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Effects Of Petroleum Hydrocarbons On Salt Marsh Communities

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EFFECTS OF PETROLEUM HYDROCARBONS ON SALT MARSH COMMUNITIES

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This dissertation is submitted in partial fulfillment
of the requirements for the degree of
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ABSTRACT

The effects of petroleum hydrocarbons on salt marsh grasses and gastropods were examined under three different circumstances. In the first study reported, a small pocket marsh was repeatedly dosed with small amounts of a No. 2 fuel oil. The second study investigated the effects of a large accidental spill of No. 6 fuel oil. The third study involved single doses of a fresh and a weathered crude oil on artificially enclosed segments of marsh.

In the chronic oil spill study, *Spartina alterniflora* was the only grass species showing effects of the oil dosing. The result is attributed to the grass's location in the marsh. A substantial proportion of the *S. alterniflora* complement in the marsh was killed by the oil dosing. The remaining proportion evidenced sublethal effects including delayed development in the spring, increased densities and reduced mean weights per stem. The second annual cohort of shoots, usually produced in late summer and early fall, was suppressed almost entirely. Decay rates of *S. alterniflora* in the oiled marsh were found to be higher and to peak later than decay rates in the control marsh. Oil which entered the roots and rhizomes of dead *S. alterniflora* was maintained in a relatively undegraded state for at least seven months after dosing stopped.

The population size and distribution of the snails, *Nassarius obsoletus*, *Littorina irrorata*, and *Melampus bidentatus* were also monitored in the chronically oiled marsh. *N. obsoletus* showed some alterations in its normal distribution pattern, attributed to the oil dosing. The *L. irrorata* population was the most severely affected by the oil. Changes in both the size and distribution of its population closely followed the changes in the marsh grass community. The population of *M. bidentatus* was unaffected by the oiling due to its location in the marsh. Several hypotheses to explain the observed effects are developed and discussed.

The large spill of No. 6 fuel oil affected both the *S. alterniflora* population and the *L. irrorata* population of the impacted marshes. The *S. alterniflora* evidenced an increased net production increased density, decreased mean height, and increased flowering relative to controls. Immediately following the spill, the population of *L. irrorata* was significantly reduced in size and showed a marked change in distribution compared to controls. The relative effects of the spill and the subsequent cleanup effort are discussed and hypotheses to explain the observations are developed.

Experimental doses of fresh and weathered crude oil affected S. alterniflora in identical manners, contrary to expectations. Large proportions of the grass within the experimental enclosures were killed by both oils, but the surviving grass showed little or no evidence of sublethal effects.

In summation the variety and complexity of oil pollution effects on salt marsh communities is seen to confound attempts to develop predictive models. The research suggests sublethal effects of oil may have importance in determining relationships between marshes and interacting coastal systems, but additional work is indicated.

The design of each of the studies is discussed, indicating relative merits and drawbacks for future studies. Specifically discussed are project formats for studying natural marsh systems, selection of the scope of the research, and design of the sampling program.

EFFECTS OF PETROLEUM HYDROCARBONS ON SALT MARSH COMMUNITIES

Section 1

GENERAL INTRODUCTION.

GENERAL INTRODUCTION

The value of salt marshes to estuarine and coastal ecosystems is generally accepted, if not always well understood. They serve as habitat and food source for large numbers of indigenous and transient species. Marshes also play important roles as geologic agents, functioning as shoreline stabilizers, sediment filters, and flood buffers (Wass and Wright 1969).

Because of their location, salt marshes are affected not only by a variety of natural factors, but also by many of man's activities. Among the activities which have raised concerns for the well-being of marsh communities are the purposeful and accidental discharges of petroleum hydrocarbons into the environment. The concern has fostered a variety of investigations into the fate and effects of petroleum hydrocarbons in salt marshes.

Among the earliest studies were those conducted in Louisiana sponsored by the oil industry, to investigate charges the industry's activities were adversely affecting fisheries in the Gulf coast estuaries (Mackin 1950 a, b). The studies involved experimental dosing of some marsh grasses with crude oil. Portending the variety of effects to be found in future research, the results indicated grasses were moderately affected by a single dose of oil, killed by repeated dosses, and apparently stimulated to increase production when allowed to recover from the oiling.

In subsequent years, investigations were usually either small experimental studies, or followup investigations of accidental oil spills (e.g. Cowell 1969). The results of all these studies seemed to confirm that marshes were able to withstand or recover quickly from single doses of oil. Multiple doses, however, could produce extensive damage. The extent of effects from any one spill depended on the type and amount of oil, the species of plant, and the time of year.

Much of the experimental work on the effects of oil on salt marshes has been done in the United Kingdom by Baker. Working in Milford Haven, a major center of petroleum refining and transportation, she undertook a number of small studies involving single and multiple applications of oil to marsh plots. She found that following a single dosing there may be reduced germination and flowering, a reduced population of annuals, and growth stimulation of some species (Baker 1971a). In a study of successive spillages (Baker 1971b) the grasses were found to exhibit varying degrees of resistance to oil. Most recovered quickly from up to four monthly doses, but recovery from more doses was seen as a long process, lasting several years. By spraying plots with crude oil at different times of the year, Baker (1971c) demonstrated apparent seasonal variations in the susceptibility of marsh plants to oil. In general, there was some evidence oils were more damaging during hot, sunny weather.

Several investigators have noticed the apparent growth stimulation of plants following oiling first reported by Mackin

(Cowell 1969, Baker 1971d). Hypotheses to explain the response include: (1) increased water retention of the oiled soil, (2) release of nutrients from oil-killed animals, (3) nutrients or growth regulators contained in the oil, and (4) increased nitrogen fixation in the sediments due to oil-degrading microbes. A definite explanation has not yet been found. Also, the response does not appear to be typical of all pollution incidents.

Chronic pollution as found around oil refineries or terminals can have decidedly deleterious effects on salt marshes. The only investigations of this type of pollution have been field studies around a refinery outfall, conducted by Baker (1971e, 1976) and Dicks (1976). Baker concluded both salt and fresh water marsh vegetation are capable of surviving at least some chronic oil pollution. Death, when it occurs, is probably the result of repeated thin coatings of oil stranded on the plants by the tides. In a study of a marsh recovering around an outfall, Dicks concluded one of the long term effects in a chronically polluted marsh may be the establishment of a different vegetative community when the marsh is finally allowed to recover.

Effects of oil pollution on salt marsh organisms other than plants have not usually been studied. A spill of fuel oil at West Falmouth, Massachusetts, killed a wide variety of organisms and led to extensive studies of the hydrocarbon content of organism tissues (Burns and Teal 1971), benthic organisms (Sanders et al. 1972, Michael et al. 1975), and organism physiology and ecology (Burns 1975, 1976, Krebs and Burns 1977). Results indicated contamination in the marsh was widespread and persistent for several years following the spill of No. 2 fuel oil. Lytle (1975) studied several species in a salt marsh-pond experimentally dosed with crude-oil. She reported marked short-term effects on the marsh grasses and no observable effects on the populations of fiddler crabs.

Based on a review of the literature several questions concerning the effects of oil pollution on salt marshes were still unanswered or insufficiently studied. In general, marsh grasses, as the most important component of the marsh system, required much additional investigation. Also, the effects of oils on gastropod populations inhabiting marshes, a significant macrobiotic component of the system, had not been studied at all.

Previous works involving marsh grasses had consisted of either cataloguing losses of live grass following spills, or determining relative susceptibilities in simple experiments. A detailed analysis of effects in a natural grass community was missing. Specifically, the sublethal effects of oil pollution on salt marsh grasses had not been fully addressed.

The studies reported here investigate natural marsh grass communities in three different oil pollution situations. In the first investigation, four grass species typical of the salt marshes of the Atlantic and Gulf coasts were studied in a marsh repeatedly dosed with small amounts of No. 2 fuel oil. The analyses not only catalogue the loss of grass due to the oiling, but also monitor changes in the physical characteristics of the remaining live grasses. The second study employs similar techniques to analyze the dominant grass, Spartina alterniflora, in a marsh impacted by a apill of No. 6 fuel oil. The third study also investigates S. alterniflora, this time in experimentally enclosed areas of marsh dosed with a fresh and a weathered crude oil.

Of the two dominant macrofaunal groups resident in marshes, fiddler crabs and snails, the crabs have been studied in some detail by Burns (1975, 1976) and Krebs and Burns (1977). Snail populations, however, have not been studied previously. The studies reported here include analyses of the popualtion size, distribution, and composition of the resident snail species in the marsh chronically dosed with No. 2 fuel oil and in the marsh affected by the spill of No. 6 fuel oil. Effects on the populations in the marsh enclosures dosed with fresh and weathered crude oil have been reported by Bender et al. (1977).

A secondary aspect of the work in this dissertation was the comparative analysis of the three basic techniques for conducting field studies of oil pollution. The first study reported involved experimental dosing of a small naturally defined system. This technique had not previously been used for oil pollution studies in marshes. Reports in the literature all involve either experimental doses applied to small, artificially defined areas, as in the third study reported here, or follow-up studies of accidental discharges of oil, as in the second study reported here.

Each of these investigations has associated investigations covering other aspects of the response of the impacted systems. The reports on effects of the chronic No. 2 fuel dosing are complimented by a study on the fate of the oil in the marsh system (Lake 1977). The No. 6 fuel oil spill study was complemented by studies on the nearshore (R. J. Orth unpublished) and offshore benthos (D. F. Boesch and R. J. Diaz unpublished). Other biological components of the marsh enclosures dosed with fresh and weathered crude oil were studied by Bender et al. (1977) and Kator and Herwig (1977). The fate of the crude oil within the pens was studied by Bieri et al. (1977).

Section 2

EFFECTS OF CHRONIC OIL POLLUTION ON SALT MARSH GRASS COMMUNITIES.

INTRODUCTION

With a large and potentially expanding petroleum based industry in the coastal waters of the United States the environmental effects of oil pollution are of real concern to an increasing number of people. The desire to encourage expansion of petroleum refining and transportation activities must be tempered by the realization that accidental discharges are almost inevitable. Within a nine month period during 1976, the U. S. Coast Guard office in Norfolk, Virginia, which has responsibility for the lower Chesapeake Bay, had more than 460 pollution incidents reported. In sixty nine of those incidents, the initial estimate of the volume of petroleum products spilled was 50 gallons or more. The remainder involved either lesser amounts or unknown quantities. It is evident, therefore, that the oil pollution problem involves not only the large catastrophic spills, but also the smaller, far more frequent discharges.

Oil naturally concentrates at the top and the bottom of a water column, making communities in both these positions of particular interest in studies of oil pollution effects. Within the coastal zone, marshes occupy both locations simultaneously. Their location plus their generally accepted, if incompletely understood, value to the estuarine system lend strong impetus to studies of marshes receiving petroleum hydrocarbons.

Previous works have investigated marshes following acute spills (Stebbings 1968, Burns and Teal 1971, Lytle 1975, Bender et al. 1977, Hershner and Moore 1977), repeated experimental doses of crude oil (Baker 1971b) and the effects of refinery effluents (Baker 1971e, 1976a, b, Dicks 1976). Results have indicated that marsh grasses can survive single, light to moderate doses of oil. Multiple doses however, are frequently very deleterious.

Multiple doses have only been studied by repeatedly spraying very small plots with crude oil or by field studies around refineries. The goal of this investigation was to study a small natural community as it was repeatedly dosed by oil slicks. In order to eliminate some of the physical effects of oil residues which had been identified and studied elsewhere, a No. 2 fuel oil was used in this investigation.

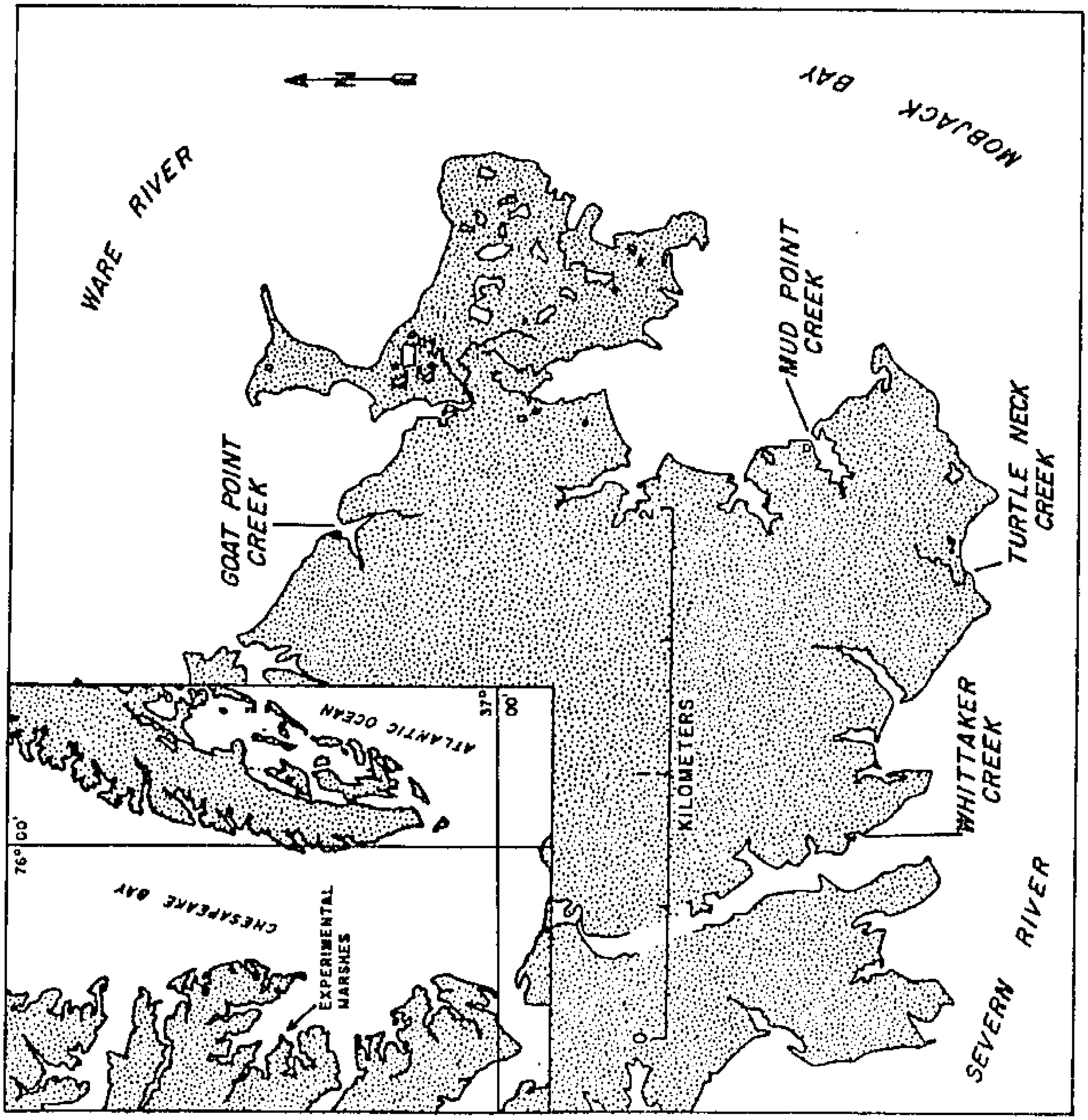
SITE

The reported investigations were carried out in two small pocket marshes, Whittaker Creek and Goat Point Creek, located in the lower Chesapeake Bay (see Figure 1). Each marsh was comprised of a tidal pond surrounded by marsh grasses and bounded on three sides by pine forest (primarily loblolly pine, *Pinus taeda*). The fourth side of each marsh is occluded by natural spits vegetated by saltmarsh cordgrass, *Spartina alterniflora*; marsh elder, *Iva frutescens*; and sea myrtle, *Baccharis halimifolia*. The remaining narrow channel, approximately 5 meters wide in both cases, handles all the exchange taking place with the adjacent tidal river. There is no fresh water input other than runoff from the surrounding forest. Salinities range between 15 and 22 ppt and water temperatures vary seasonally from 2 to 32° C. The mean tide range in the area is 0.69 meters.

Goat Point Creek was used as a control site throughout the course of the investigation. The only alterations made to the site included the erection of a small (4 foot by 8 foot) sampling platform in the entrance channel and the setting of approximately fifty survey stakes throughout the marsh for reference during sampling.

Whittaker Creek was used as the experimental site for the investigation. Alterations in this marsh included a sampling platform, approximately thirty survey stakes, and a semi-permanent

Figure 1. Location of experimental marshes.



retaining wall constructed on the river side of the two spits. The wall was constructed of PVC roofing panels. It paralleled the two natural spits, with a 5 meter opening at the channel connecting the pond and river. The wall was constructed to retain any slicks resulting from the experimental dosing. Three months into the study the wall proved unnecessary and infeasible to maintain. It was dismantled.

Whittaker Creek was dosed twice a month with 45 liters of a No. 2 fuel oil. Dosing began November 1, 1973 and ended August 10, 1974. For each dosing the oil was introduced at the mouth of the pond by siphoning into the water column on a flooding tide.

The sampling program was designed to monitor the physical configuration of the marshes, the net production of the marsh grasses, the decay rates of the dead plant material, the physical characteristics of the grasses, and the hydrocarbon content of *S. alterniflora* and the sediments.

METHODS

Physical configuration of the marshes.

Both marshes were mapped using a plane table and alidade to establish the aerial extent of the grass zone (see Figures 2 and 3). Resurveys were completed at three month intervals to quantify any changes in the marsh configuration. Topographic changes were monitored by reference to the survey stakes situated throughout each marsh.

Net production of the marsh grasses.

Grass samples were collected using a clipped quadrat technique in a stratified random sampling design. At each sampling effort twelve sample sites were selected within two constraints. First, the twelve samples were stratified so that four samples were collected in each of the three principal grass zones (i.e.: 1- _S_. _alterniflora_, 2- _J_. _roemerianus_, 3- _D_. _spicata_ and _S_. _patens_). Second, no sample was collected from a previously sampled location. All sample sites were selected prior to entering the marsh by refering to a random numbers table and the first set of vegetation maps (November 1973).

A 0.1 m^2 circular quadrat was used for all samples. All material within a quadrat above the sediment surface was removed.

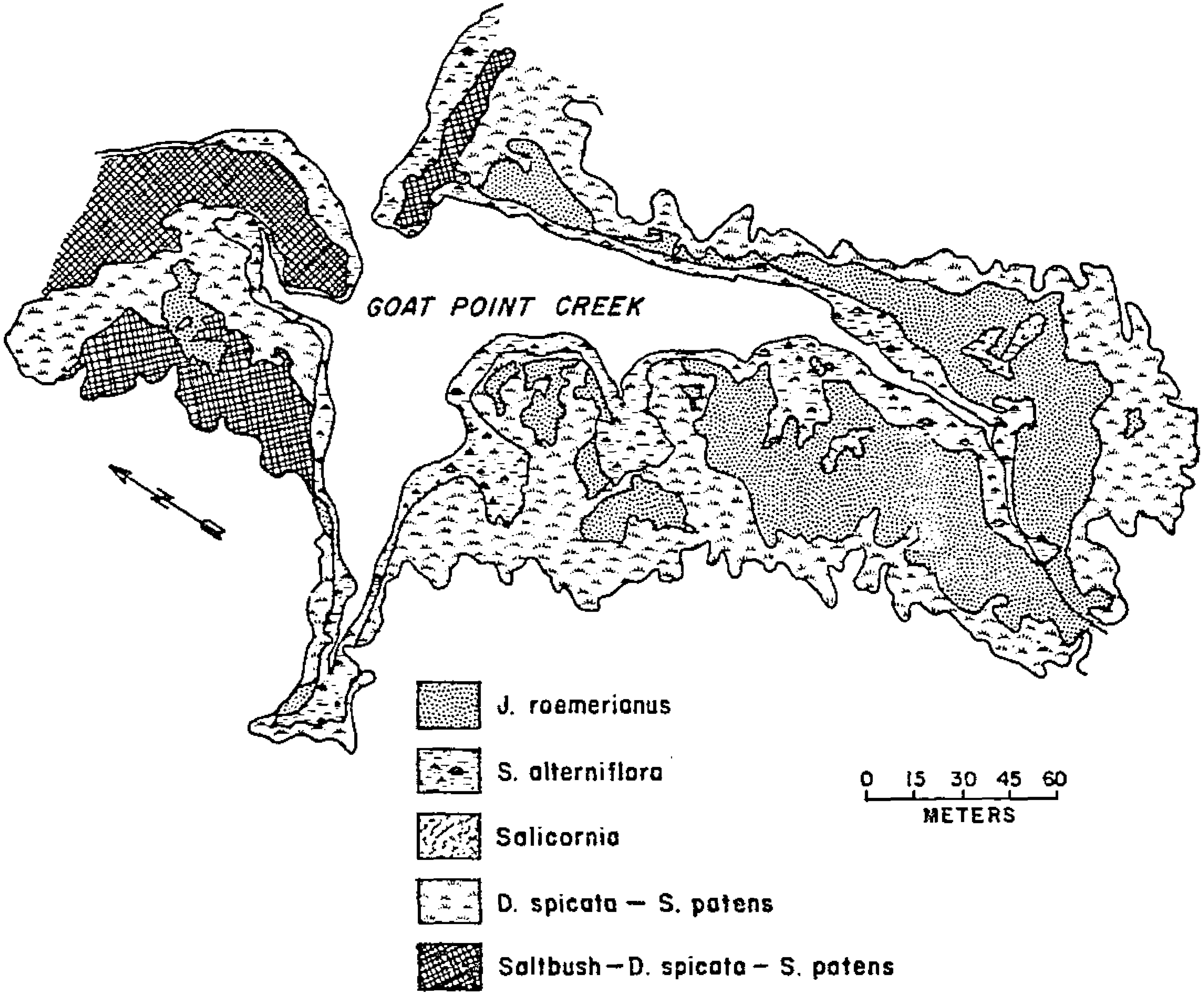
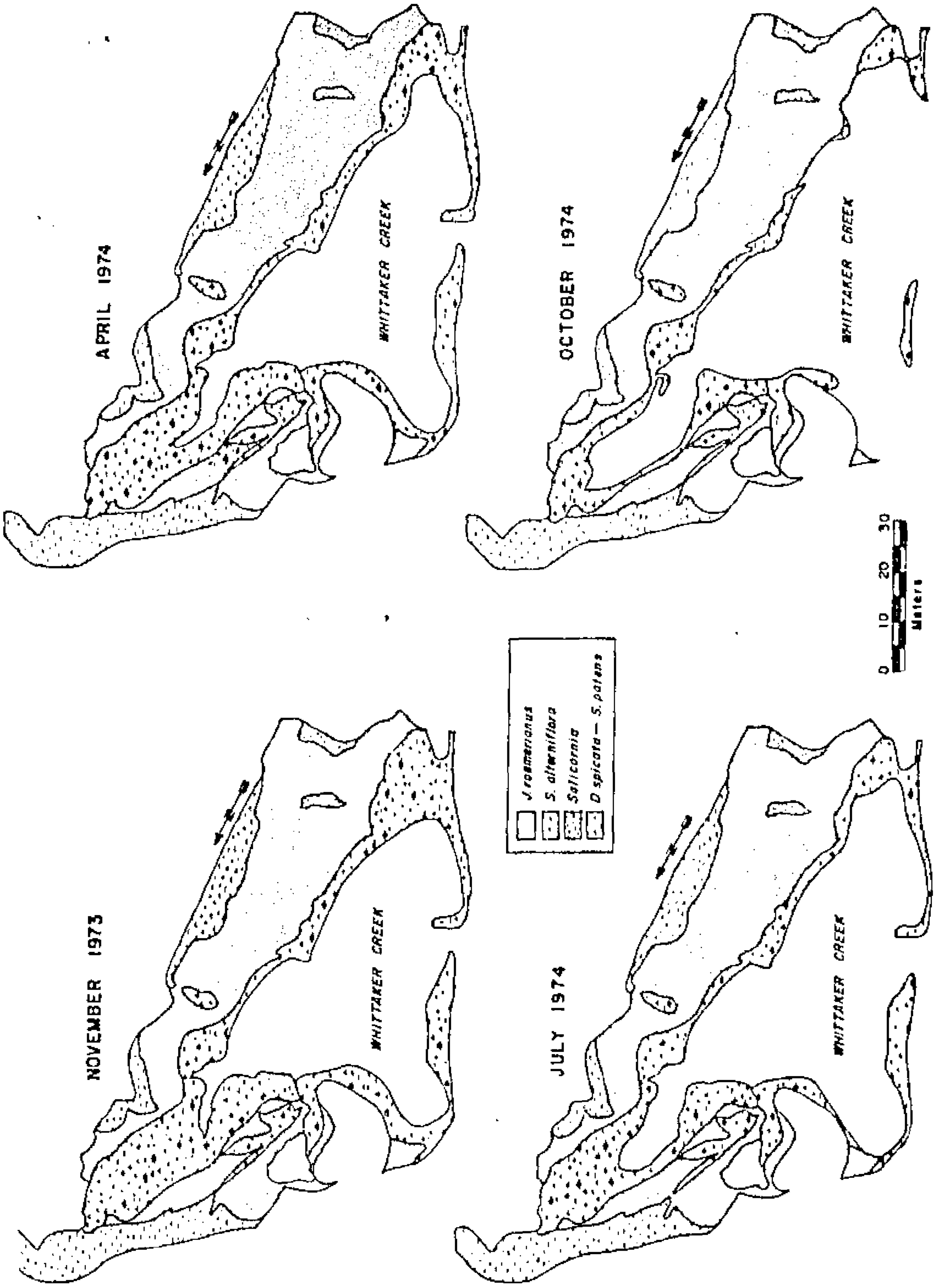


Figure 2. Vegetation map of Goat Point Creek (control marsh).

Figure 3. Vegetation map of Whittaker Creek (oiled marsh) at four times during the study.



The material was sorted by species into live and dead standing stock and dried to a constant weight. All values reported are dry weight measurements.

Production estimates for each of the four species of grass were calculated by the method proposed by Weigert and Evans (1964). The method utilizes changes in the standing crops of live and dead material and an estimate of the rate of disappearance of dead material (calculated from litter bag experiments in this work, as explained in the following section).

Using their notation:

t_i = time interval (in days)

a_{i-1} = standing crop of dead material at start of a time interval.

a_i = standing crop of dead material at the end of a time interval.

b_{i-1} = standing crop of live material at start of a time interval.

b_i = standing crop of live material at end of time interval.

r_i = specific daily rate of disappearance of dead material during interval in mg/g/day.

If x_i equals the amount of dead material disappearing during interval t_i due to in situ decomposition, then

$$x_i = [(a_i + a_{i-1})/2]\ r_i t_i \qquad (1)$$

The changes in the live and dead standing crops of a grass species are, respectively:

$$\Delta b_i = b_i - b_{i-1} \qquad (2)$$

and

$$\Delta a_i = a_i - a_{i-1} \qquad (3)$$

The mortality of live material (d_i) during an interval will equal the amount of material disappearing from the dead standing crop (x_i) plus the change in the dead standing crop (a_i):

$$d_i = x_i + \Delta a_i \qquad (4)$$

Net production of a grass species during an interval equals:

$$y_i = \Delta b_i + d_i \qquad (5)$$

Net production of a grass species throughout the study period equals:

$$Y = y_1 + y_2 + \ldots + y_n \qquad (6)$$

where n = the number of sampling intervals.

Negative solutions for equations 4 and 5 were treated as zeros for all subsequent calculations, after the summation method proposed by Smalley (1958).

Litter bag experiments to estimate loss through decay.

Corrections for loss of dead material through decomposition during sampling intervals were calculated for each species from data obtained in litter bag experiments. Each month, for each species, two nylon mesh bags (2.5 mm mesh) containing approximately 10 grams dry weight of the previous month's dead standing crop were staked on the marsh surface. The bags were placed at randomly selected sites within the appropriate grass zones and left for a three month period. Four sets of bags were used in a staggered rotation so that three sets were always on the marsh. (Except for the beginning and the end of the experiment.)

The disappearance rate of material in the bags was calculated for each bag as:

$$r_b = [\ln (w_0/w_1)]/[\Delta t] \qquad (7)$$

where: r_b = the specific daily rate of disappearance from bag "b"

w_0 = dry weight of material in bag at start of exposure

w_1 = dry weight of material in bag at end of exposure

$\Delta t = t_1 - t_0$ (in days)

For each species, the disappearance rate used to correct the production estimate for a sampling interval was taken as the mean of all rates calculated for those bags present on the marsh during that interval. Thus:

$$r_i = \sum_{b=1}^{h} r_b\,[f(b)] \Big/ \sum_{b=1}^{h} f(b) \qquad (8)$$

where: r_i = the mean specific daily rate of disappearance of dead material during interval t_i

$f(b)$ = binomial function which is equal to 1 when litter bag "b" was on the marsh during interval t_i, and equal to 0 when it was not present.

For purposes of comparison, the r_b values for each species in both marshes were reduced to a single disappearance rate, r_m, for each month. This required the calculation of a weighted mean since not all r_b values considered in any given month resulted from observations extending over the entire month. The individual bag r_b values considered were weighted by multiplying the rate by the number of days in the month during which the bag was in place on the marsh.

The sum of all weighted values was then divided by the total number of bag-days to provide the mean for the month.

Physical characteristics of the grasses.

Data on the physical characteristics of the four grass species studied was gathered by analyzing the clipped quadrat samples prior to drying. After separating the species, all live stems were measured for greatest length and sorted into 5 cm size classes. Stem density was calculated as the mean number of stems per sample divided by the quadrat size (0.1 m^2). Mean height per stem was calculated by taking the median height for a size class multiplied by the number of stems in that size class, summing the resultant values for all size classes and dividing by the total number of stems. Mean weight per stem was calculated by dividing the dried weight of all live stems in a sample by the number of stems in that sample.

The ratio of mean weight per stem to mean height per stem was calculated as a simple index of plant vigor. The ratio was used to facilitate comparisons of results from the experimental and control marshes.

Hydrocarbon content of sediments and *S. alterniflora*.

The distribution of saturated petroleum hydrocarbon concentrations in sediments of the experimental marsh was mapped using samples collected in October of 1974, six weeks after the final dosing. Sediment cores taken to a depth of 30 cm were divided into 10 cm sections for analysis. The sections were mixed and pentane extracted in an ultrasonic bath for 5 minutes. The resulting emulsion was centrifuged and after removal of the supernatant, the residue was extracted a second time. The extracted sediments were oven dried and weighed. The two extracts were combined and reduced to 0.5 ml in a rotating flask held in a warm water bath (30° C). The extract was then charged onto a 5% water deactivated silica gel column. The first fraction through the column, eluted with 30 ml of pentane, contained the saturates analyzed in this study. The elution was reduced in volume under a stream of nitrogen and analyzed on a 15 foot column of 10% SE-30 on chromosorb P, 80 - 100 mesh DMCS, in a Perkin Elmer 900 gas chromatograph.

Concentrations were obtained by comparing planimetered areas of the chromatograms with areas obtained for known standards. Compound identifications were made based on retention times. Ratios of n-C_{17}/pristane and n-C_{18}/phytane were calculated as indicators of biodegradation of n-alkanes in the oil.

Sediment samples collected in conjunction with the study of petroleum hydrocarbons in _S. alterniflora_ roots and rhizomes, were analyzed by the same procedure.

0.05 m^2 blocks of _S. alterniflora_ roots and rhizomes were excised from the most severely affected areas of the experimental marsh 2 months, 5.5 months, and 7.5 months after the last oil dosing. Uncontaminated plant material was collected from the control marsh. All sediment was rinsed from the roots and rhizomes, which were then macerated and pentane extracted in an ultrasonic bath. The extracted plant material was dried and weighed. The column separation and gas chromatographic analysis of the plant extracts were identical to the procedures used for sediments.

RESULTS

Physical configuration of the marshes.

The vegetation maps of Goat Point Creek and Whittaker Creek are shown in Figures 2 and 3 respectively. Aerial extent of the grass zones are summarized in Table 1. Goat Point Creek, the control marsh, remained unaltered throughout the course of the experiment. Whittaker Creek demonstrated marked effects of the oil dosing in the *Spartina alterniflora* surrounding the pond.

During spring 1974 the growth of *S. alterniflora* initiated simultaneously and proceeded equally in both marshes. When the grass reached 15 to 20 cm in height, in mid-April, the first visible signs of dosing appeared as a well defined chlorotic band on the stems and leaves at about the high tide level. The effect was first noticed in the corners of the pond where oil tended to concentrate as a result of wind directed drift. In a matter of weeks, chlorosis became widespread around the periphery of the pond. By the end of April, small areas of *S. alterniflora* began to die back. This process is mapped in Figure 3 and recorded in Table 1 as a loss in the aerial extent of *S. alterniflora*. By the end of the first growing season, and after ten months of dosing, Whittaker Creek had lost 52.5% of its *S. alterniflora* complement. None of the other grasses suffered a similar loss. In fact, with

Table 1. Experimental marsh areas.

Goat Point Creek (control marsh)

total area	33862.5 m^2	8.36 acres
pond	7470.0 m^2	1.85 acres
J. roemerianus	9339.5 m^2	2.31 acres
D. spicata/S. patens	11790.0 m^2	2.90 acres
S. alterniflora	5265.0 m^2	1.30 acres

Whittaker Creek (oiled marsh)

total area	5035.0 m^2	1.25 acres
pond	1485.0 m^2	0.37 acres
J. roemerianus	1637.6 m^2	0.40 acres
D. spicata/S. patens	549.9 m^2	0.14 acres
S. alterniflora (11-73)	1362.5 m^2	0.34 acres
(04-74)	1197.5 m^2	0.30 acres
(07-74)	1017.5 m^2	0.25 acres
(10-74)	647.5 m^2	0.16 acres

the exception of a small patch of *Juncus roemerianus* at the west corner of the pond, there were no visible effects of the oil on any of the other three species.

As a consequence of the disappearance of a vigorous S. *alterniflora* stand, the topography of Whittaker Creek also began to alter. The most noticeable effects occurred on the two vegetated spits which formerly separated the pond from the adjacent river. They were apparently a stable feature through the recent history of the area (as judged by a review of aerial photographs and maps covering 30+ years). Subsequent to the defoliation caused by dosing, both spits began eroding along their western, or exposed, sides. The erosion process first removed the top 1 to 2 cm of accumulated sand-silt sediment from the spit surface. Blocks of exposed and undercut roots and rhizomes were then broken free and washed away. The result was a loss of 8 to 10 cm from the vertical profile of the spits and 50 to 100 cm from the horizontal profile. Unoiled marshlands, immediately adjacent to Whittaker Creek, with the same orientation, suffered no similar erosion.

Concurrently with the erosion of the spits, sandy sediment, previously trapped by the *S. alterniflora*, was deposited over the very soft organic ooze on the pond bottom behind the spits. The net result of the erosion and accretion which occurred in Whittaker Creek was a slight migration of the spits into former pond area and a general lowering of the spits' profile.

Net production of the marsh grasses.

Spartina alterniflora (Table 2)

The average production of *S. alterniflora* in Whittaker Creek was 925.6 g/m^2 during the time period November 1, 1973 to September 26, 1974. In Goat Point Creek, from November 6, 1973 to September 26, 1974, average net production of the grass was 1040.1 g/m^2. These values indicate net production of *S. alterniflora* in the oiled marsh was 89.0% of the net production found in the control marsh. A preliminary study conducted in June of 1973, prior to the initiation of the experiment, found the average standing stock of *S. alterniflora* in the oiled marsh to be 90.9% of that in the control marsh.

During the course of the experiment, the oiled marsh showed a net loss of both live (-54.4 g/m^2) and dead (-96.0 g/m^2) standing stocks of *S. alterniflora*. The control marsh showed a net gain in the live standing stock (+84.8 g/m^2) and a loss in the dead standing stock (-171.0 g/m^2). The reduced standing stocks of live material in the oil marsh during the last months of the study reflect the loss of *S. alterniflora* due to the oiling.

Juncus roemerianus (Table 3)

Net production of *J. roemerianus* during the experimental period was 1019.9 g/m^2 in Whittaker Creek and 1491.2 g/m^2 in Goat Point Creek. These values indicate the oil marsh produced 68.4% as much of the grass per unit area as did the control marsh. Preliminary studies of plant material standing stocks found the oil marsh to have 74.7% as much *J. romerianus* material as the

Table 2. Calculations of *S. alterniflora* net production.
(in grams per m^2) (see text for heading definitions)

Whittaker Creek (oiled marsh)

date	b_i	Δb_i	a_i	Δa_i	r_i	t_i	x_i	d_i	y_i
Nov 01	112.8		293.8						
		-97.4		+113.6	06	33	69.4	183.0	85.6
Dec 04	15.4		407.4						
		-9.0		+173.7	06	29	86.0	259.7	250.7
Jan 02	6.4		581.1						
		-6.4		-289.0	06	52	136.2	-152.8	0.0
Feb 21	0.0		292.1						
		+16.8		+85.8	06	41	82.3	168.2	185.0
Apr 03	16.8		377.9						
		+140.4		+74.7	06	29	72.3	147.0	287.4
May 02	157.2		452.6						
		-61.2		-295.8	09	79	216.6	-79.2	0.0
Jul 20	96.0		156.8						
		+11.5		-39.4	11	21	31.7	-7.7	11.5
Aug 10	107.5		117.4						
		-49.1		+80.4	10	47	74.1	154.5	105.4
Sep 26	58.4		197.8						

net production = 925.6

Goat Point Creek (control marsh)

date	b_i	Δb_i	a_i	Δa_i	r_i	t_i	x_i	d_i	y_i
Nov 06	78.4		344.2						
		-57.1		-53.2	06	25	47.6	-5.6	0.0
Dec 01	21.3		291.0						
		-9.2		+42.2	06	33	61.8	104.2	95.0
Jan 03	12.1		333.4						
		-7.2		+117.8	06	64	150.6	268.4	261.2
Mar 08	4.9		451.2						
		+6.5		-145.8	06	30	68.1	-77.7	6.5
Apr 07	11.4		305.4						
		+116.9		-23.6	07	25	51.4	27.8	144.7
May 02	128.3		281.8						
		-3.9		-124.4	07	32	49.2	-75.2	0.0
Jun 03	124.4		157.4						
		+149.9		+39.8	07	37	45.9	85.7	235.6
Jul 10	274.3		197.2						
		+150.2		+69.0	07	48	77.9	146.9	297.1
Aug 27	424.5		266.2						
		-261.3		-92.8	06	30	39.6	-53.2	0.0
Sep 26	163.2		173.4						

net production = 1040.1

Table 3. Calculations of *J. roemerianus* net production.
(in grams per m^2) (see text for heading definitions)

Whittaker Creek (oiled marsh)

date	b_i	Δb_i	a_i	Δa_i	r_i	t_i	x_i	d_i	y_i
Nov 01	462.2		585.9						
		-173.8		-101.1	02	33	35.0	-75.1	0.0
Dec 04	288.4		475.8						
		+75.6		+70.6	02	29	29.6	100.2	175.8
Jan 02	364.0		546.4						
		-172.1		-50.0	02	52	54.2	4.2	0.0
Feb 21	191.9		496.4						
		+32.9		+77.8	00	41	0.0	77.8	110.7
Apr 03	224.8		574.2						
		-31.7		-72.8	01	29	15.6	-57.2	0.0
May 02	193.1		501.4						
		+63.0		+168.4	03	79	138.8	307.2	370.2
Jul 20	256.1		669.8						
		-99.9		-239.6	03	21	34.6	-205.0	0.0
Aug 10	156.2		430.2						
		+100.9		+188.4	03	47	73.9	262.3	363.2
Sep 26	257.1		618.6						
								net production =	1019.9

Goat Point Creek (control marsh)

date	b_i	Δb_i	a_i	Δa_i	r_i	t_i	x_i	d_i	y_i
Nov 06	558.0		808.9						
		-222.6		-224.5	02	25	34.8	-189.7	0.0
Dec 01	335.4		584.4						
		-0.6		-92.3	02	33	35.5	-56.8	0.0
Jan 03	334.8		492.1						
		+258.3		+389.7	02	64	87.9	477.6	735.9
Mar 08	593.1		881.8						
		-258.1		-194.2	02	30	47.1	-147.1	0.0
Apr 07	335.0		687.6						
		-4.4		+286.4	02	25	41.5	327.9	323.5
May 02	330.6		974.0						
		+115.0		-377.0	02	32	50.3	-326.7	115.0
Jun 03	445.6		597.0						
		-29.6		+11.2	02	37	44.6	55.8	26.2
Jul 10	416.0		608.2						
		-120.3		-213.8	02	48	48.1	-165.7	0.0
Aug 27	295.7		394.4						
		-74.5		+315.4	03	30	49.7	365.1	290.6
Sep 26	221.2		709.8						
								net production =	1419.2

control marsh.

In estimating the mean live and dead standing stocks of *J. romerianus*, the dead material collected in one March sample from the control marsh was not considered. This was done when random sampling positioned the sample on a float mat of dead grass material. The resultant value (1996.8 g/m^2) was anomalously high for these marshes. The value estimated from the remaining samples (881.8 g/m^2) is indicated in parantheses in Table 3.

Spartina patens (Table 4)

From November 1, 1973 to August 10, 1974 the net production of *S. patens* in Whittaker Creek was estimated to be 669.6 g/m^2. In Goat Point Creek, net production between November 6, 1973 and August 27, 1974 was estimated to be 589.7 g/m^2.

S. patens was not found in monospecific stands in either marsh. It occurred in mixed meadows with *Distichlis spicata* and *Fimbristylis spadicea*. Other consocies included *Borrichia frutescens*, *Iva frutescens*, and *Baccharis halimifolia*.

Distichlis spicata (Table 5)

In Whittaker Creek, the net production of *D. spicata* from November 1, 1973 to August 10, 1974 was estimated to be 506.6 g/m^2. In Goat Point Creek, net production from November 6, 1973 to August 27, 1974 was estimated to be 702.3 g/m^2.

D. spicata in both marshes occurred in association with *S. patens* and the other species listed previously.

Table 4. Calculations of *S. patens* net production.
(in grams per m^2) (see text for heading definitions)

Whittaker Creek (oiled marsh)

date	b_i	Δb_i	a_i	Δa_i	r_i	t_i	x_i	d_i	y_i
Nov 01	91.3		161.1						
		+107.7		+4.7	02	33	10.8	15.5	123.2
Dec 04	199.0		165.8						
		-187.3		+89.3	02	29	12.2	101.5	0.0
Jan 02	11.7		255.1						
		-4.3		+163.4	02	52	35.0	198.4	194.1
Feb 21	7.4		418.5						
		-1.4		-341.4	02	41	20.3	-321.1	0.0
Apr 03	6.0		77.1						
		+12.2		+280.5	02	29	12.6	293.1	305.3
May 02	18.2		357.6						
		+47.0		-239.2	02	79	37.6	-201.6	47.0
Jul 20	65.2		118.4						
		-10.4		-75.6	02	21	3.4	-72.2	0.0
Aug 10	54.8		42.8						

net production = 669.6

Goat Point Creek (control marsh)

date	b_i	Δb_i	a_i	Δa_i	r_i	t_i	x_i	d_i	y_i
Nov 06	11.6		129.4						
		+1.7		+102.9	02	25	9.0	111.9	113.6
Dec 01	13.3		232.3						
		-5.5		-131.1	02	33	11.0	-120.1	0.0
Jan 03	7.8		101.2						
		-1.1		+254.7	02	64	29.3	284.0	282.9
Mar 08	6.7		355.9						
		+4.9		-288.7	02	30	12.7	-276.0	4.9
Apr 07	11.6		67.2						
		+4.8		+0.6	02	25	3.4	4.0	8.8
May 02	16.4		67.8						
		+45.2		+101.6	02	32	7.6	109.2	154.4
Jun 03	61.6		169.4						
		+25.1		-59.9	02	37	10.3	-35.4	25.1
Jul 10	86.7		109.5						
		-41.8		+81.1	02	48	14.4	27.4	0.0
Aug 27	44.9		190.6						

net production = 589.7

Table 5. Calculations of *D. spicata* net production.
(in grams per m^2) (see text for heading definitions)

Whittaker Creek (oiled marsh)

date	b_i	Δb_i	a_i	Δa_i	r_i	t_i	x_i	d_i	y_i
Nov 01	26.4		461.9						
		-15.6		-2.7	02	33	30.4	27.7	12.1
Dec 04	10.8		459.2						
		-7.6		-11.4	02	29	26.3	14.9	7.3
Jan 02	3.2		447.8						
		-2.4		-83.6	02	52	42.2	41.4	39.0
Feb 21	0.8		364.2						
		+3.0		+40.4	02	41	31.5	71.9	74.9
Apr 03	3.8		404.6						
		-0.1		-150.4	02	29	19.1	-131.3	0.0
May 02	3.7		254.2						
		+181.9		+140.2	02	79	51.2	191.4	373.3
Jul 20	185.6		394.4						
		-93.8		-190.5	02	21	12.6	-177.9	0.0
Aug 10	91.8		203.9						

net production = 506.6

Goat Point Creek (control marsh)

date	b_i	Δb_i	a_i	Δa_i	r_i	t_i	x_i	d_i	y_i
Nov 06	23.7		325.2						
		-9.0		+195.2	02	25	21.1	216.3	207.3
Dec 01	14.7		520.4						
		-12.3		-197.4	02	33	27.8	-169.6	0.0
Jan 03	2.4		323.0						
		+0.9		-102.6	02	64	34.8	-67.8	0.9
Mar 08	3.3		220.4						
		+1.1		+209.2	04	30	39.0	248.2	249.3
Apr 07	4.4		429.6						
		+60.8		-53.5	04	25	40.3	-13.2	60.8
May 02	65.2		376.1						
		+4.3		-149.1	03	32	28.9	-120.2	4.3
Jun 03	69.5		227.0						
		+105.1		+46.8	03	37	27.8	74.6	179.7
Jul 10	174.6		273.8						
		-81.4		-115.2	02	48	20.8	-94.4	0.0
Aug 27	93.2		158.6						

net production = 702.3

Litter bag experiments to estimate loss through decay.

Table 6 lists the specific daily decay rates (r_m) for the four species of marsh grasses considered in this study.

Estimates of S. alterniflora mean decay rates in the control marsh varied from 5.00 mg/g/day to 7.39 mg/g/day. The high value occurred during June with values declining steadily to November. In the oil marsh estimated values began at 5.50 mg/g/day in February and peaked at 10.69 mg/g/day in July. Decay rates in the oil marsh were significantly greater ($p<0.05$) than rates in the control marsh from July through October.

Mean decay rates of J. romerianus were highest for the control marsh in September at 2.56 mg/g/day. The lowest rate estimated was 1.50 mg/g/day in March. Oil marsh decay rates for J. roemerianus began at 0.00 mg/g/day in February and climbed steadily to 5.00 mg/g/day in November. No significant difference could be detected between control marsh and oil marsh J. romerianus decay rates any time during the study.

S. patens mean decay rates varied in the control marsh from 2.23 mg/g/day in April to 1.65 mg/g/day in August. Low values in the oiled marsh of 1.50 mg/g/day were found in February and November. A high value of 2.70 mg/g/day was estimated in May. No significant difference could be found between oiled marsh and control marsh values any time during the study.

D. spicata mean decay rates ranged from a high of 3.50 mg/g/day in March and April to a low of 2.02 mg/g/day in October. Oiled marsh rates rose to a high of 1.89 mg/g/day in July and

Table 6. Specific daily decay rates (r_m) of marsh grasses calculated from litter bag experiments. *

		S. alterniflora			J. roemerianus			S. patens			D. spicata		
month	marsh	mean rate	std error	sig dif	mean rate	std error	sig dif	mean rate	std error	sig dif	mean rate	std error	sig dif
Feb	oiled	5.50	1.50		0.00	0.00		1.50	0.50		1.50	0.50	
	control	--	--		--	--		--	--		--	--	
Mar	oiled	5.50	1.50	ns	0.00	0.00	ns	1.50	0.50	ns	1.50	0.50	ns
	control	6.00	1.00		1.50	0.50		2.00	1.00		3.50	0.50	
Apr	oiled	5.97	0.75	ns	0.95	0.73	ns	1.74	0.26	ns	1.50	0.30	**
	control	6.60	0.78		1.96	0.42		2.23	0.51		3.50	0.30	
May	oiled	7.79	1.30	ns	2.14	1.08	ns	2.70	0.42	ns	1.50	0.22	*
	control	7.18	0.67		1.68	0.42		1.85	0.48		2.86	0.51	
Jun	oiled	10.17	1.40	ns	2.63	0.93	ns	2.51	0.35	ns	1.81	0.30	ns
	control	7.39	0.90		2.16	0.64		1.99	0.50		2.83	0.48	
Jul	oiled	10.69	1.26	*	2.67	0.92	ns	2.50	0.34	ns	1.89	0.33	ns
	control	6.99	1.07		2.20	0.66		1.73	0.52		2.35	0.48	
Aug	oiled	10.43	1.12	*	2.78	1.17	ns	2.05	0.24	ns	1.89	0.39	ns
	control	6.70	1.00		2.25	0.65		1.65	0.49		2.36	0.49	
Sep	oiled	9.86	1.20	*	2.90	1.33	ns	1.83	0.17	ns	1.80	0.41	ns
	control	5.80	0.76		2.56	0.51		1.97	0.31		2.25	0.58	
Oct	oiled	8.47	0.89	*	3.55	1.95	ns	1.74	0.26	ns	1.48	0.50	ns
	control	5.48	0.76		2.24	0.49		1.76	0.25		2.02	0.73	
Nov	oiled	7.50	1.50	ns	5.00	4.00	ns	1.50	0.50	ns	1.00	0.00	ns
	control	5.00	0.84		2.00	1.00		2.00	0.00		2.50	1.50	

(a) mean rates in mg $(g)^{-1}(day)^{-1}$
significant difference between mean rates: ns = no significant difference; * = significantly different at 5% level; ** = significantly different at 1% level

August, then declined to 1.00 mg/g/day in November. Mean decay rates were significantly greater in the control marsh compared to the oiled marsh during April ($p<0.01$) and May ($p<0.05$).

Physical characteristics of the grasses (Tables 7 and 8).

Spartina alterniflora

Analysis of the live standing stock of *S. alterniflora* in the control marsh found the number of stems per unit area to vary from a low of $82/m^2$ in December to a high of $345/m^2$ in May. The mean weight of a stem increased from 0.43 g in March, at the beginning of the growing season, to a maximum of 13.80 g in late August as the grass flowered. The mean height of a stem similarly increased from 7.7 cm at the March sampling to a maximum of 41.2 cm in the July sample.

The size class frequencies of stem heights indicated the appearance of a second cohort of new shoots during August. Through June and July stems equal to or less than 15 cm in height comprised 4% or less of the sample population of shoots. In August the size classes equal to or less than 15 cm in height represented 20% of the population. The ratio of mean weight to mean height of stems produced values lowest in March, at 0.06 g/cm, and highest in August, at 0.40 g/cm.

In the oiled marsh, *S. alterniflora* live standing stocks differed from the control in several instances. The density of live stems varied from $0/m^2$ in February to a maximum of $808/m^2$ in May. The oiled marsh maximum density was more than twice the control

Table 7. Physical characteristics of *S. alterniflora* and *J. roemerianus* in the experimental marshes. *

		S. alterniflora				*J. roemerianus*			
sample date	marsh	$\frac{\text{stems}}{\text{m}^2}$	mean wt	mean ht	$\frac{\text{wt}}{\text{ht}}$	$\frac{\text{stems}}{\text{m}^2}$	mean wt	mean ht	$\frac{\text{wt}}{\text{ht}}$
Nov 01	oiled	192	5.86	28.4	0.21	578	8.00	67.5	0.12
Nov 06	control	158	4.98	23.3	0.21	810	6.89	60.3	0.11
Dec 04	oiled	112	1.37	15.5	0.09	480	6.01	58.8	0.10
Dec 01	control	82	2.58	19.0	0.14	465	7.21	60.4	0.12
Jan 02	oiled	63	1.01	12.0	0.08	463	7.86	58.8	0.13
Jan 03	control	137	0.88	10.9	0.08	517	6.48	59.8	0.11
Feb 21	oiled	0	0.00	0.0	0.00	340	5.64	52.5	0.11
Mar 08	control	115	0.43	7.7	0.06	708	8.38	67.8	0.12
Apr 03	oiled	140	0.49	16.5	0.03	418	5.39	47.2	0.11
Apr 07	control	110	0.92	14.6	0.06	595	5.63	50.1	0.11
May 02	oiled	808	1.95	23.6	0.08	472	4.09	40.0	0.10
May 02	control	345	3.72	30.6	0.12	450	7.35	62.1	0.12
Jun 03	control	205	6.07	32.0	0.19	808	5.52	52.8	0.10
Jul 20	oiled	120	8.00	41.0	0.20	418	6.13	56.6	0.11
Jul 10	control	288	9.54	41.2	0.23	552	7.53	64.7	0.12
Aug 10	oiled	272	3.94	31.8	0.12	270	5.79	54.7	0.11
Aug 27	control	308	13.80	34.7	0.40	442	6.68	54.6	0.12
Sep 26	oiled	140	4.17	31.7	0.13	382	6.72	59.3	0.11
Sep 26	control	200	8.16	28.8	0.28	368	6.02	57.0	0.11

* mean weight per stem in grams, mean height per stem in centimeters, mean weight/mean height in grams per centimeter

Table 8. Physical characteristics of S. patens and D. spicata in the experimental marshes. *

		S. patens				*D. spicata*			
sample date	marsh	stems/m^2	mean wt	mean ht	wt/ht	stems/m^2	mean wt	mean ht	wt/ht
Nov 01	oiled	238	3.84	46.3	0.08	168	1.58	24.8	0.06
Nov 06	control	120	0.97	23.3	0.04	198	1.20	16.7	0.07
Dec 04	oiled	87	2.30	26.9	0.08	135	0.80	14.0	0.06
Dec 01	control	78	1.72	24.8	0.07	255	0.58	11.8	0.05
Jan 02	oiled	90	1.30	20.7	0.06	78	0.41	7.4	0.06
Jan 03	control	132	0.73	13.9	0.05	102	0.23	7.3	0.03
Feb 21	oiled	53	1.39	22.8	0.06	20	0.38	8.1	0.05
Mar 08	control	135	0.49	11.8	0.04	92	0.35	6.6	0.05
Apr 03	oiled	55	1.10	20.4	0.05	58	0.65	10.5	0.06
Apr 07	control	165	0.70	16.7	0.04	145	0.30	9.5	0.03
May 02	oiled	158	1.15	26.9	0.04	60	0.62	15.6	0.04
May 02	control	240	0.69	21.7	0.03	1185	0.55	12.2	0.05
Jun 03	control	666	0.92	30.1	0.03	732	0.95	21.6	0.04
Jul 20	oiled	200	3.26	46.2	0.07	1105	1.68	26.0	0.06
Jul 10	control	500	1.73	38.8	0.04	1245	1.40	26.3	0.05
Aug 10	oiled	155	3.53	48.6	0.07	540	1.70	27.7	0.06
Aug 27	control	173	3.23	43.4	0.07	437	2.13	27.8	0.08

* mean weight per stem in grams, mean height per stem in centimeters, mean weight/mean height in grams per centimeter

maximum which occurred simultaneously. The mean weight per stem ranged from 0 g (in the absence of any live shoots in the February sample) to a maximum 8.00 g in the July sample. Flowering in the oiled marsh occurred in August as it did in the control marsh, but the effects of the oiling were widespread by August and the *S. alterniflora* was undergoing a general decline evidenced by the lower mean weight per stem. The mean height per stem in the oiled marsh closely paralleled the control data with a minimum in February and a maximum height obtained in July. Although the mean heights did not differ greatly from the control, the population of heights exhibited less variation in the oiled marsh. Using August samples as an example, in the oiled marsh the mean height was 31.8 cm with a standard deviation of 12.5 cm. In the control marsh the mean height was 34.7 cm with a standard deviation of 21.2 cm.

The size class frequencies of stem heights indicate the second cohort of shoots which appeared in the control marsh in August, was suppressed in the oiled marsh. In July shoots equal to or less than 15 cm in height constituted 4% of the sample population in both the control and the oiled marsh. In the control marsh the percentage of the population equal to or less than 15 cm in height increased to 16% in August and 20% in September. In the oiled marsh the same size shoots constituted only 5% of the population in August and 4% in September.

The ratio of mean weight to mean height of live shoots in the oiled marsh showed a close parallel with values from the control marsh until April when the first visible signs of the dosing appeared on the grass. Shoots in the oiled marsh were consistently less robust than shoots in the control marsh. The difference was particularly striking in August and September when the typical oiled marsh shoot was approximately as tall as a control shoot, but weighed considerably less.

Juncus romerianus

The physical characteristics of the live standing stock of J. roemerianus did not vary greatly during the experiment in either the control or the oiled marsh. Shoot densities in the control marsh varied irregularly with a maximum estimated to be 810/m^2 in November and a minimum, 368/m^2 in September. The oiled marsh also varied irregularly with a maximum shoot density of 578/m^2 in November and a minimum of 270/m^2 in August. Mean weights varied from 8.38 g to 5.52 g in the control marsh and from 8.00 g to 4.09 g in the oiled marsh. Mean heights varied from 67.8 cm to 50.1 cm in the control marsh and from 67.5 cm to 40.0 cm in the oiled marsh. The mean weight:mean height ratios remained almost constant in both marshes, all values falling within the range of 0.10 to 0.13 g/cm.

Spartina patens

Estimates of S. patens shoot densities varied widely from 78/m^2 to 666/m^2 in the control marsh and 53/m^2 to 238/m^2 in the oiled marsh. A broad trend toward higher shoot densities in late summer and fall was indicated in both marshes. Mean weights and mean heights indicated a similar trend in both marshes. In the control marsh the minimum mean weight (0.49 g) and the mean height (11.8 cm) were found in March and the maximum mean weight (3.23 g) and mean height (43.4 cm) were found in August. The oiled marsh closely paralleled the control with a minimum mean weight (1.10 g) and mean height (20.4 cm) found in April and a maximum mean weight (3.84 g) in November and maximum mean height (48.6 cm) in August. The mean weight:mean height ratios from both marshes were also very similar, ranging from 0.03 g/cm to 0.08 g/cm. Minima were found in spring and early summer and maxima were found in late summer and fall.

Distichlis spicata

Samples of D. spicata indicated mean shoot densities in the control marsh ranging from $92/m^2$ in March to $1245/m^2$ in July. The oiled marsh samples indicated a minimum mean shoot density of $20/m^2$ in February and a maximum of $1105/m^2$ in July. The mean weight per shoot was 0.23 g for the control marsh in January. The value rose to a maximum of 2.13 g in August. In the oiled marsh the minimum mean weight was 0.38 g in February and the maximum was 1.70 g in August. Mean heights in the control marsh rose from 6.6 cm in March to 27.8 cm in August. In the oiled marsh mean heights went from 7.4 cm in January to 27.7 cm in August. Shoot density, mean weight and mean height in both marshes were minimal in early spring and maximal in late summer. The mean weight:mean height ratio varied from 0.04 g/cm to 0.06 g/cm in the oiled marsh and the control marsh varied from 0.03 g/cm to 0.08 g/cm.

Hydrocarbon content of sediments and _S_. _alterniflora_.

The distribution of saturated petroleum hydrocarbons in the surface sediments of the oiled marsh is depicted in Figure 4. The pattern of high concentrations in the corners of the pond is a result of wind directed slick movement after release at the pond mouth. The map presents the generalized pattern of concentrations in the marsh. It does not reflect the very patchy distribution of concentrations. Concentrations of saturated hydrocarbons in samples taken within several centimeters of one another could be found to differ by two orders of magnitude.

Analysis of sediment cores detected no saturated petroleum hydrocarbons below 20 cm depth. Five and one half months after dosing ended, hydrocarbon concentrations in the 0 to 10 cm core section ranged up to 241 ppm (dry weight). The 10 to 20 cm core sections had 2 ppm (dry weight) or less.

The analysis of S. _alterniflora_ root and rhizome tissue from the control marsh produced chromatograms characterized by a dominance of n-alkanes with odd numbers of carbon atoms. The n-alkane series began at C_{19}. Chromatograms of _S_. _alterniflora_ tissues from the oiled marsh showed the presence of n-alkanes beginning with n-dodecane, indicating contamination by the introduced oil. (see Figure 5)

The #2 fuel oil used in this study had an n-C_{17}/pristane ratio of 1.43 and an n-C_{18}/phytane ratio of 2.58. Root and rhizome tissues collected from heavily contaminated areas of the oiled marsh two months after dosing ceased exhibited an n-C_{17}/pristane

Figure 4. Concentration of the saturate fraction of the experimental oil in the sediments of Whittaker Creek in October 1974.

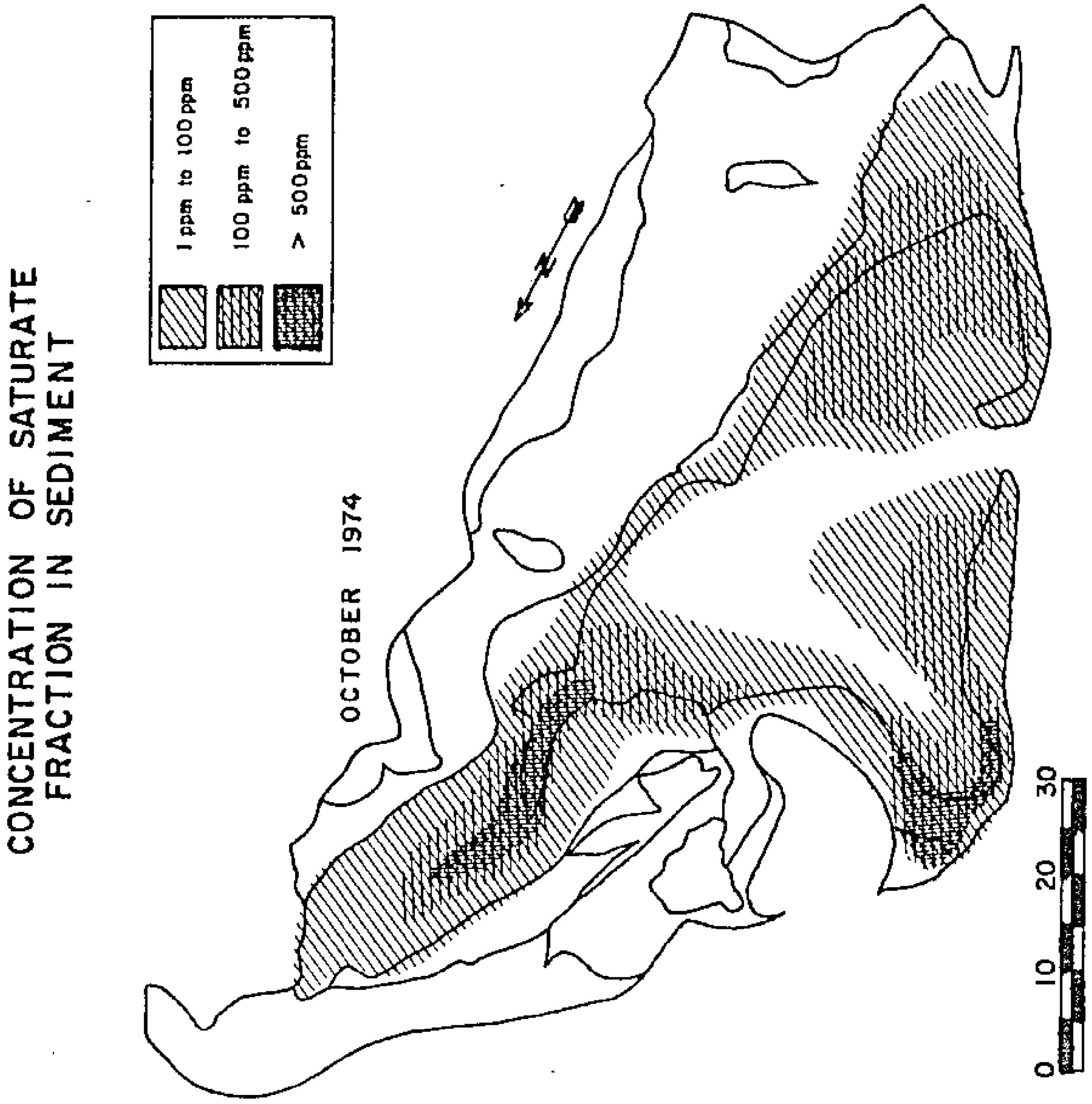
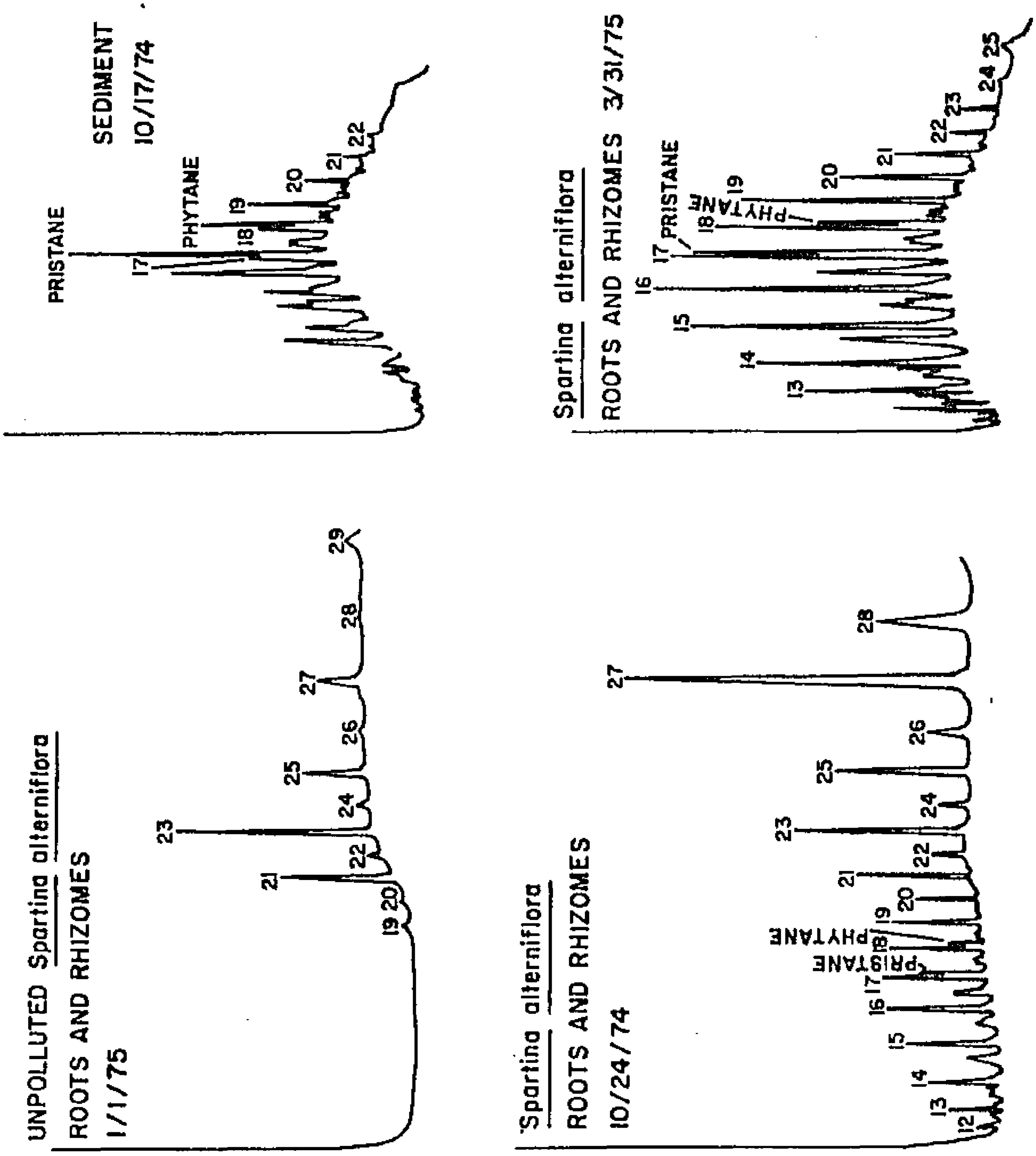


Figure 5. Chromatograms of the saturate fractions extracted from sediment and S. _alterniflora_ roots and rhizomes in the oiled and control marshes.



ratio of 1.31 and an n-C_{18}/phytane ratio of 2.12. Five and one half months after dosing ended tissue samples had an n-C_{17}/pristane ratio of 1.25 and an n-C_{18}/phytane ratio of 2.03. Seven and one half months after dosing ended the ratios were 1.55 and 2.53 respectively.

Sediment collected adjacent to the root and rhizome samples five and one half months after dosing ended contained no measurable n-C_{17} or n-C_{18}, although the isoprenoids pristane and phytane could still be identified.

DISCUSSION

Selection of dosage rate.

The dosage rate of 45 liters of #2 fuel oil twice a month can be roughly compared to other studies as follows. The average volume of water entering Whittaker Creek marsh on a high tide was estimated to be 750,000 liters. If the oil had been evenly dispersed throughout that volume of water, the concentration of oil in water would have been approximately 60 ppm. The American Petroleum Institute (1963) listed the average oil concentration in refinery wastes as 57 ppm. Lytle (1975) used a concentration of 250 ppm to study effects of a single dose of crude oil on a salt marsh pond. Nadeau and Roush (1973) used a single dose of #2 fuel oil at a concentration of 1000 ppm in a study of a marsh microcosm. Within Whittaker Creek the effective concentrations most certainly varied widely due to uneven dispersion of the oil. Additionally the marsh was flushed 25 to 35 times between dosings. Effective water column concentrations are consequently difficult to predict.

In a study of the effects of successive spillages, Baker (1971b) sprayed 10 m^2 marsh plots with 4.5 liters of fresh Kuwait crude oil. The effective dose through time was 0.45 liters of oil/m^2 per month. If all the oil introduced to Whittaker Creek on each dosing ended up evenly spread through the S. alterniflora zone of the marsh, the effective dose in this study would have been 0.066 liters of oil/m^2 per month, or approximately one sixth the dose used by Baker.

Physical configuration of the marshes.

The changes in the physical configuration of the oiled marsh were very significant. They occurred within a relatively short time span and effectively prevented any possibility of rapid recovery. Restoring the original character of the marsh-pond system will require recolonization of the spits. The spits, however, have been eroded to a point below the normal occurrence of S. alterniflora in the area. It is probable therefore, that any reformation of those features will have to occur by a slow invasion from the former terminus on the marsh proper. The process will take years, lasting long after the direct effects of petroleum hydrocarbons have subsided.

Net production of the marsh grasses.

The net production estimates for grasses in the control marsh are in general agreement with estimates previously reported in the literature on this subject. Results are not directly comparable because of differences in techniques. Estimates in this study are higher than some others because decay losses have been included.

The calculations for each sampling interval are based on a simple two compartment model. All production occurs in a live compartment and eventually enters the dead compartment. Loss from the dead compartment can occur either as small particles which have undergone some decay or as large particles flushed from the marsh by tides. The division between the two particle sizes is arbitrarily set by the selection of the litter bag mesh size. This differs from the original conceptualization by Weigert and Evans (1964). In their model, loss from the dead compartment could only occur through decay. Mathematically that meant the mortality of live material (d_i) as estimated by equation 4, could never be negative. In the present model a negative solution represents an export of material from the dead compartment, greater than the amount which could have decayed. Nevertheless, a negative solution is treated as a zero, since it also implies there was no net input from the live compartment to the dead compartment.

A general north to south trend of increasing productivity has been suggested for S. alterniflora. Reported values along the Atlantic coast range from about 500g/m^2/yr in Rhode Island and Long Island to well over 2000 g/m^2/yr in Georgia. In the middle Atlantic region, previous estimates include 361 to 572 g/m^2/yr (Mendelssohn 1973), 1332 g/m^2/yr (Wass and Wright 1969) and 329 to 1296 g/m^2/yr (Stroud and Cooper 1969). The estimate in this study of 1040.1 g/m^2/yr falls well within the range of reported values.

The estimate of net production in the oiled marsh was not greatly reduced compared to the control marsh. In fact, the brief preliminary study would seem to indicate that the relative production is very similar to predosing levels. A close look at the data used in production estimates finds that the value for the oiled marsh has been maintained high largely as an artifact of the calculation method.

Estimation of net production by the method used in this study depends on measurements of the live standing stock, the dead standing stock and the decay rate. In the marsh affected by oil, changes in these values apparently compensated each other during the first growing season. The live standing stock decreased, reflecting both a loss of productive area and a decreased vigor of the plants. The dead standing stock increased as did the decay rates. As a result much of the estimated production was being measured as loss from the living compartment rather than gains. This is reflected in the net decrease of the live standing stock in the oil marsh compared to a net increase in the control marsh.

The implication is that the marsh would be unable to sustain the level of production during the next growing season, even in the absence of further oiling. If the oiling had continued, it is very likely that the marsh would have lost its entire complement of _S_. _alterniflora_.

The population of _J_. _romerianus_ in both marshes maintained large and comparatively stable standing stocks of live and dead plant material. The production estimates (1019.9 g/m^2 in the oiled marsh and 1491.2 g/m^2 in the control marsh) are somewhat higher than values reported for other studies in the middle Atlantic region. Wass and Wright (1969) reported 650 g/m^2 in Virginia. In North Carolina reported values include 895 g/m^2 (Waits 1967), 560 g/m^2

(Foster 1968), 850 g/m^2 (Williams and Murdoch 1969), and 796 g/m^2 (Stroud and Cooper 1969).

There was no visible effect of oiling of the J. roemerianus population with the one exception mentioned previously. The difference in production between the oiled marsh and the control marsh appeared from preliminary work, to be a natural difference.

Estimates of the net production of S. patens in this study (669.6 g/m^2 in the oiled marsh and 589.7 g/m^2 in the control marsh) fall within the range of values reported elsewhere. Nixon and Oviatt (1973) estimated production to be 430 g/m^2 in Rhode Island and Waits (1967) reported 1367 g/m^2 in North Carolina. Any comparisons must be qualified by the fact that this study involved mixed stands of S. patens and other grasses. Production in pure stands would undoubtedly be higher.

Differences in S. patens production between the two marshes cannot be attributed to the oil dosing. The grass occurs at an elevation infrequently flooded. Consequently there were no visible signs of oil effects and no oil could be found in the sediments where S. patens occurs.

The estimated net production of D. spicata in this study (506.6 g/m^2 in the oiled marsh and 702.3 g/m^2 in the control marsh) is in the general range of previously reported values. Wass and Wright (1969) found 360 g/m^2 in Virginia and Udell et al. (1969) reported 647.5 g/m^2 on Long Island. The higher estimates reported here can be partially attributed to the inclusion of decay losses in the production calculations. There were no apparent effects of the oiling on the population of *D. spicata*.

Litter bag experiments.

The study of decay rates reported here differs from many previous works in the selection of material enclosed in the litter bags. Other workers have enclosed entire leaves, stems, or both. This study was not designed to look at the disappearance rate of fresh litter fall. Instead, the goal was to measure the disappearance rates of the dead standing stock over a sequence of time intervals. The most directly comparable studies are those using the paired plots design (e.g. Reimold et al. 1975). Comparisons with that type of study should be made with cognizance of the limitations of the litter bag technique. The principal difficulty is the impedance or exclusion of decomposers and grazers.

The failure to indicate significant differences in decay rates between marshes where apparent differences were found (in the final sampling period) is a consequence of the reduced number of samples.

S. alterniflora decay rates reported in this work are slightly lower than values reported by Reimold et al. (1975). They found a yearly average rate of 7 mg/g/day in the tall-form *S. alterniflora* and 18 mg/g/day for short-form *S. alterniflora*. The varation between the two studies is probably due in part to technique and latitudinal differences.

A discrete peak in decay rates of *S. alterniflora* was found in this study in contrast to the results of Reimold et al. (1975). As in this study, Kirby (1971) working in Louisiana found a high monthly decay rate in June. His value, for a stream side location is approximately double the value reported here (14.6 mg/g/day compared with 7.39 mg/g/day).

The significant increase in the decay rate of the S. alterniflora dead standing stock in the oiled marsh can probably be attributed to some combination of the following factors. Exposure to physical forces may have been increased as the vigor and amount of live material decreased. The material entering the dead stock on the marsh may have been in a less refractory condition than normal. The microbial community effecting breakdown of dead plant material may have been substantially altered.

Increased exposure of decaying plant material most certainly occurred as the living S. alterniflora was affected by the oil. The changes in the physical configuration of the marsh attest to that fact. The litter bags themselves, however, serve to prevent much physical reworking of the enclosed plant material.

Both the qualitative and the quantitative observations of the living S. alterniflora in the oiled marsh attest to its declining vigor as the oiling continued. VanOverbeek and Blondeau (1954) stated the effect of oils in plants was primarily a physical chemical solubilization which acted on the plasma membrane. Such an effect might readily enhance the breakdown of plant material. Any changes occurring in the material entering the dead stock would have been reflected in the litter bags since the previous month's dead standing stock was always used to fill them.

The hypothesis that changes in the microbial community could account for increased decay rates was not tested in this study. Walker et al. (1975) found fuel oil induced little noticeable effect on yeast and fungi populations in sediment previously free of oil. However, bacterial growth was limited by fuel oil. Kator and Herwig (1977) suggested that chronic or acute oil released in a marsh might lead to a reduction in cellulose decomposition as the microbial community shifted to hydrocarbon degradation. Conversely, they also suggested that increased bacterial protein derived from hydrocarbon degradation might aid decomposition by other components of the detrital food web. These works indicate the microbial community, in particular the bacteria, probably were not responsible for the observed increases in decay rates. However, changes in the bacterial population may have secondarily caused the increased rates by providing substrate for other fauna in the food web.

Increased decay rates in the oiled marsh were not a result of increased activity by large invertebrate grazers. The populations of Littorina irrorata and Uca minax which inhabited the S. alterniflora marsh zone were both markedly depressed by the oil (Hershner and Lake, in prep. and unpublished data). If invertebrate grazers were responsible for the observed rate increases, their sizes must have been between those of bacteria and the mesh size of the litter bags (2.5 mm).

Decay rates for J. roemerianus, S. patens, and D. spicata were lower and more constant than the S. alterniflora rates. Unlike the results reported by Reimold et al. (1975) decay rates for J. roemerianus in this study were highest during the fall. Also, the rates reported here for J. roemerianus are considerably less than the yearly average 7 mg/g/day Reimold et al. reported.

Decay rates in the oiled marsh differed significantly from rates in the control marsh only for D. spicata, and then only during April and May. No unstrained explanation for the differences could be developed.

Physical characteristics of the marsh grasses.

Physical characteristics of the marsh grass species were studied as simple indices of plant morphology and/or condition. Plants are known to respond to stress, both physical and chemical, with altered forms. In particular, researchers looking at the effects of oils on marsh plants have reported a variety of responses. Mackin (1950 a,b) reported D. spicata was killed but later regrew vigorously after applications of crude oil. Stebbings (1968) found Juncus maritimus, among other species, to be thriving after the "Torrey Canyon" spill. Baker (1971d) presented evidence of increased shoot lengths and dry weights in the salt marsh grasses Puccinellia martima and Festuca rubra following doses of Kuwait crude oil. Hershner and Moore (1977) reported increased density, production, and flowering success, but decreased height in S. alterniflora following a spill of No. 6 fuel oil.

The physical characteristics measured in this study are certainly not the only ones responsive to oil pollution. However, they serve to indicate some of the sublethal effects oil dosing may produce in marsh grasses. The ratio of mean weight to mean height, which is used here as a simple index of plant vigor, is insensitive to the distribution of growth forms within a sample. Nevertheless, for comparative purposes it provides satisfactory representation of general effects.

S. alterniflora was the only grass to show sublethal effects on the growth form which could be attributed to the oil dosing. The marked increase in the density of shoots in the oiled marsh during spring months is a response found in other studies (Mackin 1950a,b, Hershner and Moore 1977). The mechanism producing the effect is unknown. A chemical or physical blocking of development in young shoots by oil may produce an effect similar to mowing. Additional shoots are initiated at secondary points along rhizomes, resulting in a much denser stand. The subsequent decline in density may be a result of either mortality due to oil or self-thinning.

During this study the *S. alterniflora* population in the control marsh was apparently affected in several ways. During the fall and winter months, as the grass naturally declined, there was little evidence of any effects due to the oil dosing. In the spring, development of the first cohort of shoots was delayed briefly, but long enough to encourage the development of secondary shoots. The result was a relatively dense stand of light shoots. During the succeeding months large areas of *S. alterniflora* exhibited chlorosis and died. The remaining live stands became less dense and markedly less vigorous than control areas. The production of the usual second cohort of shoots during the late summer and fall was almost entirely suppressed by the oiling.

The physical characteristics of *J. roemerianus* evidenced no changes which might be attributed to the oil dosing. The species maintained a remarkably stable population in both marshes. Growth and dieback are year-round processes in *J. roemerianus* (Foster 1968), and the constancy of the mean weights and mean heights reflects the degree of balance between the two processes.

Data for *S. patens* and D. *spicata* exhibited expected seasonal trends, but no differences between the oiled and control marshes attributable to the oiling.

Hydrocarbon content of *S. alterniflora*.

The principal finding of the hydrocarbon analyses was the maintenance of relatively undegraded saturated petroleum hydrocarbons in dead *S. alterniflora* roots and rhizomes some time after oil in the adjacent sediments had been largely degraded. Other researchers (Burns and Teal 1971, Lytle 1975) have also found oil in *S. alterniflora* for extended periods of time following oil spills. The mechanism of entry into the plant in this case is not specifically known. Oil was not found in roots and rhizomes of living plants, suggesting that entrance to those tissues was gained primarily after the plant died.

The penetration of oil into the sediment in this study was not nearly as great as has been reported in other studies involving marshes. Burns and Teal (1971) found oil 70+ cm deep during studies of the West Falmouth oil spill. Lytle found oil as far as 42 cm into marsh sediments following an experimental spill of crude oil. Tidal amplitude cannot explain the differences in results. The marsh in which this study was conducted seems relatively young (in geologic time), therefore the depth of marsh peat and nature of the underlying sediment may contribute to observed differences in oil penetration.

SUMMARY

In a natural salt marsh community, repeatedly dosed with a No. 2 fuel oil:

-*S. alterniflora* was the only grass species to show effects of the oil dosing during the course of the study. This was attributed to its fringing location within the marsh. The other grass species showed no effects, presumably not because of any resistance, but rather because they were never exposed to the oil.

-Within one year, during dosing, the oiled marsh lost 52.5% of its complement of *S. alterniflora*.

-Net production of *S. alterniflora* was maintained at near control levels in the first growing season during dosing. Losses in both the live and dead standing stocks of *S. alterniflora* indicate it would be unable to sustain the level of production in succeeding years.

-Sublethal effects of the oiling on *S. alterniflora* included delaying development of the season's first cohort of new shoots, which resulted in a denser than normal stand of lighter than normal shoots.

-The second cohort of new shoots, usually produced in the fall, was suppressed almost entirely.

-Those shoots which survived the entire season were generally as tall but much lighter than shoots from the control marsh.

-Decay rates of *S. alterniflora* were found to be higher and to peak later in the year than values from the control marsh.

-Secondary effects of the oiling included major changes in the configuration of the marsh as a consequence of the loss of *S. alterniflora*. Reformation of the marsh is foreseen as a long and uncertain process.

-Oil which entered the roots and rhizomes of dead *S. alterniflora* was maintained in a relatively undegraded state for at least seven months after dosing stopped.

Section 3

EFFECTS OF CHRONIC OIL POLLUTION ON SALT MARSH GASTROPOD POPULATIONS.

INTRODUCTION

Gastropods in salt marshes are generally viewed as consumers which, through their grazing activities, may regulate microbial and algal communities (Smalley 1958, Wetzel 1976). As such their potential influence on the detritus-based system is great. Changes in gastropod population size or distribution may result in significant changes in cycling of organic compounds in the marsh-estuary system.

In the presence of oil pollution, gastropods have proven among the least sensitive organisms to soluble aromatic hydrocarbons. Moore et al. (1973) estimate the concentration of soluble aromatic hydrocarbons causing toxicity in gastropods to be in the range of 10-100 ppm. Hyland and Schneider (1976) using those values calculate the toxicity range for No. 2 fuel oil to be 50-500 ppm and proportionately higher for other types of oil and petroleum products.

In chronic oil pollution situations, where concentrations may not be high enough to cause toxicity, sublethal effects are of concern. In laboratory experiments Jacobson and Boyland (1973) showed feeding responses in Nassarius obsoletus could be affected at concentrations as low as 1 ppb of water soluble Kerosene components. Hyland and Miller (in prep) also found a significant reduction in chemosensory perception of food in N. obsoletus at concentrations of 10 ppb of No. 2 fuel oil. It is therefore probable that gastropod populations in natural environments are affected by sublethal concentrations of oil.

This investigation was designed to study the effects of low-level chronic oil pollution on the population size and distribution of three naturally occurring salt marsh gastropods, *Nassarius obsoletus*, *Littorina irrorata* and *Melampus bidentatus*. The goal was to describe effects observed and to develop explanatory hypotheses as guidelines for further work.

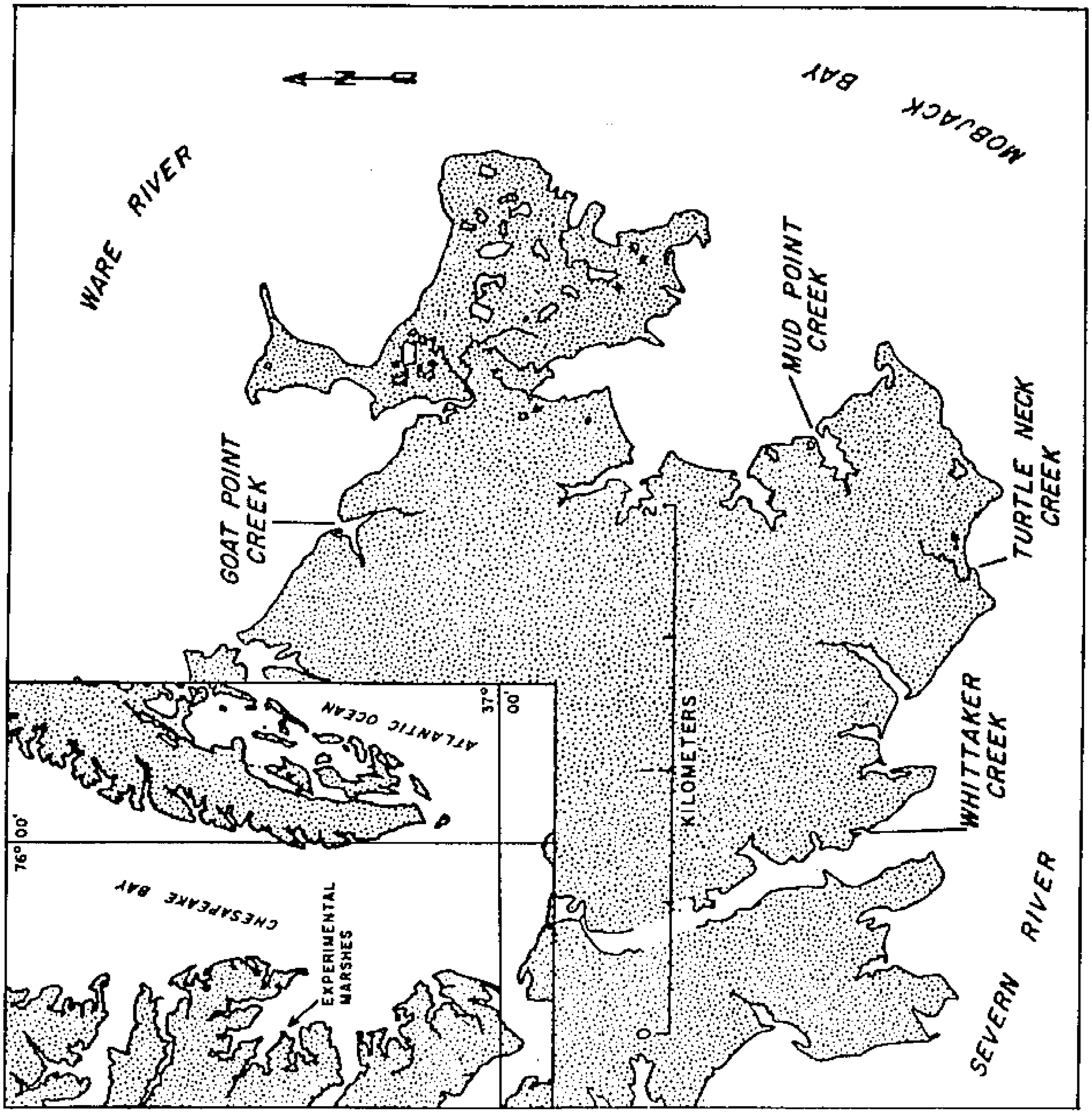
SITE

This study was conducted in two small pocket marshes in the Mobjack Bay area of the lower Chesapeake Bay (Figure 6). Goat Point Creek marsh (Figure 7) and Whittaker Creek marsh (Figure 8) are both comprised of a small tidal pond, surrounded by a marsh which grades quickly into a surrounding pine forest. The pond bottoms are soft mud. The majority of each pond bottom lies between neap tide and spring tide low water marks. Salinities ranged from 15 to 22 ppt and water temperatures varied seasonally from 2 to 32°C. The mean tide range is 0.69 meters.

The surrounding marsh consists of a fringe of *Spartina alterniflora*, backed by *Juncus roemerianus* and salt meadows dominated by *Spartina patens* and *Distichlis spicata*. Vertical profiles of the marshes typically showed a steady grade from the pond up to the surrounding forest. No berms or dykes were present at the pond edge. A more complete description of the vegetative community is provided in Section 2 of this dissertation.

Goat Point Creek was used as the control site throughout the course of the investigation. Whittaker Creek was dosed twice a month with 45 liters of a No. 2 fuel oil. Dosing began November 1, 1973 and ended August 10, 1974. The oil was introduced at the mouth of the pond by siphoning it into the water column on a flooding tide. The selection of the dosing schedule is explained in Section 2 of this dissertation.

Figure 6. Location of experimental marshes.



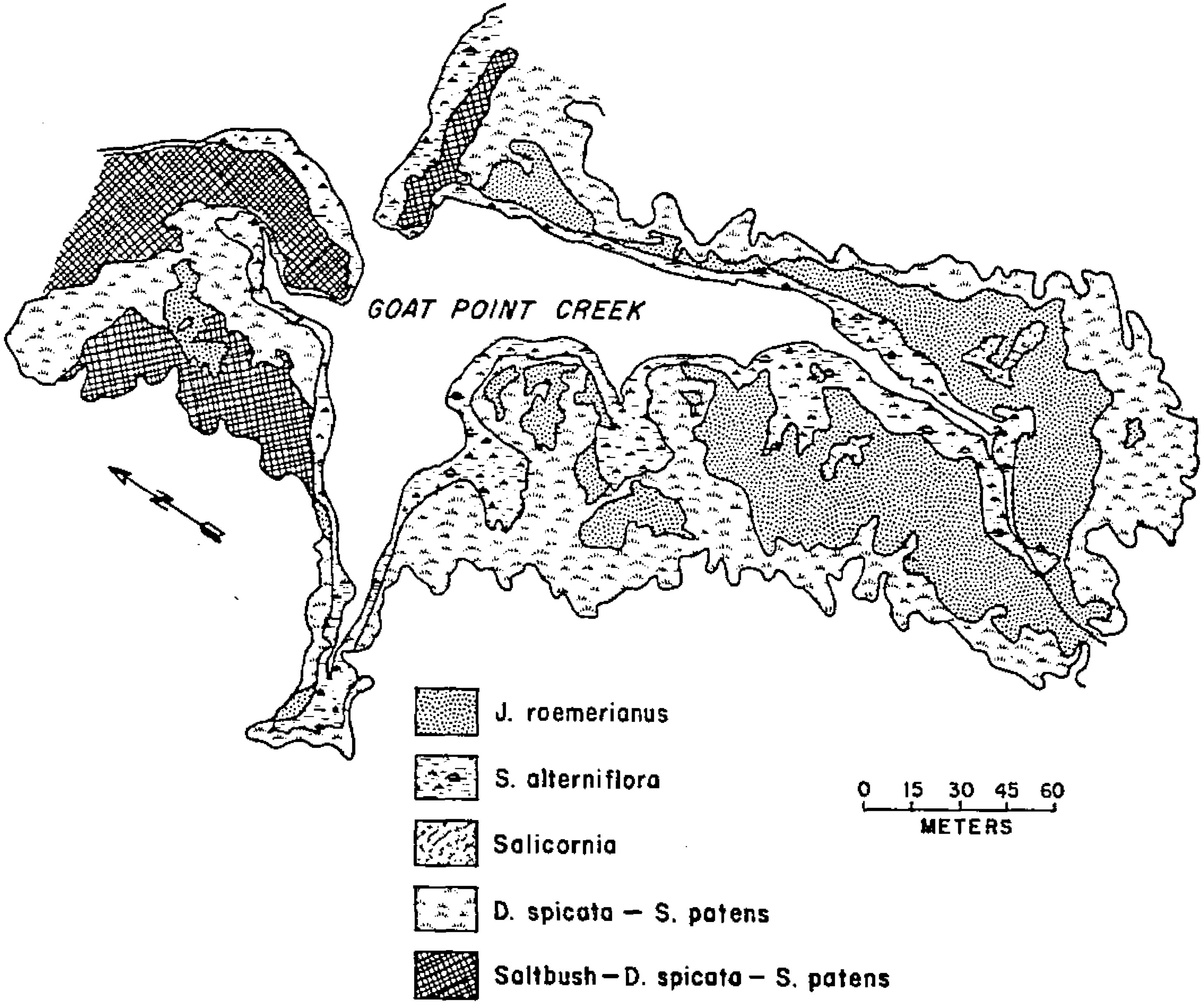
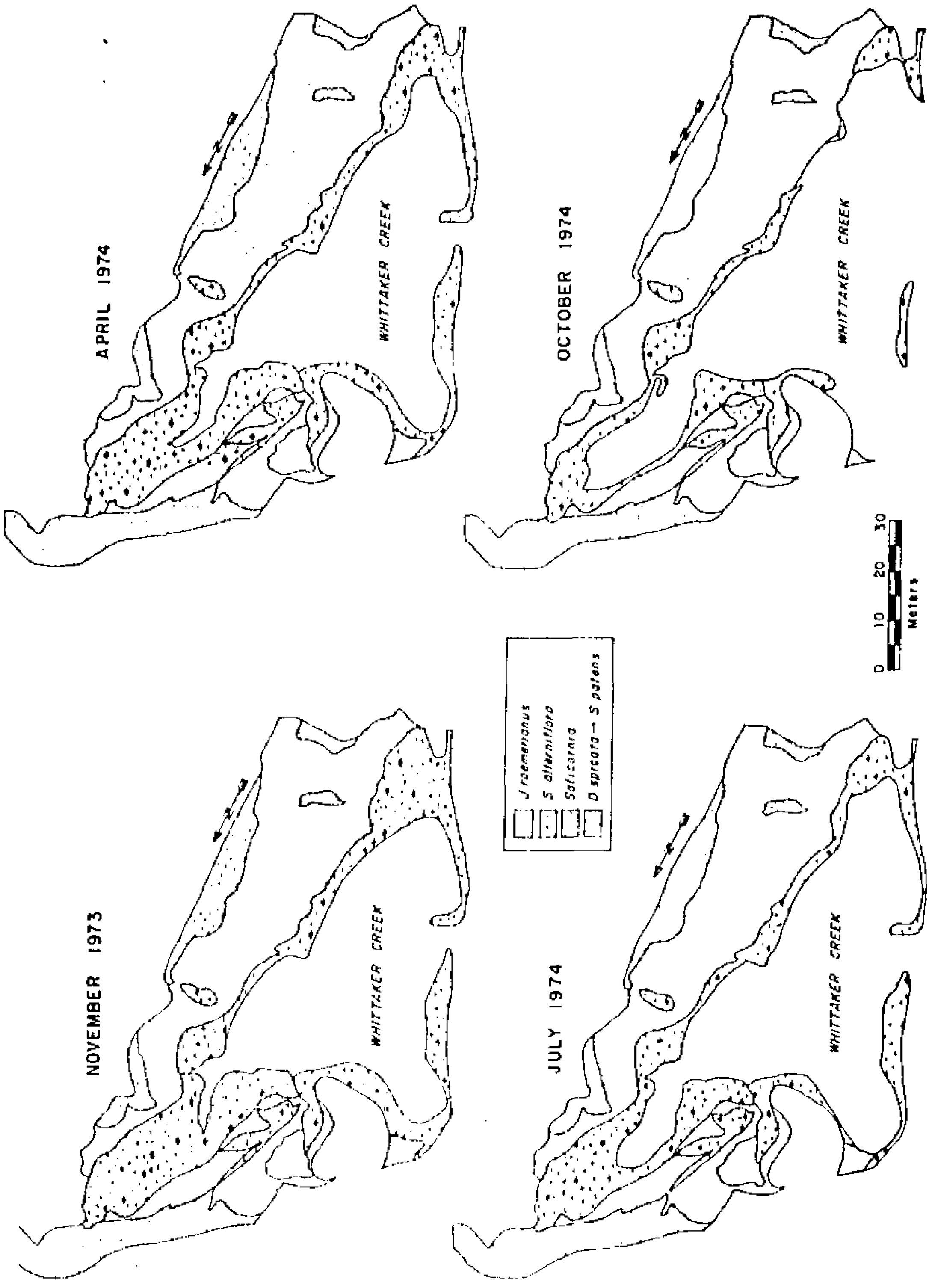


Figure 7. Vegetation map of Goat Point Creek (control marsh).

Figure 8. Vegetation maps of Whittaker Creek (oiled marsh) four times during the study.



METHODS

Populations of *Nassarius obsoletus*, *Littorina irrorata*, and *Melampus bidentatus* were sampled monthly in both the control marsh and the oiled marsh. Sampling involved counting all snails found within a quadrat placed on the sediment surface. A 1.0 m^2 quadrat was used for N. *obsoletus* samples and a 0.1 m^2 quadrat was used for *L. irrorata* and *M. bidentatus* samples. Quadrat locations were selected prior to each sampling effort by referring to maps of the marshes and a random numbers table. The sampling effort was stratified by vegetation zones. Each month, ten sites were chosen in the pond, four in the *Spartina alterniflora* zone, four in the *Juncus roemerianus* zone, and four in the *Spartina patens*-*Distichlis spicata* saltmeadows.

Estimations of population size for *N. obsoletus* were derived by multiplying the monthly mean densities of samples from the pond by the total pond area. Additionally, seasonal population size estimates were developed from maps of the population density. To accomplish this, three levels of density were identified (10/m^2, 10 to 20/m^2, and 20/m^2) as representative of the range of values recorded by monthly samples. Isopleths were plotted on maps of each marsh to define areas with densities 10/m^2 and 20/m^2. Population estimates were then derived by multiplying the density represented by an isopleth by the area enclosed.

The distribution of individuals in the N. obsoletus population was tested for agreement with a negative binomial distribution using techniques suggested by Elliott (1971). In order to have a sufficiently large sample size ($n = 40$) for distribution analyses, samples gathered over several months were combined to form two data sets for each marsh. In the control marsh, samples collected in November, December, January, and March formed one set and samples collected in June, July, August, and September formed the second set. The two data sets for the oiled marsh included November, December, January, and February samples in one set, and June through September in the second set.

The k values for samples were estimated by moments,

$$\text{estimate of } k = \hat{k} = \bar{x}^2/(s^2 - \bar{x}) \qquad (1)$$

where $\bar{x}$ = sample mean and s^2 = sample variance. $\hat{k}$ was then entered in the following equation as a first estimate in an iterative process to refine the estimate of k.

$$\hat{k} \log (1 + \bar{x}/\hat{k}) = \log (n/f_0) \qquad (2)$$

where n = number of samples, f_0 = frequency of zero counts.

Agreement with a negative binomial distribution was then tested by the statistic U.

$$U = s^2 - [\bar{x} + (\bar{x}^2/\hat{k})] \qquad (3)$$

Elliott states agreement with the negative binomial is accepted at the 95% level of greater when U is less than its standard error.

$$\text{standard error of } U = a\ (10/\sqrt{n}) \qquad (4)$$

where a = value interpolated from a log:log plot of $\bar{x}$ vs $\bar{x}/\hat{k}$ (see Elliott page 61).

Once agreement with the negative binomial had been ascertained Morisita's index of dispersion was developed for each set of samples. The index I_δ, assumes a value of one for random distributions and values greater than one for contagious distributions.

$$I_\delta = n[(\Sigma(x^2) - \Sigma x)/((\Sigma x)^2 - \Sigma x)] \qquad (5)$$

According to Elliott (1971), departures from randomness are judged significant at the 95% level when the value of

$$I_\delta(\Sigma x-1) + n - \Sigma x \qquad (6)$$

is outside the appropriate 5% significance levels of χ^2 with n-1 degrees of freedom.

L. irrorata populations were estimated by three techniques. Monthly estimates were developed by multiplying the mean density of *L. irrorata* in all samples collected in the marsh by the total area of the marsh. A second set of monthly estimates was developed by estimating the population size in each of the three marsh grass zones and summing the individual estimates. The third technique involved mapping the population densities in the same manner reported for *N. obsoletus*. For *L. irrorata* the appropriate density ranges for the mapping appeared to be $<50/m^2$, 50 to $100/m^2$, and $>100/m^2$.

The *M. bidentatus* populations were estimated by the same three techniques used for *L. irrorata*.

RESULTS

Nassarius obsoletus.

During the course of this investigation the population of *N. obsoletus* in both marsh ponds exhibited seasonal changes in distribution. The populations were small and concentrated in the deepest portions of the ponds during the winter and early spring. In the summer, each population increased in size and range. A decrease in the population size and range occured with the onset of winter.

In the control marsh, population size estimates based on the monthly sampling ranged from a low of 4,482 to a high value of 54,531 (Table 9). There was, however, no obvious seasonal pattern to the estimates.

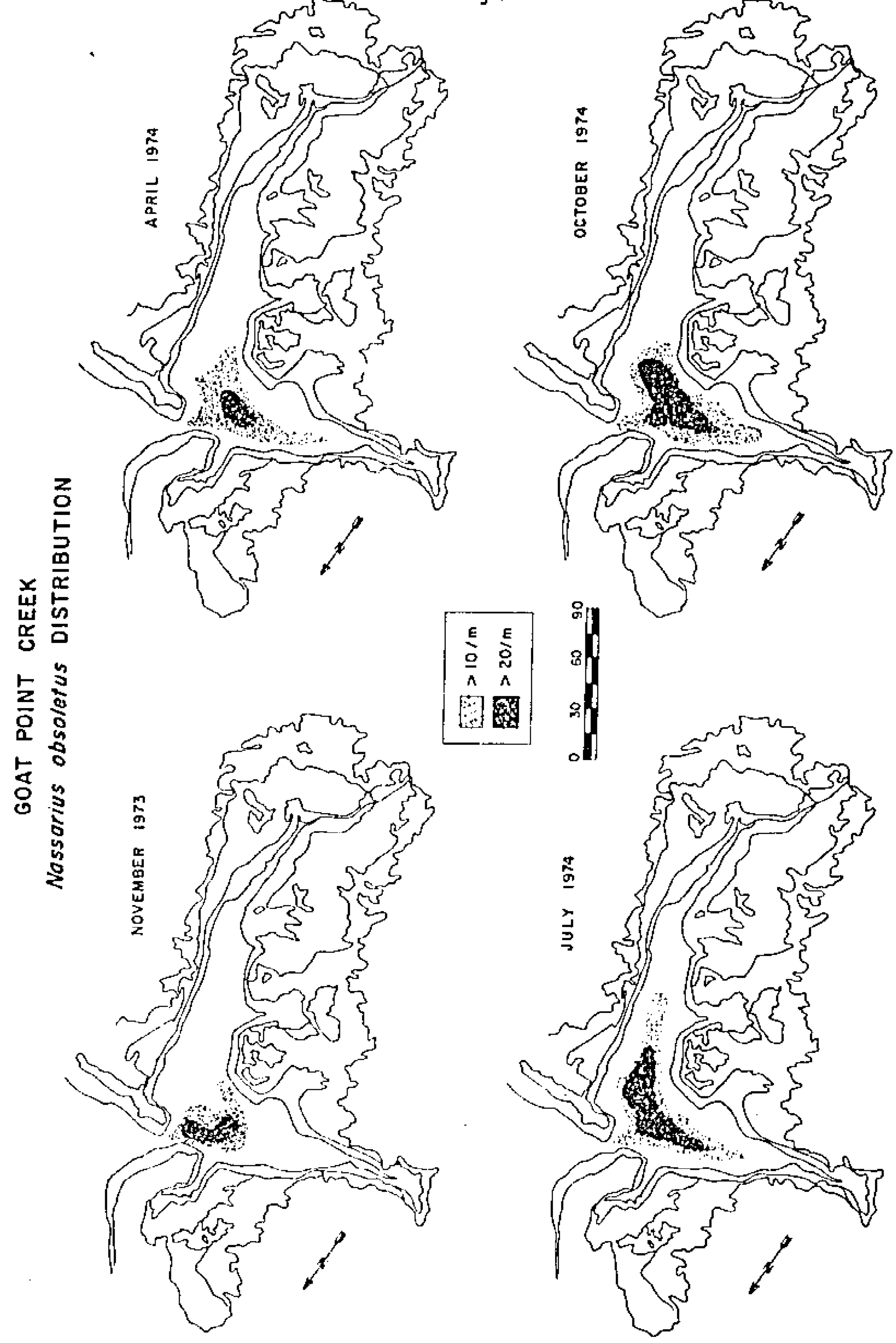
When density estimates were contoured on a map of the control marsh, a pattern consistent with the general field observations emerged (Figure 9). The population size estimates derived from the distribution maps are presented in Table 10. The estimates range from a low of 15,975 during November to a high of 39,150 during July. These values should be low estimates because they only represent population levels within the $10/m^2$ isopleth. Even within that area, the lowest density was assumed for calculations.

Population densities of *N. obsoletus* in the control marsh, ranged from $0.6/m^2$ to $7.3/m^2$ when averaged over the entire pond. When

Table 9. Population size and density estimates for *Nassarius obsoletus*.

control marsh			oiled marsh		
date	mean $\#/m^2$	pop est	date	mean $\#/m^2$	pop est
Nov 06	1.9	14193	Nov 01	12.7	18860
Dec 01	3.6	26892	Dec 04	7.4	10989
Jan 03	3.9	29133	Jan 02	5.6	8316
Mar 08	1.4	10458	Feb 21	7.5	11138
Apr 07	1.8	13445	Apr 03	17.1	25394
May 01	0.6	4482	May 02	18.4	27324
Jun 03	4.5	33615	Jun 03	10.6	15741
Jul 10	7.3	54531	Jul 20	22.6	33561
Aug 27	3.0	22410	Aug 10	12.8	19008
Sep 26	4.5	33615	Sep 26	3.5	5198

Figure 9. Distribution of Nassarius obsoletus in Whittaker Creek (oiled marsh) four times during the study.



averaged over just the area they inhabited (corresponds to the shaded area in Figure 9) the densities varied from $11.8/m^2$ to $15.3/m^2$. The maximum recorded density for a single sample was $390/m^2$ in July.

Analysis of data from combined November, December, January, and March samples, showed the _N. obsoletus_ population distribution fit a negative binomial model. The k value was estimated to be 0.13 and the U statistic (-17.2) was considerably smaller than the standard error of U (31.6). Morisita's index of dispersion for the November through March sample was 6.24. The distribution was significantly different from a random one at the 95% level.

The June through September sample also fit the negative binomial distribution. The k value was estimated to be 0.37. The U statistic (-4.7) was considerably smaller than the standard error of U (31.6). Morisita's index of dispersion for this data set was 3.45 and the distribution was significantly different from a random pattern at the 95% level.

In the oiled marsh, the population of _N. obsoletus_ estimated from monthly samples, varied from 5,198 to 33,561 (Table 9). No seasonal trends were obvious in the monthly data sets. As in the control marsh, however, when the data were plotted on a map and isopleths constructed, a general pattern of seasonal occurrence was detected (Figure 10). Population size estimates derived from the distribution maps were lowest in November at 9,025 and highest in July at 16,225 (Table 10). Again, these values should be considered low estimates because of the manner in which they were derived.

Population densities of _N. obsoletus_ in the oiled marsh

Figure 10. Distribution of Nassarius obsoletus in Whittaker Creek (oiled marsh) four times during the study.

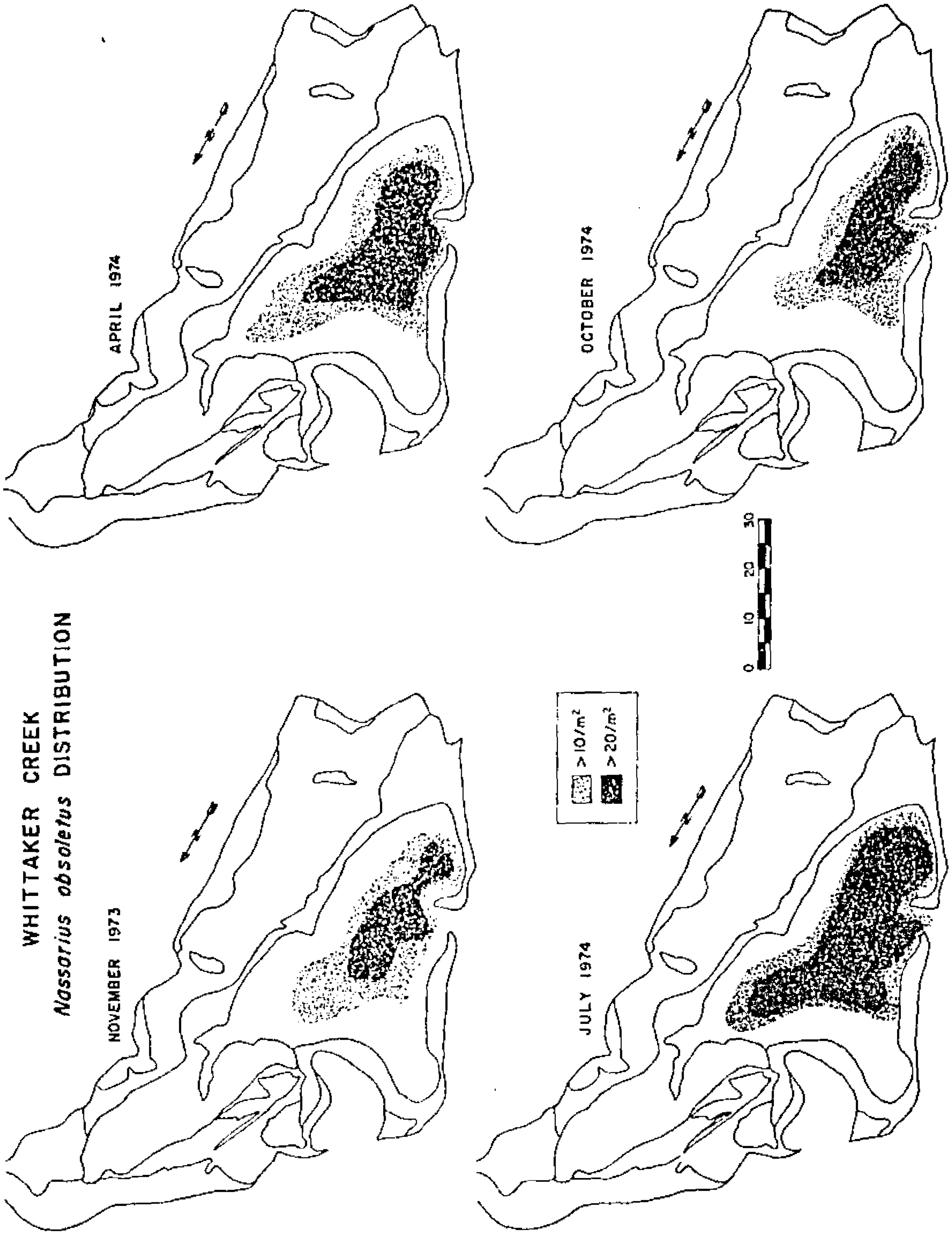


Table 10. Population density and size estimates derived from distribution maps.

control marsh

	N. obsoletus		*L. irrorata*		*M. bidentatus*	
date	mean #/m^2	pop est	mean #/m^2	pop est	mean #/m^2	pop est
Nov 06	13.7	15975	67.4	526500	13.6	223875
Apr 07	11.8	23175				
Jul 10	14.3	39150				
Sep 26	15.3	32625				

oiled marsh

	N. obsoletus		*L. irrorata*		*M. bidentatus*	
date	mean #/m^2	pop est	mean #/m^2	pop est	mean #/m^2	pop est
Nov 01	13.9	9025	69.4	91750	14.8	27125
Apr 03	15.2	12050	67.6	78125		
Jul 20	17.1	16225	68.6	79375		
Sep 26	14.6	9475	65.5	54875		

ranged from $3.5/m^2$ to $22.6/m^2$ when averaged over the entire pond (Table 9). When averaged over just the area they inhabited (corresponds to the shaded area in Figure 10), the densities varied from $13.9/m^2$ in November to $17.1/m^2$ in July.

The distribution analysis of N. obsoletus samples in the oiled marsh from November through February showed the population fit a negative binomial distribution. The k value was estimated to be 0.29. The U statistic (-115.6) was smaller than the standard error of U (94.9). Morisita's index of dispersion for the data set was 2.73, and the distribution was significantly different than a random one at the 95% level.

The June through September data set for the oiled marsh presented some difficulties in the distribution analysis. The k value was estimated to be 0.21, however the U statistic (164.1) was not smaller than the standard error of U (134.4). Therefore, the data did not appear to agree with a negative binomial distribution even though the variance of the data set (905.7) was much larger than the mean of the data set (12.4). The complicating factor was a single sample, taken in July, which contained 184 individuals. This value was approximately one order of magnitude greater than any other sample collected.

In order to permit analysis of the remainder of the data set, the anomalous value was disregarded. The mean of the remaining 39 samples was used to estimate the missing value for subsequent calculations. With the altered data set, the k value was estimated to be 0.25. The U statistic was -131.28 and the standard error of U was considerably larger (94.9). Morisita's index for the altered data set was significantly different from a random one at the 95% level.

Littorina irrorata.

In the control marsh, the *Littorina irrorata* population showed no evidence of seasonal differences in horizontal distributions. During the winter months the snails generally remained on the marsh surface, clustered around *S. alterniflora* stems. With the onset of spring and summer the population became more active, moving freely up and down grass stems. However, there was no noticeable expansion of the population's horizontal range. In general, *L. irrorata* was confined to the *S. alterniflora* zone and the lower part of the *J. roemerianus* zone (Figure 11).

Monthly estimates of the population size based on the mean density of *L. irrorata* over the entire marsh are given in Table 11. Estimates range from 118,775 to 1,562,554, with no obvious trends through time. Population size estimates based on the sampling stratified by grass zones, ranged from a low value of 106,557 to a high value of 1,164,126.

Monthly estimates of *L. irrorata* population densities averaged over the entire marsh, ranged from $4.5/m^2$ in September to $59.2/m^2$ in May. The stratified sampling found a maximum average density in the *S. alterniflora* zone in May at $92.5/m^2$. The minimum average density for that zone was reported as $2.5/m^2$ in September. In the *J. roemerianus* zone the maximum average density of $72.5/m^2$ was found in May and the minimum of $2.5/m^2$ occurred in August. *L. irrorata* was not found in the *S. patens*-*D. spicata* salt meadows.

Figure 11. Distribution of *Littorina irrorata* in Goat Point Creek (control marsh).

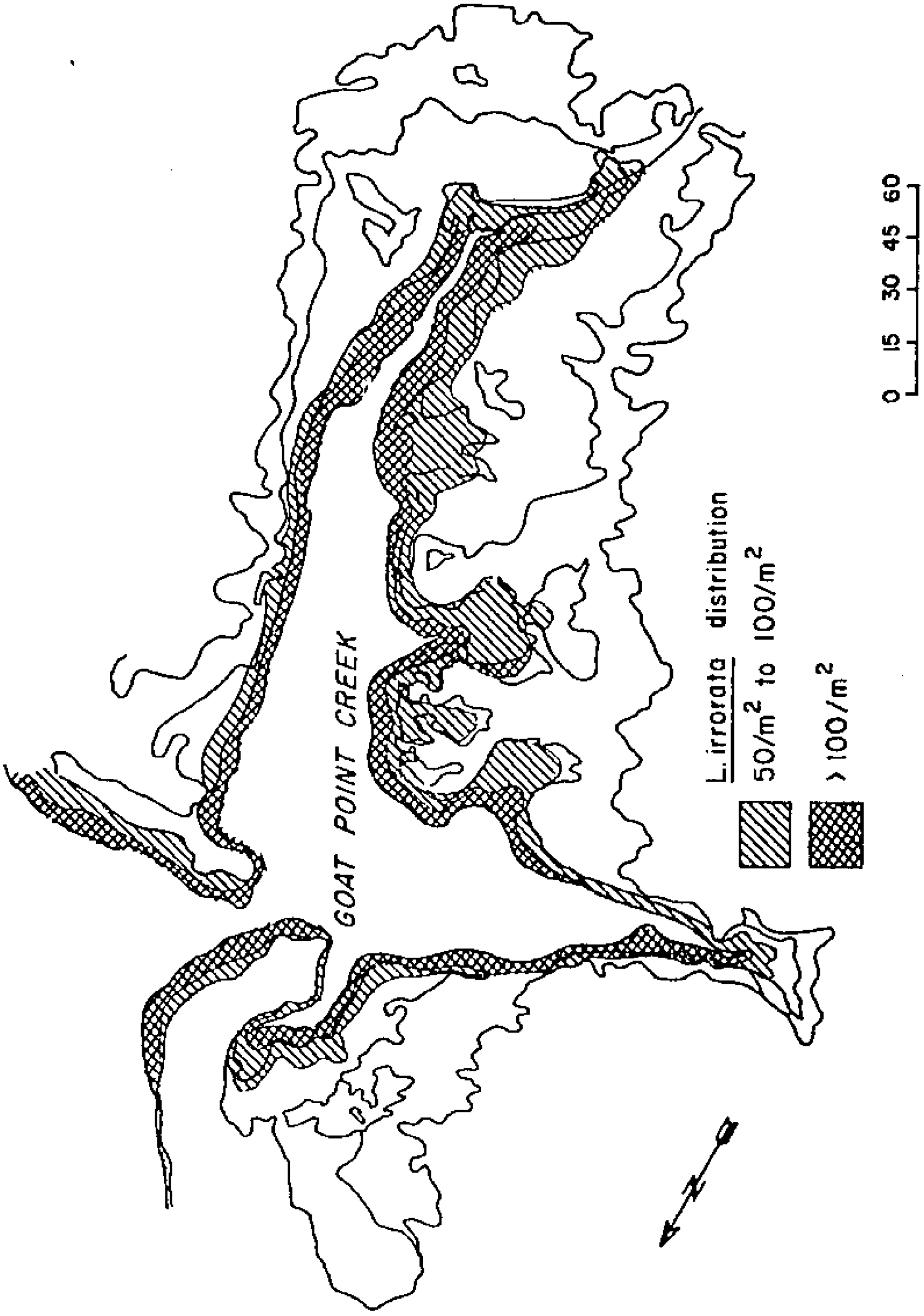


Table 11. Population size and density estimates for _Littorina irrorata_.

control marsh							oiled marsh						
	entire marsh		stratified sampling					entire marsh		stratified sampling			
date	mean #/m^2	pop est	grass zone*	mean #/m^2	pop est	total pop	date	mean #/m^2	pop est	grass zone*	mean #/m^2	pop est	total pop
Nov 06	38.2	1008270	S	47.5	250088	763760	Nov 01	38.3	135965	S	85.0	115812	164940
			J	55.0	513672					J	30.0	49128	
Dec 01	21.7	572761	S	47.5	250088	413529	Dec 04	45.8	162590	S	100.0	136250	197660
			J	17.5	163441					J	37.5	61410	
Jan 03	18.2	480380	S	30.0	157950	407315	Jan 02	68.2	242110	S	213.3	290621	350721
			J	26.7	249365					J	36.7	60100	
Mar 08	29.2	770719	S	75.0	394875	464921	Feb 21	35.0	124250	S	50.0	68125	150005
			J	7.5	70046					J	50.0	81880	
Apr 07	21.7	572761	S	20.0	105300	525578	Apr 03	40.0	142000	S	80.0	109000	174504
			J	45.0	420278					J	40.0	65504	
May 01	59.2	1562554	S	92.5	487012	1164126	May 02	27.5	97625	S	70.0	95375	115845
			J	72.5	677114					J	12.5	20470	
Jun 03	28.3	746964	S	67.5	355388	472132	Jun 03	24.2	85910	S	45.0	61312	105855
			J	12.5	116744					J	27.2	44543	
Jul 10	15.8	417033	S	62.5	329062	702642	Jul 20	34.2	121410	S	45.0	61312	65406
			J	40.0	373580					J	2.5	4094	
Aug 27	17.3	456625	S	40.0	210600	233949	Aug 10	30.0	106500	S	90.0	122625	122625
			J	2.5	23349					J	0	0	
Sep 26	4.5	118775	S	2.5	13162	106557	Sep 26	20.0	71000	S	22.0	29975	100883
			J	10.0	93395					J	43.3	70908	

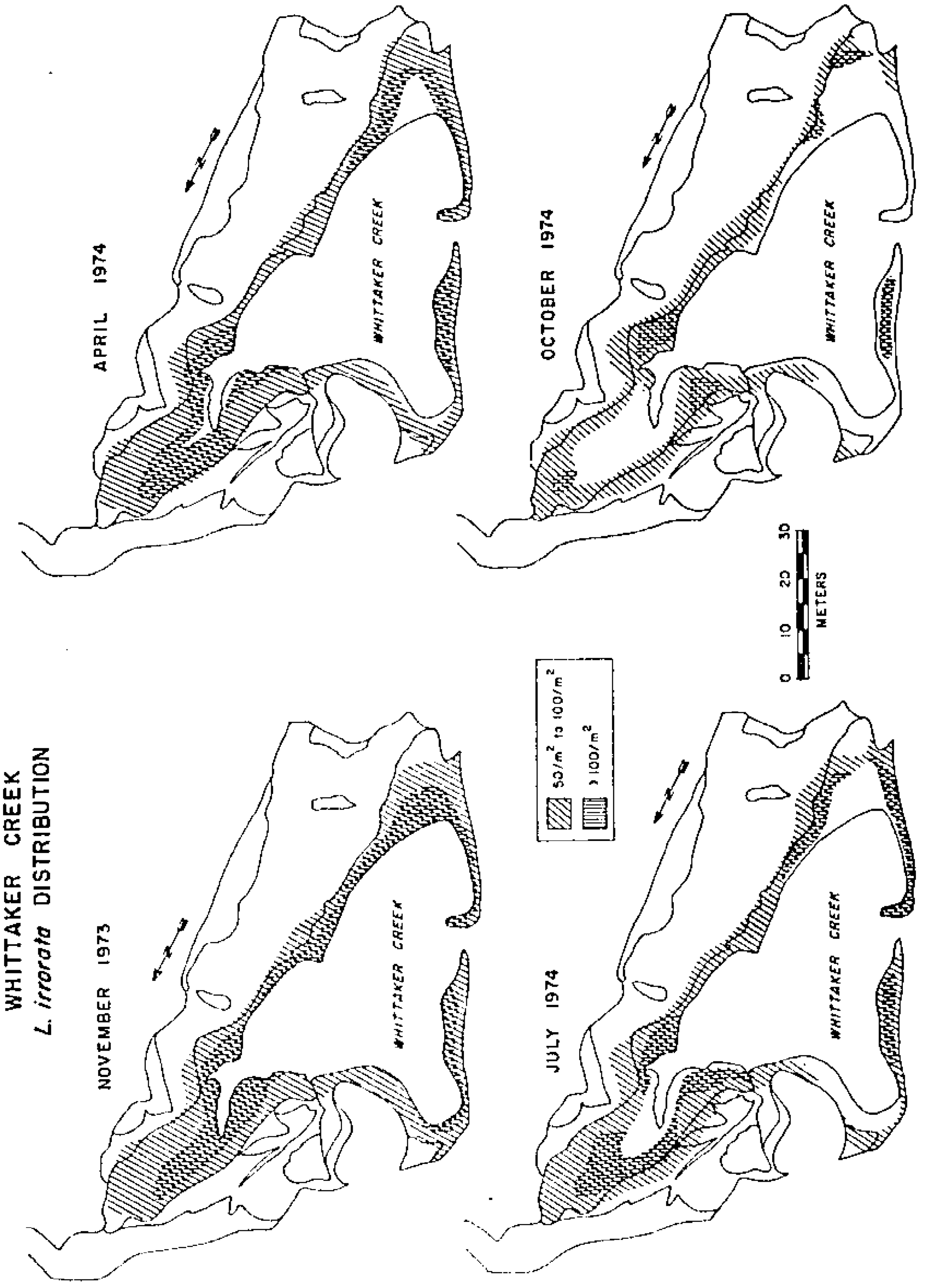
* grass zones: S = _Spartina alterniflora_ zone
J = _Juncus roemerianus_ zone

The population estimate produced by the third method, mapping density distributions was 526,500 (Table 10). That value closely matched the mean values of the other estimates (530,084 for average densities over the entire marsh and 525,451 for the stratified sampling estimates).

In the oiled marsh, the *L. irrorata* population underwent some dramatic changes during the course of the investigation. At the beginning of the investigation the distribution of the population was similar to the pattern in the control marsh. *L. irrorata* was found primarily in the *S. alterniflora* zone and in the lower portions of the *J. roemerianus* zone. Approximately six months after the oil dosing began, the first visible signs of effects on the grass community appeared as a chlorosis of *S. alterniflora* stems and leaves. As the oiling continued *S. alterniflora* began to die. By the end of the investigation 52.5% of the original complement of *S. alterniflora* had been lost (see Figure 8). (Refer to Section 2 of this dissertation for a more complete description of effects on the grasses.) The distribution and size of the *L. irrorata* population closely paralleled the dieback of *S. alterniflora* (see Figure 12).

Monthly population size estimates reflect the decreases observed in the field (Table 11). Based on average density over the entire marsh, estimates peaked at 242,110 during January and then declined to 71,000 in September. Estimates derived from stratified sampling peaked at 350,721 during January and declined to 65,406 in July. The latter estimate rose to just above 100,000 during the final two months of the study.

Figure 12. Distribution of Littorina irrorata in Whittaker Creek (oiled marsh) four times during the study.



Population density estimates averaged over the entire marsh showed a general decline from $68.2/m^2$ in January to $20.0/m^2$ in September. The density estimates derived from the stratified sampling provide some evidence corresponding to general field observations. During the dieback of *S. alterniflora*, the *L. irrorata* population did not appear to decrease steadily. As additional areas of grass were visibly affected by the oiling, densities of *L. irrorata* in adjacent unaffected areas seemed to increase temporarily. The general impression was that the *L. irrorata* population was retreating from affected areas and concentrating in unaffected areas.

The density estimates provide some support for this observation. Although the general trend of densities in the *S. alterniflora* zone was decreasing from January's estimate of $213.3/m^2$ to September's $22.0/m^2$, at least twice during that interval, the sampling indicated increased densities. These were found in April at $80.0/m^2$, up from $50.0/m^2$ in February, and August at $90.0/m^2$, up from $45.0/m^2$ in June and July. It must be remembered that these values are averages over the entire original complement of *S. alterniflora*. Consequently, while an increased estimate may not represent an actual increase in the total population, due to an insufficient number of samples, increases do indicate that the sampling had encountered a particularly high density. The August samples provide a good example. Of the four samples taken in the *S. alterniflora* zone that month, two were situated in severely affected areas of the marsh and two were in relatively unaffected areas. The population densities found in the former two were $0/m^2$ and $10/m^2$. In the latter two the values were

$70/m^2$ and $280/m^2$. The value of $280/m^2$ was higher than normal for unaffected areas of the marsh. In the beginning of the investigation the most heavily populated areas typically had densities of up to $150/m^2$. The marked increase in density encountered in the one August sample was sufficient to raise the average density reported for that month. The one sample was also representative of the population crowding observed in the field.

Additional, non-random counts, not included in any of the calculations reported here, confirmed another general impression, displayed in the distribution maps (Figure 12). If the population was retreating before the oil effects, it was not simply retreating to higher ground. Close inspection of the maps in Figure 12 shows that there was very little invasion of the *J. roemerianus* zone of the marsh despite the loss of inhabitable *S. alterniflora*.

The distribution maps in Figure 12 provide a generalized representation of the changes the *L. irrorata* population underwent during the oil dosing. Population estimates derived from the maps reflect the decreasing number of snails surviving the oiling and/or its effects. The November estimate of 91,750 was the highest value. By September, the estimate had declined to 54,875. This represents an estimated loss of 40.2% of the population.

Melampus bidentatus.

Monthly estimates of the population size of *Melampus bidentatus* in the control marsh varied widely from a low of 65,986 in July to a high of 1,760,513 in August (Table 12). There was no obvious seasonal trend in the data. Stratified sampling produced

Table 12. Population size and density estimates for *Melampus bidentatus*.

	control marsh							oiled marsh					
	entire marsh		stratified sampling					entire marsh		stratified sampling			
date	mean $\#/m^2$	pop est	grass zone*	mean $\#/m^2$	pop est	total pop	date	mean $\#/m^2$	pop est	grass zone*	mean $\#/m^2$	pop est	total pop
Nov 06	10.0	26394	S	2.5	13162	294503	Nov 01	23.3	82715	S	0	0	111913
			J	17.5	163441					J	67.5	110538	
			m	10.0	117900					m	2.5	1375	
Dec 01	9.2	242829	S	7.5	39488	269162	Dec 04	0	0	S	0	0	0
			J	2.5	23349					J	0	0	
			m	17.5	206325					m	0	0	
Jan 03	3.6	95020	S	0	0	122870	Jan 02	16.4	58220	S	0	0	24746
			J	10.0	93395					J	0	0	
			m	2.5	29475					m	45.0	24746	
Mar 08	5.0	131972	S	2.5	13162	160537	Feb 21	2.5	8875	S	0	0	9563
			J	0	0					J	5.0	8188	
			m	12.5	147375					m	2.5	1375	
Apr 07	13.3	351047	S	0	0	459348	Apr 03	5.0	17750	S	0	0	24564
			J	5.0	46698					J	15.0	24564	
			m	35.0	412650					m	0	0	
May 01	6.7	176843	S	0	0	223548	May 02	1.6	5680	S	2.5	3406	4781
			J	5.0	46698					J	0	0	
			m	15.0	176850					m	2.5	1375	
Jun 03	16.7	440788	S	0	0	509859	Jun 03	16.7	59285	S	0	0	62845
			J	32.5	303534					J	32.5	53222	
			m	17.5	206325					m	17.5	9623	
Jul 10	2.5	65986	S	0	0	88425	Jul 20	38.3	135965	S	0	0	79554
			J	0	0					J	15.0	24564	
			m	7.5	88425					m	100.0	54990	
Aug 27	66.7	1760513	S	0	0	2217096	Aug 10	8.3	29465	S	0	0	30063
			J	57.5	537021					J	15.0	24564	
			m	142.5	1680075					m	10.0	5499	
Sep 26	11.7	308816	S	0	0	326882	Sep 26	12.5	44375	S	0	0	81880
			J	35.0	326882					J	0	0	
			m	0	0					m	0	0	

* grass zones: S = *Spartina alterniflora*, J = *Juncus roemerianus*, m = salt meadow (*S. patens* & *D. spicata*)

a similar irregular pattern with a low estimate of 99,425 in July and a high estimate of 2,217,096 in August.

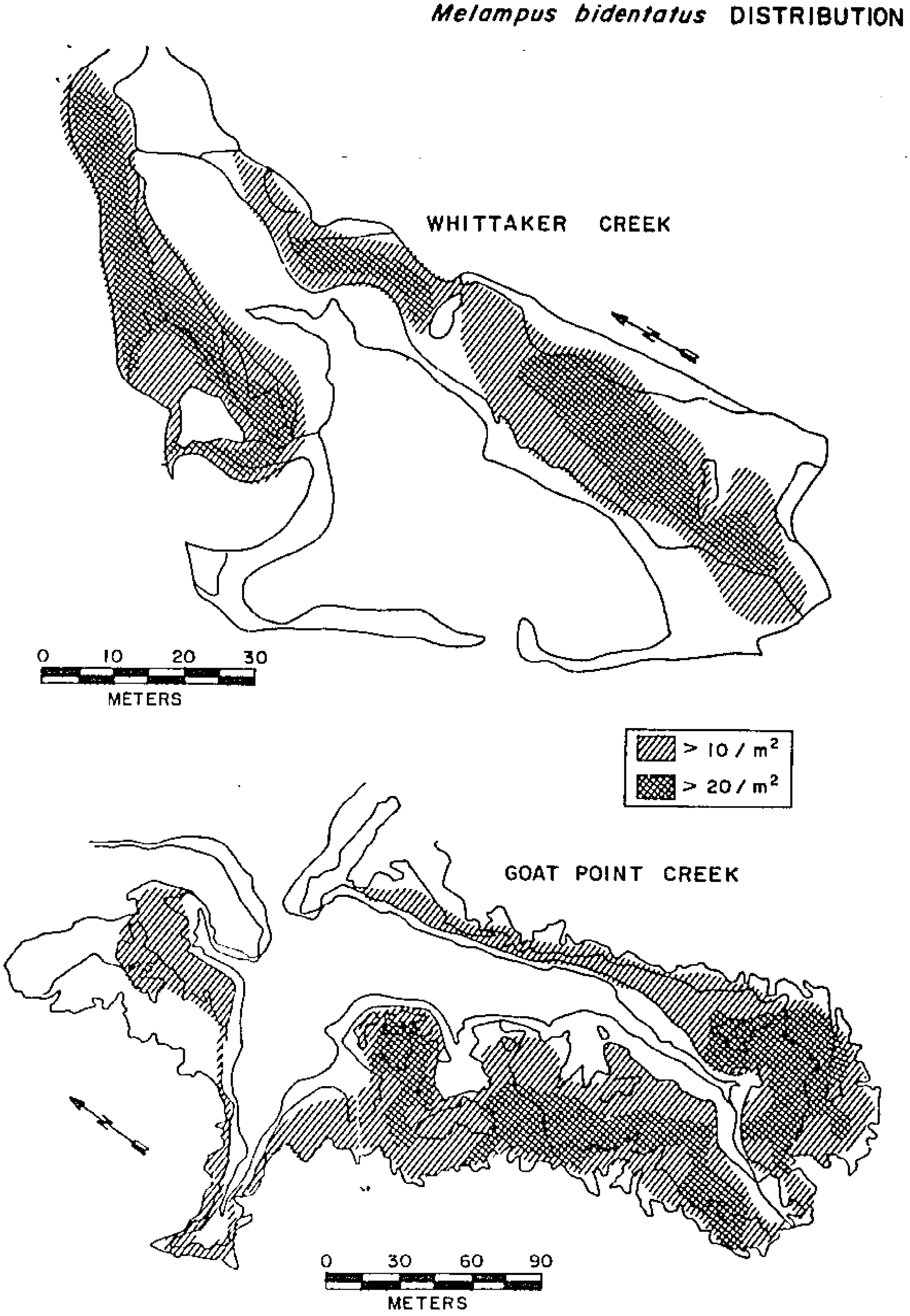
Density estimates averaged over the entire marsh ranged from 2.5/m^2 to 66.7/m^2. Within the S. patens-D. spicata salt meadows density estimates varied from 0/m^2 to 142.5/m^2. In the J. roemerianus zone values ranged from 0/m^2 to 57.5/m^2. In the S. alterniflora zone estimated densities varied from 0/m^2 to 7.5/m^2.

General field observations indicated M. bidentatus was usually found in the J. roemerianus zone and the S. patens-D. spicata salt meadows. The distribution, however, was very patchy. No seasonal trends in distribution were apparent from field observations in the control marsh. The distribution map (Figure 13) presents a general representation of the M. bidentatus population in the control marsh. Based on the map, the average density over the inhabited area (represented by the shaded area) was 13.6/m^2. The estimated population size was 223,875.

In the oiled marsh, monthly population size estimates also varied widely and irregularly. A low value of 0 was found in December and a high of 135,965 in July. Stratified sampling similarly developed estimates ranging from 0 in December to 111,913 in November, with no obvious seasonal trends.

Density estimates varied widely from 0/m^2 to 38.3/m^2 when averaged over the entire marsh. The stratified sampling estimates for the S. patens-D. spicata salt meadows ranged from 0/m^2 to 100/m^2. Estimates in the J. roemerianus zone ranged from 0/m^2 to 67.5/m^2 and values in the S. alterniflora zone varied from 0/m^2 to 2.5/m^2.

Figure 13. Distribution of Melampus bidentatus in Whittaker Creek (oiled marsh) and Goat Point Creek (control marsh).



As in the control marsh, general field observations detected no seasonal trends. Additionally, no effects of the oil dosing could be detected. The distribution map of _M_. _bidentatus_ in the oiled marsh (Figure 13) indicated an average density over the inhabited area of $14.8/m^2$ and a total population size of 27,125.

DISCUSSION

Nassarius obsoletus.

Comparisons of the *N. obsoletus* population data from the control and the oiled marsh indicate that chronic oil pollution may superimpose some alterations on the normal seasonal distribution pattern. Figure 9 and the corresponding data in Table 10 for the control marsh indicate *N. obsoletus* expands it local range and numbers in shallow waters during summer months. During this process, however, the mean population density within the inhabited area is maintained at a relatively constant level ($11.8/m^2$ to $15.3/m^2$ in this study). Throughout the year the population has a significantly contagious dispersion. The degree of contagion apparently decreases slightly as the population expands into shallower areas. This was indicated by a decrease in Morisita's index from 6.24 to 3.45. The decrease in contagion is also demonstrated by the increase in k values from 0.13 to 0.37.

In comparison, the oiled marsh *N. obsoletus* population appeared to follow a similar pattern with slight modifications. The population expanded its range locally and increased its size as shown in Figure 10 and Table 10. However, while the population density in the inhabited area remained relatively constant, the area inhabited was a proportionately greater part of the available habitat (i.e.

the pond). As a consequence the population density over the available habitat (Table 9) was considerably larger than control values. The degree of contagion within the population also did not show the decrease observed in the control marsh. As measured by Morisita's index of dispersion, the contagion in the oiled marsh population of *N. obsoletus* was slightly less than control values for both data sets analyzed. The dispersion as measured by k values was also stable, but intermediate when compared to the control values.

The implications regarding the effects of chronic oil pollution on the size and distribution of *N. obsoletus* populations are thus equivocal. It appears that oiling may somehow attract individuals to the affected marsh. This might explain the proportionately greater inhabitation of the oiled pond. There were no apparent differences in the physical characteristics of the ponds' sediments or the degree of tidal immersion which might otherwise explain the data. Chemosensory perception, such as this hypothesis requires, has been demonstrated in *N. obsoletus*. Crisp (1959) found that individuals could be attracted to other *N. obsoletus* or to suitable substrates by some water born cue. However, Jacobson and Boylan (1973) working with the water soluble fraction of Kerosene, found the oil components interfered with *N. obsoletus's* chemically mediated attraction to food extracts.

The constancy of the population densities within inhabited areas suggests individuals are able to sense and respond to one another either directly or indirectly. Crisp (1969) and Jenner (1959) both commented extensively on the ability of *N. obsoletus* to detect and respond to one another. In both works, however, the densities discussed far exceeded any of the values reported here. If N. obsoletus can detect other individuals in the population, even at the density levels found in this study, the results suggest chronic oiling does not interfere with the process.

The dispersion pattern of N. obsoletus did appear to be modestly altered by oiling. Within a negative binomial distribution, which proved an appropriate model for the N. obsoletus populations, the degree of contagion in the control marsh apparently decreased during summer months as the population became more active. By comparison dispersion in the oiled marsh was less contagious at the beginning of the study and the degree of contagion did not decrease during the summer months. If dispersion patterns are the result of an organism's perception of its environment and other individuals in that environment, and if there appears to be little effect of oiling on conspecific associations in N. obsoletus, then changes in normal dispersion patterns may be a result of oil altering the organism's perception of its environment.

This investigation was not designed as a behavioral study, consequently no direct evidence is presented in defense of this hypothesis. Nevertheless, altered perception of the environment provides a simple explanation of the observations. If No. 2 fuel oil contains compounds or analogs of compounds which are used by N. obsoletus in unpolluted environments to cue or direct population responses, the oil might cause alterations in the normal timing and/or degree of the responses. In this particular case, No.2 fuel oil may have caused the population inhabiting the dosed area to enter a potential habitat earlier and more extensively than normal. While Jacobson and Boylan's (1973) work seems contradictory, laboratory studies on artificial substrate with only a fraction of the whole oil and with distinct local stimuli may not be amenable to extrapolation to natural systems. A more detailed investigation is certainly indicated.

The anomalous value, disregarded in the distribution analyses, suggests another area worthy of investigation. The very high density recorded in that one sample might have fit a compound distribution pattern, as suggested by Pielou (1973). In this particular case the N. obsoletus population may have actually been randomly dispersed through a subset of habitats within the oiled marsh pond. The inhabited subset may have represented the habitable space on the pond bottom, and the complementary subset of spaces may have represented uninhabitable areas. The assumption is that the uninhabitable areas have been rendered so by the oiling. This hypothesis requires that the oil be distributed in a patchy manner over the pond bottom. Analysis of marsh sediments reported in Section 2 of this dissertation, found exactly that type of pattern. Agreement with a compound distribution could not be tested with the present data set because of an insufficient number of samples.

The hypothesis of a multiple distribution caused by uninhabitable spaces is intriguing as a possible explanation for the apparent increase in range within the oil pond. N. obsoletus may have been forced to expand its range moderately by the occurrence of inhospitable areas within its preferred range. However, the hypothesis does not explain the constancy of the population density. If areas could not be inhabited, one would expect the density to decline.

A converse hypothesis may explain more of the observations and results. If oil dosing creates areas which attract rather than repel _N_. _obsoletus_, the range expansion observed might be predicted. Also, if the attractant is mimicking a natural stimulant which arises in the snail's environment outside of conspecific communications, the constancy of the population densities might be predicted. Again, this hypothesis is at odds with reported laboratory studies. The discrepancy warrants further investigation.

Littorina _irrorata_.

The population of _Littorina_ _irrorata_ in the control marsh was relatively stable throughout the investigation. The fluctuations in population size reported in Table 11 can be attributed to insufficient sample numbers. A larger quadrat size would probably be more suitable. The 0.1 m^2 quadrat used in this study was selected in deference to the work force available and because larger quadrats proved troublesome at bondaries between vagetation zones. With the small quadrat, samples rarely spanned two zones, simplifying the data analysis.

As a result of problems inherent in the sampling, the effects oiling produced in the _L_. _irrorata_ population are seen more clearly in the distribution maps (Figures 11 and 12) than in the monthly population data. The observations and results showing the population receded as the effects of the oiling on _S_. _alterniflora_ spread, suggest L. irrorata can detect and respond to either direct or indirect effects of chronic oil dosing.

Direct sublethal effects of oiling would include behavioral responses such as avoidance, increased motility, or combinations of responses, all of which might tend to concentrate the population in relatively unaffected areas.

Avoidance requires the organisms be given a choice between oiled and unoiled substrates. Preliminary laboratory tests demonstrated L. irrorata may exhibit an avoidance response to fresh No. 2 fuel oil when confronted with very clear alternatives. Within the marsh however, the existence of avoidance responses may be a moot point since the snails were never really presented distinct alternatives. The oil was relatively unbiquitous and, compared to the snail populations, the water born slicks were highly mobile.

Increased motility in the presence of oil is a theoretically satisfying hypothesis. If L. irrorata was more active in the presence of oil than in unpolluted areas, the population should tend to concentrate in relatively unaffected areas through time. Preliminary studies of this hypothesis, however, were not promising. No differences in motility were indicated between individuals on oiled or unoiled substrates.

Hypotheses of compound direct effects to explain the distribution of snails are difficult to construct and do not meet the requirement for the simplest plausible hypothesis.

Indirect effects of oiling on the L. irrorata population could include loss of cover and/or changes in the substrate. Preliminary investigations of these hypothesis were not conducted. One of them, however, is particularly interesting. If the oil dosing caused an alteration in the epiphytic community on which L. _irrorata_ grazes, and if _L. irrorata_ can detect and respond to those changes the observations and results reported here might be predicted.

Walker et al. (1975) have shown that oil dosing can reduce the levels of microorganisms including proteolytic, lipolytic, chitinolytic, and cellulolytic bacteria. Decreases in these populations, even with corresponding increases in hydrocarbonoclastic bacteria, may change the _L. irrorata_ feeding substrate sufficiently to cause the snails to search elsewhere for food.

With this hypothesis, the decrease in population size could result from either direct mortality due to the oil or intraspecific competition for food resource. The relatively constant densities reported in Table 10 suggest the population was at or near the carrying capacity of the marsh. Losses in suitable habitat would thus force reductions in population size. A slight reduction in mean density might also be expected as the population inhabited some less desirable areas.

Melampus bidentatus.

The _M. bidentatus_ population in both marshes appeared relatively stable. Seasonal changes in population size and/or distribution were not observed, and no effects attributable to the oil dosing were found. The population appeared to have a clumped distribution but there was an insufficient number of samples to permit a meaningful analysis.

SUMMARY

-*Nassarius obsoletus* in the control marsh exhibited a seasonal pattern of occurrence in the shallow marsh pond. The population distribution can be described by a negative binomial model and it exhibits a significant degree of contagion.

-In the chronically oiled marsh the *N. obsoletus* population exhibited the same seasonal pattern of occurrence seen in the control marsh. The distribution and local range were modified slightly from normal expectations. It is hypothesized that oil components mimicking environmental stimuli might produce effects in a *N. obsoletus* population similar to those reported.

-The *Littorina irrorata* population in the control marsh appeared stable in both size, distribution and density throughout the investigation.

-In the oiled marsh, the *L. irrorata* population decreased in both size and inhabited area. The decrease closely followed losses of live *Spartina alterniflora*. The population appeared to retreat before the spreading effects of the oil, creating temporary areas of high density. With time, however, the densities decreased to near normal levels within the remaining unaffected areas of *S. alterniflora*. It is hypothesized that the changes in the *L. irrorata* population resulted from direct mortality due to the oil and from losses of preferred substrate in affected areas of the marsh.

-The *Melampus bidentatus* populations appeared stable throughout the investigation. No effects attributable to the oiling were observed.

Section 4

EFFECTS OF THE CHESAPEAKE BAY OIL SPILL ON SALT MARSHES OF THE LOWER BAY.

INTRODUCTION

In early February 1976, the barge STC-101 discharged approximately 250,000 gallons of No. 6 fuel oil while being towed on the lower Chesapeake Bay. The majority of the oil was carried across the Bay to its eastern shore in Northampton County, Virginia. There it was stranded intertidally on the beaches and fringing marshes. A cleanup effort was undertaken immediately. Most of the oil was removed from the beaches by scraping off the top layer of sand. In the marshes the cleanup was limited to harvesting the standing dead stems and physically removing as much oil as possible without disturbing the marsh peat.

A study to determine the effects of the spill was begun in March 1976, as the cleanup operations concluded. The primary objective of the study was to assess the biological impact on the marshes at the population level. Specifically investigated were the effects of the spill and cleanup on the growth and production of the dominant marsh grass, *Spartina alterniflora*, and changes in the population size, distribution and makeup of the macrobenthos (*Littorina irrorata*, *Modiolus demissus*, and *Crassostrea virginica*). Due to the value of the marshes as erosion buffers, attention was also given to monitoring both the extent and profile of the marsh surface.

The investigation faced two difficulties at its inception. The first was a lack of any background data from the impacted area. It was not possible to sample before the oil washed ashore. The second was a lack of desirable control sites. The oil coated such an extent of the Bay shoreline that to the south of the impacted area there were no more fringing marshes, and to the north, the plant species within the marshes were different. The problem was resolved by selecting a control site which was not part of the Bay shoreline, but which is in the immediate vicinity of the affected marshes.

SITE

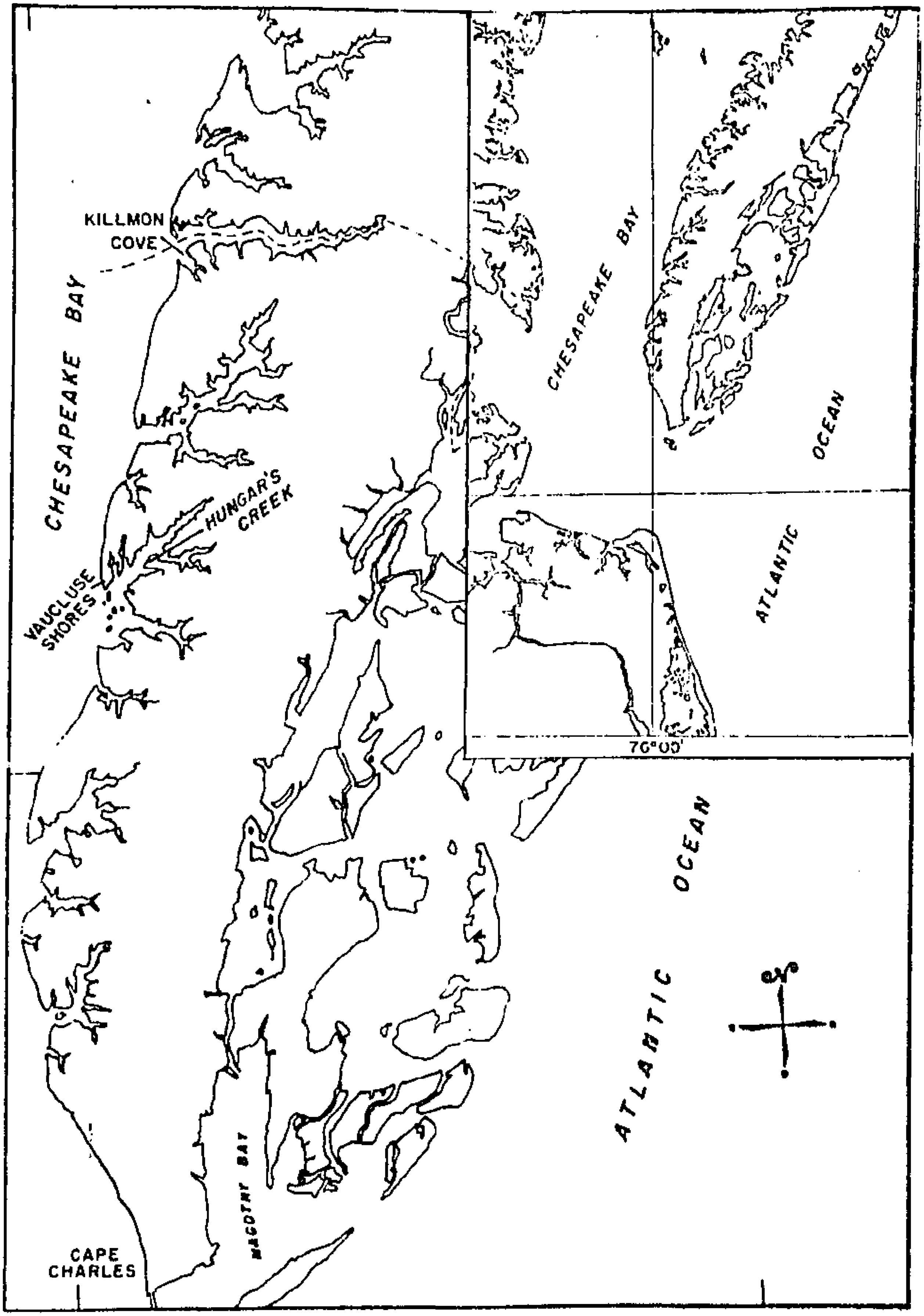
Within the affected area (Figure 14) the Chesapeake Bay shoreline is alternately sand beach and fringing marsh, both of which grade abruptly to the fastlands. The nearshore topography is characterized by a wide sand flat which slopes gently into a shallow trough and then back up onto a sand bar which lies several hundred meters offshore. The marshes are dominated by the saltmarsh cordgrass, *Spartina alterniflora*, up to the mean high water line and contain sizeable populations of snails, *Littorina irrorata*, mussels, *Modiolus demissus*, and intertidal oysters *Crassostrea virginica*.

Two sites were selected for comparison: Vaucluse Shores as an oiled site (Figure 15); and Hungar's Creek as a control site (Figure 16). Vaucluse Shores was heavily coated by oil over the lower one-half of its width. The cleanup operation removed much of the oil and most of the grass stems, but left a layer of oil on the peat up to several centimeters thick. The Hungar's Creek site by virtue of its location within the creek was apparently uncontaminated. Otherwise it closely resembled Vaucluse Shores in all apparent physical and biological aspects.

During the study period of March through October, 1976, observed surface water temperatures at the two sites were comparable, ranging between a low of 8.5°C in March to a high of 30.5°C in July.

Salinities at Vaucluse Shores and Hungar's Creek were always within 2 ppt of each other, ranging between 14 and 22 ppt during the study.

Figure 14. Location of experimental sites of the eastern shore of the Chesapeake Bay, Virginia.



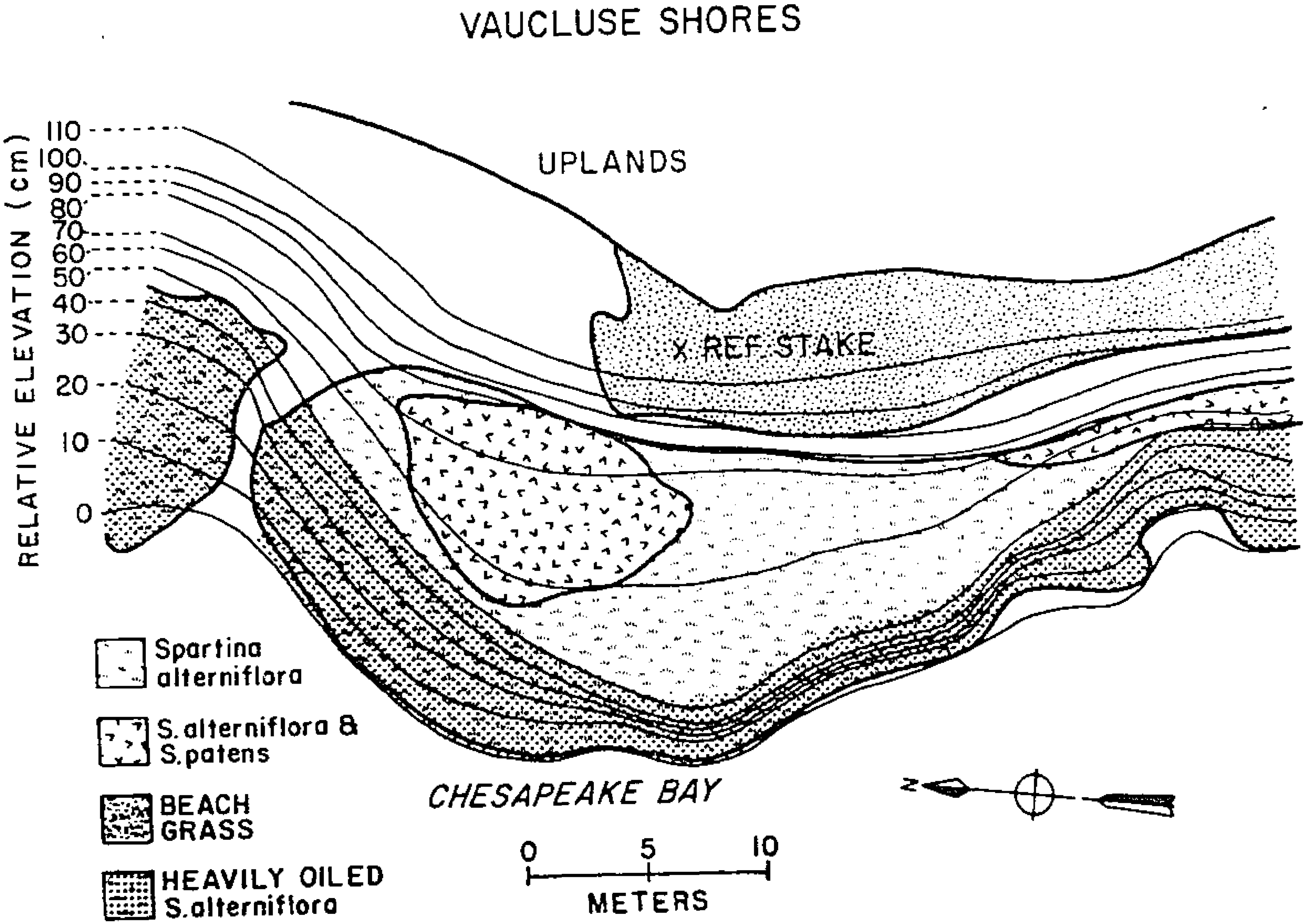
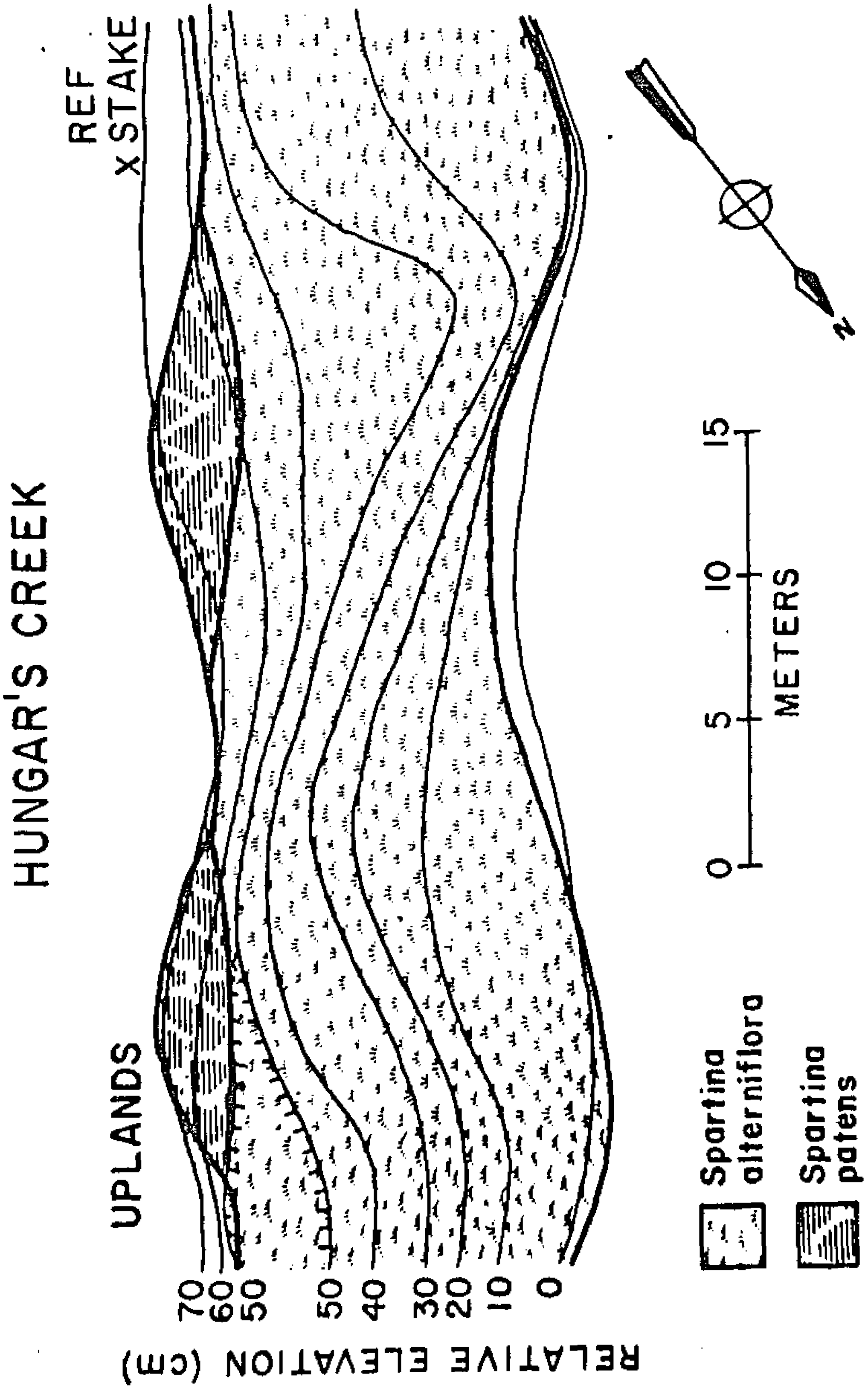


Figure 15. Vegetation and contour map of oiled marsh site on Vaucluse Shore. (elevations are relative to an arbitrary reference stake)

Figure 16. Vegetation and contour map of control site on Hungar's Creek. (elevations are relative to an arbitrary reference stake)



METHODS

To account for variations in marsh biota due to elevation, and to document any changes in distribution, all samples were collected along transects perpendicular to the shoreline. Samples for grass and macrobenthos were collected by harvesting or counting everything within adjacent $0.25m^2$ circular quadrats along a transect. Transects were run from the lower limit of the marsh to the upper extent of _S_. _alterniflora_.

Marsh grass production and growth measurements were made by harvesting everything above ground level within each quadrat. Stems were sorted into living and dead crops by species, and all live stems were counted and measured for total height. Production was estimated by drying the living and dead crops to a constant weight and measuring changes in each from one sampling date to the next. Summing the net increases between sampling times after the method of Smalley (1958) yeilded an estimate of net production during the study. Flowering success in _S_. _alterniflora_ was estimated by recording the number of seed heads present in the final set of samples (September 23 at Vaucluse Shores and October 5 at Hungar's Creek).

The population of _Littorina_ _irrorata_ was estimated by collecting all the snails within each quadrat harvested for grass samples. Snails were measured for greatest length between the apex and outer lip of the shell. Hand sorting in the lab proved efficient for the collection af all snails over approximately 0.30 cm.

Populations of mussels and oysters were censused within the same quadrats used for grasses and snails. Individuals were counted *in situ* and their location along the transect was recorded.

The topography of each site was monitored by referring to stakes driven into the marsh at several locations. Vegetation maps were produced using a plane table and alidade. A transit was used to establish contour lines at each site by referring to permanent stakes assigned arbitrary elevations.

RESULTS AND DISCUSSION

Topography.

Despite the historically dynamic nature of the study areas there have been no significant changes in either the horizontal extent or vertical profile of any of the marsh sites. Shortly after the spill, some oil, agglutinated with sand particles, was buried to a depth of approximately 10 cm in the beaches that are interspersed between the marshes along the shoreline. In the normal course of seasonal erosion and deposition of these beaches it is expected that this oil will be re-exposed sometime during the winter of 1977. However the amount of oil is so small that it should be of little consequence to the biota. Oil which was left on the marsh after the cleanup, weathered to a tarry residue and eroded away within a matter of months. By the end of the study, visible oil could be found only in the most heavily covered areas.

Crassostrea virginica.

There are extensive intertidal populations of the oyster _Crassostrea virginica_ in the affected area, and many of these were heavily oiled. Observations of mature oysters in the marshes recorded no significant mortalities which could be attributed to the spill. However, Dr. Craig Rudell (VIMS) made a qualitative observation in May, 1976, that oyster spat settled in Hungar's

Creek (control site) seemed to be faring quite well, while spat settled in oiled marshes had suffered significant recent mortalities. A histo-pathologic examination of these spat to indicate causes of the mortality was planned.

Modiolus demissus.

Beyond a small percentage of the population which was crushed or mowed off during the cleanup operation, the ribbed mussel, *Modiolus demissus*, seems to have survived well. There appeared to be a slight increase in the mortality rate of mussels in the most heavily oiled areas as temperatures began to rise in April and May. This observation was not quantified, however, and can only coincidentally be attributed to the oil. Recent work by Gilfillian (1975) and Gilfillian et al. (1976) suggests that increasing temperatures and petroleum hydrocarbons may act synergistically to produce a marked physiological stress in mussels. Such difficulties might account for the observed increase in mortalities.

Littorina irrorata.

The periwinkle *Littorina irrorata* is quite abundant in the *S. alterniflora* dominated fringing marshes throughout the Bay. The population density is usually highest within the first few meters from the marsh edge and then is reduced towards the uplands. Table 13 presents the densities recorded over the first part of a transect in both Hungar's Creek and Vaucluse Shore at three times during the study. The data for Hungar's Creek show densities typical of the lower edge of the marsh from spring through fall.

Table 13. *Littorina irrorata* population density (#/m^2) along transect (marsh edge to uplands)

distance inland (m)	Hungar's Creek Apr	Hungar's Creek Jun	Hungar's Creek Aug	Vaucluse Shores Apr	Vaucluse Shores Jun	Vaucluse Shores Aug
0.3	92	224	84	0	88	80
0.9	128	208	108	0	68	184
1.5	164	140	176	20	76	184
2.1	120	232	172	20	60	72
2.7	148	132	228	40	60	108
3.3	212	116	212	32	128	92
3.9	312	252	156	32	136	80
4.5	272	188	148	152	104	64
5.1	248	136	152	100	24	8
5.7	160	28	232	140	–	28

Numbers in this area are relatively constant throughout most of the year. The Vaucluse Shores transects show evidence of one effect of the cleanup operation. Oil coated about a four meter width of marsh where these transects were taken. During the cleaning operation all the oiled grass stems in these areas were mowed down and raked up for disposal. As a result, the populations of snails within the oiled areas were decimated due to their physical removal with the grass. The effect of the removal is apparent in the April census, which shows densities in the affected part of the marsh to be approximately one-fifth or less of values from the control marsh.

During the first year after the spill Littorina have reinvaded the affected part of the marsh, reestablishing a nearly normal density distribution (see Table 13). This has been accomplished by a redistribution of those snails remaining after the spill and by a very successful recruitment into the small size classes. Table 14 lists both the mean densities and the size class distribution of the populations at each site during the course of the study. The data from Hungar's Creek reflect a relatively stable population size throughout the spring and summer with a moderate increase in the fall due to recruitment into the two smallest size classes (those snails 1.00 cm in length). On the oiled marsh at Vaucluse Shores, the data likewise reflect a stable but much reduced population size until the fall recruitment. The size class distribution indicates that the redistribution of snails perpendicular to the shore involved primarily the larger size classes. In August and September there was a marked increase in the number of small

Table 14. *Littorina irrorata* size class distribution (values in % of total population)

	date	mean #/m^2	size classes (cm) 0.00 0.50	0.51 1.00	1.01 1.50	1.51 2.00	2.01 2.50	2.51 +
Hungar's Creek (control)	Apr	132	0.0	4.4	16.2	30.2	49.1	1.9
	May	190	2.9	3.6	16.5	35.2	41.3	0.4
	Jun	152	3.1	2.0	16.4	35.3	43.0	0.2
	Jul	113	0.8	2.4	14.3	33.5	48.6	0.3
	Aug	145	0.6	9.8	12.2	25.6	51.9	0.0
	Oct	222	2.8	14.3	11.9	31.1	39.2	0.7
Vaucluse Shores (oiled)	Apr	30	0.0	0.5	9.6	38.9	51.0	0.0
	May	40	2.5	0.0	7.5	34.8	54.7	0.5
	Jun	40	0.0	2.8	9.5	42.5	44.7	0.6
	Jul	51	0.0	3.0	10.9	36.4	49.2	0.5
	Aug	57	18.7	2.1	7.3	26.3	45.0	0.6
	Sep	143	26.6	14.1	9.4	17.7	32.0	0.2

snails present on the oiled marsh. This recruitment was sufficient to boost the population density back into the range found in the control marsh. The increase also produced a bimodal size distribution which should tend towards normal distribution during the suceeding years.

Spartina alterniflora.

Estimates of the live and dead standing crop of saltmarsh cordgrass, *Spartina alterniflora*, are shown in Table 15. The oil coated the marshes while they were relatively dormant. Consequently, the initial impact of the spill and subsequent cleanup effort was only observed in the reduction, by harvesting of the dead standing crop. Removal of the dead stems produced an effective loss to the system of a large part of the previous year's net primary production. The standing crop of dead material in the oiled marsh remained significantly less than control values throughout the study. The effect on net production, however, was more than compensated for by a significant increase in the live standing crop.

Net production of *S. alterniflora* in the control marsh was estimated to be 466 g/m^2 during the study period. This figure compares favorably with estimates made by other researchers using similar techniques in unpolluted marshes of the middle Atlantic region: Mendelssohn (1973), 361 to 572 $g/m^2/yr$, and Keefe and Boynton (1973), 427 to 558 $g/m^2/yr$. Production in the oiled marsh, Vaucluse Shores, was estimated to be 602 g/m^2 during the study period. The apparent increase of production on the oiled marsh is in contrast to the findings in Sections 2 and 5 in this dissertation and to work

Table 15. *Spartina alterniflora* standing crop (values in $g/0.25m^2$)

	date	mean live	std error of mean	mean dead	std error of mean
Hungar's Creek (control)	Apr 12	13.77	1.22	77.34	11.46
	May 25	51.61	4.19	88.34	16.58
	Jun 22	71.11	3.63	44.93	8.19
	Jul 20	94.06	4.63	(a)	-
	Aug 23	94.03	6.70	46.77	9.18
	Oct 05	117.52	8.14	46.23	3.59
Vaucluse Shores (oiled)	Apr 13	13.82	1.15	16.53**	6.21
	May 26	62.05	4.72	25.81**	1.80
	Jun 23	74.04	6.62	22.33*	3.52
	Jul 20	110.99*	7.89	(a)	-
	Aug 23	125.42**	10.72	16.86**	1.91
	Sep 23	143.14	13.90	28.72**	2.62

** value sig. dif. from control at 1% level
* value sig. dif. from control at 5% level
(a) samples lost in lab accident (i.e. fire)

done by Baker in the United Kingdom (personal communication).

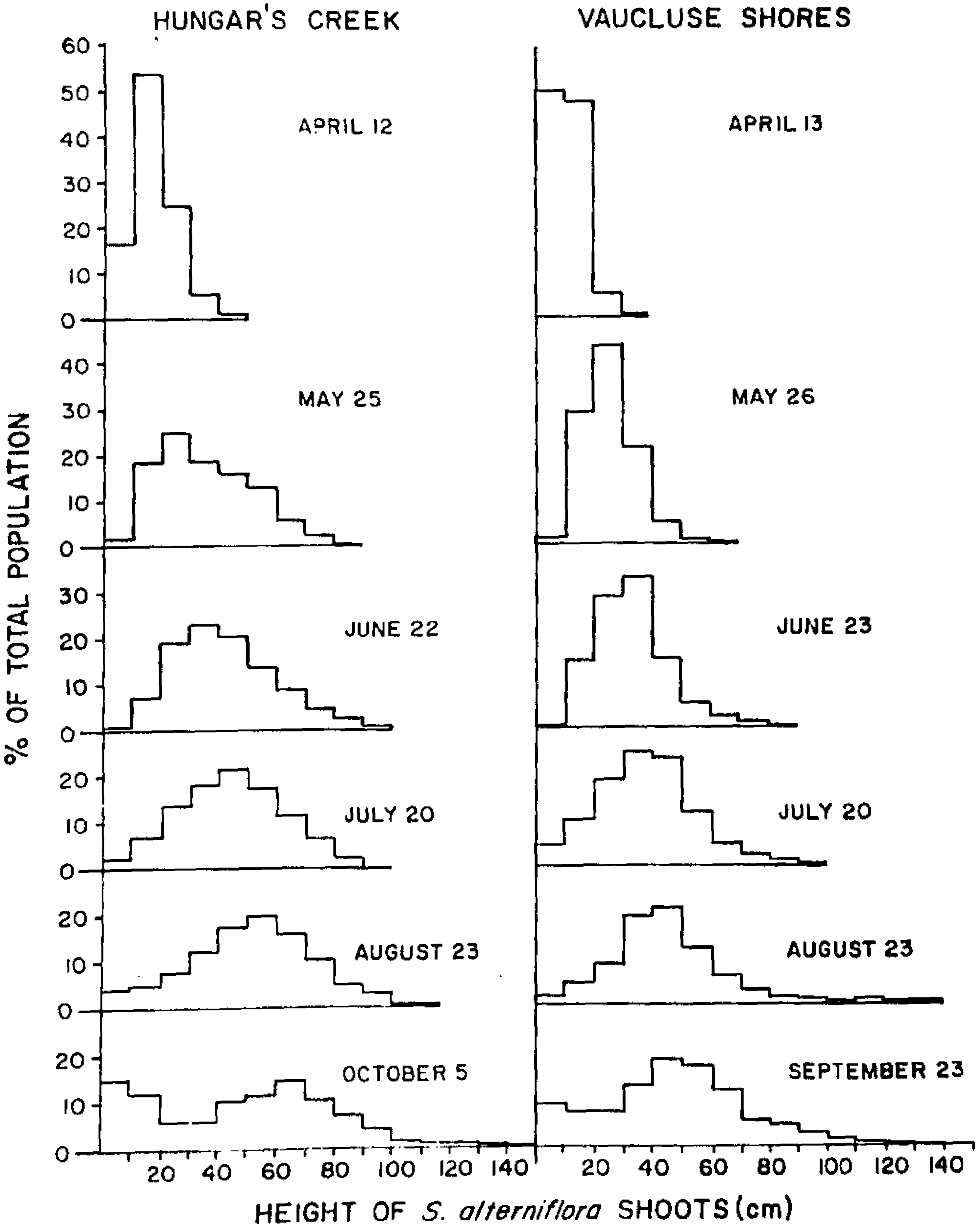
Work done with other species of grass which showed growth stimulation following oiling (Mackin 1950a, Mackin 1950b, Baker 1971d) has noted changes in number of shoots per unit area and length frequency distributions in addition to increases in standing crop. An analysis of these parameters in this study yielded the data presented in Table 16 and Figure 17. Table 16 gives estimates of the mean number of shoots per 0.25 m^2 on each marsh for every month during the study. The densities recorded for Vaucluse Shores are significantly greater (at the 1% level) than values from the control marsh for every sampling effort. Because the densities are averaged over the entire range of _S. alterniflora_ at each site, they do not reflect the extreme values encountered in the presence of the heaviest oil concentrations. At the edge of the oiled marsh, in areas containing a thick coating of oil even after the cleanup, densities typically reached 200-300 shoots/0.25m^2. During the course of the growing season densities on the control marsh remained relatively constant. On the oiled marshes a general decrease in densities was observed. This trend was more exaggerated in the most heavily oiled areas where densities decreased from approximately 500/0.25m^2. The decreases are most likely a result of self-thinning within the stand, rather than a toxic effect of the oil. This conclusion is based on a failure to observe any general reduction in the vigor of the stand.

Figure 17 shows the height frequency distribution of _S. alterniflora_ shoots in both the control marsh and the oiled marsh

Table 16. *Spartina alterniflora* shoot density (# shoots/0.25m^2)

	Hungar's Creek (oiled)		Vaucluse Shores (control)	
date	mean	std error of mean	mean	std error of mean
Apr	49	4.8	168	19.8
May	72	5.8	206	27.9
Jun	70	6.0	143	28.0
Jul	72	5.5	154	18.4
Aug	60	2.2	131	12.6
Sep	67	4.4	134	13.0

Figure 17. Spartina alterniflora shoot height frequency distributions.



during the study. The data from Hungar's Creek demonstrates the normal distribution of size classes during an annual cycle. New shoots and a few which have over-wintered begin elongating in very early spring, usually during March in this area. These shoots form a distinct cohort which continues to grow through late August or September when the flowering heads appear. Beginning in September a second cohort of shoots appears and grows to a mean height of about 10 cm.

At the Vaucluse Shores site the effects of the oil spill and subsequent cleanup operations became evident in early April. As a consequence of the mowing, trampling, and/or the oiling, development of the primary cohort of shoots was retarded. While there were more shoots present, the mean height and the variance of the population of heights were reduced relative to the control marsh. Figure 17 also shows that the second cohort of shoots developing in the oiled marsh represented a much less significant proportion of the total population than the same cohort in the control marsh.

During the September sampling, counts were made of the number of stems flowering in both the control and the oiled marshes. In the control marsh the percentage of the total population of stems which flowered and produced seed heads was calculated to be 6.0%. In the oiled marsh a significantly greater proportion, calculated to be 29.9%, produced seed heads.

In summary, an average quadrat in the marsh which was oiled and then cleaned, when compared to the same size quadrat in the control marsh, contained more stems, which uniformly grew to a shorter mean height and produced more seed heads. The result was an increase in net productivity.

The causes of the observed differences between oiled and control marshes are not definitely known. However, some hypotheses can be generated from the literature. Hubbard (1970) in experiments on repetitive cutting of *S. anglica* reported growth following the cessation of cutting was "...more uniform in height, denser and earlier in flowering than in control plots." Baker (1971f) reported increased shoot densities following cutting of unoiled plots of *S. anglica*. These findings suggest the cleanup operation may have been primarily responsible for the differences between oiled and control sites.

Discussions with other researchers have suggested the oil may produce an effect similar to harvesting by blocking development of the earliest shoots. This could have been accomplished by either chemical or physical means (see Baker 1971g, for a review of possible mechanisms). The experimental design provided no data to substantiate this hypothesis. Nevertheless, oil left after the cleanup was rapidly eroded from the marsh surface, and this fact, plus the apparent delay in development of the first cohort of grass shoots, is certainly compatible with the suggestion that the oil served as a temporary barrier.

The tremendous increase in flowering success is in contrast to findings reported by Baker (1971c) for plants oiled during flower bud development (June and July in her work). It is possible that oiling of a much earlier stage of development could lead to the observed results, however, the similarity between these results and results reported by Hubbard (1970) suggest a different hypothesis. His experiments and observations with _S_. _anglica_ indicate physical disturbance somehow produces the effect of increased flowering. This would mean most of the increase found in the oiled marsh may have been a result of the cleanup effort.

SUMMARY

-Populations of mussels and oysters in the marshes appear to have survived both the spill and the cleanup with no significant short-term effects.

-The snail, *Littorina irrorata*, suffered a reduction in population size, but by approximately eight months after the spill, the population had returned to near normal densities. The normal age/size structure of the population may be reestablished within the next few years.

-The marsh grass, *Spartina alterniflora* apparently underwent some stress from either the spill or the cleanup or both. The short-term result has been an increase in net production and a modification of the normal growth form.

-Overall, it seems several factors acted to minimize the impact of this particular spill on the marshes. First, the oil was relatively nontoxic, as compared to a lighter fuel oil or a crude oil. Second, the spill impacted the marshes at a time of the year when most of the biota was relatively inactive. Third, the impacted marshes were part of a comparatively high energy system, which reduced the oil's residence time in the marsh.

Section 5

EFFECTS OF FRESH AND WEATHERED CRUDE OIL ON *SPARTINA ALTERNIFLORA*.

INTRODUCTION

Experimental applications of crude oils have produced a variety of results in past studies. Mackin (1950b) spilled crude oil over small plots of marsh grass and reported the short-term effect was death of the grasses. However, he found some of the grasses, Distichilis spicata in particular, regrew more vigorously than they had prior to the dosing. Baker (1971a) sprayed small marsh plots with fresh Kuwait crude oil. She reported effects including inhibition of germination, growth stimulation, and reduction of flowering. She concluded that, in general, single doses of crude oil were not devastating to marsh grasses. Recovery usually occurred from two months to two or three years depending on the amount and type of oil, the time of year, and the species of plant. Lytle (1975) dosed a small estuarine pond with Empire Mix crude oil and reported a reduction in the productivity of marsh plants.

Baker (1971h) also reported that fresh crude oil was more toxic than residue. Her findings agreed with observations previouslyz reported by Cowell (1969) and have since been considered an acceptable generalization.

The present study was designed to investigate the effects of known amounts of characterized fresh and weathered crude oils on defined segments of marsh. Studies of the oil's fate in this experimental marsh have been reported by Bieri et al. (1977). General ecological effects and effects on the microbial community have been reported by Bender et al. (1977) and Kator and Herwig (1977) respectively. This report details the investigation of the crude oil spill's effects on the population of *Spartina alterniflora*, the dominant marsh grass in the experimental site.

SITE

The study site was located in Cub Creek, a tributary of the York River in the lower Chesapeake Bay. Cub Creek is bounded by a broad flat marsh dominated by *Spartina alterniflora*. Consocies include *Distichlis spicata*, *Spartina patens*, and *Scirpus robustus*. The *S. alterniflora* in the marsh occurs in both the tall form, along the creek bank, and the short form, generally in the middle of the marsh. None of them occurs in monospecific stands.

For this investigation five segments of marsh were enclosed by walls (Figure 18). The construction of the walls permitted exchange between the enclosed and unenclosed systems only through subtidal openings in the creek. Each enclosed system contained approximately 695 m^2 of marsh, 100 m^2 of open water, and 15 m^2 of intertidal mud flat. The enclosure on the upstream end of the site was used as a control for the investigation.

On September 22, 1975, three barrels (570 l) of fresh South Louisiana crude oil were introduced into each of the two downstream enclosures. The oil was pumped onto the water surface during a flooding tide. Three barrels of weathered South Louisiana crude oil were applied to the two middle enclosures on September 25, 1975.

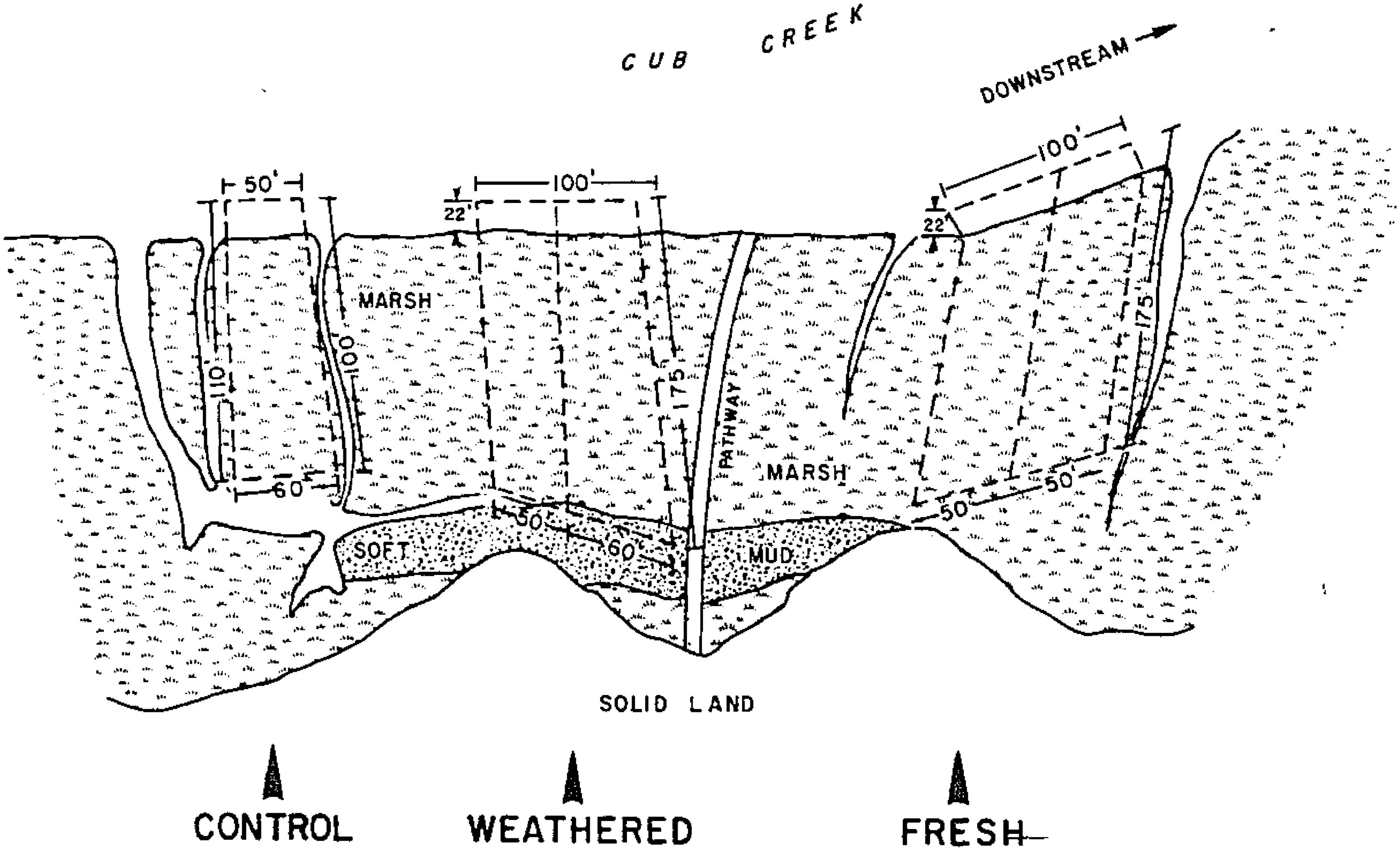


Figure 18. Experimental site design at Cub Creek.

METHODS

The grass community was sampled four times over one year to determine the net production and physical characteristics of the *Spartina alterniflora* population in each enclosure. Samples were also collected from the marsh outside the enclosures for the same analysis. The sampling pattern in each enclosure consisted of regularly spaced sites along two transects established parallel to the side walls of the enclosures. In each case one transect was located in the middle of an enclosure and the second, approximately 2 meters from the left side (facing the marsh from the creek). Ten samples were collected in each enclosure. Samples collected outside the enclosures were arranged in a similar pattern along two transects parallel to the enclosures.

Sampling involved harvesting all the plant material within a 0.25 m^2 quadrat at ground level. Each sample was sorted into live and dead material by species, and then dried to a constant weight. Net production was then estimated by the summation technique detailed by Smalley (1958).

The physical characteristics of *S. alterniflora* were investigated in the three samples collected after the dosing. The height of all live stems collected in the production samples were measured to the nearest centimeter. The mean height for an enclosure was calculated as the sum of all the stem heights collected from that enclosure divided by the number of stems collected for that enclosure. The mean weight of a stem was calculated for each enclosure as the sum of the live standing stock of _S. alterniflora_ for all samples from an enclosure divided by the number of _S. alterniflora_ stems collected from that enclosure. Mean density of _S. alterniflora_ stems in an enclosure was calculated as the total number of stems collected in that enclosure divided by the total area of the samples collected in that enclosure.

The ratio of mean weight per stem to mean height per stem was calculated at each sampling interval as a simple index of plant vigor.

Flowering success was estimated in the final sampling effort by recording the number of live stems bearing seed heads. For comparative purposes, the number of flowered stems is reported as a percentage of the total population of live stems in each enclosure.

The process used to weather the oil introduced to the two middle enclosures is explained by Bieri et al. (1977). It involved placing the oil in an exposed tank and agitating it with York River water for a period of two to three days.

RESULTS

Following the dosing, effects of the marsh grasses became apparent in the early spring of 1975. By March some areas of grass were chlorotic or already dead. The effects appeared to be limited to the peripheries of the four dosed enclosures. A brief topographic survey of the area, confirmed the impression that areas visibly affected by the oil were predominately the lowest areas in each enclosure. The oil apparently moved around the small natural dyke at the creek bank, passing through areas trampled during the construction of the walls. The survey indicated the rear portion of each enclosure was slightly lower than the front by approximately 10 cm. Consequently oil in each of the dosed enclosures was effectively trapped in the rear. The visible effects attest to the situation. In each dosed enclosure the most affected areas of marsh could be found in the rear portions. By the end of the study much of the grass community in these areas was completely dead.

Net production of _S_. _alterniflora_ (Tables 17, 18, 19).

The net production of _S_. _alterniflora_ inside the control enclosure (Table 17) was estimated to be 799.5 $g/m^2/yr$. Outside the enclosures net production was estimated to be 481.2 $g/m^2/yr$. In the enclosures dosed with fresh crude oil (Table 18), net production was estimated to be 376.7 $g/m^2/yr$ in one and 358.9 $g/m^2/yr$ in the second. These values are 47.1% and 44.9% of the control enclosure estimate respectively. Net production in the enclosures dosed with weathered crude oil (Table 19) was estimated to be 368.3 $g/m^2/yr$ in one and 259.2 $g/m^2/yr$ in the other. Compared to the control enclosure these estimates are 46.1% and 32.4% of the control value.

Physical characteristics of *S. alterniflora* (Table 20).

The estimated density of *S. alterniflora* stems outside of the enclosures averaged $285/m^2$. Inside the control enclosure the estimated density averaged $546/m^2$. In the enclosures dosed with fresh crude oil the average estimated density was $153/m^2$. Densities were lower in samples collected in June and September when the marsh grasses had died in parts of the enclosures. In the enclosures dosed with weathered crude oil, the average estimated density was $251/m^2$. There was an obvious difference between the two replicate enclosures, however. The first enclosure always had densities more than double those estimated for the second replicate. The average density in the first replicate was $392/m^2$ while the second replicate only averaged $109/m^2$.

The mean weight per stem outside the enclosures peaked in September at 1.47 g. Inside the control enclosure the mean weight also peaked in September at a slightly lower value of 1.21 g. In the enclosures dosed with fresh crude oil, the mean weights were consistently higher than control values inside or outside the enclosures. The enclosures dosed with weathered crude oil also produced mean weights which were larger than the control enclosure values for June and September. However, compared to mean weights

Table 17. Net production of *Spartina alterniflora* inside and outside control enclosures (in g/m^2).

control (inside)

date	live	Δlive	dead	Δdead	net prod
Sep 19	425.6		189.2		
		-388.9		+126.7	0.0
Mar 29	36.7		315.9		
		+417.4		+179.2	596.6
Jun 15	454.1		495.1		
		+202.9		-53.7	202.9
Sep 17	657.0		441.4		
				net production =	799.5

control (outside)

date	live	Δlive	dead	Δdead	net prod
Mar 29	75.7		576.4		
		+308.1		-205.2	308.1
Jun 15	383.8		371.2		
		+37.3		+135.8	173.1
Sep 17	421.1		507.0		
				net production =	481.2

Table 18. Net production of *Spartina alterniflora* dosed with fresh crude oil (in g/m^2).

fresh crude oil (replicate 1)

date	live	Δ live	dead	Δ dead	net prod
Sep 19	376.0		314.3		
		−358.2		+18.8	0.0
Mar 29	17.8		333.1		
		+108.4		+182.7	291.1
Jun 15	126.2		515.8		
		+85.6		−303.2	85.6
Sep 17	211.8		212.6		

net production = 376.7

fresh crude oil (replicate 2)

date	live	Δ live	dead	Δ dead	net prod
Sep 19	298.4		268.1		
		−279.3		+443.8	164.5
Mar 29	19.1		711.9		
		+131.7		−52.3	131.7
Jun 15	150.8		659.6		
		+62.7		−355.9	62.7
Sep 17	213.5		303.7		

net production = 358.9

Table 19. Net production of *Spartina alterniflora* dosed with weathered crude oil (in g/m^2).

weathered crude oil (replicate 1)

date	live	Δlive	dead	Δdead	net prod
Sep 19	517.7		305.8		
		-419.5		+330.3	0.0
Mar 29	26.2		636.1		
		+193.5		-193.1	193.5
Jun 15	219.7		443.0		
		+174.8		-96.3	174.8
Sep 17	394.5		346.7		

net production = 368.3

weathered crude oil (replicate 2)

date	live	Δlive	dead	Δdead	net prod
Sep 19	416.4		290.3		
		-409.0		+341.5	0.0
Mar 29	7.4		631.8		
		+229.4		+29.8	259.2
Jun 15	236.8		661.6		
		-99.7		-323.2	0.0
Sep 17	137.1		338.4		

net production = 259.2

Table 20. Physical characteristics of _Spartina alterniflora_. *

date	enclosure	stem/ m^2	mean weight	mean height	weight/ height
Mar 29	fresh 1	202	0.22	22.0	0.01
	fresh 2	301	0.16	18.1	0.01
Jun 15	fresh 1	69	1.82	46.3	0.04
	fresh 2	98	1.68	55.5	0.03
Sep 17	fresh 1	131	1.61	43.0	0.04
	fresh 2	115	1.86	45.7	0.04
Mar 29	weather 1	713	0.09	16.4	0.01
	weather 2	158	0.12	15.7	0.01
Jun 15	weather 1	194	1.13	50.4	0.02
	weather 2	88	1.55	54.0	0.03
Sep 17	weather 1	270	1.46	41.8	0.03
	weather 2	82	1.67	45.3	0.04
Mar 29	control (in)	681	0.13	14.6	0.01
	control (out)	-	-	-	-
Jun 15	control (in)	412	1.10	53.0	0.02
	control (out)	284	1.35	54.9	0.02
Sep 17	control (in)	545	1.21	41.5	0.03
	control (out)	286	1.47	46.1	0.03

* mean weight in grams, mean height in centimeters, mean weight/mean height in grams/centimeter

for stems outside the enclosures one replicate enclosure had estimates consistently larger and the other consistently smaller than the control values.

The mean height per stem in all the enclosures peaked in June and decreased slightly in September. The decrease reflected the appearance of the second annual cohort of shoots and not an actual decrease in height of any stems. There was very little difference between the mean heights of any of the treatments or controls.

The ratios of mean weight per stem to mean height per stem all fell in the range of 0.01 to 0.04 g/cm. The ratios in all the treatments and controls increased through the growing season. The ratio seemed to peak in June for stems in the enclosures dosed with fresh crude oil while other areas reached maximum values in September. By September the ratio in the fresh crude oil enclosures was 0.04 g/cm while the control values were 0.03 g/cm.

Table 21 presents the percentages of the live _S. alterniflora_ stems less than or equal to 15 cm in height in the June and September samples. In June the oiled enclosures appeared to have slightly more short stems than the control areas. Except for replicate 1 of the fresh crude oil treatment, the differences were not great. The September samples show the appearance of the second annual cohort of shoots. Shoots less than or equal to 15 cm in height comprised approximately 30% of each of the study populations. The exception appeared in replicate enclosure 2 of the weathered oil treatment. Only 12.2% of the _S. alterniflora_ population in that enclosure was

Table 21. Percentage of live stems less than or equal to 15 cm in height.

enclosure		June 15	September 17
fresh	rep 1	12.5	31.4
	rep 2	5.6	26.1
weather	rep 1	3.9	33.9
	rep 2	0.8	12.2
control	in	0.7	33.2
	out	1.2	29.2

less than or equal to 15 cm in height.

Flowering success was achieved by only 2.0% of the population of live stems outside the enclosures. Inside the control pen 4.5% of the population flowered. In the presence of fresh crude oil, 2.4% of the stems in replicate enclosure 1 and 0.7% of the stems in replicate enclosure 2 flowered. In the presence of weathered crude oil the percentage of stems flowering was 5.3% for replicate enclosure 1 and 2.4% for replicate enclosure 2.

DISCUSSION

Net production.

The results of this investigation indicate the _S. alterniflora_ population was affected by both the oil and the enclosures. The estimate of net production of _S. alterniflora_ outside the enclosures, 481 $g/m^2/yr$, is comparable to Mendelssohn's (1973) estimate of 572 $g/m^2/yr$ which was developed in a similar marsh using the same techniques. A marked increase in net production, estimated inside the control enclosure, appears to result from the containment. Variations in the grass community within the experimental site cannot entirely explain the observed difference between the enclosed and unenclosed marsh productivity. Since the regular unenclosed marsh samples were not collected next to the control enclosure, a peak standing crop estimate of net production was completed in September immediately adjacent to the control enclosure, to test intra-site variations. The estimate was 552.4 g/m^2, still considerably less than the 799.5 $g/m^2/yr$ estimated inside the enclosure. The effects of the oiling are therefore measured against the enclosed control.

The reductions in net production reported for the oiled enclosures reflect the loss of viable areas in each. There was no uniform decline over any of the enclosures. As mentioned previously the oil effects were patchily distributed. Each enclosure contained some areas of grass which appeared completely unaffected.

Differences between the fresh and the weathered oil were not clearly demonstrated. One of the weathered oil replicates was much less productive than any of the other enclosures, but it is unclear whether the weathered oil was more toxic or whether it simply covered a larger area of the enclosure. Bender et al. (1977) noted the weathered crude oil was more viscous and more rapidly sorbed by the marsh and sediments than the fresh crude oil. They hypothesized that relatively more weathered oil was thus available to interact with the biota, indicating the weathered oil was probably less toxic than the fresh oil on a per volume basis.

Physical characteristics.

The physical characteristics measured in this study indicate the increased production observed inside the control enclosure relative to the marsh outside, resulted from increased densities. The average stem inside the enclosures was slightly shorter and weighed slightly less than the average stem outside the enclosures. However, the increased number of stems inside the enclosures resulted in greater production per unit area. The cause of the increased density was not investigated in this study. The most obvious hypotheses would involve the impediment of water circulation presented by the wall.

Compared to stems in the control enclosure, stems in the oiled enclosures were approximately as tall but slightly heavier. The fresh crude oil appeared to produce greater differences than the weathered oil. However, none of the differences were very large.

The ratios of mean weight to mean height for S. alterniflora stems in this study were considerably smaller than those reported in Section 2 of this dissertation (study of chronic oil pollution). The reduced values are a result of much lower mean weights per stem in this study.

Unlike the other studies of oil pollution effects on S. alterniflora reported in this dissertation (Sections 2 and 4), there was no evidence of an increased density in the spring cohort of shoots. In contrast, densities in this study were depressed in the oiled enclosures. Also, no suppression of the second annual cohort of shoots could be clearly demonstrated. What appeared to be a relative suppression of the cohort in replicate enclosure 2 of the weathered oil treatment, still represented a marked increase from the size class frequency in June.

Relative flowering success among the living stems did not appear to be affected by the presence of either fresh or weathered crude oil. The percentages flowering in this study ranged from 0.7% to 5.3% and do not appear to differ markedly from the 6.0% reported for pristine marshes elsewhere (Hershner and Moore 1977).

Most of the results are reported as average conditions within the experimental enclosures. As such they do not adequately represent the dichotomous appearance of the enclosed marsh segments. Within the oiled enclosure some areas appeared unaffected while adjacent areas were devastated. It seems improbable that the crude oil was only capable of producing complete toxicity. Rather, the oil was apparently not dispersed enough throughout the enclosures to produce extensive areas of intermediate or sublethal effects.

Regardless of the type of effects produced by the oil, it is significant that there was a great deal of similarity between effects produced by the fresh crude oil and those produced by the weathered crude oil. As noted by Bender et al. (1977) this finding is contrary to the general supposition that weathering reduces the toxicity of an oil.

SUMMARY

-The population of _S_. _alterniflora_ in this investigation was affected by both enclosure and the oil dosing.

-Enclosure resulted in increased density but little change in the growth form of _S_. _alterniflora_. This resulted in increased net production inside the enclosures.

-The fresh and weathered crude oil affected the _S_. _alterniflora_ populations similarly. Net production within the oiled enclosures was reduced due to death of large areas in each enclosure. The physical characteristics of the remaining live stems were similar to the controls.

-There was no evidence of increased densities in the spring due to oiling. Neither was there any evidence of suppression of the second annual cohort of shoots.

-Relative flowering success appeared unaffected by the oiling.

Section 6

GENERAL DISCUSSION.

GENERAL DISCUSSION

General conclusions - short term effects.

It is unfortunate, but not entirely unexpected that the results of these studies did not lend themselves to a simple conclusion. The results confirm the variety and complexity of oil pollution's effects on salt marsh communities. In general, the studies have demonstrated the presence of sublethal effects of oil on dominant macrobiota. The results also indicate, however, that those effects are neither uniform nor universal for all types of oil.

Studies of the grass community found growth stimulation in only one of the pollution incidents, the No. 6 fuel oil spill. Flowering was also increased in the No. 6 fuel oil spill but unaffected in the other instances. The growth form of *S. alterniflora*, in terms of mean weight and mean height of individual stems, was affected by chronic doses of the No. 2 fuel oil and the large acute dose of No. 6 fuel oil. The crude oil study however, did not produce any evidence of similar effects. The production of the second annual cohort of *S. alterniflora* shoots was suppressed by both the No. 2 and No. 6 fuel oils, but apparently not affected by the crude oil.

Studies of the snail populations found evidence of sublethal effects, in terms of altered distributions only in the chronic dosing study. Effects observed in the acute spill of No. 6 fuel oil were interpreted as resulting from direct mortality or physical removal of portions of the population. In the crude oil spill, the population of Melampus bidentatus was apparently affected primarily by the physical enclosure, with little or no effects attributable to the oil dosing (Bender et al. 1977).

In summary, the No. 2 fuel oil and the crude oil, the two lighter oils in this study, produced the most devastating short-term effects on the marsh community. This finding might have been predicted from the general literature on oil pollution which asserts lighter oils, those with greater aromatic hydrocarbon contents, are relatively more toxic than other oils (Moore and Dwyer 1974, National Academy of Sciences 1975). Sublethal effects, however, were elicited only by the No. 2 and No. 6 fuel oils. This finding should not be interpreted to mean crude oil cannot produce sublethal effects, rather such effects were simply not evidenced in this study.

General conclusions - implications for long-term effects.

The types of effects observed in each study have some interesting implications for the long-term recovery and/or effects of subsequent recontamination of marsh systems. As discussed below, each of the pollution incidents produced a slightly different prognosis for the system's recovery. The results of each study also indicate oil pollution can affect the resistance of the marsh system to additional stresses in at least two basic ways.

Potentials for recovery.

In the acute spills of No. 6 fuel oil and crude oils the marsh's potential for recovery seems good. In both pollution situations a substantial proportion of the marsh remained relatively unaffected by the oils. This provides convenient and healthy parent populations for any recolonization necessary. In the case of the No. 6 fuel oil spill, the grass community in particular was not as severely affected as it was by the other oils studied. Regrowth was almost immediate, occurring in all but the most severely polluted areas of marsh. The crude oil spills produced longer lasting effects, but, one year following the dosing, regrowth has begun to occur in many of the affected areas. An interesting note in the crude oil study was the appearance of a few species of plants not typically found in the marsh prior to dosing (Hershner, unpublished data). The finding is similar to that reported by Dicks (1976) in marshes recovering from impacts of refinery effluents. On a more massive scale, the invading species might alter the character of the vegetative community sufficiently to delay or prevent a return to pre-spill conditions.

The prognosis for the marsh chronically dosed by No. 2 fuel oil is the least favorable. As noted in the discussion of the study on grasses, recovery will probably be a long and uncertain process. It is entirely possible the marsh will never regain its fomer configuration and complements of _Spartina alterniflora_ and _Littorina irrorata_. In the two years immediately following the cessation of dosing there was very little regrowth of grasses in the affected areas of the marsh. There has also been no resurgence of the _L. irrorata_ population and there probably will not be one before the grass community has reestablished.

Effects on resistance to additional stresses.

The resistance of a marsh system may be altered by an oil pollution incident in at least two manners. In the first, oil pollution can produce a combination of lethal and sublethal stresses in the system which persist for varying lengths of time. Coping with those stresses may reduce the system's ability to resist additional impacts. In the second, pollution may alter the composition of a marsh system such that the community's resistance to additional stress is also changed.

In the crude oil spills, large parts of the experimental enclosures were apparently highly stressed for at least a year following dosing. This was evidenced by the small amounts of regrowth occurring in those areas. Additional oil pollution incidents in the enclosures might reasonably be expected to have additive or synergistic effects, further reducing the rate of regrowth and increasing the size of severely affected areas by increasing the stresses on surviving plants. The duration of this type of reduced resistance within the system will certainly last until the concentrations of oil in the sediments are lowered to sublethal levels. The potential for additive effects probably exists for some time after that degree of depuration has been achieved. No work has been reported to date on the subject, however. The second manner in which oil pollution may reduce resistance to subsequent stresses is evidenced in the Chesapeake Bay oil spill study. The net effect of the spill was to alter the normal distribution of developmental stages of individuals in at least the _S. alterniflora_ and the _L. irrorata_ populations. The grass population in the oiled marsh was markedly uniform and the second annual cohort of shoots was suppressed. The L. irrorata population was affected such that younger individuals made up a relatively large proportion of the population compared to normal age(size) distributions. If resistance at the level of an individual changes during the normal life cycle, altered composition of a population may result in significant changes in resistance at the population level. It is not presently known whether such changes are beneficial or deleterious in the event of subsequent stresses. It is reasonable to expect such a determination to depend on the degree of change in the population and the type of stress. In any case, the results reported here indicate oil pollution can result in either increased or decreased phenotypic diversity within a population. In the case of S. alterniflora phenotypic diversity was decreased. In the case of L. irrorata the diversity was increased.

Additional research needs.

A number of research questions have been posed either directly or indirectly by the investigations reported and discussed here. Basically the questions fall in two general categories, long-term effects and sublethal effects. Research in both these areas will advance understanding of the potential impacts of oil pollution.

The short-term effects of oil on most components of the macrobiota in marsh systems have been studied in detail under a variety of situations. The significance of short-term effects in natural communities when viewed over a time period encompassing recovery have not yet been analyzed. (The West Falmouth oil spill studies come closest to achieving this goal.) Such studies will help place the effects of various oils in perspective with the marsh systems' abilities to resist and/or recover.

A major area for future work is the nature and significance of sublethal effects of oil pollution. The research reported here details some of the sublethal effects. The next steps in the investigation of those effects can lead in two directions. Searches for causative factors in the oil and their modes of action offer one possible avenue of research. The other involves studies to determine significance of the sublethal effects to both the internal and external relationships of the marsh system. The former will require extensive use of controlled experiments. The latter type of research will entail continued detailed field investigations.

Selection of research design.

In preparation for additional investigations of both long-term consequences and sublethal effects of oil pollution in marshes, several factors must be considered in the research design. These include the basic format of the research, the scope of the project, and the selection of sampling design. A brief analysis of each of these factors as they apply to these studies will permit a better understanding of the results reported here and will also provide some guidance by example for future research efforts.

Project format.

There are three basic formats amenable to the investigation of oil pollution in natural systems: (1) experimental dosing of a naturally defined system, (2) experimental dosing of an artificially defined system, and (3) studies of an area impacted by an accidental discharge. Each has its relative merits. All have inherent difficulties.

Experimental dosing of a naturally defined system offers all the benefits of a discrete system which functions as a natural unit. The system can be comparatively well characterized and monitoring fluxes of matter and/or energy through the system may not require construction of artificial boundaries. For the study of oil pollution in marshes, the major drawback is the lack of control beyond the amount of oil introduced. However, this point can also work in favor of the investigation for it offers a large degree of insurance that results can be extrapolated to similar natural systems. The use of this format in the chronic pollution studies reported here was particularly successful because very similar control systems were available.

Experimental dosing of artificial enclosures has many of the advantages of naturally defined systems as far as monitoring influxes and efluxes. It is also easier to locate systems so that control and experimental areas are similar. The principal drawbacks are those due to enclosure. This problem was evidenced in the studies with crude oil reported here. It can be difficult to insure enclosed areas still function in a natural manner. The use of this format in the studies reported here was complicated by enclosing differing grass communities, which prevented a more complex analysis of results.

Followup studies of accidental discharges is probably the most frequently used format. It can provide useful information if sufficient background information on the impacted area is possessed or if suitable controls can be found. The principal drawback of the format is the total lack of control over time, amount of oil and area covered. It does have the advantage however of being precisely the situation most other designs try to mimic.

In review, the experimental dosing of naturally defined systems and the followup study formats were best suited to the goal of this dissertation, which was the description of oil pollution effects on natural communities. For future research however, which aims to investigate the significance of those effects and to describe chronic pollution effects, the experimental dosing of naturally and artificially defined systems should prove most appropriate.

Selection of research scope.

These studies addressed only the dominant macrobiota of the system. It has been argued that investigations of effects which only address dominant organisms tend to obscure important effects (Butler and Sleeter 1977). In the case of salt marshes this may be an unwarranted assertion. Marshes are relatively simple systems, dominated by a few species. Therefore, studies of a few important species may provide an adequate indication of the real and potential effects of any consequence to the community.

Teal (1962) reasoned the low diversity of the marsh system resulted from a restricted input of organic matter and a relatively small variety of exploitable resources. The input of organic matter is restricted primarily by the export of much of the marsh's primary production by tidal flushing. The variety of exploitable resources is diminished by the virtual monoculture of the macroflora. As a consequence, even though marshes have existed long enough to permit extensive adaptive radiation of the endemic species, those species have been forced to establish themselves in a few broad niches rather than many narrow ones.

Within the relatively simple marsh system, the grasses are by far the most important component. Teal (1962) found the respiration of producers alone to account for 77% of grass production. The bacterial community respired 11% of the gross production. Ten percent of the gross production was exported from the system, and the remainder was utilized by the consumer species, which in that study consisted primarily of crabs and nematodes.

Day et al. (1973) found the major primary producers, the marsh grasses, respired 82% of the gross production. They estimated bacterial respiration accounted for 7% of the gross production. Nine percent of the gross production was estimated to be exported and approximately 1% was respired by consumers such as polychates, snails, crabs, and mussels.

Nixon and Oviatt (1973) working with a marsh-embayment system in New England, reported the marsh community in general was less productive than more southern marshes. The densities of the consumer populations in the marsh were also much lower than more southern areas. They suggested the marsh grasses respired less of the gross production than Teal reported, and they calculated a smaller percentage of the net production of the marsh was exported. Even with these alterations the resultant energy flow through the marsh was influenced primarily by the grasses.

All of these studies demonstrated the significance of the role producers play in the marsh system. Also, each reports a relatively simple energy flow pattern within the marsh system. It is apparent therefore that if the goal of an investigation is to describe the effects of an oil spill on a marsh, the experimental design should center on studies of the primary producers, particularly the marsh grasses. The next most significant population, in terms of energy flow, is the bacterial community. This group has been shown responsive to oil pollution, but is less tractable than some of the other marsh biota. The macroconsumers such as snails, mussels, and crabs are the third most significant group in the marsh. While the amount of energy passing through their population is less than the amount affected by the bacterial community, many of the macroconsumers are important information inputs to the marsh system. The snails for example have been indicated as regulators of the microbial populations (Smalley 1958, Wetzel 1976).

Analysis of the dominant organisms is therefore appropriate in efforts to detect significant effects of oil pollution on marsh systems. Priorities for selection of the research scope in marshes should probably begin with the macroflora and macrofauna, and expand to other components as the available sampling effort increases.

Selection of sampling design.

The sampling designs in the reported studies were each slightly different from the others. The unifying factor was the requirement that the design be able to detect developing or progressive effects of a pollutant in a naturally changing system. An analysis of the design in hindsight is instructive for both proper interpretation of the results and for future research activities.

Chronic oil pollution study - In the chronic oil pollution study the study site was well segregated into vegetative zones. Since the maximum sampling effort was effectively fixed by available manpower, the partitioning of the sampling effort was crucial to the study. Mendelssohn (1973), when faced with a similar system, chose a modified random co-ordinate sampling design which ensured samples would be spread over most of the experimental site. The random sampling design has some advantages for statistical analysis, however it can not assure samples will be placed in areas of special interest at each sampling effeort. This latter consideration is important when attempting to study progressive effects which may not be uniformly distributed throughout the experimental system.

The chronic oil pollution study, which obviously involved a study of progressive effects, indicates oil pollution will probably not affect all portions of a natural marsh system equally. In this situation the stratified random sampling program is very appropriate. While a uniform sample number in each stratum would enhance analysis of variance between strata, if attempted, a proportional allocation of the sampling effort can reduce the standard error of the sample mean, thus providing a better estimate of the population mean. Proportional allocation in a marsh can be easily achieved on the basis of areas, placing a percentage of the samples in each zone proportional to the percentage of the total area represented by that zone. The sample can then be self-weighting, using the sample number to weight the sample mean for each stratum.

Theoretically, an even better estimate can be obtained if the allocation of the sampling effort among the strata is proportional to the standard deviations for each strata. This is rarely possible *a priori*. The chronic oil pollution study, however, indicates the standard deviations within affected grass zones (or strata) can be expected to increase as the study progresses. The best estimate of the population mean for all grass zones can thus be obtained by increasing sampling efforts in the affected zones relative to other areas.

In future studies with a fixed sampling effort, a continuing reallocation of the effort may improve estimates of effects. In view of the present study, a far simpler experimental design would limit the study site to just those areas most likely to be affected by the oil.

The study of salt marsh gastropods clearly demonstrated the difficulties of estimating population parameters with a small fixed sampling effort. Random sampling and stratified random sampling both produced highly variable estimates. The graphical analysis based on distribtuion maps produced the most realistic estimates. Fixed quadrat sampling at nodes of a grid pattern is probably better suited to the development of distribtuion maps than the random sampling design.

Future studies with limited sampling effort should either adopt a regular sampling pattern or limit the study to permit increased sampling effort in one area of the marsh.

Chesapeake Bay oil spill study - The marshes involved in this study were narrow fringing marshes dominated by *Spartina alter-*

niflora. In this case, stratification on the basis of vegetation zones was not particularly practical or necessary because the marsh was monospecific over large areas. Instead the decision was made to limit the study to *S. alterniflora* and some of the co-occurring macrofauna.

A brief sampling survey and visual observation immediately following the spill indicated the grass and the macrofauna were not uniformly or randomly distributed throughout the marsh. There was an apparent relationship between the parameters chosen for study and either distance from the lower limit of the marsh or elevation. The latter two were positively correlated but not in a regular fashion. As a consequence stratification on either basis would have been difficult.

In order to develop estimates of the various population means which would be sensitive to both distance from the marsh edge, elevation, and the proportions of the marsh in each possible combination of factors, a transect pattern was selected. At randomly selected points along the shoreline transects perpendicular to the general shoreline were established. By harvesting as many adjacent quadrats as would fit along the transect from the lower limit of the marsh to the upper limit of *S. alterniflora*, sample means for each study parameter could be developed. The means reflected the variation in the population along the transects and were self-weighting for the proportion of marsh surface at each combination of elevation and distance inland.

The sampling pattern was more appropriate than a completlely random one because affected areas were always included in estimates of the marsh's condition. If the sampling effort had been more restricted than it was, greater distances between samples along the transects may have been utilized. If the marsh had been more homogeneous a random sampling pattern may have been more appropriate.

Crude oil spill study - The sampling design used to study the effects of crude oil spills in the Cub Creek enclosures was a combination of the two previous designs. The marsh community within each enclosure was a heterogeneous mix of several grass species, dominated by _S. alterniflora_. The _S. alterniflora_ population assumed different growth forms depending on location within the enclosure. While the change over the front half of all the enclosures was identical, the transitions through the back halves varied widely from one enclosure to the next. Attempts to stratify on the basis of the _S. alterniflora_ growth form were inappropriate because the occurrence of secondary species made the strata heterogeneous. Stratification based on the distribution of the secondary species was inappropriate because of the resultant unequal coverage between enclosures.

The most suitable design seemed to be one which would forego inter-enclosure comparisons of strata within enclosures, in favor of developing a single sample mean for each enclosure. To accomplish this transects were established in each pen paralleling the long axis. The transect thus covered the entire range of _S. alterniflora_ height forms. Samples were then spaced along the transect so that the various grass communities present in each enclosure were proportionatley represented. In this particular case, slight alterations of the transect length allowed even sample spacing to provide the required apportionment.

Given the location of the enclosures, little could have been done to ameliorate the sampling problems. The best possible alternative would have been to greatly reduce the size of the enclosures so that only one or possibly two grass zones were contained. The front one third of each marsh segment would have been best suited for enclosure. That portion of the marsh was particularly uniform between enclosures. Reducing the enclosure size might also have avoided or reduced the problem of uneven distribution of the oil, producing a more uniform affect on the grasses.

LITERATURE CITED

American Petroleum Institute. 1963. Biological treatment of petroleum refinery wastes. American Petroleum Institute, Washington, D. C. 73p.

Baker, J. M. 1971a. The effects of a single oil spillage, p. 16-20. In E. B. Cowell (ed.), The Ecological Effects of Oil Pollution on Littoral Communities. Institute of Petroleum, London.

Baker, J. M. 1971b. Successive spillages, p. 21-32. In E. B. Cowell (ed.), The Ecological Effects of Oil Pollution on Littoral Communities. Institute of Petroleum, London.

Baker, J. M. 1971c. Seasonal effects, p. 44-51. In E. B. Cowell (ed.), The Ecological Effects of Oil Pollution on Littoral Communities. Institute of Petroleum, London.

Baker, J. M. 1971d. Growth stimulation following oil pollution, p. 72-33. In E. B. Cowell (ed.), The Ecological Effects of Oil Pollution on Littoral Communities. Institute of Petroleum, London.

Baker, J. M. 1971e. Refinery effluent, p. 33-43. In E. B. Cowell (ed.), The Ecological Effects of Oil Pollution on Littoral Communities. Institute of Petroleum, London.

Baker, J. M. 1971f. Effects of cleaning, p. 52-57. In E. B. Cowell (ed.), The Ecological Effects of Oil Pollution on Littoral Communities. Institute of Petroleum, London.

Baker, J. M. 1971g. The effects of oils on plant physiology, p. 88-98. In E. B. Cowell (ed.), The Ecological Effects of Oil Pollution on Littoral Communities. Institute of Petroleum, London.

Baker, J. M. 1971h. Comparative toxicities of oils, oil fractions, and emulsifiers, p. 78-87. In E. B. Cowell (ed.), The Ecological Effects of Oil Pollution on Littoral Communities. Institute of Petroleum, London.

Baker, J. M. 1976a. Investigation of refinery effluent effects through field surveys, p. 201-225. In J. M. Baker (ed.), Marine Ecology and Oil Pollution. John Wiley and Sons, New York.

Baker, J. M. 1976b. Experimental investigation of refinery effluents, p. 247-254. In J. M. Baker (ed.), Marine Ecology and Oil Pollution. John Wiley and Sons, New York.

Bender, M. E., E. A. Shearls, R. P. Ayers, C. H. Hershner, and R. J. Huggett. 1977. Ecological effects of experimental oil spills on eastern coastal plain ecosystems, p. 505-509. Proceedings of Oil Spill Conference. American Petroleum Institute, Washington, D. C.

Bieri, R. H., V. C. Stamoudis, and M. K. Cueman. 1977. Chemical investigations of two experimental oil spills in an estuarine ecosystem, p. 511-515. Proceedings of Oil Spill Conference. American Petroleum Institute, Washington, D. C.

Burns, K. A. 1975. Distribution of hydrocarbons in a salt marsh ecosystem after an oil spill and physiological changes in marsh animals from the polluted environment. Ph. D. Dissertation, Massachusetts Institute of Technology, Cambridge, Mass. 101p.

Burns, K. A. 1976. Hydrocarbon metabolism in intertidal fiddler crab Uca pugnax. Marine Biology (Berl.) 36:5-11.

Burns, K. A. and J. M. Teal. 1971. Hydrocarbon incorporation into the salt marsh ecosystem from the West Falmouth oil spill. Technical Report 71-69. Woods Hole Oceanographic Institution, Woods Hole, Mass. 23p.

Butler, J. N. and T. D. Sleeter. 1977. Hydrocarbons. Draft of report to Center for Natural Areas for Bureau of Land Management Literature Review 1972-1977. Coastal Region: Bay of Fundy to Cape Hatteras. Harvard Univ., Cambridge, Massachusetts. 84p.

Cowell, E. B. 1969. The effects of oil pollution on salt marsh communities in Pembrokeshire and Cornwall. J. appl. Ecol. 6:133-142.

Crisp, M. 1969. Studies on the behavior of Nassarius obsoletus (Say) (Mollusca, Gastropod). Biol. Bull. 136:355-375.

Day, J. W. (Jr.), W. G. Smith, P. R. Wagner, and W. C. Stowe. 1973. Community structure and carbon budget of a saltmarsh and shallow bay estuarine system in Louisiana. Pub. No. LSU-SG-72-04. Louisiana State University. Baton Rouge, Louisiana. 79p.

Dicks, B. 1976. The effects of refinery effluents: the case history of a salt marsh, p. 227-245. In J. M. Baker (ed.), Marine Ecology and Oil Pollution. John Wiley and Sons, New York.

Elliott, J. M. 1971. Some methods for the statistical analysis of samples of benthic invertebrates. Scientific Publication No. 25. Freshwater Biological Association. 144p.

Foster, W. A. 1968. Studies on the distribution and growth of Juncus roemerianus in south eastern Brunswick County, North Carolina. M. S. Thesis, North Carolina State Univ. 72p.

Gillfillian, E. S. 1975. Decrease of net carbon flux in two species of mussels caused by extracts of crude oil. Marine Biology 29:53-57.

Gillfillian, E. S., D. Mayo, S. Hanson, D. Donovan, and L. C. Jiang. 1976. Reduction in carbon flux in Mya arenaria caused by a spill of No. 6 fuel oil. Marine Biology 37:115-123.

Hershner, C. and K. Moore. 1977. Effects of the Chesapeake Bay oil spill on salt marshes of the lower Bay, p. 615-620. Proceedings of Oil Spill Conference. American Petroleum Institute, Washington, D. C.

Hubbard, J. C. E. 1970. Effects of cutting and seed production in Spartina anglica. J. Ecol. 58(2):329-335.

Hyland, J. L. and D. Miller. (in preparation). The effects of No. 2 fuel oil on chemically evoked feeding behavior of the mud snail, Nassarius obsoletus. (manuscript) U. S. Environmental Protection Agency, Narragansett, R. I.

Hyland, J. L. and E. D. Schneider. 1976. Petroleum hydrocarbons and their effects on marine organisms, populations, communities, and ecosystems. Proceedings of symposium on sources, effects, and sinks of hydrocarbons in the aquatic environments. AIBS, Washington, D. C.

Jacobson, S. M. and D. B. Boyland. 1973. Seawater soluble fraction of Kerosene effects on chemotaxis in a marine snail, Nassarius obsoletus. Nature 241:213-215.

Jenner, C. E. 1958. An attempted analysis of schooling behavior in the marine snail Nassarius obsoletus. Biol. Bull. 115:337.

Kator, H. and R. Herwig. 1977. Microbial responses after two experimental oil spills in an eastern coastal plain estuarine ecosystem, p. 517-522. Proceedings of Oil Spill Conference. American Petroleum Institute, Washington, D. C.

Keefe, C. S. and W. R. Boynton. 1973. Standing crop of salt marshes surrounding Chingoteague Bay, Maryland-Virginia. Ches. Sci. 14:117-123.

Kirby, C. J. 1971. The annual net primary production and decomposition of the salt marsh grass Spartina alterniflora Loisel. in Barataria Bay estuary of Louisiana. Ph. D. Dissertation, Louisiana State University, Baton Rouge. 74p.

Krebs, C. T. and K. A. Burns. 1977. Long-term effects of an oil spill on populations of the salt marsh crab, Uca pugnax. Science 197(4302):484-487.

Lake, J. L. 1977. Fate of oil pollution on a salt marsh community. Ph. D. Dissertation, The College of William and Mary, Williamsburg, Va. 130p.

Lytle, J. J. 1975. Fate and effects of crude oil on an estuarine pond, p. 595-600. Proceedings of Oil Spill Conference. American Petroleum Institute, Washington D. C.

Macking, J. G. 1950a. A comparison of the effects of application of crude petroleum to marsh plants and to oysters. Project 9 Report. Texas A. and M. Research Foundation. 4p.

Mackin, J. G. 1950b. Report on a study of the effects of applications of crude petroleum on saltgrass, Distichlis spicata (L.) Greene. Project 9 Report. Texas A. and M. Research Foundation. 8p.

Mendelssohn, I. A. 1973. Angiosperm production of three Virginia marshes in various salinity and soil nutrient regimes. M. S. Thesis, The College of William and Mary, Williamsburg, Va. 102p.

Michael, A. D., C. R. VanRaalte, and L. S. Brown. 1975. Long-term effects of an oil spill at West Falmouth, Massachusetts, p. 573-582. Proceedings of Oil Spill Conference. American Petroleum Institute, Washington, D. C.

Moore, S. F., R. L. Dwyer, and A. M. Katz. 1973. A preliminary assessment of the environmental vulnerability of Machias Bay, Maine to oil supertankers. Report MITSG 73-6, Massachusetts Institute of Technology, Cambridge, Mass. 162p.

Nadeau, R. J. and T. H. Roush. 1973. A salt marsh microcosm: an experimental unit for marine pollution studies, p. 671-683. Proceedings of Oil Spill Conference. American Petroleum Institute, Washington, D. C.

Nixon, S. and C. Oviatt. 1973. Ecology of a New England salt marsh. Ecol. Monogr. 49(4):463-498.

Pielou, E. C. 1969. An Introduction to Mathematical Ecology. Wiley-Interscience, New York. 286p.

Reimold, R. J., J. L. Gallagher, R. A. Linthurst, and W. J. Pfeiffer. 1975. Detritus production in coastal Georgia salt marshes, p. 217-228. In L. E. Cronin (ed.), Estuarine Research Vol. I. Academic Press, Inc., New York.

Sanders, H. L., J. F. Grassle, and G. R. Hampson. 1972. The West Falmouth oil spill. Part I. Biology. Technical Report 72-20. Woods Hole Oceanographic Institution, Woods Hole, Mass. 36p.

Smalley, A. E. 1958. The role of two invertebrate populations, Littorina irrorata and Orchelium fidicinium in the energy fow of a salt marsh ecosystem. Ph. D. Dissertation, Univ. of Ga., Athens. 126p.

Stebbings, R. E. 1968. "Torrey Canyon" oil pollution on salt marshes and a shingle beach in Brittany 16 months after. Nature Conservancy, Furzebrook Research Station, U. K. 12p. (unpub.)

Stroud, L. M. and A. W. Cooper. 1969. Color-infrared aerial photographic interpretation and net primary productivity of a regularly-flooded North Carolina salt marsh. Report No. 14. University of North Carolina Water Resources Research Institute. 86p.

Teal, J. M. 1962. Energy flow in the salt marsh ecosystem of Georgia. Ecology 43(4):614-624.

Udell, A. F., J. Zarudsky, and T. E. Doheny. 1969. Productivity and nutrient values of plants growing in the salt marshes of the Town of Hempstead, Long Island. Bull. Torrey Bot. Club. 96:42-51.

VanOverbeek, J. and R. Blondeau. 1954. Mode of action of phytotoxic oils. Weeds 1:55-65.

Waits, E. D. 1967. Net primary productivity of an irregularly flooded North Carolina salt marsh. Ph. D. Dissertation, North Carolina State University at Raleigh. 113p.

Walker, J. D., P. A. Seesman, and R. R. Colwell. 1975. Effects of a South Louisiana crude oil and a No. 2 fuel oil on growth of heterotrophic microorganisms, including proteolytic, lipolytic, chitinolytic, and cellulolytic bacteria. Environ. Pollution 9:13-33.

Wass, M. L. and T. D. Wright. 1969. Coastal wetlands of Virginia. Interim report to the Govenor and General Assembly. SRAMSOE No. 10. Virginia Institute of Marine Science, Gloucester Point, Va. 154p.

Wetzel, R. L. 1976. Carbon resources of a benthic salt marsh invertebrate Nassarius obsoletus Say (Mollusca: Nassariidae), p. 293-308. In Estuarine Processes. Vol. II. Academic Press, Inc. New York.

Wiegert, R. G. and F. C. Evans. 1964. Primary production and the disappearance of dead vegetation on an old field in south eastern Michigan. Ecology 45(1):49-63.

Williams, R. B. and M. B. Murdoch. 1968. Compartmental analysis of production and decay of Juncus roemerianus in a North Carolina salt marsh. Ches. Sci. 13:69-79.

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