocarbons in the water column (quite shallow) and clam and oyster tissue, no spill-related hydrocarbons were found in extracts of the sediment. The analytical techniques used by these investigators were quite sensitive and sophisticated, but background levels in the area were relatively high. Another report of high sediment hydrocarbon levels showed a correlation between sample locations and a creek which had received fuel oil from a leaking storage tank (MacLeod, *et al.*, 1977). These investigators also used very sensitive chemical methods to show the

dramatic differences between oil components in the contaminated creek area and a relatively pristine area (Dungeness Bay, Washington). Levels of all hydrocarbons measured were generally higher in Port Angeles (creek site), but a list of compounds worthy of use in monitoring hydrocarbon contamination included naphthalenes, phenanthrenes, fluoranthrene, chrysene, pyrene, benzpyrene and others. Concentrations in the polluted sediment of phenanthrene, fluoranthrene and pyrene were generally on the order of 200 to 1000 ng/g dry weight (ppb). For means of comparison, concentrations of total naphthalenes and phenanthrenes in the initial sediments of installation I were 110 and 30 ppm, respectively. It would appear that concentrations in our experimental sediments were well above those encountered in even a highly polluted substrate.

Field sediment experiments with oil including detailed hydrocarbon component analyses are virtually non-existent. Lee and Anderson (1977), have described an experiment in CEPEX bags including some data on naphthalenes, but this involved the fate and effects of these compounds in the water column not the benthic environment. Shaw *et al.* (1976) conducted field exposures to oiled substrate using the clam, *Macoma balthica* and showed an effect of surface oiling on mortality. Application of 5.0 µl oil/cm^2 produced significant mortality and total oil concentrations (gravimetrically determined) in final sediment samples ranged from 760 to 3,890 ppm (dry weight). Since this animal is a deposit feeder, it is expected that surface oiling would result in high contamination of tissue and mortality. Uptake studies using the same sediment trays described in this paper have shown the bioavailability of specific

petroleum hydrocarbons to *Macoma inquinata* (Roesijadi *et al.*, 1977, 1978). The effects of oiled sediment on the mortality of *Macoma* reported by Shaw *et al.*(1976), were derived from rather heavy doses with exposure times up to 44 days. A report by Armstrong *et al.*, (1977) provides information on the effects of chronic hydrocarbon exposure on a benthic community. An effluent from a separator platform in Galveston Bay, Texas, introduces hydrocarbons into a 3 meter water column at levels of about 25 ppm, of which approximately one half are aromatics. While the water contained only about 10 ppb aromatics, sediment beneath the platform contained a total of 96 ppm and 34 ppm aromatics. The aromatics measured were primarily naphthalenes, biphenyls, fluorenes and phenanthrenes. While the concentrations of these aromatics in this fine clay substrate, were less than those reported in this study, the chronic condition reduced the numbers of species and individuals of benthic organisms living within 500 feet of the platform and abundance was correlated with naphthalenes content along three transects.

Conclusions

The rates of depuration of hydrocarbons in the two installations of coarse sediments, which received a surface oiling were quite rapid and very similar. Let us consider only the methylnaphthalenes (MNs) and methylphenanthrenes (MPs) which have been shown to be as toxic as any compounds tested (Neff *et al.*, 1976). The concentrations in the sediments decreased from 25-30 ppm MNs and 12 ppm MPs by nearly

an order of magnitude in 100 days and were not detectable in 200 days. On the other hand, when a smaller amount of oil was mixed into the sediment concentrations only decreased from 4 ppm MNs and 1 ppm MPs, to 50% or less in 100 days and detectable levels were still present after 290 days.

The hydrocarbon contamination in all three installations were therefore very similar after the first 100 days of depuration in the field (2-4 ppm MNs and 1-3 ppm MPs). This factor perhaps helps to explain the rather similar results obtained in benthic organism recruitment. It is still surprising that a greater inhibition of recruitment did not occur, when initial concentrations of hydrocarbons were quite high and levels after 100 days would appear to deleterious. With the approach used in this study, it is still possible to follow variations in populations over many more months to observe long-term effects. The apparent depression in bivalve populations may prove to be significant at later intervals. It is indeed difficult or impossible to compare the effects (or lack of it) discussed in this study with those of other field investigations where data on the composition of hydrocarbon contamination is not described. Armstrong *et al.*, (1977) found the levels in mud near a separator platform to contain 12.4 ppm total naphthalenes and 2.2 ppm total phenanthrenes, and the benthos was depressed. Based on these findings, the recruitment measured in this study should have been affected by the oil. These differences in results are likely due to the numerous variations in environmental factors which have made comparisons between spill studies so difficult. We have clearly demonstrated the effects of different types of oil introduction to sediment. Obviously, the composition of the substrate

markedly influences the rate of oil depuration and the effects on biota. The longest duration of oil effects on benthic populations has been 7 years, as recently reported by Michael *et al.* (1977). The type of oil, means of introduction, and habitat affected at West Falmouth, Massachusetts, would seem to be the worst possible combination for damaging the ecosystem. The coarse sediment in much of the intertidal zone of the Pacific Northwest has demonstrated a much more rapid recovery rate, based on hydrocarbon depuration. Effects on recruitment in this environment appear to be minimal at present, but more thorough analyses of organisms and continued sampling must be conducted before a definitive conclusion can be made.

Acknowledgements

We wish to express our appreciation to Laurie Rasmussen, Jennie Linth, Dana Woodruff and Kathy Probasco for compiling the extensive amount of biological and chemical data presented in this manuscript. This research was funded through the EPA/ERDA Pass-through Program on Contract No. 77BCF-EHA540-MI.B.3.a.

American Petroleum Institute. 1958. Determination of volatile and non-volatile oil material: Infrared spectrometric method. American Petroleum Institute, Publication No. 733-48.

Anderson, J. W., L. J. Moore, J. W. Blaylock, D. L. Woodruff, and S. L. Kiesser. 1977. Bioavailability of sediment-sorbed naphthalenes to the sipunculid worm, *Phascolosoma agassizii*, pp. 276-285, *In:* Fate and Effects of Petroleum in Marine Organisms and Ecosystems, D. A. Wolfe, ed., Pergamon Press, New York.

Armstrong, H. W., K. Fucik, J. W. Anderson and J. M. Neff. 1977. Effects of oilfield brine effluent on benthic organisms in Trinity Bay, Texas. American Petroleum Institute, Publication No. ____.

Beynon, L. R. and E. B. Cowell, eds. 1974. Ecological Aspects of Toxicity Testing of Oils and Dispersants. John Wiley and Sons, New York.

Biere, R. H. and V. C. Stamoudis. 1977. The fate of petroleum hydrocarbons from a No. 2 Fuel Oil spill in a seminatural estuarine environment, pp. 332-344, *In:* Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, D. A. Wolfe, ed., Pergamon Press, New York.

Blumer, M. and J. Sass. 1972. Oil Pollution: Persistence and degradation of spilled fuel oil. Science, 176, 1120-1122.

EXXON Production Research Company. 1974. Research Needed to Determine Chronic Effects of Oil on the Marine Environment. Workshop Proceedings, Houston, Texas.

Krebs, C. T. and K. A. Burns. 1977. Long-term effects of an oil spill on populations of the salt-marsh crab *Uca pugnax*. Science 197, 484-487.

Lee, R. F. 1977. Accumulation and turnover of petroleum hydrocarbons in marine organisms, pp. 60-70, *In:* Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, D. A. Wolfe, ed., Pergamon Press, New York.

Lee, R. F. and J. W. Anderson. 1977. Fate and effect of naphthalenes: controlled ecosystem pollution experiment. Bulletin of Marine Science 27:127-134.

MacLeod, W. D., D. W. Brown, R. G. Jenkins, L. S. Ramos, and V. P. Henry. A Pilot Study on the Design of a Petroleum Hydrocarbon Baseline Investigation for Northern Puget Sound and Strait of Juan de Fuca. Report submitted to Puget Sound Energy-related Research Project, Marine Ecosystem Analysis Program, Environmental Research Laboratory, Seattle, Washington.

MacLeod, W. D., D. W. Brown, R. G. Jenkins and L. S. Ramos. 1977. Intertidal sediment hydrocarbon levels at two sites on the Strait of Juan de Fuca, pp. 385-396, *In:* Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, D. A. Wolfe, ed., Pergamon Press, New York.

Michael, A. D. 1977. The effects of petroleum hydrocarbons on marine populations and communities, pp. 129-137, *In:* Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, D. A. Wolfe, ed., Pergamon Press, New York.

Michael, A. D., C. R. Van Raalte and L. S. Brown. 1975. Long-term effects of an oil spill at West Falmouth, Massachusetts, pp. 573-582, *In:* Conference on Prevention and Control of Oil Pollution, American Petroleum Institute, Washington D. C.

McAuliffe, C. D., A. E. Smalley, R. D. Groover, W. M. Welsh and G. E. Jones. 1975. Chevron Main Pass Block 41 Oil Spill: Chemical and biological investigations, pp. 555-566, *In:* Conference on Prevention and Control of Oil Pollution, American Petroleum Institute, Washington D. C.

Neff, J. M., J. W. Anderson, B. A. Cox, R. B. Laughlin, Jr., S. S. Rossi and H. E. Tatem. 1976. Effects of petroleum on survival, respiration and growth of marine animals, pp. 515-539, *In:* Sources, Effects & Sinks of Hydrocarbons in the Aquatic Environment. American Institute of Biological Sciences, Arlington, Virginia.

Roesijadi, G., J. W. Anderson and D. L. Woodruff. 1977. Bioavailability of naphthalenes from marine sediments artificially contaminated with Prudhoe Bay Crude oil. Accepted for publication May 16, 1977, Environmental Pollution.

Roesijadi, G., J. W. Anderson and J. W. Blaylock. 1978. Uptake of hydrocarbons from marine sediments contaminated with Prudhoe Bay Crude oil: Influence of feeding type of test species and availability of polycyclic aromatic hydrocarbons. To be presented at the International Symposium Oil/Environment, 1977, October 11-14, 1977, Halifax, Nova Scotia Canada.

Shaw, D. G., A. J. Paul, L. M. Cheek, and H. M. Feder. 1976. *Macoma balthica:* An indicator of oil pollution. Marine Pollution Bulletin 7, 29-31.

Vandermeulen, J. H. and P. P. Keizer. 1977. Persistence of non-alkane components of Bunker C oil in beach sediments of Chedabucto Bay, and lack of their metabolism by molluscs, pp. 469-474, *In:* Oil Spill Conference, American Petroleum Institute, Washington D. C.

Wormald, A. P. 1976. Effects of a spill of marine diesel oil on the meiofauna of a sandy beach at Picnic Bay, Hong Kong. Environmental Pollution 11, 117-130.

Figure Legends

Figure 1. Gas capillary chromatogram of saturate fraction of Prudhoe Bay Crude oil: $C_{11} \rightarrow C_{26}$, saturate straight chain hydrocarbons, Pr = pristane, Ph = phytane, Is = 2, 6, 10-trimethyldodecane internal standard.

Figure 2. Gas capillary chromatogram of aromatic fraction of Prudhoe Bay Crude oil.

Figure 3. Concentrations of docosane, naphthalene, and phenanthrene in sediment of installation I, as a function of depuration time.

Figure 4. Concentrations of compound classes in sediments of installation I as a function of depuration time.

Figure 5. Concentrations of docosane, naphthalene, and phenanthrene in sediment of installation II, as a function of depuration time.

Figure 6. Concentrations of compound classes in sediments of installation II, as a function of depuration time.

Figure 7. Concentrations of docosane, naphthalene, and phenanthrene in sediment of installation III, as a function of depuration time.

Figure 8. Concentrations of compound classes in sediments of installation III, as a function of depuration time.

Figure 9. Relationship between the number of recruitment organisms in sediment cores and time after sediment oiling in three installations. Installations I and II received oil on the surface of coarse sediment (< 12 mm), while 0.1% oil was mixed with a finer sediment (< 2 mm) in installation III.

Figure 10. Relationship between the abundance of *Mysella tumida* in sediment cores of 3 installations and season. Solid lines represent control (C) sediments while exposed (E) substrate is shown with a dashed line. The duration in the field is shown above the time intervals, except for installation III, which had only been in place for 110 days in February, and 190 days in May.

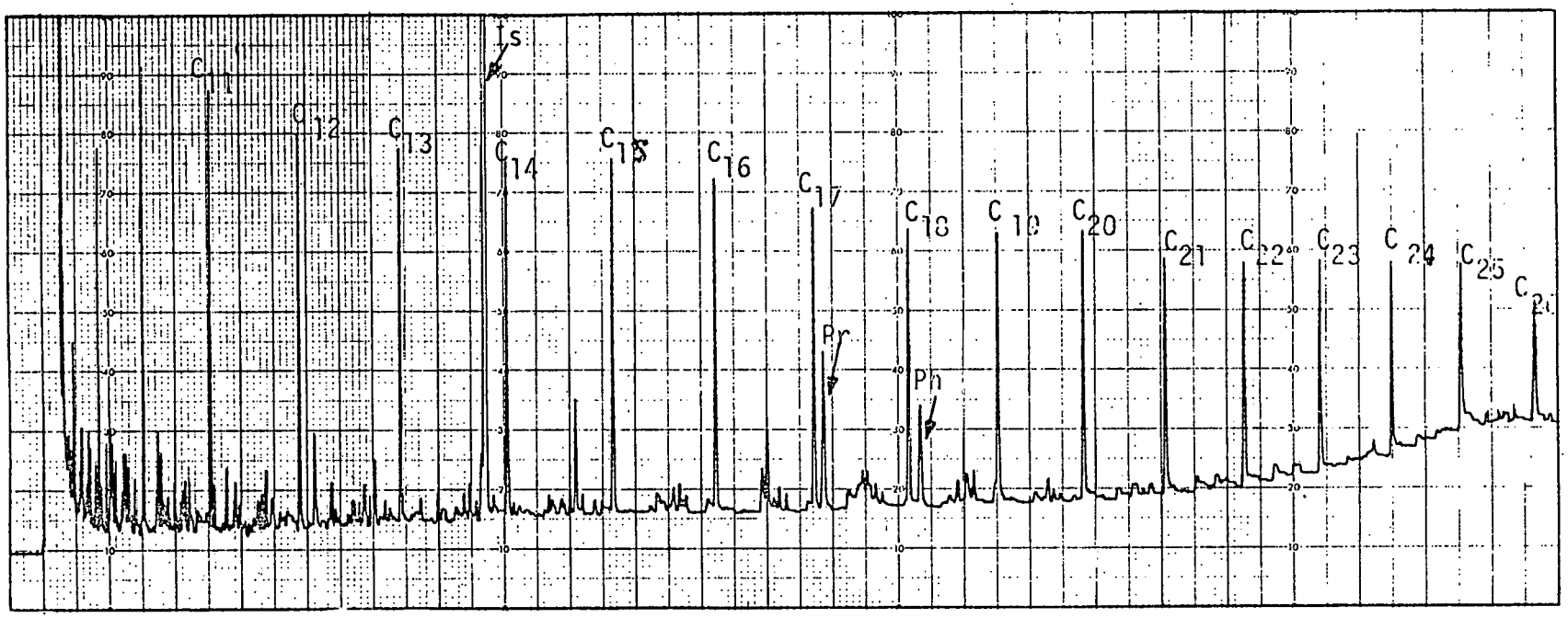


Figure 1. Gas capillary chromatogram of saturate fraction of Prudhoe Bay Crude oil: $C_{11} \rightarrow C_{26}$, saturate straight chain hydrocarbons, Pr = pristane, Ph = phytane, Is = 2, 6, 10-trimethyldodecane internal standard.

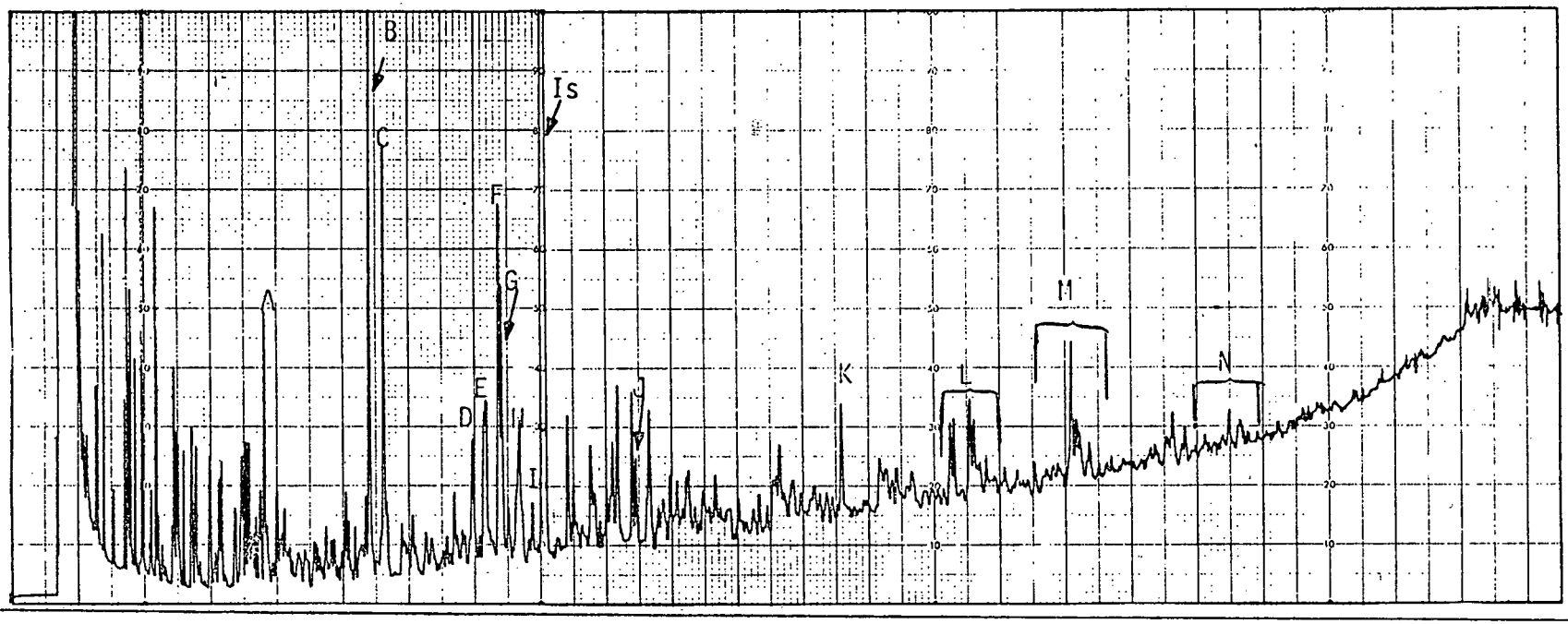
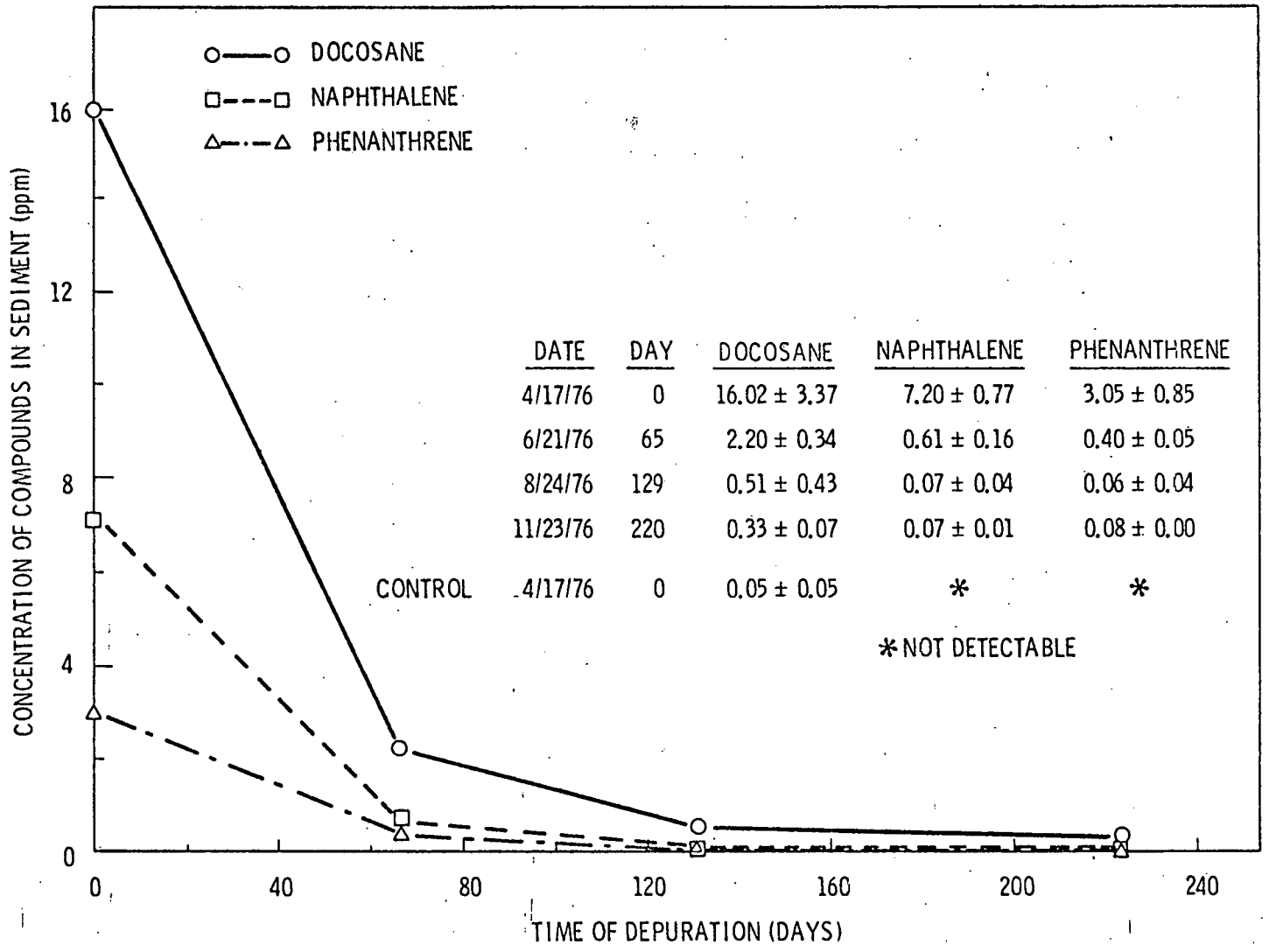


Figure 2. Gas capillary chromatogram of aromatic fraction of Prudhoe Bay Crude oil.

A. Naphthalene
B. 2-Methylnaphthalene
C. 1-Methylnaphthalene
D. 1-Ethyl + 2-Ethylnaphthalene
E. 2,6 + 2,7*-Dimethylnaphthalene
F. 1,3 + 1,6-Dimethylnaphthalene
G. 1,7*-Dimethylnaphthalene
H. 1,4 + 2,3 + 1,5-Dimethylnaphthalene
I. 1,2-Dimethylnaphthalene
J. 2,3,6-Trimethylnaphthalene
K. Phenanthrene
L. Methylphenanthrenes
M. C_2-Phenanthrenes
N. C_3-Phenanthrenes
Is = Hexamethylbenzene internal standard

*2,7 and 1,7 are suggested structures based on published literature on the law resolution gas chromatographic separation of dimethylnaphthalenes.



DATE	DAY	DOCOSANE	NAPHTHALENE	PHENANTHRENE
4/17/76	0	16.02 ± 3.37	7.20 ± 0.77	3.05 ± 0.85
6/21/76	65	2.20 ± 0.34	0.61 ± 0.16	0.40 ± 0.05
8/24/76	129	0.51 ± 0.43	0.07 ± 0.04	0.06 ± 0.04
11/23/76	220	0.33 ± 0.07	0.07 ± 0.01	0.08 ± 0.00
CONTROL 4/17/76	0	0.05 ± 0.05	*	*

*NOT DETECTABLE

Figure 3. Concentrations of docosane, naphthalene, and phenanthrene in sediment of installation I, as a function of depuration time.

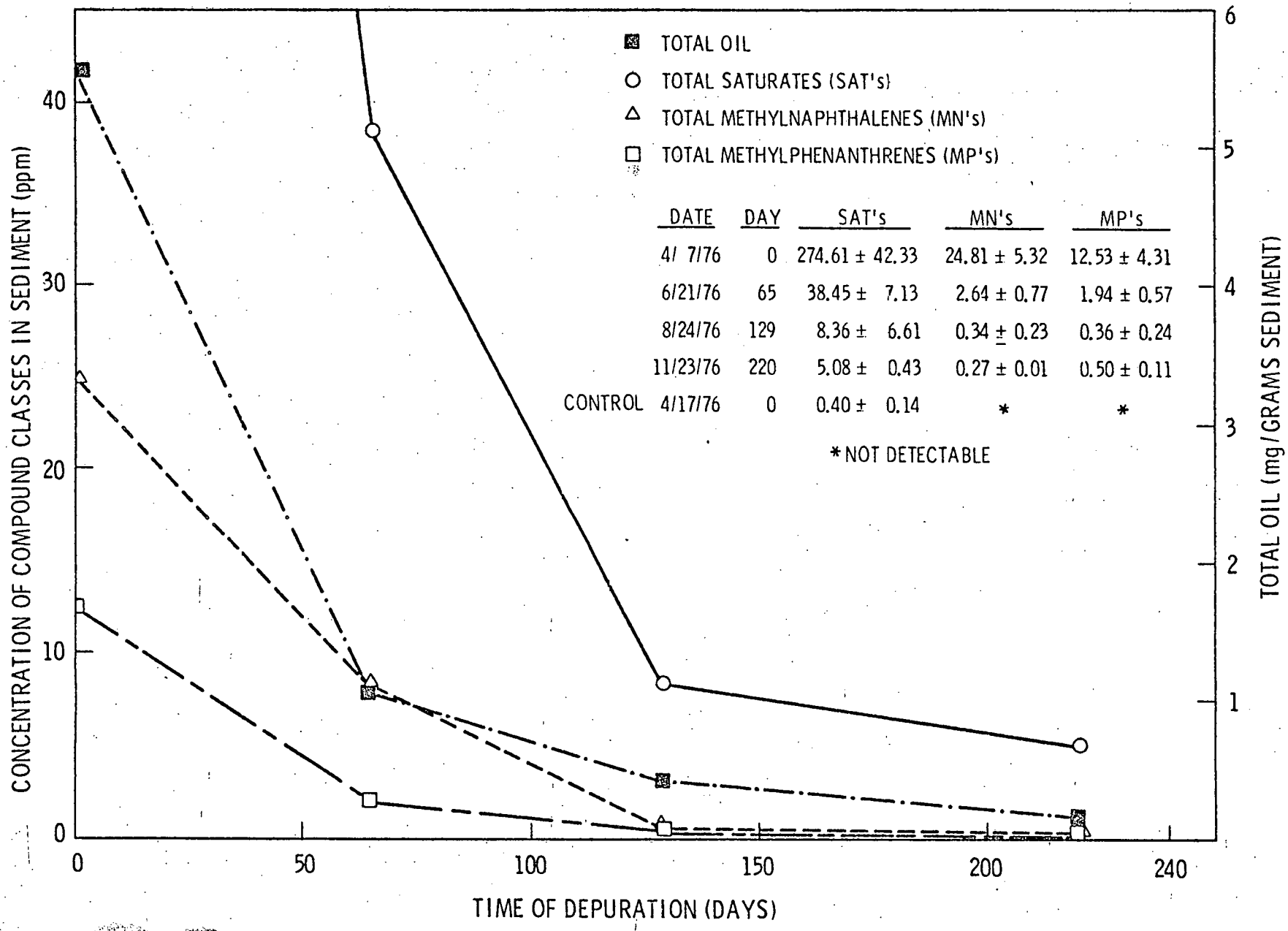
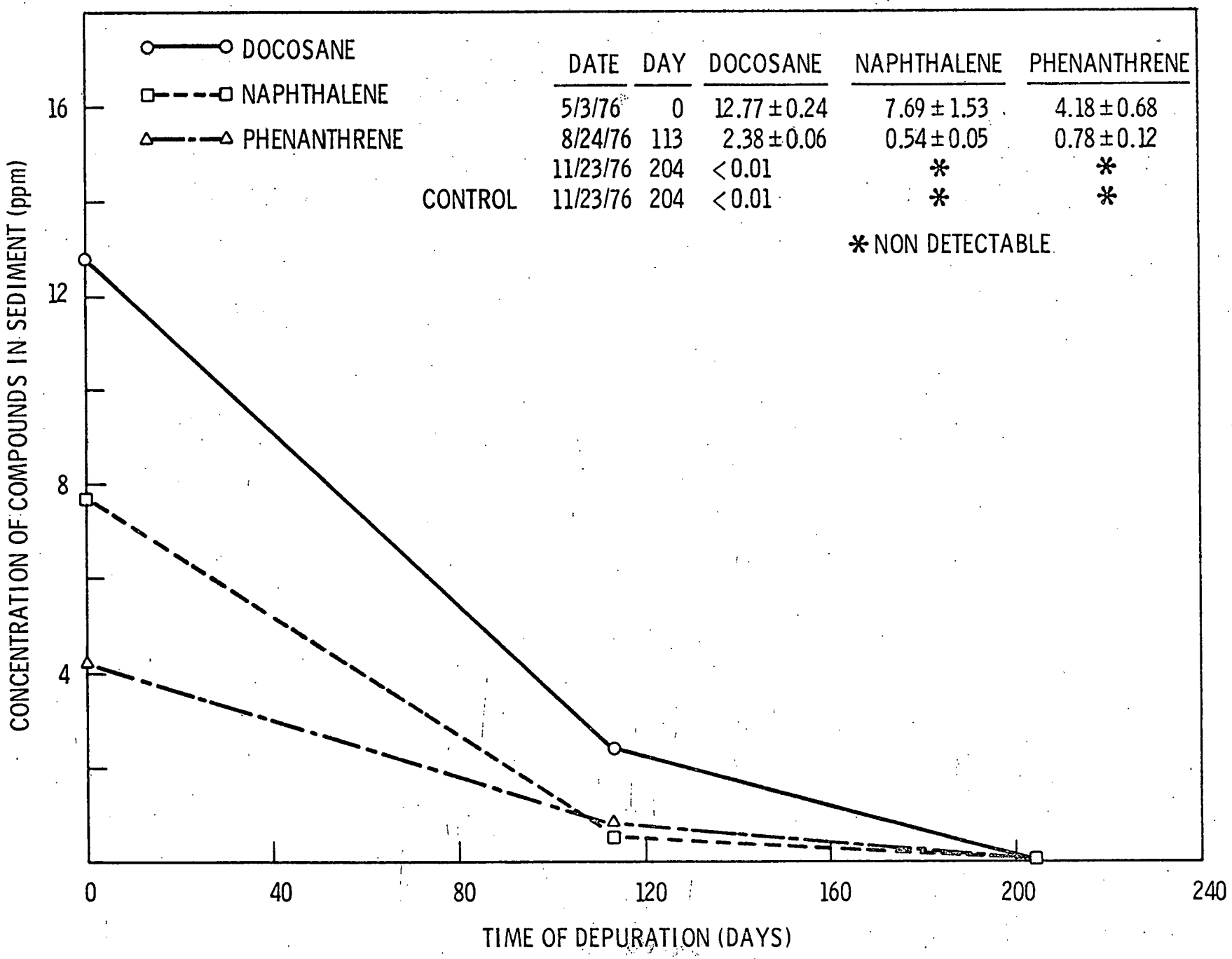
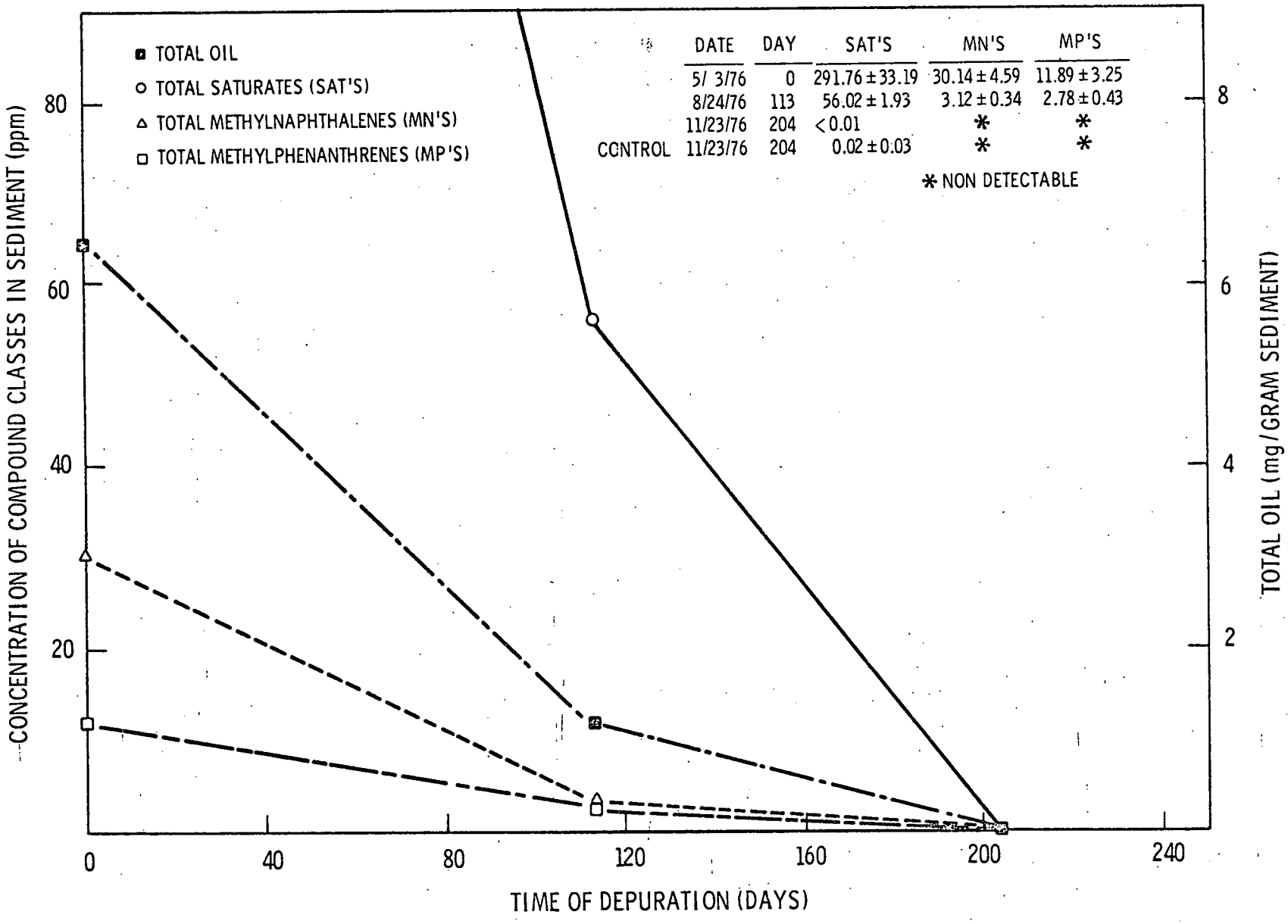


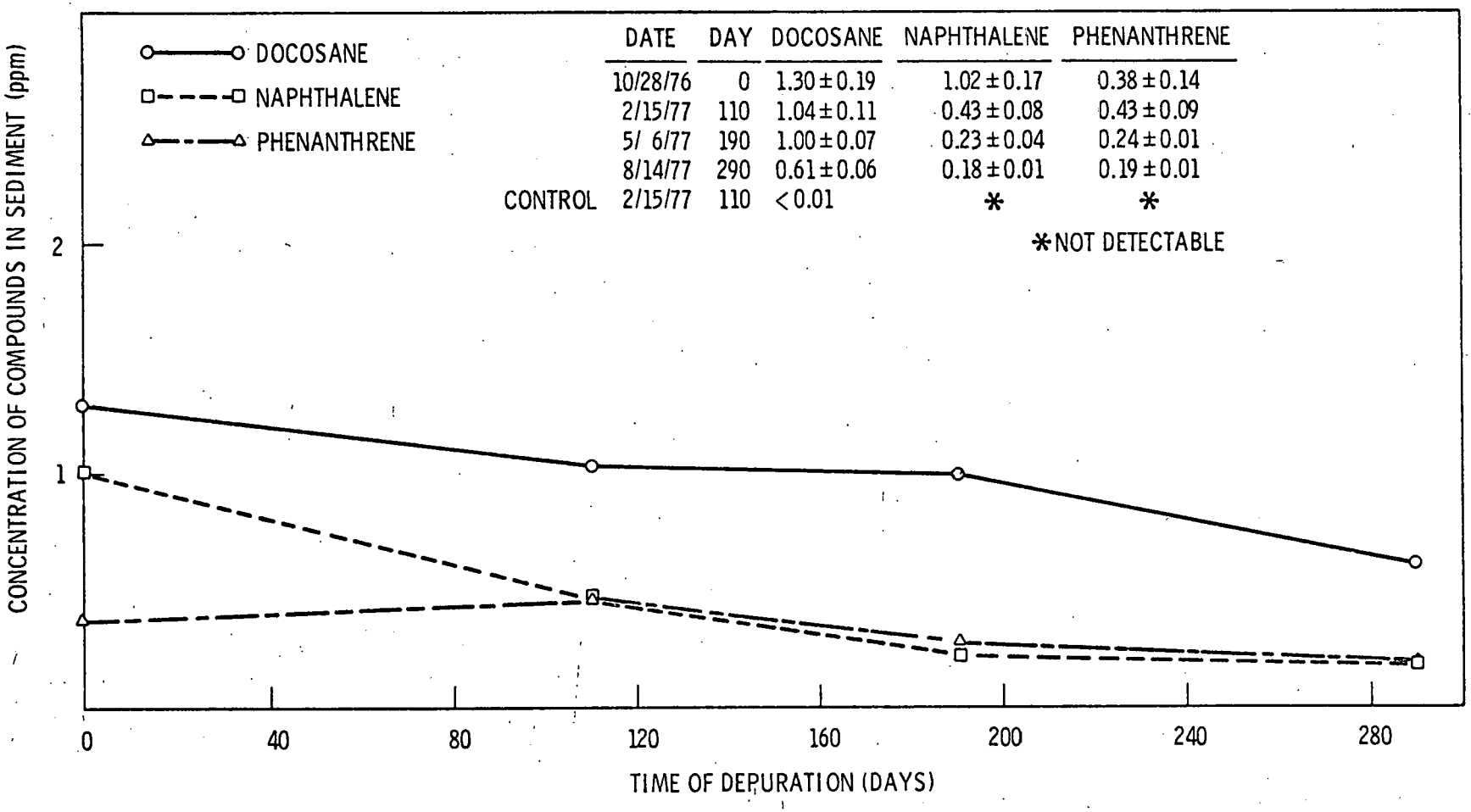
Figure 4. Concentrations of compound classes in sediments of installation I, as a function of depuration time.



	DATE	DAY	DOCOSANE	NAPHTHALENE	PHENANTHRENE
	5/3/76	0	12.77 ± 0.24	7.69 ± 1.53	4.18 ± 0.68
	8/24/76	113	2.38 ± 0.06	0.54 ± 0.05	0.78 ± 0.12
	11/23/76	204	< 0.01	*	*
CONTROL	11/23/76	204	< 0.01	*	*

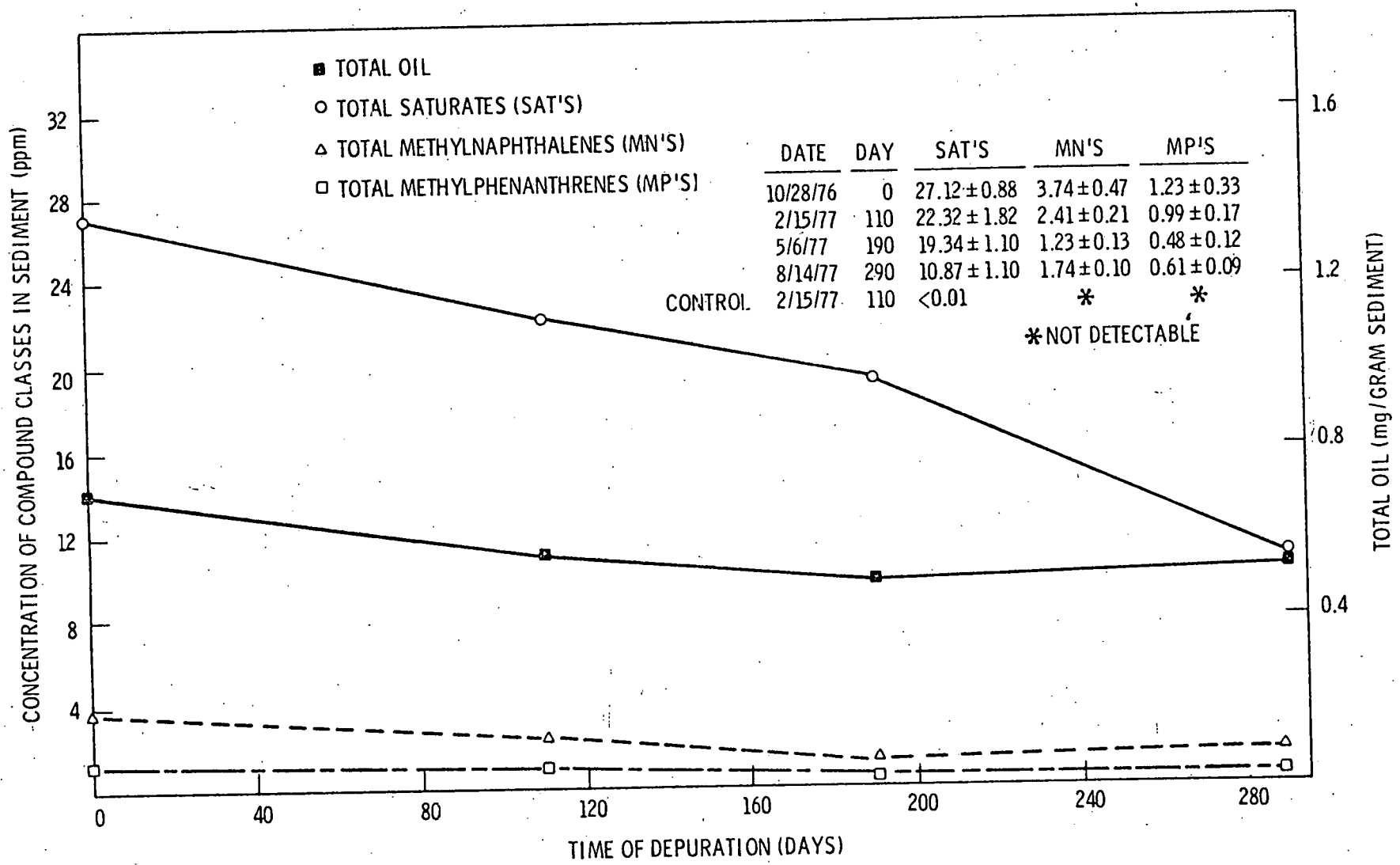
* NON DETECTABLE


TOTAL OIL
TOTAL SATURATES (SAT'S)
TOTAL METHYLNAPHTHALENES (MN'S)
TOTAL METHYLPHENANTHRENES (MP'S)
DATE DAY SAT'S MN'S MP'S
5/ 3/76 0 291.76 ± 33.19 30.14 ± 4.59 11.89 ± 3.25
8/24/76 113 56.02 ± 1.93 3.12 ± 0.34 2.78 ± 0.43
11/23/76 204 < 0.01 * *
CONTROL 11/23/76 204 0.02 ± 0.03 * *
* NON DETECTABLE
CONCENTRATION OF COMPOUND CLASSES IN SEDIMENT (ppm)
TOTAL OIL (mg/GRAM SEDIMENT)
TIME OF DEPURATION (DAYS)
0
20
40
60
80
2
4
6
8
0
40
80
120
160
200
240



	DATE	DAY	DOCOSANE	NAPHTHALENE	PHENANTHRENE
	10/28/76	0	1.30 ± 0.19	1.02 ± 0.17	0.38 ± 0.14
	2/15/77	110	1.04 ± 0.11	0.43 ± 0.08	0.43 ± 0.09
	5/ 6/77	190	1.00 ± 0.07	0.23 ± 0.04	0.24 ± 0.01
	8/14/77	290	0.61 ± 0.06	0.18 ± 0.01	0.19 ± 0.01
CONTROL	2/15/77	110	< 0.01	*	*

*NOT DETECTABLE



DATE	DAY	SAT'S	MN'S	MP'S
10/28/76	0	27.12 ± 0.88	3.74 ± 0.47	1.23 ± 0.33
2/15/77	110	22.32 ± 1.82	2.41 ± 0.21	0.99 ± 0.17
5/6/77	190	19.34 ± 1.10	1.23 ± 0.13	0.48 ± 0.12
8/14/77	290	10.87 ± 1.10	1.74 ± 0.10	0.61 ± 0.09
CONTROL 2/15/77	110	<0.01	*	*

*NOT DETECTABLE

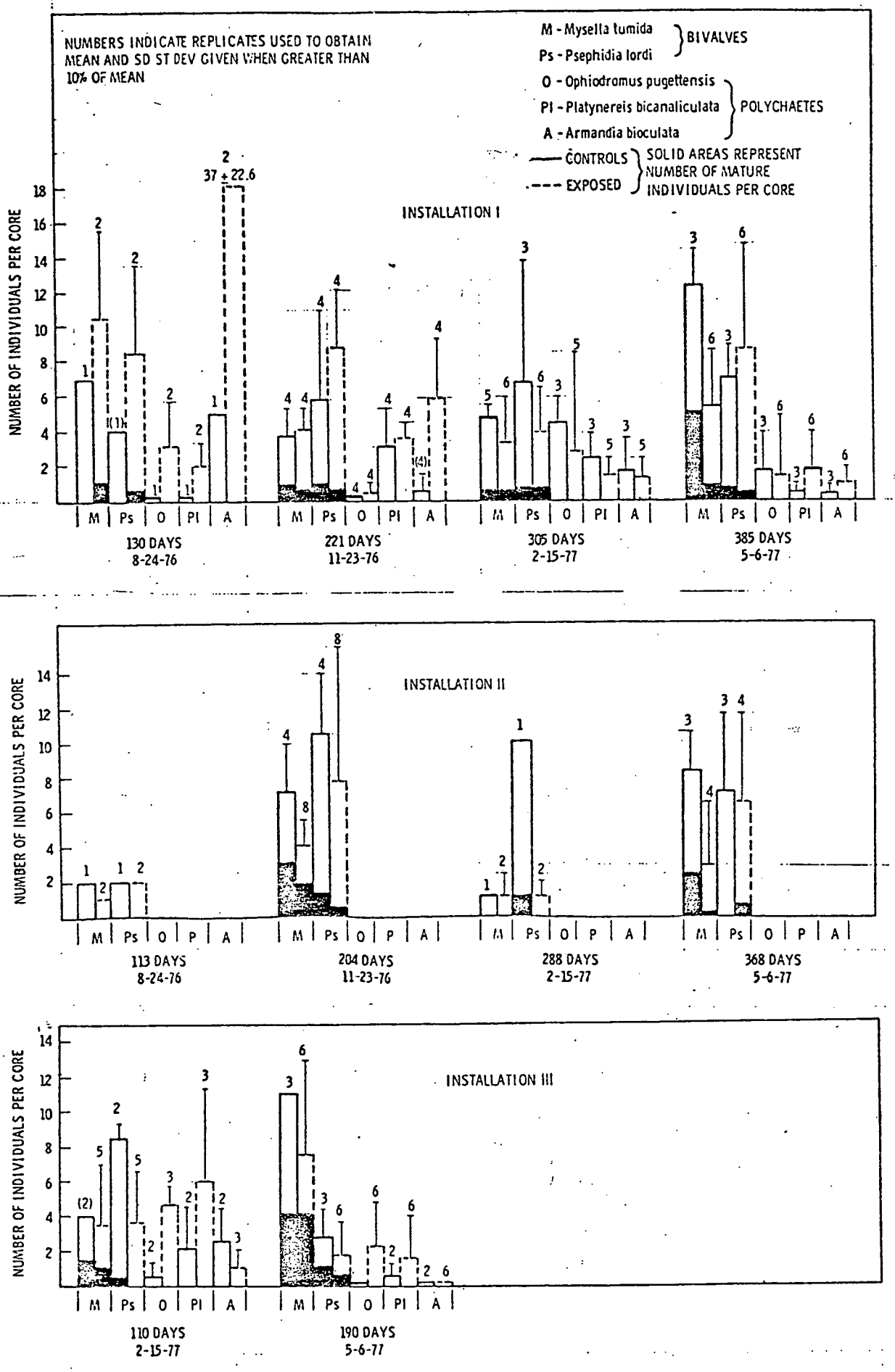
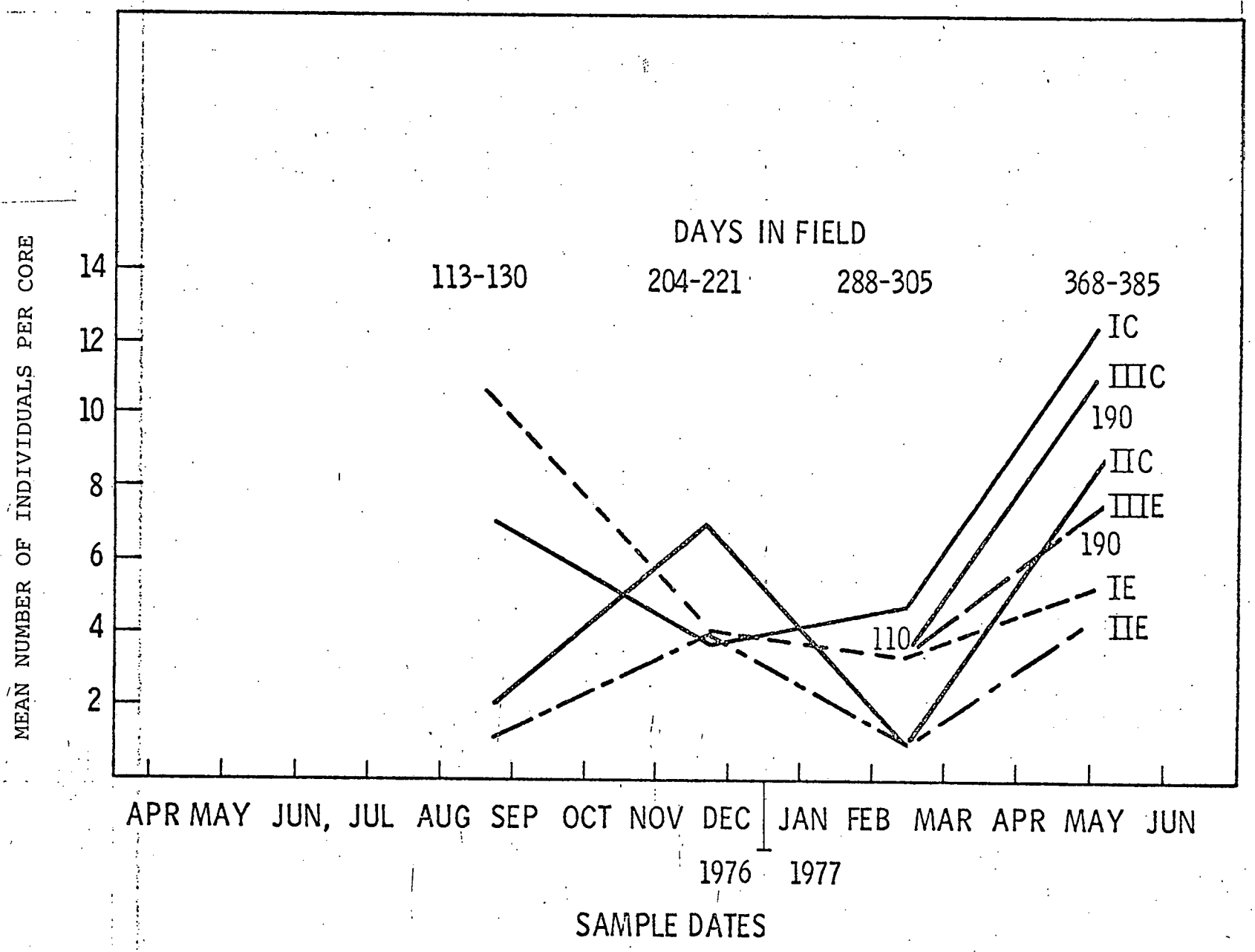

NUMBERS INDICATE REPLICATES USED TO OBTAIN MEAN AND SD ST DEV GIVEN WHEN GREATER THAN 10% OF MEAN
M - Mysella tumida
Ps - Psephidia lordi
BIVALVES
O - Ophiodromus pugettensis
PI - Platynereis bicanaliculata
A - Armandia bioculata
POLYCHAETES
CONTROLS
EXPOSED
SOLID AREAS REPRESENT NUMBER OF MATURE INDIVIDUALS PER CORE
INSTALLATION I
NUMBER OF INDIVIDUALS PER CORE
37 ± 22.6
M Ps O PI A
130 DAYS 8-24-76
221 DAYS 11-23-76
305 DAYS 2-15-77
385 DAYS 5-6-77
INSTALLATION II
M Ps O P A
113 DAYS 8-24-76
204 DAYS 11-23-76
288 DAYS 2-15-77
368 DAYS 5-6-77
INSTALLATION III
110 DAYS 2-15-77
190 DAYS 5-6-77

Figure 9.

Figure 10.


MEAN NUMBER OF INDIVIDUALS PER CORE
14
12
10
8
6
4
2
DAYS IN FIELD
113-130
204-221
288-305
368-385
IC
IIIC
190
IIC
IIIE
190
IE
IIE
110
APR MAY JUN, JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN
1976
1977
SAMPLE DATES