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Ecological effects of experimental oil spills in eastern coastal plain estuaries

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by

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CHAPTER I

Ecological Effects

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Introduction

The importance of tidal saltwater marshes as feeding grounds for wildfowl has long been known (Cowell 1969). More recent articles summarize their value for shoreline protection and fish nursery areas (Blum et al., 1978) and their roles as major suppliers of nutrients (Flemer et al., 1978; Valielda et al., 1978) and energy (Mendelssohn and Marcellus 1976; Shisler et al., 1978) to the estuarine environment. Tidal marshes may also serve as nutrient sinks, preventing estuarine algal blooms and the alteration of energy pathways (Broome et al., 1973; Valielda et al., 1975).

The effects of oil spills on marine and estuarine systems have been evaluated mainly by investigations of actual spill accidents. Although considerable understanding of ecosystem effects has been gained from these studies, uncertainties still exist with regard to: 1) the quantity of oil actually impacting the area; 2) previous conditions at the site; and 3) whether the control site chosen can be used to extrapolate to the spill site during recovery. A method used by several investigators to overcome these difficulties has been to apply oil experimentally to various field environments (Schenk et al., 1972; Lytle 1975). This approach was chosen for the present study.

The objective of this project, for which results from four years of post spill studies are reported here, has been to determine the ecological effects of weathered and unweathered South Louisiana crude oil spilled into eastern coastal plain estuaries. Ecological effects have been evaluated at all trophic levels.

Methods

The study site chosen was a natural estuarine marsh-creek habitat, Cub Creek, located at Cheatham Annex, Naval Supply Center, just off the York River which empties into the Chesapeake Bay.

Five 810 m^2 areas (695 m^2 of marsh, 100 m^2 of open water, 15 m^2 of intertidal mud flat) were enclosed by water-tight pens made of transite sheeting supported by exterior salt-treated wooden frameworks (Fig. 1). Continuity of tidal flow was maintained by openings below mean low tide level at the creek end of each pen. Three barrels (570 liters) of fresh South Louisiana crude were pumped through a manifold into the two downstream pens over a one hour period at mid-flood tide on 22 September 1975. Artificially weathered oil (Bieri et al., 1977) was applied in the same manner to the two neighboring upstream pens on 25 September. The pen farthest upstream served as a control. The volumes of oil applied to the experimental pens were sufficient to provide a uniform 0.7 mm thick layer over the enclosed areas.

Randomized procedures were used to sample the areas within each pen and an outside area situated between the two experimental treatments. Grid networks were employed throughout to insure no successive samples were taken from the same spot. Land based samples (grasses, snails) were taken along transects oriented perpendicular to the creek. Overall water quality was monitored by taking dissolved oxygen, salinity, alkalinity, nutrient, and temperature measurements at two or four week intervals for the majority of this study.

Dissolved oxygen concentrations were determined by the azide modification of the Winkler method (EPA 1974; Strickland and Parsons 1972). Salinity was measured with a model RS 7B Beckman induction salinometer, alkalinity measured with a model 36 Fisher automatic titrometer. Grab samples for nutrient analysis (nitrate, nitrite, ammonia, and orthophosphate) were preserved with mercuric chloride and analyzed with a model AAII Technicon AutoAnalyzer (EPA 1974; APHA et al., 1971). In 1979, for reasons of economy, only total nitrate and nitrite were monitored.

Widemouth jars were used to obtain 1 liter grab samples for phytoplankton enumeration. Samples were preserved with Lugol's solution and cells counted with an inverted microscope (Lund et al., 1958). Until 1978 water column primary productivity was assessed by the ^{14}C light-dark bottle technique (Vollenweider 1969) with 1 u $CiNaH^{14}CO_3$ added to 100 ml samples and radiation counts read on a Beckman LS 150 liquid scintillation counter, utilizing an internal standard and a 5g/liter PPO, 100g/liter Napthalene in Dioxane scintillation cocktail. Because differences in primary production between sampled areas were apparent only during the first few weeks of this experiment, the more economical, less time and effort consuming oxygen production method (Vollenweider 1969) was substituted in 1978. Particulate organic carbon (Vollenweider 1969) was also monitored in conjunction with primary production in 1978. ATP was routinely extracted from the water column (Holm-Hansen and Booth 1966) during the first few months of this experiment and quantified

on a JRB, Inc. ATP photometer.

Artificial substrate techniques (Sladeckova 1962) were used to survey effects of the oil spill on the periphyton community. Initially, two series of 2.5 x 11.0 x 0.3 cm transite strips were attached to wooden poles and placed just below mean low water level, one oriented in a horizontal plane relative to the water surface, the other in a vertical plane. Plates were changed at intervals dependent on projected periphyton growth rates, with a separate set of horizontal plates employed to monitor cumulative growth. Three, 2 cm^2 areas were scraped (one section from each orientation) and chlorophyll a extracted using 90% acetone (Strickland and Parsons 1972) and measured by the fluorometric method (Holm-Hansen et al., 1965) using a model III Turner fluorometer. Values were corrected for the presence of pheophytin (APHA et al., 1971). Triplicate 1 cm^2 areas from each orientation were scraped and extracted with boiling Tris buffer for ATP content (Holm-Hansen and Booth 1966) and ATP values determined with a JRB, ATP photometer. Although triplicate samples for ATP were taken from 1976-78, colonization was poor, and the samples had to be combined for analysis. The validity of the values from the cumulative growth plates during 1975 was rapidly compromised due to sedimentation, and sampling of these plates was discontinued during the second month of the study. In addition to the sample for ATP and chlorophyll analysis, a 4 cm^2 subsample was scraped from each strip and preserved with Lugal's solution for species identification.

Benthic populations were sampled until July 1977, with a standard Ekman grab measuring 22.8 x 22.8 cm. Three samples were taken in each

sampling area. Since July 1977, five replicates per sampling area have been obtained with a modified post hole digger-type grab measuring 10.2 x 15.2 cm, to provide a greater amount of replication. Samples were washed through a 0.5 mm screen and stained with either Rose Bengal or Phloxine B added to the 5% formalin preservative. Benthic organisms were identified to the species level where possible.

The standing crop of marsh grasses was estimated at the beginning, mid-point, and end of each growing season for the first three years following the oil spills. Ten or twenty 0.25 m^2 circular quadrats, or twenty 0.1 m^2 circular quadrats, per sampling unit were clipped and everything above the marsh surface removed. Grasses from each quadrat were grouped by species into living and dead components then dried to constant weight at 100°C and weighed on a Sartorius top loading balance. Sampling during the fourth year was conducted at the end of the growing season only.

The aerial extent and degree of dominance of the major grass species within each pen were estimated visually at the end of each growing season and vegetation maps were drawn.

Snail populations were estimated by counting all specimens on the marsh surface and marsh grasses in ten randomly located 1 m^2 quadrats per sampling area during the first year following the oil spills. Since that time, 0.25 m^2 quadrats have been employed.

Live boxes originally stocked with 200 Fundulus heteroclitus were installed in each sampling area and monitored daily for the first two months following the oil spills, and then on regularly scheduled sampling trips afterwards. Oysters (Crassostrea virginica) (120/sampling area) and clams (Mercenaria mercenaria) (110/sampling area) were also stocked and

monitored.

Statistical comparisons of treatments were made with one-way analysis of variance and by the Student-Newman-Kuels test (Sokal and Rohlf 1969).

Results and Discussion

General Conditions - The bulk of the weathered crude oil applied at Cub Creek disappeared after one week, with only traces remaining after two weeks. Fresh crude was visible in one of the replicate pens for five weeks and was apparent in both replicates for three weeks.

Aromatic hydrocarbons in the water column reached maximum levels in the weathered pens six hours after oil application and were maximum in the fresh crude pens 76 hours after application (Bieri et al., 1977).

Dissolved oxygen concentrations ranged between 2.1 mg/l in late summer to 13.6 mg/l during the winter (Appendix M). Oxygen levels in the oiled units were slightly lower than control values during the first three weeks following the spills. These depressions were most probably the result of the surface film interfering with reaeration and possibly some additional biochemical oxygen demand exerted by the oil.

Water temperatures ranged from below 0°C, during January and February of 1977 when 15 to 20 cm of ice covered the marsh, to as high as 30.8°C in summer (Appendix N).

Alkalinities ranged from 76.3 to 195.3 mg/l (Appendix O) and salinities from 0.2°/oo, to 18.7°/oo, with the majority of values between 2 and 6°/oo (Appendix P).

Dissolved nitrogen concentrations varied between 1.5 and 82.0 ug/l

for NO_3 (Appendix Q), from 0.6 to 9.5 ug/l for NO_2 (Appendix R), with NH_4 values ranging between 0.01 and 1.0 mg/l (Appendix S). Orthophosphate concentrations were between 1.2 and 120 ug/l with values usually less than 40 ug/l (Appendix T).

During the first month following the oil spills, both ammonia and orthophosphate levels in the fresh oil pens were elevated slightly above control levels, a trend not evident in the weathered pens. Nitrate and nitrite values were slightly elevated in the oiled pens during the first four days immediately following the oil spills. For the remainder of the study, nutrient levels have varied about control values, displaying spring-summer minimums and late summer maximums.

Primary productivity has varied from $<$1.0 to 59.3 ug C/hr. (Appendix U). The fresh oil depressed production on the first day following the spill, but by the next day a stimulation in production over the control was observed. This overshoot in production continued for the first week at which time primary production returned to control levels and remained at or near control levels. A similar pattern was observed in the weathered oil units, but inhibition was both more severe and prolonged (Fig. 2). A slight stimulation appears to have occurred on the seventh day following the spill, and by the fourteenth day levels were similar to control production. Crude oils have been shown to decrease photosynthesis and cell division in phytoplankton (Mommaerts-Billet 1973; Kust 1978). The aromatics appear to be the most toxic components of crude oils to phytoplankton (Soto et al., 1975; O'Brien and Dixon 1976) with sensitivity dependent upon the plant species and origin of the oil (Mironov 1971;

Pulich et al., 1974). Inhibition of productivity before loss of aromatics and stimulation following a decrease in the levels of aromatic residues has been previously reported (Kass and Hutchinson 1975).

ATP levels measured in the water column ranged between 0.3 and 1.0 ug/l and were in general agreement with primary productivity data (Appendix V). The data indicated early stimulation in the fresh crude pens with a return to control levels in seven days, and decreased levels in the weathered crude as late as ten days following oil spillage. Four additional months of ATP analysis failed to detect any significant differences between pens, so monitoring of ATP in the water column was discontinued.

Differences in total phytoplankton counts (Appendix W) between pens have never been statistically significant, although experimental pen counts were below control values during the first week of this study. Approximately 250 species were recorded during the first month of this study, with the most striking feature of the phytoplankton being the consistent dominance (about 50% of the total counts) of 4 u and 8 u cryptophytes. Pennate diatoms also were abundant with Nitzschia longissima and N. reversa being the most common. No differences in species composition were observed between pens. Samples of phytoplankton collected from all experimental units in March and July of 1976 were similar in species composition and abundance. Based on these results and previous observations, it was decided to discontinue observations on this community.

Periphyton plates were colonized by barnacles of the genus Balanus in approximately the same concentrations. All substrates developed diatom mats composed predominantly of the pennate genera Nitzschia

and Navicula. Filamentous members of a Melosira species also were found in all pens. Control and weathered oil pen plates had sparse growths of Ulva lactuca by the eighteenth week.

Periphyton ATP values (Appendix X) during the first 18 weeks following the spill ranged from 0.47 to 5.2 ug/cm^2, with greater values in experimental pens and significant differences between control and experimental (α 0.05) at weeks 1, 12, and 18. During this period weathered oil values were four times greater than those of the control, while fresh oil values were 2 to 3 times greater than the control. Increased microbial biomass may have accounted for these elevated ATP levels. The increased biomass might be due to utilization of hydrocarbons as a food source and/or decreased predator populations normally limiting periphyton growth. After 14 May 1976 ATP values ranged from $<$ 0.01 to 1.8 ug ATP/cm^2 with gradually decreasing values in all pens and no consistent differences between pens. There was no repetition of the elevated levels encountered earlier.

Periphyton chlorophyll a concentrations (Appendix Y) ranged from 0.2 to 8.7 ug/Chla/cm^2 during the spring and summer of 1976, with the majority less than 3.0 ug/Chla/cm^2. During this period no effects were attributable to the oil. From December 1976 to August 1978, values ranged from 0.04 to 10.7 ug/Chla/cm^2 with no consistent between-pen differences.

Marsh Grasses

Summaries of live and dead marsh plant biomass are presented in Tables 1 & 2, Figures 3 & 4 and Appendices G-L. There was a significant

effect from enclosure, with greater crops inside the control pen than outside. Differences decreased with time, however, the oiled units must be compared to the control to assess the effects of the oil on plant dynamics.

The plant biomasses were within ranges summarized elsewhere (Flemer et al., 1978; Hopkenson et al., 1978 and Shisler et al., 1978) and followed the seasonal live-dead inverse relationship described by other investigators (Kirby, 1972; Mendelssohn and Marcellus, 1976 and Blum et al., 1978) with dead biomass maximum in winter and a gradual decrease to a late summer-fall minimum, the time of peak live biomass.

The effects of the oils on the standing crop of marsh plants, comprised predominantly of Spartina alterniflora, were severe during the first year following oil application (Fig. 3). Exposed areas produced less than half the biomass of the control during 1976, and substantial differences were still evident during the second year following the spills. Growth during the third year was much improved with both oil treatments showing standing crops equal to or greater than the control, although the peak value for grasses in the fresh oil treatment were still below that of the control pen. Both treatment pens had less live standing crop than the control pen at the end of the 1979 growing season. Minimum live biomass values were usually very similar between pens throughout this study. Prior to treatment all pens had comparable live standing crops, although dead biomass in both experimental pens was greater than that found in the control pen.

Net primary production (Table 3) was estimated by the summation technique of Smalley (1958).

Table 3

Estimated Net Production (g/m 2/year)

Year	Control	Fresh	Weathered	Outside
1976	752	349	292	693
1977	529	458	368	361
1978	506	490	571	415

Differences in net production between pens were similar to those observed in standing crops, with the greatest effects evident the year following the spills. During 1977, production was estimated to be higher in the units dosed with the fresh oil than those receiving the weathered treatments, although peak standing crops were quite similar. In 1978, production estimates closely paralleled the standing crop measurements with the weathered treatment exceeding the control in both parameters.

The initial reductions in net production in the oiled pens reflected the loss of viable areas, depressed stem densities (in the oiled pens with respect to the control) and the suppression of the second cohorts of shoots in both oiled treatments (Hershner 1977).

To supplement the standing crop estimates made on the marsh plants in each unit, the aerial extent of the major grasses in each enclosure was estimated visually. Monospecific stands were considered to exist if a species comprised at least 85% of the vegetative cover. Distichlis spicata and Spartina patens were considered as a group because they occur in close association (Silberhorn 1976; Driemeyer and Zieman 1979) and because the dead forms cannot be realistically separated (Hopkinson et al., 1978; Lesser et al., 1976).

Vegetation coverages which are determined at the end of the growing season in each enclosure are shown in Figures 5-9. Prior to the oil treatments there were no monospecific stands of Scirpus or the Distichlis - S. patens group. S. alterniflora was the dominant grass in all areas.

The transition in vegetative cover in the control pen from 1976-79 is shown in Figure 5. As shown in the figure, there were gradual increases in mixed Distichlis - S. patens areas and mixed Scirpus into monospecific stands of S. alterniflora. After the second year of containment, limited quantities of Amaranthus canabinus, Aster sp., Pluchia purpurascens, Salicornia virginica and Baccharus halimifolia were also found in the control enclosure. Over the next two years, increased abundance of both Aster sp. and Amaranthus was noted.

Table 4 list the commmon and scientific names of plant species found in the enclosures during the study.

Vegetation patterns for the two fresh oil replicates (pens 1A and 1B) are presented in Figures 6 and 7. The large non-vegetated areas present one year after the oil spill were gradually recolonized over the next three years. The year following the spills (1976) pen 1A had no stands of monospecific S. alterniflora short form, but did have a stunted stand of Distichlis - S. patens. Enclosure 1B had developed large monospecific and mixed stands of Scirpus. The following year (1977) both pens had greater monospecific stands of S. alterniflora short and tall forms, as well as additional areas dominated by Distichlis - S. patens. No monospecific stands of Scirpus remained in pen 1B, which had developed a very complex vegetation pattern. During the next two years there was an

increase in mixed areas of Distichlis - S. patens and Scirpus as they spread into areas previously dominated by S. alterniflora. Small quantities of Amaranthus cannabinus, Aster sp., Pluchea purpurascens, Salicornia virginica, Baccharis halimifolia, Kosteletskya virginica, and Chenopodium sp. appeared in both pens in 1977. Aster and Amaranthus became more widespread over the next two years as they did in the control enclosure.

Vegetation patterns for the two weathered oil replicates (pens 2A and 2B) are presented in Figures 8 and 9. As in the fresh oil pens, the large non-vegetated areas found in 1976 gradually were recolonized until little remained in 1979. S. alterniflora was scattered throughout both pens from 1976-77, with complex patterns of mixing in 1977. There were steady increases in mixed Distichlis - S. patens and Scirpus stands in both pens throughout the study, and a monospecific stand of Scirpus appeared in pen 2B in 1979. There were somewhat larger areas of mixed Distichlis - S. patens and Scirpus in these pens initially than in any other pens. There was more Aster present in 1977 than in any of the other units, which along with Amaranthus increased in 1978. Baccharis, Pluchea, Salicornia, Kosteletskya, and Chenopodium also appeared in 1978. In 1979 Amaranthus and Aster were scattered throughout both pens, although greater amounts of Aster were present in pen 1. Atriplex patula was also present in 1979.

In all oiled units non-vegetated areas were first colonized by tall form S. alterniflora. Plants growing near the edges and at the rear of the enclosures were somewhat stunted due to the greater penetration of oil

along the perimeter walls where ditches had been dug.

Differences were evident in the vegetative patterns of the control and oiled enclosures. Although there were increases in the amount of Scirpus and mixed areas in the control over the 4 year post-spill period, the control pen never developed the monospecific stands of Scirpus or stands dominated by mixtures of Distichlis - S. patens as were found in the treatment enclosures. The complex vegetation patterns found in both oiled treatments never developed in the control unit.

During the study period, increases in coverage by Scirpus and Distichlis - S. patens were not observed outside the enclosures, thus implying that increases in these species in the control pen resulted from altered physio-chemical or biological conditions which were caused by enclosure. Both Distichlis spicata (Hansen et al.,1976) and Scirpus robustus (Palmisano and Newsom 1967) have been described as primary invaders of disturbed sites, suggesting that both have the capability to respond rapidly to changing conditions.

Recent work indicates that vegetation patterns in unperturbed environments are results of complex interactions of environmental and possibly biotic factors (Barbour, 1978; Eleuterius and Eleuterius, 1979). No single physical gradient (Weiss et al., 1979), microtopographic feature, microenvironmental variable (Silander 1979), or soil characteristics (Hackney and de la Cruz 1978) have been shown responsible for determining species associations. Genetic variability within salt marsh plant species is well established, with a significant genetic component involved in the differential response to nutrients between lower and upper marsh plants

of the same species (Jefferies and Perkins 1977). The range of genetic variability present in populations can be quite high and is related to environmental harshness (Silander 1979). Therefore the amount of genetic variability initially present in a specie or species at the time of an environmental perturbation may be a major factor determining response to environmental change and may account, in large part, for the differential responses (recovery time) by marsh plant communities to oil spills.

The differences between the control pen and the outside area were almost certainly due to effects produced by alterations in drainage and groundwater patterns inside the pen, while differences between the control pen and experimental pens were due to effects of the oils. Detrimental effects of oils on salt marsh plant communities are summarized elsewhere (Boesch et al., 1974; Baker 1978) and include destruction of shoots and seeds, disruption of metabolic activity, including alteration of respiration rates and reduction of transpiration and translocation, and smothering effects that restrict gas exchange and ultimately kill oil coated plants.

Other investigators have observed that fresh oils were more toxic to marsh vegetation than weathered oils (Cowell 1969 and Baker 1970, 1971). In this study both fresh and weathered oils exhibited similar apparent toxicities. The average standing crops of grasses in the units followed each other very closely (Figure 3). Production was estimated to be greater in the fresh units during 1976 and 1977 than in the weathered enclosures.

We believe that differences in physical nature of oils were probably responsible for the observation that the weathered oil was as toxic as the fresh. The weathered oil adsorbed to the plants and bottom substrates much more readily than its fresh counterpart, resulting in actually higher doses per unit area to the plants which were exposed.

The complex patterns and monospecific vegetation stands that developed in the experimental pens probably resulted from a variety of oil related stimuli.

Direct toxicity varies depending upon the plant species and the amount of oil exposure (Baker 1978; Boesch et al., 1974). Because of slight elevation differences inside the pens, neither oil was evenly distributed over the vegetation, resulting initially in relatively large non-vegetated areas due to smothering. Recolonization of these areas initially by Spartina might have resulted, in part, because Spartina shoots are known to have a large bulk storage of air (Ranwell, 1972) providing for rapid initial recovery from smothering.

A frequent result of acute mortality of marsh grasses is erosion of the substrate where the stabilizing root masses have been killed (Hershner 1977; Hampson and Moul 1978). If the erosion is not too severe the lowered elevations provide an opportunity for territorial expansion of those species inhabiting neighboring low lying areas. In addition, since some plant species maintain their dominance by root or rhizone exudates that are harmful to other species (Driemeyer and Zieman 1979), a decrease in plant root density will dilute this barrier to invading species.

Populations of the pulmonate snail, Melampus bidentatus were monitored for the first three years of this study (Appendix Table Z). Peak populations occurred in June-July with maximum densities of $128/m^2$, $42/m^2$, and $72/m^2$ in 1976, 1977 and 1978, respectively. Snail populations decreased immediately following the spills in both treatments. Control

populations averaged 7/m^2 one month after the spills, with populations in the weathered pens of 0.4/m^2, while the fresh oil units averaged 5/m^2. By the 40th week following the spills, the fresh oil populations had recovered, while the weathered population recovery lagged by eight weeks. Populations in both treatments were not subsequently different from those of the control. These population levels were higher than those reported in another Virginia salt marsh (Kerwin 1972), but lower (Shisler and Jobbins 1977) or very similar to those reported in other East Coast marshes (Lesser et al., 1976).

Benthos

Significant differences between control and experimental pen benthic populations as determined by the Student-Newman-Keuls test are presented in Figures 10-16. (See Appendix Table F for the computer program utilized to obtain this data.) When control populations were greater than experimental ones, control values were divided by experimental values. If experimental populations were greater than those of the control, the negative reciprocal was used. A zero was plotted when no significant difference between control and experimental pens occurred at the 0.05% level.

Benthic biomass (Figure 17) was consistently greater in the control pen than in any experimental pen, significantly so on 6 of the 17 dates sampled. The greatest adverse effects were seen in the polychaete, amphipod, and oligochaete components of the benthic community. The molluscs, predominantly Macoma sp., were unaffected, reflecting the high degree of resistance to oil pollution reported elsewhere (Leppakoski and Lindstrom 1978; Shaw et al., 1976; Kasymov and Aliev 1973; Baker 1973).

Polychaete Community

Of the 14 species found in Cub Creek during this study (Table 5), four displayed oil-related population fluctuations when compared to control populations. Both nereid populations showed immediate effects of the oils.

After one week, numbers of Nereis succinea (Figure 10) in the fresh crude treatments were one-tenth those of the control, numbers in the weathered crude were one-fourth of the control. This decrease is somewhat surprising in view of the reported high resistance to oil pollution of N. succinea (Bender et al., 1974; Grassle and Grassle 1974) and other nereid species (Leppakoski and Lindstrom 1978; Kasymov and Aliev 1973), and may be partly due to synergism with a physical or chemical variable encountered in the experimental pens (Tatem et al., 1978; Rossi and Anderson 1977; Reynolds et al., 1975). The control population, N. succinea, remained consistently higher than treatment populations until almost three years (week 148) after the spills, with significant differences at week 103, 114, and 135. The Laeonereis culveri population (Figure 11) in the fresh crude pen dropped to one-seventh that in the control after one weeks' exposure and remained consistently lower than control values for the next year and a half (week 74), with significant differences at weeks 1, 11, 20, 26, and 56. From week 74 to week 91, control and fresh crude populations were comparable. From week 103 to week 181, control levels were higher with significant differences on two of the sampling dates. Although numbers of L. culveri in the weathered oil pens were also consistently lower than control values until week 74, there was no corresponding immediate

response to the weathered oil nor were population depressions as severe as those found in the fresh oil units. This may be related to the lower levels of aromatics in the weathered oil. Populations in the weathered oil units were higher than those in the control until week 126, when they decreased relative to the control as did the populations in the fresh crude treatment. Recovery of these populations appears to be complete after the end of the third year.

Lysippides grayi (Figure 12) control populations were generally higher than any experimental population during the course of this study, with significant differences on 10 of the 20 dates sampled. The first significant population decrease occurred four weeks after the spills.

Populations of the capitellid, Heteromastus filiformis, (Figure 13), remained essentially the same as control pen populations until week 20, when the weathered oil population was significantly higher than the control, and until week 26 when both fresh oil and weathered oil populations were significantly higher than the control. From week 74 on, control populations of H. filiformis were generally higher than either of the experimental ones, significantly higher than the fresh oil populations on five occasions, and significantly higher than the weathered oil on two occasions.

Streblospio benedicti populations were uniformly small in all pens until week 49 when they increased dramatically (Figure 14). Fresh oil populations were frequently higher than control populations from that time on and significantly so at weeks 114 and 148. Weathered populations were generally comparable to controls.

Populations of the dominant amphipod, Leptocheirus plumulosus,

(Figure 15), were significantly higher in the control pen than either oiled pen for the majority of samples taken during the first two years of the study. Recovery occurred during the third year with comparable populations in the oiled and control units during most of the year. Significantly higher control pen populations were evidenced only on occasion at week 148. Fresh oil populations were significantly greater than weathered oil populations at weeks 125, 136 and 181.

The oligochaete populations (Peloscolex spp.) (Figure 16), were significantly greater in the control pen than in the oiled pens the majority of the time. Oiled pen populations remained relatively constant during the first two years, while control populations displayed greater fluctuations. All populations showed marked increases during the third year, and the smaller differences between control and experimental populations occurred during the last two years, implying recovery.

Chironomid larval populations were depressed in the oiled units after one weeks' exposure and remained so during the next six months (Bender et al., 1977). These insect larvae were not present again in any of the areas sampled for a period of almost two years. To our knowledge, insect control measures were not conducted during this period and the reason for their absence is unknown.

Initial tolerance to oil pollution and tolerance to the continued presence of oil pollution are entirely different things (Grassle and Grassle 1974) and may explain the delayed response of Lysipiddes grayi and Heteromastus filiformis and the delay of significant differences between control populations and experimental populations of other benthic

invertebrates.

The common members of the Cub Creek benthos are tube-dwellers and/or burrowers, and with the exception of the nereids and Eteone, they are all deposit-feeders. The explanations for development of an exclusively deposit-feeding soft bottom community in marsh areas are discussed by Levinton (1972) and Sanders (1958) and have pertinent implications for this study. Burrowing activity may be hindered by oil pollution, resulting in detrimental effects on feeding patterns and efficiencies as well as increasing susceptibility to predation (Wharfe 1975). Short of catastrophic oil effects, specialization within an exclusively deposit-feeding community (Levinton 1972) might be expected to make it difficult for invading opportunistic species (Virnstein 1976; Grassle and Grassle 1974) to become established, let alone dominant. At Cub Creek, however, almost all species have been recognized as opportunistic. Only Streblospio benedicti displayed true opportunism in the oiled pens, becoming firmly established at week 49.

The importance of benthic communities in terms of ecosystem structure and function is briefly reviewed elsewhere (Virnstein 1976), as are the pathways by which oils enter sediments (Percy 1977). Microbial degradation is probably the major factor in removal of petroleum hydrocarbons from the environment (McAuliffe 1977; Walker and Colwell 1977), with breakdown occurring at markedly reduced rates once incorporated into aerobic sediments (Percy 1977; Shelton and Hunter 1974). Anaerobic degradation occurs at even slower rates (Blumer and Sass 1972), if at all (Davis 1968). Activities of benthic tube dwellers, burrowers, and deposit feeders accelerate the rate of microbial degradation (Gordon et al., 1978; Leppaskoski and Lindstrom 1978). Although

South Louisiana crude may be one of the more susceptible oils to microbial degradation (Walker et al., 1976), when all factors limiting such biodegradation are considered (Atlas 1978), there remains an evident potential for long-term contamination of marsh benthic communities by any oil. Oil incorporated into sediments may not persist for many years, but may be resuspended by storms and tides (Blumer and Sass 1972), and by the activities of deposit feeding benthos (Rhoads and Young 1971).

Fish

This continual presence of oil in the sediment was probably the main factor responsible for the long-term effects on the benthos found in this study. The fish retained in live boxes in both weathered oil units exhibited a rapid short-term response. Individuals began to die late on the fourth day following the oil spill. All were dead by the ninth day. Lesions and discoloration preceded death. Fish were restocked in the weathered oil pens the following day. Neither control nor unweathered oil unit fish died.

The anticipated corresponding mortalities in the fresh oil unit a week later (since the weathered oil had been weathered for a week prior to being spilled) did not occur. There was a slight increase in mortality in the unweathered units and the fish that died (14 of 200) exhibited the symptoms seen earlier. No further mortalities were observed in any pens during the following two months.

All F. heteroclitus examined in the study area had heavy gill infestations of metacercariae of the digenetic trematode, Ascocotyle angrense, a common Fundulus parasite found in the Chesapeake Bay region (Stunkard

and Uzmann 1955). It is probable that the parasite had little to do with the mortality seen in the weathered pens, since heavy gill infestation reportedly does not impair Fundulus activities (Sogandares-Bernal and Lumsden 1963) and all fish in the weathered pens died, regardless of degree of infestation. Furthermore, no massive mortalities were seen anywhere else in the study area and no mortalities attributable to oil were observed in the captive clam or oyster populations.

Three bioassay experiments utilizing the original fresh and weathered oil were conducted during 1977, and an additional bioassay using a newly weathered batch of South Louisiana crude was performed in 1978. In each experiment, a 0.5 cm thick layer of oil was assayed with 90 liters of Cub Creek water and 20 Cub Creek Fundulus heteroclitus in replicate tanks. The aquaria were aerated moderately. No mortalities attributable to the oils were observed in any bioassay during the two to three weeks the bioassays were maintained.

Summary and Conclusions

It is the general consensus that the aromatic fraction of petroleum oils, the fraction that persists the shortest time after an oil spill, is the most toxic fraction to biota (Tatem et al., 1978; Rossi et al., 1976; Anderson et al., 1974; Moore and Dwyer 1974; Blumer et al., 1973; Nelson-Smith 1973; Crapp 1971). However, the fresh crude utilized in this study was clearly more toxic than the weathered crude to only three members of the marsh benthic community. The fresh oil had greater initial effects on Nereis succinea and perhaps greater sustained effects on Laeonereis culveri and Leptocheirus plumulosus. The weathered oil had greater initial effects

on the phytoplankton community (lowered primary productivities and ATP concentrations) and the snail community and was more toxic to the captive fish populations. Other than these, there were no appreciable differences between the effects of the oils. We believe the comparatively rapid disappearance of the weathered oil from its enclosure was due to a more rapid sorption of the oil to sediments and biota than was achieved by the fresh oil. This might mean that, compared to the fresh oil dosings, in the long term, more of the weathered oil was available to interact with flora and fauna. If so, the similarity of effects caused by the two oils might actually indicate the weathered oil was less toxic than the fresh oil on a per volume basis.

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TABLE 1

MARSH GRASSES - Total Live (L)
and Dead (D) Standing Crop (G/M^2)

DATE		CONTROL	1A	1B	$\bar{x}$ Pen 1	2A	2B	$\bar{x}$ Pen 2	Outside
9-19-75	L	427	387	370	377	526	426	476	-
	D	195	407	309	358	317	348	333	-
3-29-76	L	37	25	19	22	28	8	18	81
	D	418	421	764	593	699	661	680	617
6-15-76	L	454	126	151	139	220	136	178	384
	D	498	516	660	588	443	662	552	371
9-17-76	L	672	360	244	302	420	163	292	499
	D	404	365	340	353	429	470	450	565
5-3-77	L	190	81	94	87	134	85	110	154
	D	625	375	208	292	368	407	387	565
7-26-77	L	385	362	278	320	377	215	296	307
	D	359	169	138	153	167	229	198	462
8-15-77	L	478	359	342	350	376	324	350	361
	D	413	258	262	260	200	231	215	427
4-7-78	L	37	59	47	53	35	47	41	58
	D	553	395	286	340	598	468	533	586
4-24-78	L	65	133	128	131	114	107	111	125
	D	406	440	283	362	500	314	407	383
6-1-78	L	250	328	305	317	348	400	374	385
	D	364	434	239	336	506	321	414	277
8-7-78	L	503	471	381	426	520	609	564	322
	D	367	295	100	198	333	195	264	240
10-12-78	L	439	458	448	453	373	516	445	416
	D	127	167	116	142	250	141	195	158
9-20-79	L	588	548	434	491	476	437	456	455
	D	344	194	287	240	260	392	326	503

TABLE 2

Peak Live Standing Crop of Major Marsh Grasses (G/M^2)

Year	Control	1A	1B	2A	2B	Outside
			Spartina alterniflora			
1975	426	376	341	518	416	ND
1976	657	219	214	395	137	487
1977	446	228	206	342	251	304
1978	409	224	277	453	366	410
1979	554	297	289	371	256	435
			Distichlis - *S. patens*			
1975	1	10	27	7	3	ND
1976	2	104	20	20	-	-
1977	4	122	70	16	46	52
1978	16	204	115	29	105	3
1979	1	199	82	26	47	3
			Scirpus			
1975	-	-	-	-	-	ND
1976	-	-	1	-	-	-
1977	3	2	11	2	3	1
1978	-	2	1	8	56	-
1979[1]	17	9	35	41	110	-
			Aster			
1975	-	1	2	1	7	ND
1976	13	43	10	5	26	12
1977	21	7	49	11	24	5
1978	77	40	55	29	70	3
1979	16	44	17	29	24	15

[1]Estimates include dead plants because live biomass peaked earlier than the sampling date.

Table 4.

Cub Creek Marsh Grasses

Amaranthus cannabinus (Water hemp)

Aster tenifolius (Saltmarsh aster)

Atriplex patula

Baccharis halimifolia (Groundsel tree)

Borrichia frutescens (Sea oxeye)

Chenopodium sp.

Distichlis spicata (Salt grass)

Eleocharis parvula (Dwarf spikerush)

Kosteletzkya virginica (Marsh mallow)

Limonium sp. (Sea lavender)

Pluchea purpurascens (Saltmarsh fleabane)

Polygonium punctatum (Smart weed)

Sabatia stellaris (Sea pink)

Salicornia virginica (Saltwort)

Scirpus robustus (Saltmarsh bulrush)

Spartina alterniflora (Saltmarsh cordgrass)

Spartina patens (Saltmeadow hay)

Table 5.

Animals Found in the Benthic Community at Cub Creek

Oligochaetes

Peloscolex gabriellae
P. heterochaetus

Polychaetes

Notomastus latericius
Capitella capitata
Heteromastus filiformis
Lysippides grayi
Samythella elongata
Laeonereis culveri
Nereis succinea
Eteone heteropoda
Sigambra sp.
Streblospio benedicti
Scolecolepides viridis
Spio sp.
Scoloplos fragilis
Glycera dibranchiata
Travisiopsis sp. (normally pelagic)

Amphipods

Leptocheirus plumulosus
Corophium lacustre
Gammarus sp.

Isopods

Edotea triloba
Chiridotea almyra
Cyathura polita
Cassidinidea lunifrons

Tanaid

Hargeria rapax

Molluscs

Macoma mitchelli
Macoma sp.

Figure 1

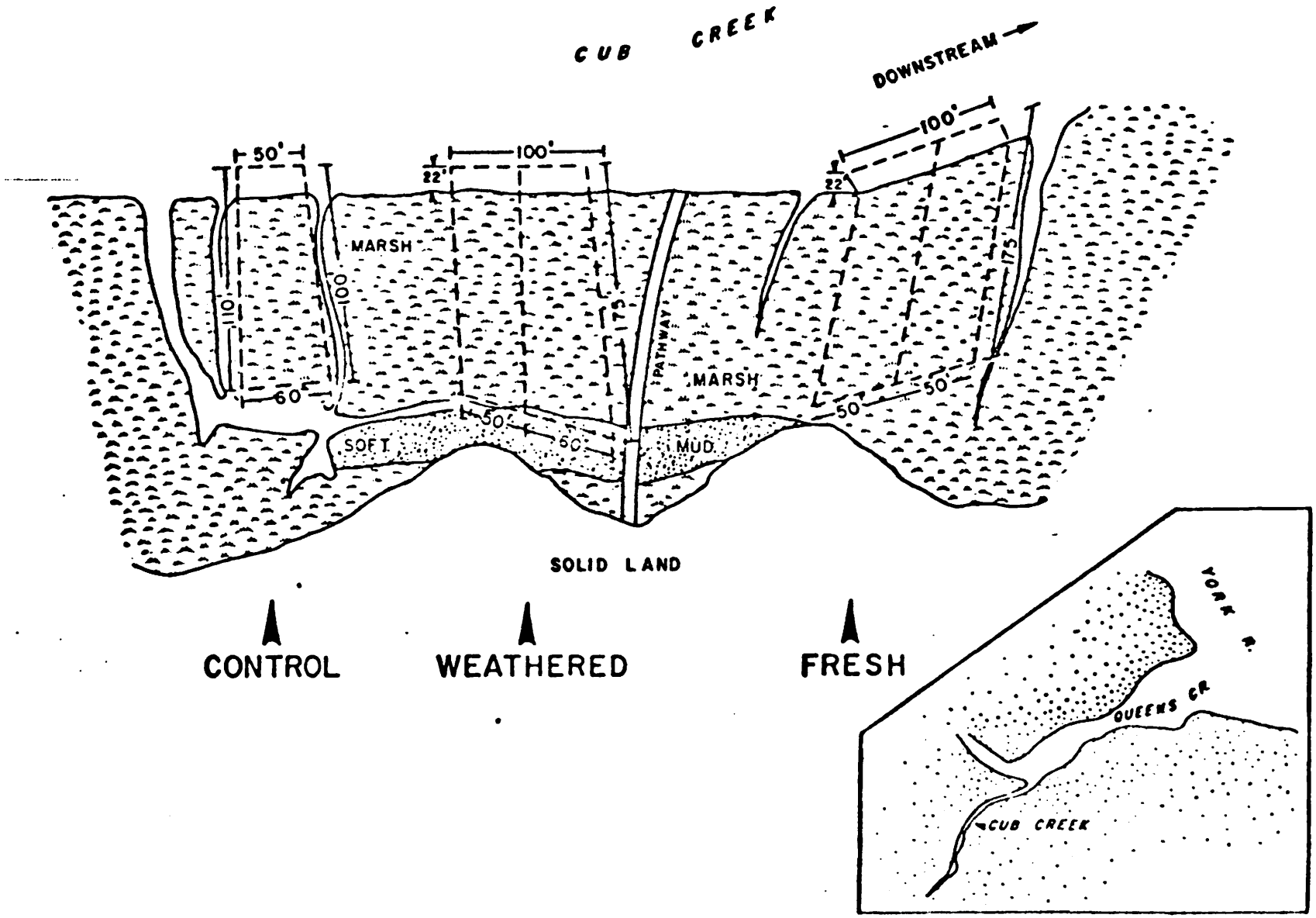

CUB CREEK
DOWNSTREAM
50'
100'
100'
22'
22'
MARSH
10'
10'
60'
175'
PATHWAY
MARSH
175'
50'
50'
50'
60'
SOFT
MUD
SOLID LAND
CONTROL
WEATHERED
FRESH
YORK R.
QUEENS CR.
CUB CREEK

Figure 2

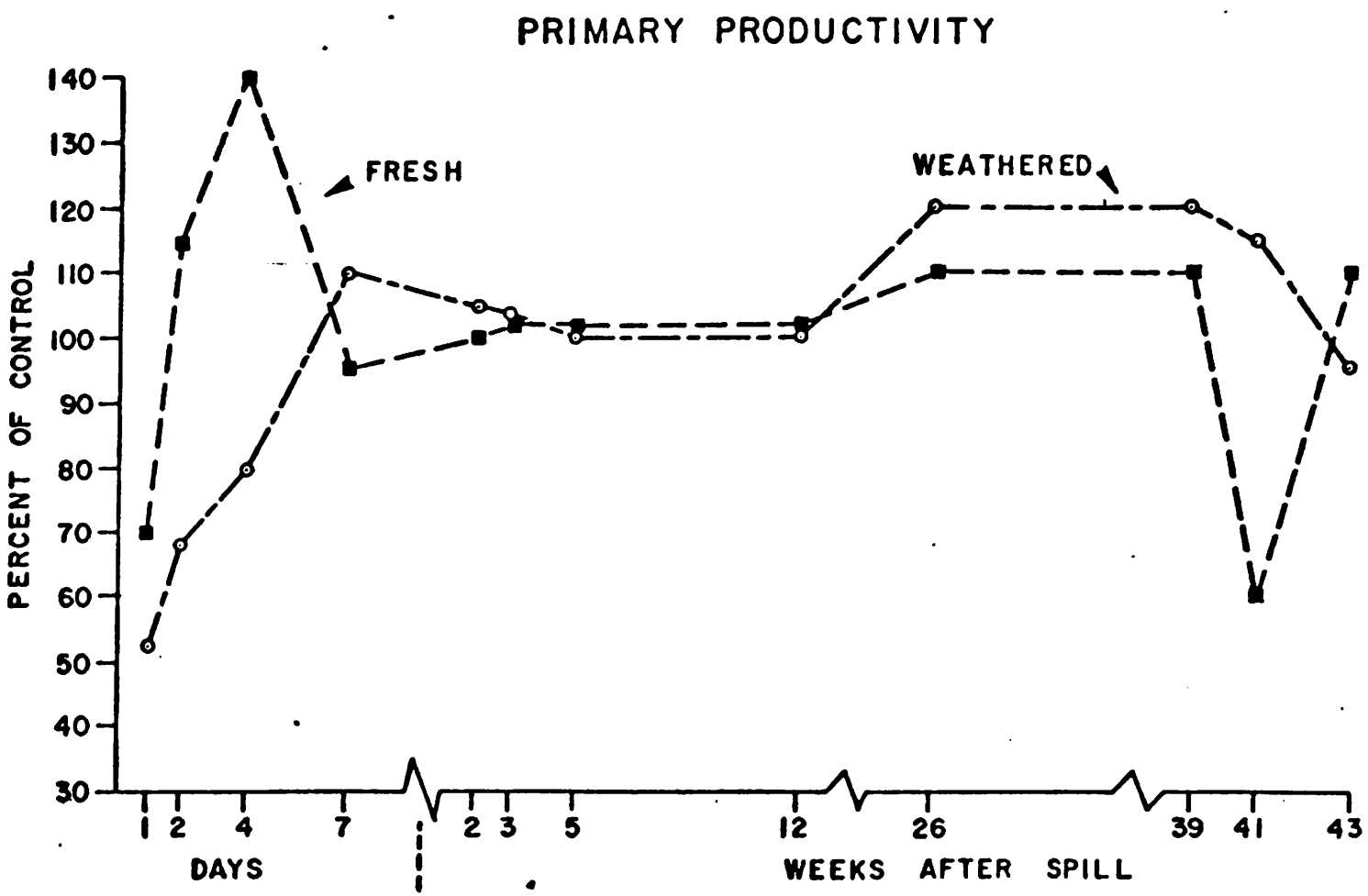

PRIMARY PRODUCTIVITY
PERCENT OF CONTROL
140
130
120
110
100
90
80
70
60
50
40
30
FRESH
WEATHERED
1 2 4 7
2 3 5
12 26 39 41 43
DAYS
WEEKS AFTER SPILL

Figure 3

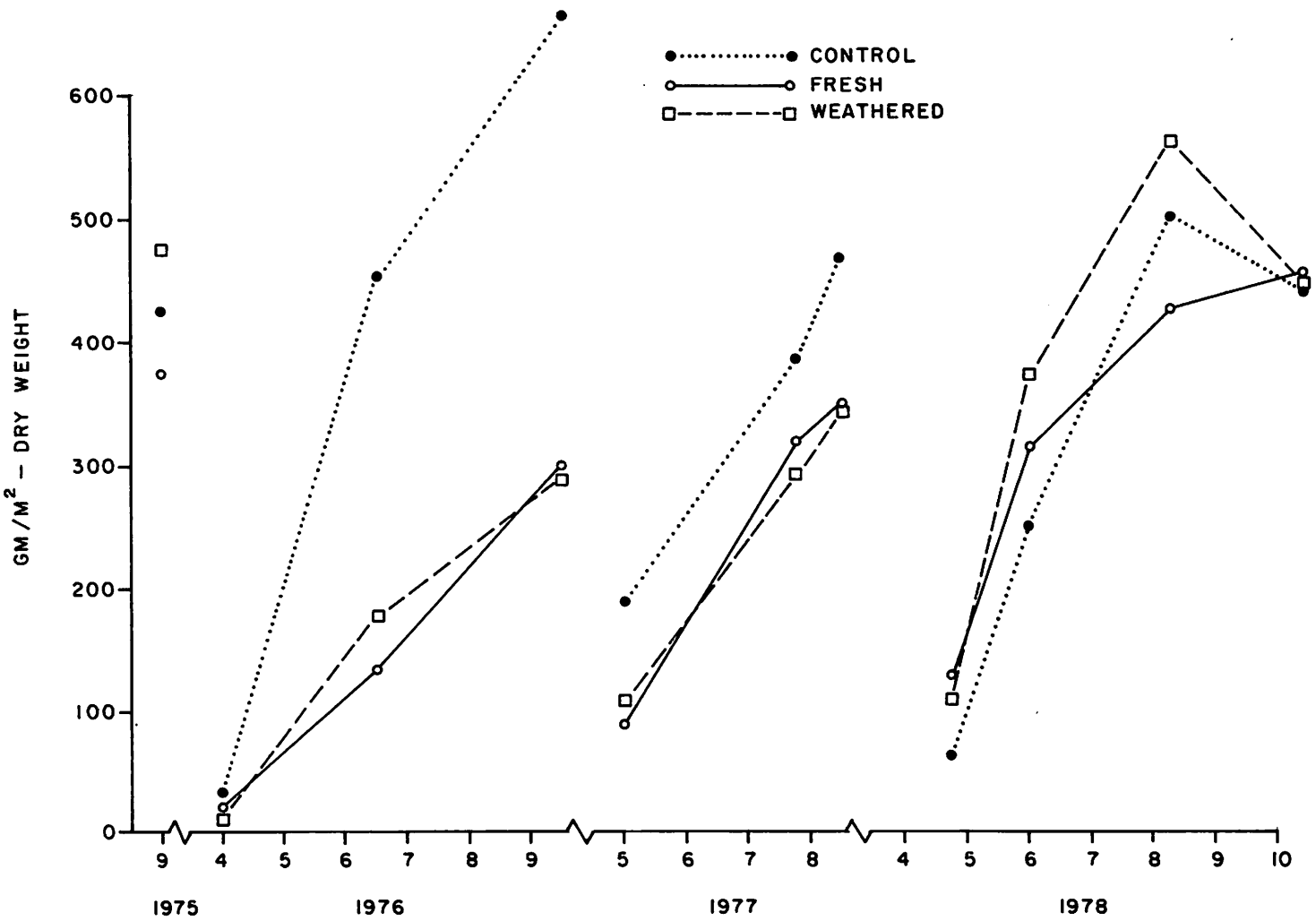

CONTROL
FRESH
WEATHERED
GM/M² - DRY WEIGHT
600
500
400
300
200
100
0
9
4
5
6
7
8
9
5
6
7
8
4
5
6
7
8
9
10
1975
1976
1977
1978

Figure 4

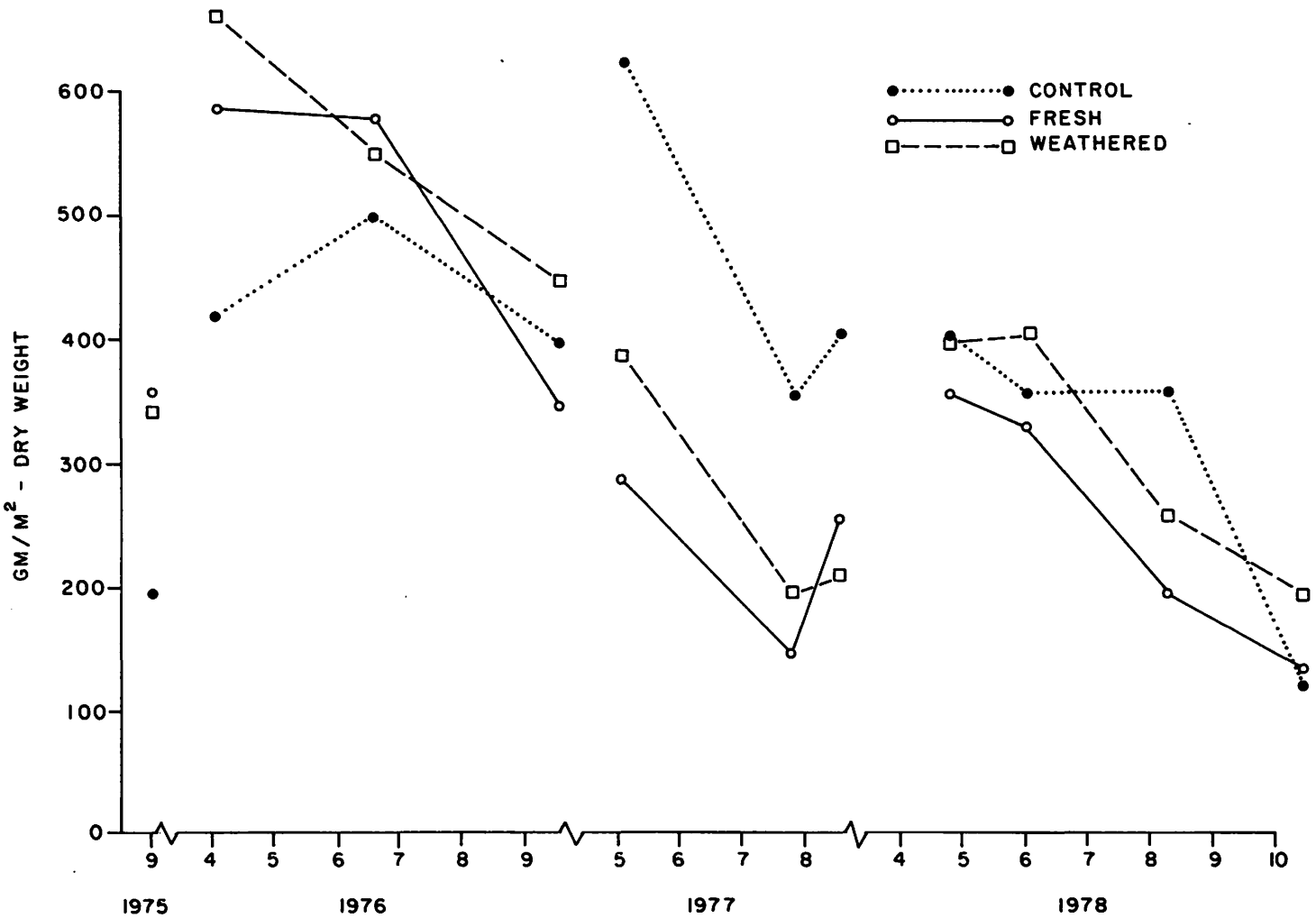

CONTROL
FRESH
WEATHERED
GM/M² - DRY WEIGHT
600
500
400
300
200
100
0
9
4
5
6
7
8
9
5
6
7
8
4
5
6
7
8
9
10
1975
1976
1977
1978

FIGURE 5

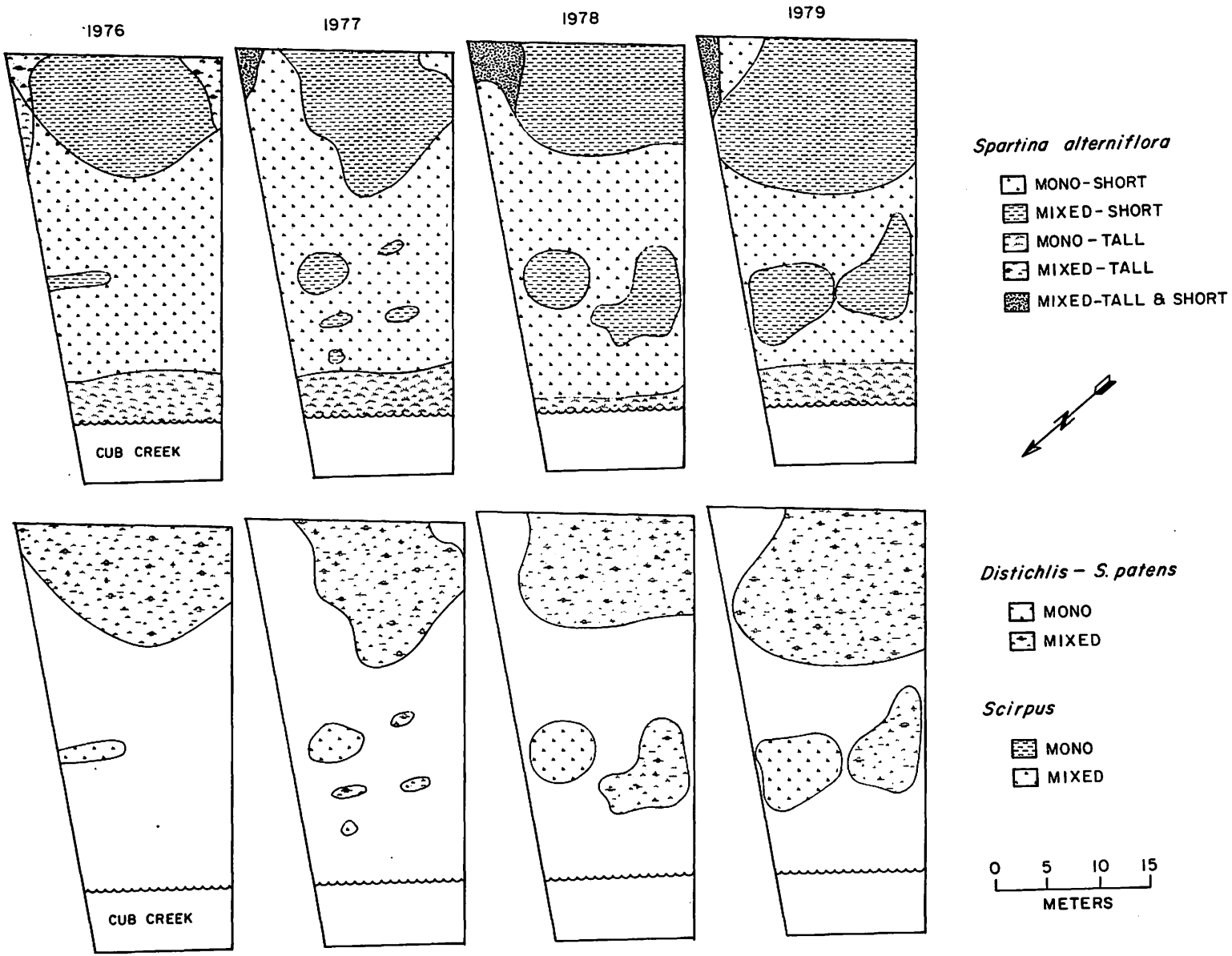

1976
1977
1978
1979
CUB CREEK
CUB CREEK
Spartina alterniflora
MONO-SHORT
MIXED-SHORT
MONO-TALL
MIXED-TALL
MIXED-TALL & SHORT
Distichlis – S. patens
MONO
MIXED
Scirpus
MONO
MIXED
0 5 10 15
METERS

FIGURE 6

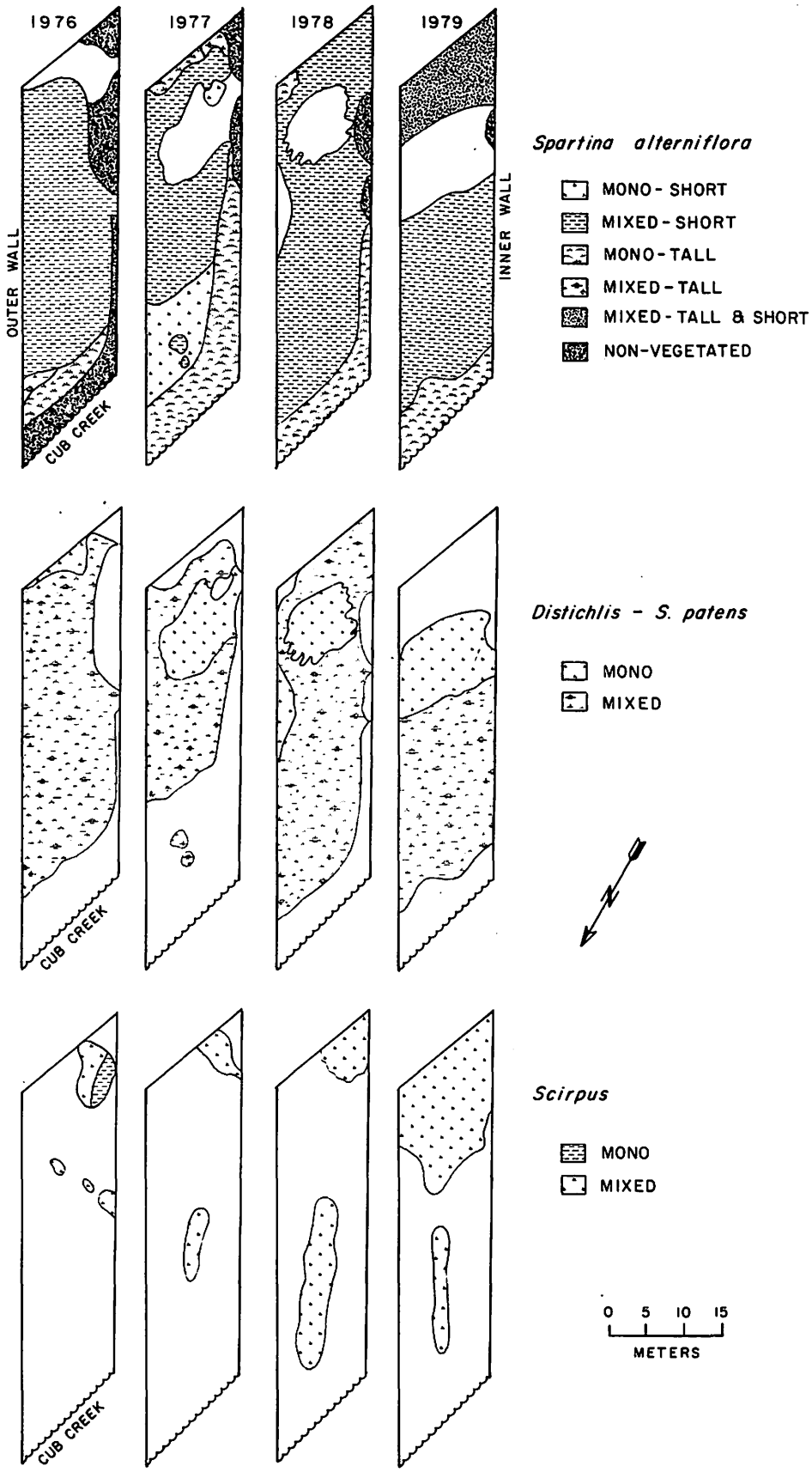

1976
1977
1978
1979
OUTER WALL
INNER WALL
CUB CREEK
CUB CREEK
CUB CREEK
Spartina alterniflora
MONO - SHORT
MIXED - SHORT
MONO - TALL
MIXED - TALL
MIXED - TALL & SHORT
NON-VEGETATED
Distichlis - S. patens
MONO
MIXED
Scirpus
MONO
MIXED
0 5 10 15
METERS

FIGURE 7

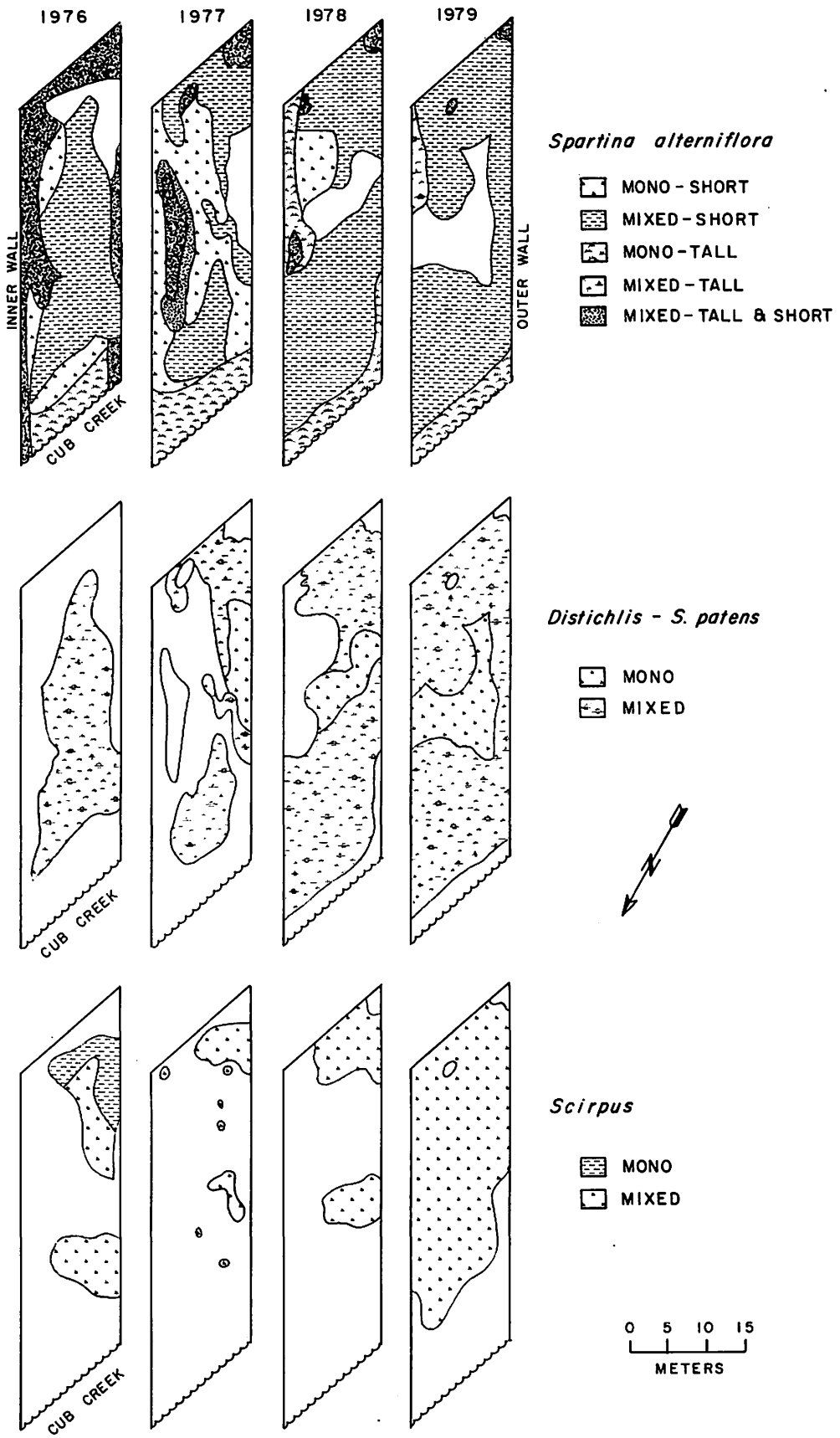

1976
1977
1978
1979
INNER WALL
OUTER WALL
CUB CREEK
CUB CREEK
CUB CREEK
Spartina alterniflora
MONO - SHORT
MIXED - SHORT
MONO - TALL
MIXED - TALL
MIXED - TALL & SHORT
Distichlis - S. patens
MONO
MIXED
Scirpus
MONO
MIXED
0 5 10 15
METERS

FIGURE 8

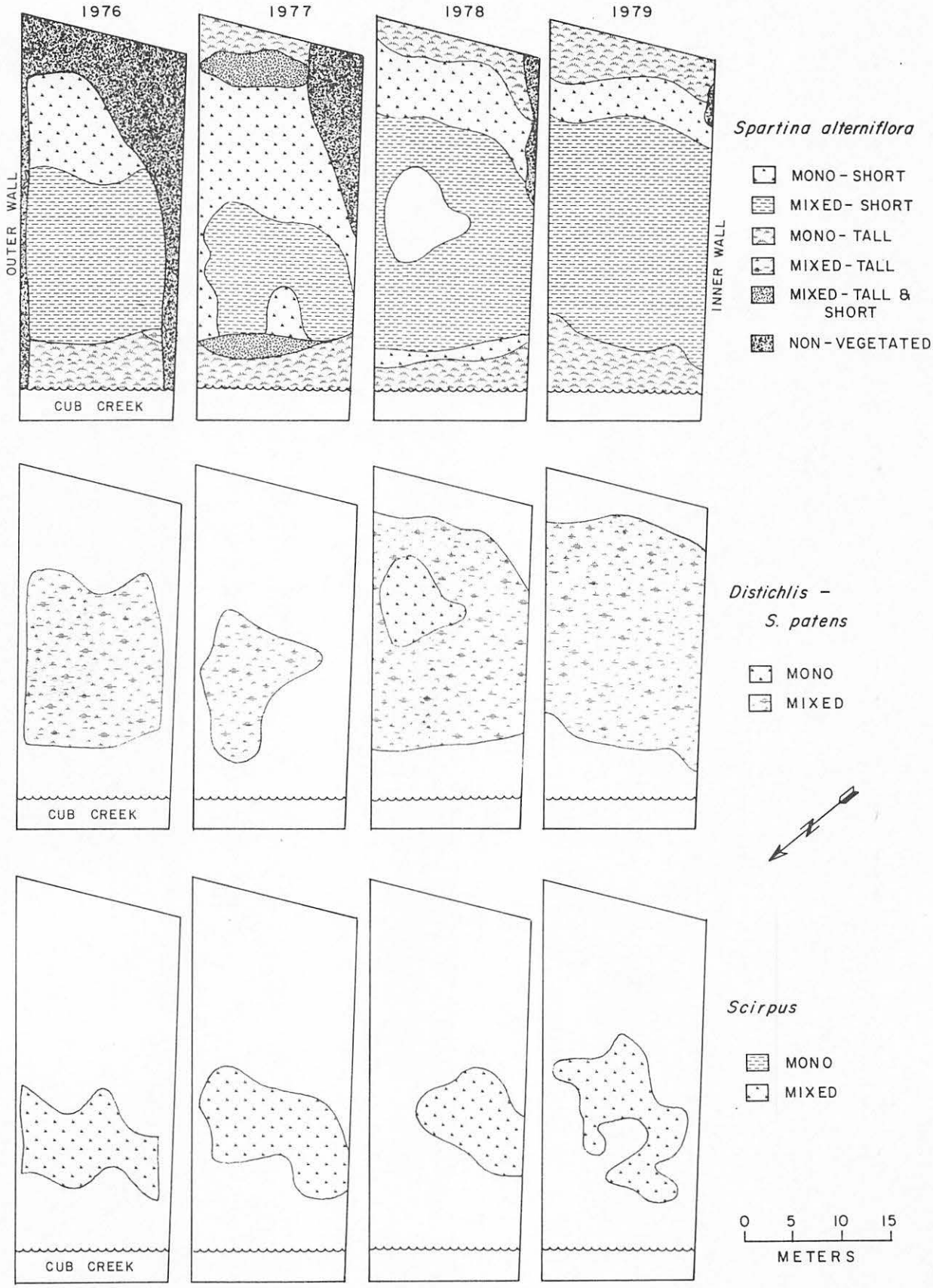

1976
1977
1978
1979
OUTER WALL
INNER WALL
CUB CREEK
Spartina alterniflora
MONO - SHORT
MIXED - SHORT
MONO - TALL
MIXED - TALL
MIXED - TALL & SHORT
NON - VEGETATED
CUB CREEK
Distichlis - S. patens
MONO
MIXED
N
CUB CREEK
Scirpus
MONO
MIXED
0 5 10 15
METERS

FIGURE 9

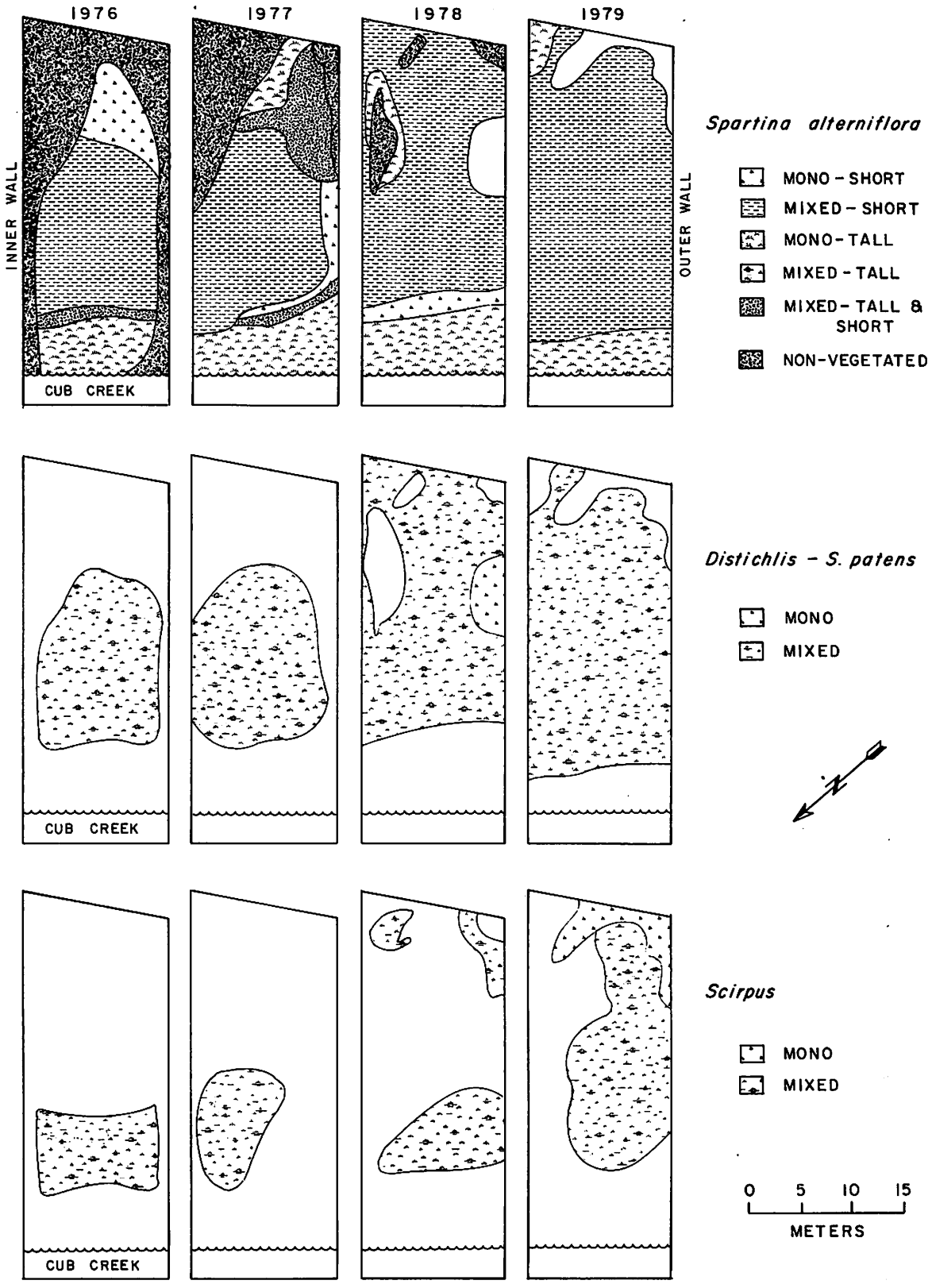

1976
1977
1978
1979
INNER WALL
OUTER WALL
CUB CREEK
Spartina alterniflora
MONO - SHORT
MIXED - SHORT
MONO - TALL
MIXED - TALL
MIXED - TALL & SHORT
NON-VEGETATED
CUB CREEK
Distichlis - S. patens
MONO
MIXED
CUB CREEK
Scirpus
MONO
MIXED
0 5 10 15
METERS

Fig. 10

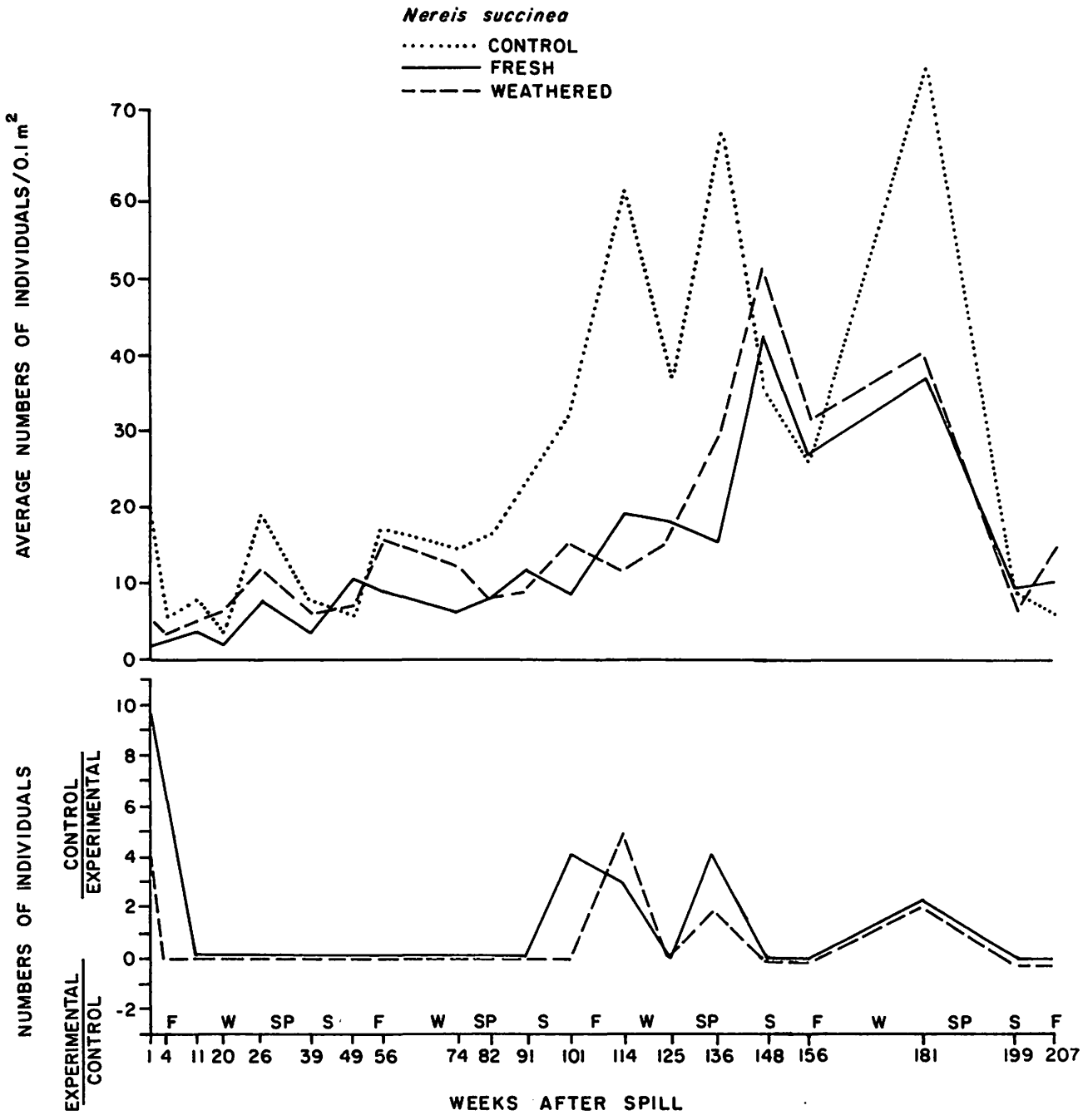

Nereis succinea
CONTROL
FRESH
WEATHERED
AVERAGE NUMBERS OF INDIVIDUALS/0.1 m²
70
60
50
40
30
20
10
0
NUMBERS OF INDIVIDUALS
CONTROL / EXPERIMENTAL
EXPERIMENTAL / CONTROL
10
8
6
4
2
0
-2
F W SP S F W SP S F W SP S F W SP S F
1 4 11 20 26 39 49 56 74 82 91 101 114 125 136 148 156 181 199 207
WEEKS AFTER SPILL

Fig 11

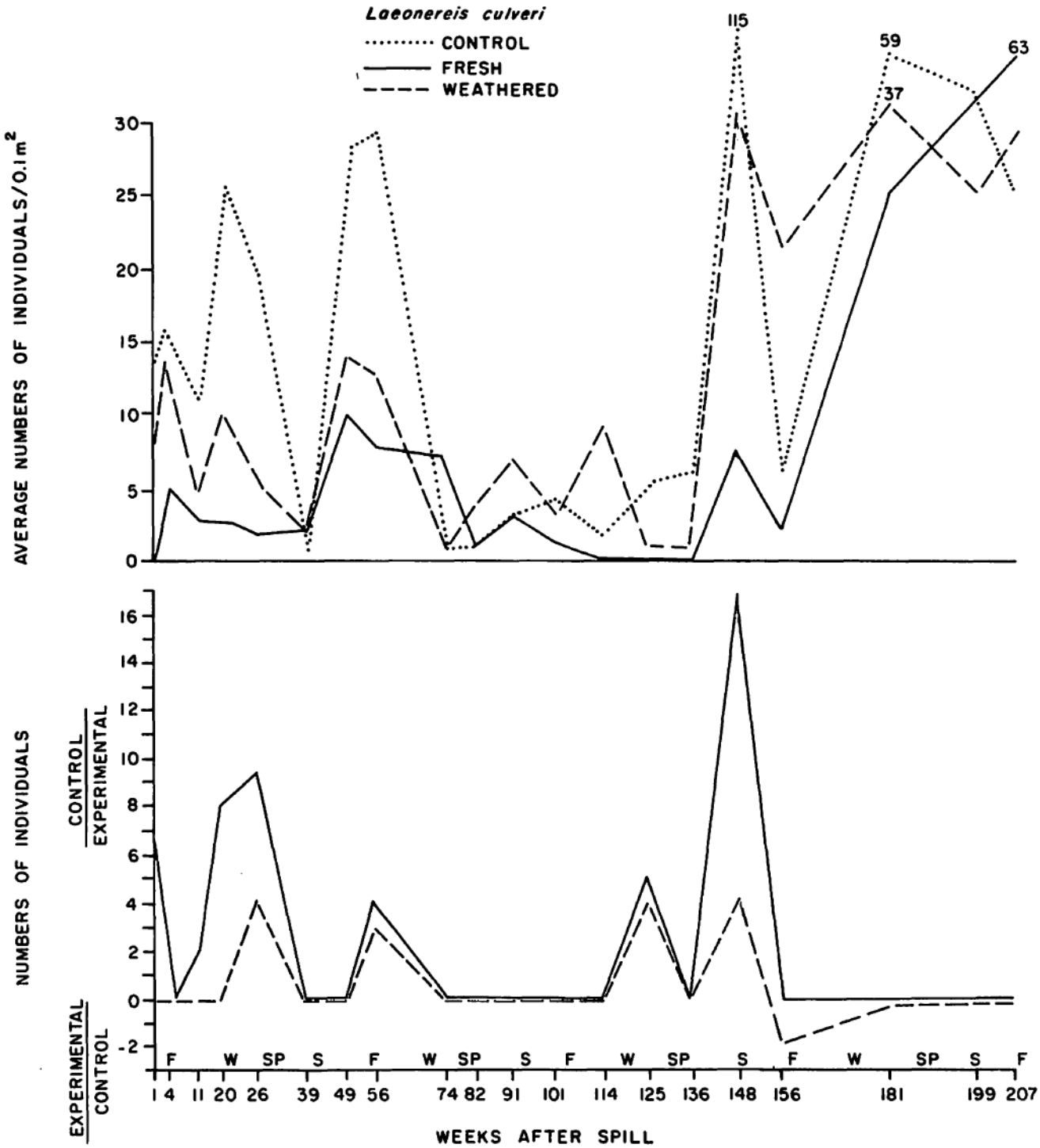

Laeonereis culveri
CONTROL
FRESH
WEATHERED
AVERAGE NUMBERS OF INDIVIDUALS/0.1m²
115
59
63
37
30
25
20
15
10
5
0
NUMBERS OF INDIVIDUALS
CONTROL / EXPERIMENTAL
EXPERIMENTAL / CONTROL
16
14
12
10
8
6
4
2
0
-2
F W SP S F W SP S F W SP S F W SP S F
1 4 11 20 26 39 49 56 74 82 91 101 114 125 136 148 156 181 199 207
WEEKS AFTER SPILL

Fig. 12

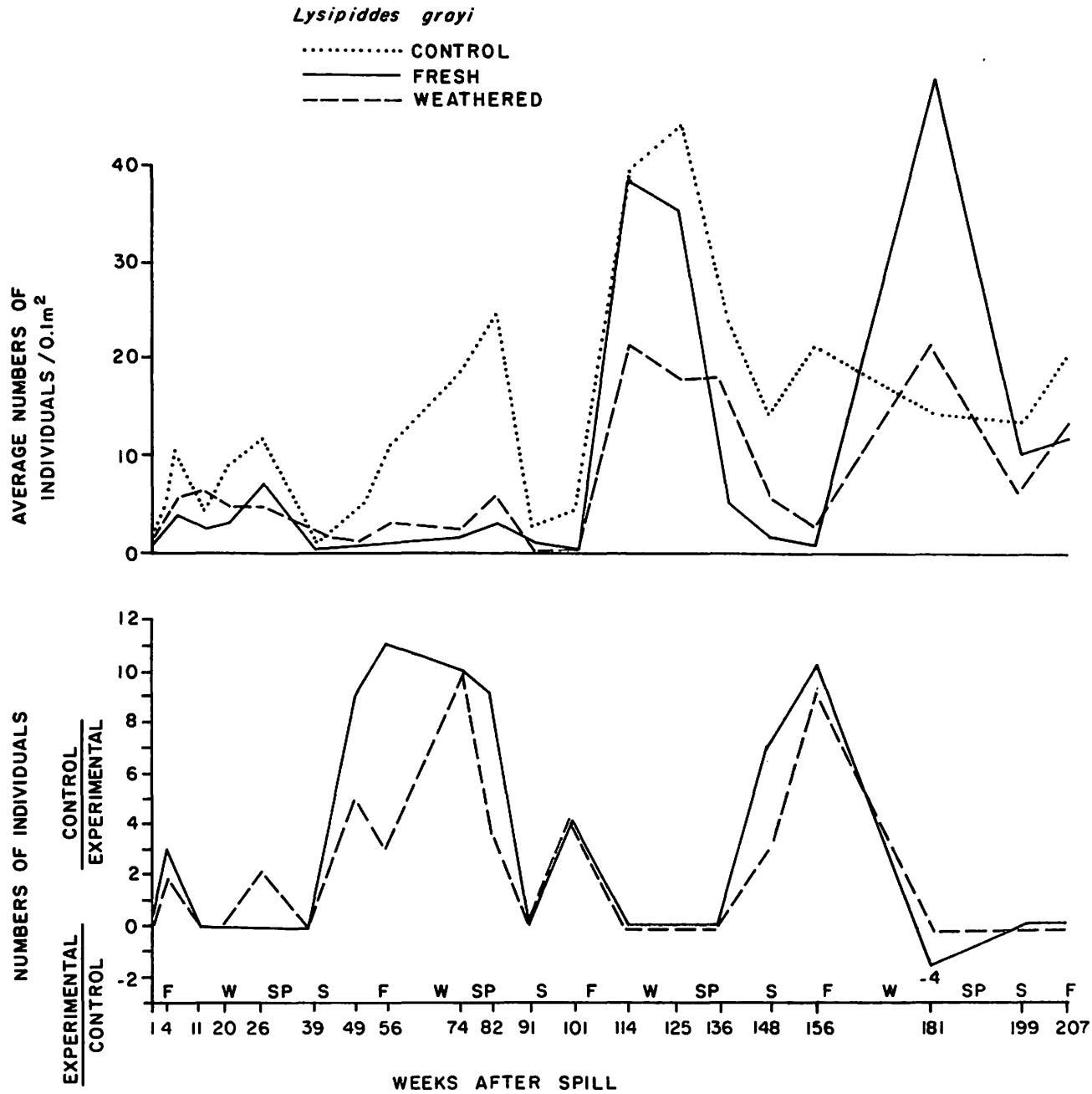

Lysipiddes groyi
CONTROL
FRESH
WEATHERED
AVERAGE NUMBERS OF INDIVIDUALS / 0.1m²
40
30
20
10
0
NUMBERS OF INDIVIDUALS
CONTROL / EXPERIMENTAL
EXPERIMENTAL / CONTROL
12
10
8
6
4
2
0
-2
-4
F W SP S F W SP S F W SP S F W SP S F
1 4 11 20 26 39 49 56 74 82 91 101 114 125 136 148 156 181 199 207
WEEKS AFTER SPILL

Fig. 13

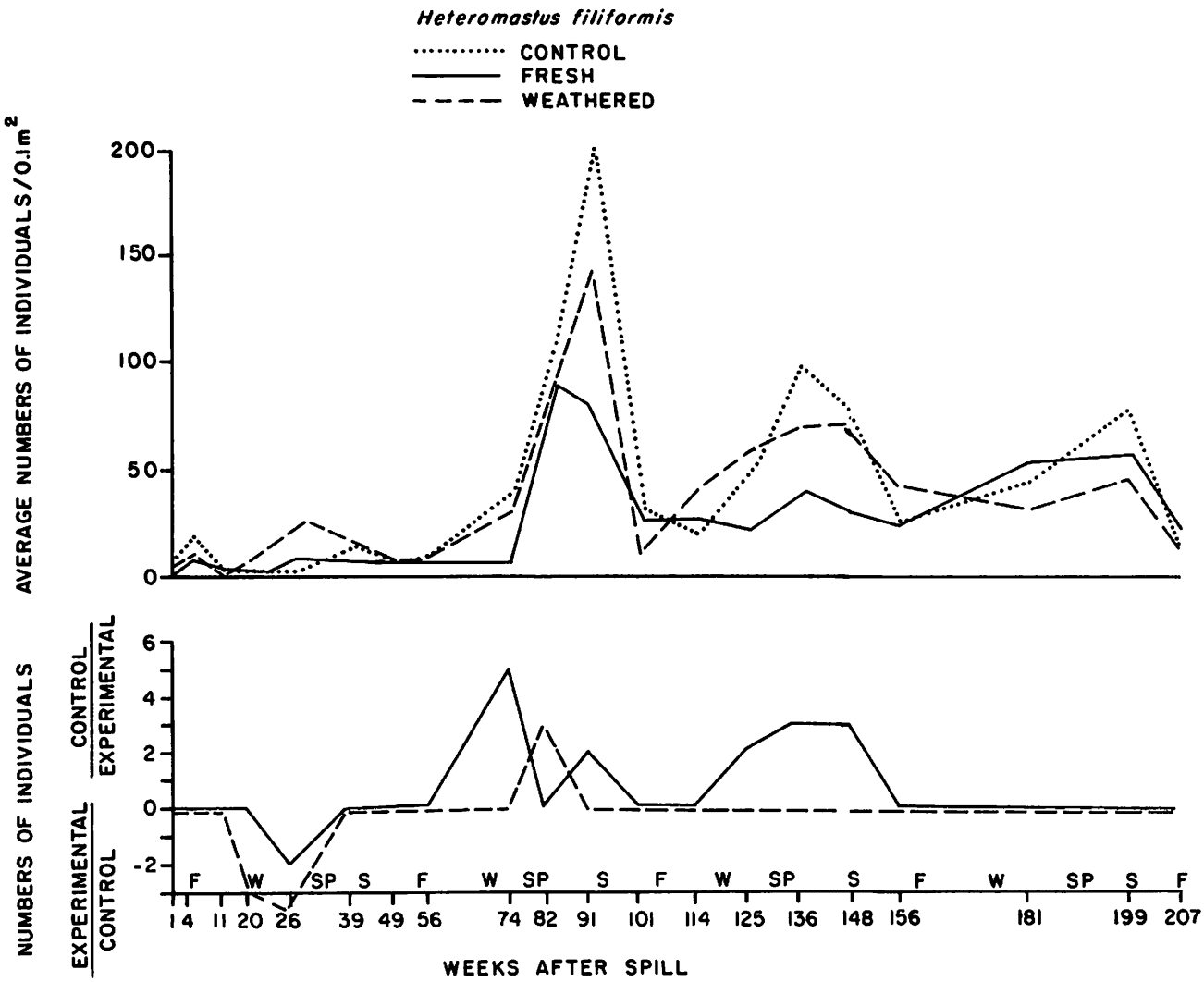

Heteromastus filiformis
CONTROL
FRESH
WEATHERED
AVERAGE NUMBERS OF INDIVIDUALS/0.1m²
200
150
100
50
0
NUMBERS OF INDIVIDUALS
CONTROL / EXPERIMENTAL
EXPERIMENTAL / CONTROL
6
4
2
0
-2
F W SP S F W SP S F W SP S F W SP S F
14 11 20 26 39 49 56 74 82 91 101 114 125 136 148 156 181 199 207
WEEKS AFTER SPILL

Fig 14

I-50

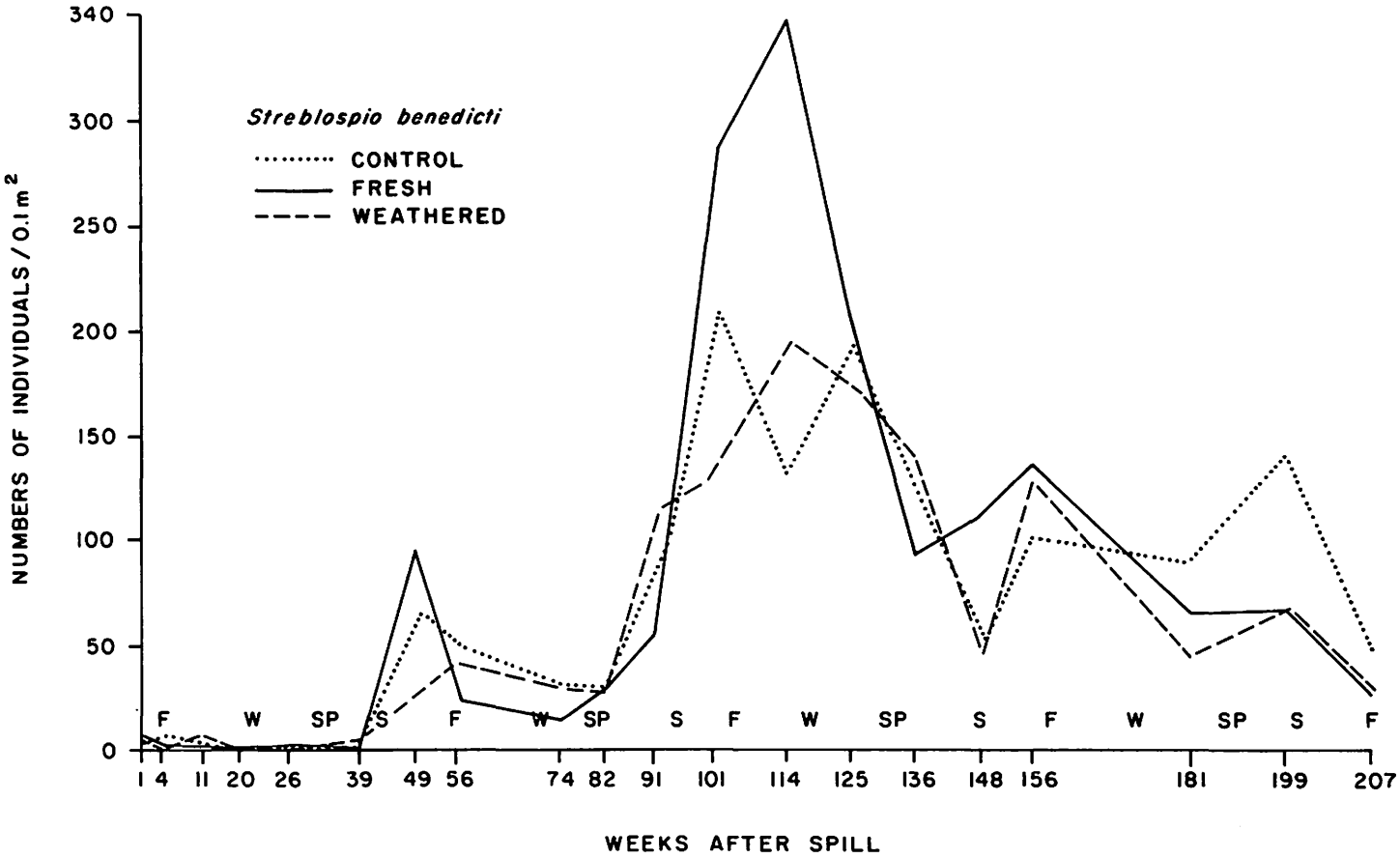

Streblospio benedicti
CONTROL
FRESH
WEATHERED
NUMBERS OF INDIVIDUALS / 0.1 m²
340
300
250
200
150
100
50
0
F W SP S F W SP S F W SP S F W SP S F
1 4 11 20 26 39 49 56 74 82 91 101 114 125 136 148 156 181 199 207
WEEKS AFTER SPILL

Fig. 15

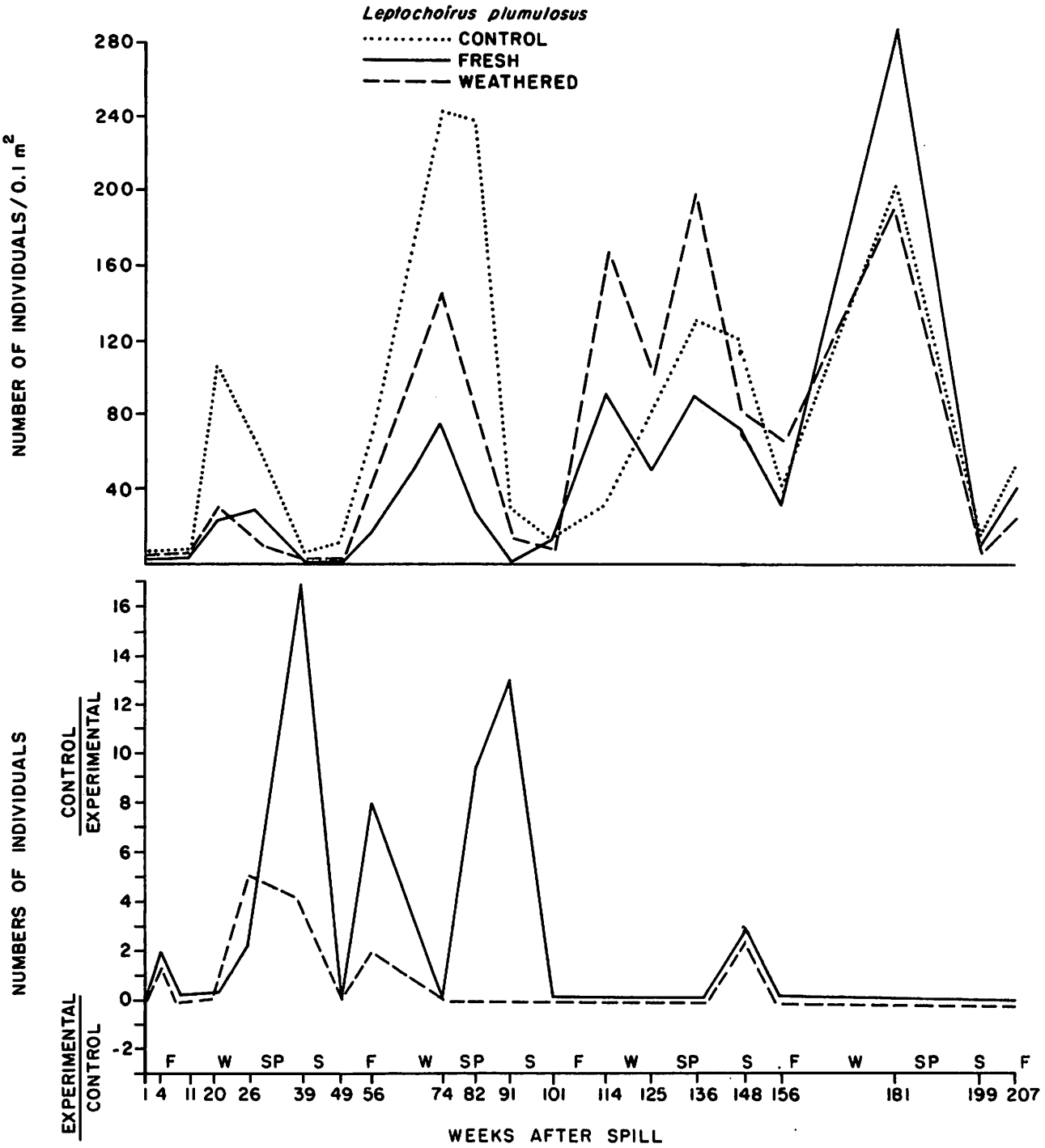

Leptochoirus plumulosus
CONTROL
FRESH
WEATHERED
NUMBER OF INDIVIDUALS/0.1 m²
280
240
200
160
120
80
40
NUMBERS OF INDIVIDUALS
CONTROL / EXPERIMENTAL
EXPERIMENTAL / CONTROL
16
14
12
10
8
6
4
2
0
-2
F W SP S F W SP S F W SP S F W SP S F W SP S F
1 4 11 20 26 39 49 56 74 82 91 101 114 125 136 148 156 181 199 207
WEEKS AFTER SPILL

Fig 16

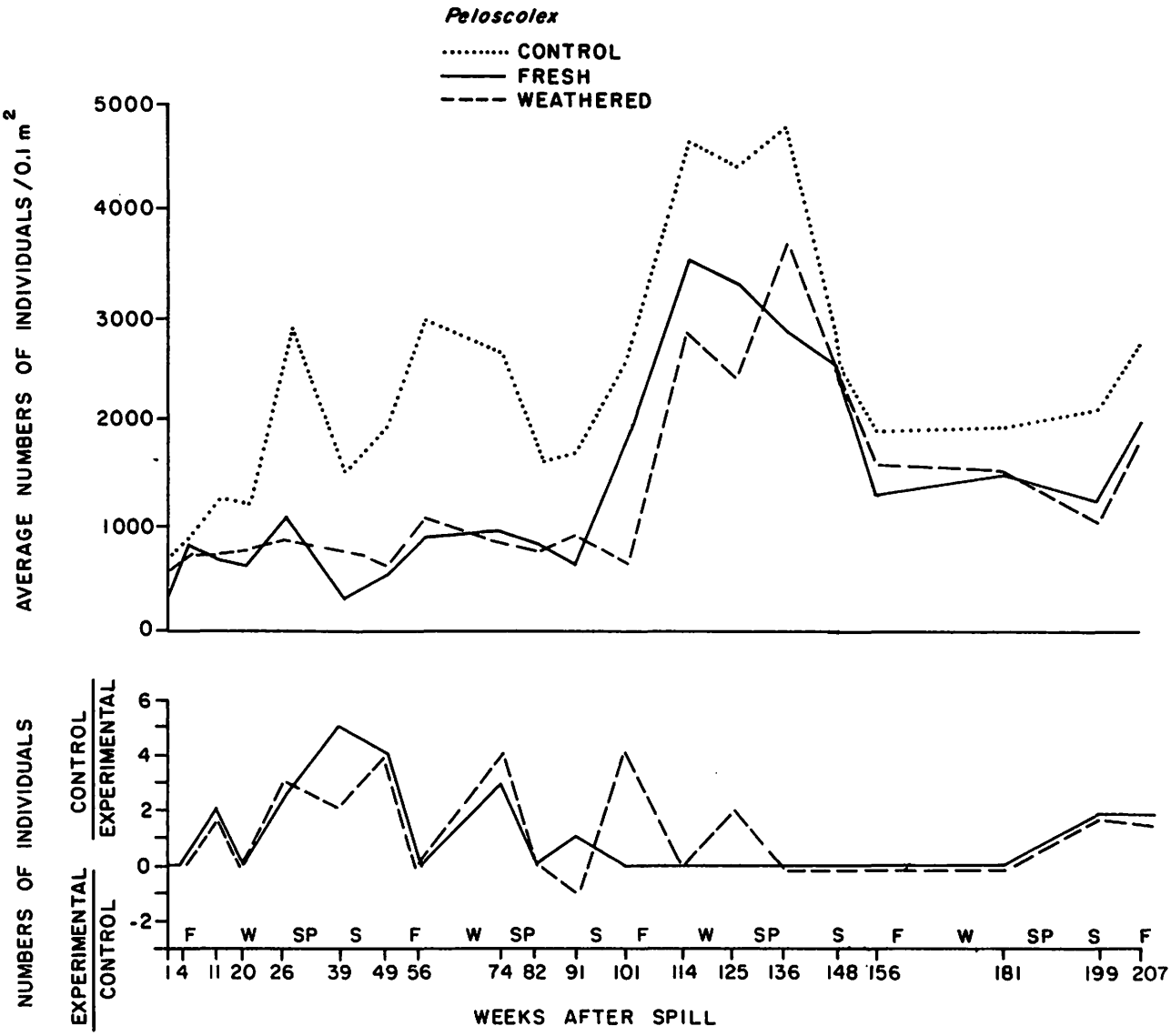
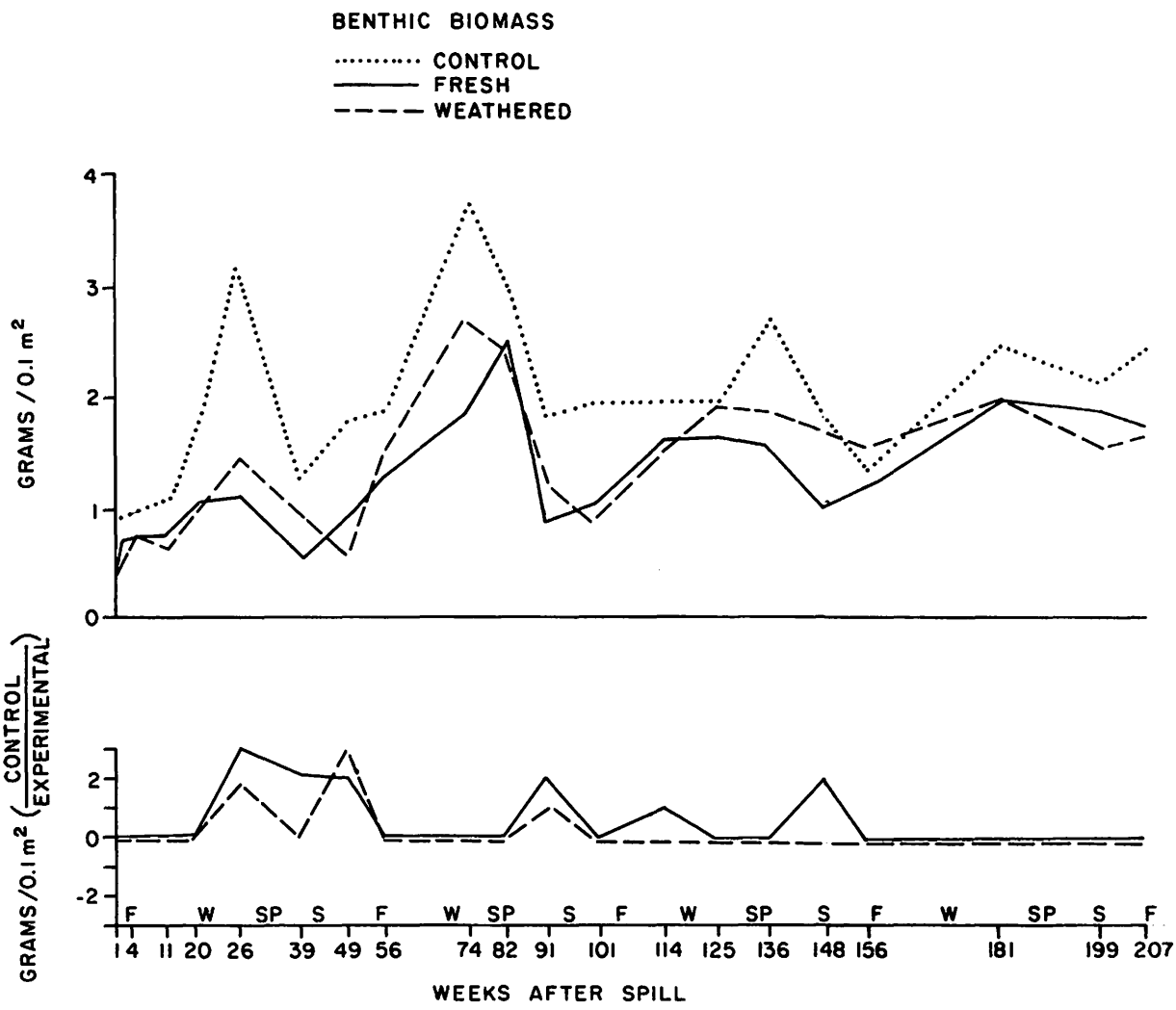

Peloscolex
CONTROL
FRESH
WEATHERED
AVERAGE NUMBERS OF INDIVIDUALS/0.1 m²
5000
4000
3000
2000
1000
0
NUMBERS OF INDIVIDUALS
CONTROL / EXPERIMENTAL
EXPERIMENTAL / CONTROL
6
4
2
0
-2
F W SP S F W SP S F W SP S F W SP S F
14 11 20 26 39 49 56 74 82 91 101 114 125 136 148 156 181 199 207
WEEKS AFTER SPILL

Fig 17


BENTHIC BIOMASS
CONTROL
FRESH
WEATHERED
GRAMS / 0.1 m²
GRAMS/0.1 m² (CONTROL / EXPERIMENTAL)
F W SP S F W SP S F W SP S F W SP S F
14 11 20 26 39 49 56 74 82 91 101 114 125 136 148 156 181 199 207
WEEKS AFTER SPILL

Appendix - Tables, Sections, and Keys.

Section 1. Benthic data

Table A. Control pen benthos
B. Fresh oil (pen 1) benthos
C. Weathered oil (pen 2) benthos
D. Outside area benthos
E. Average number of benthic organisms/pen.
F. Computer program utilized for benthic statistics
F1. Benthic Biomass
Key:

p-1	Capitella capitata
p-2	Eteone heteropoda
p-3	Heteromastus filiformis
p-4	Laeonereis culveri
p-5	Lysippides grayi
p-6	Nereis succinea
p-7	Notomastus latericius
p-8	Scolecolepides viridus
p-9	Streblospio benedicti
p-10	Sigambra sp.
p-11	Glycera dibranchiata
p-12	Scoloplos fragilis
a-1	Leptocheirus plumulosus
a-2	Corophium lacustre
a-3	Gammarus sp.
a-4	Stenothoe sp.
a-5	Caprellid amphipod
i-1	Edotea triloba
i-2	Cassinidea lunifrons
i-3	Chirodotea almyra
i-4	Cyathura polita
c-1 to 3	chironomid larvae species
c1-1	Macoma mitchelli
n-1	Nemertea sp.
o-1, 2	Peloscolex spp.
o-3	Oligochaeta sp.

Section 2. Marsh Grasses data

Table G. Weight of control pen grasses
H. Weight of fresh oil replicate (pen 1A) grasses.
I. Weight of fresh oil replicate (pen 1B) grasses.
J. Weight of weathered oil replicate (pen 2A) grasses.
K. Weight of weathered oil replicate (pen 2B) grasses.
L. Weight of outside area grasses.

Key: major groups

SA	Spartina alterniflora
DS-SP	Distichlis spicata - Spartina patens

SC	Scirpus rabustus
	others
AS	Aster tenifolius
B	Baccharis halimifolia
H	Amaranthus cannabinus
L	Limonium sp.
M	Kosteletzkya virginica
P	Polygonium punctatum
PL	Pluchea purpurascens
S	Salicornia virginica
SB	Sabatia stellaris

t = trace (one or two stems)
LA = Lab Accident

Section 3. Water parameters (physical)

Table M. Dissolved oxygen (2 replicates/pen)
N. Temperature
O. Alkalinity
P. Salinity

Section 4. Water parameters (nutrients)

Table Q. NO_3 values
R. NO_2 values
S. NH_4 values
T. Orthophosphate values

note: In 1979 analyzed total NO_3 and NO_2 (presented in Table Q).

Section 5. Phytoplankton data

Table U. Primary production
V. ATP
W. Phytoplankton counts

note: Primary production values are valuable only for comparative purposes (relative values). The number of variables and sources of error involved with determination of primary productivity raise serious questions in regard to attempts to determine absolute values (Salonen and Holopainen, 1979).

Section 6. Periphyton data

Table X. ATP values
Y. Chlorophyll "a" values

Key: H = horizontally oriented plates
V = vertically oriented plates
D = below detection limit

Installation times:

1975	at time of oil spills
1976	May 14
1977	April 11
1978	Jan 5 (but were gone when sampled Feb 21, therefore reinstalled Apr 5).

note: triplicate samples taken for ATP throughout study but individual values were so low from 1976 to 1978 the samples had to be combined for analysis.

Section 7. Snail population data

Table Z. Snail counts

Appendix Table A. Raw counts - control pen benthos.

Week # after spill	+1			+4			+11			+20			+26			+39			+49		
Replicate # / Organism Code	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
p-1																	1				
p-2																2	3	6	2	9	4
p-3	2	7	3	8	11	10	4	1	2	2	1	1	2		4	12	4	7	4	4	4
p-4	8	1	12	6	13	6	9	3	6	13	11	17	10	14	6	2			9	13	22
p-5		1	3	8	5	5	3	1	2	4	3	6	4	8	6				2	4	2
p-6				2	3	4	1	4	8	2	1	4	8	6	16	6	4	3	3	5	2
p-7		1														1					
p-8	4	7	18										51	74	73		1				
p-9			9	4	3	3		4	2	1			3						12	42	53
p-10																					
p-11																					
p-12																					
a-1	4	2		2	2	3	2	1		33	60	76	43	28	34	4	1	1	12	2	1
a-2											1		1		2		1				
a-3							1								5						
a-4																	1				
i-1								5							3						
i-2															1						
i-3																					
i-4																					1
c-1	10	5	11	6	15	1	10	8	8	19	26	34	32	8	26						
c-2	6	5	18	3	3	2	2			6	10	5	5		1						
c-3	2	7	2	3	4		1		4						1		1	1			
c-4									2												
cl-1	8	3	4	4	2	1	3	1	3	2	2	4	1	2		2	2	1			2
n-1	2	3	2	2	6	1	3	2		1	5	1	6	2	1	2	7	2	4	4	
o-1,2 0-3	120	292	712	278	466	428	720	680	644	336	638	916	2104	1110	1346	602	866	956	818	1460	760

Appendix Table A (Continued).

Week # after spill	+56			+74			+82			+91					+101				
Replicate # / Organism Code	1	2	3	1	2	3	1	2	3	1	2	3	4	5	1	2	3	4	5
p-1							4	2	7										
p-2	8	17	7			2					1	2	3	2	2	3	5		4
p-3	5	4	6	8	23	31	59	47	61	38	21	23	28	20	3	1	7	3	3
p-4	12	21	13	1			1	1					2		1			1	
p-5	5	6	6	10	9	11	19	14	6		1					1	1		
p-6	12	8	6	8	4	12	7	11	9	3	7	2	2	1	2	2	5	4	4
p-7	1												1						
p-8							2	2	1	2		1	2						
p-9	20	45	14	8	12	33	17	14	18	15	13	10	10	8	9	17	28	30	25
p-10												1		1	1	1	1	3	2
p-11		1				1				1							1	1	
p-12			1																
a-1	21	44	52	68	97	211	185	63	123	1	1	6	5	4		1		4	2
a-2		1	2	2	2	8	1	1			1								
a-3	2	1	3	2	2	2	1												
a-4																			
i-1			1				2		4		2		1						
i-2																			
i-3																			
i-4																			
c-1																	1		
c-2																			
c-3																			
c-4																			
cl-1		2		1	1	2		1	2	1	1	1	1	1	1		1	1	
n-1	2	2	4	4	2	3	1		3	3				1	1		1	1	
o-1,2	892	2274	1398	1455	1317	1373	982	762	772	206	226	113	357	98	118	235	377	234	288
o-3	21	42	31	107	118	84	54	53	37	21	19	24	30	16	8	14	48	22	20

Appendix Table A (Continued).

Week # after spill	+114					+125					+136					+148				
Replicate # / Organism Code	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
p-1														1						
p-2	4	10	8	7	8	1	1			1	1				1			1		
p-3		6	1	4	1	2	6	7	7	5	10	19	6	6	10	14	9	3	8	8
p-4		1						1	1	1		3				40	2	5	3	10
p-5	3	5	3	5	5	8	3	4	5	3	1	5		4	3	1	1	2	1	2
p-6	7	2	9	8	6	5	1	4	3	6	8	10	7	3	7	2	8	3	6	1
p-7																				
p-8											1	5		1	2	3	1		1	1
p-9	5	18	10	29	5	7	14	39	24	17	5	31	9	8	13	10	1	4	9	6
p-10											1	1			1	2				
p-11		1							1							1				
p-12																				
a-1		3	12		1	25	6	3	5	4	17	28	5	6	13	3	17	15	23	3
a-2			1								2	1		1	1					
a-3										1									1	
a-4																				
a-5																				
i-1		2	2	1	1		1					5							1	
i-2																				
i-3																				
i-4																				
c-1																		1		
c-2																3				1
c-3																2		1		
c-4																				
cl-1		3		1		2	3	3	1	1	6	4	1	4	4	5	3	3	7	1
n-1		2	2	4	3		1				1	3	1	1					1	4
o-1,2	125	436	210	818	661	404	477	719	439	225	449	371	875	177	468	334	341	122	384	186
o-3	14	28	22	53	50	7	14	24	11	4	36	41	28	16	52	18	26	7	10	10

Appendix Table A (Continued).

Week # after spill	+156					+181					+199					+207				
Replicate # / Organism Code	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
p-1																				
p-2	2	13	1	8	2															
p-3	2	4	1	2	1	2	2	3	14	1	13	8	4	6	7	3		2	1	
p-4	1	1			1	3	6	12	9	1	2	1	5	5	7	5	2	1	2	3
p-5	4	1	2	2	2		2	1	1	3	2	2	7	6	1	2	2	1	3	3
p-6	2	2	1	3	4	5	6	8	10	11		4		1					2	1
p-7																				
p-8																				
p-9	8	17	8	11	8	10	4	6	14	8	12	8	24	25	5	1	5	3	8	2
p-10		5		2	1							1	1	2						
p-11		1																		
p-12																				
a-1	1	8	2	6	1	32	24	15	14	21	2	2	1	2		3	12		6	3
a-2						1	1	1		3					2	1				2
a-3							1													
a-4																				
a-5		1																		
i-1		1					1			2						1				1
i-2																				
i-3																				
i-4																				
c-1											1	1		1		1	1	2	2	4
c-2											1		1	1	1	1			2	
c-3																				
c-4																				
cl-1	1	1		1		2			3		1	1	2	4	2	1	1	1	1	2
n-1	2	5	2	2	1	4	4	4	3	1	4	3	2	1	1	5	4	1	2	2
o-1,2	140	202	146	258	208	117	156	308	178	178	218	127	337	222	121	286	313	236	342	254
o-3	8	14	3	10	8	6	7	16	3	12	6	5	6	2	2	11	4	8	8	6

Appendix Table B. Raw counts - Pen 1 Benthos.

Week # after spill	+1						+4						+11					
Replicate #	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3
Organism Code																		
p-1																		
p-2																		
p-3	1	1	1	1		5	2	3	3	3	5	8	4	5		3	3	1
p-4							2	2	2	2	7	1	2	1		4	1	1
p-5			1		1	1	1	3	2	1	4	3	1			1	1	1
p-6		2	1		1	1	1	2		4	3	1	3	2	1	3	2	2
p-7																		
p-8																		
p-9	1	2	9	4	11	3		3	3	1	3		2	1	2			2
p-10	1		9	1	2	1							4					
p-11																		
a-1																		
a-2																		
a-3																		
a-4																		
a-5																		
i-1																	1	1
i-2	1																	
i-3																		
c-1	1	7	11	3	12	1	1	1	1	1	3		2	1		2		
c-2																		
c-3					1						1							
c1-1	3		6	4	1	1	3	1	2	5	4	4	3	3	3	3	1	3
n-1	2				2	1	2	3	1	2	3		3	1	2	3	1	
o-1,2	98	56	334	100	162	434	325	260	440	410	516	284	422	184	316	416	354	568
o-3																		

Appendix Table B (continued).

Week # after spill	+20						+26						+39					
Replicate #	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3
Organism Code																		
p-1																		
p-2															5		2	4
p-3	1			2	2	2	1	9	3	6		5	3	8		5	6	3
p-4	2	1	2	1		4	4		1		1		2		2	1	1	
p-5		1		2	1	5	3	8		3	1	7						
p-6	1	2	1	1	2	1	3	7	2	6	4	2	2	3			3	4
p-7																		
p-8							22	43	29	30	2	30						
p-9		1			1							2	1	2	2	1	2	2
p-10																		
p-11																		
a-1	9	33	2	10	7	12	4	8	2	19	14	40						
a-2				1														
a-3																		
a-4																		
a-5																		
i-1		1						2	1	1		2						2
i-2																		
i-3																		
c-1	1	12	1	1	3	6	6	5	3	8	7	6					2	
c-2								2										
c-3																		
cl-1	3	4	2	3	3	2	3	4	4	4	7	6	2	3		3		2
n-1			1		1	3	5	7	2	5	5	4	2	2	6		4	2
o-1,2	294	428	342	376	308	300	510	467	408	722	404	1016	96	170	288	206	104	84
o-3																		

Appendix Table B (continued).

Week # after spill	+49						+56						+74					
Replicate #	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3
Organism Code																		
p-1								1										
p-2	2	4	1	2	2	3	12	15	9	3	3	6			1			
p-3	1	1	8	6	8	2	1	2	2	5	6	7	3	3	4	3	6	5
p-4	2	10		5	7	6	2	3	5	3	6	7	1	7	1	6	2	4
p-5					1	1		1	1	1					3		2	
p-6	6	6	6	4	8	3	6	4	5	1	6	5	6	3	4	4	2	4
p-7																		
p-8				1														
p-9	29	87	18	39	65	57	22	22	6	14	16	8	3		23	8	10	5
p-10																		
p-11															1			
a-1	1	1	1		1		5	1	3	5	9	6	28	23	81	31	19	54
a-2															2		1	2
a-3																		
a-4																		
a-5																		
i-1																		
i-2																		
i-3																		
c-1								1						1				
c-2																		
c-3																		
c1-1			5			1		2	2				1	1	2	1		2
n-1	5		1	2	2	3	3	1	2	1	1		3	3	2	2	3	2
o-1,2	148	168	288	104	602	200	678	432	442	336	422	416	450	355	598	513	418	403
o-3							11	8	12	8	7	14	38	27	23	33	20	28

Appendix Table B (continued).

Week # after spill	+82						+91									
Replicate #	1A-1	1A-2	1A-3	1B-1	1B-2	1B-3	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5
Organism Code																
p-1	3	2	4	3	1	3										
p-2								1	1	1		1	2	3	1	1
p-3	50	39	26	60	51	64	8	8	18	5		11	9	17	9	12
p-4	1		1				1					1				1
p-5	2	1		3	1	1		1								
p-6	2	2	10	6	4	5	1		3	2		2	1		4	1
p-7																
p-8					4	7	1					1		1		
p-9	22	2	5	31	20	13	15	8	4	3		2	7	5	10	10
p-10												1				
p-11	3	1			1											
a-1	39	4	2	17	6	16	1						1			
a-2						1										
a-3	1				1											
a-4																
a-5																
i-1	4			2	1											
i-2																
i-3																
c-1																
c-2																
c-3																
cl-1	1			9	1	2		1				1	2	2		1
n-1	4	1	1	5	1	1	1	1	1	1		1	1			1
o-1,2	340	344	264	536	419	544	127	91	45	57		86	86	32	84	56
o-3	18	38	20	38	33	28	6	10	5	3		4	3	3	5	5

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Appendix Table B (continued).

Week # after spill	+101										+114									
Replicate #	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5
Organism Code																				
p-1																	2	2		
p-2						1	2		1	2	7	11	5	4	3	6	12	4	3	4
p-3	1	5	4	7	1	1	1		2	5	2	4	4	1	1	2	8	10	2	3
p-4						1														
p-5											3	4	1	5	1	6	5	10	3	2
p-6	1		1	1			1	1	1	3	1	1	1	2	2	2	4	2	2	2
p-7																				
p-8																				
p-9	10	32	27	24	23	30	49	23	34	44	14	10	10	17	1	50	90	72	57	28
p-10		1		1		4	1	2	1	1	20			4	1		58	20	3	1
p-11				1	1					1										
a-1	1					1	5	3		4	6	12	11		10	17	25	10	12	4
a-2						1	1			1		3		1			2	1		
a-3																				
a-4																				
a-5																				
i-1											1			1	2		1	5	2	2
i-2																				
i-3																				
c-1																				
c-2																				
c-3																				
cl-1	1	2	1	1	1	1	1			2	1			1	1		1	1	3	1
n-1	1			1	1	1	2			2	3		1					1	2	1
o-1,2	123	169	208	204	268	183	187	171	159	96	398	268	226	605	54	447	310	406	523	215
o-3	13	26	19	11	15	12	9	18	6	4	40	11	37	52	3	28	24	14	34	27

Appendix Table B (continued).

Week # after spill	+125										+136									
Replicate #	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5
Organism Code																				
p-1																				
p-2	1	1	2	3	3	1	2			1		1	1		1	1				
p-3	2	8	3	2	4	1	2	1	1	1	11	8	1	6	8	1	1	2	1	1
p-4							8	5	4	7										
p-5	3	3	2	1	4								1			2			2	1
p-6	1	1	1	3	2	1	1	4	3	2		1	3	1	3	2	1	1	2	2
p-7																				
p-8											2	2		1	2		1		1	
p-9	24	21	14	39	28	13	19	19	26	21	10	9	6	6	10	17	10	4	12	12
p-10																4		3		2
p-11			1			1														
a-1	2	13	14	10	4	1	7		1	5	12	9	11	6	10	17	21			11
a-2	2	7		4	5	2	5		1	3						2				1
a-3																				
a-4																				
a-5																				
i-1			1	1	1															
i-2																				
i-3																				
c-1																				
c-2																				
c-3																				
cl-1	6	7	3	9	10	2	3		4	3	1	3	10	16	2	10	5	3	5	10
n-1		2		1	1		1		1	1	1		1	1				1		2
o-1,2	317	345	467	601	259	242	266	306	401	254	222	219	263	314	164	655	367	72	234	242
o-3	8	12	18	17	11	7	3	8	12	9	12	18	31	17	21	42	46	7	18	12

Appendix Table B (continued).

Week # after spill	+148										+156									
Replicate #	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5
Organism Code																				
p-1																				
p-2	1	1							1		1	5	2	5	9		4	4	2	7
p-3	3	1	1	3	3	1	7	1	6	5	3		3	3	4	1		1	2	1
p-4							2	5						1	1					
p-5									1	1										
p-6	1	1	2	3	3	5	6	4	3	15	1	4	1	3		1	1	8	3	5
p-7																				
p-8								1												
p-9	10	2	7	6	2	18	15	9	21	30	19	3	18	6	6	12	17	34	20	7
p-10		3			1		1	1	1								5	1	1	2
p-11	1	1	1					1									1	1		
a-1	1	5	1		1	27	14	2	12	12	3		4	5	3	3	2	6	4	1
a-2		1			1	1														
a-3																				
a-4																				
a-5											1	1			1			1		
i-1						1														
i-2																				
i-3																				
c-1	2		2	1		1	1		3	1			1							
c-2	5		2	2		4	3	4		1										
c-3				1		1	4	4	4											
cl-1		4	2	7	4		2	1	5	1	2		1	1			1	1		
n-1				1				1	2	1	2		1	1		2	2	2	1	
o-1,2	353	95	362	199	273	79	309	181	204	318	154	52	80	114	108	44	150	210	132	260
o-3	20	6	8	3	14		18	6	11	5	10	5	7	12	15	4	4	7	5	9

Appendix Table B (continued).

Week # after spill	+181										+199									
Replicate #	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5
Organism Code																				
p-1																				
p-2	1																		1	
p-3	3	3	7	5	5	3	4	2	3	18	12	6	3	7	3	8	6	4	2	4
p-4	2	1	3		3	1	1	4	8	3	2	4		4	3	4	4	3	6	3
p-5	9	2	4	7	10	6	5	7	3	6		2		1			1	2	1	1
p-6	2	2	3	1		8	8	4	5	4		1	2			2	1		2	3
p-7																				
p-8																2	1			1
p-9	11	5	3	8	4	5	5	9	4	6	2	7	4	3	2	8	10	12	4	9
p-10														1			1	2		
p-11																				
a-1	37	23	39	41	42	17	26	19	21	34	1				3				1	
a-2	2	1	2		2	3	1	2	1	2			1							
a-3	1		1																	
a-4																				
a-5																				
i-1			1				1													1
i-2																				
i-3																				
c-1																			1	
c-2													1		1	2	2		2	4
c-3		1				1					3				5		2	4	6	4
cl-1	1	3	4	3		2	4	5	1	2	1	1	1	1	1	3	3	2	3	6
n-1	3	1	1	5	1	3	4	3	1	2	1	3	1	4	3	1	3	6	2	4
o-1,2	328	42	113	173	102	73	136	235	51	159	120	74	49	65	134	89	124	176	223	113
o-3	6	2	5	4	4	6	6	9	2	6	5	3	5	3	7	2	5	3	5	5

Appendix Table B (continued).

Week # after spill	+207									
Replicate #	1A-1	1A-2	1A-3	1A-4	1A-5	1B-1	1B-2	1B-3	1B-4	1B-5
Organism Code										
p-1										
p-2	1			1			1			1
p-3	1	1	2	3		1	1	4	2	2
p-4	4	1	4	2	2	2	9	1	5	3
p-5		2		1	1	1	1	1	2	1
p-6			1		1	1		4		2
p-7										
p-8										
p-9	1	2	2	3	2	1	2	4	1	2
p-10										
p-11										
a-1	1	2		5	2	5	11	1	7	2
a-2					1					
a-3										
a-4										
a-5										
i-1		1			1		1		1	
i-2										
i-3										
c-1		2		1	1					
c-2		2	1		2		2			2
c-3		1	1		1	1	2		1	1
cl-1		1	1	2	1	2	2	2	1	4
n-1	3	3	1	2	1	1	5	1	3	1
o-1,2	96	163	103	218	181	150	358	217	308	212
o-3	4	3	4	8	8	3	4	4	6	4

Appendix Table C. Raw counts - Pen 2 benthos.

Week # after spill	+1						+4						+11					
Replicate #	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3
Organism Code																		
p-1																		
p-2																		
p-3	4			4	2	3	2	7	4	1	9	6	1				1	
p-4	2	1	3	4	5	7	4	6	9	13	4	7	3	1	3	3	3	4
p-5	2			1	1		2	1	2	4	2	4	3	3	2	2	5	4
p-6	3	2	3	1	3	2	1	2	3	1	4	1	2	4	3	2	4	2
p-7																		
p-8																		
p-9	5	1	3		3	8	2		1				2		10	4		2
p-10																		
p-11																		
a-1																		
a-2																		
a-3																		
a-4																		
a-5																		
i-1								2	1			1						
i-2																		
i-3						1												
c-1	10	1		2	2	1			1				3					
c-2																		
c-3																		
c1-1	2	3	1	1	1	6	6	4	2	2	2	4	1	1		3	5	2
n-1			2	3	1	2	2	2	2	4	3	2	3	1	1	2	1	2
o-1,2	324	186	410	312	172	488	408	328	352	342	388	302	458	384	484	306	412	382
o-3																		

Appendix Table C (continued).

Week # after spill	+20						+26						+39					
Replicate #	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3
Organism Code																		
p-1																		
p-2													4	2	3	4	3	2
p-3	5	5	3	6	7	2	4	7	5	24	31	8	2	6	18	9	8	7
p-4	2	4	13	2	1	9	1		4	6		4		1			1	
p-5	2	4	8		1	1	2	2	1	4	7				6			
p-6		4	7	3	1	4	6	12	1	4	11	4	3		7		2	6
p-7							1				1							
p-8							36	32	30	25	27	6		1	1	1		
p-9													1	2	3			4
p-10																		
p-11																		
a-1	15	14	42	11	8	8	11	2	17	7	4	3		3				
a-2																		
a-3																		
a-4																		
a-5																		
i-1							1		1									
i-2																		
i-3																		
c-1	2	1	11			7	1	3	4	6	1		2					
c-2																		
c-3																		2
cl-1	3	2	4	2	4		3	2	2	4	1		1	1	3	2	2	
n-1	2	2	3	1	1	6	2	3	3	12	11	4	4	4	5	4	4	10
o-1,2	420	316	572	266	368	394	542	490	418	714	402	410	366	746	370	154	328	508
o-3																		

Appendix Table C (continued).

Week # after spill	+49						+56						+74					
Replicate #	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3
Organism Code																		
p-1													1					
p-2	1	1	3	2	1	1	10	2	5	1	9	3	1					
p-3	5	1	8	5	5	4	6	3	11	1	7	5	10	8	31	11	20	17
p-4	4	5	9	15	7	3	5	10	10	2	4	9		1	1		1	1
p-5	1				1	1				1	6	4		1	1	1	2	
p-6	4	1	7	1	5	5	11	11	14	2	6	4	2	9	10	6	5	7
p-7																		
p-8																		
p-9	12	17	50	46	36	78	33	16	33	12	16	20	19	10	26	13	8	21
p-10																		
p-11									1						4	1		
a-1	1	1				1	20	26	60	1	18	5	76	113	132	51	36	48
a-2												1		2			1	1
a-3														1				
i-1	1				1						1	1	1			1	1	
i-2																		
i-3																		
c-1																		
c-2														1				
c-3																		
cl-1							1	1	1			1	1	1	1		1	
n-1	2	2	6	1			2	3	6	1	1	1	1	3	1	1	1	
o-1,2	220	106	408	154	266	402	950	488	640	230	508	386	580	356	193	518	243	393
o-3							14	6	18	2	18	12	29	18	16	32	18	24

Appendix Table C (continued).

Week # after spill	+82						+91									
Replicate #	2A-1	2A-2	2A-3	2B-1	2B-2	2B-3	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5
Organism Code																
p-1	4		6		1											
p-2					1		1						3			1
p-3	81	42	65	32	16	21	21	20	8	13	15	28	21	27	15	24
p-4	1		1	1	5	3		2				1	2		4	
p-5	4	2	2	1	4	5										
p-6	3	3	4	7	4	4	2	1	2	1		2	1	1	1	1
p-7																
p-8	7	1	4	4	4	1			1	1	1		1	2	1	
p-9	5	29	31	18	6	13	6	9	15	20	9	23	9	25	17	13
p-10							1		2			4				
p-11	1	2										1				
a-1	27	48	43	41	83	11	2	5	4	2	1	2		1	3	
a-2						1								2		1
a-3																
a-4																
a-5																
i-1		3			1	1					3		1			
i-2																
i-3																
c-1																
c-2																
c-3																
cl-1	1				1	1		1					1			
n-1	4		1	3	3	3	2		1	1			1	1	1	
o-1,2	346	257	526	497	291	442	133	111	66	102	98	152	79	107	83	113
o-3	19	24	36	33	28	26	14	11	13	10	7	13	2	8	10	7

Appendix Table C (continued).

Week # after spill	+101										+114									
Replicate #	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5
Organism Code																				
p-1																				
p-2				1					2	2	4	4	4	5	2	2	6	1	2	2
p-3	1	4	1	1	1	1	2	1	1	2	5	4		4	8	10	3	2	2	6
p-4				1				1	1		2		1	1		4	1			
p-5											1		6	8	1	1		4	1	1
p-6	2	3	1	1	1	1	2	1	3	1		2	2	1	1	2	2	1	2	
p-7																				
p-8		1							1											
p-9	7	13	9	11	11	11	16	11	23	16	38	41	10	10	15	42	24	17	13	9
p-10											1		3			11				1
p-11					1	1				1										
a-1		6				1					50	35	7	4	24	28	11		3	13
a-2							1						3				2			
2-3																				
a-4																				
a-5																				
i-1											1		1	2			1			
i-2																				
i-3																				
c-1																				
c-2																				
c-3																				
cl-1		1	1	1						1	1	1	3	1		1		1		2
n-1		1		1	1	1	2		1	1	4			1		1	1	2		2
o-1,2	32	96	29	43	67	61	65	39	92	62	321	246	231	282	248	478	166	251	241	274
o-3	4	8	5	5	8	5	3	2	5	3	22	13	23	33	14	39	27	17	35	32

Appendix Table C (continued).

Week # after spill	+125										+136									
Replicate #	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5
Organism Code																				
p-1															1					1
p-2			3			1				1	1								3	
p-3	8	3	1	2	5	6	6	8	5	6	6	1	8	11	6	9	4	6	4	14
p-4								1		1							1			
p-5	1	3	4	6	1	1	1	2		3	1	5				5	3	2	2	2
p-6	2	1	5	1	1	1	2	3	1		7	5	3	1	2	1	3	3	1	3
p-7																				
p-8											1	1		1	5	2	2	1		4
p-9	31	11	18	18	11	17	7	39	14	19	12	11	12	10	23	14	23	5	21	12
p-10						1					4	1								
p-11	1				1			1								1				
a-1	5	26	14	3	3	5	4	6	3	34	31	77	2	4	3	17	3	14	58	6
a-2		4	13			1		1		1		1							6	1
a-3		2																		
a-4																				
a-5																				
i-1											1								2	
i-2																				
i-3																				
c-1																				
c-2																				
c-3																				
cl-1	1	3	6	3	2	10		1	2	2	11	2	19	11	5	13	10	10	13	10
n-1	1	3		2		2	3	1	1		1		1	3	1		1		2	1
o-1,2	259	278	572	254	156	318	137	194	174	181	309	276	238	422	400	418	800	276	361	419
o-3	8	4	11	6	5	10	4	6	5	3	22	26	17	29	31	36	38	17	30	24

Appendix Table C (continued).

Week # after spill	+148										+156									
Replicate #	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5
Organism Code																				
p-1					2															
p-2			1	3							2	4	5		1	3	7	8	2	1
p-3	3	3		7	8	13	7	11	13	7	2	3	2	2	1	7	7	5	7	5
p-4	8	6	1	6	3	2	4	2				2	2		2	6	2	1	8	
p-5			1					4			2									
p-6	2	6	8	8	8	5	4	3	8	3	7	3	2	3	3	2	5	2	3	2
p-7																				
p-8		1																1		
p-9	5	2	4	8	8	4	3	5	2	7	11	12	11	16	15	2	24	11	14	19
p-10			3									2		1						
p-11				2			2													
a-1	14	1	23	21	6	13	1	3	2	1	1	7	9	17	12	1	6	6	6	2
a-2				1		4			1	1		1		1						
a-3									1											
a-4																				
a-5																	1		1	
i-1	1																			
i-2																				
i-3																				
c-1			2			2	1			2										
c-2	2	1	2	7	3	2	2	2	2	2	1									
c-3	3	1			1		2				1			1						
cl-1	1	2	10	1	1	2	2	2	2	4		1	2			1				1
n-1	1	1	1	1	1	2		1	3		2	5	3	1	1	3		6	4	4
o-1,2	174	88	117	307	380	306	259	157	279	220	172	190	142	150	152	86	222	112	146	172
o-3	16	10	4	18	12	14	7	14	10	8	17	18	18	12	16	8	24	15	13	16

Appendix Table C (continued).

Week # after spill	+181										+199									
Replicate #	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5
Organism Code																				
p-1																				
p-2																			1	
p-3	1	1		2	1	10	5	3		6	2	2	2	2	7		5	4	13	
p-4	8	2	2	8	1	7	6	1	2	2	4	2	2	2	4	1	2	1	5	
p-5	1		2	3	3	3	2	1	1	3	1			1	2		1			
p-6	6	8	3	5	4		4	6	1	3	2			1	1	1			2	
p-7																				
p-8	1																			
p-9	3	5	5	8	5	4	7			7	10	5	7	9	7	9	6	2	4	
p-10											1		1	2						
p-11																				
a-1	26	24	14	48	38	6	23	7	13	4			1	1	1					
a-2				1	1		2	1												
a-3					1															
i-1																				
i-2																				
1-3																				
c-1																				
c-2		1									2				1		1		2	
c-3											3	1	2		4	3	1	3	4	
c1-1	1	1	2	3	2		2	2	2		1	1	2	2	1	3	1	2	2	
n-1	1	3	1	4	1		1	1	2		1	1	1	2	2	2	2	2	5	
o-1,2	26	91	110	83	112	267	329	163	99	171	66	120	127	61	88	102	74	117	82	
o-3		3	4	6	2	5	6	3	4	9	4	6	3	3	4	5	3	5	5	

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Appendix Table C (continued).

Week # after spill	+207									
Replicate #	2A-1	2A-2	2A-3	2A-4	2A-5	2B-1	2B-2	2B-3	2B-4	2B-5
Organism Code										
p-1										
p-2							1			1
p-3	2	1	1	1	1	2	1	1	1	2
p-4	2	3	4	1	2	4	5	5	2	2
p-5	2	1		2	1	1		1		1
p-6		1	2	3		4	1	1	2	
p-7										
p-8										
p-9		4	1	2	3	3	4	2	3	1
p-10										
p-11										
a-1	1	13	2	8	1			1	1	
a-2							1			
a-3										
i-1										
i-2										
i-3										
c-1	4	2	1	1						
c-2	3	2	2	1	2			1	1	
c-3	2			2				1		2
cl-1	1	1	2		2			1	1	2
n-1	1	1	1	2	1	2	1	3	5	
o-1,2	239	204	196	82	178	210	283	194	172	191
o-3	8	4	4	2	3	4	6	4	3	4

Appendix Table D. Raw counts - outside benthos.

Weeks after spill	+1			+4			+11			+20			+26			+39			+49		
Replicate #	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Organism Code																					
p-1																					
p-2																			1	2	1
p-3	4		1	1	2	2		1		4	1	4	4	1	3				1	2	8
p-4	1	5	2	2	3	2	10			2	2	5	3	2						1	1
p-5		3		6	19		13			6	1	6	6	3	1						
p-6	1	2	6	2	4	3	6	3		3	1	7	3	3	5				5	3	4
p-7	1																				
p-8													34	49	27						
p-9	6	22	11	5	10		5			3		1							28	25	40
p-10																					
p-11																			1	1	1
p-12																					
a-1			6		2		32	4		21	5	53	27	25	55				22	14	10
a-2				1																	
a-3							1														
a-4																					
a-5																					
i-1					1		7	3													1
i-2																					
i-3																					
i-4																					
c-1	4	8	5	9	2		4	3		1	5		7	4	12						1
c-2			1	3	2					2			2								
c-3	2	2		2																	
cl-1	1	1	2	3	3	2	4	1		1	1	4	1	2	2				2	1	2
n-1	6	3	1	1		1				1	2	1		1	1				1	1	
o-1,2	622	1028	374	288	382	264	992	618		264	204	348	360	402	304				372	174	528
o-3																					

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EKMAN GRAB BORKEN THEREFORE NO SAMPLES TAKEN

Appendix Table D (continued).

Weeks after spill	+56			+74			+82			+91					+101				
Replicate #	1	2	3	1	2	3	1	2	3	1	2	3	4	5	1	2	3	4	5
Organism Code																			
p-1					1	2	1	1											
p-2	8	2	3	2	2			1			3		6		2	1			2
p-3	10	8	5	30	7	22	26	51	8	18	20	13	16	19	4	6	8	14	6
p-4	3			3									2				1		
p-5					3			2											
p-6	8	2	9	12	6	7	4	5	3	1	1	2	1	3	2	1	3		5
p-7																			
p-8						1				1	1		1		1				
p-9	21	38	26	20	8	12	6	5	3	15	8	11	24	5	13	14	29	32	15
p-10							1			6		1	2	1		2	5	4	3
p-11			1	1	2			4		5					1		4	4	
p-12																			
a-1	69	118	76	84	62	78	25	25	73	1	1	2		2	29	32	33	35	28
a-2				1	1	1	1	1											
a-3	1	1			2	2	3		2										
a-4																			
a-5																			
i-1				1									1						
i-2																			
i-3																			
i-4				1												1			
c-1					1	3													
c-2																			
c-3																			
cl-1		2		1	1	1		1	2		1		2	1	2			2	
n-1		1	1	3	2	1		3	2	1		1	4	3	2	4	2	4	3
o-1,2	966	1128	892	1243	627	709	280	174	490	117	216	135	279	91	112	103	161	117	121
o-3	18	17	10	94	38	33	14	18	28	18	14	26	29	22			1		

Appendix Table D (continued).

Weeks after spill	+114					+125					+136					+148				
Replicate #	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Organism Code																				
p-1					1										1					
p-2	5	6	10		3		1		1	1		1		1	2					
p-3	6	9	15		9	14	3	8	1	10	11	10	14	7	6	17	5	6	4	3
p-4																2				1
p-5	4	1	1		5	3	4	2	3	4	1	5	1	1	3	1	1			
p-6	2	4	3		3	7	1	8	3	5	1	3	3	2	3	1	3	5	2	1
p-7																				
p-8											6	2	2	2	3	1	1		1	
p-9	72	34	132		89	38	74	43	17	65	16	27	17	53	25	5	5	18	2	4
p-10	15		23						1								1			
p-11	2	2				1		1							1					
p-12																				
a-1	57	69	89		68	3	10	14	6	25	38	25	90	53	74					
a-2												1								
a-3						1	1		2	1										
a-4																				
a-5														1	1					
i-1					3				1				1					1		
i-2																				
i-3																				
i-4																				
c-1														2	1					
c-2																				
c-3																				
c1-1		1	5		6	1	2	6	1	6	5	5	2	2	3	3	1	1	1	1
n-1	2	2	2		2		1	1	2	1	1	1	3	1	2		3	1	1	
o-1,2	306	351	324		353	660	278	454	230	417	621	470	419	419	472	267	231	247	155	137
o-3	15	35	31		22	17	2	10	7	15	53	43	49	22	18	11	8	5	8	13

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Appendix Table D (continued).

Weeks after spill	+156					+181					+199					+207				
Replicate #	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Organism Code																				
p-1														1						
p-2	3	3		1	1									1	1					
p-3	2	5	3	3	2	48	13	7	5	4	8	5	4	6	7	3	1	1	3	2
p-4			1		1						6	1	7	3	6	4	5	5	3	2
p-5						4	2	6	2		1	2	1	1	1					
p-6	3	11	1	1	5	11	6	7	8	2	1	2	1			2		2	3	1
p-7																				
p-8											1		1	1	2	2				2
p-9	23	7	5	23	16	58	11	13	19	8	4	7	10	2	3	2	2	1	1	4
p-10				1							1	1	1		2					
p-11		1			1															
p-12																				
a-1	3	8	1	11	5	52	33	57	33	39	14	6		1	3	18	8	15	12	8
a-2	1					3			1	1										
a-3																				
a-4																				
a-5		1																		
i-1				1																
i-2																				
i-3																				
i-4																				
c-1						1				1	2	2		1			1		1	
c-2							1				3	2	1			1	1		1	2
c-3																	2			2
cl-1		1		1	1	2	5	4	1	3	2	1	2	2	2	1	2	3	1	2
n-1		2	4	7	4		1	2	5	1	3	1	3	2	1	4	1	1	1	4
o-1,2	160	252	110	196	174	506	182	235	83	154	278	328	118	246	196	318	399	238	244	236
o-3	4	11	4	10	10	10	8	5	4	6	22	12	6	14	8	12	10	5	10	14

Appendix Table E. Average number of benthic organisms per pen - (#/0.1 m^2)

Weeks after spill	+1				+4				+11				+20				+26			
Pen	Out	Con	1	2	Out	Con	1	2	Out	Con	1	2	Out	Con	1	2	Out	Con	1	2
Organism Code																				
p-1	1	1																		1
p-2																				
p-3	3	8	3	4	3	19	8	9	1	4	5	1	6	2	2	9	5	4	8	25
p-4	5	13		7	4	16	5	14	10	11	3	5	6	26	3	10	3	19	2	5
p-5	2	2	1	1	16	11	4	5	12	4	2	6	8	8	3	5	6	12	7	5
p-6	6	19	2	5	6	6	3	4	9	8	4	5	7	4	2	6	7	19	8	12
p-7																				
p-8																	70	127	50	50
p-9	25	6	13	6	9	6	3	1	5	4	2	6	2	1	1			2	1	
p-10			4								1									
p-11																				
p-12																				
a-1	4	4			2	4			34	2			50	108	23	31	68	67	28	14
a-2					1									1	1			2		
a-3									1	1								3		
a-4																				
a-5																				
i-1					1			1	10	3	1				1			2	2	1
i-2			1															1		
i-3				1																
i-4																				
c-1	11	17	11	5	7	14	2	1	7	17	2	1	4	50	8	7	15	42	11	5
c-2	1	19			3	5				1			1	13			1	4	1	
c-3	2	7	<1		1	4	<1			3								1		
c1-1	2	10	5	4	5	4	6	6	5	4	5	4	4	5	5	5	3	2	9	4
n-1	6	4	2	2	1	6	3	5	1	3	3	3	2	4	2	5	1	6	9	11
o-1,2	1287	715	377	634	596	810	775	676	1538	1302	718	771	520	1203	653	745	678	2903	1123	947
o-3																				

Appendix Table E (continued).

Weeks after spill		+39				+49				+56				+74				+82			
Pen		Out	Con	1	2	Out	Con	1	2	Out	Con	1	2	Out	Con	1	2	Out	Con	1	2
Organism Code																					
p-1			1									1			2			1	1	8	5	3
p-2			7	4	6	2	10	4	3	8	20	15	9	3	1	1	1	1	1			1
p-3			15	8	16	7	8	8	9	15	10	7	11	37	40	8	31	54	106	92	82
p-4			1	2	1	1	28	10	14	2	29	8	13	2	1	7	1		1	1	4
p-5					2		5	1	1	2	11	1	3		19	2	2	1	25	2	6
p-6			8	4	6	8	6	11	7	12	17	9	15	16	15	7	12	8	17	9	8
p-7			1								1										
p-8			1		1			1						1					3	4	7
p-9				3	3	59	68	94	76	54	50	28	41	26	34	16	31	9	31	30	32
p-10																		1			
p-11						1				1	1		1	2	1	1	2	3		2	1
p-12											1			2							
a-1			4		1	29	10	1	1	167	74	9	41	143	239	75	145	78	236	27	81
a-2			1								2		1	2	8	2	2	1	1	1	1
a-3										1	4			3	4		1	3	1	1	
a-4			1																		
a-5																					
i-1				1		1			1		1		1	1			1		4	2	2
i-2																					
i-3																					
i-4							1							1							
c-1				1	1	1	1					1		3		1					
c-2																	1				
c-3			1		1																
cl-1			3	3	3	3	1	2		1	1	1	1	2	3	2	1	2	2	4	1
n-1			7	5	10	1	5	4	3	1	5	2	4	4	6	5	2	3	2	4	4
o-1,2			1543	302	787	684	1948	481	497	1608	2907	867	1019	1642	2639	872	727	601	1602	779	751
o-3										29	59	19	23	105	197	54	44	38	92	56	52

BROKEN EKMAN GRAB (NO SAMPLES)

Appendix Table E (continued).

Weeks after spill	+91				+101				+114				+125			
Pen	Out	Con	1	2	Out	Con	1	2	Out	Con	1	2	Out	Con	1	2
Organism Code																
p-1		2							10		4					
p-2	14	12	9	4	10	27	6	5	57	71	56	34	6	6	13	5
p-3	131	200	82	147	73	32	26	14	93	23	36	42	69	52	24	48
p-4	3	3	2	7	2	4	1	3		2		9		6		2
p-5		2	1			4			26	40	38	22	31	44	35	21
p-6	12	23	12	9	21	32	9	15	29	61	19	12	46	36	18	16
p-7																
p-8	5	8	3	5	2			2								
p-9	96	86	55	112	197	207	281	122	781	128	333	195	453	191	214	177
p-10	4	3	1	5	27	15	10		91		102	15				1
p-11	2	2		1	17	4	3	3	10	2			4	2	2	3
p-12																
a-1	9	26	2	15	300	13	13	7	676	31	102	167	111	81	54	98
a-2				2			3	1		2	7	5	2		28	19
a-3													10			2
a-4																
a-5																
i-1	2	5		3					7	12	13	5	2	2	3	
i-2																
i-3																
i-4					2											
c-1						2										
c-2																
c-3																
cl-1	6	8	6	2	8	6	10	4	14	8	9	10	31	19	45	29
n-1	14	6	5	6	29	6	8	8	19	21	8	11	10	2	7	12
o-1,2	1280	1528	568	798	1173	2379	1680	556	3185	4298	3296	2615	3895	4302	3302	2410
o-3	166	168	34	73	2	218	128	46	247	318	258	244	97	114	100	59

Appendix Table E (continued).

Weeks after spill	+136				+148				+156				+181			
Pen	Out	Con	1	2	Out	Con	1	2	Out	Con	1	2	Out	Con	1	2
Organism Code																
p-1	2	2		2				2								
p-2	8	4	4	4		2	3	4	15	141	37	32			1	
p-3	92	97	38	66	67	80	30	69	29	19	17	39	147	42	51	28
p-4		6		1	6	115	7	31	4	6	2	22	65	59	25	37
p-5	21	25	6	19	4	13	2	5		21		1	27	13	56	18
p-6	23	67	15	28	23	38	43	52	40	25	26	31		76	35	38
p-7																
p-8	29	17	9	16	6	12	1	1				1				1
p-9	264	126	92	137	65	57	114	56	142	100	136	130	208	80	57	42
p-10	2	6	9	5	2	4	17	3	2	15	8	3				
p-11	2					2	4	4	4	2	2					
p-12																
a-1	535	132	93	205		116	72	81	54	35	30	64	409	202	286	195
a-2	2	10	3	8			3	7	2			2	10	12	15	5
a-3						2		1						2	2	1
a-4																
a-5	2								2	2	4	2				
i-1	2	10	1	3	2	2	1	1	2	2				6	2	
i-2																
i-3				1												
i-4																
c-1	6					2	11	7			1		4			
c-2						8	21	24				1	2			1
c-3						6	13	7				2			2	
cl-1	32	36	62	99	13	36	25	26	6	6	8	5	28	10	24	14
n-1	15	12	6	10	10	10	5	11	32	23	11	28	17	44	23	13
o-1,2	4586	4469	2628	3742	1981	2611	2266	2184	1712	1830	1252	1482	2216	1790	1348	1386
0-3	353	330	214	248	86	136	86	108	75	83	75	150	63	84	48	40

Appendix Table E (continued).

Weeks after spill		+199				+207		
Pen	Out	Con	1	2	Out	Con	1	2
Organism Code								
p-1	2							
p-2			1	1			4	2
p-3	57	73	52	41	19	12	16	12
p-4	44	38	32	24	36	25	63	29
p-5	12	34	8	5		21	10	9
p-6	8	10	10	7	15	6	9	13
p-7								
p-8	10		4		8			
p-9	50	141	58	61	19	36	19	22
p-10	10	8	4	4				
p-11	4							
p-12								
a-1	46	13	5	3	116	46	34	26
a-2		4	1			10	1	1
a-3								
a-4								
a-5								
i-1			1			4	4	
i-2								
i-3								
i-4								
c-1	10	6	1		4	19	4	8
c-2	12	8	11	6	10	6	9	11
c-3			23	23	8		8	7
cl-1	17	19	21	16	17	12	14	10
n-1	19	21	27	20	21	27	20	16
o-1,2	2227	1958	1114	890	2740	2733	1916	1861
o-3	118	40	41	40	97	71	46	40

APPENDIX TABLE F1. Average Benthic Biomass

(ml Volumetric Displacement/Pen)

Weeks Post Spill	PENS							
	Control	1A	1B	Pen 1 Average	2A	2B	Pen 2 Average	Outside
0	1.1	1.0	0.8	0.9	0.9	1.1	1.0	1.0
1	0.9	0.4	0.9	0.7	0.5	0.5	0.5	1.1
4	1.0	0.7	0.8	0.8	0.8	0.8	0.8	1.0
11	1.1	0.8	0.8	0.8	0.8	0.7	0.8	1.0
20	1.7	0.8	1.4	1.1	1.0	1.2	1.1	0.9
26	3.2	1.1	1.2	1.2	1.5	1.5	1.5	0.9
39	1.3	0.9	0.3	0.6	0.8	1.0	0.9	BG
49	1.8	1.1	0.7	0.9	0.4	1.0	0.6	1.4
56	1.9	1.1	1.4	1.3	1.5	1.2	1.4	1.8
74	3.7	1.8	LA	1.8	2.7	LA	2.7	2.9
82	3.2	2.3	2.7	2.5	2.1	2.6	2.4	1.8
91	1.8	0.4	1.2	0.8	1.1	1.5	1.3	1.3
101	2.0	1.1	1.0	1.0	1.0	0.5	0.8	1.6
114	2.0	1.3	2.1	1.7	1.4	1.9	1.6	1.9
125	2.0	1.4	2.0	1.7	1.9	2.0	2.0	2.1
136	2.7	1.7	1.5	1.6	1.5	2.3	1.9	1.9
148	1.8	0.6	1.4	1.0	1.9	1.5	1.7	1.1
156	1.4	1.2	1.2	1.2	1.5	1.7	1.6	1.4
181	2.4	1.9	2.0	2.0	1.8	2.2	2.0	2.6
199	2.1	1.8	2.0	1.9	1.8	1.5	1.6	2.4
207	2.4	1.6	1.9	1.8	1.6	1.8	1.7	2.5

LA = samples lost in storage

BG = Broken Grab

Appendix Table F. Computer Program Utilized for Benthic Statistics.

Name: Statistical Package for the Social Sciences
SPSS for DS/360, Version H, Release 8A, August, 1978

Tests provided: One way analysis of variance
F ratio and probability
Homogeneity of variances
Cochrans C and probability
Bartlett - Box F and probability
Multiple Range Test
Student-Newman-Keuls

Appendix Table G. Raw weight of grasses in control pen (g/0.25 or 0.1 m^2)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
9-19-75(0.25)	253.8					58.2				
	96.3					30.4				
	66.2	0.6		0.7(AS)		16.3				
	77.4					70.6				
	68.7	0.7				12.3	8.1			
	76.0					96.2				
3-29-76(0.25)	18.1					147.1				
	12.2					103.9				
	8.1	0.2		t(L)		94.8	1.2			
	7.1					78.3		1.3		
	9.6					129.9				
	11.4					92.7	0.7			
	3.4					72.8				
	10.3					139.6				
	4.7					100.5				
	6.9			t(L)		81.1				
6-15-76(0.25)	133.7					159.1				
	167.6					148.4				
	183.6					204.2				
	119.4					151.6				
	79.1					137.6				
	86.8					114.9				
	83.5					129.0				
	93.7					99.5				
	81.6					84.9				
	111.1					39.9				
	104.7					87.0				
	117.5					136.4				
9-17-76(0.25)	204.9					183.3				
	200.0					170.6				
	70.0			20.0(AS)		46.1	5.7			
	143.6			0.9(AS)	4.6(P)	26.0				
	145.0					104.2				
	148.7			0.3(AS)		98.0				
	183.2					67.4				
	190.0					103.0				
	154.9			11.2(AS)		70.8				
	202.2			t(AS)		134.4				
5-3-77(0.25)	64.9					108.3				
	62.9					215.2				
	40.6	2.0				118.4				
	30.1					122.6				
	37.6					143.6				
	37.0			2.2(L)		148.0				
	49.6					125.1				
	41.0					195.8				
	48.6			0.5(L)		151.2				
	57.2					233.5				

Appendix Table G (cont.)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
7-26-77(0.25)	84.7			0.4(AS)	32.3(H)	57.4			4.9(AS)	
	89.9					138.9				
	75.7		17.7	14.9(AS)	0.2(H)	96.0		12.2	3.4(AS)	
	72.2			14.5(AS)		74.4				
	103.4					90.1				
	63.6			3.1(AS)		106.7				
	86.0			8.2(AS)		80.6				
	64.3			4.3(AS)		109.8				
	124.3					52.1				
	95.6			7.6(AS)		70.1				
8-15-77(0.25)	Two replicates of 10 samples each									
	137.7					74.0				
	128.0			4.0(AS)		114.0		2.2		
	62.1	1.1	16.7	38.2(AS)		73.1	0.5		4.9(AS)	
	72.4			20.6(AS)	0.1(H)	124.8			2.8(AS)	
	85.2					110.2				
	126.8					110.1				
	161.5					94.3		113.0		
	169.2					126.6				
	173.7					158.6				
	52.3					33.6				
	101.5					49.5				
	135.7			1.4(AS)		128.3			1.1(AS)	
	83.1	5.1				88.1			0.8(AS)	
	66.0	0.3		17.8(AS)		130.6			0.3(AS)	
	91.2	3.3				133.7				
	99.9					111.9				
	138.2	1.4		4.1(AS)		123.3				
	128.9	7.7				109.0				
	133.3			19.7(AS)		19.8				
	84.9			0.5(AS)		26.9				

Samples taken for productivity estimates:

	Live	Dead		Live	Dead
4-7-78(0.1)	3.6	54.4	6-1-78(0.1)	7.7	49.9
	3.8	48.8		6.8	32.3
	4.7	47.6		7.8	35.1
	3.5	57.0		4.5	38.9
	2.8	68.5		5.5	47.0

Date	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
6-1-78(0.1)	31.0									
	3.7	5.5		16.3(AS)		7.2	1.7			
	5.1	3.0		15.2(AS)		12.6	6.1		6.0(AS)	

Appendix Table G (cont.)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
6-1-78(0.1)	7.8	2.4		11.7(AS)		38.0				
	9.7	4.0		2.5(AS)		35.8	4.1			
	17.9					56.1			0.8(AS)	
	27.0			7.9(AS)		43.0			2.9(AS)	
	27.2	1.6				49.2	0.7			
	25.6	1.6				45.2	t			
	23.6					54.2				
8-7-78(0.1)	53.9			5.8(AS)		41.7				
	13.8	1.6		32.0(AS)		5.2	6.5			
	25.0	7.5		16.0(AS)		8.8	4.6			
	27.2	7.1		21.2(AS)		14.8	0.1		0.8(AS)	
	43.9					48.7				
	39.6					33.7				
	42.8					37.7				
	47.2					59.3				
	56.9					40.1				
	58.6			3.4(AS)		64.6				
10-12-78(0.1)	Two replicates of 10 samples each									
	60.9					15.5				
	68.2					16.9				
	41.5			5.1(AS)		21.7				
	47.2	4.3		1.4(AS)		18.4	1.2			
	12.7	0.4		43.4(AS)		10.9	0.3			
	10.9					4.2				
	8.6			31.5(AS)		3.6			8.6(AS)	
	18.4			7.5(AS)		8.1				
	27.4	0.4		18.5(AS)		15.2			3.0(AS)	
	38.2	2.1				9.1	0.6			
	45.4			11.4(AS)		11.5				
	40.3			6.3(AS)		9.7			1.1(AS)	
	42.6					14.3				
	51.1	1.7				10.1	0.3			
	22.3					24.6	0.8			
	27.8					4.4				
	12.3			67.2(AS)		7.6				
	18.8					2.2				
	39.8					13.3				
	41.3					16.4				
9-20-79(0.1)	Two replicates of 10 samples each									
	77.6					47.2				
	67.8			0.7(AS)	1.1(H)	39.2			0.5(AS)	
	57.2			0.5(AS)	2.1(H)	30.4				
	45.4	0.3		1.0(AS)	0.5(H)	22.3	0.5	8.9	1.1(AS)	
	32.1			2.0(AS)	3.2(H)	26.2	2.6	25.7	0.3(AS)	
	51.7				1.0(H)	24.2				
	67.8					8.0				
	55.7					35.0				

Appendix Table G (cont.)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
9-20-79(0.1)	44.9				0.5(H)	34.8				
	42.6			1.6(AS)		34.3				
	125.7					42.7				
	60.8					41.0				
	96.5			12.3(AS)		39.6			3.2(AS)	
	43.4	0.7		2.0(AS)	0.4(H)	33.8	4.5		0.2(AS)	
	33.0			2.2(AS)	0.3(H)	20.8	9.2		2.5(AS)	
	38.6			1.0(AS)	0.1(H)	22.0	6.3		3.0(AS)	
	49.4	1.3		0.5(AS)		9.6				
	52.3					35.3				
	41.8			7.0(AS)		25.9	5.2		1.6(AS)	
	24.0			2.0(AS)		39.4			1.0(AS)	

Appendix Table H. Raw weight of grasses in pen 1A (g/0.25 or 0.1m^2)
(Fresh oil i)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
9-19-75(0.25)	160.8					80.2				
	273.5					108.4				
	47.6	15.0		0.9(AS)		41.6				
	36.7					128.5	139.0			
	30.7			0.5(AS)		107.6				
	14.7					5.2				
3-29-76(0.25)						206.5				
	0.3					215.0				
	11.4					65.9				
	5.0	1.4		0.3(L)		56.8	68.8			
	3.5					67.3				
	10.1			t (L)		81.0				
	2.0	3.4		0.2(L)		37.9	31.9			
	2.5	11.7				25.1	53.9			
	1.8	2.1				9.6	53.0			
	7.0					67.6	12.2			
6-15-76(0.25)	14.4					185.1				
	-					321.1				
	-					204.1				
	-					392.7				
	77.5					145.9				
	86.2					62.7				
	69.8					70.7				
	27.2					33.0				
	28.2					40.6				
	5.9					12.2				
	9.0					22.4				
	60.6					56.8				
9-17-76(0.25)	31.7					38.1				
	2.3					176.9				
	93.0	10.4		1.7(AS)		82.1	11.8			
	62.6	32.6		12.0(AS)		26.5	50.8			
	111.0	15.1		6.8(AS)		41.7	25.9			
	43.3	22.2		28.4(AS)		53.7	39.7			
	63.0	18.8		20.2(AS)		31.6	29.6			
	18.4	89.2		10.7(AS)		12.8	104.0			
	34.3	35.2		12.8(AS)		20.5	56.9			
	69.8	37.3		14.4(AS)		47.5	63.3			
5-3-77(0.25)	16.6					118.0				
	24.2	0.5				120.4	0.3			
	23.6	0.3				84.3	0.1			
	19.2	0.2				132.1				1.3(L)
	19.9	0.5		0.2(L)		72.0				1.3(L)
	11.2	t				71.7				12.3(L)
	20.9	2.2		0.2(L)		75.5				3.1(L)

Appendix Table H (Continued)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
	5.1	1.4		0.2(L)		80.9	0.7		3.6(L)	
	26.8			0.5(L)		61.8			6.2(L)	
	26.7	2.0				84.8			6.6(L)	
7-26-77(0.25)	81.4	21.8				3.7	0.1	0.1		
	71.8			8.5(AS)		34.7				
	68.5			3.4(AS)		52.4			2.4(AS)	
	88.1	2.2				17.1				
	84.2			2.4(AS)		73.0			5.1(AS)	
	93.2					44.7				
	86.3			0.2(AS)		43.9			4.3(AS)	
	98.1			0.8(S)		18.2				
	96.6	1.1		8.0(AS)		34.1			5.3(AS)	
	63.1	24.2		0.8(AS)		73.5	8.7		1.0(AS)	
8-15-77(0.25)	Two replicates of 10 samples each									
	239.7					11.2				
	62.6					57.8				
	31.5	21.7		6.5(AS)		59.6	12.6		1.8(AS)	
	33.2	27.7		2.4(AS)		31.4	18.5		0.6(AS)	
	22.9			6.0(AS)		83.8	12.0		3.0(AS)	
	19.7	25.4		6.0(AS)		25.9	28.0		1.2(AS)	
	1.2	56.0		0.9(AS)	t(S)	17.2	22.7		1.1(AS)	
	8.6	44.3		0.1(AS)		44.8	9.1		3.3(AS)	
	49.1	79.6		0.3(AS)		47.3	61.1		0.4(AS)	
	75.6	0.4				97.3				
	171.3					47.2				
	111.5			1.3(AS)	5.7(H)	67.4				
	109.7					48.3				
	82.5	2.1				82.0				
	21.4	15.0		0.5(AS)		90.5	19.0			
	20.1	55.8		1.3(AS)		23.8	22.7		1.1(AS)	
	20.2	33.2		2.4(AS)		42.8	18.3		4.6(AS)	
	5.6	114.2				-	17.8			
	24.3	64.9		1.9(AS)		44.3	24.3		5.2(AS)	
	27.2	71.8	7.5	7.3(AS)		53.6	17.8	8.3		

Samples taken for productivity estimates

4-7-78(0.1)	Live	Dead
	7.7	32.3
	3.8	36.4
	9.3	48.2
	5.4	38.5
	3.3	42.1

4-24-78(0.1)	Live	Dead
	15.2	40.8
	13.9	43.2
	13.4	42.5
	12.1	45.8
	12.1	47.6

	Live				Dead			
	SA	DS-SP	SC	Other	SA	DS-SP	SC	Other
6-1-78(0.1)	28.9				76.4			
	49.2				10.2			
	41.2	0.9			76.7	1.4		
	20.8	16.3			18.8	3.9		

Appendix Table H (Continued)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
	8.0	9.8		1.1(AS)		31.4	22.2		0.4(AS)	
	8.2	14.3		0.3(AS)		9.1	13.5			
	3.2	14.5		1.8(AS)		12.1	25.9		0.4(AS)	
	3.2	34.1		0.1(AS)		5.8	29.3			
	6.2	14.1	7.7	0.6(AS)		10.6	28.9	3.5	0.4(AS)	
	8.6	23.5	11.5	0.1(AS)	0.1(B)	24.5	23.5		4.2(AS)	
8-7-78(0.1)	44.8			1.8(H)		35.6				
	25.0	22.4		7.9(AS)		18.0	12.7			
	15.4	14.0		8.2(AS)		24.2				
	24.9	18.4		6.1(AS)		-	6.1			
	31.3	1.4				10.7				
	18.1	17.0		2.1(AS)		14.8	5.9			
	16.3	20.0	1.2	6.2(AS)		10.4	13.6			
	-	86.1		1.5(AS)		-	61.9			
	9.8	20.4	0.6	8.3(AS)	0.6(H)	19.2				
	38.3	4.2				62.3				
10-12-78(0.1)	Two replicates of 10 samples each									
	52.3					30.1				
	48.2					26.3				
	28.3			14.9(AS)		12.5			0.6(AS)	
	34.2			3.0(AS)		14.8				
	53.4			3.6(AS)		25.5				
	61.4	4.3		4.7(AS)		16.4	1.4			
	40.3	10.7		5.1(AS)		9.6	5.6			
	33.8	6.2				10.2	4.1			
	27.9	3.7		2.7(AS)		17.4	5.0			
	26.3	1.2		2.8(AS)		15.9	0.7			
	41.7	8.2		5.5(AS)		13.5	6.7		3.0(AS)	
	44.2	7.2		3.9(AS)		8.5	5.2		0.9(AS)	
	23.0	13.2		1.5(AS)		7.1	10.4			
	20.6	8.6				7.3	1.9			
	9.8	25.8		2.4(AS)		10.2	2.8		0.8(AS)	
	14.3	20.2		3.2(AS)		8.7	2.6			
	31.6	50.0		2.1(AS)		2.6	3.5		0.9(AS)	
	27.8	17.3				6.7	4.2			
	22.7	4.2		3.5(AS)		12.1	4.8	0.3	1.5(AS)	
	26.9	2.6		4.9(AS)		10.3	0.2		1.6(AS)	
9-20-79(0.1)	Two replicates of 10 samples each									
	65.7	2.4		1.2(AS)		53.1	0.3			
	68.5	13.2		2.4(AS)		16.1	1.7		1.3(AS)	
	9.2	29.3		12.5(AS)	0.6(H)	9.0	3.3		1.8(AS)	
	5.7	9.3		20.4(AS)		4.8	16.1		1.2(AS)	
	13.5	25.0		2.3(AS)		2.4	3.3		1.4(AS)	
	20.5	33.2		6.0(AS)		17.9	3.9		0.7(AS)	
	29.6	34.1		6.2(AS)		18.4	26.9			
	-	-				-	-			
	-	61.7				4.2	50.3			
	31.5	20.8				9.0	2.3			

Appendix Table H (Continued)

Date(m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
	110.3	1.0				33.2				
	64.3			3.5(AS)		6.4				
	26.0	22.5		9.1(AS)		4.2	9.9	0.6		
	26.7	22.5				28.4				
	16.8	2.9				7.0				
	41.5	10.9		0.4(AS)		9.0		2.8	0.4(AS)	
	28.3	4.5		7.1(AS)		7.5		1.4		
	15.9	27.9		2.0(AS)		2.4		2.0		
	9.9	54.4		1.0(AS)		8.1		8.5	1.0(AS)	
	10.0	22.9		13.5(AS)	0.5(H)	2.8		2.2		

Appendix Table I. Raw weight of grasses in pen 1B (g/0.25 or 0.1 m^2) (Fresh 2)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
9-19-75(0.25)	133.5					53.6				
	131.4					78.2				
	65.9					105.0				
	28.6	30.2		1.6(AS)		10.1	48.7			
	53.3	13.9		0.3(AS)		51.4	29.5	3.5		
	118.4			1.5(AS)		49.5				
	67.8					108.0				
	83.4					80.4				
3-29-76(0.25)	1.0					144.4				
	12.8					230.7				
	17.7	0.3		0.8(L)		155.8	6.2			
	--					254.3				
	3.8	0.1		0.1(L)		135.7	0.6	3.7		
	--					278.8				
	5.0	0.3				89.6	43.8			
	0.4					248.0				
	7.1	3.0				65.6	85.3			
	--					176.8				
6-15-76(0.25)	49.7					190.3				
	24.1					294.9				
	49.7					207.2				
	12.2					225.7				
	72.3					149.2				
	5.8					181.0				
	55.4					128.8				
	--					266.6				
	107.2					96.1				
	29.4					133.8				
	29.7					55.5				
	16.8					49.6				
9-17-76(0.25)	127.7					160.7				
	70.0			3.7(AS)		53.3				
	130.9	10.8		14.4(SB)		85.9	1.7			
	26.6	4.4				61.7	1.4			
	49.3	10.7		0.6(AS)		68.0	12.0	1.3		
	0.8					83.3				
	75.0	8.7		3.9(AS)		86.0	21.1			
	--		2.4			19.2				
	52.0	15.1		1.6(AS)		69.0	54.1			
	1.5					72.1				

Appendix Table I. (cont.)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
5-3-77(0.25)	39.1					39.4				
	39.9	t				62.0				
	12.6	6.8		0.2(L)		71.4	14.0		4.5(L)	
	3.9	7.6				2.3	18.5			
	15.9	7.7	3.7			54.6	10.8	1.0		
	8.9	1.6	0.5			12.2	3.4	t		
	9.0	9.2		0.4(L)		42.5	31.4		11.3(L)	
	28.6					52.2				
	9.1	18.5		0.5(L)		31.5	35.8		0.4(L)	
	10.8					21.2				
7-26-77(0.25)	95.4			t(AS)		41.7				
	95.8			0.1(AS)	21.3(H)	18.0				
	66.7	2.2		0.9(AS)		27.3			0.3(AS)	
	21.0	8.4	4.0	11.6(AS)		31.3		1.6	6.6(AS)	
	22.8	1.5				2.0				
	24.7	2.9	2.7	5.2(AS)	0.1(H)	20.4		8.6	4.0(AS)	
	76.5					36.8				
	57.7	10.8		11.8(AS)		83.3			12.9(AS)	
	65.0			15.9(AS)	1.6(H)	13.8				
	57.7			11.4(AS		31.2		3.7	0.8(AS)	
8-15-77(0.25)	Two replicates of 10 samples each									
	93.0			1.8(H)	0.4(S)	17.8				
	71.0			10.9(AS)		75.0				
	15.7	5.5		22.1(AS)		74.1	2.6		18.9(AS)	
	6.0	15.1		0.8(AS)		66.6	4.0			
	50.7	20.4	1.3	4.4(AS)		53.8	4.2	10.3		
	73.9	10.1		0.4(S)		37.2	3.4			
	87.3					55.3			0.4(AS)	
	72.5					66.1				
	10.5	19.3		36.5(AS)	2.7(H)	22.8	10.9		0.4(AS)	
	80.9	13.6	8.4	32.2(AS)	2.2(H) 8.7(S)	22.2	1.5	3.7		
	245.0			1.0(AS)		29.6				
	95.0	11.4		20.4(AS)	2.6(H)	151.1	1.8		0.2(AS)	
	28.8	38.1	6.0			85.0	8.4		1.6(AS)	
	25.4	21.4	3.0	12.5(AS)		54.0	39.2	15.6	5.4(AS)	
	16.5	19.7	6.1	26.7(AS)		46.5	8.7	1.3	1.6(AS)	
	11.6	37.1		5.8(AS)	0.2(PL)	17.0	34.8		0.4(AS)	
	4.6	30.3		21.7(AS)		60.3			5.4(AS)	
	3.8	79.3		14.6(AS)		31.4	39.9	2.0		
	10.5	24.4	1.4	32.4(AS)	0.5(PL)	33.6	8.8	9.9		
	27.1	2.0	0.9	1.0(AS)	15.4(PL)	36.6	13.5	14.1		

Appendix Table I. (cont.)

Samples taken for productivity estimates:

4-7-78(0.1)	Live	Dead
	4.0	32.4
	2.3	26.1
	4.4	29.4
	6.1	29.8
	6.6	25.1

4-24-78(0.1)	Live	Dead
	18.8	37.6
	10.5	24.1
	9.8	26.7
	9.1	28.6
	15.6	24.6

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
6-1-78(0.1)	58.8					19.1				
	12.1			13.4(AS)		18.5			1.6(AS)	
	0.2	10.6		1.8(AS)		17.1	20.0	1.3		
	15.0	5.7	7.0	4.1(AS)		15.8	0.5	1.4	0.4(AS)	
	12.5	7.3		3.8(AS)		34.7	0.5			
	8.5	1.8		0.1(AS)		19.8			0.8(AS)	
	6.6	12.3		3.8(AS)		19.5	18.4			
	27.1	0.8	8.7	0.1(AS)		15.7	0.9	0.7	0.2(AS)	
	16.2	5.8	10.2	2.7(AS)	t(H)	19.9	4.3	0.6	0.4(AS)	
	10.8	3.6	32.4	1.0(AS		3.7		2.3	0.6(B)	
8-7-78(0.1)	40.1			0.4(AS)		7.7				
	29.6	4.6		22.5(AS)		8.4				
	18.7	17.4		15.3(AS)	0.6(H)	7.8				
	1.9	17.1	4.8	21.0(AS)		5.5	2.2	5.8		
	50.8	1.9				26.4				
	2.5	19.1		6.8(AS)		2.0	4.7			
	11.2	4.2		25.3(AS)	0.4(H)	5.4				
	11.8	20.2		13.7(AS)		9.6	2.4			
	0.3	1.2	2.8			4.6				
	5.9	3.3	4.3	0.9(AS)	0.1(H)	4.6		3.1		
10-12-78(0.1)	Two replicates of 10 samples each									
	50.9					21.0				
	58.3					26.2				
	51.7			1.3(H)		9.0				
	46.3					7.4				
	8.8	0.2		45.4(AS)		8.5	0.1			
	16.7	4.8		18.6(AS)		14.5	0.9		3.1(AS)	
	16.0	34.9		0.6(AS)		3.4	1.2			
	12.4	18.7				5.6				
	14.1	21.7		2.3(AS)		1.3	3.3			
	21.2	16.3		8.2(AS)		8.3	2.6		1.1(AS)	
	58.1	15.0				11.1				
	48.2	10.1				8.8				
	36.4	8.6		1.0(AS)		12.3	10.4		3.5(AS)	
	39.9	12.7		2.2(AS)		7.8				
	9.8	27.1				3.9	8.4			
	15.2	14.4		4.1(AS)		2.7				
	6.7	26.7	0.9	15.0(AS)		4.4	2.2		0.6(AS)	
	10.1	10.2		6.4(AS)		5.4			0.9(AS)	
	18.1	3.8		2.1(AS)		12.3	0.6	2.5	7.0(AS)	
	15.8	5.4	0.3	4.0(AS)		6.8	1.2		2.1(AS)	

Appendix Table I. (cont.)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
9-20-79(0.1) Two replicates of 10 samples each										
	96.3					47.2				
	22.7	7.4		4.5(AS)		20.6	9.7			
	22.2	9.8		1.4(AS)		13.5	13.9		1.6(AS)	
	36.4	7.3		1.4(AS)		21.5	12.2			
	40.7	4.0		1.5(AS)	3.4(H)	17.3	6.2			
	26.1	2.4		0.7(AS)	1.3(H)	10.6	11.6	12.9		
	27.2					31.4				
	26.1					26.1	3.1			
	21.5	2.2		3.6(AS)		25.4	9.2		2.2(AS)	
	6.2	0.4		1.6(AS)		0.8	8.7	23.1		
	132.0			2.5(AS)		6.8				
	33.2	23.7		2.4(AS)	0.7(H)	9.0	11.5			
	11.3	10.0			2.2(H)	7.6	16.4	12.5		
	35.6	10.4		0.4(AS)	2.5(H)	4.0	7.9	1.6	0.7(AS)	
	4.1	11.8		6.0(AS)		8.9	28.4		1.0(AS)	
	22.0	40.0		0.5(AS)		7.6	12.9		2.9(AS)	
	6.4	16.9		0.8(AS)	3.0(H)	8.0	11.1	5.4	0.8(AS)	
	--	5.9		0.9(AS)	8.5(M)	--	22.6	9.6	1.4(AS)	
					1.2(H)					
	4.2	10.2		5.4(AS)	1.4(H)	9.2	9.1	3.5	2.7(AS)	
	25.4	1.1		1.0(AS)	0.6(H)	11.6	7.8	2.3		

Appendix Table J. Raw weight of grasses in pen 2A (g/0.25 or 0.1m^2 (weathered 1)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
9-19-75(0.25)	221.6					64.8				
	128.7					161.5				
	120.1					47.0				
	119.4	2.7		1.9(AS)		56.5		12.6		
	81.8	3.7		0.3(AS)		35.0		9.0		
	168.1	7.6		0.4(AS)		89.3				
	70.9					95.0				
	124.8					62.4				
3-29-76(0.25)	3.1					211.3				
	0.6					214.9				
	9.9	0.5		0.7(L)		156.4	9.5			
	10.6	0.8		0.1(L)		156.0	28.8			
	10.8			0.4(L)		130.3		6.8		
	4.3	0.4				59.3	112.7			
	12.6					173.0				
	11.2			0.2(L)		190.2				
	1.4					98.0				
	1.3					200.8				
6-15-76(0.25)	75.7					43.2				
	64.2					206.4				
	61.9					135.7				
	51.7					161.6				
	60.5					115.1				
	42.9					67.4				
	54.6					57.0				
	26.7					60.7				
	71.5					82.9				
	78.1					133.8				
	13.7					155.8				
	57.6					109.4				
9-17-76(0.25)	206.6					272.7				
	153.2					49.8				
	58.0	3.2		5.6(AS)		44.4	63.5			
	63.8	47.7		5.2(AS)		50.9	56.1	(6.9)		
	110.8			0.1(AS)		70.3	0.9	7.8		
	51.4			2.5(AS)		41.9	71.1	t		
	108.7					54.9				
	144.6					74.1				
	12.1					14.0				
	77.1					193.9				
5-3-77(0.25)	40.3					94.8				
	18.0					127.9			1.8(L)	
	28.2		0.6	t(L)		101.0		0.6		
	21.9	0.9	2.0	0.1(L)		90.6	2.1	1.9	0.7(L)	
	22.5	0.1	1.8	0.2(L)		122.7		3.3	0.2(L)	
	28.4	3.2	1.4	0.2(L)		89.3	5.6		0.8(L)	

Appendix Table J (continued)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
	29.9					62.5				
	43.8	0.8				107.4	t			
	52.7					58.0				
	37.6			0.3(L)		47.6			0.5(L)	
7-26-77(0.25)	71.2			11.7(H)		24.6				
	227.5			91.1(H)		37.6				
	47.9	2.6	6.3	3.5(AS)		29.5	0.2	7.4	2.4(AS)	
	56.6			25.7(AS)		57.3			2.8(AS)	
	69.0			0.8(H)		61.0			2.5(AS)	
	74.5			3.3(H)		39.1				
	115.6					61.4				
	39.6					1.3				
	81.3					86.5				
	13.1			0.3(H)		3.7				
8-15-77(0.25)	Two replicates of 10 samples each									
	122.8					81.7				
	37.1	31.7		7.3		7.2				
	27.0	26.3		4.6(AS)		45.2	2.0	5.4		
	60.1	6.0		8.7(AS)	0.4(H)	71.0	0.7	9.7		
	79.7	11.6		8.2(AS)		74.9				
	151.7			0.3(H)		86.6				
	202.1			0.2(AS)		85.6				
	28.6					17.3				
	204.4					11.2				
	121.0			16.7(H)		79.8				
	195.7			0.1(H)		111.0				
	21.5			11.8(AS)		7.0				
	74.2	0.5		16.2(AS)	0.3(H)	119.0			1.3(AS)	
	18.4	2.4	7.8			20.9			1.9(AS)	
	107.1			3.1(AS)		25.5				
	158.3			0.6(H)		62.5				
	63.2					21.0				
	35.5			5.6(H)		49.5				
	-					-				
	-					-				
	341.7									

4-7-78(0.1) Samples taken for productivity estimates

Live	Dead	4-24-78(0.1)	Live	Dead
7.0	54.2		17.4	59.5
2.3	44.9		6.6	33.2
1.4	49.9		10.5	52.6
3.9	54.5		11.2	48.7
2.9	94.8		11.5	55.9

	Live					Dead			
	SA	DS-SP	SC	Other		SA	DS-SP	SC	Other
6-1-78(0.1)	54.0					62.0			
	-	1.3		5.8(AS)	t(H)	13.1	2.2		16.7(AS)
	8.5	11.4	10.2	0.4(AS)		11.6	2.2	1.4	12.1(AS)

Appendix Table J (continued)

	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
	6.2	1.8	14.6	5.5(AS)		23.5	3.0	7.1	5.2(AS)	
	15.0	2.8	13.5	2.4(AS)		33.7	0.7	5.5	0.6(AS)	
	30.4					60.1				
	31.8					52.7				
	36.1					58.1				
	65.2					77.7				
	30.5			0.8(H)		56.7				
8-7-78(0.1)	49.0					64.2				
	17.4	18.0	1.0	4.8(AS)		9.3	11.6			
	25.1	1.9		10.0(AS)		3.8	6.0		0.3(AS)	
	15.1	5.1	1.9	6.4(AS)		3.4	5.7	5.5	0.4(AS)	
	33.0	3.7	4.9	7.4(AS)	1.2(H)		4.4	2.6		
	62.0					42.6				
	66.5					41.9				
	11.7					63.9				
	80.8				0.1(H)	26.0				
	92.2				0.5(H)	41.9				
10-12-78(0.1)	Two replicates of 10 samples each									
	58.0					35.0				
	53.7					30.2				
	52.1					47.1				
	56.9					41.2				
	19.2	6.5				7.0	1.9			
	17.4	5.2				10.2	2.2			
	13.2	1.3	0.6	16.0(AS)		30.7	2.6	2.0	0.1(AS)	
	21.0	3.6		8.3(AS)		22.4	2.4		1.4(AS)	
	5.5			6.1(AS)		12.5	5.2	9.0	0.8(AS)	
	18.5			3.2(AS)		9.9			1.9(AS)	
	25.6	5.1		16.4(AS)		9.2	1.1			
	25.8	8.1		22.1(AS)		12.1	1.3		4.1(AS)	
	26.2			1.5(AS)		22.8				
	19.9			3.6(AS)		12.7			0.5(AS)	
	19.4					13.6				
	26.7					18.2				
	31.5			2.2(H)		49.2				
	28.4					26.3				
	26.0			63.2(H)		28.8			3.1(H)	
	29.2					21.2				
9-20-79(0.1)	Two replicates of 10 samples each									
	54.0					36.5				
	22.3	3.4		6.6(AS)		18.5	16.7		2.8(AS)	
	19.3	13.8		6.4(AS)		6.0	13.0	8.8		
	-	4.4		12.7(AS)	0.9(H)	2.4	24.6	4.4		
	0.4	1.5		2.4(AS)	0.8(H)	1.2	22.7	3.6	1.0(AS)	
	21.5	8.0		1.4(AS)	2.7(H)	6.4			1.8(AS)	
	61.1				4.0(H)	20.2				
	43.9					15.9				
	85.1			3.8(AS)	7.3(H)	14.7				

Appendix Table J (continued)

Live					Dead				
SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
38.2			1.1(AS)		6.2				
31.4			0.5(AS)		10.3				
50.7					22.8				
46.2	0.8	1.0	19.1(AS)		30.4		7.6	0.3(AS)	
39.7					28.2				
29.0	1.5	0.7	1.3(AS)	1.2(H)	1.2	1.3	14.9	0.2(AS)	
3.0	2.3		0.6(AS)	3.2(H)	8.9	0.2	7.6		
18.2	8.9		0.4(AS)		30.2	7.3	16.4		
28.2	6.4		1.1(AS)		28.6	2.5	11.8		
80.8		5.3			36.6				
68.0					25.5				

Appendix Table K. Raw weight of grasses in pen 2B (g/0.25 or 0.1m^2)
(weathered 2)

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
9-19-75(0.25)	75.8					12.8				
	188.7					65.2				
	132.0	4.7		1.6(AS)		38.8		21.7		
	69.4	0.6		1.8(AS)		60.1	8.75	14.8		
	64.7			1.5(AS)		85.8	33.3			
	87.7			9.3(AS)		48.4	37.8			
	124.6					132.0				
	89.7					137.4				
3-29-76(0.25)	5.2					143.5				
	2.8					150.2				
	6.2	0.2		t(L)		23.1	37.3	11.3		
	4.0	0.1				95.0	2.9	6.7	0.3(L)	
	-					82.8				
	-	t				121.1	15.2			
	0.4					169.4				
	-					265.6				
	-					245.8				
	-					283.1				
6-15-76(0.25)	82.8					68.2				
	85.2					173.4				
	109.0					136.3				
	62.8					75.1				
	46.0					49.8				
	15.7					154.3				
	6.1					67.6				
	1.3					165.2				
	-					236.8				
	-					402.0				
	-					194.3				
	-					261.5				
9-17-76(0.25)	129.7			3.7(AS)		118.7				
	113.0			55.4(AS)		89.2				
	57.1			0.8(AS)		39.0	12.6	23.6		
	22.0					94.8	22.6	43.8		
	18.5			4.3(AS)		9.7	79.7			
	-					100.7	(145.8)			
	2.6					97.1				
	-					129.4				
	-					23.8				
	-					143.4				
5-3-77(0.25)	77.5					211.8	12.5		0.1(L)	
	42.3					116.6				
	13.4	14.3	5.2	0.6(L)		61.8	10.8	17.6	1.0(L)	
	1.7		7.0	0.2(L)		105.8	3.6	24.1	20.0(L)	
	14.5	3.4		0.4(L)		63.9	24.8		1.2(L)	
	5.5	1.0		10.1(L)		73.0	2.8			
	4.2			10.2(L)		72.9	3.5		0.7(L)	
	t			0.2(L)		127.4			0.1(L)	

Appendix Table K. Raw weight of grasses in pen 2B (g/0.25 or $0.1m^2$) (weathered 2).

Date (m^2)	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
5-3-77(0.25)	0.1					21.4				
	0.2			t(L)		39.9				
7-26-77(0.25)	108.4			0.6(AS)		102.4				
	101.3			5.7(AS		87.1				
	32.5	1.3		1.4(AS)		74.7	0.2	27.2		
	35.8	1.7		2.4(AS)	0.1(H)	76.6		10.0		
	24.5	4.8		32.3(AS)	12.4(H)	53.3			0.2(AS)	
	36.9	13.3		22.5(AS)		43.8			0.2(AS)	
	1.0					44.1			1.0(AS)	
	84.1					15.9				
	3.0					7.6				
	8.9		2.5	t(AS)		29.0		t		
8-15-77(0.25) Two replicates of 10 samples each										
	122.4					223.7				
	62.0	6.9		0.5(AS)		69.8		14.0		
	43.2	12.4		4.4(AS)		51.4	5.1	7.6		
	28.3	16.9		1.4(AS)		38.6	11.7	12.2	0.5(AS)	
	9.6	57.3		7.0(AS)		2.0				
	5.5	40.4		3.1(AS)	29.1(H)	24.1				
	27.0	15.8				116.1				
	1.2					7.4				
	-		14.2			11.2		7.5		
	-					-				
	226.6					105.6				
	105.9			11.6(AS)		97.0		5.4		
	56.2	7.1		2.1(AS)		62.6	0.2	5.2	0.1(AS)	
	32.8	4.1		4.9(AS)		58.2	5.9	6.6		
	25.5	1.8		12.6(AS)		43.0	16.4			
	24.2	46.5		37.9(AS)		30.1				
	134.7	19.7				54.2				
	134.6			t(AS)		26.4				
	127.9					29.2				
	89.4			34.1(AS)		3.6				

Samples taken for productivity estimates

	Live	Dead		Live	Dead
4-7-78 (0.1)	5.8	103.0	4-24-78(0.1)	12.6	56.5
	2.3	28.9		7.3	22.2
	2.3	24.7		11.4	20.0
	7.8	40.5		7.4	22.9
	5.2	36.9		15.0	35.2

Appendix Table K (continued)

Date	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
6-1-78(0.1)	38.8					29.1				
	31.8		5.6	0.8(AS)		28.4		0.7	0.6(AS)	
	10.2	6.1	11.4	2.4(AS)		23.1	26.8	4.1		
	11.9	8.5	12.6	4.2(AS	0.1(H)	9.4	7.5	2.0		
	7.5	11.9	7.1	1.1(AS)	0.8(H)	8.7	11.8	0.9		
	3.3	27.5		1.1(AS)	t(H)	2.1	9.2		0.2(AS)	
	5.5	6.8		5.4(AS)	0.2(H)	24.5	7.2		5.9(AS)	
	46.4			0.7(AS)		--			39.0(AS)	
	46.7					--				
	55.9		26.5			79.0		0.8		
8-7-78(0.1)	92.0					15.3				
	24.3	3.8	11.6	5.2(AS)	0.3(H)	13.3	5.7	4.3	0.4(AS)	
	26.6	10.0	5.7	12.8(AS)		20.9				
	17.0	9.5	7.2	11.5(AS)		3.6	11.0			
	13.5	18.9	11.8	14.2(AS)		6.0	14.8			
	6.7	30.4		11.8(AS)	4.7(H)	--	20.3		0.9(AS)	
	3.9	27.8		15.0(AS)	3.8(H)	14.9	2.4			
	51.2	4.4	6.3			34.2				
	60.2		10.0	2.8(H)		7.2		4.9		
	71.0		3.2			14.9				
10-12-78(0.1)	Two replicates of samples each									
	67.0					31.1				
	74.3					38.5				
	78.0					14.8				
	70.1					20.2				
	14.1	1.5		14.7(AS)		6.5		5.8		
	10.2	4.3		14.0(AS)		8.8			4.2(AS)	
	16.8	3.9		13.3(AS)		4.3	3.8	2.7		
	20.2	4.8		20.1(AS)		3.2	1.2	0.2	4.0(AS)	
	16.0	12.6		15.7(AS)		4.8		3.6	0.1(AS)	
	16.3	11.4				4.2	2.9			
	26.5	7.4		18.3(AS)		1.0	11.8	7.0	0.2(AS)	
	21.2	8.2		9.9(AS)		6.4	6.4		1.1(AS)	
	15.0	33.6		21.2(AS)		1.0	2.0		0.5(AS)	
	20.8	32.1		18.3(AS)		4.2				
	45.5	7.1		16.1(AS)		1.4	3.0			
	37.7	6.1		10.4(AS)		1.7	1.9		0.5(AS)	
	65.3			3.1 (AS)		20.0				
	48.6			1.8(AS)		18.2				
	30.8			0.2(AS)		12.6		1.2		
	28.1					14.6				

Appendix Table K (continued)

Date	Live					Dead				
	SA	DS-SP	SC	Other	Other	SA	DS-SP	SC	Other	Other
9-20-79 (0.1) Two replicates of 10 samples each										
	86.4					12.8				
	51.7	1.8	0.6	0.9(AS)		33.4	2.0	4.8	0.2(AS)	
	21.3	3.2		2.6(AS)		23.8	5.9	10.8	3.1(AS)	
	12.6	10.4		2.6(AS)		25.5	9.6	12.0	0.4(AS)	
	14.6	18.4	0.3	2.4(AS)		3.2	17.6	1.1		
	9.0	19.9		6.0(AS)	1.5(M)	2.0	7.2			
	9.6	24.8		5.9(AS)	1.3(H)	1.3	13.5			
	5.8	4.1		12.0(AS)		0.8	8.3	15.4	4.7(AS)	
	6.4					20.5		54.4		
	6.7		0.2			--		48.9		
	92.4					70.6				
	43.3					32.7		1.8	0.1(AS)	
	16.0	1.2		3.6(AS)		10.2	23.2	16.8	2.3(AS)	
	14.9	3.6		2.1(AS)		5.6	18.7	16.8	1.2(AS)	
	6.8	0.9		0.8(AS)		3.9	19.7	11.4	3.4(AS)	
	4.6	3.0		5.8(AS)	2.2(H)	10.6	28.5		1.2(AS)	
	12.4	2.4		1.9(AS)	1.3(H)	5.9	37.4		1.0(AS)	
	48.1		2.0	1.5(AS)	1.1(H)	42.8	8.4	1.9		
	31.8		2.1			20.3		14.2		
	17.0			1.4(H)		27.0		3.8		

Appendix Table L. Raw weight of outside grasses (g/0.25 or $0.1m^2$)

Date (m^2)	Live				Dead			
	SA	DS-SP	SC	Other	SA	DS-SP	SC	Other
3-29-76(0.25)	9.7				114.5			
	18.7	0.3		0.3(L)	162.2	2.0		
	16.3				154.5			
	27.2				144.7			
	21.9				164.2			
	19.3				162.9			
	22.2	1.1		1.3(L)	162.3	4.7		
	11.4	7.5		t (L)	72.8	86.8		
	20.6	2.5		t (L)	149.9	7.3		
	22.0				153.0			
6-15-76(0.25)	149.7				123.5			
	102.2				108.0			
	LA				LA			
	69.2				122.9			
	82.3				103.4			
	111.0				87.4			
	147.2				98.9			
	120.4				91.4			
	70.3				88.7			
	30.9				27.8			
	79.2				57.0			
	93.2				111.7			
9-17-76(0.25)	Two replicates of 10 samples each							
	177.2				136.6			
	49.9			12.6(AS)	116.0	14.2		
	82.8				121.1			
	107.7				147.7			
	129.4			4.5(AS)	204.4			
	174.2				169.7			
	84.3			11.5(AS)	110.6	6.9		
	43.4			6.7(AS)	51.2	56.6		
	112.8			2.9(AS)	87.9	(3.0)		
	91.0			1.2(SB)	124.4	1.0		
	178.7				198.4			
	196.8				204.7			
	94.5			5.7(AS)	106.2			
	131.1			8.6(AS)	132.1	t	t	
	107.8			0.5(AS)	100.1	(6.5)	12.1	
	86.6			6.2(AS)	102.0	11.3	8.9	
	146.0				128.7		2.7	
	143.2				168.2			
	139.6				136.6			
	156.7				156.3			

Appendix Table L (cont.)

Date (m^2)	Live				Dead			
	SA	DS-SP	SC	Other	SA	DS-SP	SC	Other
5-3-77(0.25)	55.6				141.1			
	30.0				173.5			
	26.7	0.4		0.4(L)	133.9	14.1		1.4(L)
	39.5			0.3(L)	160.5	5.1		1.7(L)
	33.0	1.7		0.1(L)	52.4	60.3		t (L)
	31.6				161.9			
	33.8				118.3	13.5		0.9(L)
	34.5				141.0			
	40.5	1.1			81.4	4.8		
	55.8				145.7			
7-26-77(0.25)	105.9				138.1			
	76.0				126.3			
	55.9			0.9(AS)	108.0			0.7(AS)
	73.3	3.2		0.8(AS)	135.3			
	65.1				125.8			
	106.1	3.2		0.3(AS)	85.0	3.5		
	56.9				157.4			
	83.3			0.2(AS)	105.1			
	93.2				62.9			
	43.0				105.9			
8-15-77(0.25)	Two replicates of 10 samples each							
	259.6				27.2			
	85.0	3.0		t (AS)	88.5	1.1		
	43.0	22.2		0.4(AS)	89.8	10.7		
	42.4	26.7		0.2(AS)	72.7	3.6		
	25.3	10.0		1.1(AS)	66.2	28.2	1.3	0.4(AS)
	24.0	38.1		0.1(AS)	1.4	69.8		1.6(AS)
	45.7	24.3		0.3(AS)	115.4			
	107.6	43.0	4.7	0.2(AS)	146.1	3.0		0.2(AS)
	85.9	1.5			112.6			
	64.6				137.9			
	232.5				212.2			
	53.5				106.4			
	46.2			0.5(AS)	122.6	20.8		
	57.4	14.7		0.4(AS)	111.2			
	36.2	23.7			61.2	31.4		0.3(AS)
	13.8	17.2		21.0(AS)	2.2	82.6		t (AS)
	46.8	22.0			52.5			
	49.2	12.1			102.8	24.5		0.6(AS)
	95.8				129.9			
	104.0				93.5			

Appendix Table L (cont.)

Date (m^2)	Live				Dead			
	SA	DS-SP	SC	Other	SA	DS-SP	SC	Other
Samples Taken for Productivity Estimates								
4-7-78(0.1)	Live	Dead				Live	Dead	
	6.9	102.2		4-24-78(0.1)		11.9	65.1	
	3.4	60.6				10.3	23.4	
	4.1	36.6				16.7	38.9	
	4.3	47.5				8.8	28.9	
	10.3	46.1				14.7	35.1	
6-1-78(0.1)	38.3				44.5			
	18.2				24.8			
	8.8	18.2		0.3(AS)	5.5	6.8		
	10.6	9.0	15.4		10.5	5.3	2.6	
	16.7	19.3			14.4	11.0		
	43.2				27.3			
	43.8				32.1			
	50.4				16.6			
	44.3				42.0			
	48.2				33.9			
8-7-78(0.1)	34.0				8.4			
	33.8	1.6		0.7(AS)	13.2			
	47.5				12.6			
	27.2	2.6			34.2			
	42.5				38.7			
	31.2				9.3			
	21.4				13.2			
	15.6				38.1			
	29.5				33.8			
	34.5				38.4			
10-12-78(0.1)	Two replicates of 10 samples each							
	31.0				7.2			
	33.1				6.1			
	25.6				4.2			
	26.5				8.3			
	28.5				15.9			
	23.2				12.2			
	34.3			3.3(AS)	16.0			
	39.7				21.2			
	34.6			2.2(AS)	23.6			
	26.8				18.4			

Appendix Table L (cont.)

Date (m^2)	Live				Dead			
	SA	DS-SP	SC	Other	SA	DS-SP	SC	Other
	46.0				27.0			
	51.4				22.3			
	62.2				26.6			
	60.0	4.9			31.1			
	38.7	2.1			15.1			
	44.9				8.8			
	57.2				10.8			
	49.2				12.2			
	47.1				14.4			
	60.1				14.6			
9-20-79(0.1)	44.6				50.0			
	36.6			11.5(AS)	47.6			
	51.0			2.5(AS)	52.8			
	35.5			0.3(AS)	51.0	24.0		
	32.7			0.4(AS)	38.2			
	24.5				38.0	30.0		
	59.5	2.8		3.1(H)	44.2	0.8		
	54.8				52.3			
	44.4				31.3			
	51.2				43.2			

Appendix Table M. Dissolved Oxygen Data

(mg/l; 2 replicates/pen)

Date/pen	Control	1A	1B	2A	2B	Outside
9-17-75	5.7	6.7	6.4			
	5.8	7.3	6.6			
9-23-75	6.0	5.3	5.0	4.8	5.1	
	5.9	4.7	5.1	4.8		
9-24-75	5.7	5.9	5.5	6.2		
	5.9	5.8	5.4	5.3		
9-26-75	4.6	2.8	3.8	3.4	3.7	4.8
	4.7	2.6	4.1	3.3	3.5	4.3
9-27-75	6.4			5.9	6.0	
	6.2			6.1	6.3	
9-29-75	8.0	7.8	7.7	8.2	8.4	8.2
	8.2	7.7	7.7	8.1	8.3	8.5
10-2-75	5.9			5.8	5.2	5.4
	6.1			5.8	5.0	6.0
10-15-75	8.7	6.1	7.3	5.1	5.3	
	8.7	6.2	7.4	5.0	5.7	
10-16-75	7.7	6.5	7.1	7.0	7.2	8.0
	6.9	6.3	7.5	6.6	7.3	8.5
10-29-75	6.1	6.6	6.5	6.2	6.9	7.3
	6.4	6.7	6.5	6.5	6.8	7.7
12-11-75	10.7	10.6	10.3	11.9	11.2	10.8
	11.2	10.3	10.7		11.4	12.0
1-26-76	12.7		12.0	12.0	12.0	
	12.8		11.8	12.6	12.1	
2-10-76	12.0	11.4	11.6	11.7	12.0	11.8
	11.9	11.2	11.7	11.8	11.9	11.7
3-24-76	17.7	15.6	20.1	12.5	13.9	14.5
	14.4	17.3	16.3		17.4	12.9
5-6-76	10.3	9.8	9.8	9.6	10.8	9.3
	10.1	11.3	9.5	9.3	11.5	9.2
6-24-76	10.1	12.1	10.6	10.3	12.4	7.6
7-8-76	9.9	6.5	9.4	10.2	9.7	11.8
	9.8	6.8	9.6	10.2	10.4	11.1
7-22-76	4.4	3.8	3.7	3.8	4.7	4.2
	4.5	3.9		4.0	4.6	6.1
8-4-76	7.5	6.6	6.7	7.5	6.9	7.3
		5.8	6.6	7.0	7.5	7.5
8-18-76	6.3	5.2	5.1	5.0	6.3	6.2
	6.6	5.0	5.0	4.6	6.0	6.8
8-31-76	4.6	3.8	4.1	4.1	4.5	4.3
	4.9	3.6	4.3	4.2	4.6	4.1

Appendix Table M. (cont.)

Date/pen	Control	1A	1B	2A	2B	Outside
9-17-76	5.7	6.3	4.0	5.2	5.5	6.6
	5.9	6.0	3.8	5.2	5.6	6.4
10-14-76	6.4	6.8	5.8	6.5	6.4	6.2
	6.3	6.6	6.2	6.6	6.4	6.3
10-28-76	9.0	8.8	8.7	8.5	8.5	9.1
	8.8	8.8	8.5	8.6	8.7	9.1
12-15-76	10.7	10.6	10.0	10.6	10.4	10.9
	10.4	10.3	10.8	10.3	10.2	10.7
3-2-77	13.0	11.0	12.2	13.3	12.8	
	13.5	12.4	13.1	13.6	12.6	
4-11-77	10.4	9.6	9.3	9.2	9.8	9.7
	9.8	9.4	9.0	9.1	9.6	9.7
4-26-77	5.2	7.4	8.3	6.8	5.8	5.4
	4.9	8.0	6.9	7.5	5.9	5.6
6-1-77	6.4	6.7	6.8	6.7	6.6	6.3
	7.0	6.7	5.6	6.4	6.0	6.3
6-14-77	4.4	5.7	5.3	4.8	6.4	5.7
	5.3	6.7	4.9	4.8	7.0	5.9
6-29-77	2.4	4.0	2.7	5.4	5.2	5.4
	2.1	4.2	2.3	4.5	3.9	3.8
7-26-77	5.3	5.0	4.5	5.3	6.8	5.8
	5.0	5.9	4.4	4.5	6.0	6.0
8-11-77	6.6	8.2	5.1	9.6	4.8	4.3
	8.3		3.5		4.8	4.9
8-29-77	2.3	4.6	4.2	4.0	4.7	3.2
	3.1	4.5	4.1	3.7	4.7	3.4
9-21-77	2.9	3.3	3.1	3.3	3.9	3.5
	3.1	6.9	3.6	3.5	3.7	3.5
10-20-77	8.9	9.7	8.0	8.7	9.0	7.4
	9.1	10.0	8.5	8.8	9.2	7.2
11-15-77	8.1	8.6	7.9	8.5	8.3	8.5
	8.7	8.9	8.6	8.6	8.5	8.2
12-5-77	7.9	8.7	6.7	6.0	6.9	8.0
	7.1	8.0	7.5	6.2	6.9	7.4
1-5-78	11.4	11.3	11.0	10.9	11.0	11.2
	11.3	11.6	11.7	11.2	11.1	11.4
3-21-78	10.8	10.5	10.5	10.4	10.9	10.9
	10.5	11.5	10.8	10.3	11.4	10.7
4-5-78	8.6	8.5	8.9	9.2	8.4	9.0
	8.6	9.2	8.9		8.6	9.0
5-1-78	7.6	6.5	6.7	7.3	7.8	6.8
	6.7	6.7	5.9	7.5	9.2	7.0
5-17-78	8.0	11.1	10.1	6.2	7.5	10.4
	7.8	7.9	7.1	7.6	7.5	7.8

Appendix Table M. (cont.)

Date/pen	Control	1A	1B	2A	2B	Outside
6-1-78	8.3	7.6	7.2	7.5	8.8	6.9
	7.0	6.4	9.5	7.2	8.3	6.7
6-13-78	6.2	5.0	4.7	6.2	6.4	6.0
	5.7	5.1	5.5	6.7	6.4	5.5
6-28-78	6.3	7.6	6.7	10.0	6.5	5.3
	6.4	6.2	5.3	10.0	7.4	6.3
7-12-78	4.2	5.4	4.0	5.4	5.8	5.0
	4.3	5.2	3.9	5.0	6.1	4.8
7-25-78	6.2	6.5	5.1	4.9	5.8	6.5
	5.4	5.7	5.8	5.4	5.0	6.4
8-16-78	6.0	5.5	4.4	5.3	5.8	5.7
	6.1	2.9	5.2	5.4	7.9	5.5
9-12-78	4.0	5.7	3.4	5.8	4.2	3.9
	3.9	4.0	3.7	4.9	4.4	4.1
9-28-78	5.6	6.6	7.6	9.1	7.0	6.3
	6.2	5.8	6.3	7.8	8.7	7.5
3-20-79	8.3	8.3	8.2	7.5	8.3	8.8
	9.0	8.7	7.5	8.3	8.7	9.1
5-17-79	6.3	6.0	11.1	6.5	4.9	5.9
	5.5	5.9	5.9	6.8	6.3	5.9
6-28-79	5.7	5.9	4.9	5.6	6.6	6.3
	5.8	7.9	4.9	4.7	5.8	4.5
7-26-79	7.8	7.2	6.3	6.6	6.1	5.4
	7.9	5.5	5.0	6.6	8.2	6.8
8-22-79	6.4	6.8	6.0	6.2	5.5	6.3
	5.8	6.3	5.8	6.1	6.1	

Appendix Table N. Temperature data (°C)

Date/Pen	Control	1A	1B	2A	2B	Outside
9-17-75	21.0	21.5	21.4	21.2	21.0	19.8
9-23-75	21.8	21.5	21.5	22.0	21.8	21.5
9-24-75	23.0	22.0	22.5	22.5		23.2
9-26-75	24.2	24.0	24.0	24.0	23.8	24.2
10-2-75	20.9		20.5	20.0	20.5	20.5
10-15-75	22.5	22.0	22.0	20.2	21.1	
10-16-75	22.5	22.0	22.2	22.0	22.0	22.0
10-29-75	17.0	17.6	17.4	17.3	17.5	17.2
12-11-75	7.0	7.0	6.3	6.0	6.0	7.2
1-26-76	9.5	10.1	11.0	11.5	11.8	
2-10-76	6.2	5.0	4.8	5.0	6.4	6.1
3-24-76	17.8	16.5	16.4	17.2	17.1	17.0
5-6-76	21.2	19.1	20.0	18.5	23.5	19.5
6-24-76	30.4	30.4	30.1	30.0	30.2	30.2
7-8-76	30.2	29.9	30.8	29.8	30.7	29.9
7-22-76	29.0	29.0	29.0	29.0	29.0	29.0
8-4-76	26.0	25.0	25.3	24.9	25.0	25.1
8-18-76	25.2	25.0	26.0	25.0	25.8	25.5
9-17-76	24.0	24.2	24.2	24.1	24.1	24.1
10-14-76	13.9	14.2	14.2	14.0	14.0	14.0
12-15-76	4.1		3.0	3.1	3.0	4.5
3-2-77	10.0	10.0	10.1	10.0		
4-11-77	17.0	16.0	15.0	14.0	14.2	16.0
4-26-77	17.3	17.0	17.1	16.3	15.1	16.5
6-1-77	25.0		25.0	24.8	25.0	25.0
6-14-77	24.5	24.3	24.2	24.0	24.2	24.0
6-29-77	27.0	27.0		27.1	27.2	27.0
7-26-77	29.0	28.0	28.3	28.0	28.8	
8-29-77	26.5	26.5	26.3	25.9	26.4	26.2
9-21-77	24.3	24.5	24.0	25.0	24.6	24.8
10-20-77	14.0	13.6	13.8	14.0	14.2	14.2
11-15-77	7.0	7.1		7.4	6.9	7.0
12-5-77	9.0	8.3	9.1	8.3	8.9	9.0
1-5-78	3.0	3.0		3.2		3.0
1-25-78	4.0	4.2		4.0		3.8
3-21-78	16.2	16.0	16.0	15.9	16.1	15.9
4-5-78	20.0	20.8	19.9	20.0	19.3	19.7
5-1-78	15.0	16.0	16.0	15.5	15.8	15.3
5-17-78	22.0	21.8	21.3	21.6	21.6	21.8
6-1-78	30.0	29.8	29.3	30.1	29.8	29.4
6-13-78	22.0					

Appendix Table N. (cont.)

Date/Pen	Control	1A	1B	2A	2B	Outside
6-28-78	29.5	28.5	28.3	28.0	28.3	29.0
7-12-78	25.0	24.6	25.0	24.8	24.8	25.0
7-25-78	26.0	26.2	25.8	25.4	25.9	25.7
8-16-78	26.8	27.0	27.2	26.8	26.9	27.0
9-12-78	25.6	25.2	24.7	25.7	25.3	25.5
9-28-78	25.0	24.2	24.6	24.2	24.4	24.6
3-20-79	8.8	8.2	8.6	8.5	8.5	8.0
5-17-79	20.7	21.2	19.5	20.9	20.7	19.5
6-28-79	21.0	21.0	21.4	21.4	21.2	20.1
7-26-79	26.0	25.0	25.3	26.0	26.0	25.1
8-22-79	25.9	25.7	26.7	25.7	26.5	26.2
9-20-79	22.2	22.0	23.0	22.2	22.8	22.5

Appendix Table O. Alkalinity data (mg/l)*.

date/pen	control	1A	1B	2A	2B	Outside
9-17-75	109.5	111.0		111.5		105.5
9-23-75	112.0	117.0	84.5	101.0	111.0	114.0
9-24-75	119.5	116.5	117.0	117.5	112.0	
9-26-75	119.0	115.5	118.5	116.5		121.0
9-27-75	96.0			98.5	96.5	99.0
9-29-75	114.0	111.5	109.0	113.5	111.5	110.0
10-2-75	111.5			108.0	100.0	144.0
10-16-75	136.5	138.0	137.0	138.5	132.5	136.5
10-29-75	136.5	133.5	137.0	136.0	135.5	137.5
12-11-75	128.0	133.0		130.0		138.0
2-10-76	119.0	112.0	116.5	117.5	118.5	124.0
3-24-76	174.5	191.0	188.5	197.5	184.0	195.5
5-6-76	138.0	141.5	138.5	140.0	142.0	157.0
6-24-76	123.5	113.0	112.5	117.0	100.5	121.0
7-8-76	153.0	147.5	154.5	155.5	149.5	154.0
7-22-76	168.0	155.5	153.0	154.5	147.5	154.0
8-4-76	142.0	134.0	135.5	130.5	135.0	137.0
8-18-76	119.0	115.5	115.0	118.5	115.5	133.0
8-31-76	139.5	136.0	141.0	140.5	141.0	147.0
9-17-76	107.0	109.0	111.5	103.0	113.5	113.5
10-14-76	125.5	113.0	120.5	115.0	117.5	117.0
10-28-76	119.5	119.0		120.5	118.0	128.5
12-15-76	141.5	138.0	140.0	142.0	140.5	140.5
3-2-77	136.5	129.0	133.0	136.0		140.0
4-11-77	151.5	142.0	142.0	171.5	148.0	146.0
4-26-77	93.5	99.0	93.5	89.5	76.5	119.5
6-1-77	117.5		113.0	116.0	120.5	110.5
6-14-77	102.0	121.0	123.0	121.0	113.0	112.5
6-29-77	106.5	127.5	111.5	118.0	121.0	
7-26-77	168.0	160.0		161.0	168.0	162.5
8-11-77	139.0	135.0	140.5	136.5	142.5	144.0
9-21-77	162.0		150.5	149.0	163.0	
11-15-77	187.5	179.5	186.5	184.0		181.5
12-5-77	178.5	183.0	178.0	176.5	175.5	169.5
1-5-78	136.0	123.5	134.0	142.5	140.0	143.0
3-21-76	97.0	124.5	107.5	98.0	115.0	94.0
5-17-78	142.0	140.5	148.5	139.5	139.0	140.5
6-13-78	126.5	128.0	129.0	130.0		130.0
7-12-78	121.5	97.5	114.0	120.0	112.0	131.5
7-25-78	109.0	104.5	100.0	68.0	108.5	107.5
8-16-78	125.5	136.0	125.0	118.5	131.0	130.0
9-28-78	142.0	140.0	139.0	136.0	135.0	141.5
3-20-79	123.5	97.5	108.5	114.5	100.0	118.5
5-17-79	146.0	141.0	139.0	140.0	133.5	142.0
6-28-79	130.5	124.0	124.5	115.5	122.5	137.5
7-26-79	110.5	88.0	98.5	102.5	90.0	114.5
8-22-79	127.5	122.5	130.0	118.5	120.5	131.0
9-20-79	135.0	125.0	131.5	130.5	143.0	138.5

*rounded to nearest 0.5 mg/l

Appendix Table P. Salinity values (‰)

Date/pen	Control	1A	1B	2A	2B	Outside
9-17-75	1.0	0.9		0.9		
9-23-75	2.4	2.0	10.0	6.4	4.4	
9-24-75	0.6	0.6	0.6	0.6		
9-26-75	2.1	2.5	2.0	2.4	2.6	2.3
9-27-75	0.2			0.3	0.3	
9-29-75	0.4	0.4	0.4	0.3	0.4	0.4
10-2-75	1.2			1.5	1.6	1.5
10-15-75	1.0	1.0	1.2			
10-16-75	1.0	1.4	1.4	0.9	0.9	1.6
10-29-75	1.4	1.8	1.5	1.4	1.5	1.4
12-11-75	1.8	3.0	2.6	1.8	1.8	1.0
1-26-76	2.8		3.3	3.2	4.1	
2-10-76	0.9	0.8	0.8	1.0	1.4	1.1
3-24-76	3.7	2.7	2.8	1.7	2.0	2.5
5-6-76	2.3	4.0	3.2	2.8	3.1	3.2
6-24-76	3.8	5.5	5.8	4.2	6.0	4.9
7-8-76	5.3	6.2	5.2	5.1	5.5	4.8
7-22-76	9.2	10.7	10.0	9.9	9.4	9.0
8-4-76	11.3	12.6	12.5	12.0	12.1	11.8
8-18-76	9.8	9.0	11.6	10.3	10.4	5.6
8-31-76	14.6	15.3	14.7	14.9	14.9	14.5
9-17-76	1.7	4.0	3.8	2.4	2.6	2.1
10-14-76	10.0	10.1	10.0	10.2	9.9	7.0
10-28-76	3.7	3.7	3.6	3.9	3.6	2.1
12-15-76	2.2	2.0	2.1	1.8	2.0	1.9
3-2-77	1.8	3.0	1.9	1.5	1.6	
4-11-77	1.6	2.7	2.4	2.1	2.1	2.7
4-26-77	6.6	4.7	6.3	5.7	5.7	3.4
6-1-77	6.9	7.2	8.2	7.2	7.5	7.1
6-14-77	9.5	10.0	9.8	9.1	9.8	9.5
6-29-77	14.7	14.3	14.7	15.1	14.1	15.1
7-26-77	10.8	12.6	12.2	11.9	11.6	12.0
8-29-77	13.9	13.7	13.1	12.5	13.5	16.6
9-21-77	18.4	18.7	18.5	18.4	18.4	18.2
10-20-77	16.0	14.8	15.1	15.2	15.6	13.8
11-15-77	7.7	7.3	7.3	6.5	9.1	11.4
12-5-77	15.3	10.1	10.5	9.5	10.6	5.9
1-5-78	4.6	6.1	5.4	3.4	6.2	5.1
1-25-78	2.5	3.5	3.6	3.0	3.0	2.6
3-21-78	5.3	2.9	3.9	2.1	4.2	5.4
4-5-78	1.4	1.7	1.9	1.5	1.6	1.8

Appendix Table P. (cont.)

Date/pen	Control	1A	1B	2A	2B	Outside
5-17-78	1.0	1.3	0.8	0.8	1.1	0.9
6-1-78	0.9	0.8	0.7	0.9	1.0	0.8
6-13-78	1.5	2.5	2.7	1.9	3.1	1.5
6-28-78	2.8	3.6	3.9	3.5	3.8	3.4
7-12-78	6.9	7.2	6.9	6.6	7.2	4.6
7-25-78	1.5	1.8	1.6	1.4	1.9	1.5
8-16-78	1.4	2.4	1.9	2.4	2.0	1.9
9-12-78	10.1	11.1	10.1	10.3	10.5	10.2
9-28-78	9.1	9.5	9.3	9.0	9.5	6.8
3-20-79	0.8	1.4	1.5	1.4	1.4	0.8
5-17-79	0.7	0.8	0.7	0.7	0.7	0.7
6-28-79	1.0	1.6	1.7	1.1	1.6	1.5
7-26-79	3.2	4.8	6.0	2.8	4.2	4.2
8-22-79	5.1	4.9	5.0	5.3	5.5	4.8
9-20-79	7.1	8.0	7.7	7.7	7.3	7.0

Appendix Table Q. NO_3 levels (µg/l) in water column

Date/pen	Control	1A	1B	2A	2B	Outside
9-24-75	32	49	58	47	44	
9-26-75	25	30	32	35	35	
9-29-75	24	27	23	22	22	23
10-2-75	15			14	16	14
10-16-75	21	16		21	20	24
10-29-75	44	42	44	43	44	
2-10-76	65	82	75	59	81	65
3-24-76		samples lost during pre-processing storage				
5-6-76		samples lost during pre-processing storage				
6-8-76		samples lost during pre-processing storage				
6-24-76	<5	5	8	<5	7	5
7-8-76	7	<5	6	<5	15	<5
7-22-76	5	<5	9	6	5	<5
8-4-76	<5	<5	6	<5	<5	<5
8-18-76	16	19	19	15	16	21
8-31-76	11	10	9	8	11	11
10-14-76	59	55	67	68	54	49
10-28-76	62	59	64	63	60	73
12-15-76	61		66	58	61	63
3-2-77	15	5	9	11	9	
4-11-77	32	20	16	26	19	16
4-26-77	21	24	28	44	43	26
6-14-77	6	<5	<5	<5	<5	5
7-26-77	8	8	6	6	8	6
8-11-77	17	15	8	18	15	7
9-21-77	20	27	32	21	25	26
10-20-77	42	42	54	40	40	45
11-15-77	53	49	31	29	46	50
12-5-77	64	42	51	48	64	53
1-5-78	36	61	37	48	57	53
1-25-78	43	36	50	44	45	35
3-21-78	16	19	11	17	11	17
4-5-78	13	16	8	13	8	13
5-1-78	19	11	<5	12	12	19
5-17-78	8	20	20	10	20	10
6-1-78	<5	<5	<5	<5	<5	
6-13-78	10	9	7	7	8	10
6-28-78	7	7	7	<5	<5	6
7-12-78	10	7	6	<5	6	7
8-16-78	<5	<5	<5	<5	<5	<5
9-12-78	<5	6		8	6	<5
9-28-78	7		7	8	7	10

Appendix Table R. NO_2 levels (µg/l) in water column

Date/pen	Control	1A	1B	2A	2B	Outside
9-24-75	8	7	7	7	8	
9-26-75	7	8	7	8	8	
9-29-75	6	6	6	6	7	6
10-2-75	6			5	5	6
10-16-75	7	6	6	5	5	7
10-29-75	6	6	6	6	7	
2-10-76	5	<5	7	<5	<5	<5
3-24-76	samples lost during pre-processing storage					
5-6-76	samples lost during pre-processing storage					
6-8-76	samples lost during pre-processing storage					
6-24-76	(all <5)					
7-8-76	(all <5)					
7-22-76	<5	<5	<5	<5	5	<5
8-4-76	(all <5)					
8-18-76	(all <5)					
8-31-76	(all <5)					
10-14-76	8	9	9	9	7	5
10-28-76	(all <5)					
12-15-76	(all <5)					
3-2-77	(all <5)					
4-11-77	(all <5)					
4-26-77	(all <5)					
6-14-77	(all <5)					
7-26-77	(all <5)					
8-11-77	(all <5)					
9-21-77	<5	<5	<5	5	<5	<5
10-20-77	13	7	14	5	8	7
11-15-77	<5	<5	<5	<5	<5	6
12-5-77	<5	<5	<5	<5	5	<5
1-5-78	<5	<5	<5	<5	5	<5
1-25-78	(all <5)					
3-21-78	(all <5)					
4-5-78	(all <5)					
5-1-78	(all <5)					
5-17-78	(all <5)					
6-1-78	(all <5)					
6-13-78	(all <5)					
6-28-78	(all <5)					
7-12-78	(all <5)					
8-16-78	(all <5)					
9-12-78	(all <5)					
9-28-78	(all <5)					

Appendix Table S. NH_3 levels (ppm) in water column.

Date/Pen	Control	1A	1B	2A	2B	Outside
9-24-75	0.02	0.06	0.08	0.03	0.03	
9-26-75	0.02	0.05	0.04	0.05	0.04	
9-29-75	0.02	0.02	0.04	0.02	0.02	0.02
10-2-75	0.02			0.02	0.03	0.03
10-16-75	0.03	0.03	0.03	0.02	0.03	0.02
10-19-75	0.04	0.05	0.04	0.07	0.06	
2-10-76	0.05	0.04	0.04	0.04	0.04	0.04
3-24-76	samples lost during pre-processing storage					
5-6-76	"	"	"	"	"	
6-8-76	"	"	"	"	"	
6-24-76	0.02	0.02	0.03	0.04	0.05	0.02
7-8-76	0.07	0.03	0.04	0.06	0.10	0.03
7-22-76	0.10	0.08	0.06	0.08	0.12	0.04
8-4-76	0.07	0.05	0.08	0.03	0.04	0.03
8-18-76	0.05	0.05	0.05	0.04	0.05	0.05
8-31-76	0.04	0.04	0.04	0.04	0.04	0.04
10-14-76	0.12	0.10	0.13	0.13	0.10	0.12
10-28-76	0.05	0.06	0.05	0.04	0.04	0.05
12-15-76	0.08	0.08	0.12	0.08	0.06	0.08
3-2-77	0.03	0.02	0.08	0.03	0.02	
4-11-77	0.09	0.03	0.03	0.09	0.04	0.03
4-26-77	0.02	0.08	0.03	0.04	0.12	0.03
6-14-77	0.04	0.03	0.05	0.03	0.02	0.04
7-26-77	0.03	0.04	0.04	0.03	0.05	0.03
8-11-77	0.13	0.04	0.04	0.04	0.04	0.03
9-21-77	0.08	0.08	0.09	0.07	0.08	0.08
10-20-77	0.18	0.10	0.23		0.10	0.10
11-15-77	0.12	0.10	0.08	0.07	0.10	0.09
12-5-77	0.05	0.03	0.04	0.03	0.05	0.02
1-5-78	0.06	0.11	0.05	0.06	0.09	0.08
1-25-78	0.01	$<$0.01	$<$0.01		$<$0.01	0.04
3-21-78	0.01	0.01	0.01	0.01	0.01	0.01
4-5-78	0.01	0.02	$<$0.01	0.01	$<$0.01	0.02
5-17-78	0.06	0.07	0.07	0.04	0.09	0.04
5-1-78	0.07	0.04	0.06	0.03	0.01	0.02
6-1-78	0.11	0.14	0.10	0.12	0.09	0.07
6-13-78	0.04	0.04	0.03	0.04	0.02	0.04
6-28-78	0.03	0.03	0.03	0.03	0.06	0.04
7-12-78	0.12	0.06	0.07	0.06	0.07	0.18
8-16-78	0.04	0.04	0.04	0.03	0.04	0.04
9-12-78	0.04	0.06	0.03	0.04	0.03	0.03
9-28-78	0.05	0.04	0.07	0.07	0.03	0.03
3-20-79	0.01	0.01	0.02	0.01	0.01	0.02
5-17-79	0.01	0.02	0.03	0.04	0.03	0.03
6-28-79	(All $<$ 0.05)					

Appendix Table T. Ortho-P levels (µg/l) in water column.

date/pen	control	1A	1B	2A	2B	Outside
9-24-75	22	29	25	26	24	
9-26-75	31	32	31	32	31	
9-29-75	22	31	26	22	25	24
10-2-75	32			31	30	33
10-16-75	34	37		31	32	37
10-29-75	25	27	27	25	27	
2-10-76	15	11	12	13	17	18
3-24-76	samples lost during pre-processing storage.					
5-6-76	samples lost during pre-processing storage.					
6-8-76	samples lost during pre-processing storage.					
6-24-76	73	45	87	54	82	47
7-8-76	110	67	77	77	112	82
7-22-76	120	84	94	112	109	93
8-4-76	42	36	38	41	43	35
8-18-76	84	82	90	89	90	57
8-31-76	50	45	35	42	45	33
10-14-76	27	33	40	30	27	26
10-28-76	25	28	26	24	24	25
12-15-76	30		31	28	27	28
3-2-77	14	5	10	16	18	
4-11-77	24	15	27	14	17	15
4-26-77	6	9	14	10	1	2
6-14-77	12	9	11	10	9	15
7-26-77	53	61	33	42	43	51
8-11-77	82	74	62	91	95	48
9-21-77	42	37	30	45	37	33
10-20-77	94	33	79		28	25
11-15-77	22	26	19	17	15	19
12-5-77	24	14	17	17	20	14
1-5-78	19	24	19	20	27	20
1-25-78	11	11	12	11	10	11
3-21-78	10	9	7	11	7	10
4-5-78	20	30	28	44	10	52
5-1-78	44	40	36	40	48	64
5-17-78	10	18	10	11		12
6-1-78	10	10	20	18	20	10
6-13-78	20	28	37	30	46	30
6-28-78	22	20	18	27	33	11
7-12-78	36	27	27	30	10	12
8-16-78	40	40	48	69	50	32
9-12-78	62		29	13	23	10
9-28-78	42	32	20	32	22	20
3-20-79	10	20	20	20	10	20
5-17-79	90	93	90	95	66	93
6-28-79	93	78	104	76	57	60

Appendix Table U. Primary productivity values (μC/l/hr)[1].

date/pen	Control	1A	1B	2A	2B	Outside
9-17-75	31.0	31.9		28.0		
9-23-75	13.4	8.1	8.8	17.0	18.7	
9-24-75	13.3	15.6	15.0	13.9		
9-26-75	26.4	37.3	36.7	13.0	14.1	21.3
9-27-75	20.0			14.4	16.7	
9-29-75	23.8	19.2	25.0	21.6	33.3	14.6
10-2-75	30.8			32.5	32.3	23.9
10-16-75	11.4	9.9	11.3	8.8	14.4	10.9
10-29-75	8.9	7.5	8.5	8.7	9.5	16.7
12-11-75	0.3	0.3	0.1	0.5	0.3	0.1
3-24-76	6.9	6.0	10.5	8.3	7.4	6.1
6-24-76	13.9	12.7	20.9	10.8	19.9	15.4
7-8-76	44.9	68.2	34.0	27.9	31.8	19.9
8-4-76	48.0	51.4	40.2	59.3	49.2	41.0
8-31-76	32.9	31.8	29.2	28.2	28.9	18.0
10-14-76	10.2	9.8	9.6	11.4	9.3	9.6
6-14-77	8.1	8.7	8.2	8.8	7.6	7.2
8-11-77	37.3	31.0	36.3	29.9	37.8	
7-12-78	4.0	4.4	2.6	2.7	-0.1	2.1
8-22-78	3.7	5.2	2.9	1.2	2.5	5.1

[1]1975-77 C^{14} method
1978 Winkler oxygen production method

Appendix Table V. ATP values (µg/l) in the water column.

date/pen	Control	1A	1B	2A	2B	Outside
9-22-75	0.81	0.82	0.76			0.93
9-25-75	0.86			0.78	0.72	0.82
9-26-75	0.74	0.95	1.03			0.82
9-29-75	0.43	0.35	0.37	0.82	0.76	0.27
10-2-75	0.93	0.84	0.84	0.88	0.84	0.83
10-5-75	0.89			0.96	0.80	0.97
10-15-75	0.84	0.84	0.80	0.89	0.84	0.82
10-29-75	0.60	0.54	0.55	0.61	0.53	0.74
12-11-75	0.31	0.27	0.29	0.30	0.27	0.29
1-26-76	0.63	0.63	0.66	0.60	0.72	0.79

Appendix Table W. Phytoplankton counts (10^3/ml); 3 replicates/pen.

date/pen	Control	1A	1B	2A	2B	Outside
9-22-75	69.4	80.4	119.2			
	179.6	117.0	84.7			
	122.7	99.3	100.4			
9-26-75	47.2	108.0	106.0	33.6	58.5	93.6
	126.0	54.4	112.0	97.2	76.7	88.6
	97.3	82.3	46.8	64.8	77.3	99.3
9-29-75	150.0	49.0	117.7	127.2	123.2	154.4
	105.6	147.2	89.6	95.2	83.6	95.2
10-2-75	120.8			99.6	133.2	
	185.0			172.4	103.2	
10-16-75	39.2	31.6		51.6		
	76.8	26.4		34.8		
	31.6			28.2		
3-23-76	62.6	73.8	68.9	49.8	52.0	38.7
	54.6	50.4	60.3	64.8	58.4	41.2
7-8-76	71.2	68.2	63.8	75.5	66.7	
	63.4	65.8	82.3	61.5		

Appendix Table X. Periphyton ATP values (μg ATP/cm^2) - triple replicates/pen.

date (H-V)/pen	Control	1A	1B	2A	2B	Outside
9-30-75	0.89	0.85	1.46	1.92	1.75	plates
	0.80	1.21	1.20	1.83	1.60	destroyed
	0.98	0.94	1.24	1.08	1.61	in first
10-7-75	0.87	2.46	1.30	2.02	0.62	week
	0.89	1.79	1.12	1.93	1.94	
	1.12	1.36	1.63	1.28	1.75	
10-14-75	0.47	0.89	1.72	2.22	1.02	
	0.75	1.98	1.90	2.08	1.08	
	1.21	2.11	1.54	1.54	1.78	
12-11-75	0.63	3.01	2.77	3.38	3.82	
	0.77	3.16	2.57	3.37	4.00	
	1.42	1.84	3.69	3.56	5.21	
1-26-76	0.81	1.33	0.95	3.75	3.61	
	1.13	0.74	1.44	3.83	4.12	
	0.89	0.91	1.11	5.18	3.45	
5-27-76(H)	0.96	0.17	0.89	1.83	0.39	1.13
(V)	0.26	0.46	0.30	0.85	0.26	0.33
6-21-76(H)	0.06	0.08	0.06	0.01	0.15	missing
(V)						
7-8-76 (H)	0.72	0.10	0.28	0.85	0.65	0.48
(V)	0.44	0.46	0.56	0.43	0.82	0.02
7-22-76(H)	0.10	0.20	0.79	0.03	0.15	0.62
(V)	0.07	0.28	0.13	0.09	0.09	0.06
8-5-76 (H)	0.04	0.03	0.08	0.09	0.07	0.04
(V)	0.03	0.05	0.02	0.12	0.02	0.03
8-31-76(H)	0.20	0.11	0.06	0.14	0.17	0.06
(V)	0.06	0.38	0.26	0.37	0.07	0.07
9-17-76(H)	0.07	0.04	0.03	0.05	0.03	0.02
(V)	<0.01	0.07	0.09	0.02	<0.01	<0.01
10-14-76(H)	<0.01	<0.01	0.01	0.02	0.02	<0.01
(V)	<0.01	0.06	0.05	0.01	<0.01	<0.01
10-28-76(H)	<0.01	0.04	<0.01	0.02	0.01	
(V)	<0.01	<0.01	<0.01	<0.01	<0.01	
12-15-76(H)	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
(V)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
4-26-77(H)	all <0.01				missing	missing
(V)	all <0.01					missing
6-1-77 (H)	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
(V)	<0.01	<0.01	<0.01	<0.01	<0.01	B.D.
7-7-77 (H)	0.05	<0.01	0.01	0.05	0.05	0.04
(V)	0.08	0.03	0.03	0.06	0.04	0.07
7-20-77(H)	0.01	<0.01	<0.01	<0.01	<0.01	0.02
(V)	0.01	<0.01	0.01	<0.01	0.01	0.01
8-29-77(H)	0.01	0.08	0.04	0.07	0.08	0.02
(V)	0.04	0.06	0.02	0.02	0.04	0.05
9-27-77(H)	<0.01	0.05	0.04	0.02	0.05	0.02
(V)	0.02	0.02	<0.01	0.02	0.04	0.04
12-5-77 (H)	<0.01		<0.01	<0.01	B.D.	0.01
(V)	B.D.		<0.01			B.D.

Appendix Table Y. Periphyton chl. a values (μg chl. a/cm^2)

date (H-V)/pen	Control	1A	1B	2A	2B	Outside
5-27-76(H)	1.70	1.20	1.04	1.61	0.83	1.31
(V)	1.20	1.62	1.16	1.23	0.93	1.75
6-21-76(H)	2.72	1.18	1.86	1.30	2.12	missing
7-8-76 (H)	1.32	0.96	1.20	1.20	1.08	1.50
(V)	1.68	2.82	1.80	4.74	3.00	4.92
7-22-76(H)	0.72	0.88	0.60	1.50	0.78	1.06
(V)	0.80	0.76	0.42	0.27	0.53	1.06
8-5-76 (H)	1.18	0.92	1.43	0.65	1.27	2.81
(V)	0.80	0.60	1.38	0.97	0.46	2.03
8-18-76(H)	2.22	1.05	1.16	1.98	2.20	2.28
(V)	3.01	4.91	3.16	8.72	5.72	7.84
9-17-76(H)	0.20	0.83	0.59	0.50	1.71	0.59
(V)	0.20	0.21	0.37	0.27	0.44	2.47
10-14-76(H)	0.29	0.07	0.33	1.00	0.28	0.14
(V)	0.34	0.26	0.56	0.51	1.32	1.31
10-28-76(H)	0.11	0.09	0.11	0.11	0.09	
(V)	0.08	0.04	0.04	0.08	0.11	
12-15-76(H)	0.52	0.51	1.08	1.53	2.84	1.17
(V)	0.72	0.26	1.29	0.36	0.70	5.66
4-26-77(H)	0.34	0.36	0.62	0.46		
(V)	1.66	0.48	2.30	1.62	3.07	
6-1-77 (H)	0.47	3.42	0.98	3.06	1.35	0.80
(V)	4.74	1.88	1.04	1.50	1.29	10.07
7-7-77 (H)	1.01	0.86	1.38	1.08	0.96	1.91
(V)	2.39	1.22	1.61	1.48	1.69	1.18
7-20-77(H)	0.40	0.38	0.43	0.53	0.44	0.71
(V)	1.30	0.52	0.97	1.24	1.41	3.20
8-29-77(H)	1.44	1.52	4.67	2.12	1.66	1.82
(V)	3.02	3.33	2.71	1.86	1.74	6.34
9-27-77(H)	0.65	0.63	0.48	1.33	0.70	0.97
(V)	0.88	0.92	0.65	0.92	1.04	1.05
12-5-77 (H)	0.61		1.30	0.78	0.44	1.36
(V)	0.59		0.75			0.51
5-1-78 (H)	0.45	0.46	0.35	0.28	0.56	0.73
(V)	0.71	0.58	0.66	0.46	0.72	0.49
5-12-78(H)	0.24	0.23	0.24	0.20	0.20	0.36
(V)	0.33	0.38	0.34	0.27	0.36	0.36
6-13-78(H)	0.44	0.44	0.35	0.52	0.43	0.81
(V)	0.91	0.13	0.14	0.13	0.13	0.52
7-25-78(H)	0.66	0.80	0.61	0.74	0.44	0.86
(V)	0.77	0.76	0.52	0.47	0.50	0.78
8-16-78(H)	1.31	1.28	1.75	1.62	0.92	1.48
(V)	1.68	2.02	1.74	1.60	1.54	1.94

Appendix Table Z. *Melampus bidentatus* counts (10 samples/pen; top count is sample nearest creek.

Date/Pen	Out	Cont	1A	1B	2A	2B	Date/Pen	Out	Cont	1A	1B	2A	2B
9-29-75		4	0	0	0	0	5-14-76	0	0	0	1	0	0
10-2-75		0	1	1	0	1		0	1	1	0	0	2
		4	7	5	6	5		7	4	2	10	2	1
		4	6	4	0	6		9	3	3	22	16	6
		0	0	4	4	3		29	13	7	26	19	43
		8	11	7	12	7		31	3	48	88	12	67
		12	10	13	17	15		15	20	77	13	14	42
		11	9	5	13	4		8	14	138	18	16	8
		6	0	1	0	1		2	32	99	93	5	16
		4	0	0	0	0		1	35	32	104	10	40
10-9-75	1	3	0	0	1	1	6-24-76	4	3	10	37	26	2
	17	0	0	0	0	10		19	9	28	0	14	25
	5	3	0	2	1	6		42	14	75	33	25	17
	15	3	6	3	5	4		11	2	28	69	100	47
	27	3	3	6	3	4		9	83	55	59	95	16
	35	7	8	6	0	2		58	47	14	44	239	152
	16	8	18	14	1	0		8	98	139	77	16	39
	6	18	0	5	0	0		6	112	119	189	13	45
	0	0	6	5	0	0		19	148	80	95	10	28
	0	0	0	0	0	0		19	99	92	183	13	16
10-29-75	1	6	0	1	0	2	7-22-76	0	0	18	40	29	0
	6	2	1	1	1	0		2	3	88	36	76	32
	0	2	0	0	0	0		27	41	184	51	24	33
	4	3	3	1	1	0		9	138	197	59	63	48
	11	1	5	0	0	1		43	143	139	153	61	74
	6	5	1	6	1	2		32	154	236	25	31	80
	9	8	8	7	0	0		12	126	286	133	62	44
	2	16	9	10	0	0		21	93	285	102	86	24
	0	22	12	14	0	0		21	267	190	73	38	36
	0	6	3	0	0	0		7	218	243	173	30	30
5-7-76	0	0	0	0	0	0	8-4-76	0	0	1	0	14	35
	0	0	2	0	7	2		0	14	7	6	0	34
	0	4	4	0	13	7		2	24	71	31	10	51
	3	49	5	0	7	60		2	61	56	44	35	117
	6	18	19	16	6	44		9	74	47	86	18	78
	18	54	7	62	0	65		4	52	70	161	37	173
	10	32	21	42	16	64		00	22	137	45	21	113
	5	63	107	75	0	50		3	49	83	210	12	14
	8	158	78	175	0	160		9	106	186	57	8	59
	4	87	7	72	0	9		3	96	34	11	4	22
8-18-76	0	0	0	19	24	27	4-26-77	1	0	0	0	0	12
	5	0	0	12	3	39		0	4	0	7	0	5
	13	16	43	22	2	58		0	0	26	11	0	15
	32	6	37	30	64	141		2	2	25	9	1	16
	5	94	86	48	95	166		4	4	12	9	6	12
	16	46	82	188	71	151		3	16	3	0	1	12
	3	37	138	138	42	116		3	18	16	36	1	13
	0	123	123	128	47	227		3	38	43	62	2	3
	12	42	164	145	23	55		2	16	34	28	1	10
	0	116	224	30	19	13		1	28	18	16	2	11

Appendix Table Z (continued).

Date/Pen	Out	Cont	1A	1B	2A	2B
8-31-76	0	0	0	3	5	11
	3	5	10	53	76	6
	13	14	41	36	64	19
	8	26	26	55	122	27
	4	27	67	36	89	41
	2	48	55	30	29	19
	3	41	68	65	63	3
	2	37	52	15	3	28
	1	34	56	27	12	18
	2	22	102	72	0	0
10-14-76	0	0	0	0	0	0
	0	1	0	0	1	0
	0	39	6	13	3	2
	3	20	4	9	4	4
	1	77	16	11	16	12
	6	9	4	0	2	1
	0	37	22	16	1	3
	2	50	9	3	2	8
	0	65	28	37	0	0
	0	30	24	28	0	2
4-11-77	0	0	0	5	0	0
	0	0	2	7	1	16
	3	11	19	9	3	23
	2	5	14	3	2	33
	0	4	8	4	1	18
	2	0	10	6	0	7
	3	0	7	3	0	7
	0	48	4	8	0	3
	0	12	68	10	0	4
	1	17	28	16	0	0
5-18-78	0	0	0	1	0	8
	1	1	0	0	0	0
	4	0	4	8	1	12
	3	6	6	9	1	16
	0	7	0	15	7	11
	2	8	4	16	11	11
	2	11	11	20	1	3
	1	8	6	21	7	4
	1	9	10	2	10	2
	0	4	9	0	0	0
6-14-78	0	0	0	0	2	1
	0	0	0	2	4	0
	0	13	9	4	7	1
	1	18	11	5	4	8
	0	3	6	4	2	10
	0	9	6	6	0	6
	1	8	2	8	0	4
	1	4	5	5	5	2
	1	5	9	8	2	0
	0	2	4	6	0	0

Date/Pen	Out	Cont	1A	1B	2A	2B
6-14-77	0	0	0	0	0	0
	2	3	10	6	10	3
	8	31	8	7	5	16
	13	19	0	38	13	14
	14	12	16	22	6	21
	7	14	7	18	9	30
	5	23	19	21	5	26
	2	47	36	22	8	41
	48	19	44	26	58	44
	17	35	57	15	6	0
7-26-77	0	9	0	0	2	6
	0	3	4	2	2	0
	0	8	12	18	5	8
	4	2	8	14	2	5
	10	6	9	38	12	27
	2	3	5	8	1	11
	1	3	7	4	0	4
	0	1	4	7	0	13
	0	7	18	12	1	3
	0	43	30	14	14	2
9-21-77	0	4	1	0	0	0
	0	1	0	2	0	1
	0	3	0	0	1	4
	3	6	3	6	5	2
	1	3	4	19	7	0
	0	4	8	13	6	8
	1	2	5	17	0	3
	3	2	15	6	0	0
	0	0	0	4	4	2
	0	1	2	3	0	6

Appendix Table Z (continued).

Date/Pen	Out	Cont	1A	1B	2A	2B
6-28-78	0	0	0	0	0	0
	4	7	8	8	5	14
	5	14	22	30	12	3
	0	20	13	12	18	20
	2	17	17	10	7	42
	4	9	12	7	10	8
	2	14	12	1	3	5
	0	15	14	9	5	3
	1	18	9	6	6	0
	1	4	3	0	2	0
8-16-78	16	0	0	0	1	0
	0	0	0	0	4	1
	3	3	14	16	8	7
	8	30	52	23	10	12
	7	0	14	42	3	2
	0	4	21	6	10	10
	0	3	45	15	2	2
	1	2	30	36	9	0
	0	1	9	7	2	0
	0	2	22	10	0	0

CHAPTER II

Chemical Fate

by

R. H. Bieri, M. Kent Cueman & V. C. Stamoudis

INTRODUCTION

Natural oil spills are unpredictable in both time and space and, therefore, are not well suited for research on the fate and effects of hydrocarbons. Conversely, laboratory experiments often are too abstract in their design and omit many factors possibly influencing a natural spill. To be useful, an experiment must retain much of the natural complexity, but simultaneously allow a measure of experimental control. The design must permit the oil to interact with natural populations of microbes, plankton, plants, and animals. Animals transplanted for exposure should not be subjected to additonal stress. Tidal fluctuations should cause dispersal, deposition, and redistribution of the oil within the experimental area.

The complexity of oil spills also must be taken into account in the chemical analysis. Thus, the oil must be characterized down to the lowest environmental levels encountered in as much detail as possible. For the toxicologically important aromatic fraction, this requires the use of computerized GC/MS systems.[1,2,3,4,5] Likewise, as a consequence of the complex composition of petroleum, it is logical to use the highest possible resolving power for the gas chromatographic separation.

We report findings of a large-scale experiment which seeks to fulfill these conditions. Measured amounts of both fresh and artificially weathered South Louisiana crude oils were spilled on the creek surface of a tidal marsh enclosed by transite structures.[6] Large openings below the lowest tide level allowed free exchange of water but prevented the oil film from escaping. This research is an extension of an earlier experiment, performed on a less ambitious scale using No. 2 fuel oil, by Bieri and Stamoudis[7] (1976).

EXPERIMENT

Details of the experimental design and size of the oil spills studied here can be found in a paper by Bender, et al.[6]

Artificial weathering of the oil.

Fresh South Louisiana crude oil (SLAC) was received in 190-L barrels from Exxon Company. Eight barrels (1,520-L) of this oil were artificially weathered by dumping 380 L at a time into a 4,000-L fishtank (resin-coated fiberglass) filled with water from the York River. The tanks were agitated by a jet of water directed at the floating oil from a submerged pump. After two to three days of exposure, the water was drained for disposal, the tank was refilled with fresh river water, and the process repeated. Air temperature during the weathering was between 30° and 35°C, but due to absorption of radiation, the temperature of the water at the end of the daily insolation was up to 5°C above the air temperature. All eight barrels were blended before the spill.

Sample collection and pretreatment.

In all samples collected, the prevention of cross-contamination by the thick layer of oil originally present at the surface was of major concern. The problem was most severe for water samples, but was solved quite successfully by the design of a sampler employing Teflon®-stoppered one-gallon jugs (regular solvent bottles) that could be opened under water for filling and closed again before retrieving the sample through the oil layer. Water was extracted without prior filtering. All samples were collected on receding tide, with the water running off the marsh.

Fundulus heteroclitus, kept in wire cages, were collected by hand after lifting the cage close to the surface. Care was taken to prevent contact

of the fish remaining for exposure with the oil layer. As an additional safety measure all peripheral tissues were cleaned carefully by rinsing with tap water, methanol, and hexane before homogenization. Special attention was given to mouthparts and gills. An aliquot of a homogenate of about seven to eight fish (average weight, 3 g ea.) per sampling was used for analysis.

Unconsolidated sediments were collected with a sampler of special design. It consisted of an aluminum disc (25 centimeters diameter) attached to a long handle by tripod legs. A glass and Teflon^{R} tube ran up the handle. The lower end of the tube was suspended about 5 cm above the center of the aluminum disc. The upper end of the glass tube was connected to a 25-L jug. After the aluminum disc was placed on the bottom and the flask evacuated with a hand pump, the jug filled with detritus-ladden water. When the color of the water indicated a low suspended-particulate content, the disc was moved to another position and pumping continued. While one certainly may design more sophisticated samplers, we believe that it essentially delivered the samples we sought. About 20-L of water with suspended-particulate matter was collected in each sampling.

Oysters were picked from their exposure trays by hand. The trays, normally resting on the sediment, were lifted close to the surface for the collection of samples. They were, however, carefully kept away from the surface slick to prevent contamination of the oysters left in the tray for continued exposure. Contact with the surface layer for collected oysters could not always be prevented, but was judged to be minimal since during the upward movement of the hand, the slick usually broke and was pushed away by gravity flow. Additional precautions to prevent contamina-

tion were taken in the sample preparation step. After collection, the oysters were stored in plastic bags and kept on ice.

Extraction and Separation Methods

The detailed extraction and separation of hydrocarbons into aliphatic and aromatic fractions is given in Appendix 1.

Analysis

The gas chromatograph (GC) used for quantitative analysis was a Varian Model 2740. Both, packed (stainless steel, 2-m long, 3.2 mm OD, chromosorb G-AW-MDCS, coated with 1.5% OV-17) and wall-coated, open tubular, glass capillary columns (20-m long, .32 mm ID, deactivated with Carbowax 20-M, and coated with SE-52) were used (Grob & Grob[8], 1976). For the glass capillary column operation, the instrument was equipped with a Grob Injector System operated in the splitless mode. The temperature programming rate was 50 to 250°C at 12°C/min for the packed columns, and 50-250°C at 8°C/min for the capillary columns. The GC/MS system used was a Du Pont Model 492 B with 094B data systems and a Varian Model 2700 GC. Sample admission to the source was via a jet separator for packed columns. For capillaries, the GC effluent was directly fed into the MS source through a platinum capillary interface. The coupling of the GC to the interface capillary occurred at atmospheric pressure in a helium flushed volume. The GC columns, their conditioning, and the injection system were the same as specified for the GC analysis.

The use of gas chromatography and GC/MS to derive quantitative information on specific compounds in complex mixtures, from a purely analytical point of view, is tied to three requirements that ideally must be fulfilled: 1) the

compound must be identifiable and measurable in the chromatograms, 2) the relative concentration of a particular compound in a chromatographic peak must be close to 100%, and 3) the unresolved background of the chromatogram must be small compared to the resolved peaks because it is impossible to measure the penetration of the peak into the background response ("unresolved background" cannot be assumed to be a smooth, featureless hump).

In crude oil related samples, a chromatographic peak usually contains several different compounds with concentrations of similar order of magnitude. This was particularly true for data derived from packed-column GC analysis. The use of wall coated glass capillary columns improved this problem. But is has to be noted that probably no resolution will ever be high enough to cope successfully with the tremendous complexity of crude oils. These problems are compounded in the heavy molecular weight region, especially for aromatic hydrocarbons. While it is possible to identify the different components present in a chromatographic peak by mass spectrometry and to quantify such a peak, a comparison of mass chromatograms with the reconstructed gas chromatograms often reveals that major compounds coincide with valleys. Thus, actual conditions are less than ideal.

In addition to the difficulties related to the complex composition of oils, other factors also affect accuracy in a basic way. They include cross contamination, local inhomogeneities of oil within the spill area, temporal variations in the concentration of oil due to physical and chemical processes, biological parameters affecting uptake, depuration and degradation, and variability due to sample nature and extraction yields. Although we have taken every possible precaution to prevent cross-contamination, we cannot exclude its occasional occurrence.

Anyone who has seen an oil spill will realize that homogeneity certainly is not a characteristic property. Temporal changes in the concentration of hydrocarbons may vary over vastly different time intervals. Eddies in the water column can change the concentration within seconds and by their nature are irregular. Tidal cycles will introduce periodic fluctuations. Evaporation, dissolution, dispersion, and chemical reactions, finally, must cause changes that are aperiodic and occur over intervals of days to weeks. In the results the net effect of these changes are superimposed.

While we know little about the biological parameters as they affect tissue concentrations, there is at least one consolation: because the residence times of hydrocarbons in biota are on the order of hours to days,[9,10,11,12] short-term fluctuations will be integrated and results should be more consistent. When analyzing for hydrocarbons only, degradation cannot be observed directly, but is indicated by the disappearance of certain compound classes.

Variability resulting from the extraction of samples is difficult to pinpoint. There appears to be no relation to extraction yield in the 50% to 100% range. For a thorough discussion of the use of internal standards, refer to Bieri and Stamoudis[7] (1976). Because of limitations in sample size and time, we could not get enough re-extractions for statistical evaluation. However, reprocessed samples indicate that aliphatic concentrations are accurate within 15% and aromatic concentrations at most 50%. These error margins apply to concentrations from different samples. Individual concentrations within a sample have a precision of 15% for aromatics. The later discrepancy is related to the presence of numerous fused or incompletely separated peaks in the aromatic fraction.

RESULTS

Comparison of Weathered and Fresh Oil

The aromatic fractions of both weathered SLAC oil and fresh SLAC oil were found to be identical, except for concentration differences related to loss of volatile compounds. This was confirmed by comparing the retention times and the mass spectra of 225 peaks (molecular weight region between naphthalene and pyrene) in high resolution chromatograms of the aromatic fractions of both oils.

Fundulus and Water Samples

Quantifiable organic compounds identified in the aromatic fractions from Fundulus tissue at various times after both spills are presented in Table 1. The concentrations of the aromatics found in water samples are given in Table 2.

Compounds more volatile than naphthalene have been omitted because they evaporate too quickly. It is also difficult to control the loss of th se volatile components during the concentration of pre-column extracts and column chromatography eluates. For the less volatile substituted benzenes, indans and tetrahydronaphthalenes, the presence of large numbers of isomers leads to excessive superimposition that prevents the assessment of discrete compounds. This is also true for the heavy molecular weight region whose mass spectra indicate the presence of many different compoun s and isomers at roughly equal concentration in all chromatogram peaks.

In comparing the quantified aromatic hydrocarbons in Tables 1 and 2 with those in crude oil, it is observed that the grouping of the peaks in the chromatograms in some cases is different. These differences are due to the

fusing of peaks, caused by changes in the concentration of neighboring compounds and it is shown graphically in Fig. 1 and 2.

As the unresolved envelope (UCM) rises with time after the spill, individual peaks begin to shift and new components begin to appear as it is apparent from the mass spectra. This creates difficulties not only in recognizing peak groups quantified in previous samples, but also in making comparisons. In other cases, peaks originally present may disappear altogether and new peaks may appear instead. For example, 2-methylnaphthalene, which is clearly present in Fundulus samples 155, 156, and 158, is replaced 216 hours after the spill (sample 161) by a compound that according to the mass spectrum is a C_3-tetralin.

The results for the aromatic fraction extracted from Fundulus (Table 1) exposed to fresh SLAC show the presence of the full spectrum of aromatic petroleum hydrocarbons only six hours after the spill. This is followed by an increase in the concentrations of all aromatics in the +31 hour sample, where the C_2- and C_3- naphthalenes are at a maximum. All other compounds reach their maximum near +76 hours. With the exception of naphthalene and 2-methylnaphthalene, all compounds still are present 216 hours after the spill.

Similar trends are observed in the aliphatic fraction, (Table 3) but all n-alkanes reach their maximum concentration 76 hours after the spill.

No aromatics could be found in the six hour water samples (Table 2). The corresponding aliphatic fractions (Table 4) are composed of a homologous series of even-numbered n-1-alkenes extending from n-1-hexadecene to n-1-triacontene, which also are present in all control

samples. These olefins have been positively identified, and are not artifacts either. The absence of aromatic hydrocarbons in the six hour fresh SLAC water sample (or their presence at an undetectably low level), compared with the presence of both aliphatics and aromatics in the six hour Fundulus sample either may be related to basic difficulties in the collection of representative water samples or may indicate that the fish acquired these hydrocarbons by ingested material.

Contrary to the observations in Fundulus, all water-accommodated aromatic hydrocarbons from fresh SLAC (Table 2), except those eluting past fluorene, C_3-biphenyls and C_4-naphthalenes, reach a maximum 31 hours after the spill. Naphthalene, the methylnaphthalenes and maybe also the dimethylnaphthalenes are disappearing very rapidly between the 31- and 76-hour samples, probably as a result of their volatility and relatively high solubility, leading to rapid depletion in the oil pool. This fact is supported by analyses of surface layer samples. In general, the concentration of individual hydrocarbons in the water is below their solubility[9,13] and much lower than in Fundulus. If one calculates ratios from the concentrations found for individual hydrocarbons in Fundulus and water, one finds values of about 1,000 or 2,700 respectively at +31 and +76 hours for aromatics, and of 290 or 220 respectively for aliphatics. These ratios in part reflect the biomagnifications of hydrocarbons[14,15] but the discrepancy between aromatic and aliphatic compounds probably cannot be explained by this mechanism alone.

The results of Fundulus and water samples for the weathered SLAC oil spill are given also in Tables 1 and 2. All but a few Fundulus in this spill area were found to be dead a few hours after collection of the 120 hour sample.

Beginning with the aromatic fraction, it is noted that petroleum-related aromatics six hours after the spill not only are present in Fundulus (Table 1), but already have reached their maximum concentration in water (Table 2). Individual amounts at +6 hours compare well with those at 31-76 hours in the fish exposed to fresh crude. This may coincide with some general observations that have been made: two days after the spill of weathered SLAC (when the +45 hour samples were collected), it was noticed that much of the water surface was free of oil except for some thick, isolated patches. In the patches the oil had a "bubbly" appearance (emulsified?), possibly caused by heavy rain. The rapid disappearance of the weathered SLAC was very much in contrast to the trends noted in the fresh SLAC spill-area, where this crude oil covered much of the water surface of the sampling area for more than 20 days. Obviously, while the fresh crude kept sloshing back and forth with the tides, the weathered crude for some reason (viscosity, marsh topography?) was deposited over a large surface of the marsh. This could have accelerated the integrated accomodation fluxes of hydrocarbons into the water, from which they were available to the fish. Indeed, the concentrations of aromatic hydrocarbons found in water from the weathered SLAC spill area also show a maximum at +6 hours (this maximum occurred at +76 hours for fresh crude). Extracts of samples collected at

+45 hours and +120 hours in both fish and water contained lower amounts of aromatic hydrocarbons.

n-Alkane concentrations in water (Table 4) have trends similar to the aromatics. Maximum concentrations are found in samples collected six hours after the spill. At +45 and +120 hours respectively, the concentrations are approximately equal and lower than in the six hour samples. In Fundulus, however, the maximum concentrations occur at +120 hours and the minimum at +6 hours. These trends have been confirmed by reextracts. At their respective maxima, the n-alkane concentrations in Fundulus exposed to weathered SLAC agree well with those in fresh SLAC for those alkanes that were not already depleted by the weathering process. Concentration ratios (Fundulus/water) for aromatics are close to 1,000 for all samples. Ratios for normal alkanes vary from 70 at +6 hours to 230 at +45 hours and 790 at +120 hours. Compared to the results from fresh SLAC, the high ratio at 120 hours seems unusual. However, this value results from the coincidence of the maximum concentration in fish with the minimum in water.

Oyster and Unconsolidated Sediment Samples

Tables 5 and 6 present the results (derived by glass capillary-column chromatography) of aromatic hydrocarbons identified in the oyster samples from the fresh SLAC oil spill and the weathered SLAC oil spill, respectively. The data in these tables show that in oyster, maximum concentrations for naphthalene and methylnaphthalene are reached between +6 and +31 hours after the fresh SLAC spill, and between +6 and +45 hours after the weathered SLAC spill. However, for the higher alkylated naphthalenes, the maxima are reached later. In addition, we point out that the maximum concentrations of C_2 and C_3-naphthalenes are about an order of magnitude larger in the weathered SLAC spill than in the fresh SLAC spill samples (500 ppb vs. 60-70 ppb).

The tissue concentrations of individual compounds clearly drop with increasing exposure time, but most are still detectable past 10,000 hours. However, the tables are misleading insofar as they only contain select compounds that were identifiable in relatively unaltered oil (in samples collected shortly after the spills). The estimates for the unresolvable complex matrix (UCM) indicate that the total aromatic concentrations first rise with time and then remain at an essentially constant level. This trend is also evident in gravimetric determinations.

Finally, we note a considerable background in the pre-spill samples of oysters exposed to artificially weathered oil. We believe this not to be an artifact of sample contamination, but a cross-contamination between the neighboring spill areas (for details of the spill areas and the spill experiments, see ref. 6). Such contamination has also been observed in samples collected in the control-pen. Since communication between spill areas at subtidal levels via Cub Creek was possible, and since the weathered SLAC was spilled 3 days after the fresh oil, this is not really surprising. However, identification of compounds in the pre-spill samples has been made on the basis of absolute retention alone (the concentrations were too low to produce reliable mass spectra) and for this reason some of the assignments may be incorrect.

Tables 7 and 8 present the results (derived from glass capillary-column chromatography) of aromatic hydrocarbons identified in the unconsolidated sediment samples from the fresh SLAC oil spill and the weathered SLAC spill respectively. The results show that unconsolidated sediments contain hydrocarbons at much higher levels than the oysters. As in Tables 5 and 6, compounds are abbreviated by summing individual isomers of discrete hydrocarbons. Also

listed in Tables 7 and 8 is the sum of all chlorinated hydrocarbons (semi-quantitative determination) that have been detected. They consist mainly of di-, tri-, tetra-, and penta-chlorobiphenyls, but some unidentifiable structures are also included. (A few chlorinated hydrocarbons were also encountered in some oyster extracts, but their concentration was not sufficient to warrant inclusion of this information in Tables 5 and 6).

In the fresh oil spill area, the highest concentration (5 ppm) is found for naphthalene at +6 hr after the spill, followed by the methyl-naphthalenes and C_2-naphthalenes. Methylphenanthrenes also build up to ppm-levels, but at a later time the methylphenanthrenes are most prominent, followed by phenanthrene itself. It is evident that the data contain a considerable amount of scatter. These samples are probably quite inhomogenous, not only due to the method of collection, but also because of possible variations in the ratio of organic detritus to minerals (this ratio is dependent on sampling location and on runoff conditions). This may account for the most serious discrepancies.

Amounts of sample available for analysis also were quite small: between 1-2 g (dry weight) of solids were collected on fiberglass filters from approximately 20 liters of slurry. While the UCM in oysters typically is between 2 and 5 ppm, it is between approximately 40 to 700 ppm in the unconsolidated sediment. As in oysters, some cross-contamination is indicated. It should be remembered that the concentrations in oysters are based on wet weight; reduction to dry weight would increase the numbers by approximately a factor of 10).

DISCUSSION

If the reason for the Fundulus mortality observed shortly after 120 hours of exposure (in the weathered SLAC spill only) is in the results presented in Tables 1 and 2, it certainly is not obvious. Since the maximum concentrations in Fundulus and in water for both oils are roughly the same, concentration per se is an unlikely explanation. A fundamental difference, however, is seen in the exposure as a function of time. While the accommodation of aromatic hydrocarbons in water and the uptake in fish show a relatively slow start, with concentrations in the fresh crude spill reaching their peak between +31 and +76 hours, accommodation and uptake were very rapid in the case of the weathered crude. This may be significant in that the Fundulus had little time to adjust. Although most fish appear to be able to metabolize aromatic hydrocarbons by transforming them to compounds that allow their transfer to the bile from where they are excreted, they may need some time to reach enzyme levels sufficient to cope with the situation.

Alternatively, since our analyses are limited essentially to hydrocarbons, there remains the possibility that the mortality of fish was caused by some derivatized product formed while the oil was deposited in the marsh and exposed to solar radiation and microbial degradation. Dissolved oxygen at insufficient levels to sustain breathing also could have caused the mortality. In this case the oil would have been involved only in an indirect way. Unless an effort is made to also include more polar heterocompounds (hydrocarbons containing sulfur, nitrogen, or oxygen) in the extraction and analysis, such basic ambiguities in the interpretation will remain to be a problem. Note that analysis of polar fractions was not part of this study.

The results from the exposure of fish to petroleum hydrocarbons in a natural environment can be interpreted to reflect both uptake and depuration.

While the general trends agree with those from laboratory studies,[9,11] there are differences that are characteristic of a natural spill situation. Similar to observations made in oysters exposed to a No. 2 fuel oil,[7] it appears that hydrocarbon concentrations in fish mainly reflect the composition of the exposure-mixture that exchanges with tissue (e.g., water accomodated hydrocarbons that exchange directly from water into tissue, or sorbed hydrocarbons on ingested material that exchanges with tissue of the digestive tract). Thus, the apparent time dependence of tissue concentrations[7] essentially is determined by the residence time of a particular compound available to the animal in the environment, not by the biological residence time that is measured in depuration experiments. The environmental residence time[7] of a hydrocarbon is determined by evaporation, dissolution, biodegradation, photochemical oxidation, adsorption, etc., while the biological residence time is related to the exchange across tissue and the rate of metabolism. The assessment of residual concentration in tissue then depends on the compound or compounds that are used. For example, the tables show that naphthalene in fish exposed to fresh SLAC goes through a maximum six to 31 h after the spill and then disappears in less than 200 h. In the water column, naphthalene cannot be determined past +76 h. However, an analysis of the total aromatic or aliphatic fraction shows a maximum concentration at +552 h, followed by a slow decrease that probably is attributable to metabolism since the same fractions in oysters remain at a constant level.

Compared to the hydrocarbon concentrations in Fundulus heteroclitus, the concentrations in oysters are low in both spill areas. For fresh SLAC the maximum concentrations of most compounds occur at +76 h, as was observed in Fundulus. Exceptions are naphthalene, the methylnaphthalenes, and the

C_2-naphthalenes, which reached their maximum concentrations at +6 h and +31 h, respectively. All compounds remained at a very low essentially constant level between 200 and 10,000 h.

The observed constancy of individual compounds over time intervals of 10,000 h and compositional details would suggest that this results from the continued availability of these compounds (large environmental residence times) to the oysters, although their concentration in water is below detectability. It also reflects on the limited capacity of the environment to cleanse itself of this hydrocarbon fraction via natural processes such as biodegradation, photolysis, and chemical reaction, in the absence of effective dispersion.

In the weathered oil spill area (where concentrations in Fundulus were found to reach maximum levels +6 h after the spill), the oysters show maximum tissue concentrations at +45 h for most compounds except naphthalene and methylnaphthalenes.

Acquired concentration levels are higher than those observed for the fresh oil. Past the maximum, tissue concentrations appear to decrease more slowly as compared to the fresh oil spill area, but after +318 h, again not much change is observed. In general, the tissue concentrations in the exposed oysters appear to parallel the trends of those in Fundulus. Considering that these animals may acquire the hydrocarbons from different sources, the replication of these trends is as close as one could expect.

In the design of the spill experiment, the collection and analysis of unconsolidated sediment (which preferably should have been organic detritus) was added because we hoped to be able to correlate the hydrocarbons in this material with those in oysters. A previous experiment[7] using No. 2 fuel oil

suggested that most of the uptake in oysters occurred via ingestion. Positive evidence for hydrocarbon uptake in oysters from ingested material could be established if characteristic compositional hydrocarbon changes in ad- or absorbed fractions would also be reflected in the oyster extracts.

As the concentrations listed in Tables 5 and 6 show, clear-cut systematic compositional changes with time are not outstanding features of the unconsolidated sediment samples that were analyzed. An exception are some of the more volatile naphthalenes in unconsolidated sediment from the fresh oil spill area. Analytical problems related to the presence of a very large UCM (Σ/UCM<0.06), inadequate resolution of UCM-superimposed peaks, and sample inhomogeneity are some of the reasons mentioned before. In addition, the amount of oil spilled (570 liter/enclosure), was so overwhelming that the total amount that could have been biodegraded -- even after long exposure times -- remained elusive (lack of evidence for biodegradation was noted in chromatograms of hexane fractions, where branched and isoprenoid alkanes remained essentially constant relative to n-alkanes).

While we cannot detect any distinct influence of unconsolidated sediment particles (mineral and organic detritus) in the hydrocarbon composition of oyster tissue, there are indications to the contrary. For example, many chlorinated biphenyls and other chlorinated compounds (only the sum of individual concentrations is listed in Tables 5 and 6) at concentration levels higher than, or of the same order of magnitude as those of petroleum hydrocarbons, simply could not have been overlooked during peak-by-peak identification of GC/MS output from unconsolidated sediment samples. But in oysters, only a pentachlorobiphenyl isomer was found in several samples, plus a few chlorinated hydrocarbons that had an unidentifiable mass-spectrum.

Although we do not want to imply that the unconsolidated sediment samples contained chlorinated biphenyls and other chlorinated hydrocarbons that were absent in oyster tissue, the fact that we did not encouter them is difficult to understand. As can be inferred from Neely[14] et al., partition ratios for chlorinated hydrocarbons in lipids are substantially higher than those of their parent hydrocarbons. Unless the chlorinated biphenyls observed in unconsolidated sediments were sorbed to particles discriminated against by the oysters, their concentrations should for this reason stand out even more in tissue.

Aside from these observations, the hydrocarbons in unconsolidated sediments must be interpreted as being essentially constant over a time span of nearly 10,000 h (although systematic changes with time could be hidden behind the large scatter in these data). This is in disagreement with the observation of maxima in oyster tissue. Thus, it must be concluded that neither the composition nor the concentration versus time response in oyster tissue seem to be strongly related to the hydrocarbons in unconsolidated sediment that was sampled, and the question of the principal origin of the hydrocarbons acquired by the oyster cannot be answered.

In any discussion of the fate of spilled oil, one cannot help but overemphasize those parts of the analysis that have received the most attention -- in our case, the concentration of individual compounds or the sum of isomers. The results presented in Tables 7 and 8 and Fig. 3, however, give a clear indication of the importance of the UCM. We did not attempt to characterize chemically the UCM of the oyster and unconsolidated sediment samples. However, analysis of the UCM of aromatic fractions from surface marsh soils six to eighteen months after the spills (see Appendix 2) revealed the presence

of classes of compounds identical to those found in aromatic fractions from SLAC oil with the major contributors (75%) being higher alkylated benzenes, tetralins, and dihydronaphthalenes (all monoaromatics). Note that pH-fractionations (to extract possible acidic or basic components) or derivatization experiments proved not successful to identify such polar compounds in the aromatic fractions of these surface marsh soils. While the hydrocarbon composition of surface marsh soils (thick layers of oily material from the back of the marsh) cannot be directly related to the unconsolidated sediment or oyster tissue, it was hoped that they at least would give us some clues about the nature of enviornmentally resistant hydrocarbons. Since a portion of the UCM in the aromatic fractions of unconsolidated sediment samples is due to chlorinated hydrocarbons which were not detected before the spill, a hypothesis on how natural hydrocarbons and pollutants may be concentrated by the spillled oil is formulated in the concluding section.

Summary and Conclusions

The concentrations of individual aromatic hydrocarbons in water, Fundulus heteroclitus, Crassostrea virginica and unconsolidated sediment, resulting from surface spills of fresh and artificially weathered South Louisiana crude oil in an itertidal marsh area have been investigated. Sediments samples showed evidence of direct surface-slick deposition and were not pursued further. Hydrocarbon concentrations in clams were very low and all animals, including the controls, died after a few weeks of exposure. It is suspected that the exposure of clams in trays is not feasible and may prevent these animals from functioning properly. An ealier experiment[7] with clams exposed by this method was also unsuccessful (the results were the same). Analyses of surface-slick samples were so inhomogenous that they could not be interpreted in a meaningful way. A few samples of marsh soil were analyzed and the results are presented in Appendix 2.

Based on knowledge extrapolated from laboratory assay studies, some of the results are difficult to comprehend. If results of laboratory assay studies are often too abstract and narrowly defined to be useful for the prediction of the fate and the effects of oil spills in the natural environment, semi-natural spill experiments may suffer from too many influences that remain undefined and leave considerable room for ambiguities in the interpretation of results. That environmental fate is difficult to predict is demonstrated in two sets of semi-natural experimental oil spills in different environments, one employing a small amount of #2 fuel oil (discussed in an earlier report), the other large amounts of SLAC oil as discussed in this report. Although some of the parameters, excerting a rather powerful influence on the experiment in principle, could have been assessed -- for example, the mixing and the homogeneity of the water within the sampling area with water from the creek could have been determined by the use of dyes -- it would have demanded a considerably larger effort than

was feasible. Other parameters, such as the effect of oil viscosity, marsh topography, and rain on the distribution of oil between marsh and biota exposure area, would be almost impossible to measure.

Despite such inherent flaws in the smi-natural spill experiment, it is believed that much has been learned. Among the more important findings are:

a) Despite the large quantities of oil spilled, the water-accommodated oil components remained extremely small and considerably below solubility. Since high-frequency turbulence in an intertidal marsh is very low, solubility is the main determinant of hydrocarbon concentration in the water column, with emulsification playing a minor role. An exception may be indicated by the apparently more rapid accommodation rates of artificially weathered SLAC in water (maximum concentrations found 6 h after the spill, compared to approximately 31-76 h for fresh oil) which may have been caused by the formation of emulsions during a heavy rain, before the 6+h sample collection. Hydrocarbons in water were further diluted by mixing with relatively uncontaminated water from Cup Creek, with which it could freely exchange through openings at a subtidal level.

b) In a natural system, petroleum hydrocarbons may be available to an animal in many different forms: dissolved, emulsified, absorbed on live or dead organic tissue, or on minerals, etc. Thus, uptake can be expected to be complex and unpredictable. Although, Fundulus heteroclitus and oysters were exposed under similar conditions, the hydrocarbon concentration of individual compounds were substantially higher in fish than in oysters. For aromatic

compounds in artificially weathered oil, there was a difference in the time interval to reach maximum tissue concentrations: 6 h for Fundulus and 45 h for oysters. For fresh SLAC, most aromatics reached a maximum concentrations after close to 76 h of exposure (for more volatile compounds, the maximum occurs at 31 h).

c) It is difficult to relate the composition of the spilled oil to the composition of hydrocarbons to which the animals were exposed. The amount of oil spilled was so large that biodegradation, photochemical reactions, possible mineral catalyzed reactions, etc., could have affected only a very small fraction of the total mass. Thus, such chemical changes were not revealed in bulk analyses. The results in Tables 5, 6 and especially 7 and 8 indicate additional processes which may attribute to such changes. In unconsolidated sediment from the fresh spill area (Table 7) no UCM is present in aromatic fractions from prespill and 6 h samples, but all later stations contain UCM's that, relative to the sum of individual compounds clearly attributed to the spilled oil, dominate the chromatograms (Fig.3). Although our sampling does not allow us to draw a mass balance, the fact that the UCM so suddenly dominates the composition of the extract makes an origin from degraded oil unlikely. Chlorinated hydrocarbons which appear simultaneously with the UCM point in the same direction and are perhaps even more indicative: they certainly were not present in the crude oil and are unlikely to be derived from a degradation of the oil. It appears, thus, more likely that at least some of the UCM and most of the chlorinated hydrocarbons have their origin in the marsh, but were

brought to the surface by the spilled oil by a process approximating solvent extraction. While it is not difficult to explain the presence of chlorinated hydrocarbons in the marsh (previous pollution), the presence and the origin of compounds in the UCM is more difficult to pinpoint. Most common biogenic hydrocarbons are aliphatic in nature and do not elute in the aromatic fraction, but some olefins and most of the polyolefins do. Since a marsh typically is a strongly reducing environment, a preponderance of polyolefins may be possible, but we were unable to prove it. (A discussion about the composition of the UCM in the aromatic fractions from surface marsh soil samples is given in Appendix 2). Analysis of unconsolidated sediments from an artificially weathered spill area (Table 8) show similar results, but a strong UCM and high concentrations of chlorinated hydrocarbons already are present in the 6 h sample. We cannot explain this difference.

A UCM also suddenly appears in oysters exposed to fresh SLAC (Table 5), beginning with the 76 h sample; in oysters exposed to artificially weathered SLAC, it is present already in the pre-spill sample and persists throughout the experiment. Although a few chlorinated hydrocarbons were also found in those oyster extracts, they were not as abundant in concentration as well as variety as those in the unconsolidated sediment samples. Since oysters would be expected to bioconcentrate chlorinated hydrocarbons more strongly than pure hydrocarbons, we are at a loss to explain this observation. If toxic effects are observed under these circumstances, there are two basic questions:

1) What are the components of the exposure mixture which contribute to most of the toxicity and how does the animal acquire them?
2) Are the classes of compounds that were analyzed (depending on the "state of the art") relevant with respect to observed toxicities?

To answer question 1) would require detailed knowledge about major uptake mechanisms. It is believed that, while we have at the present an acceptable general understanding of such mechanisms, the details remain obscured. With respect to 2), unless one or several of the analyzed compounds is recognized as being toxic at encountered concentrations, the question of relevancy remains elusive as long as it cannot be ascertained what the non-analyzable part (UCM or polars not studied here) contains. In addition, there are the problems of synergism and of secondary causes.

d) Hydrocarbon concentrations in animal tissues mainly reflect the environmental residence time of the spilled oil. Compared to environmental residence times, biological residence times are short, causing a phase-shift only in the tissue concentrations versus time trends.

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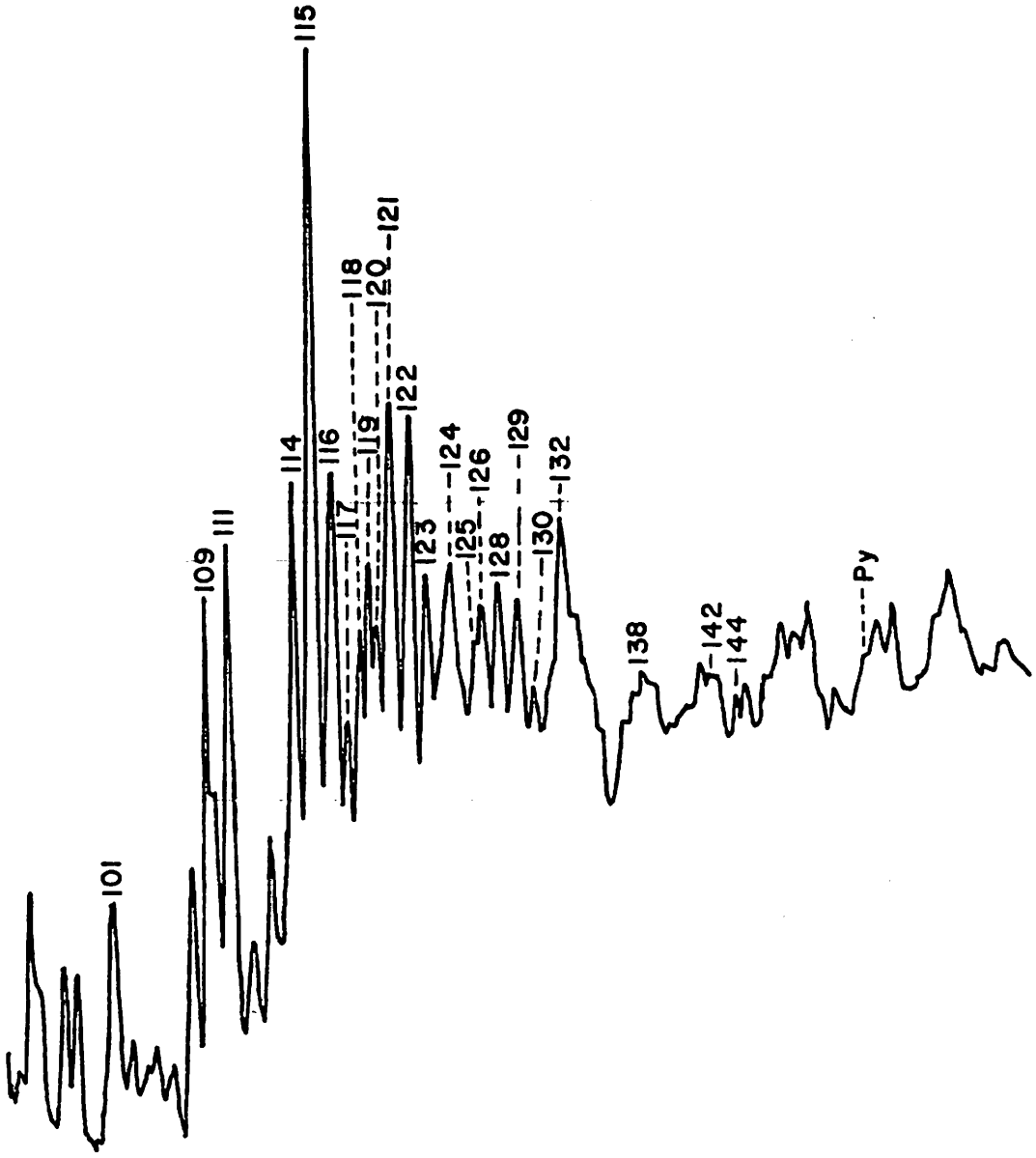
Figure Captions

Fig. 1: Chromatogram of an aromatic fraction extracted from Fundulus 6 hours after it was exposed to fresh South Louisiana crude oil. Note the differences in the elution pattern relative to fresh SLAC in Fig. 2, which are due mainly to concentration changes. Numbers in chromatograms refer to the following compounds: 101 - Naphthalene; 109 - 2-Methylnaphthalene; 111 - Methylnaphthalene; 114 - Biphenyl+2,6-Dimethylnaphthalene*, 115 - 1,3-Dimethylnaphthalene*; 116 - 1,5-Dimethylnaphthalene*; 117 - 2,3-Dimethylnaphthalene*; 119 - 3-Methylbiphenyl+C_3-Naphthalene; 120 - 4-Methylbiphenyl+C_3-Naphthalene; 121 - C_3-Naphthalene; 122 - Methylbiphenyl+C_3-Naphthalene; 123 - 2,3,5-Trimethylnaphthalene; 124 - C_3-Naphthalene+C_2-Biphenyl; 125-127 - Fluorene+C_4-Naphthalenes; 128 - C_4-Naphthalene+C_2-Biphenyl+C_5-Naphthalene; 129 - C_4-Naphthalene+C_2-Biphenyl+C_3-Biphenyl; 132 - Methylfluorene+C_4-Naphthalene+C_3-Biphenyl; 137 - Dibenzothiophene+C_4-Biphenyl+C_5-Naphthalene; 138 - Phenanthrene; 142 - 3-Methylphenanthrene; 144 - 1-Methylphenanthrene

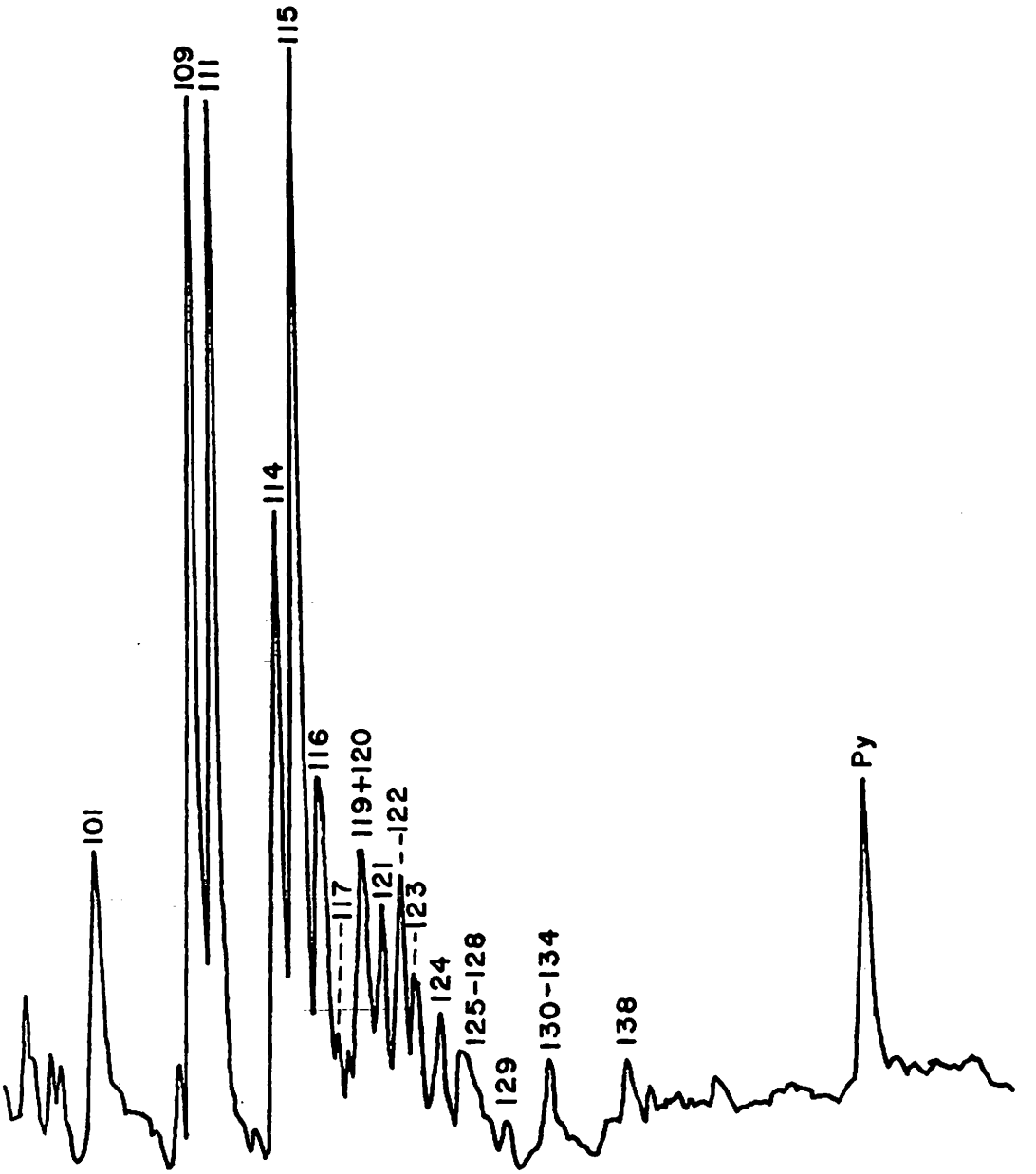
Fig. 2: Chromatogram of an aromatic fraction of fresh South Louisiana crude oil. For the meaning of numbers, refer to text of Figure 1.

Fig. 3: Chromatograms of two aromatic fractions from unconsolidated sediment extracts collect in the fresh South Louisiana crude oil spill area. The chromatograms were developed from 20 m long, 0.32 mm i.d. wall coated (SE52) glass capillary columns. A: sample collected 6 hours after spill, B: sample collected 1370 hours after spill, 1: Naphthalene; 2: 2-Methylnaphthalene; 3: 1-Methylnaphthalene; 4: Biphenyl; 5: C_2-Naphthalenes; 6: Fluorene; 7: Phenanthrene; 8: Fluoranthene. The interval between a and b in the lower chromatogram was recorded with higher attenuation (x2).



CHEMICAL INVESTIGATIONS OF OIL SPILLS

FIGURE 1



CHEMICAL INVESTIGATIONS OF OIL SPILLS

FIGURE 2

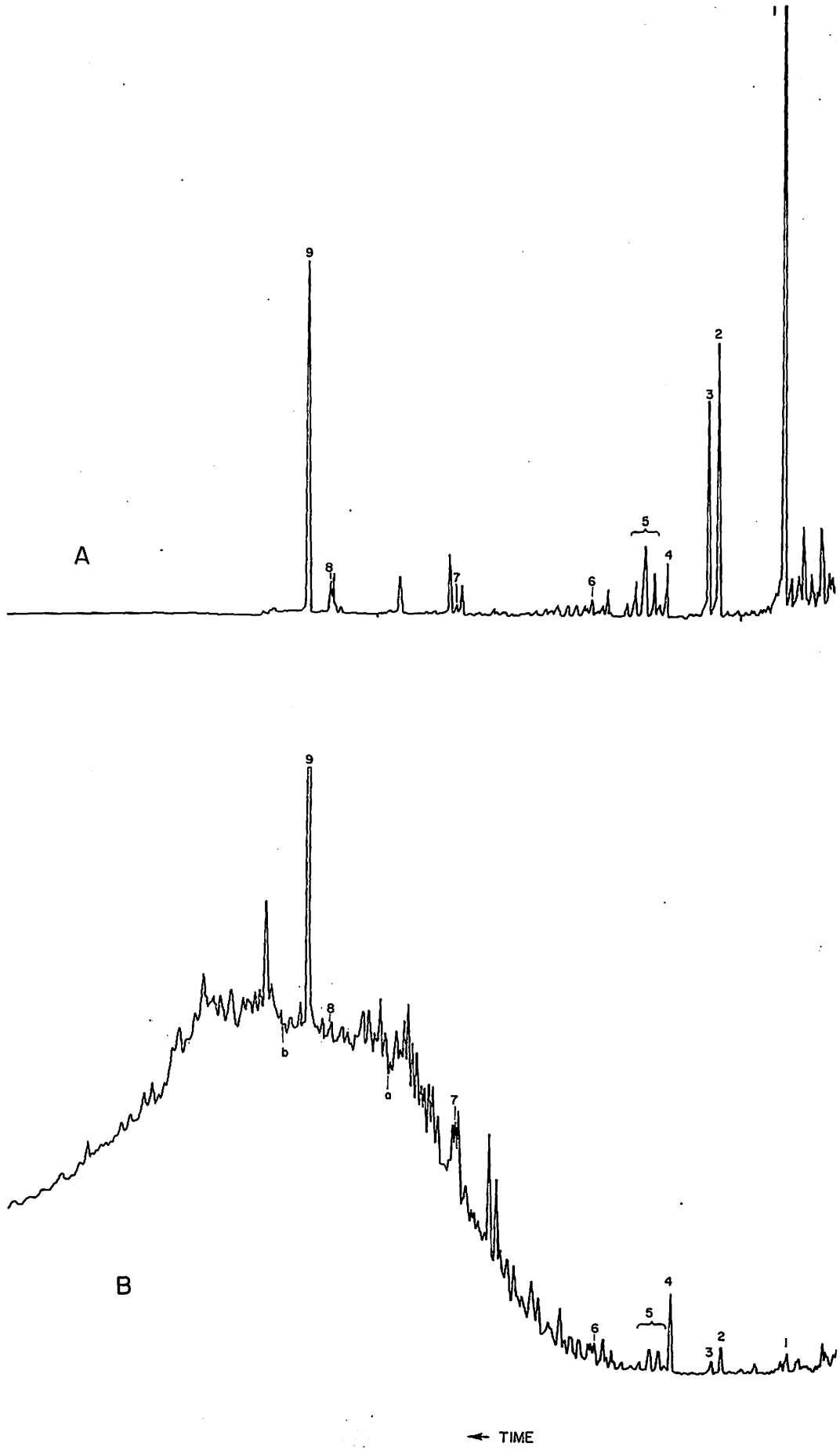


Figure 3

Table 1. Time Dependence of Aromatic Hydrocarbon Concentrations (ppb) in Fundulus after Experimental Spills of South Louisiana Crude Oil

	Fresh Crude Spill				Weathered Crude Spill		
Time after Spill (hrs)	+6h	+31h	+76h	+216h	+6	+45h	+120h
Sample Number	155	156	158	161	158	159	160
Compounds							
Naphthalene	0.25	0.68	0.14	n.d.	0.56	n.d.	n.d.
2-Methylnaphthalene	0.34	1.56	1.12	n.d.	1.85	0.1	0.11
1-Methylnaphthalene	0.34	1.26	0.98	0.13	1.42	0.2	0.13
Biphenyl +2,6-Dimethylnaphthalene*	0.25	0.81	0.99	0.25	1.05	0.3	0.27
1,3-Dimethylnaphthalene*	0.36	1.18	1.54	0.40	1.48	0.4	0.48
1,5-Dimethylnaphthalene*	0.13	0.37	0.47	0.16	0.44	0.1	0.17
2,3-Dimethylnaphthalene*	0.04	0.05	0.03	0.05	0.06	>0.1	not id.
3-Methylbiphenyl+C_3-Naphthalene + 4-Methylbiphenyl	0.10	0.27	0.56	0.14	0.40	0.1	0.21
C_3-Naphthalene	0.09	0.20	0.42	0.12	0.28	0.1	0.19
Methylbiphenyl+C_3-Naphthalene	0.07	0.17	0.44	0.09	0.31	0.1	0.18
2,3,5-Trimethylnaphthalene	0.07	0.13	0.25	0.08	0.18	0.1	0.13
C_3-Naphthalene+C_2-Biphenyl	0.03	0.09	0.22	0.03	0.2	>0.1	0.15
Fluorene+C_4-Naphthalenes+ C_2-Biphenyl+C_5-Naphthalene	0.04	0.11	0.45	0.05	0.3	0.1	0.16
C_4-Naphthalene+C_2-Biphenyl+ C_3-Biphenyl	0.03	0.04	0.11	0.03	0.1	-	0.04
Methylfluorene+C_4-Naphthalene+ C_3-Biphenyl+	0.11	0.23	0.54	0.13	0.2	0.2	0.11
Dibenzothiophene+C_4-Biphenyl+ C_5-Naphthalene	0.01	0.02	n.d.	n.d.	0.1	0.2	0.11
Phenanthrene	0.08	0.11	0.26	0.08	0.1	0.2	0.11

* Other isomers of C_2-naphthalene may be superimposed

n.d. Peak not detectable

Table 2. Time Dependence of Aromatic Hydrocarbon Concentrations (ppb) in Water after Experimental Spills of South Louisiana Crude Oil

Time after Spill (hrs)	Fresh Crude Spill				Weathered Crude Spill		
Sample Number	+6h 155	+31h 156	+76h 158	+216h 161	+6 158	+45h 159	+120h 160
Compounds							
Naphthalene	n.d.	1.80	n.d.	n.d.	1.13	n.d.	n.d.
2-Methylnaphthalene	n.d.	1.05	0.18	n.d.	0.96	n.d.	n.d.
1-Methylnaphthalene	n.d.	1.04	0.20	n.d.	1.04	0.35	0.31
Biphenyl +2,6-Dimethylnaphthalene*	n.d.	0.50	0.19	n.d.	0.65	0.17	0.35
1, 3-Dimethylnaphthalene*	n.d.	0.71	0.31	n.d.	1.02	0.29	0.57
1, 5-Dimethylnaphthalene*	n.d.	0.29	0.09	n.d.	0.47	0.13	0.25
2, 3-Dimethylnaphthalene*	n.d.	0.10	0.07	n.d.	0.15	0.05	0.07
3-Methylbiphenyl+C_3-Naphthalene+ 4-Methylbiphenyl	n.d.	0.19	0.15	n.d.	0.37	0.11	0.26
C_3-Naphthalene	n.d.	0.18	0.16	n.d.	0.33	0.13	0.21
Methylbiphenyl+C_3-Naphthalene	n.d.	0.13	0.11	n.d.	0.41	0.11	0.17
2,3,5-Trimethylnaphthalene	n.d.	0.13	0.11	n.d.	0.26	0.10	0.16
C_3-Naphthalene+C_2-Biphenyl	n.d.	0.09	0.08	n.d.	0.16	0.05	0.12
Fluorene+C_4-Naphthalenes+ C_2-Biphenyl+C_5-Naphthalene	n.d.	0.17	0.15	n.d.	0.31	0.11	0.22
C_4-Naphthalene+C_2-Biphenyl+ C_3-Biphenyl	n.d.	0.08	0.07	n.d.	0.15	0.07	0.12
Methylfluorene+C_4-Naphthalene+ C_3-Biphenyl+	n.d.	0.20	0.28	n.d.	0.31	0.15	0.20

* Other isomers of C_2-naphthalene may be superimposed

n.d. Peak not detectable

Table 3. Concentration of n-alkanes in Fundulus Exposed to Experimental Oil Spills of South Louisiana Crude Oil (in ppm)

No. of Carbons in n-alkane	Fresh Crude Spill				Weathered Crude Spill		
	+6h	+31h	+76h	+216h	+6h	+45h	+120h
	155	156	158	161	158	159	160
15	0.17	0.76	1.30	0.38	0.15	0.19	0.70
16	0.21	0.77	1.42	0.53	0.19	0.25	1.10
17	0.30	0.84	1.62	0.60	0.25	0.32	1.30
19	0.35	0.77	1.50	0.64	0.31	0.37	1.55
20	0.36	0.72	1.45	0.61	0.33	0.39	1.50
21	0.41	0.75	1.50	0.66	0.37	0.43	1.55
22	0.37	0.71	1.42	0.59	0.34	0.42	1.45
23	0.33	0.63	1.25	0.51	0.31	0.37	1.23
24	0.27	0.48	1.02	0.42	0.26	0.31	0.99
25	0.21	0.40	0.80	0.33	0.21	0.26	0.80

Table 4. Concentration of n-alkanes in Water Exposed to Experimental Spills of South Louisiana Crude Oil (in ppb)

No. of Carbons in n-alkane	Fresh Crude Spill				Weathered Crude Spill		
	+6h	+31h	+76h	+216h	+6h	+45h	+120h
	155	156	158	161	158	159	160
15	n.d.	0.50	2.15	n.d.	1.35	0.29	0.34
16	n.d.	0.84	4.02	0.11	2.22	0.64	0.84
17	n.d.	1.53	5.04	0.21	2.96	1.11	1.39
19	n.d.	2.07	6.35	0.40	3.94	1.45	1.63
20	n.d.	2.33	6.63	0.51	4.68	1.62	1.70
21	n.d.	2.49	6.80	0.65	5.37	1.87	1.78
22	n.d.	2.49	6.57	0.70	5.37	1.83	1.78
23	n.d.	2.11	5.44	0.63	4.85	1.64	1.58
24	n.d.	1.76	4.65	0.57	4.19	1.43	1.46
25	n.d.	1.40	3.40	0.51	3.33	1.14	1.16

n.d. Homologuous series of even n-1- alkenes present only.

Table 5 Concentrations in ppb (wet weight) of aromatic compounds in oyster samples from the fresh SLAC oil spill

Time after spill (hrs.)	Prespill 154	+6 155	+31 156	+76 158	+216 161	+552 163	+1370 168	+6938 195	+10610 201
Compounds									
Naphthalene	1	30	15	1	--	--	3	--	--
2-Methylnaphthalene	2	28	31	6	--	--	2	1	2
1-Methylnaphthalene	1	20	25	6	--	--	1	1	1
C_2-Naphthalenes	10(?)	29	75	65	1	1	5	4	2
C_3-Naphthalenes	3(?)	21	57	62	20	9	14	11	5
C_4-Naphthalenes	--	<3	4	11	<9	5	<9	5	3
Biphenyl	1	14	11	36	--	<1	3	3	2
Methylbiphenyls	15(?)	5	18	20	5	3	--	2	2
C_2-Biphenyls	--	<2	6	6	<9	<2	5	>3	>1
C_3-Biphenyls	--	--	--	3	2	<1	4	3	1
Dibenzofuran	--	3	8	10	4	<2	<3	<3	<1
Methyldibenzofuran	--	>1	7	9	>4	2	<4	<1	<1
Fluorene	--	1	5	9	1	<1	--	--	--
Methylfluorene	--	1	5	7	6	2	2	2	1
C_2-Fluorenes	--	--	--	4	2	1	2	1	1
Phenanthrene	<1	<2	6	7	5	1	2	1	2
Methylphenanthrenes	--	--	--	17	16	4	4	<4	>2
C_2-Phenanthrenes	--	1	1	6	5	<3	<3	2	1
C_3-Phenanthrenes	--	--	--	--	--	3	3	3	1
Fluoranthene	<23	<10	<2	<24	<6	<14	<10	<9	<5
Σ, Sum of total above	--	171	275	309	95	55	79	59	33
UCM	0	0	0	2000	2000	1000	1000	5000	2000
Σ/UCM	--	--	--	0.15	0.05	0.06	0.08	0.01	0.02

Table 6 Concentrations in ppb (wet weight) of aromatic compounds in oyster samples from the artificially weathered SLAC oil spill

Time after spill (hrs.)	Prespill	+6	+45	+120	+318	+603	+1323	+4106	+6891	+10563
Sample Number	157	158	159	160	162	165	168	191	195	201
Compound										
Naphthalene	7(?)	67	4	5	9	7	4	2	--	--
2-Methylnaphthalene	4	116	37	5	9	<1	2	--	--	--
1-Methylnaphthalene	2	88	32	3	9	<1	<1	--	--	--
C_2-Naphthalenes	13	188	529	53	94	16	8	--	--	--
C_3-Naphthalenes	6	100	519	108	184	24	18	--	--	--
C_4-Naphthalenes	2	10	45	24	39	13	12	<3	<3	<3
Biphenyl	5	28	32	7	20	6	6	--	<1	2
Methylbiphenyls	3	40	135	30	53	<5	<2	--	5	2
C_2-Biphenyls	--	12	71	17	33	7	4	2	1	2
C_3-Biphenyls	--	2	11	6	12	4	5	2	1	4
Dibenzofuran	2	16	53	21	34	6	6	2	2	<1
Methyldibenzofuran	--	<12	<51	<10	<25	<6	<4	<2	<1	--
Fluorene	--	9	25	5	11	1	<1	--	--	--
Methylfluorenes	1	7	38	12	25	8	4	2	1	1
C_2-Fluorenes	1	3	8	6	10	4	<3	1	1	<3
Phenanthrene	2(?)	9	39	7	17	3	3	--	--	--
Methylphenanthrenes	--	10	65	28	38	<11	<6	<3	<3	<4
C_2-Phenanthrenes	1	2	12	9	10	1	4	<1	2	4
Fluoranthene	<4	<10	<11	<2	<23	<9	<9	<40	<19	<5
Σ, Sum of total above	--	729	1717	358	655	133	102	56	40	31
UCM	1000	1000	5000	2000	5000	2000	4000	3000	2000	2000
Σ/UCM	--	0.73	0.34	0.18	0.13	0.07	0.03	0.03	0.02	0.02

Table 7 Concentrations in ppb (dry weight) of aromatic compounds in unconsolidated sediment from the fresh SLAC oil spill

Time after spill (hrs.)	Prespill	+6	+31	+76	+216	+1370	+4150	+6938
Sample Number	154	155	156	158	161	168	191	195
Compounds								
Naphthalene	--	4999	166	--	104	119	92	--
2-Methylnaphthalene	--	2270	104	--	66	170	54	--
1-Methylnaphthalene	--		62	--	28	93	23	--
C_2-Naphthalenes	--	1874	228	--	208	425	191	--
C_3-Naphthalenes	--	242	93	35	104	<405	239	--
C_3 & C_4-Naphthalenes	--	83	41	10	38	136	107	--
C_4-Naphthalenes	--	--	--	--	--	--	--	--
C_5-Naphthalenes	--	--	<83	<49	<159	<644	<399	19
Biphenyl	--	437	643	--	609	543	629	187
Acenaphthene	--	187	41	8	33	127	69	--
Methylbiphenyls	--	83	83	5	55	255	130	--
C_2-Biphenyls	--	21	21	10	16	<102	107	--
C_3-Biphenyls	--	--	--	--	--	--	--	--
Dibenzofuran	--	104	42	10	38	153	77	--
Methyldibenzofuran	--	--	21	33	16	<85	54	--
Fluorene	--	<83	62	<20	<82	<254	<77	--
Methylfluorene	--	--	--	--	--	--	--	--
C_2-Fluorenes	--	--	21	29	<82	<245	138	19
Phenanthrene	--	90	249	<147	433	611	383	75
Methylphenanthrene	--	--	685	<284	1046	1272	<659	<169
C_2-Phenanthrenes	--	--	<187	<103	<197	<237	261	94
C_3-Phenanthrenes	--	--	--	137	--	--	--	--
Dibenzothiophene	--	--	31	20	55	54	31	19
Fluoranthene	--	<38	<435	<245	<241	<271	<368	<712
Σ, Sum of total above	--	12281	3298	1145	3610	6201	4088	1294
UCM (ppb)	--	--	55000	41000	69000	202000	191000	459000
Chlorinated HCs	--	--	1679	638	4133	2604	688	562
Σ/UCM	--	--	0.06	0.03	0.05	0.03	0.02	0.003

Table 8 Concentration in ppb (dry weight) of aromatic compounds in unconsolidated sediment from the artificially weathered SLAC oil spill

Time after spill (hrs.)	Prespill	+6	+45	+120	+318	+605	+1323	+4106	+6891
Sample Number	157	158	159	160	162	165	168	191	195
Compounds									
Naphthalene	100(?)	--	--	--	6(?)	9(?)	3	133	50
2-Methylnaphthalene	44	14	58	23	19	22	9	80	58
1-Methylnaphthalene	22	14	29	6	13	9	6	35	25
C_2-Naphthalenes	110(?)	28	189	52	38	36	<77	247	125
C_3-Naphthalenes	66	<83	294	81	49	>9	207	256	83
C_3 & C_4-Naphthalenes	22	28	102	23	26	18	87	88	33
C_4-Naphthalenes	<105(?)	28	<305	<232	103	58	<136	<318	33
C_5-Naphthalenes	44	<139	<378	<209	<77	<81	<359	<274	<91
Biphenyl	111(?)	14	87	151	193	72	93	619	224
Acenaphthene	22	28	58	23	19	18	93	80	33
Methylbiphenyls	44	28	145	35	32	111	105	159	50
C_2-Biphenyls	11(?)	14	87	23	13	<9	<49	44	17
C_3-Biphenyls	--	--	--	--	--	--	--	--	--
Dibenzofuran	22	14	87	23	26	36	80	124	33
Methyldibenzofuran	11	28	204	23	--	<9	<62	<141	12
Fluorene	<5	<55	175	<70	<64	<45	<166	<97	<41
Methylfluorenes	--	--	--	--	--	--	--	--	--
C_2-Fluorenes	--	<110	<378	<81	<71	<54	<420	<725	<522
Phenanthrene	155	<416	<1076	360	385	242	630	309	158
Methylphenanthrenes	277	<1455	2415	766	1144	443	<2045	<990	--
C_2-Phenanthrenes	<100	<914	<887	<290	<463	<125	266	265	58
C_3-Phenanthrenes	--	<499	--	--	--	--	--	--	--
Dibenzothiophene	22	<42	87	46	51	18	111	35	8
Fluoranthene	<466,	<222	<524	<348	<206	179	198	<230	348
Σ, Sum of total above	--	4173	7565	2865	2998	1603	5202	5249	2002
UCM (ppb)	--	272000	502000	672000	67000	58000	76000	133000	99000
Chlorinated HCs	--	2690	5250	1415	2055	1835	2383	1997	721
Σ/UCM	--	0.02	0.02	0.004	0.04	0.03	0.07	0.04	0.02

Appendix 1

EXTRACTION AND SEPARATION METHODS

I. Water Samples

Methylene chloride extraction (approx. time for 6 samples: 3 working days). The extraction of hydrocarbons from water (unfiltered) started immediately after return from sampling to the laboratory. The outside of the collection bottles was first washed with detergent to prevent contamination of the clean room. Next, the amount of water in the collection bottles was reduced to 3 L, and 90-100 mL of CH_2Cl_2, and a standard solution containing hexacosane and pyrene in acetone were added. The mixture was agitated by hand several times at intervals of more than 2 h and left standing for at least 24 h after the last agitation for phase separation (methylene chloride always remained broken up into globules of varying size, with each globule surrounded by organic debris). Most of the water was then transferred to another clean bottle by decantation; this water was extracted a second time with 40-50 mL of CH_2Cl_2. The mixture left in the first extraction bottle was transferred to a separatory funnel and the bottle was rinsed with 20 mL of CH_2Cl_2 which was also added to the funnel. This was done for the second crude extract as well. Then, the contents of the separatory funnel were swirled and gently swapped back and forth to bring the CH_2Cl_2 globules to coalescence. After separation into a greenish, murky looking layer of water on top, a layer of remaining CH_2Cl_2 globules and organic debris in the middle and a clear layer of CH_2Cl_2 at the bottom, the latter was drained into a 300 mL Erlenmeyer flask equipped with a ground glass stopper. This process was repeated, if necessary, with further addition of small amounts of CH_2Cl_2 and removal of water by decantation. Almost complete coalescence was

was observed when most of the water was removed. All CH_2Cl_2 fractions were combined in the Erlenmeyer flask, 10 g anhydrous soldium sulfate were added and the flask was left stoppered for at least 16 h.

II. Oyster and Clam Samples

1. Sample preparation (approx. time for 6 samples: 4 h/3 people).
Four to five oysters or clams were kept in plastic bags under ice for 4-8 h right after sampling. Each shell was (1) brushed thoroughly with warm tap water containing Alconox® (2) washed with plenty of tap water and (3) rinsed with methanol and hexane. The dry shells were opened with a shell opener. Care was taken so both juice and tissue were quantitatively transferred to a homogenizer flask equipped with a funnel. The tissue was homogenized, using a Virtis 45 homogenizer for about 30 seconds, or longer if needed, at medium or higher speed. After the appropriate amount of sample was taken to process it, the rest was kept at -20°C.

2. Digestion (approx. time for 3 samples: 3 h).
Approximately 50 g (25 g from substation A and 25 g from substation B) homogenized oyster or clam tissue was placed in a 300 mL round bottom flask using a long neck funnel and stainless steel spatula. About 7.0 g of potassium hydroxide pellets, ca. 50 g methanol, ca. 20 g water, and hexacosane and pyrene standard were added to the flask which was fitted with a Snyder type condenser and the mixture was refluxed for about 2 h in a water bath of 83-87°C.

3. Oyster Extraction (approx. time for 6 samples: 2 working days). The digested mixture was left to reach room temperature and then transferred to a 500 mL separatory funnel. About 300 mL water, ca. 12 g sodium chloride and 80 mL pentane were added to the funnel, the mixture was shaken thoroughly and left for the layers to separate, usually 1-2 h or longer if needed. Then the water layer was drained in a 1000 mL beaker. The pentane layer and/or possible emulsion were retained in the separatory funnel. If the pentane layer was emulsified or a separate emulsion layer was present, 1-3 g sodium chloride were added and the mixture was shaken and left for 1/2-1 h. Then the bottom layer with possible emulsion was drained to a beaker and the clear pentane layer was transferred to an Erlenmeyer flask, equipped with a ground glass stopper. A second extraction was then conducted the same way and a third one followed. The pentane and/or possible emulsion layer of the third extraction was washed with 350 mL H_2O and after 1-2 h, the bottom layer was discarded. The three pentane extracts were combined in the separatory funnel and the combined extract was washed 3 times with 350 mL water. If some emulsion was still present after the third washing, the pentane layer was shaken with 1-2 g sodium chloride and left for 1 h. If a water layer was formed, it was discarded and the clear pentane extract was transferred from the top of the funnel to the Erlenmeyer flask and 10 g sodium sulfate were added. The separatory funnel was rinsed with about 20 mL pentane and the latter was transferred to the Erlemeyer flask, too. The flask was then left stoppered for at least 16 h.

4. Clam Extraction (approx. time for 6 samples: 3 working days for the Soxhlet extraction plus 2 working days for pentane extraction).

a) Soxhlet Extraction. The digested mixture was left to settle and reach room temperature and then it was transferred through a coarse fritted glass Soxhlet 130 × 45 m thimble to a 500 mL round bottom flask with the help of a regular funnel. To facilitate the filtration, the clean solution was first added and after it passed through, the addition of solids followed. The flask was rinsed thoroughly with plenty of methanol. After the filtration was through (the solids in the thimble do not have to necessarily be dry), additional methanol was added to the flask to reach a total volume of ca. 200 mL. Then the thimble was placed in a Soxhlet extractor fitted with the flask containing the clear sample. The residue was then extracted with a speed of 1 1/2-2 cycles per h or up to the point the extract was not colored (usually about 18 h). To prevent foaming, the heating was occasionally turned off and the draining was performed mechanically. If foaming persisted, a new flask with fresh solvent was used.

b) Pentane Extraction. The total methanol extract (]70-220 mL) was transferred quantitatively (the flask was rinsed three times with 10 mL methanol) to a 1000 mL separatory funnel. Then, about 450 mL water, ca. 15 g sodium chloride and ca. 80 mL pentane were added to the funnel and the mixture was shaken thoroughly and left for the layers to separate, usually 1/2-1 h or longer, if needed. Then the bottom (water) layer with possible emulsion (usually very little) was drained to a 1000 mL beaker and the clear pentane layer was transferred to an Erlenmeyer flask equipped with ground glass stopper. A second extraction was then conducted the same way and a third one followed. The pentane and/or possible emulsion layer of the third extraction was retained and the three pentane extracts were combined in the separatory funnel. The combined extract was then washed 3 times with 500 mL water. If

some emulsion (usually very little) was still present after the third washing, the pentane layer was shaken with 1/2-1 g sodium chloride and it was left for 1/4-1/2 h. If a water layer was formed, it was discarded and the clear pentane extract was transferred from the top of the funnel to the Erlenmeyer flask and 10 g sodium sulfate were added. The separatory funnel was rinsed well with about 20 mL pentane and the latter was transferred to the flask, too. The flask was then left stoppered for at least 16 h.

III. Fundulus Samples

1. Sample preparation (approx. time for 3 samples: 3 h/2 persons).
Seven or eight little fish (average weight 3 g each) right after sampling were kept in quart bottles filled with river water in a cold box for 4-8 h. The fish were washed thoroughly with plenty of tap water and placed on a clean Teflon® surface. Then each fish was rinsed first with methanol and then hexane. Care was taken so that the gills were rinsed, too. The fish then were placed in a pre-weighed (second decimal point accuracy) homogenizer flask, a known amount (usually two times the weight of the fish) of methanol was added and the fish were homogenized by using a Virtis-45 homogenizer for about 1 minute, or longer if needed, at medium or higher speed. After the appropriate amount of sample was taken to process it, the rest was kept at -20°C.

2. Digestion (approx. time for 3 samples: 3 h).
Approximately 70 g (35 g from substation A and 35 G from substation B) of the homogenized mixture of fundulus and methanol corresponding to about 23 g of fundulus, wet tissue was placed in a 300 mL round bottom flask using a Long neck funnel and stainless steel spatula. About 7.0 g potassium

hydroxide pellets, 20 mL water and hexacosane and pyrene standard were added to the flask, which was fitted with a Snyder column, and the mixture was refluxed for about 2 h in a water bath of 83-85°C.

3. Pentane Extraction for Fundulus (approx. time for 6 samples: 2 working days).

The digested mixture was left to reach room temperature and then transferred to an 1000 mL separatory funnel. About 500 mL water, 15 g sodium chloride and 80 mL pentane were added and the mixture was shaken thoroughly and left for the layers to separate, usually 1-2 h or longer, if needed. Then the water layer was drained in an 1000 mL beaker. The pentane layer and/or possible emulsion were retained in the separatory funnel. Then about 2 g sodium chloride was added and the mixture was swirled firmly and left for 1/2 h. The (usually black) emulsion layer was drained to the beaker and the clear pentane layer was transferred from the top of the funnel to an Erlenmeyer flask equipped with a ground glass stopper. A second extraction was then conducted the same way, and a third one followed. The pentane and/or possible emulsion layer of the third extraction was washed twice with 500 mL water. The three pentane extracts were combined in the separatory funnel and the combined extract was washed 3 times with 500 mL water. If some emulsion was still present after the third washing, the pentane layer was shaken with 1-2 g sodium chloride, and left for 1/2 h. If a water layer was formed, it was discarded and the clean pentane extract was transferred from the top of the funnel to the Erlenmeyer flask and 10 g sodium sulfate were added. The separatory funnel was rinsed with 20 mL pentane, the latter was transferred to the Erlemeyer flask too, and the flask was left stoppered for at least 16 h.

IV. Sediment

1. Soxhlet Extraction (approx. time for 6 samples: 4 working days). The sample right after sampling was kept under ice for 4-8 h. Approximately 50 g (25 g from substation A and 25 g from substation B) wet sediment (mixed well) was placed in a pre-weighed (2nd decimal point accuracy) coarse fritted glass 130 × 45 mm thimble, hexacosane and pyrene standard were added and placed in a Soxhlet extractor, fitted with a 500 mL Soxhlet round bottom flask containing ca. 180 mL methanol and glass boilers. The sediment was extracted for 48 h with a speed of 2-2 1/2 cycles per h. If the draining was not good after 3-4 cycles, the heat was turned off and the draining was done mechanically for one complete cycle, and then the heat was turned on again for regular recycling. For some samples, like surface sediments from the back of the marsh, a second flask with fresh solvent was used usually connected after 20 h of extraction.

2. Pentane Extraction for sediment and organic detritus (approx. time for 6 samples: 1 working day). The total methanol extract (ca. 200 mL) was quantitatively (the flask was rinsed 3 times with 8 mL methanol) transferred to a 1000 mL separatory funnel. About 450 mL water, ca. 15 g sodium chloride and 80 mL pentane were added to the funnel and the mixture was shaken thoroughly and left for the layers to separate, usually 1/2-3/4 h or longer if needed. Then the bottom layer with possible emulsion (usually very little) was drained into a 1000-mL beaker and the clean pentane layer was transferred from the top of the funnel to an Erlenmeyer flask equipped with a ground glass stopper. A second extraction was then conducted the same way, and a third one followed. The pentane and/or possible emulsion layer of the third extraction was retained and the three pentane extracts were combined in the

separatory funnel. The combined extract was washed 3 times with 500 mL water. If some emulsion (usually very little) was still present after the third washing, the pentane layer was shaken with 1/2-1 g sodium chloride and left for 1/4-1/2 h. The water layer was discarded and the clean pentane extract was transferred from the top of the funnel to the Erlenmeyer flask and 10 g sodium sulfate were added to it. The separatory funnel was rinsed well with about 20 mL pentane and the latter was transferred to the flask, too. The flask was then left stoppered for at least 16 h.

V. Unconsolidated Sediment

1. Sample preparation (approx. time for 3 samples: 2 working days).
Since organic detritus was always collected with large amounts of water, the latter had to be removed in the laboratory by filtering. Glass fiber discs (Reeve Angel grade 934AH) of 11 cm diameter, after washing at 400-450°C, were weighed and placed in a Buchner funnel. The sturry was filtered by maintaining a pressure gradient of 600 mm Hg, generated by applying vacuum from an aspirator-pump to the effluent side across the filter disc. Due to pore-closing three separate filters were used per sample. The filters containing the detritus were dried in a clean room at room temperature before reweighing.

2. Digestion (approx. time for 3 samples: 3 h).
The air dried detritus with the fiberglass filters was transferred to a 300 mL round bottom flask using metallic forceps. To the flask about 45 g methanol, about 40 g potassium hydroxide and 20 mL water were added, and after the addition of hexacosane and pyrene standard, the flask was fitted

with a Synder column and placed in a water bath of 83-85°C/ The mixture then was refluxed for about 2 h at this temperature.

2. Extraction (approx. time for 6 samples: 2 working days for Soxhlet extraction plus 1 working day for pentane extraction).

a) Soxhlet Extraction: The digested mixture was left to reach room temperature and then it was transferred through a coarse fritted glass 130 × 45 mm Soxhlet thimble to a 500 mL Soxhlet round bottom flask with the help of a long neck funnel. The filter papers were transerred with the help of a pair of metallic forceps. The flask was rinsed thoroughly with plenty of methanol. After the filtration more methanol was added to the flask to reach a total volume of about 200 mL and the thimble was placed in a Soxhlet extractor fitted with the flask. The Soxhlet extraction continued for about 12 h with a speed of 2-2 1/2 cycles per h.

b) Pentane extraction: (see IV number 2)

VI. Concentration

Concentration (approx. time for 3 samples: 3 h).

The dried pentane extract was placed in a large Kuderna-Danish concentrator using Snyder column. The column was covered with a glass tube and the concentrator was wrapped with aluminum foil. The Erlenmeyer flask was first rinsed with 20 mL pentane and then 6-8 mL hexane. The concentrator was placed in a water bath (about 75-80°C) and the concentration continued until 3-7 mL volume was left. Then the Snyder column and concentrator were rinsed with 2-3 mL hexane. At this point an aliquot of 10% of the sample was placed in a pre-weighed aluminum pan (5th decimal point accuracy) for the total

pentane extractables determination. The sample was then further concentrated in a Kontes apparatus to about 1 mL volume.

VII. Column Chromatography*

Column Chromatography (approx. time: 2 h per 2 samples for column and 3-6 h per 6 samples for evaporation).

A 10 × 300 mm chromatographic column with a coarse fritted glass ring in the bottom was used. The column was packed by pouring a slurry of activated (235°C, 16 h) Bio-Sil® silica gel (100-200 mesh) with hexane. The gel was settled by gentle tapping until 17.5 cm of absorbent was achieved (about 7 g dry gel). Then the column was washed by eluting about 40 mL hexane. After the solvent level reached the top of the absorbent, the (1 mL) sample was placed at the top of the column using a Pasteu pipet. The sample tube was rinsed with about 1 mL hexane which was placed at the top of the column, too, after the former was eluted through. The collection of the fractions started immediately after the addition of the sample. The column was eluted first with hexane and fractions H_1 = 5 mL and H_2 = 13 mL are collected. Then the column was eluted with 40/60 (v/v) benzene/hexane and fractions Hb_1 = 7 mL and Hb_2 = 25 mL were collected. Fractions H_2 (aliphatic) and Hb_2 (aromatic) were then further concentrated using the Kontes apparatus in a 10 mL evaporator tube equipped with a chimney, which was covered with a glass tube (hood), to a final volume of usually 0.4-0.5 mL. The temperature

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*For removal of sulfur in sediment samples, about 2 cm of treated electroytic copper powder was placed at the top of the column. The treatment was performed in a centrifuge tube by washing the powder first with concentrated hydrochloric acid and then water (2x), methanol (3x), benzene (1x), pentane (2x), and hexane (1x), draining well (centrifugation) after each washing. The powder was kept closed under hexane. A test, using commercial sulfur, was performed always before use.

of the head of the apparatus was 94-98°C. The samples were then sealed in 1 mL vials with Teflon® covered silicone rubber seals and stored at -20°C.

VIII. Solvents and Reagents

Pentane, hexane (UV), benzene, methanol, and methylene chloride, all "distilled in glass," were purchased from Burdick and Jackson. Sodium chloride and sodium sulfate, both "analytical reagent" grade, were Soxhlet extracted with hexane and dried at 135° for 16 h. Potassium hydroxide (analytical reagent) was washed with pentane and then dried over nitrogen. Water (pretreated by reverse osmosis, charcoal treated and ion exchanged in a central system) before use was extracted with hexane (100 mL hexane per liter water). All glassware used was first brushed with soapy (Alconox®) warm water, sonicated if needed, rinsed with plenty of tap water and acetone. Then, each item was placed in dichromate cleaning solution, rinsed with plenty of tap water, distilled water, acetone (pesticide grade) and dried at 135°C. Before use, each item was rinsed with pentane.

Epilogue

Due to the low spike recovery yields especially in the aliphatic fraction of oyster and clam tissue extracts, we tried to develop a better method applicable also to all tissue samples. Below we describe a modification to the procedure which proved to be successful as far as the high spike-recovery yields are concerned. This method, which is the final we recommend, gave much better overall yields not only for aliphatic but aromatic fractions as well. The explanation lies on the fact that with this modification, we eliminate considerable amount (in some cases most) of the emulsion problems.

Modification (Unified tissue-extraction method)

The digested mixture was left to reach room temperature and then filtered through a coarse fritted glass Soxhlet thimble. The filtrate was collected in a 1000 mL separatory funnel. The flask and the thimble were rinsed well first with 15 mL methanol and then with 80 mL pentane under constant stirring. After the addition of 20 g sodium chloride and 320 mL water, the mixture was shaken thoroughly and left standing for 1-2 h. The procedure then was the same as described for oyster extraction, except that the 80 mL pentane used for the second extraction was passed through the thimble, too.

The above modification was applied only* to the following samples

Oyster[1]	Clam[1]
155 U01R	154 UC0R
157 U02R	155 UC1R
158 U01R	157 UC2R
158 U02R	158 UC1R
159 U02R	158 UC2R
160 U02R	159 UC2R
162 U02R	160 UC0R
165 U00R	161 UC1R
165 U02R	162 UC0R
168 U00R	162 UC2R
168 U01R	163 UC1R
168 U02R	165 UC2R
191 U00	
191 U01	Fundulus
191 U02	191 UF0
195 U00	191 UF1
195 U01	191 UF2
195 U02	195 UF1A[2]
201 U00	
201 U01A[2]	
201 U02A[2]	

1) v, location of sampling (Cub Creek); 0, oyster; 0, control station; 1, station 1 (fresh oil spill); 2, station 2 (weathered oil spill); C, clam; F, Fundulus.

2) A: the sample was from substation A.

* All other samples were treated as described in the methods without the modification.

APPENDIX 2

Characterization of Surface Marsh Soil Samples by Liquid Chromatography and GC/MS

The surface marsh soil samples analyzed were collected from the back of the spill areas. These samples consisted of thick layers of surface soil (0.5-1 cm) and subsurface soil (1-2 cm). Considerable amounts of marsh-grass roots were present. The samples were extracted as described in Appendix 1 and analyzed by capillary GC as described in the main text. The results described here are confined to aromatic fractions only.

Since the relative increase of unresolvable complex matrix (UCM) over resolved peaks was the most obvious change (with time) in the GC fingerprints of the aromatic (and aliphatic) fractions, it was realized that these samples cannot be characterized by GC without further chemical treatments or fractionations.

Chemical Treatments of Aromatic Fraction

The design of the silica chromatography to obtain the aromatic fraction (as described in Appendix 1) was such that this fraction would contain only aromatic hydrocarbons and not any polar material that would interfere with the analysis. The following experiments confirmed this requirement.

To test possible presence of acidic or basic components, the aromatic fraction was further fractionated into acid, base, and neutral fractions using a generally accepted pH-fractionation technique. The acid and base fractions analyzed on capillary column GC showed no peaks. The neutral fraction chromatogram was identical with the original. The sample was also tested on TLC. Most of the material lined up on the band corresponding to a variety of aromatic hydrocarbons used as standards. The marsh soil aromatic fraction also was derivatized with a potent TMS silylating reagent (Sylon-BTZ, from Supelco).

The chromatograms prior and after the treatment looked identical. From this it was concluded that the aromatic fraction contained no compounds with functional groups such as (-OH, $-NH_2$ and -NHR).

Fractionation by Liquid Chromatography

Since chemical treatments showed that the marsh soil aromatic fraction was practically free of very polar components, a known liquid chromatography technique was applied to separate the aromatics into mono-, di-, and poly-aromatics and polars (heterocyclics). An alumina (20 cm × 1 cm ID) chromatographic column was used by applying a stepwise polarity gradient (5% benzene in hexane; 20% benzene in hexane; 40% benzene in hexane; and 30:30:40, ether: methanol:benzene) to "break-up" the very complex aromatic fractions. Two surface marsh soil samples were fractionated: a 4106-h and a 6891-h sample from the weathered SLAC oil spill area. Two independent analyses on aromatic fractions, prepared from SLAC oil, were also performed using the same chromatographic conditions in parallel with the two samples for comparison purposes.

To establish meaningful cuts for the collection of fractions containing distinct classes of organic compounds, a tedious alumina-column chromatographic separation was done by collecting 60 fractions from the elution of each aromatic fraction of a marsh soil sample and SLAC oil using the polarity gradient specified above. All 60 fractions from both runs were analyzed on GC and 12 (one in every five) by GC/MS. Most of these fractions except the ones corresponding to mono-aromatics gave chromatograms with very distinct peaks without UCM. The qualitative correlations between the fractions of the marsh soil and those of the SLAC oil were very good as confirmed by the GC/MS analysis. All the GC peaks of the marsh soil fractions were readily identifiable and attributable to homologous series of aromatic classes of compounds that were also present in the SLAC oil fractions. After the cuts were established, the aromatic fractions of the two marsh soil samples

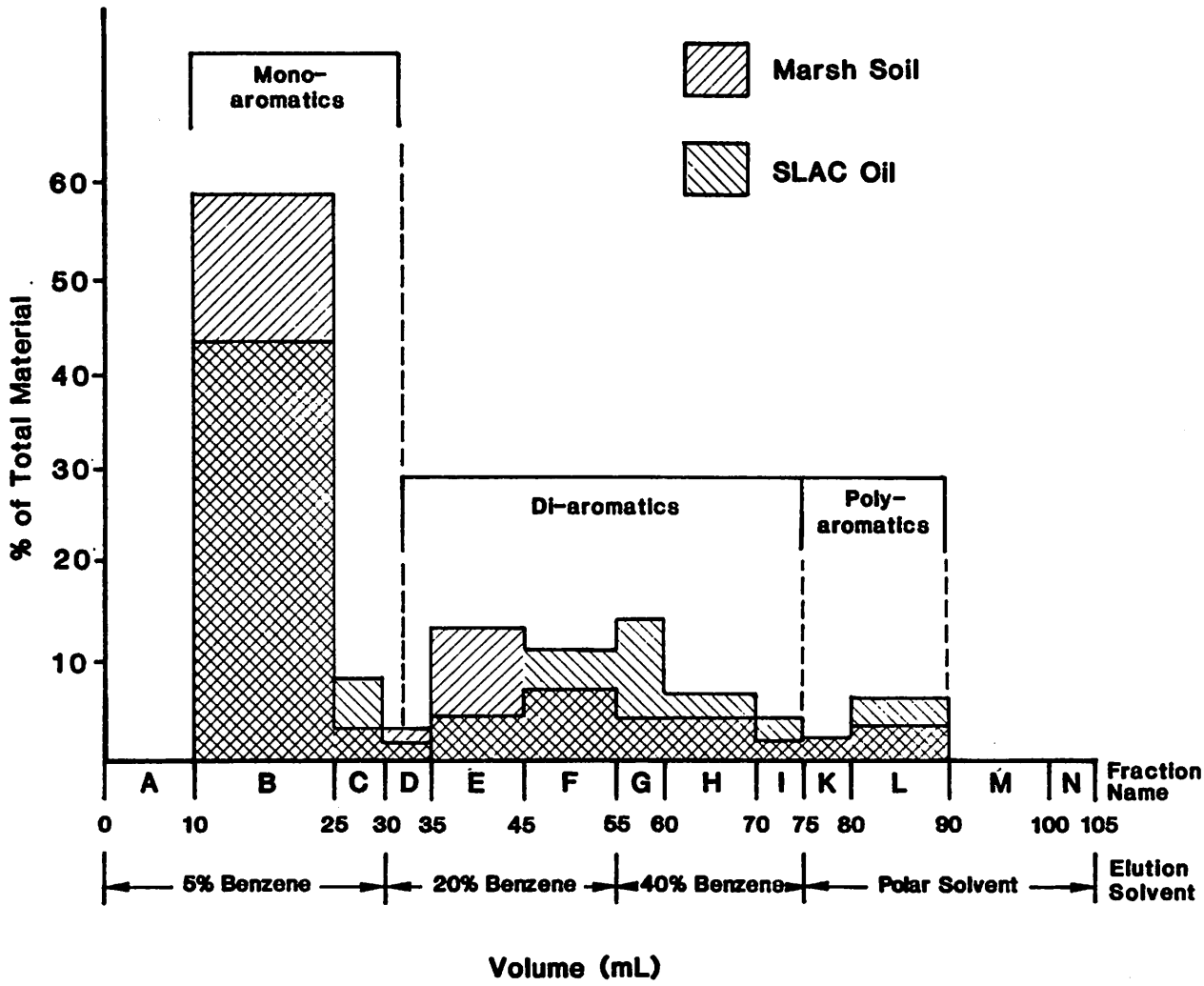

Fig. 1. Fractionation of aromatic fractions of a surface marsh soil sample and SLAC oil by alumina column.

(4106-h and 6891-h) and two SLAC oil samples were fractionated using the same alumina column (20 cm x 1 cm ID) eluted with progressively more polar solvents as described already and shown in Fig. 1. Thirteen fractions (A,B,...M,N) were collected from each fractionation and all fractions were concentrated accordingly and analyzed by capillary column GC and selected fractions (B,E,F,G,I and L) by GC/MS. The results of the liquid chromatography expressed as relative amount (as determined by planimetry of FID-GC areas) of material eluted in each fraction for one marsh soil sample and SLAC oil are shown in Fig. 1. The results of the chemicals identified in the various fractions are summarized in Table 1. The two marsh soil samples were very similar so their results are not reported separately. From the GC and GC/MS data of both the 60-fraction and the 13-fraction silica column elutions we concluded the following:

1. Most of the material is eluted in fraction B. The GC chromatogram of this fraction was the only one to contain major UCM. The marsh oil sample had a substantially greater UCM than the SLAC oil did.
2. The first set of fractions (B,C, and D) contained mainly mono-aromatics. The second set of fractions (D,E,F,G, and I) contained di-aromatics, and the third set of fractions (K and L) contained mainly poly-aromatics (including some highly substituted di-aromatics.
3. The major differences between the marsh soil sample and the SLAC oil were found to be present in the mono-aromatic fractions. Highly alkylated benzenes (Table 1), (up to C_{18}), tetralins and dihydronaphthalenes accounted for all the major peaks in the case of the marsh soil sample. The major masses of mass spectra from the UCM indicated also the presence of compounds of the same classes,

and possibly presence of alkylated indans. The SLAC oil sample showed a substantially lesser degree of alkylation.

4. In the case of the di-aromatics (fractions D,E,F,G,G,H, and I) the major difference again was the degree of alkylation, as seen in Table 1. However, both the marsh soil and the SLAC oil were composed of the same classes of compounds, and there was no visible UCM in both. An interesting feature is that longer n-alkyl chains (up to n-butyl) were clearly discernible in the early eluted di-aromatics (fraction E). The correlation of early elution and n-alkyl substitution was established by n-alkylated benzene standards. Therefore, the fact that the elution of the di-aromatics of the marsh soil (through the alumina column) peaks earlier (fraction E), whereas, the elution of the di-aromatics of the SLAC oil peaks later (fraction G) might have to do with differences in alkylation.
5. The poly-aromatics fractions (K and L) of both marsh soil and SLAC oil did not show significant differences.

The FID-GC areas of all fractions were measured by planimetry and the results summed-up as mono-aromatics, di-aromatics, and poly-aromatics are given in Table 2.

Table 2. Relative amounts (derived from FID-GC) of mono-, di-, and poly-aromatics in marsh soil samples and SLAC oil.

Aromatics	Marsh Soil Samples +4106h	+6891h	SLAC oil Run 1	Run 2
Mono-	72.8	78.4	65.5	65.6
Di-	23.3	16.7	29.6	27.8
Poly-	3.8	4.9	4.9	6.5

Table 1. Major classes of organics identified in aromatic fractions of marsh soil and SLAC oil by liquid chromatography and GC/MS

Fraction[1]	Compounds[2] Identified In	
	Marsh Soil	SLAC Oil
B and C	C_8-C_{18}-Benzenes (C_8,C_9,C_{10},C_{15})[3]	C_4-C_{12}-Benzenes (C_5,C_6,C_7)
	C_2-C_{10}-Tetralins (C_5,C_6,C_7)	C_0-C_7-Tetralines (C_2,C_3)
	C_2-C_7-Dihydronaphthalens (C_3,C_4)	C_0-C_5-Dihydronaphthalenes (C_2,C_3)
D,E, and F	C_3-C_{11}-Naphthalenes (C_4,C_5)	C_0-C_8-Naphthalenes (C_1,C_2)
	C_2-C_7-Biphenyls (C_3,C_4)	C_0-C_5-Biphenyls (C_1,C_2)
	C_2-C_6-Acenaphthenes (C_3)	C_0-C_4-Acenaphthenes (C_1,C_2)
G,H, and I	C_3-C_6-Naphthalenes (C_3,C_4)	C_1-C_5-Naphthalenes (C_1,C_2)
	C_2-C_5-Biphenyls (C_2,C_3)	C_0-C_4-Biphenyls (C_1,C_2)
	C_2-C_4-Acenaphthenes (C_2,C_3)	C_0-C_3-Acenaphthenes (C_1,C_2)
	C_1-C_3-Benzothiophenes (C_1)	C_0-C_3-Benzothiophenes (C_1)
K and L	C_0-C_4-Phenanthrenes (C_0,C_1,C_2)	C_0-C_3-Phenanthrenes (C_0,C_1)
	C_0-C_4-Fluorenes (C_0,C_1,C_2)	C_0-C_3-Fluorenes (C_0,C_1)
	C_0-C_2-Dibenzothiophenes (C_0,C_1)	C_0-C_2-Dibenzothiophenes (C_0,C_1)

1. For fraction name refer to Fig. 1.
2. C_n, refers to C_nH_{n+1} alkyl substituents with n total number of carbon atoms.
3. C_n in parenthesis indicates major homologs found in each homologous series.

Conclusions

The data from this study seem to indicate that with respect to aromatics the most important change to the SLAC oil due to natural weathering (up to 40 weeks after the spill) is loss of volatiles and solubles, and an enrichment in highly alkylated and alicyclic classes of aromatic hydrocarbons, especially in the one- and two-aromatic-ring range. Interestingly, these highly alkylated and alicyclic aromatics are known to persist in the environment, and survive from biological degradations. Perhaps the most important conclusion, however, is that most of the material identified belongs to the same fundamental classes of aromatic compounds originally present in the oil. This means that the modified (by the long weathering process) oil is still available in the environment through marsh surface sediments. Of course, since we didn't analyze the UCM of the aromatic fractions derived from animals (oysters) or unconsolidated sediments, we cannot speculate with certainty about their composition. However, the persistence of the UCM in the aromatic fractions of all these samples (oysters and unconsolidated sediments) and the fact that major masses of from these UCMs correspond to the same classes of aromatic compounds, it is reasonable to assume that there is a direct correlation of the UCM material identified in the surface marsh soil and those of the oysters and unconsolidated sediments.

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CHAPTER III

Microbial Responses

by

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INTRODUCTION

Microorganisms have been consistenly implicated in the degradation of pollutant crude oil hydrocarbons (2,6,10,21). As hydrocarbons of biogenic origin constitute a group of substrates naturally degraded by microorganisms, such observations are eminently reasonable, and elevated levels of petroleum-degrading microbes would not be unexpected in environments polluted by petroleum hydrocarbons (1,12,24).

One aspect of petroleum-related microbiology which remains unclear concerns the potential interference of hydrocarbons with the cycling of natural carbon compounds. Chet and Mitchell (7) and Bitton et al. (6) have observed that kerosene inhibited chemotaxis of non-petroleum degrading bacteria toward albumin, casein and nutrient broth. Similarly, potentially toxic petroleum components could reduce the levels of microorganisms required for biopolymer decomposition or inhibit their activities (23). Alternately, one can envision segments of heterotrophic microbial populations responding to pollutant hydrocarbons as additional substrates in a situation which might be called "competitive heterotrophy". Thus chronic or acute oil release in marshland, for example, could induce unbalanced conditions resulting in a reduction of chitin and cellulose decomposition because a significant proportion of the heterotroph population shifts to hydrocarbon degradation. However, it is also possible that increased amounts of bacterial biomass derived from petroleum degradation would aid in the decomposition of cellulose by other components of the detrital food web (17).

Biopolymers such as cellulose and chitin, sources of detrital carbon and energy produced by marsh grasses and associated organisms, are essential to the existence of complex detrital food webs in estuarine and coastal waters. Silmultaneous observations of the in situ responses to crude oil spillage of marshland microbial populations degrading petroleum and chitin or cellulose seldom have been made. A multidisciplinary experiment, designed to introduce relatively large volumes of unweathered and weathered crude oil into a Spartina salt marsh enclosed by transite structures permitting tidal exchange (4), afforded a unique opportunity to quantitate these responses in terms of viable microbial counts. Populations monitored included aerobic bacterial and fungal heterotrophs, petroleum-degrading bacteria, chitinolytic and cellulytic bacteria. Results of approximately five years of observations of these populations after oil spillage are discussed.

MATERIALS AND METHODS

Oil spillage. Measured volumes of unweathered and artificially weathered South Louisiana crude oil were spilled in transite-enclosed tidal creeks prior to flood tide. These oils were ultimately transported into the Spartina tidal marsh proper during flood tide. Details of the location, construction, and experimental procedures involving the spills have been discussed previously (4,5).

Sampling. Transite enclosures were designated A (dosed with unweathered oil), B (dosed with weathered oil), and C (control, no oil). For sampling purposes, each enclosure (Figure 1) was considered to be divided in half lengthwise and then partitioned to yield three duplicate sampling zones, i.e., creek and intertidal, mid-marsh, and backmarsh, of approximately equivalent area. A and B enclosures possessed a lengthwise divider actually constructed of transite. Each zone was identified by a code which designated the particular enclosure (A, B, or C) and a letter or number indicating the upstream of downstream side of the enclosure, e.g., AE-S, B1-W. The type of sample was designated by -S or -W, where -S = sediment, -W = water.

Different areas within each enclosure were subject to periodic submergence due to tidal flooding. Intertidal zones were characterized by tall, dense Spartina stands and sediments composed of clayey sands. Mid- and back-marsh zones, subjected to tidal inundation of shorter duration, typically contained Spartina of lesser height and, at low tide, shallow pools of water with Fundulus sp. Sediments in the mid- and back-marsh, composed of fibrous plant detritus, rhizome and root

structures and silt, possessed a thin aerobic layer (ca. 2 cm) overlying an anaerobic layer blackened by precipitated sulfides and smelling of H_2S. Precipitates, characteristic of molecular sulfur produced by Chlorobium sp., were frequently observed on sediments or in shallow pools.

Sediment samples from the Spartina rhizome area were collected from three zones within each enclosure. A random number generator was employed to determine which side would be sampled for each zone. Five random subsamples were collected and pooled for each zone sampled, yielding a total volume of approximately 10 ml. Sediment samples were collected in sterile plastic 10 or 50 cc syringes modified by removal of the luer tip to produce a mini-corer. Intertidal zones were sampled by plunging a modified 10 cc syringe into the sediment to a depth of approximately 2 cm. Five subsamples were collected using this same syringe for each zone. This micro-coring technique was not feasible for sampling in mid- or back-marsh zones owing to the fibrous, rooty nature of the "sediments." Instead, a sterile spatula was used to remove five sediment subsamples from the upper 2 cm of the marsh sediment to give a final sample volume of approximately 10 ml. Similarly, replicate, pooled sediment samples from each zone were obtained for sediment dry weight determination. Water samples were collected from the creek-intertidal zones using sterile milk dilution bottles (150 ml) inverted below the creek surface. All samples were transported on ice to the laboratory for immediate processing.

Sampling always was performed at slack water before flood on days selected initially by a geometric time scale (i.e., 0, 1, 2, 4, 8,

16, 32, and 64 days) and thereafter performed at approximately bimonthly intervals.

Media. Aerobic heterotrophic bacteria were enumerated on a heterotroph medium modified after ZoBell (26). Modifications consisted of replacing ferric phosphate with ferric citrate (0.01 g/l) and sodium glycerol phosphate (0.1 g/l) to reduce inorganic precipitates, and lowering the concentration of peptone to 1.0 g/l.

Fungi (filamentous and yeasts) were enumerated on a medium developed to enumerate fungi associated with Spartina degradation (14). Chitinoclastic bacteria were enumerated using a medium modified after Hood (13). Chitin (Calbiochem, San Diego, California, unspecified purity) ball milled at 4°C for 48 hours was "dissolved" in 50% H_2SO_4 and precipitated by the addition of large volumes of distilled water. This precipitate was centrifuged, repeatedly washed with distilled water, and neutralized to a pH of 7.0. Centrifugation was repeated, the clear supernatant discarded, and purified chitin stored under refrigeration prior to use. A bi-layer petri dish technique was employed with the base layer consisting of heterotroph medium over-layered with 10 ml of chitin-agar containing 3% chitin in aged estuarine water with 1.5% agar and 0.05% yeast extract.

Petroleum-degrading bacteria were enumerated by a three-tube most probable number (MPN) technique (11). The medium consisted of 1% (v/v) sterile South Louisiana crude oil as the sole added source of carbon in a mineral salts-enriched [1 g/l $(NH_4)_2SO_4$ and 0.1 g/l K_2PO_4] aged estuarine water (16). Membrane filter-sterilized unweathered Louisiana crude oil was used for the A and C enclosures and sterilized weathered oil for the B enclosure. Cellulytic bacteria were measured

by an MPN technique in a medium consisting of 1.0 g/l $(NH_4)_2SO_4$, 0.1 g/l K_2HPO_4, and 1.0 g/l yeast extract in aged estuarine water. A strip of No. 1 Whatman filter paper was added to each tube.

All media were adjusted to a pH of 7.6 and a salinity of 12 ppt (°/oo). Estuarine water was aged for 30 days in darkness and filtered through Whatman No. 1 paper immediately prior to use.

Enumeration procedures. All dilutions were performed using sterile, aged and filtered estuarine water (salinity = 12 °/oo). Sediment samples were weighed in the syringes, extruded into chilled Waring blenders containing 90 ml of sterile estuarine water, and blended for one minute. The empty syringes were weighed to determine the weight of the inoculum. Creek water and blended sediment suspensions were then serially diluted to appropriate dilutions.

Aerobic heterotrophic bacteria, fungi, and chitinolytic bacteria were counted by spread plating 0.1 ml of selected dilutions on appropriate media. The MPN of petroleum-degrading and cellulytic bacteria were determined by inoculating 1 ml of selected dilutions into tubed media. Chitinolytic bacteria produced a clear zone in the chitin overlayer. MPN tubes were scored positive for petroleum degradation if there was visible turbidity and oil emulsification, pellicle formation at the oil surface, or if the oil overlay had a "stringy" appearance. MPN tubes for cellulytic bacteria were considered positive if, with gentle shaking, the paper strips could be broken at the air-water surface.

All media were incubated at 20-22°C. The following incubation times were found optimal: heterotrophic bacteria,

two weeks; heterotrophic fungi, two weeks; chitinolytic bacteria, one week; petroleum-degrading bacteria, four weeks; cellulytic bacteria, four weeks.

RESULTS

Viable counts of microbial groups enumerated during the experimental period are listed in Table 1. Count data were coded as discussed for Figure 1 where - H_2O refers to samples from Cub Creek and - S to samples from sediments. Microbial counts are expressed as colony forming units (CFU/g wet sediment or /ml creek water). Relative proportions of water to sediment for all samples are shown in Table 2. Intertidal sediments contained approximately twice the water content as sediments from mid- and back-marsh zones. Mean dry weight values were used to convert viable counts/g wet sediment to viable counts on a dry weight basis.

Sediment, air, and water temperatures for each sampling interval are depicted in Figure 2. Salinity and temperature data are listed in Table 3. Salinities were usually quite low during slack before flood except during periods of very low runoff. Periodic oscillations in temperature were evident with the highest temperatures generally occurring in September and the lowest in the months of November-December-January. The lowest temperatures recorded occurred in the winter of 1978 when the creek and sediments were completely frozen over. A consideration of Figure 2 suggests that water-sediment temperatures were usually more closely coupled and stable than air temperatures, the latter tended to exhibit greater relative changes.

Geometric mean values for microbial groups enumerated are listed by enclosure as CFU/g wet or CFU/g dry sediment in Tables 4 and 5, respectively. As can be seen, expression of counts on a dry weight basis resulted in greater relative increases of viable counts from intertidal sediments compared with mid- and back-marsh sediments.

Table 1. Values of selected microbial populations (C.F.U. /ml or /g wet sediment) in salt marsh waters and sediments following spillage of Weathered and Unweathered Louisiana crude oil. Samples, coded as in Figure 1, are: A = Unweathered oil treated, B = Weathered oil treated, C = Control, untreated.

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
9/22/75					
ADH_20	1.5E06	2.4E03	1.1E04	9.8E02	1.1E04
ADS	2.3E08	4.0E05	1.6E06	6.3E04	1.9E05
AES	4.6E08	8.8E05	3.2E06	1.3E05	6.0E05
AFS	3.8E08	1.8E05	2.8E06	1.1E05	1.4E05
CDH_20	2.6E05	1.1E03	2.3E03	1.8E02	1.5E03
CDS	2.0E08	3.1E04	1.2E06	2.4E04	1.4E04
C2S	7.1E08	2.2E05	3.1E06	1.1E05	2.5E04
C3S	3.4E08	1.3E05	3.2E06	1.8E05	2.4E05
9/23/75					
$A1H_20$	2.3E05	4.6E02	2.5E03	1.7E02	1.5E03
A1S	1.0E08	5.1E04	2.2E06	2.9E04	1.4E05
AES	4.8E08	1.7E05	3.6E06	1.6E05	1.8E07
AFS	2.7E08	3.7E05	4.0E06	5.6E04	6.0E04
$C1H_20$	2.5E05	4.6E02	7.0E02	5.8E02	1.1E04
C1S	1.8E08	3.2E04	4.5E06	3.6E04	8.8E04
CES	5.3E08	1.5E05	3.2E06	1.7E05	1.5E06
C3S	5.4E08	1.4E05	2.8E06	1.3E05	2.6E05
9/24/75					
$A1H_20$	3.4E05	2.4E03	2.2E03	1.7E02	2.1E03
A1S	3.1E08	3.2E06	2.6E06	9.5E04	6.4E04
AES	5.9E08	3.9E06	5.9E06	7.6E04	2.0E06
A3S	4.6E08	1.2E06	5.2E06	5.0E04	8.7E06
$C1H_20$	2.6E04	2.4E03	2.8E03	1.2E02	2.9E03
C1S	1.6E08	1.9E04	1.3E06	4.0E04	1.9E07
CES	4.0E08	6.6E04	3.5E06	4.1E04	2.0E04
CFS	5.3E08	1.2E06	2.7E06	7.7E04	1.3E05

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
9/25/75					
BDH_20	1.9E05	2.4E03	1.6E03	1.9E02	7.5E02
BDS	8.0E07	1.6E05	2.7E06	3.8E04	5.0E03
B2S	4.9E08	3.3E06	2.5E05	5.6E05	1.1E06
B3S	2.5E08	1.9E05	3.9E06	1.6E05	1.0E06
$C1H_20$	1.1E05	2.1E02	1.5E03	4.6E02	1.2E02
C1S	5.0E07	5.7E04	5.0E05	2.0E04	1.2E05
CES	1.4E08	8.8E04	2.4E06	6.7E04	2.0E06
C3S	1.8E08	7.1E04	3.6E06	8.3E04	1.9E04
9/26/75					
ADH_20	6.1E05	1.1E04	5.4E03	4.4E02	2.1E03
ADS	2.4E08	1.7E07	2.7E06	7.2E04	7.1E05
A2S	4.7E08	3.2E06	3.1E06	8.9E04	5.2E05
AFS	4.5E08	9.0E06	2.0E06	8.6E04	1.2E05
$B1H_20$	7.1E05	2.4E04	3.7E03	1.5E02	4.3E02
B1S	5.0E07	9.7E04	2.6E06	4.1E04	2.0E05
BES	4.9E08	1.7E06	1.2E06	6.1E04	1.6E05
BFS	3.9E08	2.9E05	4.5E06	8.6E03	1.5E05
CDH_20	1.7E05	1.1E04	2.4E03	1.9E02	4.6E03
CDS	3.6E08	4.9E04	2.9E06	6.2E04	4.2E04
CES	3.9E08	6.8E04	2.9E06	2.3E04	3.5E04
C3S	4.6E08	4.9E04	2.5E06	1.1E05	8.6E04

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
9/27/75					
$B1H_2O$	3.0E05	2.4E04	2.0E03	1.9E02	1.5E03
B1S	1.8E08	1.8E07	2.9E06	9.1E04	N.A.
B2S	8.2E08	1.9E07	2.6E06	4.2E05	1.2E05
B3S	N.A.	1.7E06	3.3E06	1.0E05	6.6E04
CDH_2O	6.7E04	2.3E02	1.2E03	3.6E01	4.3E02
CDS	4.3E08	1.3E05	2.9E06	3.8E04	3.7E04
CES	4.5E08	8.3E04	2.9E06	7.2E04	3.8E05
CFS	7.8E08	8.6E04	4.7E06	3.8E05	5.0E05
9/29/75					
BDH_2O	2.9E05	4.6E03	1.1E03	3.9E01	4.3E01
BDS	2.5E08	1.7E07	1.1E06	7.5E04	6.6E04
BES	4.6E08	3.9E06	1.5E06	3.9E04	1.3E05
B3S	5.2E08	2.0E07	5.9E06	8.7E04	1.3E05
CDH_2O	1.0E05	4.3E02	6.2E02	6.2E02	9.3E01
CDS	2.0E07	N.A.	3.0E04	9.1E02	2.5E03
CES	5.7E08	5.0E05	5.0E05	1.4E05	5.3E04
CFS	5.8E08	2.1E05	1.7E06	9.1E04	3.6E04

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
9/30/75					
A1H2O	1.7E05	4.3E02	1.5E03	5.2E01	2.3E02
A1S	5.2E08	1.8E07	2.3E06	1.1E05	1.8E05
A2S	6.4E08	9.4E06	3.6E06	5.5E04	3.3E04
A3S	9.0E08	1.0E07	2.2E06	5.3E04	2.2E05
C1H2O	4.1E05	7.5E02	2.0E03	9.4E01	2.9E02
C1S	N.A.	7.4E03	1.9E05	3.3E03	1.1E04
CES	5.8E08	2.1E05	3.6E06	6.9E04	2.6E04
CFS	5.5E08	9.5E04	5.4E06	6.3E04	9.4E05
10/3/75					
B1H2O	2.1E05	2.9E03	2.0E03	5.1E01	7.5E01
B1S	3.4E08	3.7E07	2.4E06	3.6E04	N.A.
B2S	3.0E08	2.6E06	6.5E06	2.7E04	8.2E05
BFS	4.1E08	1.7E08	3.7E06	3.3E04	1.0E04
C1H2O	6.1E05	1.5E03	7.6E03	1.6E02	3.9E01
C1S	2.4E08	8.2E05	1.6E06	4.8E04	2.0E04
C2S	3.2E08	5.7E04	3.6E06	4.3E04	2.6E04
CFS	5.3E08	8.6E04	3.6E06	9.0E04	2.1E04
10/8/75					
ADH2O	3.6E05	2.4E03	1.4E03	3.7E01	9.3E02
ADS	7.5E08	2.1E07	1.7E06	4.7E04	6.5E03
AES	2.1E08	1.3E07	5.3E06	5.5E04	2.0E05
AFS	1.9E09	6.3E07	5.4E06	4.6E04	1.3E06
C1H2O	2.7E05	9.3E02	2.0E03	1.5E02	7.5E01
C1S	2.8E08	8.3E04	1.5E05	2.1E04	8.1E03
CES	1.1E09	8.4E05	1.5E07	5.0E04	8.4E04
C3S	1.1E09	2.8E05	4.5E06	1.8E05	2.8E05
10/11/75					
BDH2O	3.3E05	1.5E03	1.9E03	1.9E02	1.5E02
BDS	2.8E08	3.2E07	2.4E06	7.8E04	6.5E03
BES	3.0E08	2.4E07	3.8E06	3.3E04	4.4E03
B3S	5.2E08	1.2E07	4.4E06	4.0E04	3.8E05
CDH2O	1.9E05	4.6E03	9.0E02	6.8E02	1.1E03
CDS	4.4E08	1.9E06	3.8E06	4.8E04	7.9E04
CES	6.5E08	3.6E05	5.0E06	1.4E05	7.8E04
CFS	4.7E08	9.5E04	1.0E07	6.2E04	2.1E05

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
10/24/75					
$A1H_20$	3.3E05	2.4E03	1.2E03	8.5E01	4.3E02
A1S	8.9E08	1.5E07	2.0E06	1.8E04	6.6E03
A2S	1.5E09	7.1E06	5.7E06	1.0E04	7.1E05
AFS	2.1E09	2.9E07	6.6E06	1.9E05	2.8E05
CDH_20	1.8E05	1.5E02	1.7E03	3.7E01	4.3E02
CDS	8.4E07	7.0E04	2.5E06	9.5E03	1.1E04
C2S	1.3E09	9.9E04	4.6E06	1.4E05	9.9E04
CFS	7.4E08	2.6E05	3.9E06	3.3E04	1.0E04
10/27/75					
BDH_20	1.6E05	2.4E03	3.0E03	7.8E02	2.3E02
BDS	4.6E08	8.1E07	2.0E06	1.8E05	2.9E04
B2S	6.0E08	2.2E07	5.5E06	2.4E05	8.5E05
B3S	5.7E08	2.0E07	6.2E06	1.3E05	1.9E04
$C1H_20$	1.8E05	4.3E02	1.9E03	7.3E02	2.3E02
C1S	3.6E08	1.1E05	2.6E06	7.2E04	1.7E05
CES	4.5E08	8.3E04	3.5E06	5.7E04	3.8E05
CFS	3.8E08	1.6E05	5.1E06	5.2E04	1.6E04
11/25/75					
$A1H_20$	1.6E05	1.5E03	2.5E03	2.8E02	4.3E02
A1S	3.3E08	2.0E07	2.2E06	7.7E04	7.4E03
AES	5.6E08	8.7E06	5.2E06	1.6E05	8.7E04
A3S	6.2E08	2.2E07	1.2E06	1.3E05	8.7E04
CDH_20	2.7E05	2.3E02	1.7E03	1.4E02	9.3E02
CDS	2.1E08	7.6E04	2.4E06	3.6E04	7.4E03
C2S	9.2E08	3.9E05	4.4E07	4.8E04	3.9E05
CFS	6.3E08	1.7E06	4.6E06	1.3E05	5.4E04
11/25/75; 12/4/75*					
$B1H_20$	2.2E05	1.5E03	2.2E03	2.2E02	1.5E03
B1S	1.3E08*	3.9E07	4.1E06*	N.A.	3.0E03
B2S	6.6E08*	1.7E08	6.0E06*	N.A.	6.5E05
BFS	4.8E08*	3.7E06	1.8E07*	N.A.	6.4E04

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
2/25/76					
ADH_20	1.3E05	4.3E02	5.6E02	2.0E01	4.3E01
ADS	3.1E08	3.4E06	1.3E06	9.1E04	2.8E03
A2S	9.5E08	9.2E06	1.1E07	3.3E05	4.3E04
A3S	7.4E08	8.1E06	5.7E06	3.5E05	2.0E05
BDH_20	3.5E04	9.3E02	9.3E02	2.8E02	9.3E01
BDS	8.4E08	1.7E07	3.4E06	1.8E05	N.A.
B2S	1.0E09	1.0E08	6.7E05	7.5E05	1.1E05
BFS	1.3E09	9.6E07	5.3E06	5.0E04	1.1E05
CDH_20	5.9E04	4.3E01	9.0E02	1.0E01	3.6E00
CDS	5.6E08	1.9E06	2.7E06	6.7E04	3.7E06
C2S	7.8E08	8.6E05	7.5E06	9.0E04	3.6E05
CFS	7.8E08	2.1E06	8.6E06	1.9E05	1.4E05
5/18/76					
$A1H_20$	4.2E04	9.3E02	2.3E03	2.3E01	1.2E01
A1S	3.6E08	1.7E06	3.8E06	9.6E03	6.6E03
A2S	3.8E08	3.6E05	8.3E05	1.7E05	3.3E03
A3S	4.3E08	7.9E05	1.2E06	2.2E04	7.9E05
BDH_20	3.3E04	4.3E02	2.4E02	9.3E00	9.3E02
BDS	7.4E08	1.0E06	2.6E06	1.2E04	3.0E04
BES	5.8E08	3.8E06	2.4E06	2.9E04	3.8E04
BFS	1.1E09	3.4E07	7.8E06	9.3E04	1.1E05
CDH_20	3.5E04	4.3E02	1.7E02	1.0E01	9.3E02
CDS	3.7E08	1.6E05	1.3E06	4.1E04	2.0E04
C2S	5.4E08	3.7E05	2.2E06	2.3E04	8.1E04
CFS	6.3E08	3.9E05	2.9E06	6.7E04	3.9E04
8/3/76					
ADH_20	9.2E05	4.3E02	3.7E03	8.6E01	4.3E02
ADS	6.1E08	1.1E07	3.2E06	3.6E04	1.1E05
AES	6.3E08	8.9E05	3.8E06	4.5E04	2.2E04
A3S	1.4E09	2.9E06	1.0E07	4.2E04	9.3E04
BDH_20	1.4E06	2.3E02	3.3E03	3.0E01	3.9E02
BDS	4.7E08	1.2E07	3.3E06	2.7E04	7.6E04
BES	8.9E08	4.1E07	9.0E06	1.4E05	1.4E05
BFS	9.8E08	8.2E05	4.7E06	2.0E04	8.2E04
$C1H_20$	1.5E06	2.3E02	2.3E03	4.7E01	7.5E01
C1S	3.4E08	1.7E05	3.7E06	2.2E04	3.1E04
C2S	5.8E08	4.7E04	8.4E06	6.9E04	3.8E04
C3S	6.2E08	5.1E04	8.3E06	6.3E04	8.9E04

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
10/29/76					
A1H_2O	5.5E04	7.5E02	2.1E03	6.3E01	4.3E02
A1S	8.4E08	1.7E07	1.7E07	5.1E04	1.0E05
A2S	1.0E09	2.2E07	1.1E07	4.3E05	8.5E04
A3S	1.9E09	2.6E06	6.3E06	5.9E05	8.4E04
BDH_2O	4.5E04	4.3E02	1.0E03	6.7E01	7.5E01
BDS	7.1E08	3.3E06	4.0E05	5.7E04	5.5E03
BES	8.3E08	4.9E05	7.3E06	1.3E05	3.2E04
BFS	8.5E08	8.3E06	9.8E06	6.5E04	4.1E06
C1H_2O	4.1E04	2.3E02	1.7E03	2.5E02	2.1E02
C1S	3.7E08	5.2E05	4.8E06	4.4E04	5.2E04
C2S	4.7E08	1.9E05	2.8E06	1.6E05	1.6E04
CFS	7.5E08	1.9E04	3.0E06	5.7E04	3.5E04
1/27/77					
A1H_2O	1.2E05	N.A.	1.4E03	5.6E01	4.3E01
A1S	7.2E07	N.A.	5.0E05	2.6E03	I.D.
B1H_2O	1.9E05	N.A.	1.2E03	9.4E01	2.3E01
B1S	8.1E07	N.A.	8.9E05	6.8E03	3.5E04
C1H_2O	1.0E05	N.A.	8.0E02	1.1E02	9.1E00
C1S	4.6E07	N.A.	7.3E04	2.9E03	2.6E03
3/24/77					
ADH_2O	1.5E05	4.3E02	8.0E02	5.5E01	2.1E02
ADS	4.7E08	3.6E07	1.3E06	7.2E03	7.3E03
AES	1.6E09	6.1E07	1.7E06	2.9E05	5.2E04
A3S	1.6E09	2.9E07	1.7E07	2.0E05	1.1E06
B1H_2O	1.5E05	1.5E03	1.3E03	4.2E01	7.5E02
B1S	8.0E07	3.4E07	8.8E05	5.8E03	2.2E03
B2S	5.7E08	4.3E07	1.6E07	2.8E05	4.0E05
BFS	2.4E09	1.5E07	1.6E07	2.8E05	4.4E05
CDH_2O	1.7E05	7.5E02	1.6E03	6.9E01	4.3E01
CDS	2.3E08	1.7E05	7.0E05	1.4E04	1.7E04
CES	1.2E09	2.2E07	2.5E07	2.6E05	2.2E06
C3S	1.2E09	7.4E05	1.3E07	3.1E05	1.5E05

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
5/24/77					
A1H2O	4.0E05	1.1E04	1.7E03	1.4E01	7.5E01
A1S	1.4E08	8.8E07	2.6E06	4.5E03	2.9E03
A2S	9.2E07	3.9E05	5.8E04	2.5E05	3.6E03
A3S	6.9E08	4.7E05	3.9E06	1.8E04	8.5E03
BDH2O	6.6E05	2.4E03	1.6E03	2.8E01	7.5E01
BDS	7.3E07	6.0E06	1.3E06	3.1E03	1.9E04
B2S	1.9E09	6.9E06	2.0E07	4.8E04	3.6E05
B3S	1.8E09	1.1E06	1.2E06	7.3E04	7.4E04
CDH2O	3.7E05	2.4E03	3.6E03	2.1E01	4.3E01
CDS	1.5E08	5.8E05	1.4E06	4.7E04	1.8E04
C2S	1.2E09	2.7E06	1.8E07	9.9E04	1.1E05
C3S	3.3E08	2.2E06	1.4E07	2.2E06	4.1E05
7/25/77					
A1H2O	3.1E05	4.6E03	2.2E03	1.7E01	9.3E01
A1S	1.4E08	≥2.0E08	8.3E05	3.5E03	7.7E04
AES	1.8E08	6.3E06	2.9E07	3.1E04	6.3E04
AFS	3.2E08	3.5E06	~3.2E06	1.8E05	3.5E04
B1H2O	7.4E05	≥2.4E04	1.0E04	2.3E01	4.3E01
B1S	3.0E08	2.1E07	2.4E06	4.6E03	3.8E04
BES	1.3E08	1.6E05	1.8E07	1.2E05	6.3E04
B3S	3.1E09	≥1.4E08	5.9E06	5.7E04	≥1.4E06
CDH2O	1.1E06	4.6E03	1.3E04	2.5E01	1.5E02
CDS	1.2E08	2.0E05	1.2E06	5.1E03	4.0E04
CES	2.2E08	4.4E05	8.3E06	2.3E05	1.4E05
C3S	4.5E08	2.7E05	~1.3E07	2.3E05	9.5E04
9/19/77					
ADH2O	1.3E06	2.3E03	1.0E04	1.2E02	N.D.
ADS	1.3E08	7.9E06	3.3E06	8.0E03	N.D.
AES	3.4E08	1.2E05	~2.3E06	2.9E05	N.D.
AFS	3.6E08	3.4E06	2.7E06	1.9E05	N.D.
B1H2O	2.0E06	4.3E03	~1.0E04	4.9E01	N.D.
B1S	7.1E07	1.4E06	2.1E06	9.0E03	N.D.
B2S	1.1E09	4.7E06	~6.6E06	4.1E04	N.D.
BFS	1.9E08	3.7E06	6.5E06	3.0E04	N.D.
C1H2O	1.2E06	4.3E03	~3.0E04	4.3E01	N.D.
C1S	2.0E08	3.7E05	4.7E06	9.5E03	N.D.
CES	1.3E08	4.2E05	2.3E06	2.8E04	N.D.
C3S	1.3E08	4.7E05	6.2E06	1.1E05	N.D.

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
12/02/77					
A1H$_2$0	2.5E05	4.3E03	4.6E03	1.2E02	4.6E02
A1S	4.9E08	4.1E07	8.2E06	1.2E04	2.1E05
AES	4.2E09	2.5E06	9.7E06	5.1E05	≥2.6E06
AFS	4.8E08	4.7E06	1.4E06	3.5E04	1.7E05
B1H$_2$0	1.2E05	4.3E03	6.0E03	2.0E02	1.1E03
B1S	1.1E08	6.2E06	1.4E06	1.8E04	3.8E05
B2S	3.7E08	9.3E06	5.0E06	1.6E05	2.4E05
BFS	8.8E08	7.8E07	1.7E06	4.3E05	4.1E05
C1H$_2$0	1.1E05	4.3E03	2.2E03	8.6E01	4.6E02
C1S	2.6E08	3.0E05	1.2E06	1.1E05	3.2E05
C2S	1.8E08	6.8E05	2.8E06	6.8E04	8.0E05
C3S	2.4E08	1.0E07	3.0E06	6.7E04	5.1E05
2/28/78					
A1H$_2$0	1.9E05	7.5E03	2.6E03	2.0E02	≥2.4E03
A1S	1.3E09	8.0E06	1.5E06	1.1E05	2.0E04
A2S	8.4E08	2.6E07	~4.4E06	5.1E05	1.7E04
AFS	5.6E08	5.1E07	8.1E06	1.5E05	1.0E05
BDH$_2$0	1.7E05	1.2E03	2.1E03	1.6E02	1.1E03
BDS	4.6E08	2.2E07	1.7E07	1.3E05	8.6E04
B2S	5.7E08	1.0E07	7.4E06	2.3E05	≥2.6E06
B3S	1.1E09	≥2.6E08	7.3E06	8.3E04	2.5E04
CDH$_2$0	2.1E05	1.5E03	3.0E03	1.7E02	≥2.4E03
CDS	2.7E08	3.1E06	7.2E05	5.5E04	5.4E04
CES	1.5E08	2.6E07	1.2E06	2.4E05	1.0E05
C3S	1.3E08	4.7E06	2.3E06	1.9E05	4.7E03
5/10/78					
A1H$_2$0	4.7E04	9.3E02	2.4E03	N.A.	1.1E03
A1S	1.7E08	7.3E06	1.2E05	N.A.	3.4E04
AES	6.3E08	1.7E06	7.5E06	N.A.	5.1E05
AFS	1.2E09	2.9E07	1.8E07	N.A.	1.3E06
B1H$_2$0	4.5E05	9.3E02	6.1E03	N.A.	≥2.4E03
B1S	9.0E07	1.7E06	1.3E06	N.A.	5.6E04
BES	4.7E08	2.2E07	3.9E06	N.A.	4.3E05
B3S	1.7E09	≥2.2E08	~2.7E07	N.A.	4.2E05
CDH$_2$0	4.5E04	4.2E03	2.8E03	N.A.	>2.4E03
CDS	6.9E08	3.1E05	2.7E06	N.A.	3.7E05
CES	2.9E08	8.8E05	4.2E06	N.A.	8.8E04
CFS	4.8E08	8.2E05	4.6E06	N.A.	1.1E05

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
7/12/78					
ADH2O	7.3E05	9.3E02	6.6E03	2.0E02	2.4E02
ADS	1.4E08	1.3E07	1.1E06	2.3E04	6.3E03
AES	5.1E08	3.4E05	4.2E06	1.3E05	7.3E04
A3S	1.0E09	2.6E06	6.4E06	2.3E05	1.1E05
B1H2O	1.2E06	9.3E02	6.1E03	1.4E02	4.6E02
B1S	1.3E07	3.1E05	1.3E05	3.5E03	2.8E02
BES	4.9E08	2.5E05	8.6E06	1.7E05	5.0E04
B3S	6.1E08	4.7E06	1.9E07	7.4E04	5.0E05
CDH2O	2.2E06	4.3E03	8.1E03	1.6E02	2.1E02
CDS	5.3E08	7.0E05	9.2E06	7.6E04	4.7E04
C2S	1.6E08	2.3E05	7.6E06	8.4E04	5.0E04
CFS	2.1E08	4.7E05	6.6E06	2.2E04	8.2E04
9/8/78					
ADH2O	3.1E05	1.5E04	5.9E03	2.0E01	2.3E01
ADS	1.2E08	9.0E06	1.1E06	2.3E04	4.2E04
AES	1.6E08	4.2E05	1.3E07	2.3E04	1.5E05
AFS	3.6E08	4.2E06	2.8E07	1.8E05	2.3E05
BDH2O	5.1E05	4.3E03	2.6E03	4.2E01	2.4E02
BDS	5.2E08	7.9E06	9.4E06	1.9E04	7.9E04
BES	8.2E08	1.7E06	1.0E07	1.1E05	1.7E05
BFS	6.9E08	3.4E06	2.6E06	4.3E03	1.1E04
C1H2O	6.9E05	4.3E03	1.2E04	2.0E01	9.3E01
C1S	2.0E08	3.5E05	1.7E05	1.2E04	2.1E04
C2S	5.8E08	1.1E05	9.2E06	4.3E04	2.5E05
CFS	4.1E08	7.1E05	1.1E06	2.4E04	2.2E04
12/6/78					
C1H2O	2.2E04	9.3E02	1.1E03	3.3E01	4.3E01
C1S	7.5E07	2.1E05	1.1E06	6.2E04	3.9E03
C2S	1.4E08	4.3E05	4.8E06	8.5E04	9.2E03
CFS	1.0E08	2.2E05	1.1E06	2.1E05	7.7E03
B1H2O	3.8E04	4.3E02	9.8E02	3.6E01	9.3E01
B1S	2.0E07	1.2E06	2.4E05	3.9E03	3.5E03
BES	2.3E08	2.0E05	5.1E06	9.4E04	2.0E05
BFS	3.3E08	1.7E05	2.8E06	4.7E04	8.3E04
ADH2O	2.2E04	3.9E02	1.1E03	8.3E01	4.6E02
ADS	1.7E07	3.9E06	1.8E05	8.5E03	2.1E03
AES	4.6E08	9.7E05	3.7E06	2.4E05	3.0E04
A3S	4.5E08	2.3E06	9.2E06	8.8E06	2.4E05

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
3/5/79					
ADH2O	7.7E04	4.3E02	3.4E03	4.0E02	4.6E02
ADS	2.3E08	3.4E07	4.8E06	7.4E04	3.2E04
AES	3.1E08	4.3E05	7.8E06	1.3E05	1.5E05
AFS	4.9E08	7.4E05	9.0E06	1.8E05	4.5E05
BDH2O	1.2E05	2.3E03	2.3E03	3.1E02	1.1E03
BDS	2.8E08	2.6E06	2.8E06	1.7E05	5.6E04
B2S	4.5E08	1.6E06	1.6E07	6.7E04	1.1E05
B3S	5.8E08	4.1E06	3.6E06	7.9E04	2.3E05
CDH2O	1.4E05	7.5E02	1.7E03	2.0E02	4.6E02
CDS	2.3E08	3.1E05	3.2E06	7.1E04	1.1E05
C2S	4.1E08	4.6E05	4.9E06	7.3E04	6.9E04
CFS	3.4E08	4.0E06	5.7E06	3.8E04	7.0E04
5/17/79					
ADH2O	1.9E05	9.3E02	4.1E03	1.3E02	1.5E03
ADS	2.8E08	1.8E06	2.3E06	5.0E04	3.4E04
A2S	4.9E08	1.4E06	1.0E06	5.8E04	2.8E06
A3S	3.3E08	2.8E06	4.3E06	1.5E05	2.8E05
B1H2O	1.2E05	1.5E03	4.2E03	1.7E02	4.3E03
B1S	7.9E08	6.7E05	5.7E06	4.3E05	3.1E05
B2S	9.4E08	5.0E06	1.9E07	4.6E05	1.6E06
BFS	9.8E08	2.9E06	5.3E06	5.3E04	3.2E05
CDH2O	1.6E05	4.3E03	4.6E03	3.6E02	4.3E03
CDS	6.6E08	3.5E05	6.6E06	2.9E04	1.2E05
C2S	9.9E08	2.2E06	7.8E06	1.7E04	7.8E04
CFS	1.4E08	4.8E06	8.3E06	3.2E05	1.1E05
8/29/79					
ADH2O	3.0E05	2.3E03	4.4E03	3.6E01	9.3E01
ADS	2.5E08	7.7E05	4.4E06	9.1E03	9.9E04
A2S	4.6E08	2.0E06	7.5E06	1.6E05	1.8E05
AFS	4.3E08	1.2E06	4.3E06	2.1E04	2.0E05
BDH2O	7.3E05	2.3E03	7.0E03	1.8E01	1.5E02
BDS	1.9E08	1.9E07	1.3E04	8.8E03	3.5E04
BES	5.5E08	1.4E06	1.0E07	4.2E04	2.7E05
B3S	7.7E08	7.7E05	5.3E06	5.0E04	9.5E05
C1H2O	4.2E05	2.3E03	3.6E03	1.6E01	2.3E01
C1S	3.7E08	4.0E05	1.7E06	3.5E06	8.5E05
C2S	3.3E08	2.2E05	5.5E06	5.5E04	1.2E05
C3S	6.0E08	2.7E06	9.5E06	5.7E04	2.7E05

Table 1. Continued

Date and Station	Heterotrophic Bacteria	Petroleum Degrading Bacteria	Chitinolytic Bacteria	Fungi	Cellulytic Bacteria
10/26/79					
ADH2O	2.0E05	2.3E04	2.7E03	5.2E01	2.1E03
ADS	2.5E08	2.0E07	4.3E06	9.0E04	1.7E05
A2S	4.6E08	1.7E07	6.5E06	1.8E05	2.5E05
AFS	5.2E08	4.6E07	2.5E06	1.7E05	9.2E05
BDH2O	1.6E05	4.3E04	6.8E02	2.6E01	9.3E01
BDS	2.8E08	1.7E07	8.7E05	3.3E04	3.4E05
B2S	5.5E08	3.5E07	3.6E06	5.2E04	7.1E05
BFS	7.5E08	2.0E07	3.4E06	4.1E04	1.2E05
C1H2O	1.2E05	9.3E03	7.8E02	4.3E01	1.5E04
C1S	2.5E08	2.0E07	6.0E06	7.0E04	1.8E04
CES	2.6E08	1.6E06	1.6E06	3.1E04	6.6E04
CFS	3.5 08	9.6E06	3.1E06	5.2E04	9.6E04

Table 2

Mean values for the expression $(1 - \frac{\text{sediment dry wt.}}{\text{sediment wet wt.}} \times 100)$ by zone for all enclosures.

	Zone								
	Intertidal			Midmarsh			Backmarsh		
	A	B	C	A	B	C	A	B	C
n	23	23	30	22	22	29	22	22	29
$\bar{X}$	38.8	36.7	35.5	18.7	19.4	19.9	16.0	22.1	19.4
SD	± 3.6	± 5.4	± 6.9	± 4.1	± 4.5	± 3.8	± 4.7	± 5.3	± 5.2

Table 3

Temperature (°C) and Salinity (°/oo) Data

Sampling Date	Temperature			Salinity Enclosures		
	Air	Water	Sediment	A	B	C
09-22-75	N.A.	N.A.	N.A.	0.0	0.0	-
09-23-75	24.0	21.5	22.0	4.0	1.0	-
09-24-75	30.0	23.0	26.0	0.0	0.0	-
09-25-75	29.0	24.0	27.0	0.0	0.0	-
09-26-75	29.0	24.0	27.0	2.0	2.0	2.0
09-27-75	22.0	23.0	23.0	-	0.0	0.0
09-29-75	24.5	22.0	23.0	-	0.0	0.0
09-30-75	30.0	23.0	26.0	0.0	0.0	-
10-03-75	22.0	21.0	21.0	1.0	1.0	-
10-08-75	17.0	17.0	16.0	1.0	1.0	-
10-11-75	25.0	20.0	20.0	-	1.0	1.0
10-24-75	19.0	18.0	17.0	2.0	2.0	-
10-27-75	14.0	15.0	15.0	0.0	0.0	-
11-25-75	12.0	9.0	9.0	6.0	6.0	6.0
02-25-76	22.0	13.0	15.0	2.0	2.0	2.0
05-18-76	24.0	23.0	23.0	2.0	2.0	2.0
08-03-76	24.0	23.0	23.5	N.A.	N.A.	N.A.
10-29-76	8.0	5.0	4.0	N.A.	N.A.	N.A.
01-27-77	12.0	2.0	3.0	1.5	0.2	0.2
03-24-77	14.0	10.0	9.0	4.0	3.0	5.5
05-24-77	23.5	23.0	21.0	8.0	7.0	6.0
07-25-77	N.A.	N.A.	N.A.	12.0	12.0	12.0
09-19-77	31.0	26.0	25.0	15.0	16.0	16.0
12-02-77	17.0	10.0	11.0	N.A.	N.A.	N.A.
02-28-78	3.0	1.5	1.5	4.0	2.0	3.0
05/10/78	25.0	17.5	17.5	0.5	0.5	0.5
07-12-78	25.0	25.0	24.0	7.0	7.0	6.0
09-08-78	26.0	27.0	26.5	10.0	10.0	10.0
12-06-78	10.5	11.0	9.0	N.A.	N.A.	N.A.
03-05-79	20.0	12.0	15.0	2.0	2.0	2.0
05-17-79	21.0	19.0	20.0	1.8	1.8	1.8
08-29-79	21.0	19.0	20.0	10.0	10.0	10.0
10-26-79	9.0	12.0	9.0	4.0	4.0	4.0

Table 4

Geometric Means of Microbial Counts in Wet Sediments (/g) and Creek Waters (/ml) from API Spill Enclosures through Period September 1975 - October 1979

Enclosure	Heterotrophic Bacteria	Petroleum-Degrading Bacteria	Chitinoclastic Bacteria	Cellulytic Bacteria	Fungi
A-Unweathered Oil Spill					
Water	5.3 ± 0.5	3.3 ± 0.5	3.4 ± 0.3	2.5 ± 0.7	1.9 ± 0.5
Intertidal	8.4 ± 0.4	6.9 ± 0.7	6.3 ± 0.4	4.5 ± 0.7	4.4 ± 0.5
Mid-Marsh	8.7 ± 0.3	6.4 ± 0.7	6.6 ± 0.5	5.2 ± 0.9	5.1 ± 0.4
Back-Marsh	8.8 ± 0.3	6.7 ± 0.7	6.6 ± 0.4	5.3 ± 0.6	5.0 ± 0.4
B-Weathered Oil Spill					
Water	5.4 ± 0.5	3.4 ± 0.6	3.5 ± 0.6	2.5 ± 0.6	1.9 ± 0.5
Intertidal	8.3 ± 0.5	6.8 ± 0.8	6.2 ± 0.6	4.4 ± 0.8	4.4 ± 0.6
Mid-Marsh	8.8 ± 0.2	6.7 ± 0.8	6.7 ± 0.5	5.3 ± 0.6	5.0 ± 0.4
Back-Marsh	8.9 ± 0.3	6.9 ± 0.9	6.8 ± 0.3	5.2 ± 0.7	4.8 ± 0.4
C-Control, No Spill					
Water	5.3 ± 0.5	3.0 ± 0.6	3.3 ± 0.4	2.4 ± 0.8	2.0 ± 0.5
Intertidal	8.3 ± 0.3	5.4 ± 0.7	6.1 ± 0.6	4.7 ± 0.9	4.5 ± 0.6
Mid-Marsh	8.6 ± 0.3	5.6 ± 0.7	6.7 ± 0.3	5.0 ± 0.6	4.8 ± 0.4
Back-Marsh	8.6 ± 0.3	5.6 ± 0.7	6.7 ± 0.3	4.9 ± 0.6	5.0 ± 0.6

Table 5

Geometric means of microbial counts in sediments (expressed on a dry sediment basis (/g) and creek waters (/ml)) for API spill enclosures through period September 1975 - October 1979.

Enclosure	Heterotrophic Bacteria	Petroleum-Degrading Bacteria	Chitinoclastic Bacteria	Cellulytic Bacteria	Fungi
A-Unweathered Oil Spill					
Water	5.3 ± 0.5	3.2 ± 0.5	3.4 ± 0.3	2.5 ± 0.7	1.9 ± 0.5
Intertidal	8.6 ± 0.4	7.1 ± 0.8	6.5 ± 0.4	4.7 ± 0.7	4.6 ± 0.5
Mid-Marsh	8.8 ± 0.3	6.5 ± 0.7	6.7 ± 0.5	5.3 ± 0.9	5.2 ± 0.4
Back-Marsh	8.9 ± 0.3	6.7 ± 0.7	6.8 ± 0.4	5.5 ± 0.6	5.1 ± 0.4
B-Weathered Oil Spill					
Water	5.4 ± 0.5	3.4 ± 0.6	3.5 ± 0.6	2.5 ± 0.6	1.9 ± 0.5
Intertidal	8.5 ± 0.5	6.9 ± 0.8	6.4 ± 0.6	4.6 ± 0.8	4.6 ± 0.6
Mid-Marsh	8.8 ± 0.2	6.8 ± 0.8	6.8 ± 0.4	5.4 ± 0.6	5.1 ± 0.4
Back-Marsh	9.0 ± 0.3	7.0 ± 0.9	6.8 ± 0.3	5.3 ± 0.7	5.0 ± 0.4
C-Control, No Spill					
Water	5.3 ± 0.5	3.0 ± 0.6	3.3 ± 0.4	2.4 ± 0.8	2.0 ± 0.5
Intertidal	8.5 ± 0.4	5.5 ± 0.8	6.4 ± 0.6	4.9 ± 0.8	4.6 ± 0.5
Mid-Marsh	8.7 ± 0.3	5.7 ± 0.7	6.8 ± 0.4	5.1 ± 0.6	4.9 ± 0.5
Back-Marsh	8.7 ± 0.3	5.7 ± 0.7	6.8 ± 0.4	5.0 ± 0.6	5.1 ± 0.4

Densities of heterotrophic, petroleum-degrading and chitinoclastic bacteria, and fungi in the water column were generally three logs units smaller than corresponding densities in sediments. Cellulytic bacteria were 100X greater in intertidal and 1000X greater in mid- and back-marsh sediments compared with the water column. Heterotrophic bacteria were the most abundant group in the water column being 100X greater than petroleum-degrading or chitinoclastic bacteria. Cellulose-degrading bacteria and fungi were approximately 1000X less abundant in creek waters than heterotrophic bacteria. Standard deviations of the means were generally equal to $\pm$ 0.5 log units for bacterial groups except cellulose-decomposers which exhibited the largest standard deviations in both sediment and water column samples. Mean values for all microbial groups except petroleum-degrading bacteria were generally within $\pm$ 0.1 log units comparing oil polluted versus control creek zones. Mean counts of petroleum-degrading bacteria were larger in oil-polluted creek zones than the control creek zone.

Mean values of viable sediment populations increased when expressed on a dry weight basis. Comparison of mean viable count data from intertidal sediments with those from mid- and back-marsh sediments revealed that intertidal microbial populations were generally smaller. An obvious exception to this statement was the greater numbers of petroleum-degrading bacteria found in intertidal rather than mid- and back-marsh sediments of both oil polluted enclosures.

Geometric means of viable count data for sediment microbes calculated on a yearly basis are shown in Table 6. With few exceptions, heterotrophic bacterial means were quite consistent from year to year

Table 6
Geometric means of viable microbial counts by year, zone, and enclosure

MICROBIAL GROUP: HETEROTROPHIC BACTERIA

Zone	Intertidal			Mid-marsh			Back-Marsh		
Enclosure	A	B	C	A	B	C	A	B	C
Year									
1975	8.7 ± 0.3	8.5 ± 0.3	8.4 ± 0.4	8.8 ± 0.2	8.8 ± 0.2	8.8 ± 0.2	8.9 ± 0.3	8.7 ± 0.1	8.8 ± 0.2
1976	8.9 ± 0.2	9.0 ± 0.1	8.8 ± 0.1	8.9 ± 0.2	9.0 ± 0.1	8.8 ± 0.1	9.1 ± 0.3	9.1 ± 0.1	8.9 ± 0.1
1977	8.5 ± 0.3	8.2 ± 0.2	8.4 ± 0.3	8.8 ± 0.7	8.8 ± 0.5	8.7 ± 0.5	8.8 ± 0.3	9.2 ± 0.5	8.6 ± 0.4
1978	8.4 ± 0.7	8.2 ± 0.7	8.6 ± 0.4	8.7 ± 0.3	8.8 ± 0.2	8.4 ± 0.3	8.9 ± 0.2	8.9 ± 0.3	8.4 ± 0.3
1979	8.6 ± 0.0	8.7 ± 0.3	8.7 ± 0.2	8.7 ± 0.1	8.9 ± 0.1	8.7 ± 0.3	8.7 ± 0.1	8.9 ± 0.1	8.6 ± 0.3

MICROBIAL GROUP: PETROLEUM-DEGRADING BACTERIA

Zone	Intertidal			Mid-marsh			Back-Marsh		
Enclosure	A	B	C	A	B	C	A	B	C
Year									
1975	6.2 ± 2.4	7.1 ± 1.1	5.1 ± 0.6	6.6 ± 0.6	7.1 ± 0.7	5.3 ± 0.4	6.8 ± 0.9	6.8 ± 0.9	5.3 ± 0.4
1976	7.0 ± 0.5	6.9 ± 0.6	5.8 ± 0.5	6.5 ± 0.8	7.1 ± 1.0	5.5 ± 0.5	6.5 ± 0.4	6.5 ± 0.4	5.3 ± 0.9
1977	7.9 ± 0.5	7.1 ± 0.5	5.3 ± 0.7	6.4 ± 1.1	6.8 ± 0.9	6.3 ± 0.7	6.7 ± 0.6	6.7 ± 0.6	6.2 ± 0.6
1978	7.1 ± 0.2	6.6 ± 0.7	5.9 ± 0.5	6.2 ± 0.8	6.3 ± 0.9	6.0 ± 0.9	7.0 ± 0.6	7.0 ± 0.6	6.0 ± 0.5
1979	7.0 ± 0.8	6.9 ± 0.7	6.2 ± 0.9	6.4 ± 0.7	6.7 ± 0.7	6.0 ± 0.5	6.6 ± 0.8	6.6 ± 0.8	6.8 ± 0.2

MICROBIAL GROUP: CHITIN-DEGRADING BACTERIA

Zone	Intertidal			Mid-marsh			Back-marsh		
Enclosure	A	B	C	A	B	C	A	B	C
Year									
1975	6.5 ± 0.1	6.6 ± 0.6	6.4 ± 0.6	6.7 ± 0.1	6.7 ± 0.2	6.7 ± 0.4	6.6 ± 0.3	6.8 ± 0.2	6.7 ± 0.2
1976	6.8 ± 0.5	6.5 ± 0.5	6.6 ± 0.3	6.7 ± 0.5	6.6 ± 0.5	6.7 ± 0.3	6.7 ± 0.4	6.9 ± 0.2	6.8 ± 0.3
1977	6.5 ± 0.4	6.3 ± 0.2	6.1 ± 0.6	6.5 ± 1.0	7.1 ± 0.3	7.0 ± 0.5	6.7 ± 0.4	6.7 ± 0.5	7.1 ± 0.4
1978	5.9 ± 0.5	6.4 ± 0.9	6.3 ± 0.7	6.9 ± 0.2	6.9 ± 0.2	6.7 ± 0.3	7.1 ± 0.3	7.0 ± 0.5	6.5 ± 0.4
1979	6.8 ± 0.1	6.0 ± 1.2	6.8 ± 0.3	6.7 ± 0.4	7.1 ± 0.3	6.7 ± 0.3	6.7 ± 0.2	6.7 ± 0.1	6.9 ± 0.2

Table 6 (continued).

MICROBIAL GROUP: CELLULOSE-DEGRADING BACTERIA

Zone	Intertidal			Mid-marsh			Back-March		
Enclosure	A	B	C	A	B	C	A	B	C
Year									
1975	4.9 ± 0.8	4.5 ± 0.7	4.8 ± 0.9	5.8 ± 0.8	5.4 ± 0.8	5.2 ± 0.7	5.5 ± 0.7	5.1 ± 0.7	5.1 ± 0.6
1976	4.5 ± 0.8	4.6 ± 0.6	5.2 ± 1.0	4.4 ± 0.6	4.9 ± 0.3	4.9 ± 0.6	5.3 ± 0.5	5.5 ± 0.8	4.9 ± 0.3
1977	4.6 ± 0.9	4.7 ± 0.8	4.6 ± 0.8	5.0 ± 1.2	5.4 ± 0.4	5.7 ± 0.6	5.0 ± 0.9	5.7 ± 0.5	5.5 ± 0.3
1978	5.0 ± 0.6	4.3 ± 1.1	5.0 ± 0.7	5.0 ± 0.6	5.3 ± 0.8	4.9 ± 0.5	5.7 ± 0.6	5.0 ± 0.7	4.5 ± 0.6
1979	5.0 ± 0.4	5.3 ± 0.5	5.3 ± 0.7	5.7 ± 0.6	5.7 ± 0.5	5.0 ± 0.1	5.7 ± 0.3	5.6 ± 0.4	5.2 ± 0.3

MICROBIAL GROUP: FUNGI

Zone	Intertidal			Mid-marsh			Back-Marsh		
Enclosure	A	B	C	A	B	C	A	B	C
Year									
1975	5.0 ± 0.3	5.0 ± 0.3	4.5 ± 0.5	4.9 ± 0.4	5.1 ± 0.6	4.9 ± 0.3	5.0 ± 0.2	4.9 ± 0.5	5.1 ± 0.3
1976	4.8 ± 0.4	4.8 ± 0.5	4.8 ± 0.2	5.3 ± 0.4	5.2 ± 0.6	5.0 ± 0.4	5.1 ± 0.7	4.8 ± 0.3	5.0 ± 0.3
1977	4.0 ± 0.3	4.0 ± 0.3	4.4 ± 0.6	5.4 ± 0.5	5.1 ± 0.4	5.1 ± 0.5	5.0 ± 0.5	5.1 ± 0.5	5.5 ± 0.6
1978	4.6 ± 0.5	4.3 ± 0.7	4.8 ± 0.4	5.2 ± 0.6	5.3 ± 0.2	5.3 ± 0.5	5.4 ± 0.1	5.1 ± 0.4	4.9 ± 0.6
1979	4.8 ± 0.5	5.0 ± 0.8	4.9 ± 0.2	5.2 ± 0.2	5.1 ± 0.5	4.9 ± 0.5	5.1 ± 0.5	4.8 ± 0.1	5.0 ± 0.4

and exhibited the smallest standard deviations of all groups enumerated. Differences related to oil pollution in enclosure A and B were not apparent.

Mean petroleum-degrading bacterial densities were larger in oil-polluted enclosures compared with the control enclosure for all years except 1979. In 1979, densities of this group were actually lower in the back-marsh zones of oil polluted enclosures than in the control enclosure. Relatively large standard deviations for means of petroleum-degrading bacteria could be related to patchiness in the distribution of oil in the sediments, migration of the petroleum during tidal excursions, and through percolation of overlying water during low tide to the creek through the marsh. However, as large standard deviations also occurred in control populations, a more general mechanism perhaps related to season and temperature was suggested. Independent enumeration of the 5 subsamples normally pooled, provided a 95% confidence interval of $\pm$ 0.2 log units for the enumeration procedure.

Mean levels of petroleum-degrading bacteria were elevated approximately 100X in intertidal oil polluted sediments for three years after the spills. Mean values of petroleum-degrading bacteria in oil-polluted enclosures were generally larger in intertidal sediments compared with sediments from mid- and back-marsh zones. Note the increases in means of these bacteria in the control enclosure over the five year study interval. Petroleum-degrading bacterial levels in oil-polluted enclosures appeared to reach peak levels in either the second or third year of the study. Similar trends for heterotrophic bacteria in either control or oil-polluted enclosures were not observed.

Mean levels of chitinoclastic bacteria tended to be somewhat larger in mid- and back-marsh sediments than in intertidal sediments. Differences between mean values in oil polluted versus control sediments were not apparent.

Mean values of cellulose-decomposing bacteria tended to be larger in mid- and back-marsh sediments than intertidal sediments. Values of means as functions of oil pollution were not evident for intertidal sediments but means from mid- and back-marsh zones seemed somewhat greater than corresponding control values.

With the exception of 1975 (not actually a complete year), the only consistent trend discernible from means of fungal data was that levels in intertidal zone sediments were lower than levels in both oil-polluted and control sediments from mid- and back-marsh zones. Effects due to oil pollution were not evident.

All viable count data as a function of time are illustrated in Figures 3-22. A quasi-three dimensional graph format provides for simultaneous comparison of counts from a given zone for all three enclosures. These figures provide additional detail to augment the previous discussion and evidence for the occurrence of periodic changes in some viable count data at intervals reminiscent of periodic oscillations in ambient temperatures (Figure 2).

Periodic changes in heterotrophic bacterial densities in the water column appear at first glance related to temperature fluctuations. Densities were similar for all creek enclosures over the experimental period. Definition of periodic changes exhibited by water column viable count data tend to become less pronounced moving from the creek to intertidal sediments and then to mid-marsh and back-marsh

sediments. Thus, periodicity visible in the intertidal data curves is less evident in curves from mid- and back-marsh sediments. The relative consistency and uniformity of heterotrophic populations, especially in mid- and back-marsh sediments, is quite striking. Heterotrophic bacterial counts in oil-polluted back-marsh sediments appeared somewhat greater than those in the control enclosure.

Viable counts of petroleum-degrading bacteria in creek waters graphically depicted two trends. Firstly, an increase in levels of petroleum-degrading bacteria occurred in creek waters of oil-polluted enclosures during the initial days of each spill. This increase was maintained for approximately 160 days post spill. Thereafter, counts in oil-polluted creek waters declined. The second trend began approximately January, 1977 when plots of viable counts displayed periodicities reminiscent of the temperature curves. Whereas viable counts in mid- and back-marsh zones returned to levels similar to control sediments (note final control levels were actually greater than at the start), intertidal sediment counts from oil polluted enclosures were still elevated compared to the control at the last sampling.

Plots of chitinoclastic bacterial counts did not display obvious or consistent differences when control and oil-polluted enclosure data were compared. Although counts from the unweathered oil polluted mid- and back-marsh zones appeared uniformly larger compared with the other enclosures, the significance of this observation requires statistical analysis. Periodicities in the viable count curves, especially in the creek zones and somewhat less pronounced in the intertidal zones, were observed.

Plots of cellulose-decomposing bacteria from creek zones revealed rather well defined periodicities. Similar periodicities were visible for intertidal, mid- and back-marsh zones. Periodic changes in cell densities were best defined for this microbial group. Differences in viable counts related to oil addition were not observed for water or intertidal zones. However, counts in back-marsh zones of oil-polluted enclosures appeared larger than in the control enclosure.

Differences in viable counts of fungi from creek samples due to oil addition were not observed. However, count plots suggested greater fungal densities in oil polluted intertidal and mid-marsh sediments during the initial 160 day post spill period. Counts also appeared relatively elevated in unweathered oil polluted mid- and back-marsh zone sediments during 1978-1979.

Periodic changes in viable count plots appeared similar in appearance (shape and number of occurrences) to periodic seasonal changes in sediment, air, and water temperature plots. Closer examination revealed that sediment temperature and viable count data plots did not generally correspond by superimposition. Viable counts tended to reach and maintain maximum values 2-3 months after seasonal temperature maxima despite substantial drops in ambient temperatures. Thus, cellulose degrading bacterial densities in mid- and back-marsh sediments reached maximum values 2-3 months after the seasonal temperature maximum in September of 1977. Similar observations of an asynchrony of temperature and viable counts were observed for other microbial groups when periodicities were evident. An analysis of

of representative data using conventional regression analysis failed to reveal a significant linear relationship of viable counts and temperature.

Viable count data from sediments were further analyzed using several non-parametric tests. Two basic approaches were employed. The first was to determine if counts in sediment zones within an individual enclosure were statistically uniform (e.g., intertidal A vs. mid-marsh A vs. back-marsh A). In the second, counts from similar zones were tested for uniformity by comparison of all three enclosures (e.g., intertidal A vs. intertidal B vs. intertidal C).

Results from testing for uniformity of microbial count data within each enclosure are given in Table 7. Although the value of H for heterotrophic bacteria in enclosure A was less than the critical value required to reject H_o at $\alpha = 0.05$, this value was so close (H_o could be rejected at $\alpha = 0.07$) that nonuniformity of heterotrophic counts was suspected. Therefore, viable counts of heterotrophic bacteria were considered non-uniform within all enclosures. Similarly, chitinoclastic bacterial and fungal counts were significantly non-uniform within each enclosure.

Values of H indicated cellulytic bacterial counts were uniform in the control ($H = 3.66$ corresponds to an α of approximately 0.18) and non-uniform in both oil-polluted enclosures. Values of H for petroleum-degrading bacteria indicate non-uniformity in the unweathered oil polluted enclosure and uniformity for both control and weathered oil polluted enclosures.

Those microbial groups indicated as non-uniformly distributed within an enclosure were further tested to determine which zones

Table 7

Results of Kruskal-Wallis one-way analysis of variance by ranks to compare viable bacterial counts from all zones within each enclosure. Value of statistic H corrected for ties.

H_o: Microbial counts are similar in all zones

H_1: Microbial counts are not similar in all zones

Enclosure	MICROBIAL GROUP				
	Heterotrophic Bacteria	Petroleum-Degrading Bacteria	Chitinoclastic Bacteria	Cellulytic Bacteria	Fungi
A	5.45	10.67*	7.06	10.72*	17.36*
B	18.12*	0.53	16.63*	14.87*	8.44*
C	8.95*	1.69	11.71*	3.66	17.64*
Creek Water	1.14	3.12	1.42	2.07	0.03

*H_o rejected if $H \geq 5.99$ for $\alpha = 0.05$

Table 8

Values (z) of Mann-Whitney statistic comparing viable microbial counts from two zones within the same enclosure. Counts expressed as counts/g dry wt (sediment).

H_o: counts come from same population H_1: counts come from different populations

Enclosure	Zones Compared	Heterotrophic Bacteria	Petroleum-Degrading Bacteria	Cellulytic Bacteria	Chitinoclastic Bacteria	Fungi
		MICROBIAL GROUPS				
	x y					
	Intertidal vs mid-marsh	-1.83*	+3.24*	-2.05*	-2.56*	-3.77*
A	Intertidal vs back-marsh	-2.54*	+1.99*	-3.29*	-2.18*	-3.43*
	Mid-marsh vs back-marsh	-0.49	-1.33	-1.20	+0.07	0.82
	x y					
	Intertidal vs mid-marsh	-2.95*		-3.60*	-3.30*	-2.86*
B	Intertidal vs back-marsh	-4.00*		-3.09*	-3.75*	-1.82*
	Mid-marsh vs back-marsh	-1.85*		0.63	-0.27	+1.21
	x y					
	Intertidal vs mid-marsh	-2.19*		-1.90*	-2.97*	
C	Intertidal vs back-marsh	-2.36*		-1.50	-3.07*	
	Mid-marsh vs back-marsh	-0.04		0.68	-0.30	

*Reject H_o and accept H_1 if absolute values of $z \geq 1.64$ at significance level of $\alpha = 0.05$
Negative value indicates $\bar{y} > \bar{x}$, + value indicates $\bar{x} > \bar{y}$.

were dissimilar. Application of the Mann-Whitney statistic yielded the results shown in Table 8.

In all enclosures heterotrophic bacterial counts from intertidal sediments were significantly different from mid- and back-marsh zones. Negative values indicated counts were larger in the latter zones. Viable heterotroph counts from mid- and back-marsh zones could not be distinguished in enclosures A and C but were dissimilar in B. A negative value indicated viable counts were significantly larger in the back-marsh zone for B.

Viable counts of petroleum-degrading bacteria from intertidal sediments of enclosure A were significantly greater than either mid- or back-marsh zone counts.

Similarly, counts of cellulose-decomposing bacteria from intertidal zones of all enclosures were significantly smaller than counts from mid- and back-marsh zones. Counts from mid- and back-marsh zones were not dissimilar. Patterns of viable count data for both cellulose-decomposing bacteria and fungi were similar to those for cellulose-decomposing bacteria.

Counts in similar zones from all three enclosures were tested for uniformity. Results of Kruskal-Wallis testing are shown in Table 9. Of immediate attention are the very large values of H for petroleum-degrading bacteria in all zones. These values were significant at $p > 0.001$. Heterotrophic bacterial counts were similar in intertidal and mid-marsh zones but significantly dissimilar in back-marsh zones. Cellulose-decomposing bacteria would be dissimilar in the back marsh zones using an α of 0.10 rather than 0.05.

Viable count-zone combinations considered significantly non-uniform were further tested using the Mann-Whitney statistic (Table 10). As expected, viable counts of petroleum-degrading bacteria in enclosures A and B were significantly larger than control counts. Heterotrophic counts in back-marsh zones of enclosures A and B were also significantly larger than control counts. Similarly, viable counts of cellulose-decomposing bacteria in oil polluted enclosures were significantly different from control counts in back-marsh (A and B) and mid-marsh (B) zones. Finally, fungi in the intertidal zone of enclosure A exhibited significantly larger counts than those in the control.

Mann-Whitney statistic results are also shown for creek water zones. Significant values of z allowing rejection of the null hypothesis were only obtained for petroleum-degrading bacteria.

Table 9

Kruskal-Wallis one way analysis of variance testing

H_o: Microbial viable counts are the same in a given zone for all three enclosures

H_1: Microbial viable counts are not the same in a given zone for all three enclosures

	Microbial Group Enumerated				
Zones	Heterotrophic Bacteria	Petroleum-Degrading Bacteria	Chitinoclastic Bacteria	Cellulytic Bacteria	Fungi
Intertidal	-0.21	39.9*	0.16	0.4	0.1
Mid-Marsh	2.15	28.1*	1.93	3.1	3.0
Back-Marsh	9.37*	27.2*	1.12	5.1*	-2.3

*For df = 2, H_o can be rejected for $\alpha = 0.05$ when $H \geq 5.99$, H_o can be rejected at $\alpha = 0.10$ when $H \geq 4.60$

Table 10

Values of the Mann-Whitney Statistic testing

H_o: Microbial viable counts are the same in sediments from similar zones in oil polluted and control enclosures.

H_1: Microbial viable counts are different in sediments from similar zones in oil polluted and control enclosures

for viable counts from sediment zones indicated by the Kruskal-Wallis test as significantly non-uniform by comparison of all three enclosures. The Mann-Whitney statistic is also calculated for viable count data for creek zones shown to be significantly non-uniform.

	MICROBIAL GROUP							
	Heterotrophic Bacteria		Petroleum-Degrading Bacteria		Cellulose-Decomposing Bacteria		Fungi	
Zones	Enclosures	z*	Enclosures	z*	Enclosures	z*	Enclosures	z*
Creek			CvsA	-1.50				
			CvsB	-1.86*				
Intertidal			CvsA	-5.58*				
			CvsB	-5.13*				
Mid-Marsh			CvsA	-3.85*	CvsA	-0.25	CvsA	-1.84*
			CvsB	-4.09*	CvsB	1.93*	CvsB	-0.92
Back-Marsh	CvsA	-3.23*	CvsA	-4.19*	CvsA	-2.55*	CvsA	0.07
	CvsB	-1.52	CvsB	-4.54*	CvsB	-1.80*	CvsB	1.39

*Values of z must be $\geq \pm 1.64$ for rejection of H_o at $\alpha = 0.05$

DISCUSSION

Viable counts of selected microbial groups were monitored in salt marsh waters and sediments to evaluate the effects of crude oil spillage on microbial biomass. Microbial groups were selected on the basis of their significance to mineralization processes in the marsh, e.g., cellulose and chitin degrading bacteria, and their anticipated responses to the presence of spilled petroleum.

Despite the surfeit of reports affirming the ubiquitous existence of a subset of heterotrophic bacteria capable of degrading petroleum hydrocarbons, knowledge of the effects of petroleum on the in situ activities and/or biomass of salt marsh microbial populations is extremely limited (2). Various reports in the literature (6,7,23) have suggested that petroleum (in vivo) is either toxic or can inhibit bacterial chemotaxis and/or mineralization processes. However, few controlled in situ experiments of extended duration exist where responses of microbial biomass or activities have been determined. Although hastened participation in the spillage experiments which are the basis of this report precluded development and incorporation of activity measurements, monitoring of selected microbial viable counts have provided unique information on the responses of salt marsh microbial populations to spillage of South Louisiana crude oil.

A number of basic observations may be drawn from this intensive study. Firstly, the biomass of autochtonous mesophilic heterotrophic bacteria capable of degrading petroleum hydrocarbons increased rapidly following spillage of crude oils in enclosures A and B. Secondly, these responses were maintained over an extended

interval of time measured in years. If it is acknowledged that such differential responses constituted measures of microbial populations active on petroleum and derived substrates, then it is not unreasonable to conclude that such elevated microbial counts were evidence for the continued presence of petroleum hydrocarbons in those sediments. Visual observations of oil leaching from sediments and personal communications with Dr. Bieri indicated that hydrocarbons derived from the petroleum were present in marsh sediments during a significant portion of the experimental period and that the rapid loss of aromatic and normal alkanes from the marsh was followed by an unresolved envelope of undoubtedly great complexity and lack of detail. Hydrocarbons remaining in marsh sediments would be expected to adsorb to particulates, both inorganic and organic, be metabolized under favorable nutrient and oxygen regimes, be subject to bulk transport suspension and burial as functions of tidal currents and wind, and importantly, preserved under the anaerobic regime which characterizes salt marsh sediments. Burial of weathered petroleum in marsh sediments would result in decreased degradation since microbial hydrocarbon degradation is an obligately aerobic process (8,9). Therefore, with the exception of oxygen available at the Spartina root/sediment interface (20), degradation would be dependent on physical release and transport of oil from anaerobic sediments and oxygen availability at sediment/air interfaces especially during low tide when the sediments are exposed. Based on microbial viable count data, visual evidence of oil release and limited chemical analysis, it appeared that the marsh sediments served as sinks for crude oil, inhibiting the oxidation of oil due to the anaerobic sediment regime, and slowly releasing weathered oil during tidal excursions. Relative rates of oil release and transport from the sediments are unknown.

The comparative elevation of petroleum-degrading bacteria in the oil treated enclosures was particularly evident in sediments from the intertidal zone. How can this be explained considering the observations that oil was visually most evident in the back marsh zones? One explanation is that ebbing tidal flow carried adsorbed and/or bulk oil toward the creek as well as providing the height differential for flow or percolation of solutes through sediments (especially intertidal) to the lowered sea level. The intertidal zones would therefore be at the end of a gradient of petroleum hydrocarbons migrating creekward as well as the area where oil carried on the flooding tide would first impact.

Another explanation for the high levels of petroleum degrading bacteria is related to the fact that this zone contains "tall" Spartina plants while the mid- and back-marsh zones are characterized by "short" plants. Evidence by Valiela et al. (22) suggests that the tall farm flourishes due to the greater availability of inorganic nitrogen. This is related to the continual renewal of the sediment interstitial waters during tidal flushing, draining, and exposure of intertidal sediments to creek waters for the longest intervals. Observations that elevated levels of petroleum-degrading bacteria still exist in intertidal zone sediments suggest that weathered petroleum is still present in the marsh and the greater biomass of petroleum-degrading bacteria in such sediments could be due to this availability of inorganic nutrients and the continual renewal of oxygen in the interstitual waters.

Significant differences in microbial counts were observed when comparing zones within individual enclosures. With the notable exception of petroleum-degrading bacteria in the oil polluted enclosures, viable microbial counts were usually smaller within

intertidal sediments than in either mid- or back-marsh zones. Such non-uniformity could be related to the coarser grain size and the comparatively lower amounts of macro-organic matter present. Generally, microbial populations tend to be greater in sediments of finer grain size distributions compared with coarse (26). Finer sediments possess larger surface area and usually contain higher concentrations of organic matter. With certain exceptions, microbial counts in mid- and back-marsh zones were not significantly dissimilar. Such observations are not unexpected since both zones were uniformly covered with short Spartina and the same densely matted sediment carpet consisting of roots, rhizomes and blue green algae.

Heterotrophic bacterial counts in the back-marsh of enclosure B were significantly greater than in the mid-marsh zone. The back-marsh of this enclosure exhibited the most obvious and extensive Spartina damage due to smothering by the weathered petroleum. Elevated levels of heterotrophs were presumably due to the greater biomass of petroleum-degrading bacteria and the mineralization processes associated with the petroleum-killed Spartina. It is also possible that these responses were magnified in this enclosure owing to the larger amounts of weathered oil observed to remain in the back marsh, perhaps due to its greater "stickiness"; vicosity and decreased mobility.

Counts of cellulose decomposing bacteria were also lower in the intertidal zones of all enclosures. This is logical considering the obvious absence of densely matted sediments relatively lacking in macro-organic matter derived from Spartina rhizomes and roots, and again, the coarser grain size.

Petroleum-degrading bacteria were uniformly distributed within zones of enclosures B and C. However, the reasons for these distributions are different. Although counts were significantly larger in B, the distribution of such counts with the enclosure was uniform. This contrasts with enclosure A where counts were significantly greater in intertidal sediments. Such differences may be related to the lowered mobility of the weathered oil, resulting in a more gradual loss from the back marsh to mid-marsh and intertidal sediments over time or to a larger dosage of oil covering a larger area relative to the weathered oil. Unweathered oil was observed to be more mobile than the weathered oil and was visually lost from the marsh on ebbing tidal flow during early post-spill day. It must be realized, however, that in the absence of quantitative data on hydrocarbon concentrations in marsh sediments, speculation must be tempered by the complexity of the marsh-enclosure systems and the many possible variations in dosing, hydrodynamics, and weather.

Importantly, in the absence of spilled petroleum, counts of petroleum-degrading bacteria were uniformly distributed at lower levels in the control enclosure.

Observations related to differences in microbial activities and biomass within different zones have been previously mentioned. Such differences were related to the "type" of Spartina present, i.e. Spartina plants are characteristically "tall" in intertidal sediments and "short" in sediment zones which correspond to the mid- and back-marsh zones described in this report. Sherr and Payne (19) reported significant differences in microbial dentrification potential comparing sediments containing "short" and "tall" Spartina and discussed characteristic differences noted by other researchers for methane

evolution, total adenylates, vertical versus horizontal rhizome-root development patterns, and amount of subsurface macro-organic matter. Denitrification potential and several biomass parameters were seasonably variable in what correspond to mid- and back-marsh sediments but stable in the "tall" Spartina or intertidal sediment. Interestingly, microbial parameters appeared to couple more closely to seasonal Spartina growth patterns than to temperature (19). Our data distinctly revealed that mesophilic microbial counts tended to lay behind temperature. This effect was especially evident with cellulytic bacteria and may be a similar phenomenon related to the seasonal production of organic matter by Spartina.

Comparisons of similar zones in all three enclosures indicated highly significant responses of petroleum-degrading bacteria. Although these responses were anticipated their duration was not. Increases in viable counts of petroleum-degrading were also reflected in the significantly elevated levels of heterotrophic bacteria in the back-marsh zones of enclosures A and B. Similarly, the relatively greater numbers of cellulose-decomposing bacteria in the mid- and back-marsh zones of the oil polluted enclosures were most logically attributable to death caused by oil smothering and acute toxicity. Fungi, also recognized as cellulose-decomposings, were significantly elevated in the mid-marsh zone of enclosure A.

Effects on microbial biomass related to the potential toxicity of the crude oils spilled were not observed. On the basis of viable count data analysis, significant short or long term reductions in bacterial populations involved in the mineralization of the biopolymers chitin or cellulose were not observed. This contrasts with observations reported for in vivo batch culture experiments (using South Louisiana

crude oil) where petroleum was observed to engender a reduction in the relative numbers of cellulytic, chitinalytic, and lipid-degrading bacteria (23).

Significant changes of viable counts for microbial populations assayed during this experiment were logical and were interpreted as responses to the addition of an allochthonous substrate (petroleum) or mineralization due to damage to existing biomass (Spartina cellulose). Although no significant reduction of biopolymer degrading populations occurred (in fact enhancement was observed), it must be cautioned that biomass measurements are indirectly related to microbial activity and that the effects of petroleum on complex and interrelated processes were not determined. However, within these limitations, comparisons of viable count data obtained under the experimental conditions employed, indicated microbial population's assayed were not detrimentally affected by the spillage of South Louisiana crude oil in the salt marsh and that the use of petroleum-degrading bacteria has provided a sensitive measure of the presence and apparent longevity of petroleum-hydrocarbons in marsh sediments.

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Figure 1

SAMPLING ZONES

CREEK and INTERTIDAL – 1 or D
MID MARSH – 2 or E
BACK MARSH – 3 or F

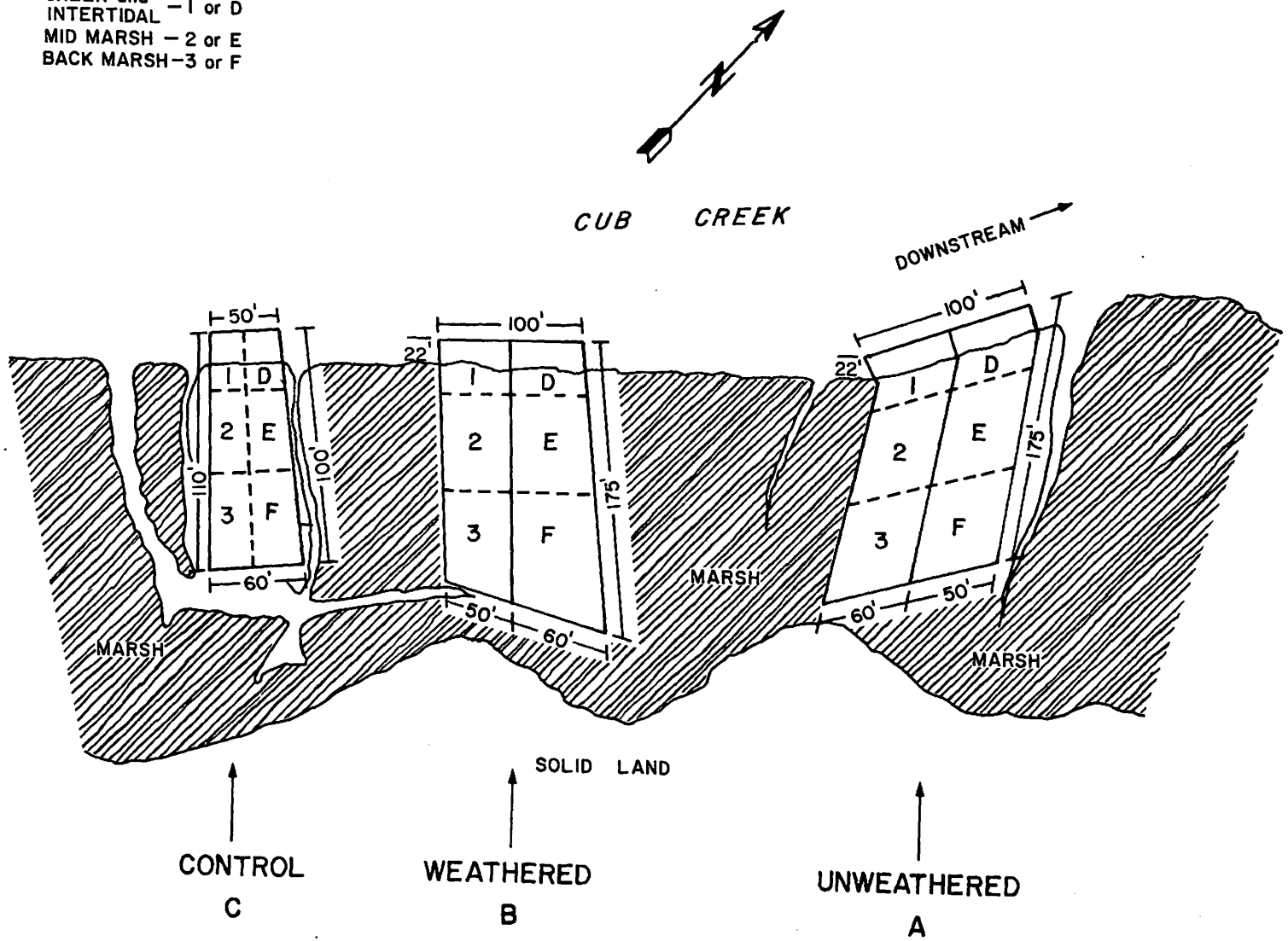
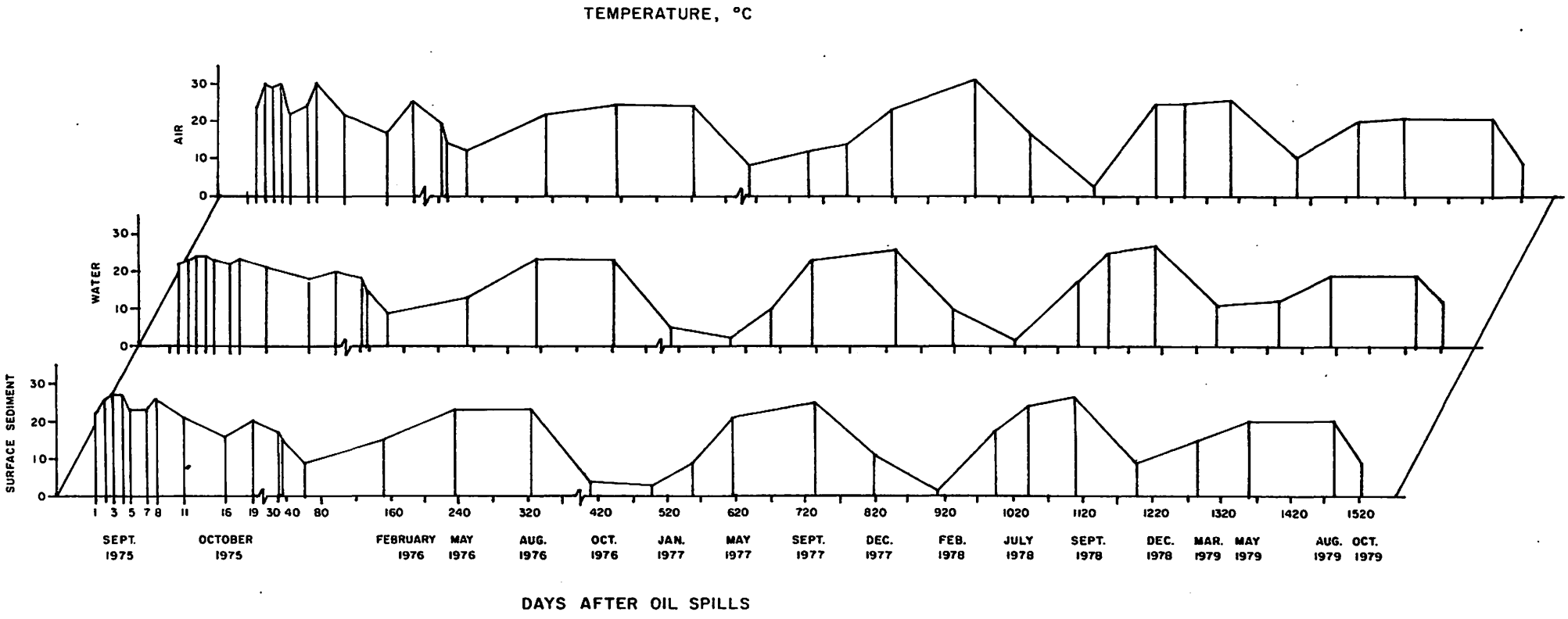


Figure 2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20
10
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SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG. 1976
OCT. 1976
JAN. 1977
MAY 1977
SEPT. 1977
DEC. 1977
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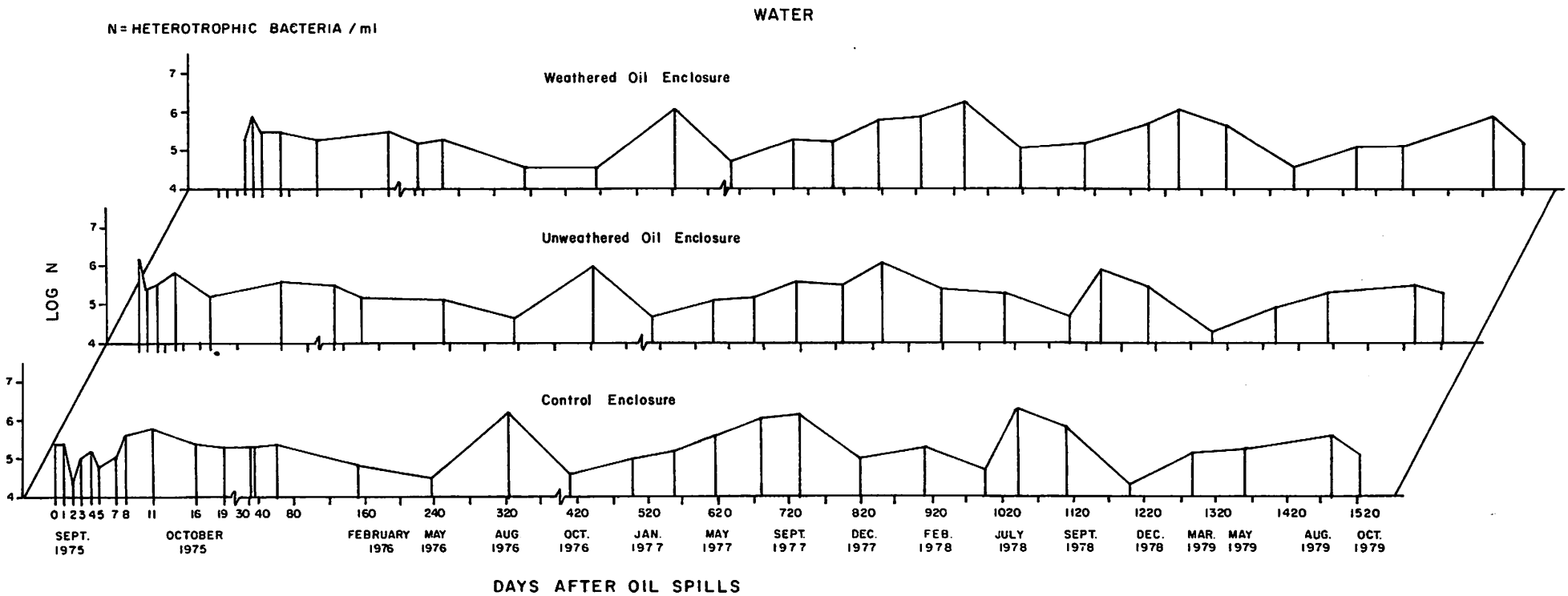
DEC. 1978
MAR. 1979
MAY 1979
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 3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Unweathered Oil Enclosure
Control Enclosure
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6
5
4
0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG 1976
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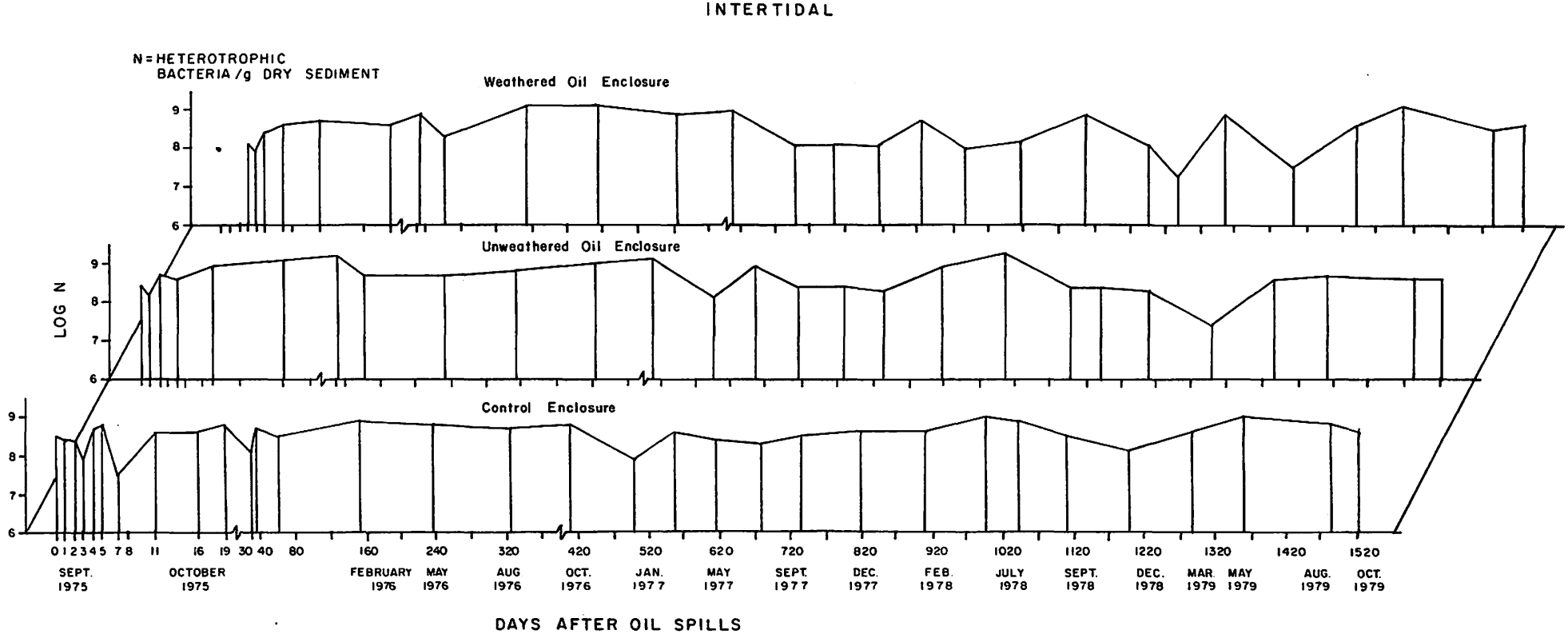
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 4


INTERTIDAL
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Weathered Oil Enclosure
Unweathered Oil Enclosure
Control Enclosure
LOG N
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8
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6
0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG 1976
OCT. 1976
JAN. 1977
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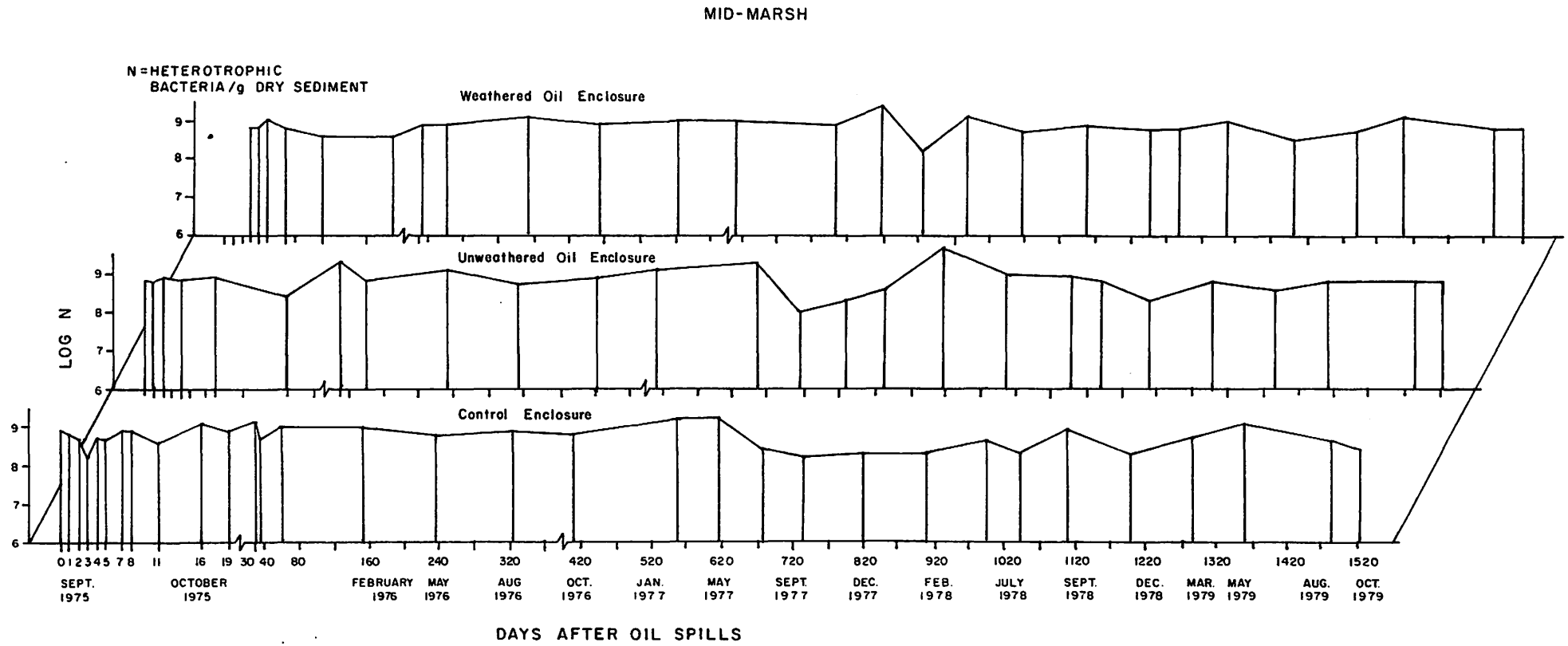
MAY 1979
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 5


MID-MARSH
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Weathered Oil Enclosure
Unweathered Oil Enclosure
Control Enclosure
LOG N
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8
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0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
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OCT. 1976
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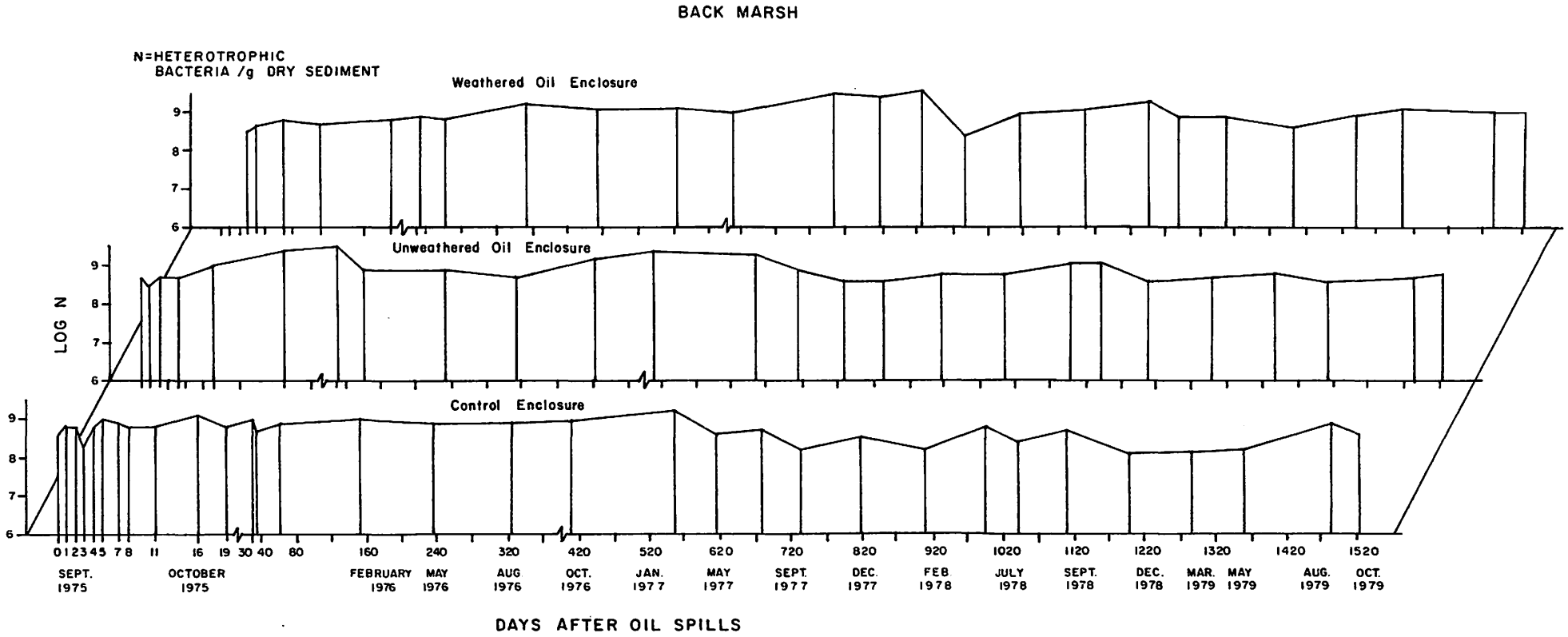
MAY 1979
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 6


BACK MARSH
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Unweathered Oil Enclosure
Control Enclosure
LOG N
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6
0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG 1976
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JAN. 1977
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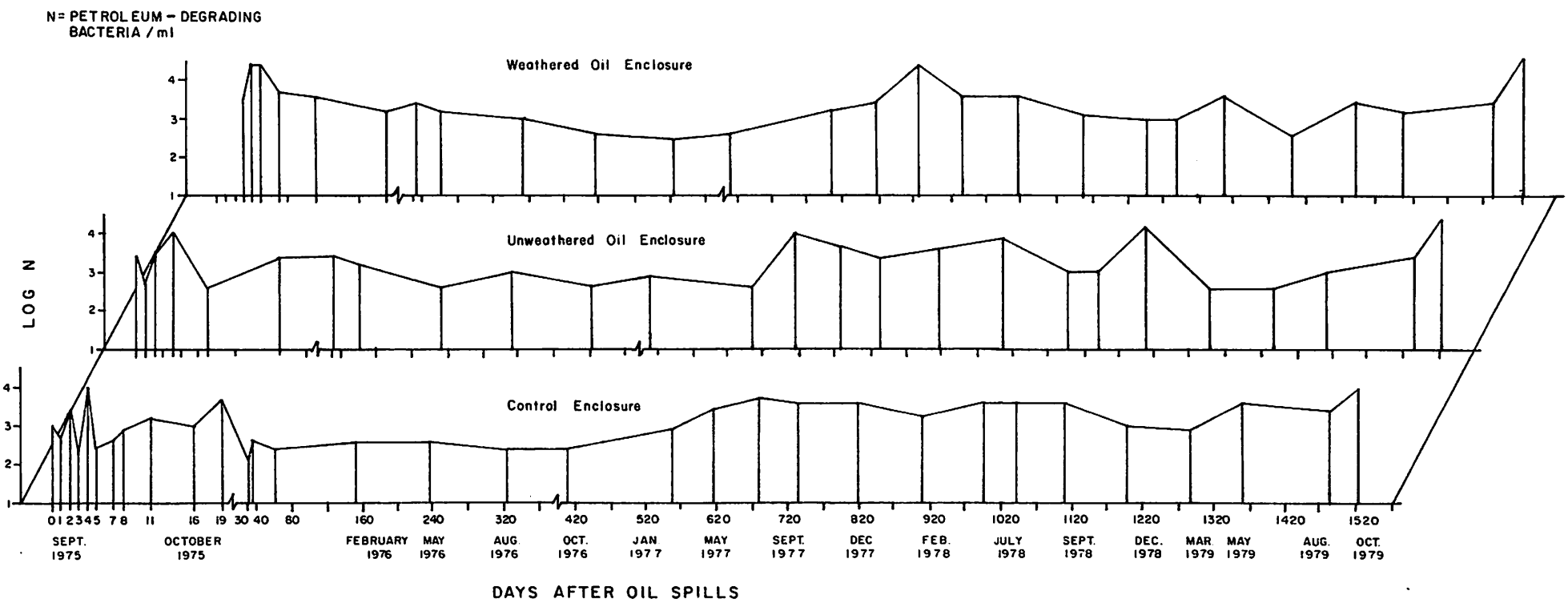
SEPT. 1977
DEC. 1977
FEB 1978
JULY 1978
SEPT. 1978
DEC. 1978
MAR. 1979
MAY 1979
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

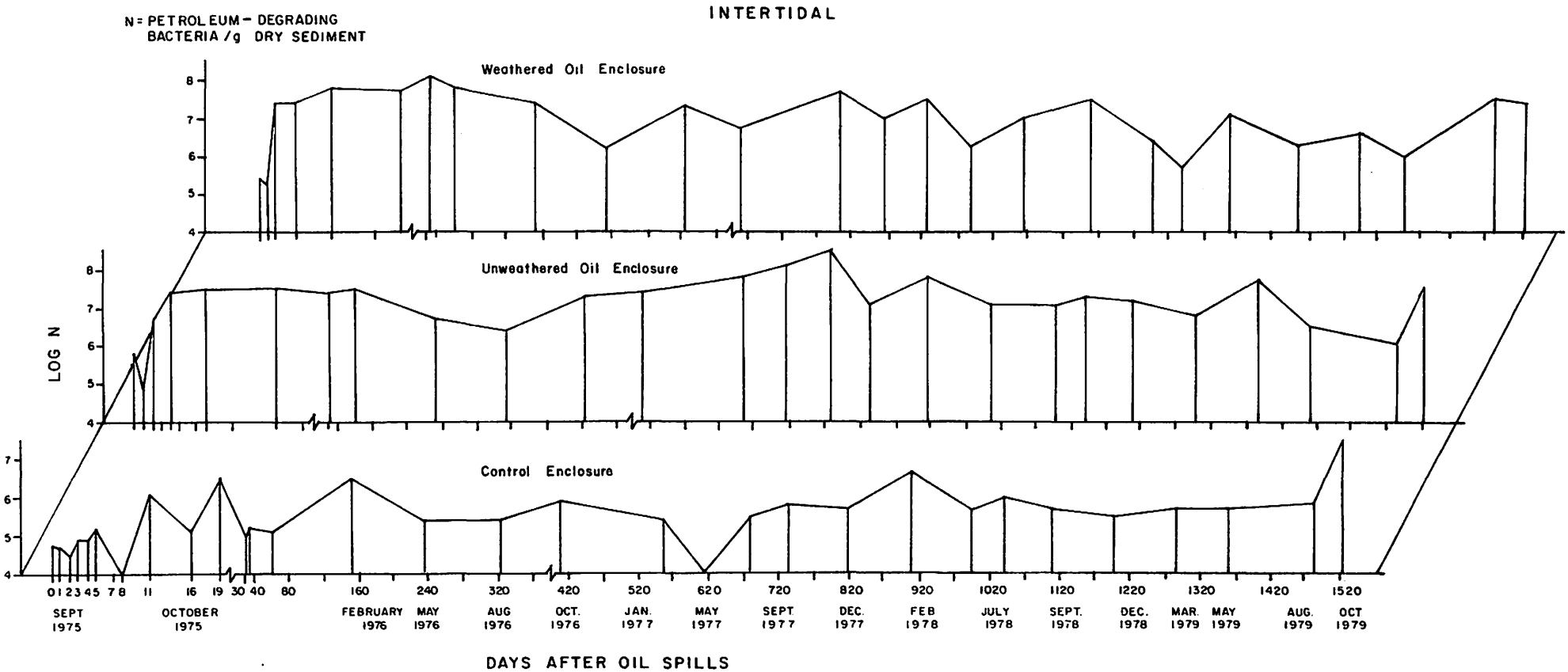
Figure 7

WATER



III-56

Figure 8


INTERTIDAL
N = PETROLEUM – DEGRADING BACTERIA / g DRY SEDIMENT
Weathered Oil Enclosure
Unweathered Oil Enclosure
Control Enclosure
LOG N
8
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0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG 1976
OCT. 1976
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MAY 1977
SEPT 1977
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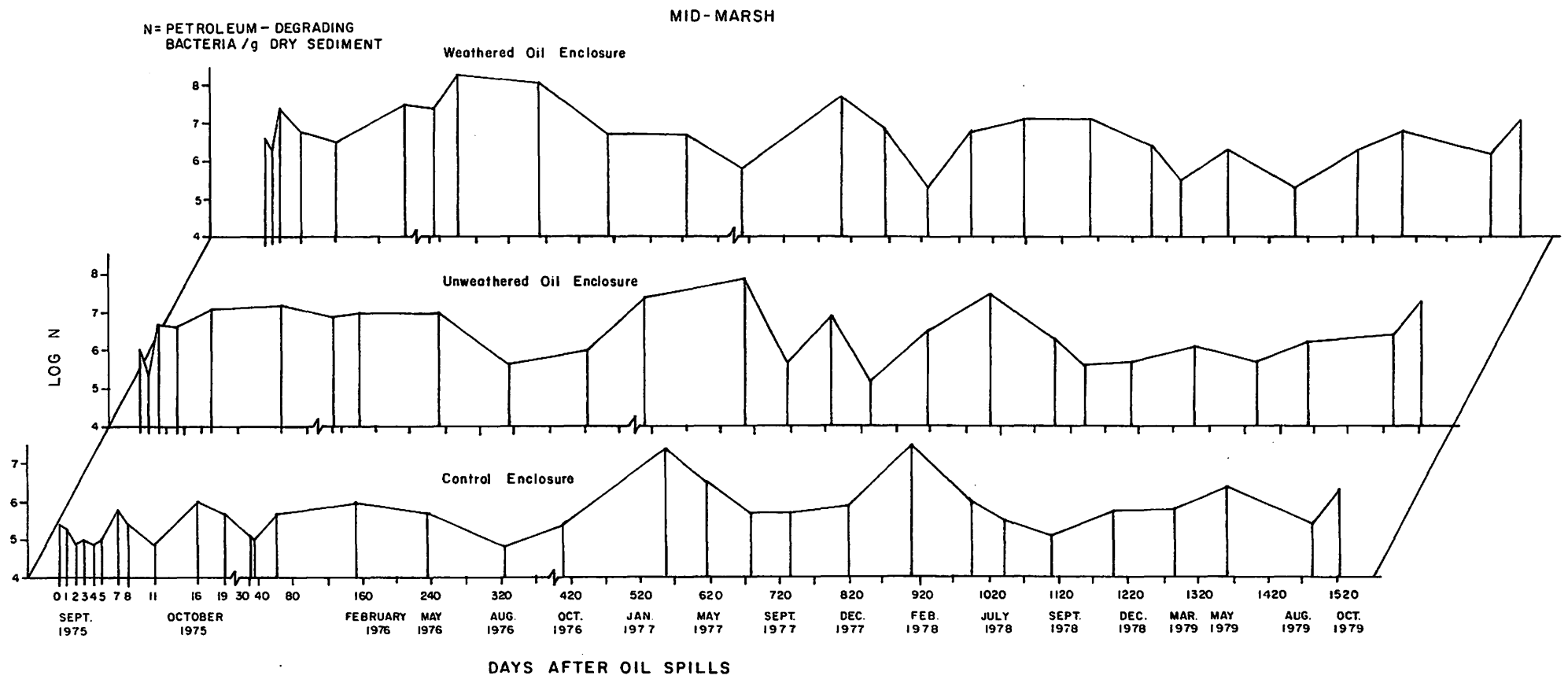
OCT 1979
DAYS AFTER OIL SPILLS

Figure 9


MID-MARSH
N= PETROLEUM - DEGRADING BACTERIA /g DRY SEDIMENT
Weathered Oil Enclosure
Unweathered Oil Enclosure
Control Enclosure
LOG N
4
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0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG. 1976
OCT. 1976
JAN. 1977
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SEPT. 1977
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MAR. 1979
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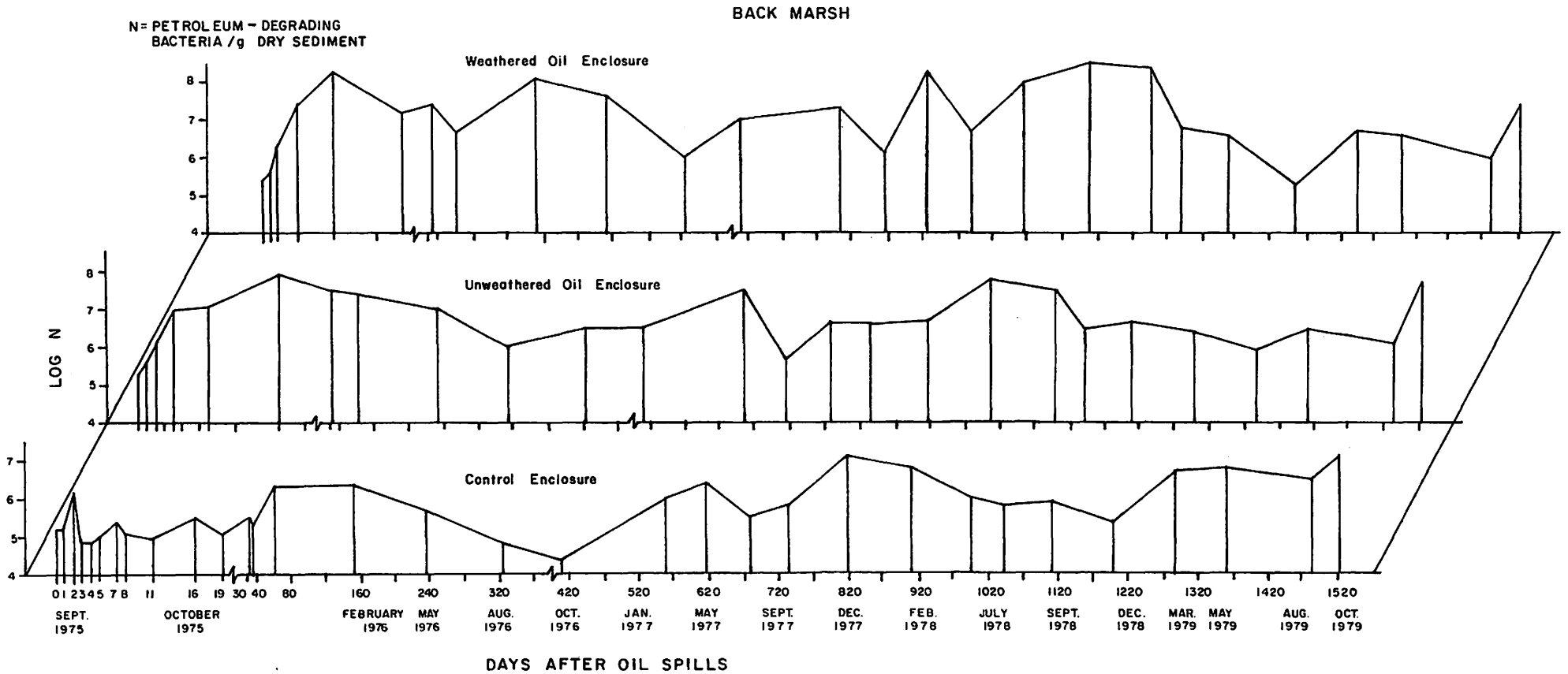
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 10


BACK MARSH
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Unweathered Oil Enclosure
Control Enclosure
LOG N
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0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG. 1976
OCT. 1976
JAN. 1977
MAY 1977
SEPT. 1977
DEC. 1977
FEB. 1978
JULY 1978
SEPT. 1978
DEC. 1978
MAR. 1979
MAY 1979
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 11

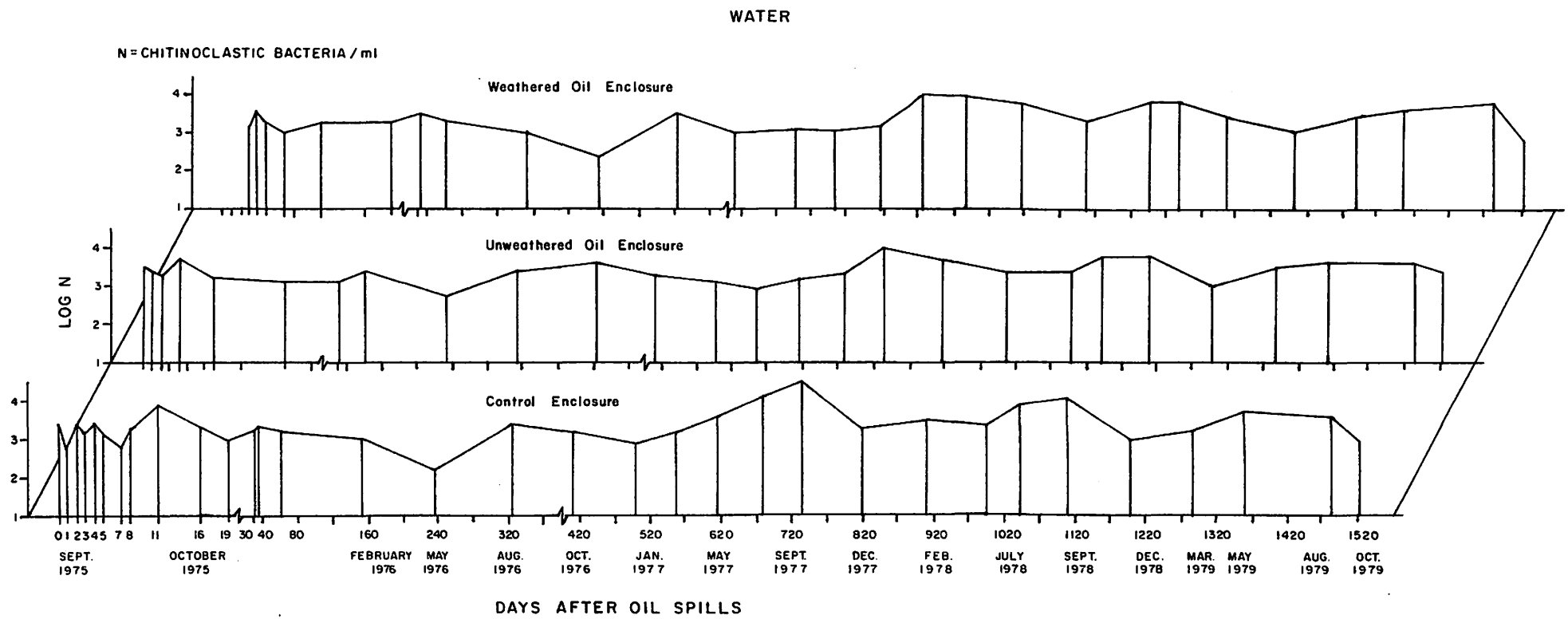

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Figure 12

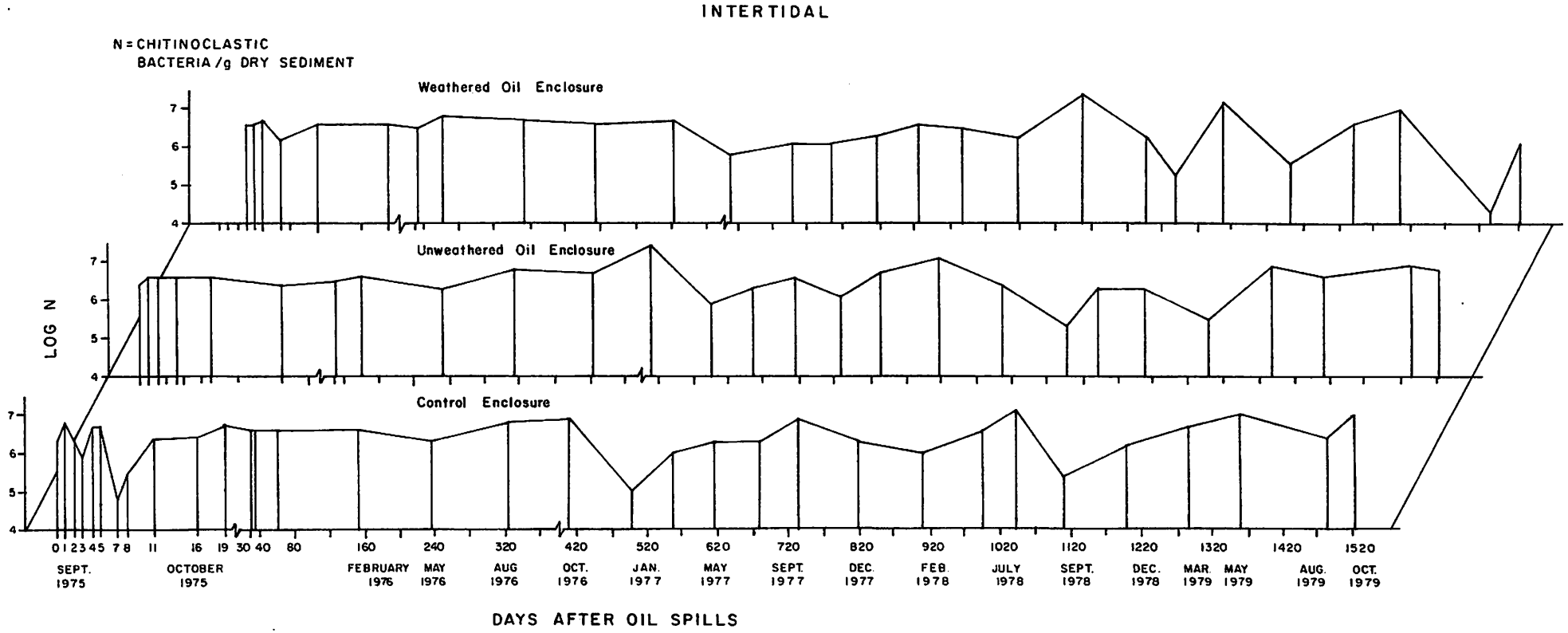
INTERTIDAL
N = CHITINOCLASTIC BACTERIA / g DRY SEDIMENT
Weathered Oil Enclosure
Unweathered Oil Enclosure
Control Enclosure
LOG N
7
6
5
4
0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG 1976
OCT. 1976
JAN. 1977
MAY 1977
SEPT. 1977
DEC. 1977
FEB. 1978
JULY 1978
SEPT. 1978
DEC. 1978
MAR. 1979
MAY 1979
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 13

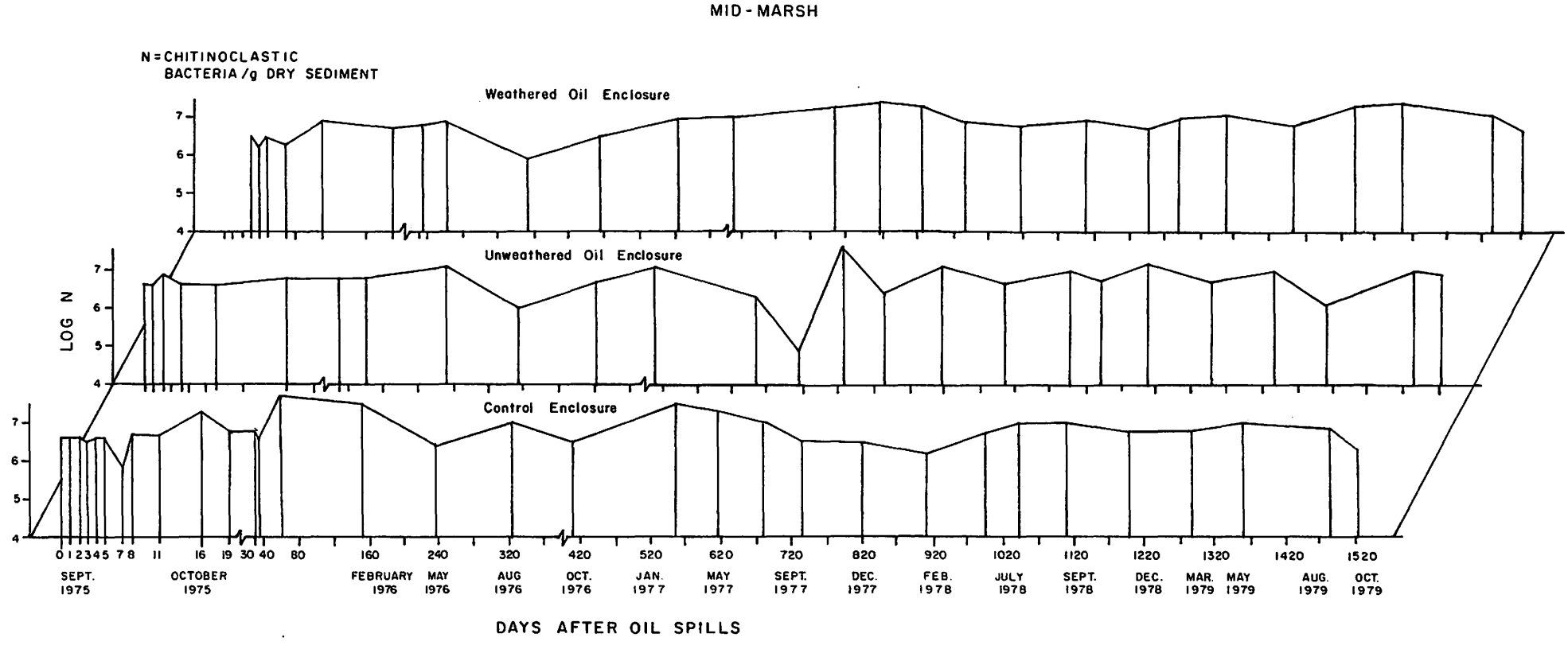
MID-MARSH
N=CHITINOCLASTIC BACTERIA/g DRY SEDIMENT
Weathered Oil Enclosure
Unweathered Oil Enclosure
Control Enclosure
LOG N
7
6
5
4
0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG 1976
OCT. 1976
JAN. 1977
MAY 1977
SEPT. 1977
DEC. 1977
FEB. 1978
JULY 1978
SEPT. 1978
DEC. 1978
MAR. 1979
MAY 1979
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 14

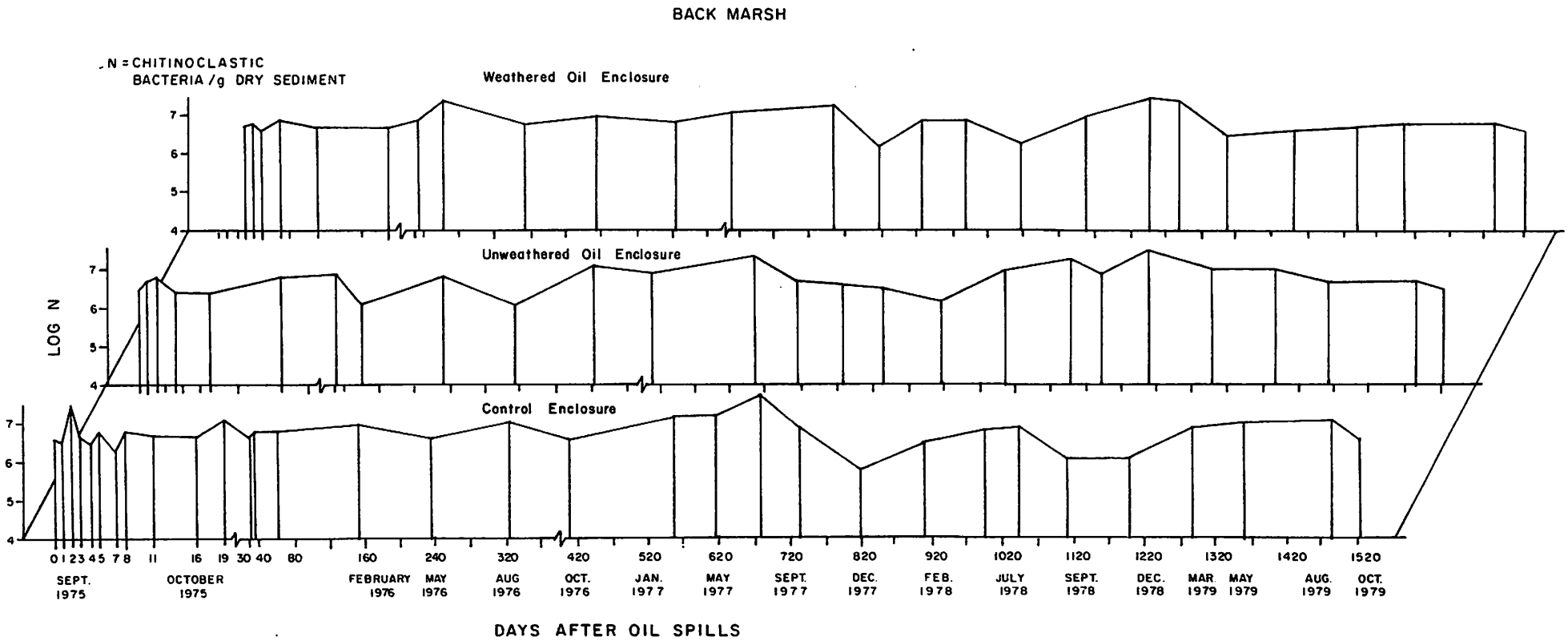
BACK MARSH
N = CHITINOCLASTIC BACTERIA /g DRY SEDIMENT
Weathered Oil Enclosure
Unweathered Oil Enclosure
Control Enclosure
LOG N
7
6
5
4
0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG 1976
OCT. 1976
JAN. 1977
MAY 1977
SEPT. 1977
DEC. 1977
FEB. 1978
JULY 1978
SEPT. 1978
DEC. 1978
MAR. 1979
MAY 1979
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 15

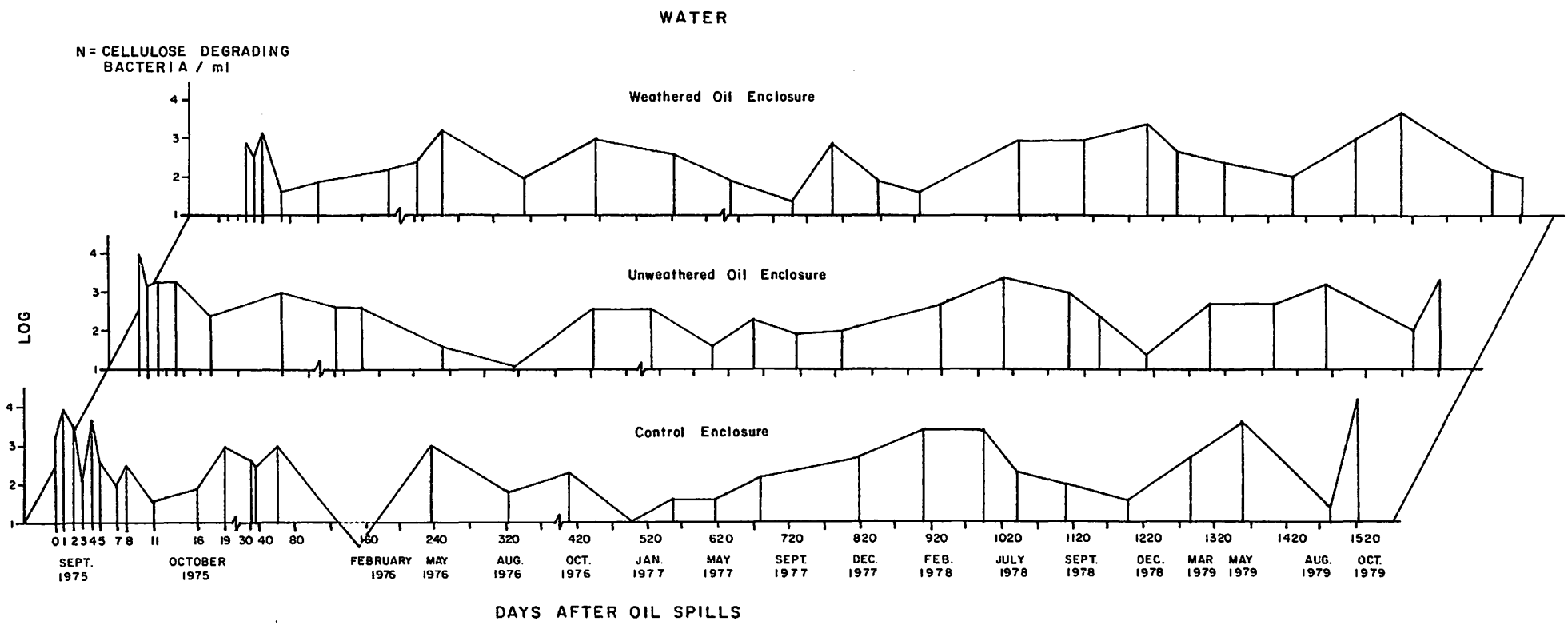

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Figure 16

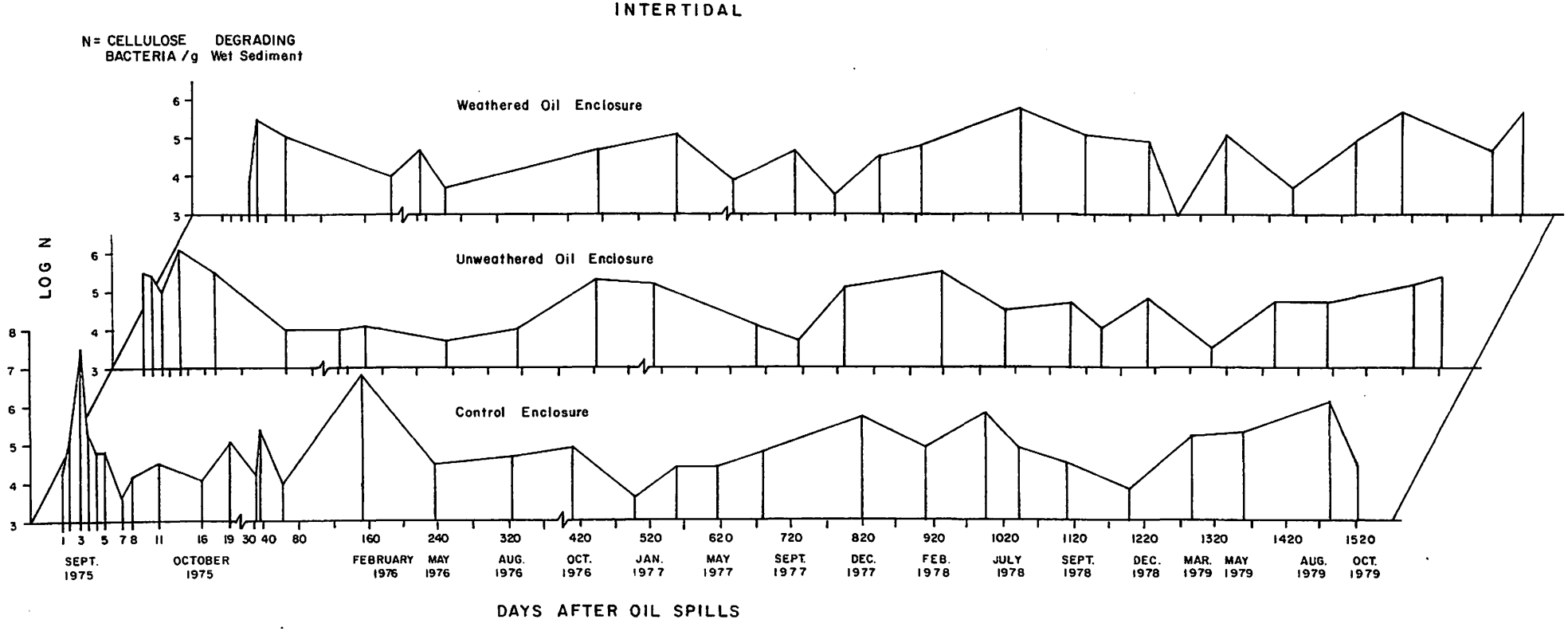

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Figure 17

MID-MARSH

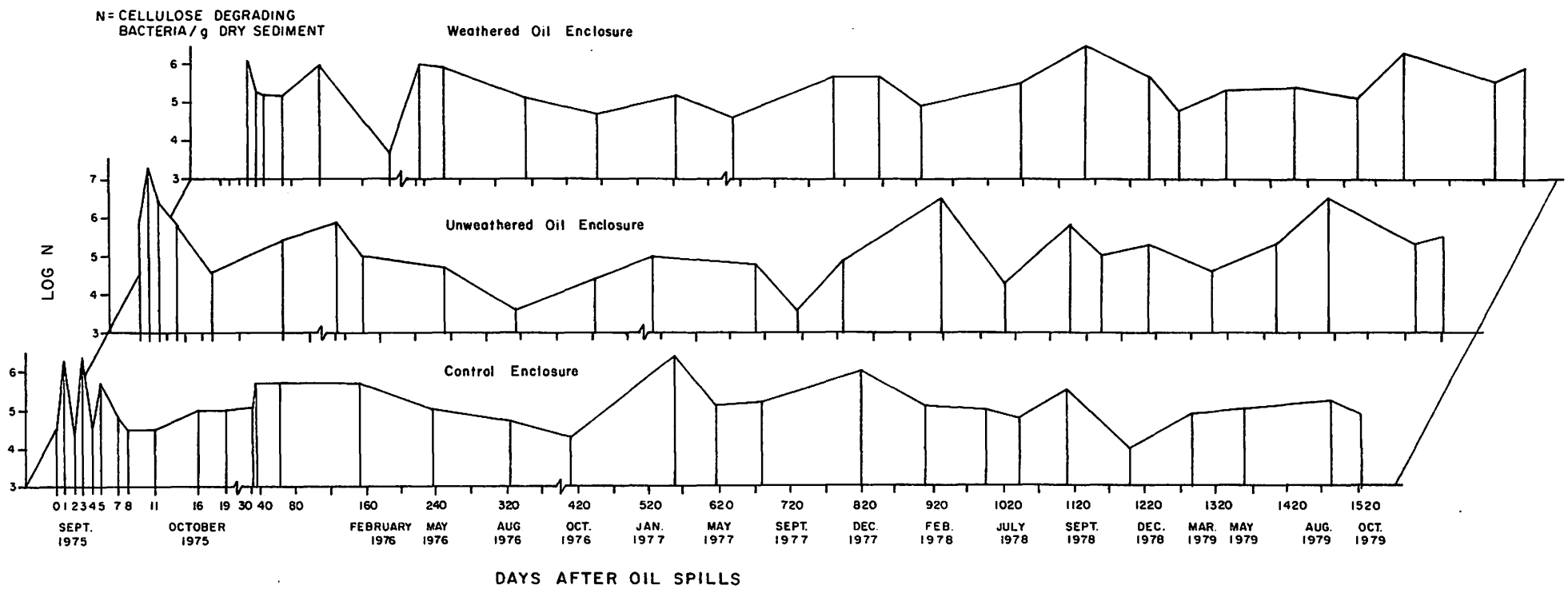

Figure 18

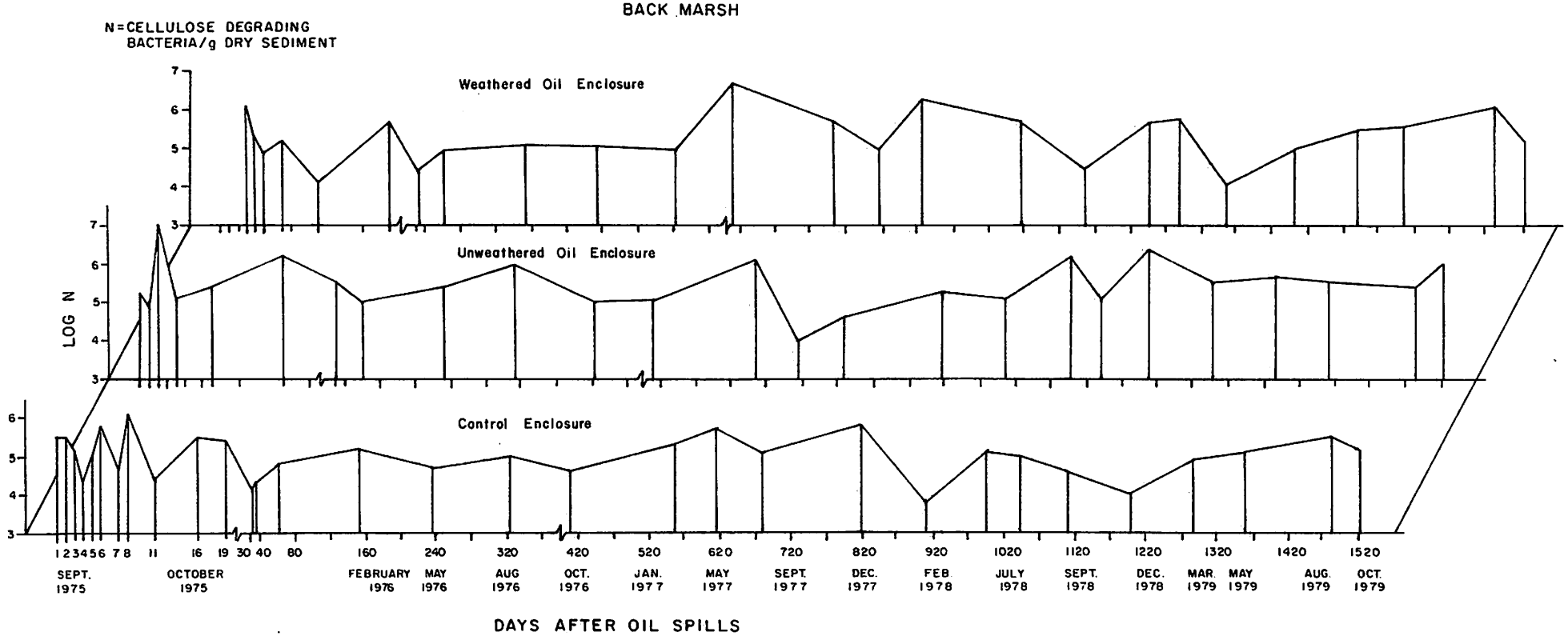

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Figure 19

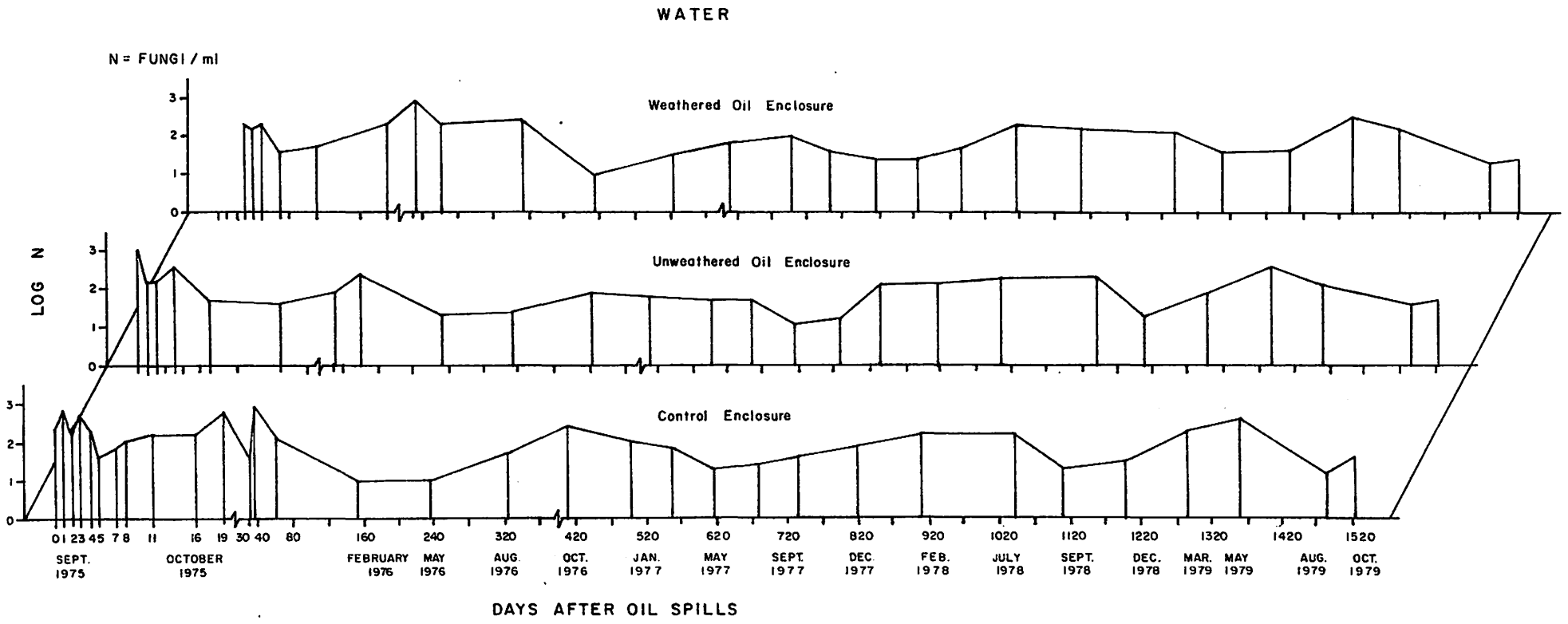

WATER
N = FUNGI / ml
Weathered Oil Enclosure
Unweathered Oil Enclosure
Control Enclosure
LOG N
0
1
2
3
0 1 2 3 4 5 7 8 11 16 19 30 40 80 160 240 320 420 520 620 720 820 920 1020 1120 1220 1320 1420 1520
SEPT. 1975
OCTOBER 1975
FEBRUARY 1976
MAY 1976
AUG. 1976
OCT. 1976
JAN. 1977
MAY 1977
SEPT. 1977
DEC. 1977
FEB. 1978
JULY 1978
SEPT. 1978
DEC. 1978
MAR. 1979
MAY 1979
AUG. 1979
OCT. 1979
DAYS AFTER OIL SPILLS

Figure 20

INTERTIDAL

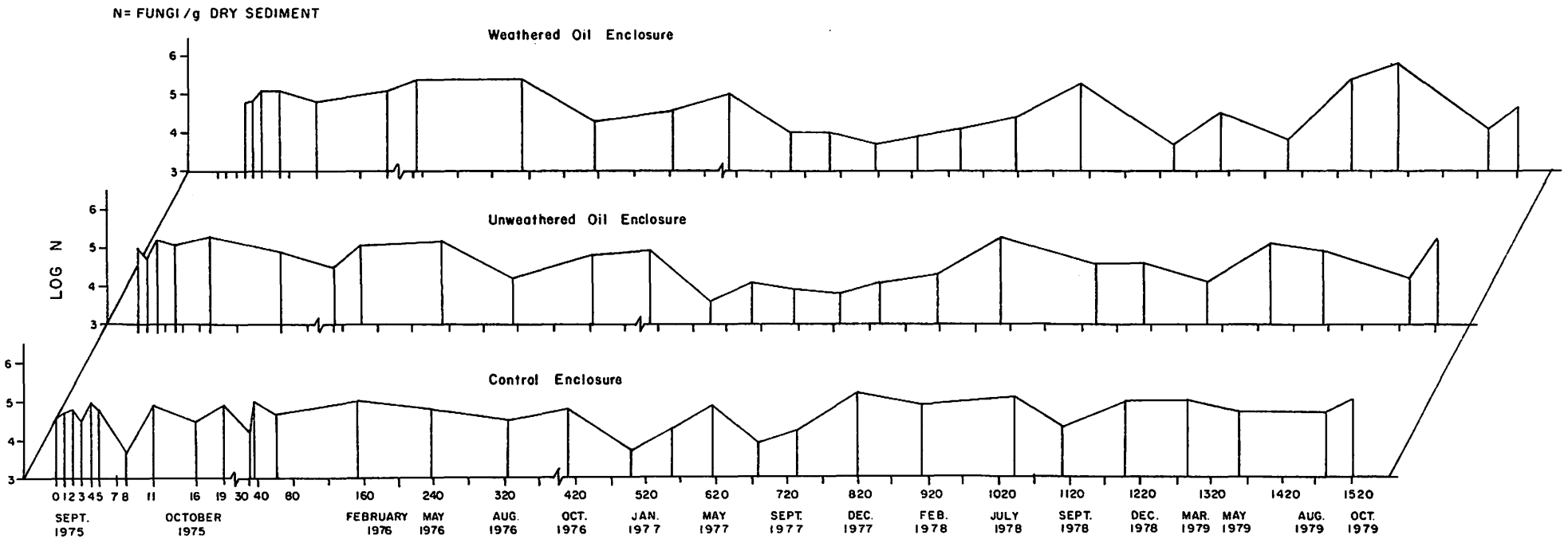


Figure 21

MID-MARSH

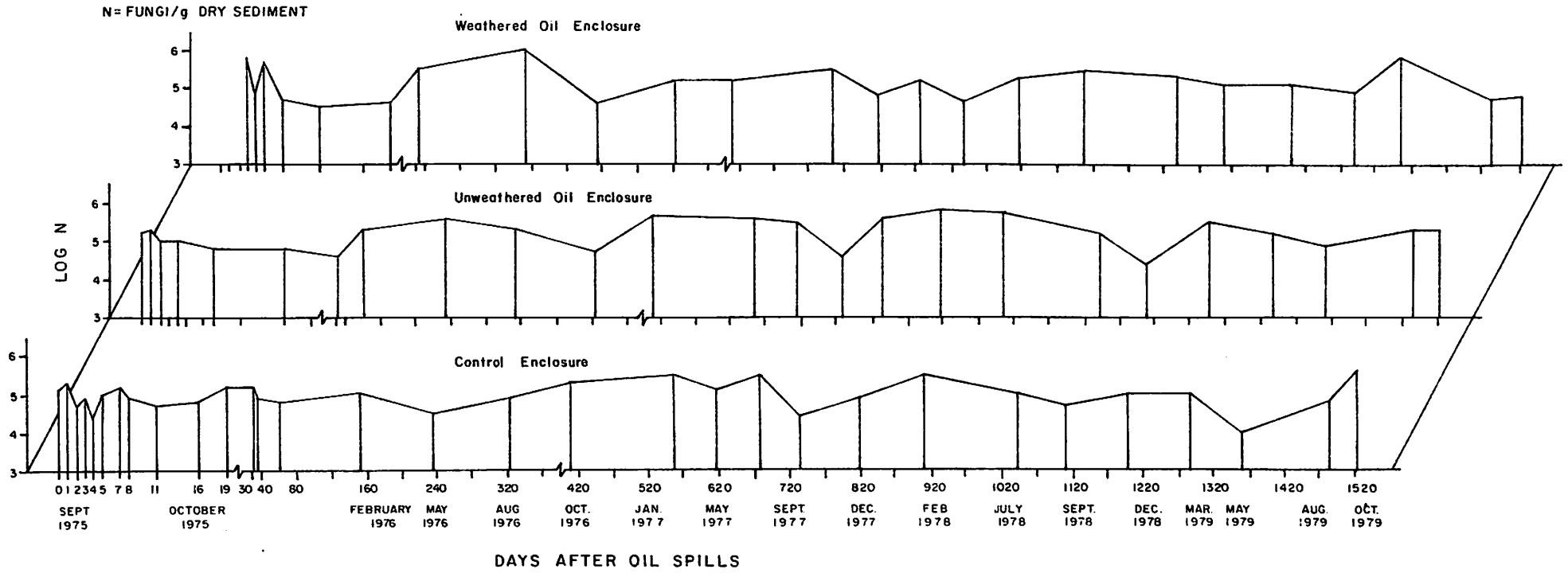
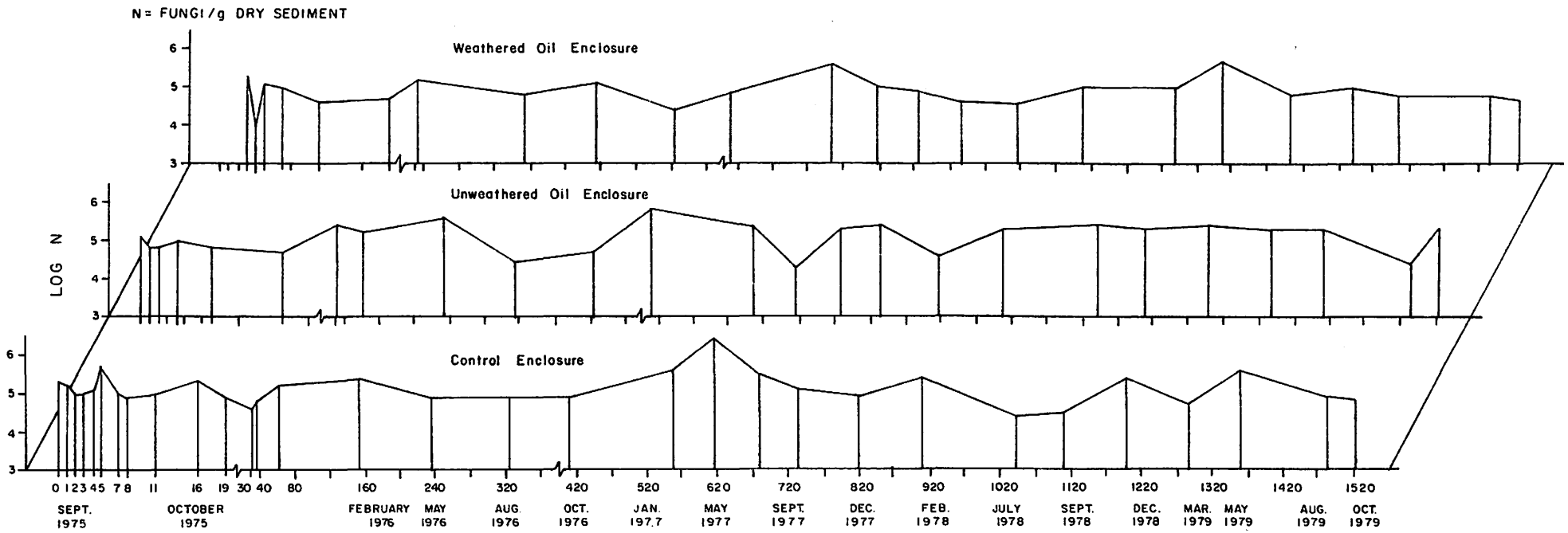


Figure 22

BACK MARSH



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