NOAA Technical Memorandum OMPA-18

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ABSTRACT

Mounting concern about the potential effects of minor oil spills in the Puget Sound region led to the design and implementation of experiments whose purpose was to determine the effects of crude oil spills on selected members of hard substrate, subtidal marine communities. By oiling an early colonizing species of replicate substrates, we simulated the effects of a one-time oiling event on an initial hard substrate colonizer, the encrusting bryozoan Parasmittina trispinosa. Subsequent community development was then monitored over a period of several months. Experimental manipulations were conducted in the laboratory on panels which prior to and after manipulation events were positioned in the natural environment. Thus panels were subjected to natural events except for those we experimentally controlled. These experimental methods on artificial surfaces were determined to be feasible for further experimental substrates of this type.

Extensive and detailed chemical analyses performed throughout the recovery period showed that only four compounds of the 170 detected in the initially applied crude oil were retained by the benthic organisms, and that at an amount less than one percent of that applied. Only two of those compounds were detected after 10 days, and then at an order of magnitude lower concentration than was applied.

Comparison of community characteristics on substrates on which the bryozoan was either removed, or oiled with different concentrations of Prudhoe Bay crude oil, were made with a series of control substrates. When the bryozoan colonies were not removed, minimal effects on species numbers or percent cover were observable. Removal of this member of the benthic community in spring resulted in statistically significant percent cover differences for only short periods of time. Panels whose bryozoan colonies were removed but received no subsequent oiling of the vacant spot had a higher percent cover 10 days after manipulations than those panels receiving oil after the bryozoan removals. These panels also eventually achieved the highest total percent coverage. The next highest total percent coverage was found on panels whose bryozoans had been removed and whose empty spots were then oiled. No statistically significant effects could be detected in terms of species richness. High and low quantities of oil directly applied to the bryozoan colonies had no statistically significant effects. Our results suggest that in spring, when temperatures are warming, the presence of a vacated space eventually allows settlement of one or more species whose ability to usurp space on the panel surface is even greater than that of early-arriving colonists. The effect of oilings and removals of this particular encrusting species at this time of year is relatively rapidly obliterated by newly arriving colonists.

1. INTRODUCTION

Although not an oil producing area, Puget Sound ranks high as a transport region because of its growing population and its established refineries. Since transport of oil tends to parallel continental margins, areas of considerable marine productivity and proximity of large urban centers, there is increasing concern in these areas over the possibility of oil spills and their effects. As transport of oil increases, so does the potential for oil pollution. The devastation caused by major oil disasters elsewhere during the past two decades, as well as attempts to disperse these spills, serve as vivid and, in some cases, well-documented reminders of this problem (e.g., Southward and Southward, 1978). While major oil disasters, which may decimate significant fractions of the biota, are relatively infrequent, spills of lesser magnitude may occur with even greater frequency. These may affect only a small geographic region or a subset of the biological community. As yet there have been no major spills in Puget Sound, but minor spills have been reported (Woodin et al., 1972). In the present study we examined the potential damage induced by a small-scale, nonchronic spill on a hard substrate community in Puget Sound.

An oil spill may directly smother the biota, inhibiting growth or feeding for a period of time, and is therefore potentially lethal (Thomas, 1973). Toxicity due to the water soluble fraction of oil is an indirect lethal mechanism of considerable consequence (Laughlin et al., 1978). The latter mechanism is more important with respect to refined oils because of the presence of heavier aromatics, than with crude oils which have lighter aromatics and a better evaporation rate (Anderson et al., 1974).

Sublethal effects of oil pollution pose problems on both physiological and behavioral levels. There is ever-increasing documentation of sublethal effects on a variety of marine organisms. Instances of incomplete moulting in several crab species attest to this (Laughlin et al., 1978). Decreases in growth rates of corals exposed to crude oil for only 1-2 hours are also reported (Birkeland et al., 1976). On the other hand, these effects may sometimes be stimulatory as well as inhibitory to algae, depending upon oil type and algal species considered (Gordon and Prouse, 1973; Pulich et al., 1974). Stimulatory effects are not necessarily beneficial; however, as Loya and Rinkevich (1979) point out, the presence of oil can induce premature release of gametes in corals.

In our experiments we simulated the possibility that a nonchronic oil spill may occur in the area on a small scale. Rather than decimate the entire biota, it may selectively inhibit activity, or at the extreme, eliminate a particular member of the community. Those species that may be affected are more susceptible to oil effects than others in the community either because of their differential intolerance to oil, or because of their chance proximity to oil while other species in the community are protected. Once this hypothetical oil event occurs it is not repeated; thus, our experiments report the results of a one-time oiling, after which the community is allowed to recover.

2. MATERIALS AND METHODS

2.1 Materials

Artificial substrates were chosen for these experiments because of the need for extensive replication with the assurance that experimental surfaces were identical. Previous experimentation over a range of panel sizes indicated that 15.3 x 15.3 cm panels were considerably better as measured by their ability to maintain total species richness, than those of smaller sizes, while nearly as good as areas several times as large (Schoener and Schoener, 1981). Various types of materials have been tested for their ability to attract and maintain fouling species (e.g., Pomerat and Weiss, 1946); textured surfaces generally are more successful than smooth surfaces. Reassuringly, recent comparisons of the processes and biotas occurring on both artificial and natural surfaces indicate that they are very similar (Sousa, 1979). Studies of natural subtidal biota from the Strait of Juan de Fuca and the west coast of Whidbey Island (Webber, 1979; Nyblade, 1978) often recorded the same species of algae, polychaetes, and mollusks present as were present on our panels. Other attributes of artificial substrates that make them attractive for experimental purposes are that experiments could be extended to other regions for comparison without introducing additional variability due to substrate differences.

Our arrays consisted of 10 identical panels apiece. They were suspended between a subsurface buoy and two cement anchors. Panels measuring 15.3 x 15.3cm, with an additional handle for convenience, were cut from 0.64cm sheets of textured formica. Each panel was consecutively numbered on its reverse side and then attached to a 122cm long PVC bar by nylon screws and nuts.

2.2 Field Methods

Seven arrays, totalling 70 panels, were simultaneously submerged in early fall 1979. At each observation time thereafter, divers removed racks holding panels from each array; these racks were transported via ship in a box kept damp with sprayed seawater until they reached the laboratory sea tables. They were then maintained in running seawater for periods of less than 3 days. After observations were completed, panels were returned to their field position, although no attempt was made to match individual racks with individual arrays, as water clarity was generally too poor to enable divers to attempt this.

2.3 Field Site

The field site is located off Burrows Island, a small island off the southwest tip of Fidalgo Island, near Anacortes, Washington (Figure 1). Since the island itself is a rock island, we expected the variety of sessile species present there to be similar to those settling on our experimental surfaces. In addition, we expected that these species would be found in other areas of the Sound as well. Data from Webber (1979) at

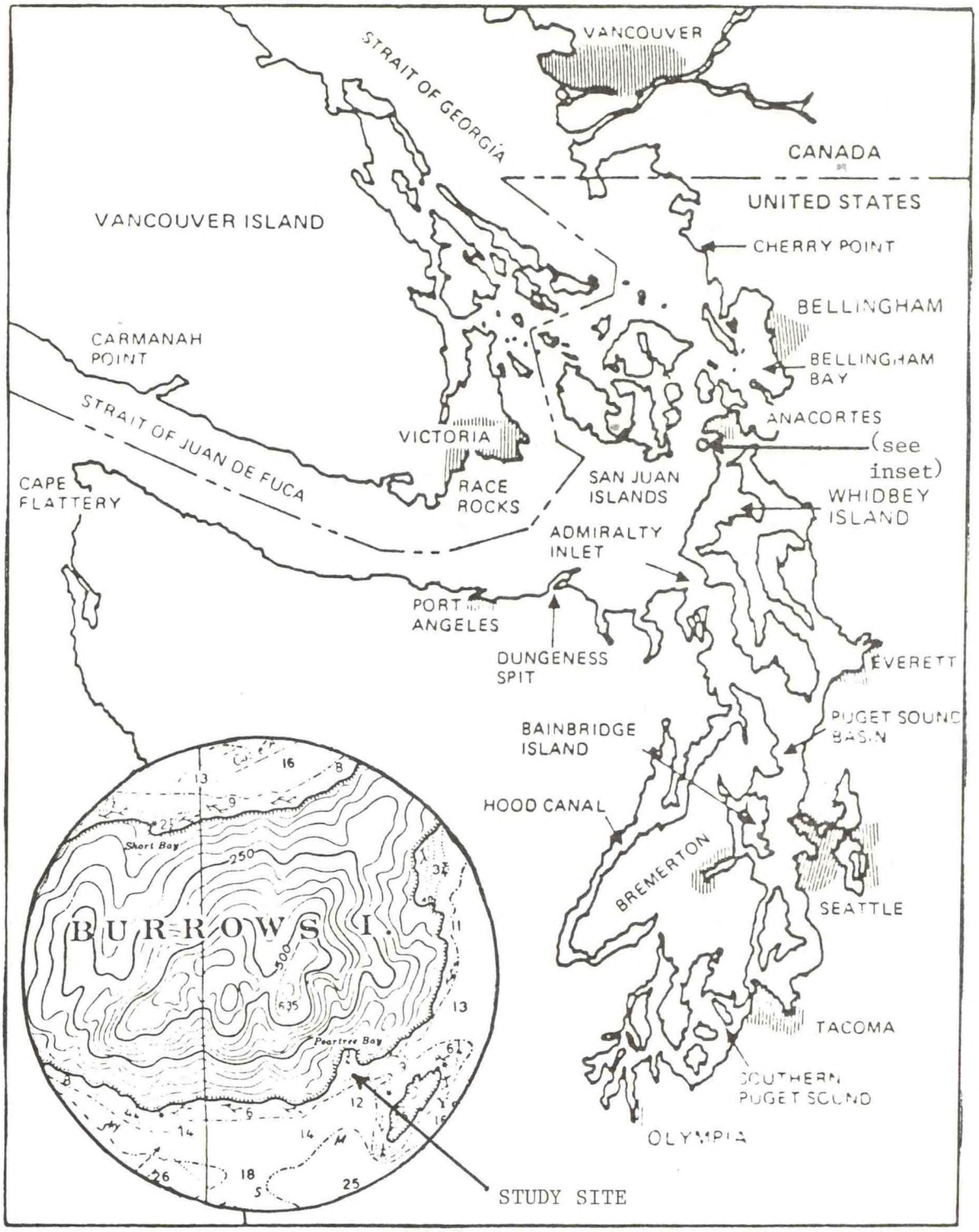

Figure 1. Map of Puget Sound, with inset showing study site.

subtidal cobble beaches in the fall have many of the same algal species, as well as some mollusks, barnacles, and polychaetes. Since bryozoans were not generally prevalent on the cobble, we can make no comparison. Nyblade's (1978) samples from 5m - 10m at Pillar Point, Morse Creek, and Tongue Point had many similarities with the species found on our panels.

Panels were submerged on September 24, 1979, in Peartree Bay, a small southfacing bay on the southeastern tip of the island, having a muddy bottom. As shown on the map (Figure 1), the bay is shallow and somewhat protected. Little current movement occurs inside the bay, although currents of ca. 2 knots are at times reported (P. Cassidy, pers. comm.). Extensive siltation generally occurred on the upper side of horizontally positioned panels. Since our studies concern only the underside of these panels, we removed this silt prior to each laboratory examination of panels.

Water depth ranged from ca. 7m to 13m in the bay where the arrays were positioned. Divers adjusted each of the 7 racks underwater so that all panels floated at depths of ca. 3m below mean tide level. Arrays held by two 45.5kg cement anchors apiece were set ca. 7m apart from one another by moving a little distance from the first array before dropping the next. Three arrays were positioned on the outer edge of the bay and four towards the inside.

To enable divers to locate arrays when turbidity was severe, a line was tied joining the base of the anchors so that upon locating the line divers could find all 7 arrays. Lines were fitted with weak links at intervals, in case anchors were snagged by boaters. We anticipated that this study site would not be fished by net-type fishermen. This site had an additional advantage in its proximity to the Sundquist Marine Laboratories. Other underwater experiments had been successfully undertaken there previously.

During the course of these experiments, salinity and water temperatures were monitored by water samples by the Sundquist Marine Laboratory, ca. 2 miles distant from the study site. Although these data are then not precisely comparable, the continuity of their records is far more complete than our own isolated sampling would have produced. Figure 2 summarizes their data for these parameters during the period of the experiment.

2.4 Laboratory Procedures

2.4.1 Sampling Schedule

Observations were made on panels initially on a monthly basis until sufficient growth developed for experimental manipulations to begin. The late fall submergence time for panels precluded their developing a growth sufficient for experimental purposes until the following spring. Therefore, although we intended to perform both a fall and a spring series of experiments, only the latter could be carried out. On June 13, 1980,

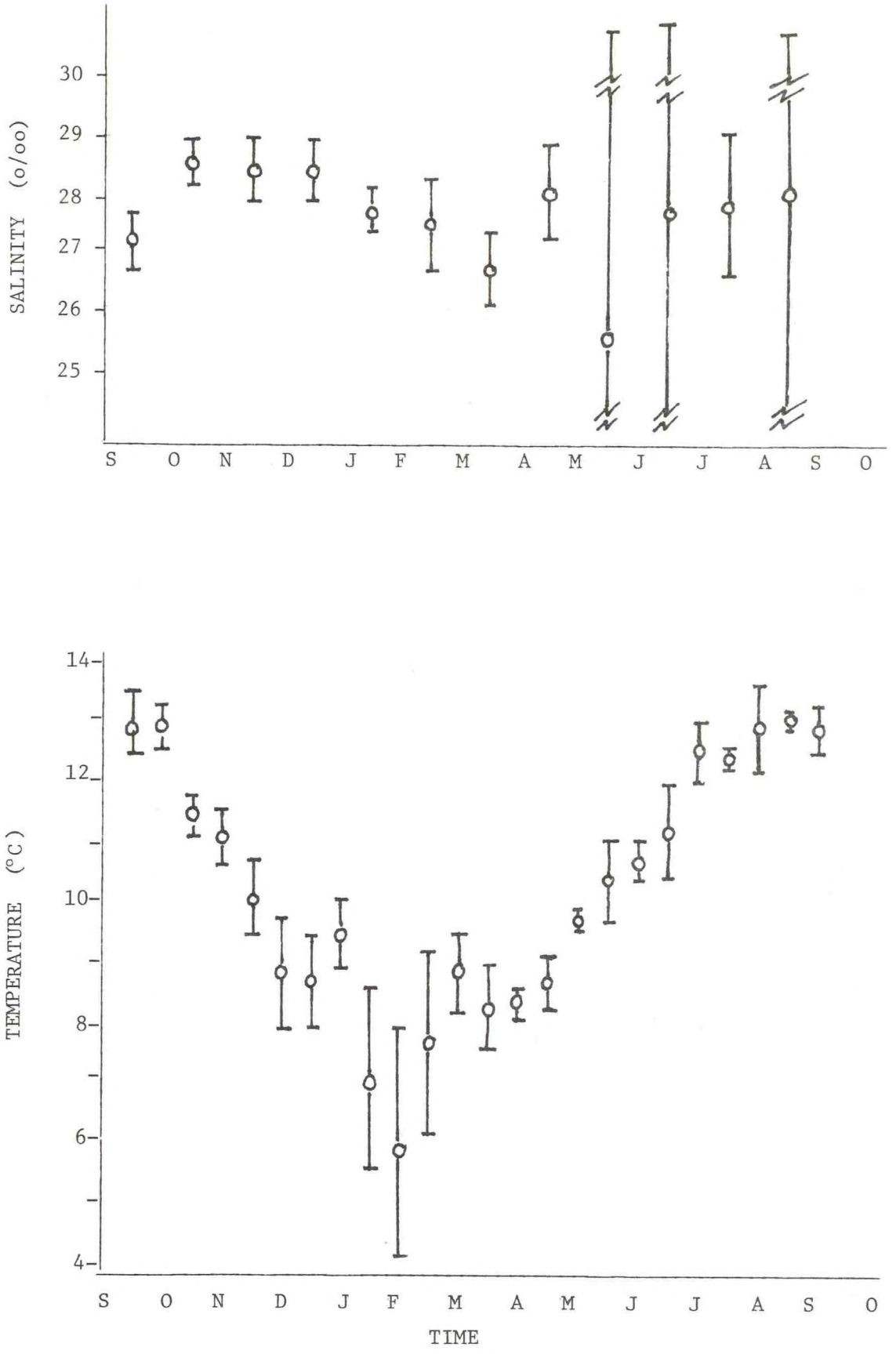

Figure 2. Physical parameters ascertained from water samples collected by P. Cassidy at Sundquist Marine Laboratory dock.

manipulations began and sampling subsequently became more frequent. The biological sampling schedule is listed in Table 1, and the schedule of oil samplings in Table 2. After manipulations were made, panels were returned to their original racks. At subsequent times censuses were made in a double-blind mode without knowledge of which panel had been treated or manipulated in what way.

2.4.2 Biological Technique

Throughout the course of this study nondestructive censuses were made on panels, i.e., panels were observed in seawater and then returned to their field site for further colonization. The identities of the attached biota and their percent cover were recorded. Organisms 0.5mm and larger were identified and representative panels photographed at each observation. Since a nondestructive sampling technique was utilized, the colonization sequence of individual panels could be followed through time. Censuses of each panel generally required less than half an hour apiece and panel biota appeared unharmed by this observation technique, as is generally noted when this procedure is employed (Sutherland, 1974; Schoener and Schoener, 1981). The number of species attached directly to panels are computed to give a measure of species richness. In order to provide an estimate of percent cover, 75 random points generated anew at each census were plotted on a clear plastic sheet. Sutherland (1974), utilizing this number of points on panels of dimensions equal to our experimental panels, determined that by tallying the number of points under which species are present, an estimate of percent cover could be obtained within 5% accuracy of the actual areas as computed by tracing outlines projected onto graph paper with a camera lucida.

Experimental manipulations were begun once growth of species on panels was under way. Prior to oil treatments all 70 panels were compared in terms of species numbers and abundance of major species covering panels. Monitoring panels monthly allowed observation of the species and their proportions on panels. Panels not colonized successfully by similar species were not considered further for experimental purposes, although they were maintained at the position on the racks. In this manner the experiment could be evaluated in a double-blind mode. From similar panels five groupings were designated and random numbers were generated in order to assign panels to one of the following categories:

1. Controls (N = 8);
2. Selected species removals with no oil added (N = 9);
3. Selected species removed and vacant spot oiled (N = 7);
4. Selected species oiled lightly (N = 6);
5. Selected species oiled heavily (N = 7).

Choice of species to be manipulated was necessarily limited to those present on a majority of the panels, as well as to those whose resistance to oil was relatively slight. In order to establish species-specific oil tolerance, we consulted the literature as well as specialists in the field. Craddock (1977) summarized an impressive literature of macroorganisms whose tolerance to petroleum have been investigated.

Table 1. Sampling schedule for biological analyses.

Time (months) after panel submergence Sept. 24-25, 1979	Date	Number of panels examined
1	Oct. 25, 1979	58
2	Nov. 27, 1979	70
3	Dec. 18, 1979	70
4	Feb. 5, 1980	58
6	March 18, 1980	70
7	April 14, 1980	48
7 1/2	May 5, 1980	48
8 1/2	June 2, 1980	48
8 3/4 experimental oiling	June 13, 1980	0
9	June 23, 1980	48
9 1/2	July 7, 1980	48
10	July 30, 1980	48
11	August 27, 1980	48

Table 2. Sampling schedule for chemical analyses.

Time after oiling of panels	Date	Number of panels collected
0 days (0.5 hrs.)	June 13, 1980	2
10 days	June 23, 1980	2
21 days	July 7, 1980	2
38 days	July 30, 1980	2
72 days	August 27, 1980	2

Although for the most part the organisms tested are motile, some data exist relevant to sessile biota, e.g., hydroids, pelecypods and barnacles. None of the species for which oil tolerance had been measured were those we observed in the present study, except for the barnacle Balanus crenatus. That B. crenatus exhibited (73%) mortality after 96 hours of exposure to oil at 30-1,000 ppm seemed to imply considerable tolerance. Their calcareous skeletons may protect them from the effects of oil. To supplement information from the literature we conducted some preliminary experiments to evaluate the effects of oil on the various members of our panel community. Oil droplets were placed on isolated individuals or colonies of test organisms, which were submerged in petri dishes of seawater. Frequent observations were made to assess the effects of direct oilings on these test organisms. From the preliminary observations (Tables 3 and 4) we chose the encrusting bryozoan Parasmittina trispinosa for removal. This bryozoan, encrusting on panel surfaces, is a filter feeder. Although possessing a calcareous skeleton, the zooids protrude through lattice-like openings during feeding. Oil was occasionally observed trapped in the zooid.

Recognition of the desirability of precisely determining the amount and type of oil present, both initially and as the experiment proceeded, led us to devote considerable attention to the chemical composition of the oil and its detection throughout the course of our experiments.

2.4.3 Chemical Technique

Prudhoe Bay crude oil was utilized in these experiments. Oil was applied to experimental panels after they drip-dried out of water for 2-3 hours. In order to enhance oil absorption, excess moisture was removed from either the bryozoan colonies or the spot left by their removal, by applying a cleansing tissue to the surface. In addition to the oiling of experimental panels, five additional panels with fouling growth were selected from the remaining panels and were oiled, slated to be sacrificed at intervals for chemical analysis of oil retention. This consisted of adding 3 drops at 9 locations on each of the formica plates with growth present. Measurement of 10 individual oil drops gave a weight of 19.7 ± 1.6gm per drop while the diameter of the spot was 1.35 ± 0.13cm whose surface area was 1.43cm^2, giving an areal loading of 41.3mg/cm^2. As the weight of the bryozoans covering such an area was determined to be 14mg, this represents an oil loading of 4.2mg oil/mg bryozoan colony for the high level oiling, and 1.4mg oil/mg bryozoan colony for the low level oilings. Although our original design called for much lower levels of oil, our initial experiments indicated that higher levels of oil were warranted. After oiling, panels were transported by ship to the experimental site, requiring an additional hour. All panels were reattached to their submerged racks and periodically sampled for oil analysis (Table 2).

2.4.4 Chemical Analysis

Our objectives were to determine the composition of the Prudhoe Bay crude oil, so that its characteristic properties could be detailed initially and its traces detected subsequently as the experiment proceeded.

Table 3. Preliminary experiments with oiling of various fouling species on panels.

SPECIES	TIME (hours elapsed after oiling)							
	0	1/4	1/2	1	24	38	45	66
Parasmittina trispinosa	+	-	-	+	+	-	-	-
Callopora horrida	+	+		+	-	+	-	-
Hippothoa hyalina	+	-		-	-	-	-	-
Balanus crenatus	+	+	+	+	+	+	+	+
Clytia sp.	+	+	+	+	+	+	+	-

+ indicates some feeding appendage functioning; - indicates absence of motion.

Table 4. Effects of oil on Parasmittina trispinosa colony.

TIME (min)	RESULT	TIME (min)	RESULT	TIME (min)	RESULT
0	+	50	+	100	-
5	-	55	+	115	-
10	-	60	+	120	_
15	-	65	_	125	-
20	_	70	_	285	-
25	+	75	-	290	-
30	-	80	-	295	-
35	-	85	-	300	-
40	-	90	-	360	-
45	-	95	-		

+ indicates >10% of zooids functioning; - indicates ≤10% of zooids functioning

The crude oil was analyzed using gas chromatography (GC) and gas chromatography (GC)/mass spectroscopy (MS)/data searching (DS) techniques. Oil was first fractioned using gel permeation chromotography (GPC) with biobeads S-X2 to separate the lipids and fats from the hydrocarbons. The hydrocarbons, as analyzed by capillary GC, were further separated using high pressure liquid chromatography (HPLC) with a normal silica column. The sequence of steps in fractionation of oil components is shown in Figure 3.

3. RESULTS

3.1 Community Composition

Initial colonists during fall included the red algae Platythamnion pectenatum and Polysiphonia sp., barnacles Balanus crenatus, and stoloniferous hydroids, Campanulina sp. and Obelia sp. Panels were settled by the encrusting bryozoan Parasmittina trispinosa during the second month's submergence, along with occasional representatives of a second encrusting bryozoan, Tubulipora sp. Additionally, Calyptraea fastigiata and Serpula sp. settled at this time. By the fourth observation period, the mussel Mytilus edulis, and the solitary tunicate Corella willmeriana were occasionally observed. Other algal species were added, e.g. Pleonosporium squarosum, Desmarestia sp. and Laminaria sp. as time progressed. Several species of algae and encrusting bryozoans characterized much of the attached growth on panels, although other sessile benthic species were present.

3.2 Initial Colonization Rates

Initial colonization curves of panels are shown in Figure 4. The number of sessile species attached to panels rose slowly when panels were submerged in fall. Three months after submergence a mean of 4 species was observed and by eight months a mean of 9 species was found. Mean species richness could also be computed for the 6 panels later used as controls and which survived the experimental period. Comparison of these values with those of the entire series of panels available until the experiment began showed similar tendencies in both groups. This subset of 6 panels could be followed into the experimental period, and suggested further increase in species numbers during the experimental period on panels which were not subject to experimental manipulations.

3.3 Effects of Species Removal and Oilings

Total species richness and total percent cover for each treatment group are given in Tables 5 and 6. One way analysis of variance techniques were employed to determine the source and significance of the values among groups after manipulations were made. These results are shown for several different times for total number of sessile species (Tables 7-10) and in Tables 11-14 for percent cover on panels. Significant sources of variation

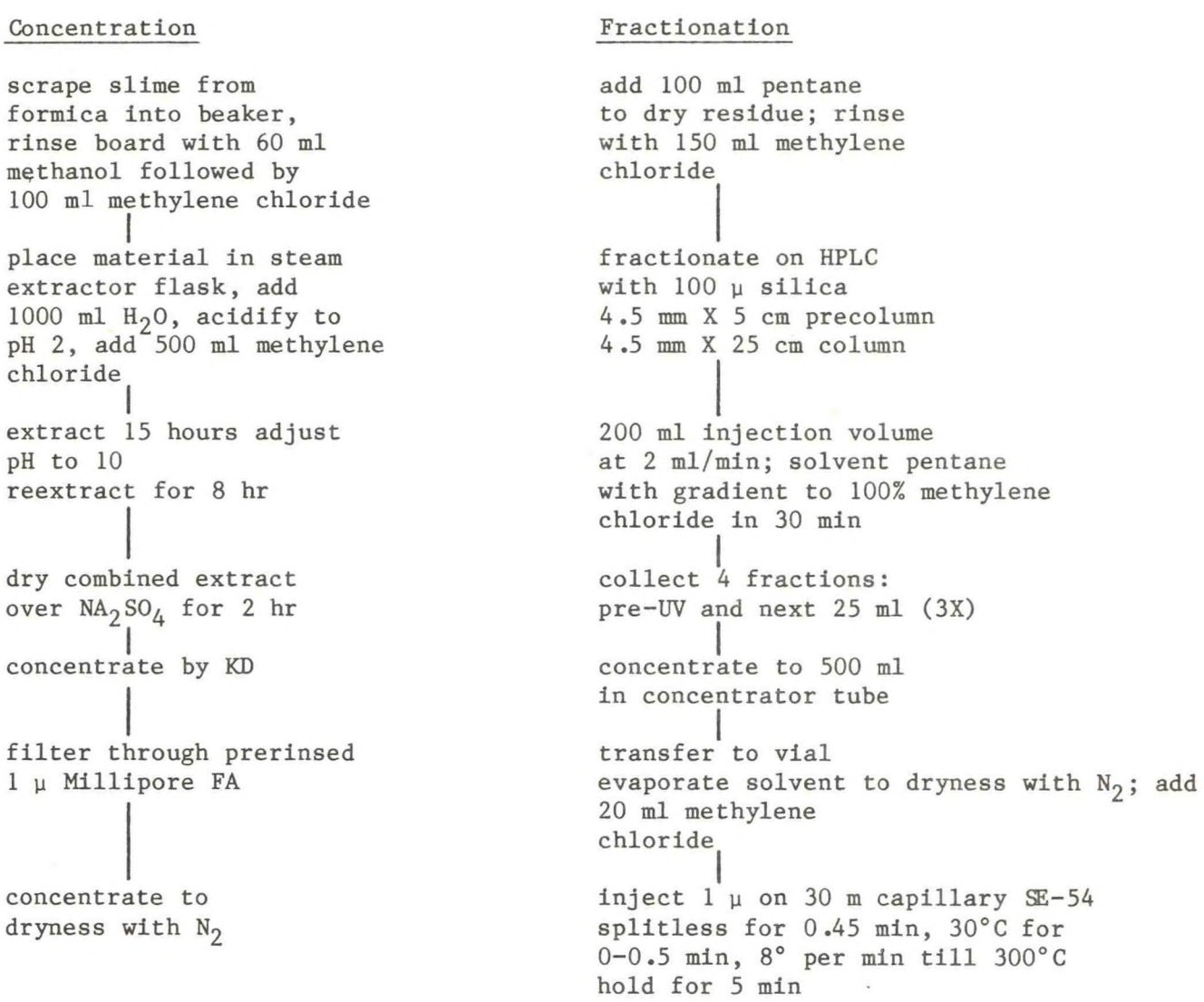

Figure 3. Sequence of steps in fractionation of oil components.

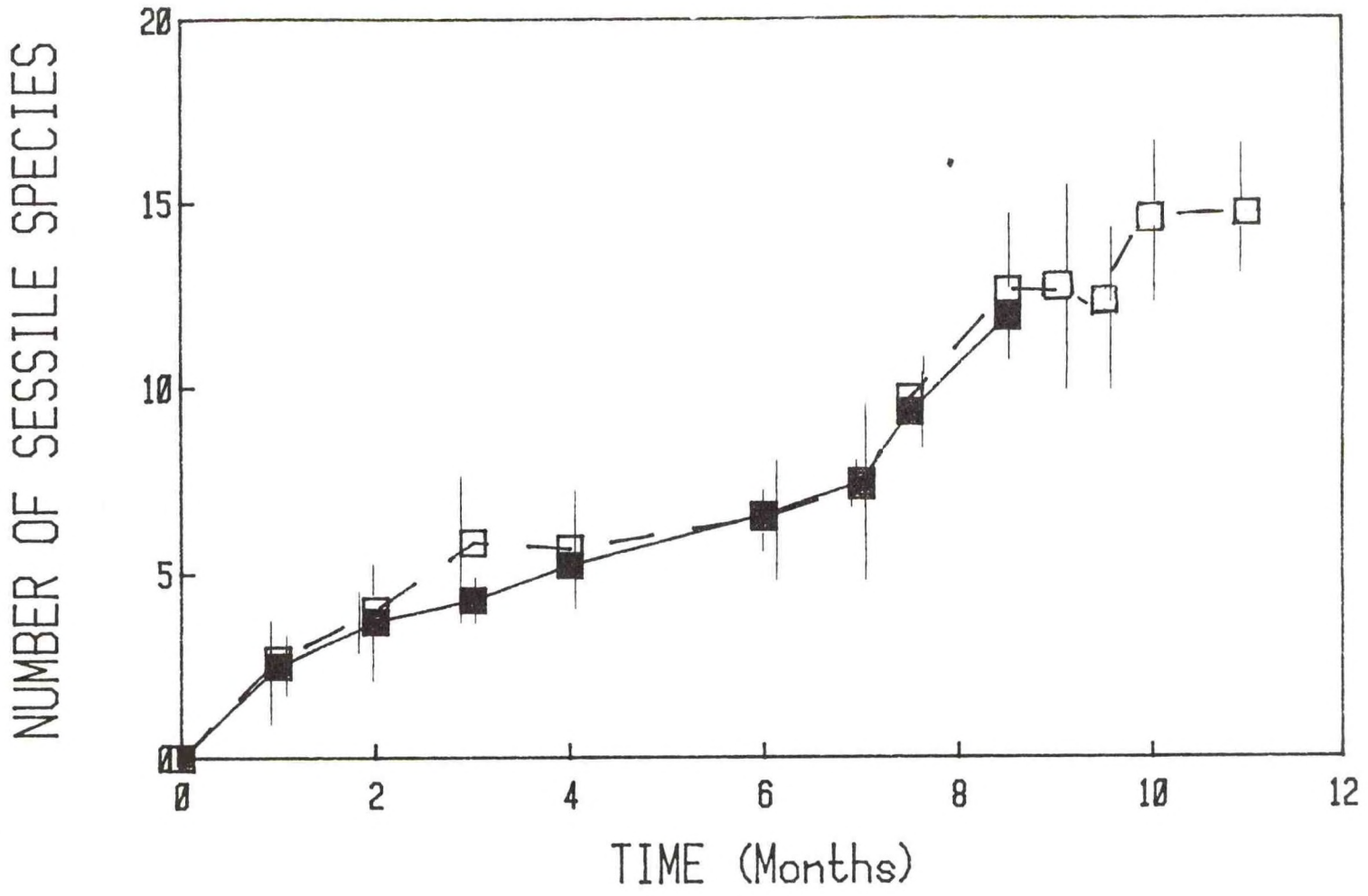

Figure 4. Initial colonization curves for panels. Dark squares are all panels prior to experimental manipulations; open squares are those panels which later became controls.

Table 5. Mean total number of species and standard deviations on panels prior to and after experimental manipulations.

SERIES	PRIOR	POST-MANIPULATIONS			
	June 2, 1980 11 days before	June 23, 1980 10 days after	July 7, 1980 24 days after	July 24, 1980 41 days after	August 27, 1980* 75 days after
CONTROLS	12.13 ± 2.1	12.00 ± 2.7	12.00 ± 1.9	13.88 ± 2.6	15.16 ± 1.9
BRYOZOANS OILED LIGHTLY	12.83 ± 2.9	12.67 ± 2.7	12.00 ± 2.0	13.40 ± 0.8	13.00 ± 2.5
BRYOZOANS OILED HEAVILY	10.86 ± 1.9	10.00 ± 1.8	10.3 ± 2.1	10.86 ± 3.1	-
BRYOZOANS REMOVED	13.44 ± 2.4	12.33 ± 2.0	12.11 ± 1.3	13.78 ± 2.1	11.60 ± 2.0
BRYOZOANS REMOVED AND SPOT OILED HEAVILY	11.29 ± 1.8	9.86 ± 2.9	10.14 ± 1.6	12.14 ± 1.6	13.75 ± 2.2

*some panels lost

Table 6. Mean total percent cover and standard deviations on panels prior to and after experimental manipulations.

SERIES	PRIOR	POST-MANIPULATIONS			
	June 2, 1980 11 days before	June 23, 1980 10 days after	July 7, 1980 24 days after	July 24, 1980 41 days after	August 27, 1980* 75 days after
CONTROLS	29.8 ± 8.5	37.2 ± 4.2	51.0 ± 5.8	63.7 ± 20.8	81.8 ± 9.2
BRYOZOANS OILED LIGHTLY	29.3 ± 10.2	33.3 ± 5.5	41.6 ± 10.8	64.9 ± 13.2	81.7 ± 8.5
BRYOZOANS OILED HEAVILY	31.6 ± 14.1	36.1 ± 13.7	41.0 ± 16.0	65.2 ± 21.7	-
BRYOZOANS REMOVED	30.2 ± 10.4	43.7 ± 10.9	50.9 ± 15.3	72.8 ± 19.1	92.7 ± 9.6
BRYOZOANS REMOVED AND SPOT OILED HEAVILY	31.6 ± 11.2	29.8 ± 4.2	38.5 ± 13.0	62.8 ± 23.4	88.4 ± 4.1

*some panels lost

Table 7. ANOVA for number of species at first observation period after manipulations (June 23, 1980).

SOURCE OF VARIATION	Degrees of freedom (df)	Sum of squares (SS)	Mean squares (MS)	F-statistic (F_s)
Among groups	32	192	6	2.125
Within groups	4	51	12.75	

*indicates significance $P<0.05$.

Table 8. ANOVA for number of species on panels at second observation period after manipulations (July 7, 1980).

SOURCE OF VARIATION	df	SS	MS	F_s
Among groups	32	101	3.16	2.294
Within groups	4	29	7.25	

Table 9. ANOVA for number of species on panels at third observation period after manipulations (July 30, 1980).

SOURCE OF VARIATION	df	SS	MS	F_s
Among groups	32	162	5.06	2.470
Within groups	4	50	12.5	

Table 10. ANOVA for number of species on panels at fourth observation period after manipulations (August 27, 1980).

SOURCE OF VARIATION	Degrees of freedom (df)	Sum of squares (SS)	Mean squares (MS)	F-statistic (F_s)
Among groups	16	75	4.69	1.92
Within groups	4	36	9	

*indicates significance P<0.05.

Table 11. ANOVA for percent cover of panels at the first observation period after manipulations (June 23, 1980).

SOURCE OF VARIATION	df	SS	MS	F_s
Among groups	32	2610	81.56	3.57*
Within groups	4	1165	291.25	

Table 12. ANOVA for percent cover of panels at the second observation period after manipulations (July 7, 1980).

SOURCE OF VARIATION	df	SS	MS	F_s
Among groups	32	5832	182.25	2.10
Within groups	4	1533	383.25	

Table 13. ANOVA for percent cover of panels at the third observation period after manipulations (July 30, 1980).

SOURCE OF VARIATION	df	SS	MS	F_s
Among groups	32	12,759	398.72	0.503
Within groups	4	803	200.75	

Table 14. ANOVA for percent cover of panels at fourth observation period after manipulations (August 27, 1980).

SOURCE OF VARIATION	df	SS	MS	F_s
Among groups	16	1615	100.94	0.523
Within groups	4	211	52.75	

*indicates significance P<0.05.

Table 15. Results of Student-Newman Keuls test (for multiple comparisons of means of unequal sizes) comparing differences in the mean change of percent cover among control and experimental treatments on June 23, 1980. Significance levels are above diagonals and differences between means are below diagonals.

TREATMENT	Bryozoan removed and space oiled	Bryozoan oiled lightly	Bryozoan oiled heavily	Control	Bryozoan removed
Bryozoan removed & space oiled	-	NS	NS	NS	*
Bryozoan oiled lightly	3.5	-	NS	NS	NS
Bryozoan oiled heavily	6.3	2.8	-	NS	NS
Control	7.4	3.9	1.1	-	NS
Bryozoan removed	13.9	10.4	7.6	6.5	-

NS = not significantly different; * P<0.05.

among treatment groups were observed ($P<0.05$) only during the first observation period 10 days after initial manipulations were begun. These significant differences were observed only with respect to percent cover (Table 11). The Student-Newman-Keuls Test (Table 15), a stepwise method using the range of the statistic to measure differences among means, was employed to determine which of these treatment means were significantly different from the others. This test indicated that both treatments which involved removal of the encrusting bryozoan (i.e., bryozoan removed, bryozoan removed and spot heavily oiled) were significantly different from each other in terms of subsequent percent cover ($P<0.05$), but not from the plates from which no species were removed. Some trends after this date were also apparent, although the total variability inherent within groups was too great to allow the other means to be distinguished statistically from one another. Panels with bryozoans removed had a higher percent cover 10 days after manipulations than those panels that received oil after the bryozoan removals. The space made available by the bryozoan removal greatly enhanced subsequent attachment, whereas oil inhibited attachment.

3.4 Time to Return to Control Composition

Figure 4 shows that at the time the experimental manipulations were initiated the colonization curve for panels was not yet approaching an asymptote. Due to this, no equilibrium species richness could be designated. From these data it is not possible to determine how long it would have taken to return to the control composition.

3.5 Estimates of Effects of Environmental Variables

Since we performed experimental removals in spring, we can only infer the effects of different temperatures and salinities on our results. Performing manipulations when temperatures were ca. 11°C and increasing may have resulted in a more rapid rate of response of settlement as compared to colder months. Unfortunately there are no comparable recovery data in our series for comparison at times of year when temperatures are lowered.

3.6 Detection of Chemical Residues

The composition of crude oil is shown before high pressure liquid chromatograph (HPLC) fractionation (Figure 5) and after fractionation in Figures 6-9. These four fractions will be later compared to those obtained by scraping the biomass from panels.

The first pre-ultraviolet absorbing fraction containing aliphatic compounds is shown in Figure 6. A mixture of aromatic compounds was present in the first UV fraction (Figure 7), while ketones were primarily present in the second UV fraction (Figure 8). Water soluble alcohols were present in the third UV fraction (Figure 9). A quantitative indication of the major component of the crude oil is presented in Table 16. A tentative identification of these 170 compounds is also shown using a forward search

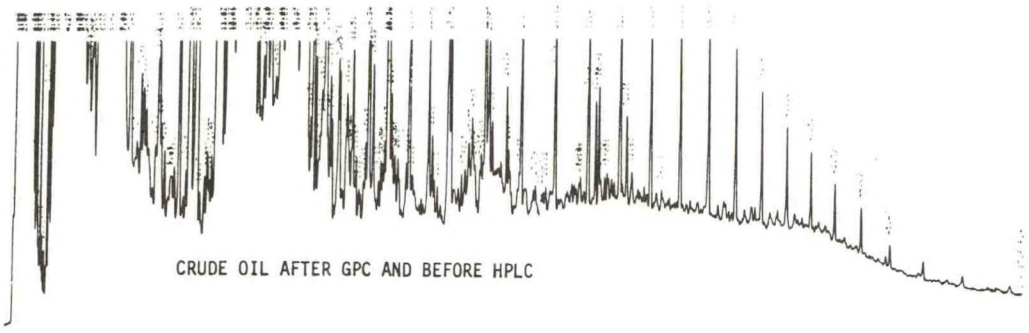

Figure 5. Gas chromatogram of oil before fractionation

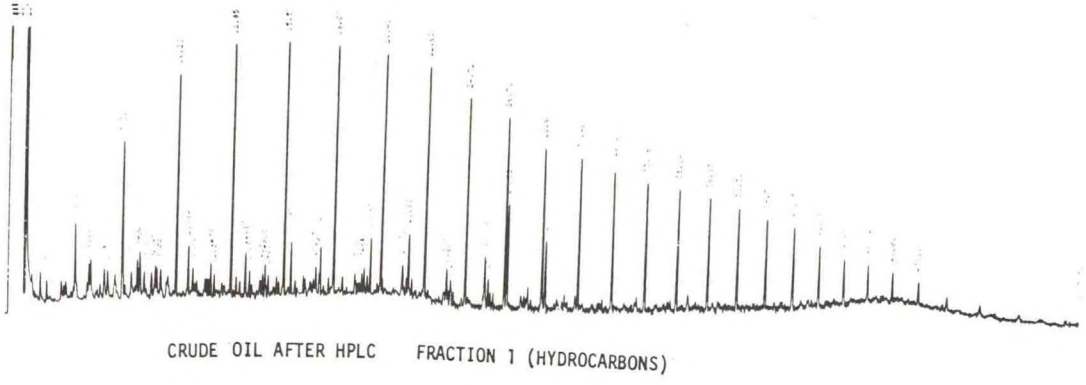

Figure 6. Gas chromatogram of first fraction after HPLC separation of oil.

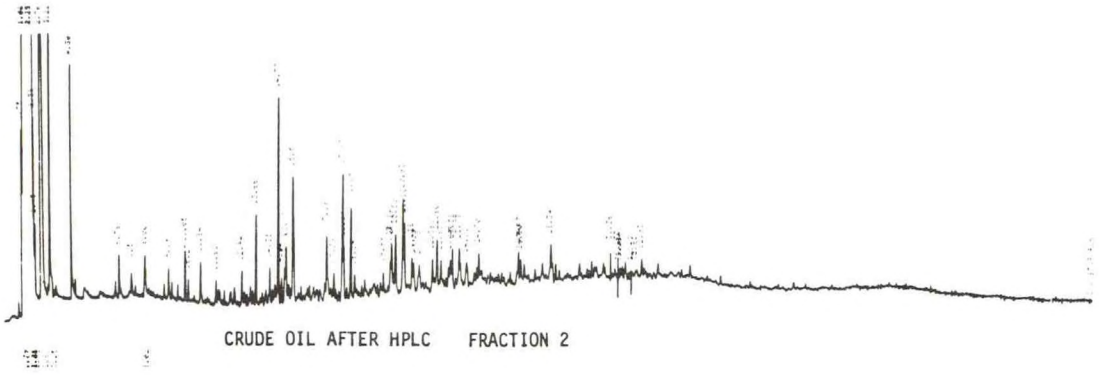

Figure 7. Gas chromatogram of second fraction after HPLC separation of oil (first UV fraction)

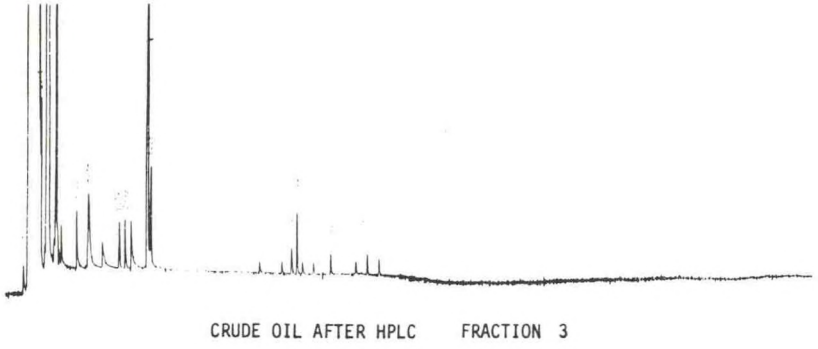

Figure 8. Gas chromatogram of third fraction after HPLC separation of oil (second UV fraction).

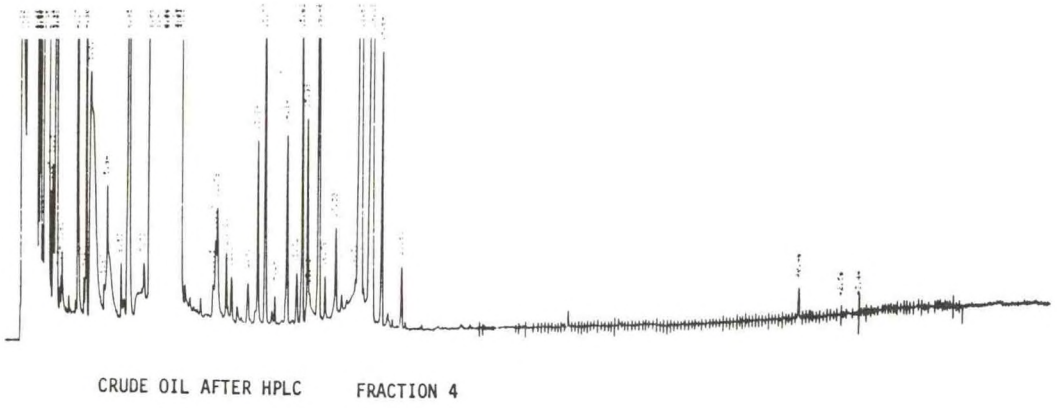

Figure 9. Gas chromatogram of fourth fraction after HPLC separation of oil (third UV fraction).

Table 16. Constituents found in the crude Alaska oil (MW = molecular weight).

Oil, First Fraction

	Name	Formula	MW	Scan	Amount (g/l)	Fit	Purity
1	tridecane	$C_{13}H_{28}$	184	828	2.3	<901	<875
2	dodecane,2,6,11-trimethyl-	$C_{15}H_{32}$	212	908	1.0	868	857
3	tetradecane	$C_{14}H_{30}$	198	930	3.7	-	-
4	cyclopentane,1 ethyl-1-methyl-	C_8H_{16}	112	982	1.1	895	752
5	hexane,3,3-dimethyl-	C_8H_{18}	114	994	3.1	933	864
6	pentadecane	$C_{15}H_{32}$	212	1031	8.8	<954	<849
7	cyclopentane,(2-methyl-propyl)-	C_9H_{18}	126	1081	2.1	919	786
8	hexadecane	$C_{16}H_{34}$	226	1125	5.9	<945	<622
9	octane,2,6-dimethyl-	$C_{10}H_{22}$	142	1168	2.7	941	860
10	cyclohexane, alkyl-	-	-	1176	1.6	-	-
11	hepta decane	$C_{17}H_{36}$	240	1216	4.7	-	-
12	penta decane,2,6,10,14-tetramethyl-(pristane)	$C_{19}H_{40}$	268	1218	4.7	934	851
13	cyclohexane, alkyl-	-	-	1265	1.5	-	-
14	octadecane	$C_{18}H_{38}$	254	1297	3.8	<940	<525
15	hexadecane,2,6,10,14-tetramethyl(phytane)	$C_{20}H_{42}$	282	1304	3.0	961	681
16	nonadecane	$C_{19}H_{40}$	268	1376	5.0	<942	<722
17	eicosane	$C_{20}H_{42}$	282	1452	3.5	<932	<596
18	heneicosane	$C_{21}H_{44}$	296	1524	2.7	<922	<719
19	docosane	$C_{22}H_{46}$	310	1593	2.5	<911	<702
20	tricosane	$C_{23}H_{48}$	324	1660	2.0	<900	<572
21	tetracosane	$C_{24}H_{50}$	338	1723	1.8	<890	<692
22	pentacosane	$C_{25}H_{52}$	352	1785	1.8	<898	<690
23	hexacosane	$C_{26}H_{54}$	368	1843	1.4	<888	<695
24	heptacosane	$C_{27}H_{56}$	382	1900	1.0	<861	<686
25	octacosane	$C_{28}H_{58}$	396	1954	.7	<815	<556
26	nonacosane	$C_{29}H_{60}$	410	2009	.6	<812	<545

Table 16 (Continued)

	Name	Oil, Second Fraction Formula	MW	Scan	Amount (g/l)	Fit	Purity
1	methane, dichloro-	CH_2Cl_2	84	207	20	906	906
2	1,3-dioxolane, 4-ethyl-	$C_5H_{10}O_2$	102	224	7.0	879	739
3	methylbenzene	C_7H_8	92	235	.4	909	906
4	2-butene-1, 4-diol-	$C_4H_8O_2$	88	256	4.5	868	827
5	formic acid, ethylester	$C_3H_6O_2$	74	283	.1	785	587
6	benzene, ethyl-	C_8H_{10}	106	324	.3	857	857
7	benzene, 1,3-dimethyl-	C_8H_{10}	106	333	1.6	980	949
8	1-propane, 3-ethoxy-	$C_5H_{10}O$	86	349	.3	731	461
9	benzene, 1,2-dimethyl-	C_8H_{10}	106	358	.5	929	929
10	1-3 dioxolane, 4 ethyl-	$C_5H_{10}O_2$	102	384	2.9	814	761
11	2(3H)-furanone,dihydro-	$C_4H_6O_2$	86	386	3.6	943	927
12	pyridine,2,3,4,5-tetrahydro-	C_5H_5N	83	399	.1	777	777
13	benzene,1-ethyl-4-methyl-	C_9H_{12}	120	437	1.1	943	936
14	benzene,1,2,3-trimethyl-	C_9H_{12}	120	445	.4	869	869
15	benzene (1-methylethyl)-	C_9H_{12}	120	459	.3	930	922
16	benzene 1,3,5-trimethyl-	C_9H_{12}	120	475	1.5	991	971
17	decane	$C_{10}H_{22}$	142	481	.7	<898	<898
18	benzene, 1,2,4-trimethyl-	C_9H_{12}	120	510	.7	926	886
19	butanethioic acid, S-methylester	$C_5H_{10}OS$	118	527	.1	751	475
20	benzene (1-methylpropyl)-	$C_{10}H_{14}$	134	544	.7	925	922
21	benzene (2-methylpropyl)-	$C_{10}H_{14}$	134	550	.3	911	724
22	benzene, 1-methyl-2-propyl-	$C_{10}H_{14}$	134	563	.3	886	886
23	benzene, 1,1-dimethylethyl-	$C_{10}H_{14}$	134	578	.5	857	857
24	benzene, 1-ethyl-2,4-dimethyl-	$C_{10}H_{14}$	134	585	.5	850	850
25	benzene, 1-ethenyl-4-ethyl-	$C_{10}H_{12}$	132	587	.2	751	716
26	undecane	$C_{11}H_{22}$	156	600	1.3	905	905
27	benzene (1,2-dimethylpropyl)-	$C_{11}H_{16}$	148	604	.1	719	719
28	propanoic acid, 2 methyl-anhydride	$C_8H_{14}O_3$	158	617	.4	794	794
29	benzene, diethylmethyl-	$C_{11}H_{16}$	148	621	.3	735	691
30	benzene, 1-methyl-3-propyl-	$C_{10}H_{14}$	134	627	.3	763	763
31	furan, 2-butyltetrahydro-	$C_8H_{16}O$	128	634	5.4	931	843

Table 16 (Continued)

Oil, Second Fraction

	Name	Formula	MW	Scan	Amount (g/l)	Fit	Purity
33	2-hexene,4,4,5-trimethyl-	C_9H_{18}	126	642	.1	741	741
34	indan, 1-methyl-2-nonyl-	$C_{19}H_{30}$	258	648	<.1	557	501
35	benzene (1,1-dimethylpropyl)-	$C_{11}H_{16}$	148	656	.4	883	883
36	benzene, 1-ethenyl-4-ethyl-	$C_{10}H_{12}$	132	662	.1	731	731
37	benzene,1,4-dimethyl(mixt)	$C_{10}H_{14}$	134	664	.7	852	762
38	naphthalene,1,2,3,4-tetrahydro-	$C_{10}H_{12}$	132	676	.2	568	568
39	2-furanol,tetrahydro-2-methyl-	$C_5H_{10}O_2$	102	683	13.1	957	914
40	butanic acid,ethenylester	$C_5H_{10}O_2$	114	690	.9	870	544
41	2,5-cydohexadiene-1,4-dione,	$C_8H_8O_2$	136	699	1.1	985	684
42	naphthalene 2-ethyl-	$C_{10}H_8$	128	703	1.8	983	859
43	benzene(1,1-dimethyl-2-propenyl)	$C_{11}H_{14}$	146	706	.2	659	579
44	benzene,1-methyl-3-propyl-	$C_{10}H_{14}$	134	711	.2	557	557
45	furan,2-butyltetrahydro-	$C_8H_{16}O$	128	716	9.1	966	842
46	benzene, (1-ethylpropyl)-	$C_{11}H_{16}$	148	718	.2	692	132
47	naphthalene,6,7-dimethyl-	$C_{12}H_{16}$	160	723	<.1	327	327
48	hexane,2,2,5-trimethyl-	C_9H_{20}	128	731	.2	740	484
49	benzene,2,4-dimethyl-1-(1-methylpropyl)-	$C_{12}H_{18}$	162	747	.2	702	680
50	propanoic acid,2-methyl-, methylester	$C_5H_{10}O_2$	102	751	.9	721	704
51	benzene(1-ethyl-1-methylpropyl)-	$C_{12}H_{18}$	162	760	.3	812	538
52	benzene(1-methylhexadecyl)-	$C_{23}H_{40}$	316	777	.1	629	629
53	benzene, hexyl-	$C_{12}H_{18}$	162	785	.2	698	666
54	benzene, (1-methyldodecyl)-	$C_{19}H_{32}$	260	794	.4	734	506
55	naphthalene, 1-methyl-	$C_{11}H_{10}$	142	827	4.6	935	764
56	ethanone,1-\4-(1-methyl-ethenyl) phenyl\-	$C_{11}H_{12}O$	160	834	.1	710	430
57	naphthalene, 2-methyl-	$C_{11}H_{10}$	142	845	3.0	956	921
58	benzene,1-(1-methylethenyl)-3-(1-methyl ethyl)-	$C_{12}H_{16}$	160	852	.5	716	607
59	cyclohexane (1-methylethyl)-	C_9H_{16}	124	874	.3	797	276
60	alkyldioxolane	-	-	879	.1	-	-
61	benzene,(1-methylnonadecyl)-	$C_{26}H_{45}$	358	885	.1	650	650
62	4-heptanone, 3-methyl-	$C_8H_{16}O$	128	893	.3	774	181
63	benzene, (butoxymethyl)	$C_{11}H_{16}O$	164	896	.2	755	755
64	3-hexanone,2,4-dimethyl-	$C_8H_{16}O$	128	906	.3	772	701
65	acenaphthelene,1,2-dihydro-	$C_{12}H_{10}$	154	916	.4	858	818
66	alkane	$C_{11}H_{24}$	156	930	2.1	911	676

Table 16 (Continued)

Oil, Second Fraction

	Name	Formula	MW	Scan	Amount (g/l)	Fit	Purity
67	2-hexene,4,4,5-trimethyl-	C_9H_{18}	126	939	.1	676	45
68	naphthalene,1,5-dimethyl-	$C_{12}H_{12}$	156	943	23	975	947
69	naphthalene,2,3-dimethyl-	$C_{12}H_{12}$	156	958	55	996	947
70	4-heptanol,2,6-dimethyl-4-propyl-	$C_{12}H_{26}O$	186	980	.3	735	521
71	1-hexene,4,5-dimethyl-	C_8H_{16}	112	988	.3	727	180
72	hexane,3,3-dimethyl -	C_8H_{18}	114	992	.6	867	840
73	naphthalene,1,4-dimethyl-	$C_{12}H_{12}$	156	994	.3	782	712
74	benzene acetic acid, 1,1-dimethylester	$C_{12}H_{16}O_2$	358	1000	.3	823	651
75	naphthalene,1-(2-propenyl)-	$C_{13}H_{12}$	168	1022	<.1	734	636
76	alkane	-	-	1028	18	-	-
77	naphthalene,2,3,6-trimethyl-	$C_{13}H_{14}$	170	1082	13	899	861
78	2,8-decadiyne	$C_{10}H_{14}$	134	1086	.2	678	611
79	naphthalene,1,3,6-trimethyl-	$C_{13}H_{14}$	170	1098	10	965	845
80	benzene (1-methyl nonadecyl)-	$C_{26}H_{46}$	358	1103	.3	716	716
81	1-hexanol,2-ethyl-2-propyl-	$C_{11}H_{24}$	172	1121	15	914	530
82	naphthalene,2-(1-methylethyl)-	$C_{13}H_{14}$	190	1129	.4	534	377
83	dibenzofuran,4-methyl-	$C_{13}H_{10}O$	182	1151	.1	724	484
84	heptadecane,2,6,10,14-tetramethyl-	$C_{21}H_{44}$	296	1165	.4	737	690
85	cyclohexane, octyl-	$C_{14}H_{28}$	196	1173	.1	700	286
86	alkylbenzene	-	-	1176	<.1	-	-
87	alkylbenzene	-	-	1179	.1	-	-
88	3-hexanone,2,4-dimethyl-	$C_8H_{16}O$	128	1209	11	900	538
89	9H-fluorene, 9-methyl-	$C_{14}H_{12}$	180	1226	<.1	685	522
90	diazene(4-methylphenyl)phenyl-	$C_{13}H_{12}N_2$	196	1266	.1	765	440
91	aromatic amine	-	188	1287	<.1	-	-
92	phenanthrene	$C_{14}H_{10}$	178	1291	.5	918	907
93	alkane	-	-	1293	.4	-	-
94	1-nonene, 4,6,8-trimethyl-	$C_{12}H_{24}$	168	1301	.4	742	580
95	9H-thioxanthene	$C_{19}H_{10}S$	198	1350	.5	806	739
96	dibenzothiophene,4-methyl-	$C_{13}H_{10}S$	198	1367	.2	670	510
97	alkane	-	-	1372	.2	-	-
98	phenanthrene, 2-methyl-	$C_{15}H_{12}$	192	1388	.2	779	751
99	benzoic acid,phenylmethylester	$C_{14}H_{12}O_2$	212	1446	.2	591	352
100	alkane	-	-	1448	.2	-	-
101	1-nonene,4,6,8-trimethyl-	$C_{12}H_{24}$	168	1519	.2	784	672
102	2-pentene,5-(pentyloxy)-	$C_{10}H_{20}O$	156	1562	.3	853	430
103	heptadecane,2,6,10,14-tetramethyl	$C_{21}H_{44}$	296	1588	.3	734	734

Table 16 (Continued)

Oil, Second Fraction

Name		Formula	MW	Scan	Amount (g/l)	Fit	Purity
104	alkane	-	-	1654	.3	-	-
105	alkane	-	-	1718	.3	-	-
106	alkane	-	-	1812	<.1	-	-
107	ether,butylpentyl penta-	$C_9H_{20}O$	144	1894	.3	682	682

Oil, Third Fraction

	Name	Formula	MW	Scan	Amount (g/l)	Fit	Purity
1	2-propen-1-ol	C_3H_6O	58	305	1.8	880	<99
2	ethane,1,1,2,2-tetrachloro-	$C_2H_2Cl_4$	166	322	1.4	994	903
3	3,4-hexanedione,2,5-dimethyl-	$C_8H_{14}O_2$	142	326	4.7	873	743
4	2(3H)-furanone,dihydro-	$C_4H_6O_2$	86	340	63.6	96	346
5	unknown	-	-	342	12.2	-	-
6	2H-pyran-2-one	$C_5H_4O_2$	96	345	1.5	943	874
7	2-propen-1-ol	C_3H_6O	58	365	.4	845	687
8	heptane, 4-(1-methylethyl)-	$C_{10}H_{22}$	142	447	1.0	908	613
9	cyclohexane,1,3-dichloro-	$C_6H_{10}Cl_2$	152	493	.5	847	821
10	2 furanol,tetrahydro-2 methyl-	$C_5H_{10}O_2$	102	563	12.9	889	889
11	furan, 2-butyltetrahydro-	$C_8H_{16}O$	128	646	5.6	973	905
12	butanoic acid, 3-methyl-2-oxo, methylester	$C_6H_{10}O_3$	130	682	1.6	926	705
13	formic acid, cyclohexylester	$C_7H_{12}O_2$	126	721	1.8	861	833
14	3,4-hexanedione,2,5-dimethyl-	$C_8H_{14}O_2$	142	1490	.3	921	434

Table 16 (Continued)

	Name	Formula	MW	Scan	Amount (g/l)	Fit	Purity
		Oil, Fourth Fraction					
1	1, 3-dioxolane, 4-ethyl –	$C_5H_{10}O_2$	102	224	6.1	876	718
2	ethane,1,1,2-trichloro –	$C_2H_3Cl_3$	132	239	1.1	936	466
3	butane, 1, 1-dichloro –	$C_4H_8Cl_2$	126	245	21.2	996	943
4	propane, 1, 3-dichloro –	$C_3H_6Cl_2$	112	342	29.7	934	620
5	ethane,1,1,2,2-tetrachloro -	$C_2H_2Cl_4$	166	387	14.4	999	649
6	1-hexanol	$C_6H_{14}O$	102	403	19.4	977	924
7	1, 3-propanediol	$C_3H_8O_2$	76	457	51.1	769	558
8	1,5-heptadiene-3,4-diol	$C_7H_{12}O_2$	128	479	99.2	853	654
9	cyclohexanol, 2-chloro -	$C_6H_{11}OCl$	134	536	22.3	887	686
10	cyclohexanol,4-chloro –	$C_6H_{11}OCl$	134	538	18.0	919	661
11	cyclohexane,1,2-dichloro -	$C_6H_{10}Cl_2$	152	569	3.3	900	809
12	1-propene,3-chloro-2-(chloromethyl)–	$C_4H_6Cl_2$	124	633	4.5	926	509
13	2-furanol,tetrahydro-2-methyl-	$C_5H_{10}O_2$	102	641	12.2	922	901
14	formic acid,1-methylethylester	$C_4H_8O_2$	88	662	.1	878	262
15	2-furan methanol,tetrahydro-, acetate	$C_7H_{12}O_3$	144	727	8.0	938	876
16	butanic acid,3-methyl-2-oxo, methylester	$C_6H_{10}O_3$	130	769	113.9	951	670
17	2-hexene, 2, 3-dimethyl-	C_8H_{16}	112	779	1.8	816	665
18	formic acid, cyclohexylester	$C_7H_{12}O_2$	128	808	9.6	901	828
19	butanal, 2-methyl –	$C_8H_{10}O$	86	866	122.9	856	790
20	2-butanol, 2, 3-dimethyl-	$C_6H_{14}O$	102	873	2.6	817	795
21	butanal, 3-hydroxy	$C_4H_8O_2$	88	891	123.4	866	602
22	pentanal	$C_5H_{10}O$	86	921	22.1	845	767
23	hepthylhydroperoxide	$C_7H_{16}O$	132	954	2.5	912	713
24	butane,1,2-dichloro-2-methyl-	$C_5H_{10}Cl_2$	140	1113	1.1	708	631

GC/MS/DS routine (Table 16). The certainty of the identification increases as both Fit and Purity approach 1,000. A semi-quantitative analysis was made using a uniform response factor.

Panels sacrificed periodically for chemical analysis were scraped clean of organisms and the resulting biomass was steam extracted with methylene chloride/methanol as shown previously (Figure 3). The unfractionated benthic extract is shown in Figure 10. HPLC fractionation resulted in fractions as above (Figures 11-14). The hydrocarbon fraction shown in Figure 11 can then be compared with its corresponding fraction derived from the crude oil (Figure 6). This comparison clearly shows that no aliphatic hydrocarbons were present in the organisms. This analytical result confirmed a visual observation which showed that the dark oil was removed from the panels directly after submergence. Comparison of Figures 7 and 12 showed few corresponding aromatic compounds. Comparison of Figures 8 and 9 with Figures 13 and 14, respectively, indicates that some aldehydes and alcohols were retained.

A computer library was assembled with each of the 170 detectable compounds in the crude oil fractions. Each oil compound was then compared with the organics found in the benthic organisms of the corresponding fraction within a given search window. Organics found through this searching routine and also identified in the initial forward searching are listed in Table 17, together with their fit with respect to the identified structure. The tentative cross comparison showed three compounds in both the third and fourth fraction, two of which were identified twice. The tentative identification showed two furans, one ester and one aldehyde. The mass spectra of the individual organics in both the oil and benthic organisms is shown in Figures 15-20. The results in Table 17 show that the agreement between the oil and the benthic fraction is comparable to the agreement between their actual spectrum and the pure compound spectrum. These organics were not found in the water column. The significance of this finding is that these four organics detected in the benthic organisms likely originated from the applied oil.

The extensive and detailed chemical analyses performed throughout the recovery period showed conclusively that only four compounds of the 170 detected in initially-applied crude oil were retained by the benthic organisms, and they were found at an amount of less than one percent of that applied. Only two of these compounds were detected after 10 days and then at an order of magnitude lower concentration than was applied (Figure 21). The four compounds which were identified at that point are all very water soluble, greatly enhancing their diffusion into the attached benthos. It should be noted that many low molecular weight aromatic compounds often found in the water soluble fraction apparently did not diffuse into the benthic organisms.

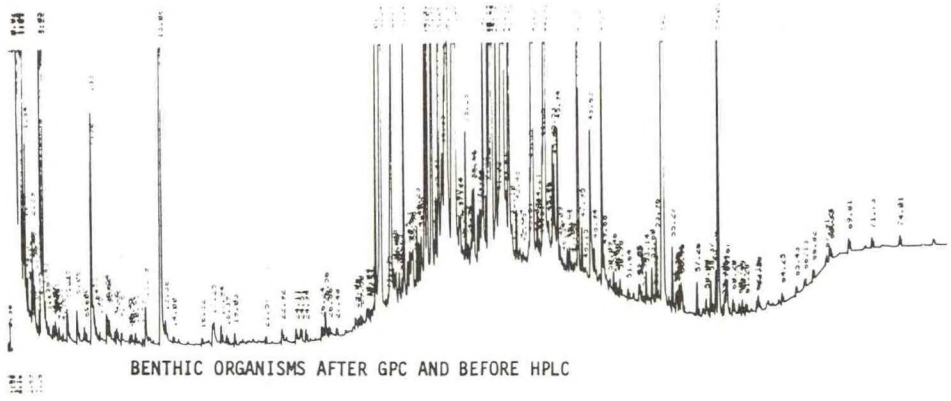

Figure 10. Gas chromatogram of benthic extract before fractionation.

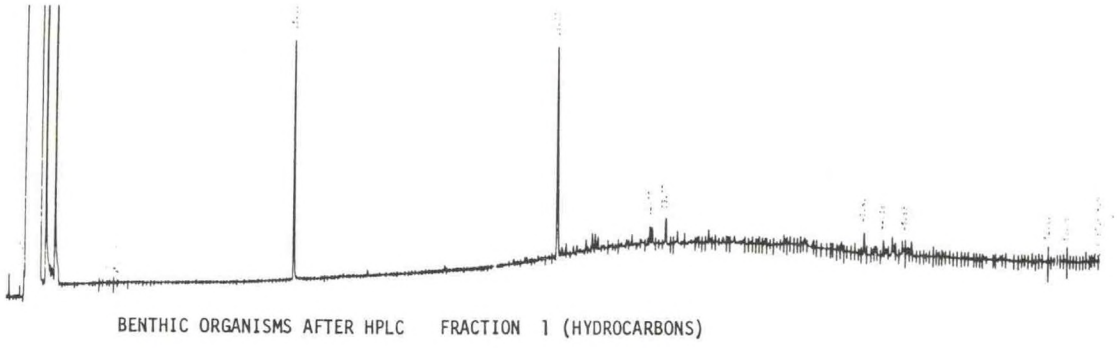

Figure 11. Gas chromatogram of first fraction after HPLC separation of benthic extract.

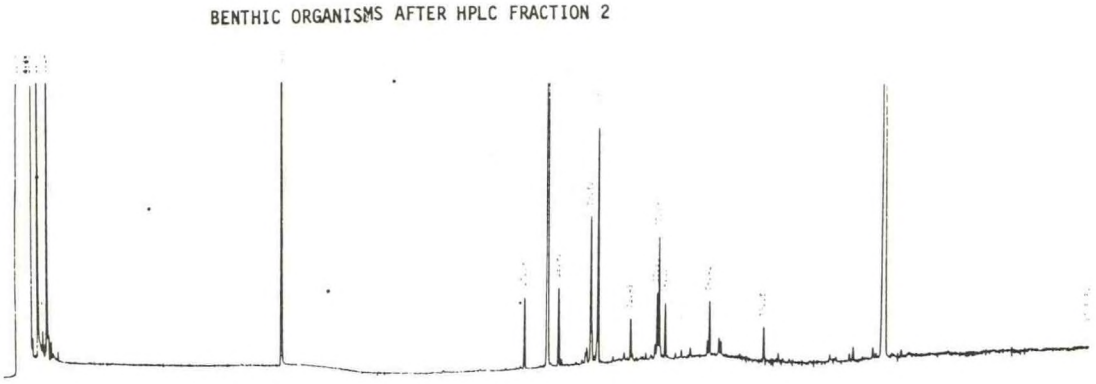

Figure 12. Gas chromatogram of second fraction after HPLC separation of benthic extract.

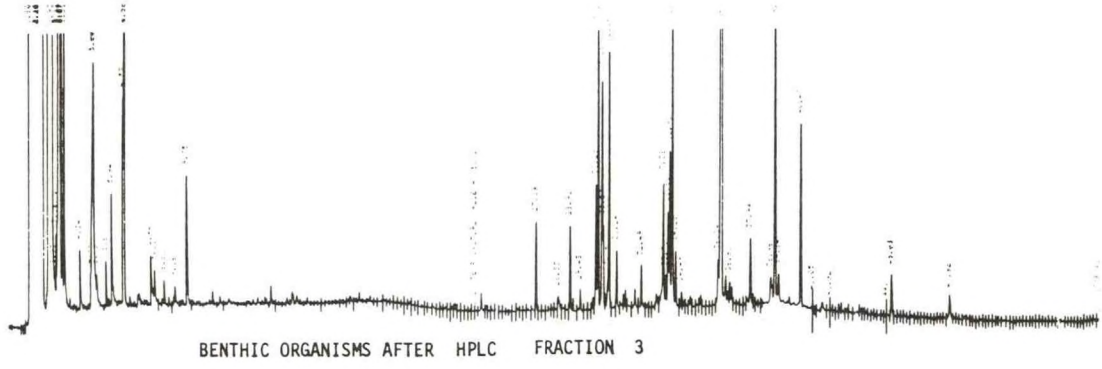

Figure 13. Gas chromatogram of third fraction after HPLC separation of benthic extract.

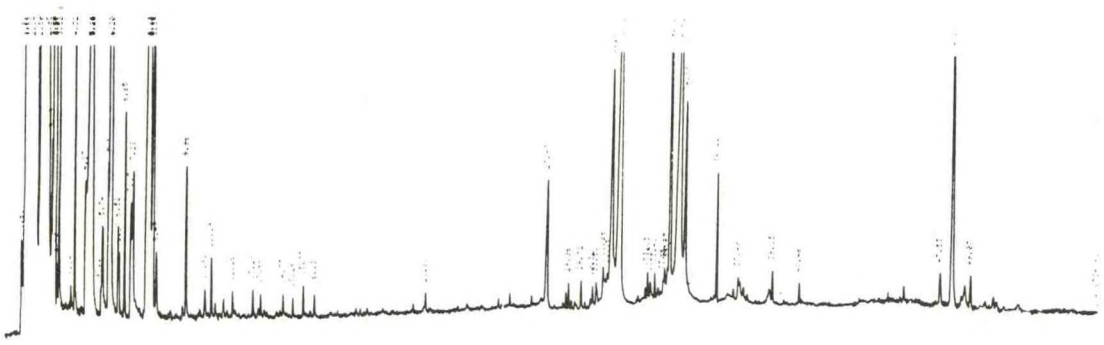

Figure 14. Gas chromatogram of fourth fraction after HPLC separation of benthic extract.

Table 17. List of compounds found both in the fractionated oil and in the benthic organisms scraped from the panels sacrificed for chemical analysis.

OIL CONSTITUENTS				BENTHIC CONSTITUENTS				
Scan	Fit	Appld. (µg)	Fit between oil and benthic cmpnd.	Compound name	Structure	MW	Fit	Recovered (µg)
Third Fraction (4 hr)								
563	889	720	970	2-furanol, tetrahydro-2-methyl-	$C_5H_{10}O_2$	102	902	1.39
646	973	3150	943	furan-2-butyltetrohydro-	$C_8H_{16}O$	128	943	9.99
682	926	916	965	butanoic acid, 3-methyl-2-oxo methylester	$C_6H_{10}O_3$	130	941	13.93
Fourth Fraction (4 hr)								
641	922	6831	926	2-furanol, tetrahydro-2-methyl-	$C_5H_{10}O_3$	102	951	6.0
769	951	63651	986	butanoic acid, 3-methyl-2-oxo methylester	$C_6H_{10}O_3$	130	952	64.5
866	856	68198	994	butanal, 3-methyl-	$C_5H_{10}O$	86	883	39.5
Fourth Fraction (10d)								
641	922	6831	910	2-furanol, tetrahydro-2-methyl-	$C_5H_{10}O_2$	102	961	1.5
769*	951	63651	940	butanoic acid, 3-methyl-2-oxo methylester	$C_6H_{10}O_3$	130	<940	3.1

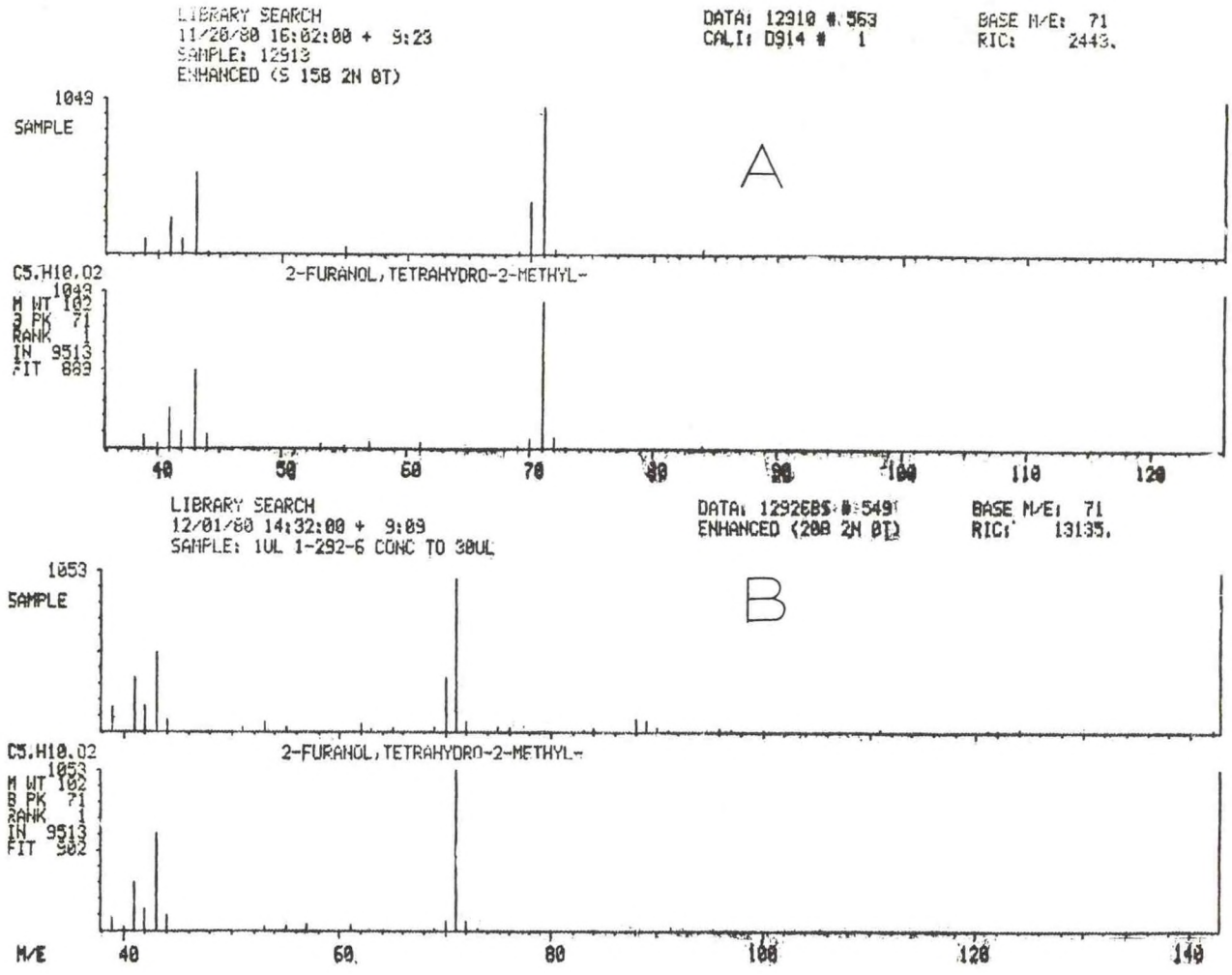

Figure 15. Mass spectrum of the tetrahydro-2-methyl-2-furanol found both in the third fraction of the benthic extract (A) and in the oil (B).

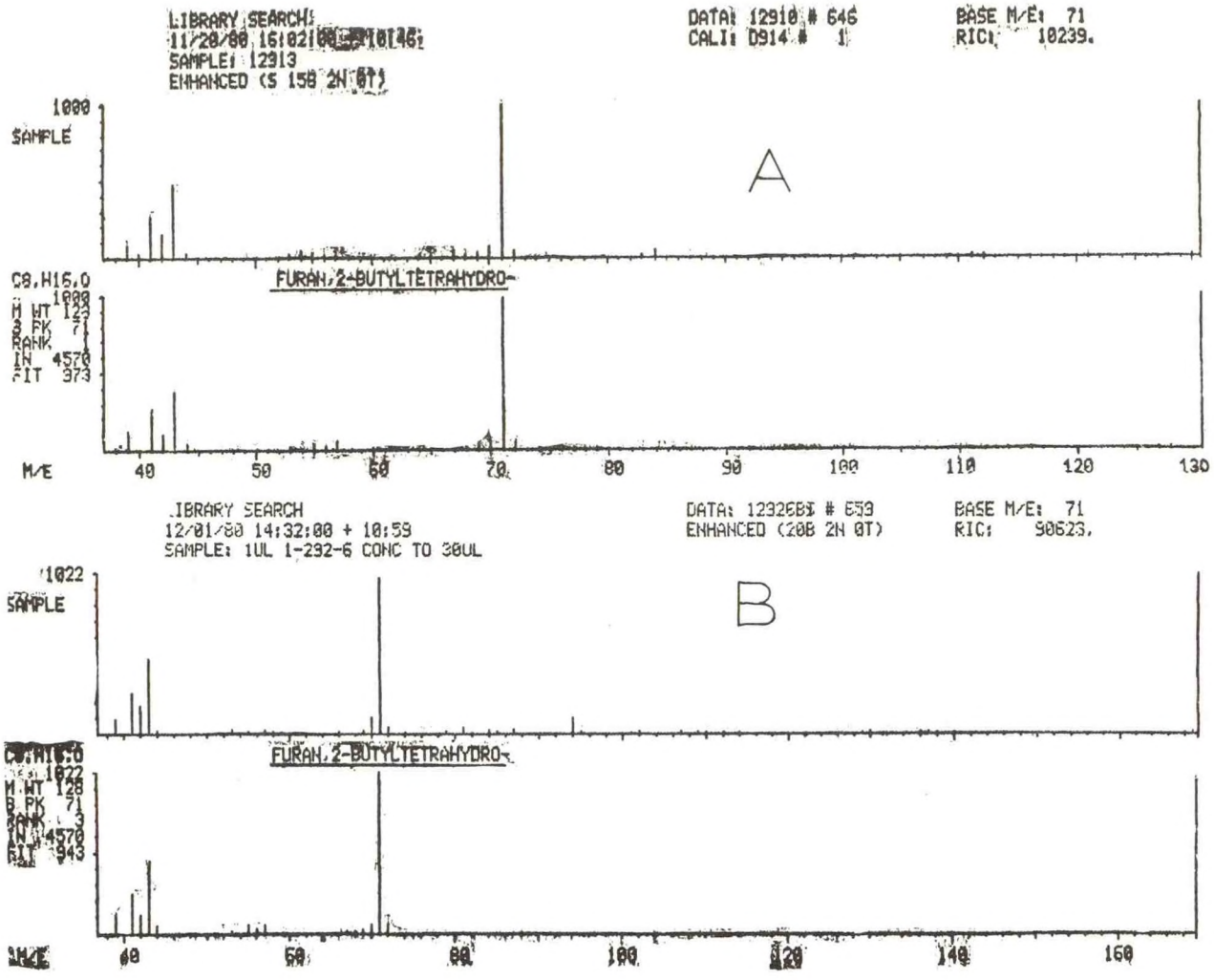

Figure 16. Mass spectrum of the 2-butyl-tetrahydrofuran found both in the third fraction of the benthic extract (A) and in the oil (B).

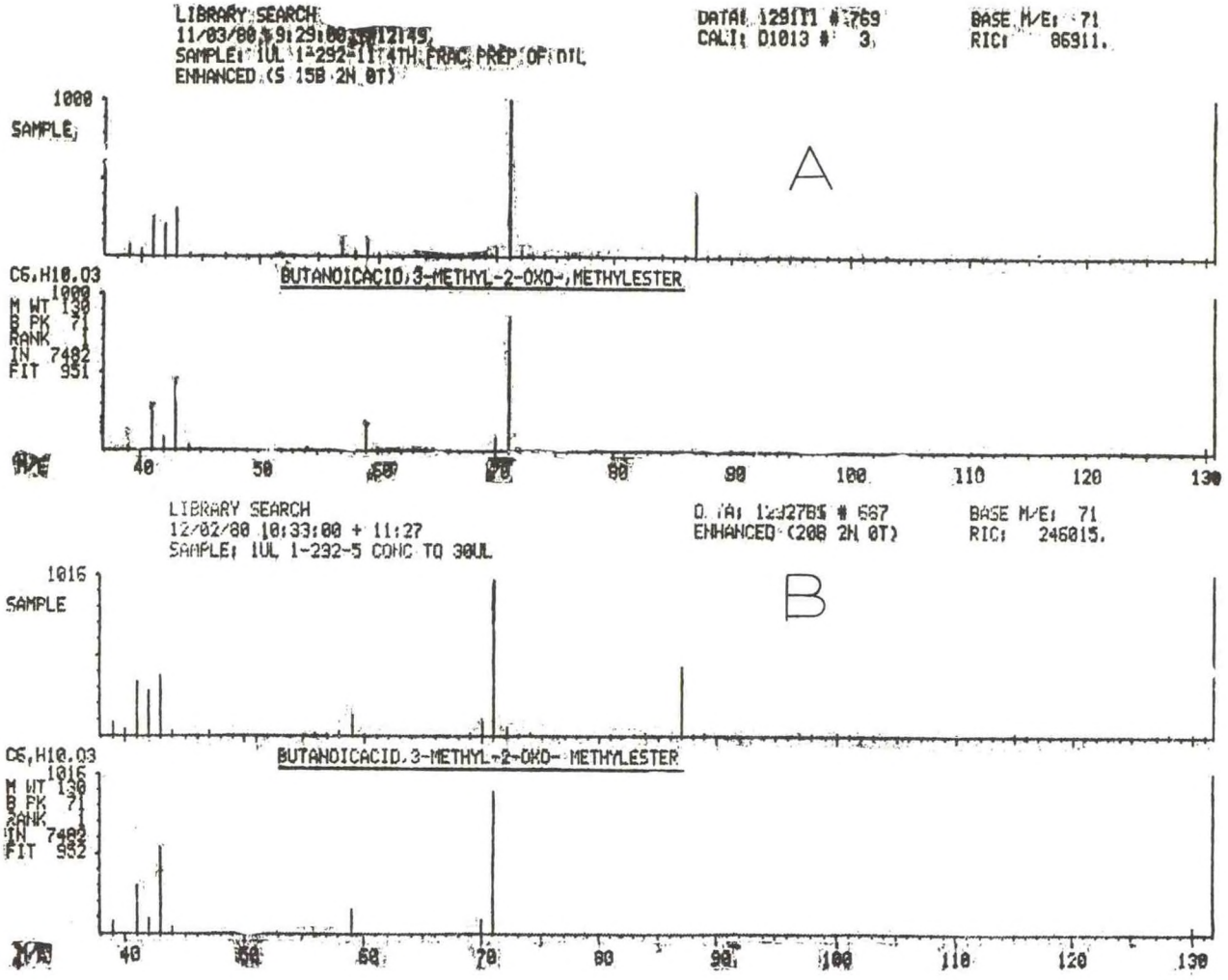

Figure 17. Mass spectrum of the 3-methyl-2-oxo-methylester of butanoic acid found both in the third fraction of the benthic extract (A) and in the oil (B).

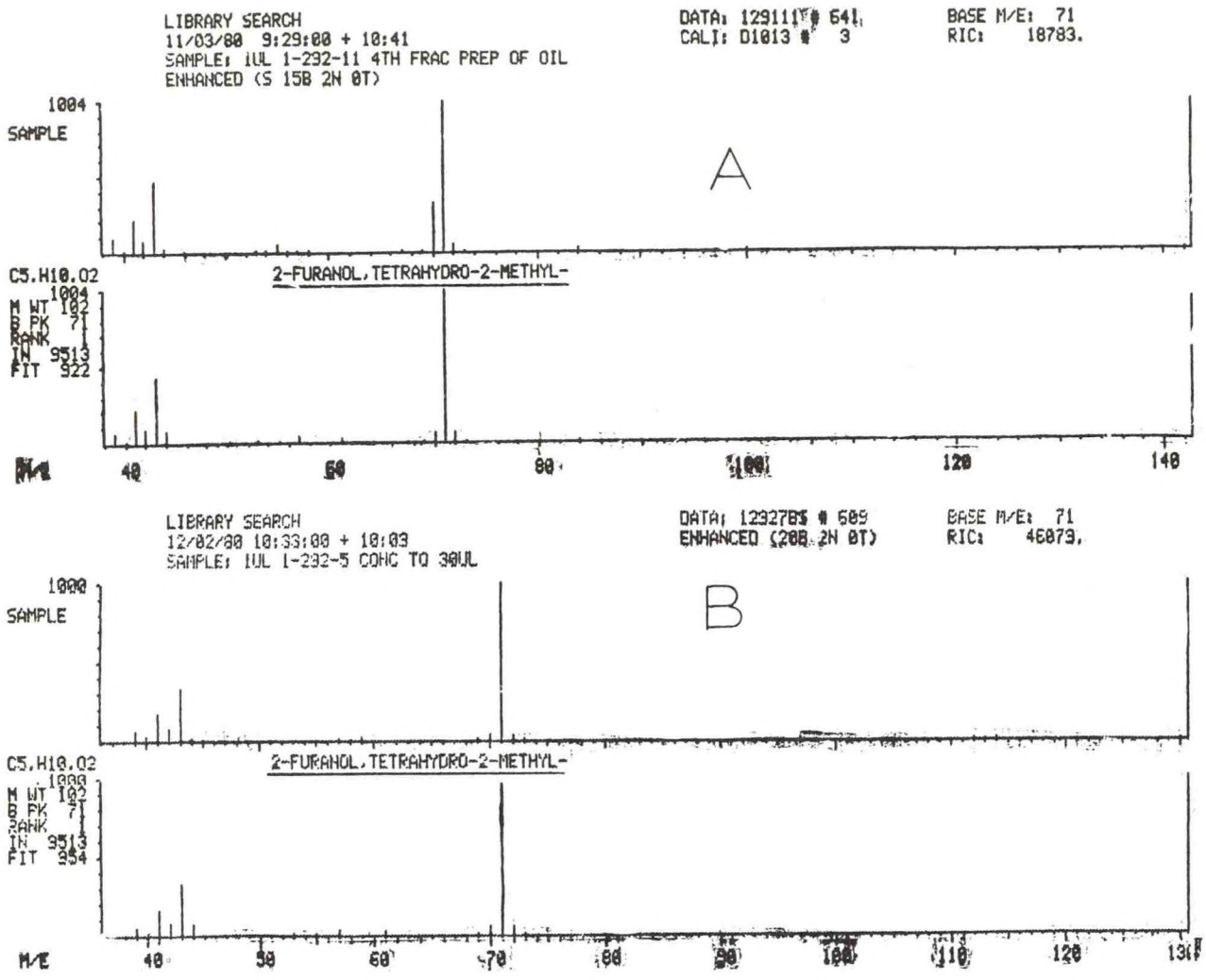

Figure 18. Mass spectrum of the tetrahydro-2-methyl-2-furanol found both in the fourth fraction of the benthic extract (A) and in the oil (B).

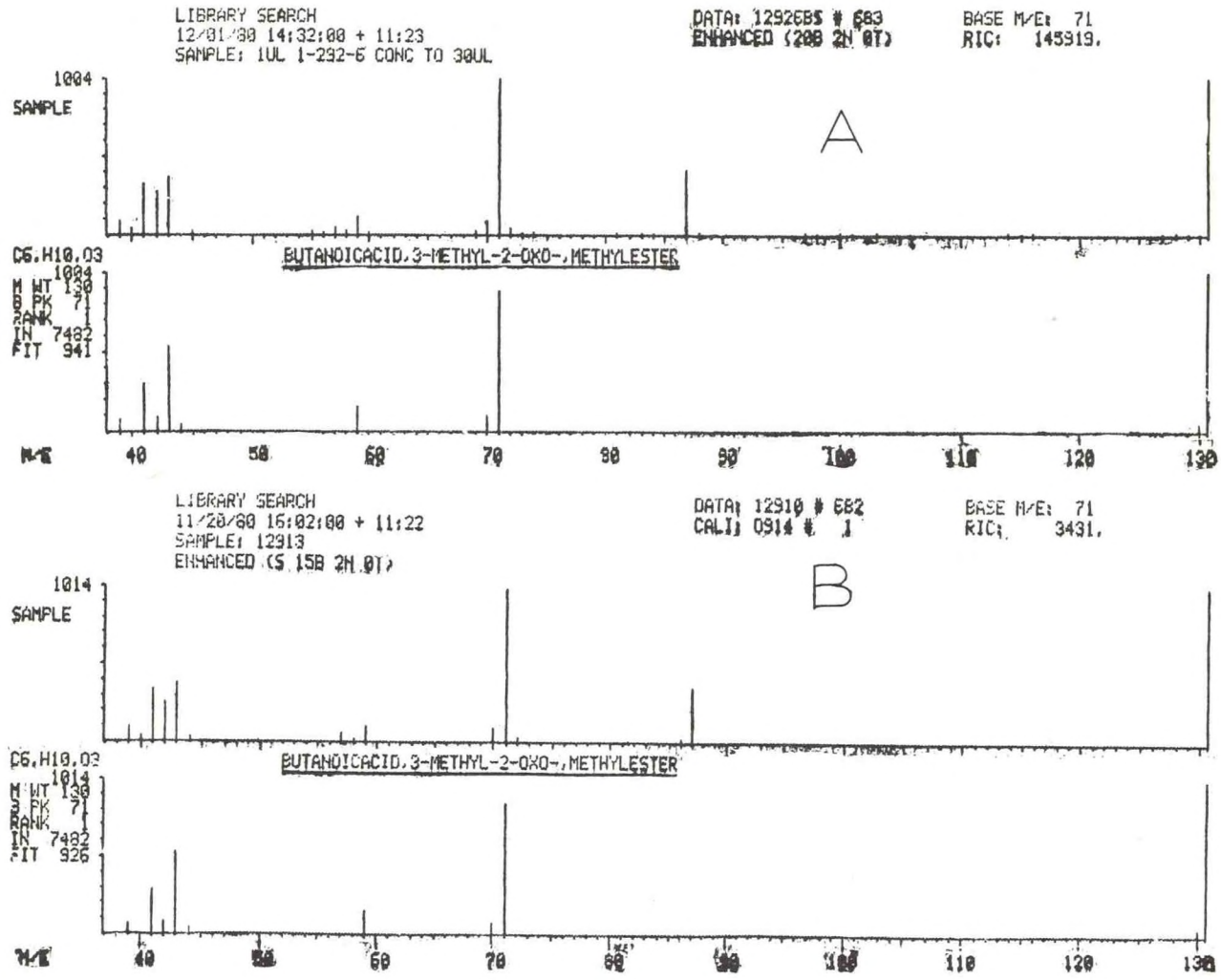

Figure 19. Mass spectrum of the 3-methyl-2-oxo-methylester of butanoic acid found both in the fourth fraction of the benthic extract (A) and in the oil (B).

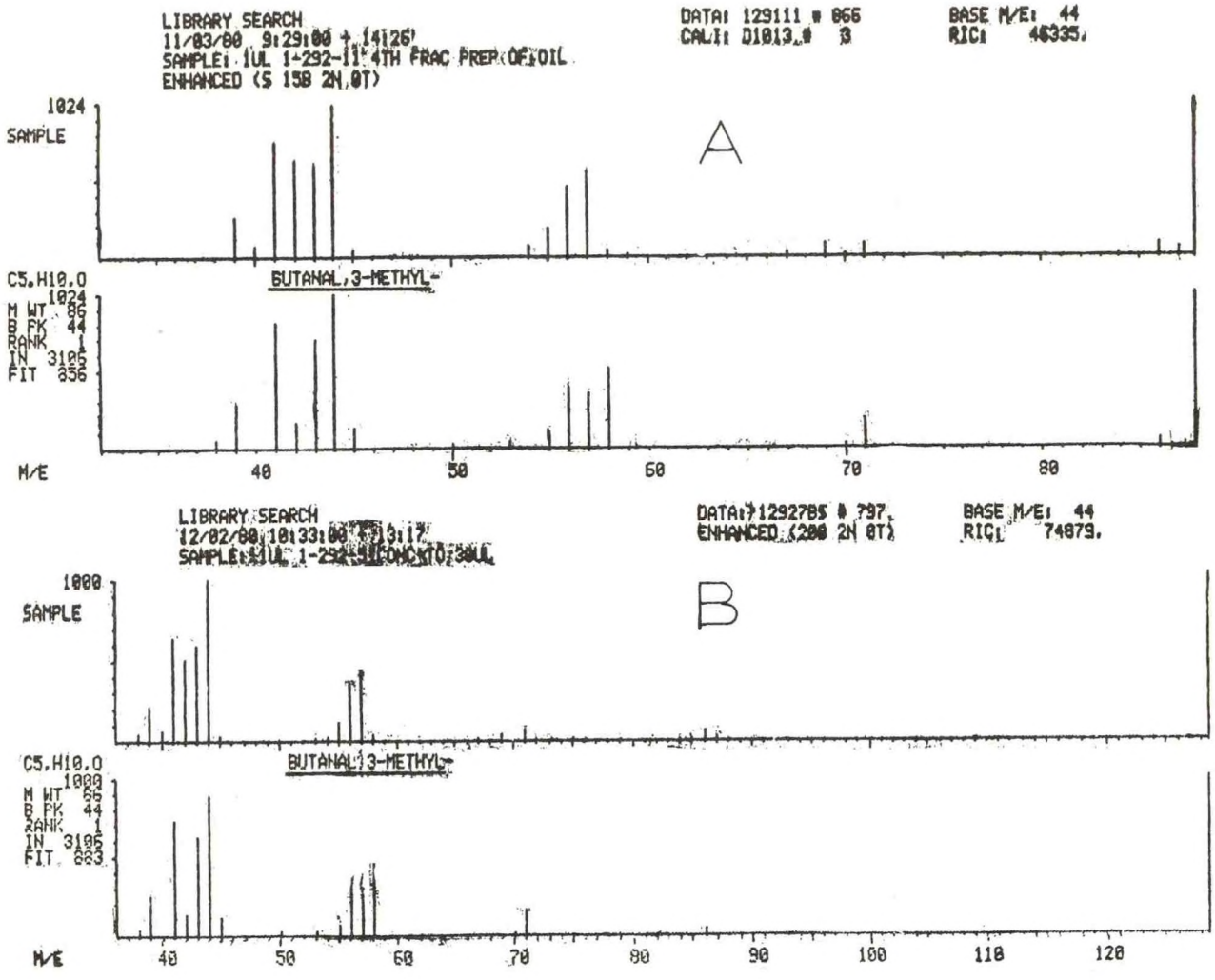

Figure 20. Mass spectrum of the 3-methyl-butanal found in both the fourth fraction of the benthic extract (A) and in the oil (B).

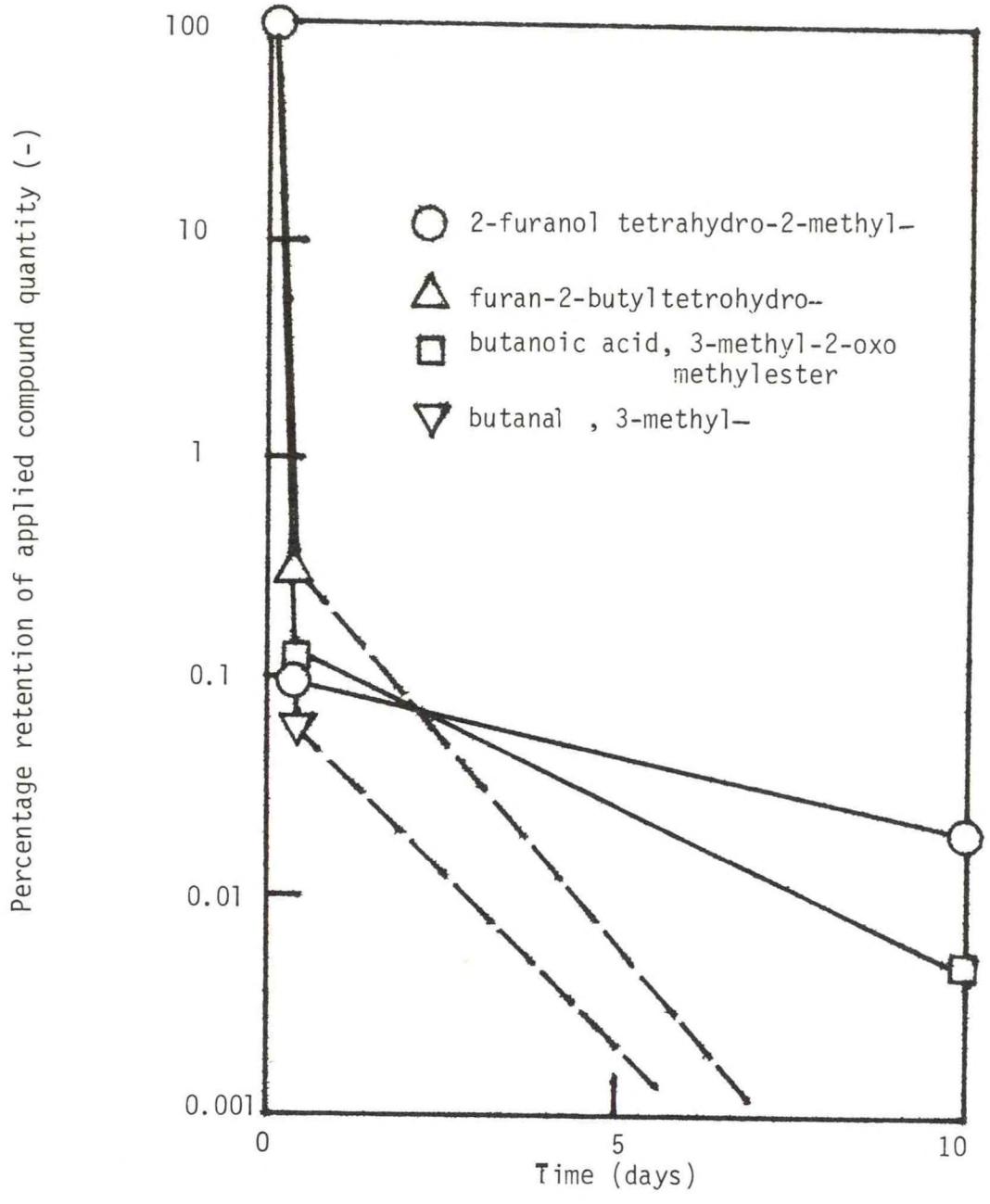

Figure 21. Retention of four oil compounds by benthic organisms following the oil application.

4. DISCUSSION

Basically the experiments we performed were divided into two categories: The first involved those in which actual removal of a space-occupying species took place; the second involved those in which no space was vacated, but the experimental species was oiled (activity was temporarily inhibited by coating the species with crude oil). Monitoring of the panels indicated that the amount of oil subsequently present after direct oiling was performed decreased substantially even within a brief period. Similarly, Vanderhorst et al. (1980) showed in a study of oiled hard brick substrates exposed in the intertidal zone, that substantial loss of oil took place during the early portion of their monitoring, although considerable variation in their percentages appeared between trials. Our surfaces could not be dried completely, since the tolerance of subtidal fouling species to exposure to air was not well known. Our experience showed that several hours' exposure to air did virtually little damage to fouling organisms and no obvious effects were noted at the first observation period. We interpret our oiling experiments as affecting the oiled bryozoan colonies most severely for only a short period of time, after which some slight quantity of water soluble fraction of oil could be detected. The rate of decrease in oil concentration is in marked contrast to that observed by Blumer et al. (1973), where stranded crude oil remained on rocks and beaches for prolonged periods.

In our experiments significant differences between the two treatment groups in which bryozoan species were removed were discernible at the first observation period 10 days following the oiling, when percent cover was considered. Panels with bryozoans removed but no oiling of the empty spot had a higher percent cover 10 days after manipulations than those panels that received oil after the bryozoan removals. The space made available by the bryozoan removal greatly enhanced subsequent attachment, whereas oil inhibited attachment. While species coverage gave significant differences, no such effects were discernible for species richness even during this period. Interestingly, both bryozoan removal treatment groups recovered at rates which could be shown to be significantly different from one another during the first observation period. Although this trend continued at subsequent dates different treatment groups were not statistically different. Our conclusion is that the major effect of the oil is observed through direct surface oiling rather than by inhibition of the organisms by toxic components in the oil. This may be attributable to the greater affinity of the oil to the dry surface as compared to the lower affinity of live and wet bryozoan colonies. High and low quantities of oil directly applied to the bryozoan colonies had no statistically significant effects. These results suggest that in spring, when water temperatures are high, bryozoan removals followed by the application of oil to the resulting vacant spot, inhibit growth or settlement of sessile benthos for at least 10 days. The panels which had their bryozoans removed followed by subsequent panel surface oiling had lower coverage on them in three subsequent weeks although this was not significant statistically. Chemical analyses confirmed the short-term presence of some water soluble organics of the crude oil in the benthic organisms. Jacobson and Boylan (1973)

indicate how a water soluble fraction of kerosene can interfere with chemotaxis in a marine snail, and perhaps some similar mechanism is involved in inhibiting settlement or growth on the oiled surfaces of our panels. At our last observation period, 72 days after oiling, this trend was no longer apparent. Panels whose bryozoans had been removed and whose vacant spots were not subsequently oiled achieved the highest total percent coverage; the next highest total percent coverage was found on panels whose bryozoans had been removed and whose vacant spots were then oiled. This suggests that at this time of year, when temperatures are fairly high, the presence of a vacated space eventually allows settlement of one or more species whose ability to usurp space on the panel surface was even greater than those early arriving colonists. At this season, the effect of the removals or oilings is relatively rapidly obliterated.

In conclusion, our experiments indicate that the use of replicate artificial surfaces can be successfully utilized in monitoring the effects of pollutants on sessile benthic organisms.

5. ACKNOWLEDGMENTS

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