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122 Saltmarsh Revisited – The Long–Term Effects of Oil and Dispersant on Saltmarsh Vegetation

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SALTMARSH REVISITED - THE LONG-TERM EFFECTS OF OIL AND DISPERSANT ON SALTMARSH VEGETATION

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SUMMARY

Saltmarshes are highly productive communities of great ecological and economic importance. Because they are flooded with seawater twice daily, saltmarshes are highly susceptible to oil spills. Unlike higher-energy coastal habitats, however, they have little opportunity for cleansing through wave action. The application of chemical dispersants to incoming oil slicks is one potential means of reducing the amount of oil entering a saltmarsh, but also increases the risk of potentially toxic dispersants contaminating saltmarsh vegetation. It is therefore necessary to examine the effects of dispersants and oil on saltmarsh vegetation to determine whether near-shore use of dispersants is an appropriate management response to oil spills.

The effects of treatment of saltmarsh vegetation with a chemical dispersant (Corexit 9527) and oil (Alberta sweet blend crude) were examined at Conrod's Beach Saltmarsh on Petpeswick Inlet, Nova Scotia (44°42'N, 63°11'W). Treatments (oil, dispersant, or oil + dispersant) were applied to three distinct marsh vegetation types (creek-edge Spartina alterniflora, mid-marsh S. alterniflora, and high-marsh S. patens) in early July, 1986, and vegetation and soil characteristics were measured in late summer of 1986, 1987, 1988 (vegetation only), and 1990. Data from 1986 and 1987 were reported previously (Lane et al. 1987; Crowell and Lane 1988). This report describes the results of 1988 and 1990 sampling periods, two and four years (or three and five growing seasons), respectively, after treatments were applied.

The following 'medium-term' effects of oil and/or dispersant on saltmarsh vegetation were observed (differences are significant at $P < 0.05$; marginal differences are significant at $0.05 \leq P \leq 0.1$):

Creek-edge

1988 Spartina on oiled plots was less dense and had less reproductive biomass than controls. On dispersant-treated plots, Spartina was taller, less dense, and had greater reproductive biomass and more flowering stems than controls.

1990 Spartina on oiled plots was shorter and had less foliar cover than controls. On dispersant-treated plots, Spartina was taller, less dense, and had greater live biomass than controls. On oil + dispersant-treated plots, Spartina was taller than controls and differed in fluorescence (both peak and 100-second difference values). One soil sample from one oiled plot (of 72 samples examined) showed low levels (5 ppb) of weathered hydrocarbons in the sediment sample.

Mid-marsh

1988 Spartina on oiled plots was shorter, less dense, and had marginally greater

reproductive biomass than controls. In dispersant-treated plots, Spartina was shorter, less dense, and had less live biomass and foliar cover than controls.

1990 Spartina on dispersant-treated plots had marginally greater foliar cover than controls and differed marginally in peak fluorescence. On oil+dispersant-treated plots, there was less detrital biomass; 100-second difference values for fluorescence were marginally different.

High Marsh

1988 Spartina on oiled plots was shorter, less dense, and had less foliar cover than controls; there was also less detrital biomass. On dispersant-treated plots, Spartina was shorter, had less live biomass, and marginally less foliar cover than controls; detrital biomass was also less.

1990 Spartina was shorter on oiled plots than on controls. On oil+dispersant-treated plots, there was less Spartina reproductive biomass, fewer flowering stems, and a difference in fluorescence 100-second difference values.

Overall, the two and four year (three and five growing season) post-treatment effects of oil ranged from relatively small but persistently negative in the creek-edge and high-marsh to negative but short-lived in the mid-marsh. Dispersant effects ranged from slightly positive in the creek-edge to acutely negative but ephemeral in the mid- and high-marshes. Oil+dispersant's effects were not measured in 1988 but in 1990 ranged from slightly positive in the creek-edge to slightly negative in the mid- and high-marsh zones.

Applying management recommendations from an experimental study to an actual spill situation is complicated by differences in scale. Although this study documents some recovery from all treatments by saltmarsh vegetation by the fifth post-treatment growing season, many of the mechanisms by which recovery occurs (e.g. seeding in and expansion of rhizome networks) might conceivably operate more quickly in small treatment plots surrounded by undamaged vegetation than in marshes suffering substantial disruption of vegetation over large areas.

Whether spilled oil should be chemically dispersed before it enters a saltmarsh is still open to question. This study suggests any benefits of dispersal in terms of more rapid vegetational recovery would be relatively small, if they exist at all. In fact, it might be expected that since both dispersant and oil+dispersant were more acutely toxic initially, they might more effectively inhibit recolonization and recovery of vegetation if a large area of saltmarsh was affected. Alternatively, oil alone might have longer lasting but less acute toxicity, which, when combined with mechanical interference over a large area, could substantially increase the time to recovery over that observed in this study.

RÉSUMÉ

Les marais salants renferment des biocénoses extrêmement productives et représentent une grande importance des points de vue économique et écologique. Submergés par l'eau de mer deux fois par jour, les marais salants sont très vulnérables aux déversements de produits pétroliers. Contrairement aux habitats côtiers à haute énergie, toutefois, ils peuvent difficilement être nettoyés par l'action des vagues. L'épandage précoce de dispersants chimiques sur les nappes est un des moyens qui permettraient de réduire la quantité de pétrole qui pénètre dans les marais salants, mais la végétation des marais risquerait alors d'être contaminée par les dispersants potentiellement toxiques. Il faut donc évaluer les effets des dispersants et du pétrole sur cette végétation afin de déterminer si l'utilisation de dispersants dans les milieux littoraux constitue une façon valable de gérer les marées noires.

On a étudié les effets d'un dispersant chimique (Corexit 9527) et du pétrole (mélange de brut non corrosif d'Alberta) sur la végétation du marais salant de Conrod's Beach à Petpeswick Inlet, en Nouvelle-Écosse (44°42'N, 63°11'O). Ces traitements (pétrole, dispersant ou un mélange de pétrole et de dispersant) ont été appliqués à trois types distincts de végétation de marais salants (*Spartina alterniflora*, en bordure de ruisseau, *S. alterniflora*, en marais médians et *S. patens*, en haut marais) au début de juillet 1986, et les caractéristiques de la végétation et du sol ont été mesurées à la fin des étés 1986, 1987, 1988 (uniquement la végétation) et 1990. Les données recueillies en 1986 et en 1987 ont déjà été publiées (Lane et col., 1987; Crowell et Lane, 1988). Le présent rapport énonce les résultats obtenus au cours des périodes d'échantillonnage de 1988 et de 1990, soit deux et quatre ans (ou trois et cinq saisons de croissance) respectivement après l'application des traitements.

On a constaté que le pétrole et le dispersant avaient sur la végétation du marais salant les effets à moyen terme suivants (les écarts sont significatifs à $P < 0,05$; les écarts marginaux sont significatifs à $0,05 \leq P \leq 0,1$) :

Bordure de ruisseau

1988 Dans les sols mazoutés, *Spartina* était moins dense et sa bioproduction plus faible que dans les sols témoins. Dans les sols traités au dispersant, *Startina* était plus haute, moins dense, présentait une bioproduction plus importante et un plus grand nombre de tiges en fleurs que dans les sols témoins.

1990 Dans les sols mazoutés, *Spartina* était moins haute et avait une surface foliaire moins étendue que dans les sols témoins. Dans les sols traités au dispersant, *Spartina* était plus haute, moins dense et présentait une biomasse vivante plus importante que dans les sols témoins. Dans les sols traités avec un mélange de pétrole et de dispersant, *Spartina* était plus haute que dans les sols témoins et différait en fluorescence (valeurs de pointe et taux de variation par intervalle de cent secondes). Un échantillon prélevé dans le sol mazouté (sur les 72

échantillons examinés) indiquait de faibles niveaux (5 ppb) d'hydrocarbures altérés dans l'échantillon de sédiment.

Marais intermédiaire

1988 Dans les sols mazoutés, *Spartina* était plus courte, moins dense et montrait une bioproduction légèrement plus faible que dans les sols témoins. Dans les sols traités au dispersant, *Spartina* était plus courte, moins dense et présentait une proportion de biomasse vivante moins importante que dans les sols témoins.

1990 Dans les sols traités au dispersant, *Spartina* présentait une surface foliaire légèrement plus étendue que dans les sols témoins et différait quelque peu de ces derniers en fluorescence de pointe. Dans les sols traités avec un mélange de pétrole et de dispersant, la biomasse détritique était moindre, et le taux de variation par intervalle de cent secondes en fluorescence étaient légèrement différentes.

Haut marais

1988 Dans les sols mazoutés, *Spartina* était plus courte, moins dense et présentait une surface foliaire moins étendue que dans les sols témoins; la biomasse détritique était également moindre.

1990 Dans les sols mazoutés, *Spartina* était moins haute que dans les sols témoins. Dans les sols traités avec un mélange de pétrole et de dispersant, la bioproduction de *Spartina* était moindre, il y avait moins de tiges en fleurs et une différence en fluorescence du taux de variation par intervalle de cent secondes.

Dans l'ensemble, les effets du traitement au pétrole sur la végétation après une période de deux et de quatre ans (ou trois et cinq saisons de croissance) variaient de relativement faibles mais nocifs de façon persistant en bordure de ruisseau et dans les hauts marais à nocifs mais provisoires, dans les marais médians. Le dispersant avait un effet qui variait de légèrement bénéfique en bordure de ruisseau à très nocif mais passager dans les hauts marais et les marais médians. Les effets du mélange de pétrole et de dispersant, qui n'ont pas été mesurés en 1988, variaient, en 1990, de légèrement bénéfiques en bordure de ruisseau à légèrement nocifs dans les zones de marais médians et de hauts marais.

Il est difficile d'appliquer les recommandations formulées dans le cadre d'une étude expérimentale à la gestion d'un déversement réel parce qu'on se situe alors à une toute autre échelle. Bien que cette étude indique un rétablissement partiel de la végétation des marais salants au terme de la cinquième saison de croissance qui suit les traitements, bon nombre des mécanismes qui régissent ce rétablissement (par exemple, ensemencement et prolongement des réseaux de rhizomes) agissent vraisemblablement plus rapidement dans de petites étendues de sol

traité entourées de végétation intacte que dans les marais dont la végétation a été perturbée sur de vastes étendues.

Doit-on disperser par des moyens chimiques les nappes de pétrole avant que ces dernières ne gagnent les marais salants? Il est difficile de trancher. Cette étude suggère que la dispersion chimique, qui semble accélérer le rétablissement de la végétation, n'aurait en fait que peu ou pas d'effets favorables. En réalité, si l'on considère que le dispersant et le mélange de dispersant et de pétrole tend à présenter une toxicité aiguë plus marquée dans les premiers temps, on peut s'attendre à ce que ces produits aient pour effet d'empêcher effectivement la recolonisation et le rétablissement de la végétation si une grande partie du marais est contaminée. Par ailleurs, le pétrole seul, qui présente une toxicité plus persistante bien que moins aiguë que le dispersant, peut, lorsque combiné à des phénomènes mécaniques qui agissent sur une vaste étendue, entraîner un temps rétablissement beaucoup plus long que les délais observés dans le cadre de la présente étude.

1.0 Introduction

Saltmarshes are highly productive communities of great ecological and economic importance. They fix large quantities of carbon, a large proportion of which is transported to estuarine and coastal waters where it fuels marine food webs. The marshes are used by invertebrates, fish, birds, and mammals as feeding, breeding, and resting areas. Most saltmarshes along the Atlantic coast of North America are dominated by the saltmarsh cord grass, Spartina alterniflora Loisel., a species tolerant of high salinity and anoxia in the sediments, yet capable of high rates of primary production.

Saltmarshes are inundated twice daily with sea water and are therefore highly susceptible to nearby oil spills. Unlike more exposed, coastal sites, however, they have little opportunity for cleansing through wave action. The sensitivity of saltmarshes to spilled oil depends on the type of oil, its toxicity and potential for mechanical interference, weathering, volume spilled, frequency of spills, seasonal timing of the spill, types of organisms and habitats exposed to the oil, and stresses experienced by organisms prior to the spill. These considerations hinder attempts to implement appropriate mitigative measures following an oil spill and are further complicated because most saltmarsh clean-up techniques have proven either ineffective or damaging in their own right (Vandermeulen and Jotcham, 1986). Lack of further intervention may sometimes be a valid option provided the aggregate adverse effect of the oil spill is less than that of the prescribed clean-up procedure. The ability to make this decision depends on prior knowledge available from oil and clean-up impact studies conducted in a variety of saltmarsh types, where different types and amounts of oil were applied at different times of year, and monitored for a number of years.

The application of chemical dispersants to incoming oil slicks is potentially a means of reducing the amount of oil which enters the marsh. However, near-shore applications of dispersant also increase the likelihood of dispersants contaminating saltmarsh vegetation. A few studies have investigated the impacts of dispersants on saltmarsh vegetation (Baker et al., 1984; Delaune et al., 1984; and Smith et al., 1984). They have demonstrated that phytotoxicity of dispersant formulations varies considerably. It is therefore important to document the effects of a variety of dispersants in order to determine which may be safely used in close proximity to saltmarsh vegetation.

Two previous studies by P. Lane and Associates Limited (Lane et al., 1987; Crowell and Lane, 1988) examined the impacts of applications of Alberta sweet blend crude oil and the dispersant Corexit 9527 on the vegetation of a Nova Scotian saltmarsh. These studies compared patterns of vegetation growth among controls and treatments in the first two growing seasons after the experimental oil and/or dispersant applications, and found that, in general, areas treated with both oil and dispersant experienced greater damage, and took longer to recover, than areas treated with oil only. The present study extends these earlier studies, and compares saltmarsh vegetation in control and oil- and/or dispersant-treated plots in the third and fifth growing seasons following treatment.

2.0 Methods

2.1 Description of the Study Site

The field study site is located at Conrods Beach on Petpeswick Inlet, Nova Scotia (44°42'N, 63°11'W; Figure 1). The Conrods Beach saltmarsh has developed behind a barrier dune system and is drained by a single channel which breaches the dune system. The vegetation of the saltmarsh is divisible into three more or less distinct zones, referred to here as creek-edge, mid-marsh, and high-marsh zones.

The creek-edge zone is characterized by a lush growth of Spartina alterniflora. Small quantities of Salicornia europea, Plantago juncoides, Suaeda maritima, and Atriplex patula are also present. This zone is usually restricted to within two metres of drainage channels in the marsh. The lush growth of Spartina alterniflora probably results from the relatively well-drained and aerated sediments of this zone.

The mid-marsh zone is characterized by a dwarf ecotype of Spartina alterniflora, with lesser amounts of Spartina patens, Salicornia europea, Suaeda maritima, Triglochin elata, Limonium nashii, and Plantago juncoides. This is the most extensive of the three zones in the Conrod's Beach saltmarsh, and is generally found in poorly drained areas of the marsh interior. The stunted growth of Spartina alterniflora results from poor aeration of the sediments.

The high-marsh zone is characterized by dense growth of Spartina patens. Small quantities of Spartina alterniflora, Salicornia europea, Triglochin elata, Suaeda maritima, and Glaux maritima are also frequently present. This zone is located on slightly elevated, better-drained, and aerated soils of the marsh.

2.2 Experimental Design

Twelve 0.5 m x 4.0 m ($2m^2$) plots were established at random locations in the marsh in each of the three vegetation zones, and four treatments (control, oil, dispersant, or oil + dispersant) were randomly assigned to each zone (Figure 2).

For the experimental applications, each plot was separated from other plots by a 10 m buffer zone to prevent or reduce cross-contamination by either oil or dispersant from the other plots.

Each quadrat was surrounded by a wall of sorbent material to prevent the escape of oil from the quadrat. Quadrats were also covered with garden netting to exclude wildlife. Treatments were applied on July 7, 1986.

"Alberta sweet blend" crude oil, weathered by evaporation and stirred for 24 hours to 15-25% weight loss, was used for all experiments. For oil-treated plots, 2.25 litres of weathered

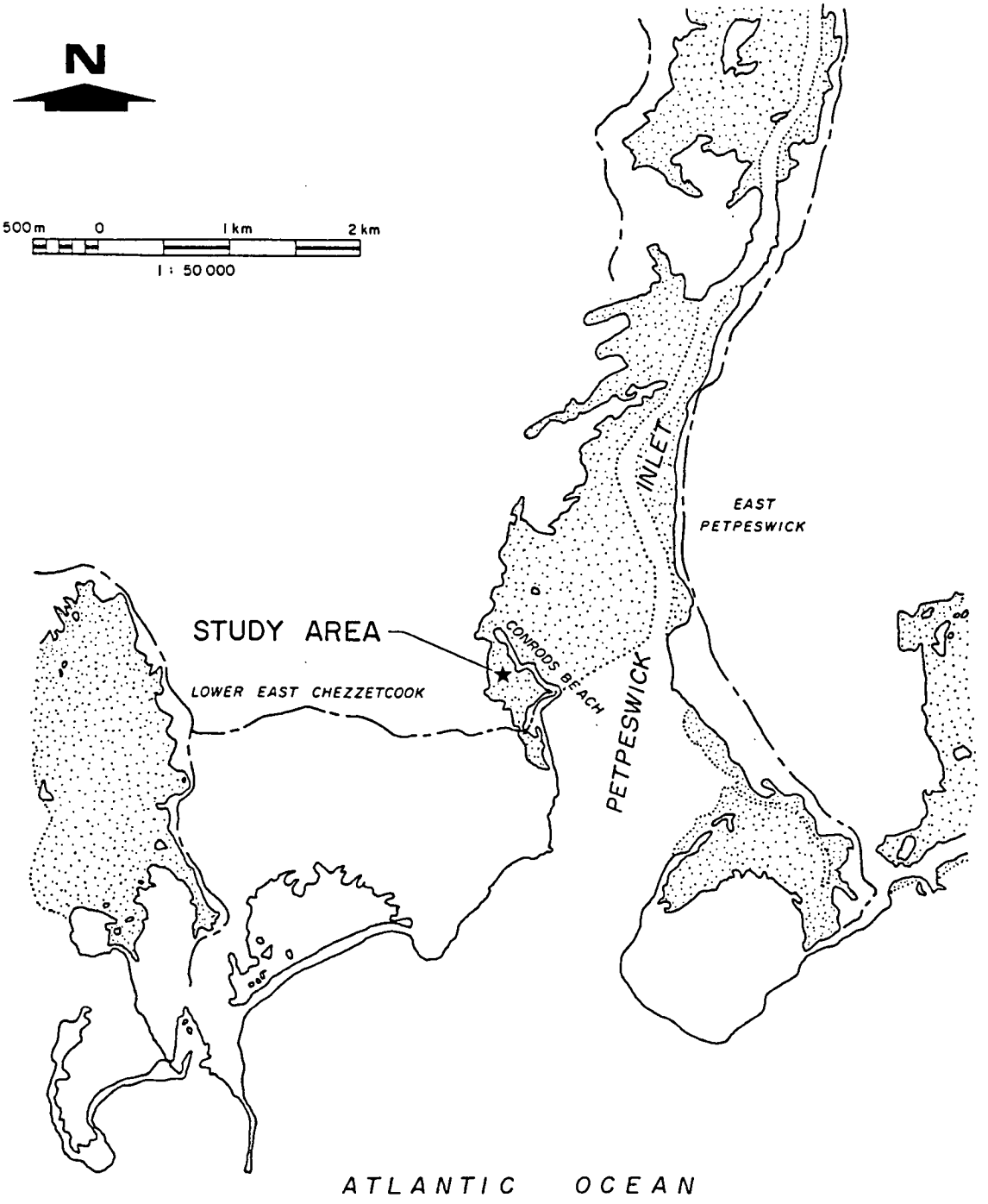

Figure 1. Map showing the location of Petpeswick Inlet and the Conrods Beach salt marsh.

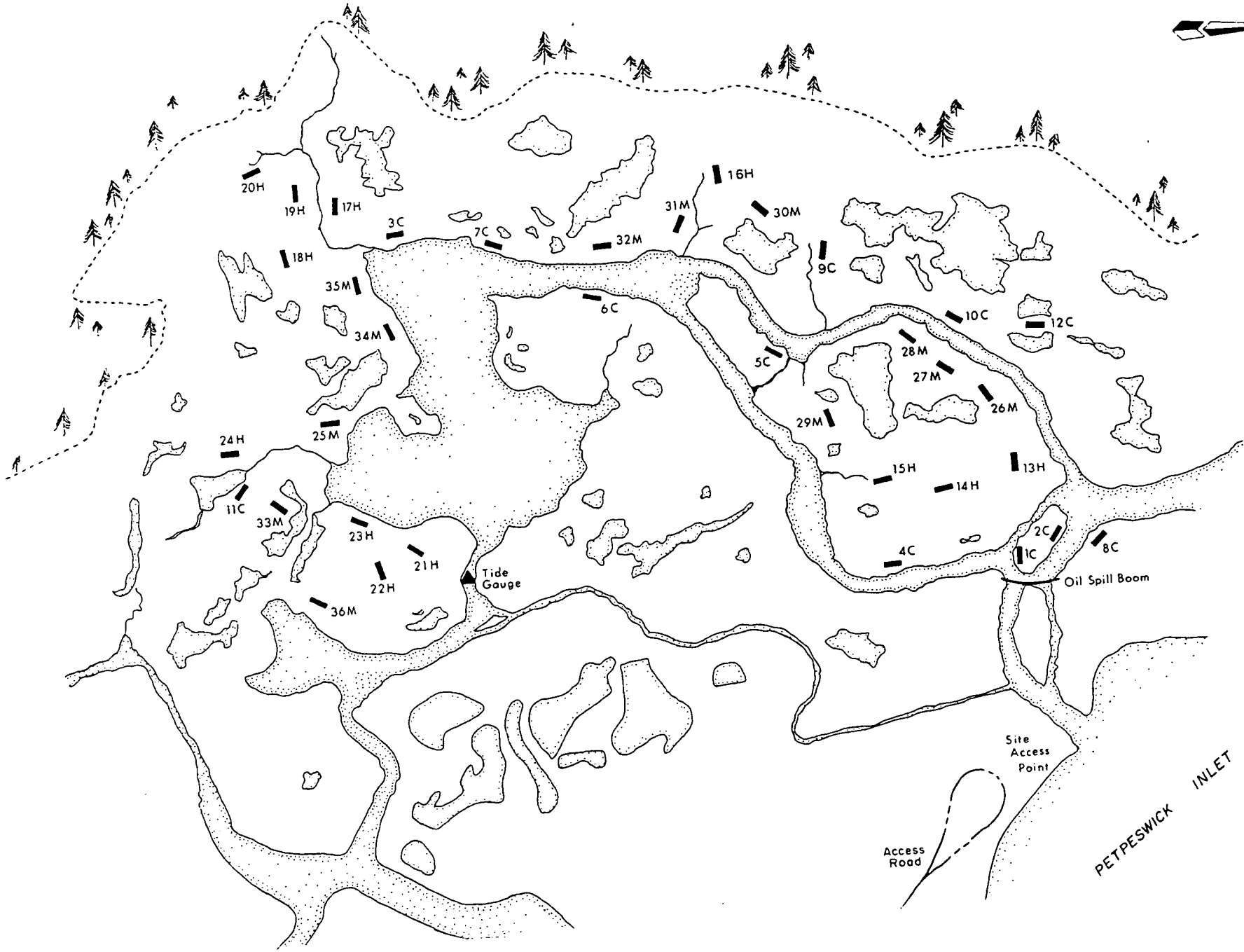

Figure 2. Map of the Conrods Beach salt marsh. The locations of study plots, tide gauge, and sorbent booms are indicated. Numbers associated with plots indicate quadrat code number. "C" = creek edge; "M" = mid marsh; and "H" = high marsh.

oil were applied from a backpack sprayer approximately two hours after the onset of the ebb, yielding a nominal 0.5 mm oil slick. On the day of treatment, the tide did not cover the entire marsh surface; therefore, each plot was sprayed with seawater prior to application. The oil spray application was done so that the spray was held near the marsh sediment surface, at about half of the mean plant height, thereby simulating contamination by a surface oil slick.

For dispersant treatments, ethylene glycol-based Corexit 9527 was mixed with water in a ratio of 1 part dispersant to 10 parts seawater. A total of 2.25 litres of the dispersant-seawater mixture was applied to each plot from a backpack sprayer. Application differed from that of the oil application in that the dispersant spray was directed at the vegetation from 50 cm above the sediment surface, simulating an operational dispersant application.

For the combined oil+dispersant treatment, 2.25 litres of Corexit 9527 was prepared as above, but was applied six hours after initial spraying with oil, yielding a 10:1 oil+dispersant application ratio. Oil was applied near the marsh sediment surface, while the Corexit 9527 was applied from 50 cm above the plants.

The vegetation and soil characteristics of 1.5 m^2 plots experimentally treated with oil, dispersant, or oil+dispersant were examined three and five growing seasons after the treatments were first applied. The methods followed as closely as possible those used by previous investigators, who studied the experimental treatments in the same year (first growing season, 1986), and one year after (second growing season, 1987) they were applied (Lane et al., 1987; Crowell and Lane, 1988). Whereas measurements were taken throughout the growing seasons of 1986 and 1987, they were taken only once in 1988 and 1990, at the end of the growing season. No measurements of any kind were made on oil+dispersant-treated plots in 1988.

The following characteristics were measured, all in late August or early September:

i) Plant Height - Within each plot, 40 Spartina stems, systematically selected at 10 cm intervals along the length of the plot, were measured. Plant height was measured as the distance from the tallest point of the plant to ground-level.

ii) Stem Density - Within each plot, twelve quadrats were systematically positioned at 30 cm intervals along the length of the plot. In creek-edge and mid-marsh (S. alterniflora) zones, 0.01 m^2 quadrats were used while in the high-marsh (S. patens) zone, 0.0056 m^2 quadrats were used. All live stems in each quadrat and all flowering stems in each plot were counted.

iii) Biomass - Biomass was collected from one 0.09 m^2 quadrat systematically positioned in one end of each plot. [In 1990, quadrats were randomly positioned within plots to avoid apparent trampling effects resulting from previous sampling.] All above-ground biomass in each quadrat was collected and dried at 80°C in a

convection oven. Live biomass was sorted according to species and weighed while dead biomass was simply weighed. All flowering stems in each entire plot were collected; reproductive portions (flowers) were separated from vegetative portions (stems) and botmh were weighed on an analytical balance. The small sample size meant that not all species could be analyzed simultaneously, so rarer species, such as Suaeda, Salicornia, Plantago, and Limonium were ignored. Measurements of the two Spartina species were combined in calculating both live and reproductive biomass, with reproductive biomass being the total of reproductive stems and flowers.

iv) Species Cover - Percent cover of each species was estimated at the plot level by examining foliage overlap of the soil, i.e., such that multiple layers of foliage could yield estimates of "aggregate" foliage cover greater than 100%. As with biomass, rare species were ignored because of the small sample size. Spartina alterniflora was analyzed in the creek-edge and mid-marsh and S. patens was analyzed in the high-marsh; density of flowering Spartina stems, also tallied at the plot level, was included in the same analysis.

v) Fluorometry (1990 only) - Three plants were randomly selected from each plot for fluorometric analysis. The upper-most fully expanded leaf from each stem was placed in a plastic bag (one per plot) into which distilled water was sprayed. The samples were then placed in a light-proof box for transport to the laboratory. Collection and preparation of the leaves took approximately 0.5 hours, and during transport, the leaves were allowed to equilibrate in the dark for 1.5 hours. Working illumination in the laboratory was provided by a 6 volt lantern fitted with multiple layers of green gel material. Fluorometric measurements were made with a Brancker Research Ltd. (Ottawa) model SF-20 portable fluorometer. The fluorescence induction response was monitored for 100 seconds and initial, transient peak, peak, and final readings were recorded (Figure 3). From these, two additional measurements were calculated: i) peak-minus-initial fluorescence; and ii) final-minus-initial fluorescence. Redundancy among the variables was analyzed using principal components analysis (PCA), with the result that only peak, peak-minus-initial, and final-minus-initial fluorescence were retained for further analyses. The principles of plant fluorescence induction measurements are briefly explained in Appendix A.

vi) Soil Chemistry (1990 only) - At each plot, two soil cores approximately 6 cm wide by 15 cm long were removed using a garden trowel. A 50 g sample of soil was taken from the middle of the core and extracted three times with 20 ml of methylene chloride. The resultant solution was dried, taken up in hexane, and cleaned up. Samples were cleaned by pouring them through a mini-column containing sodium sulphate, copper, and activated florisil. Total oil and grease values were determined with UV fluorescence analysis, scanning from 200 to 300 nm. Data were expressed as ppm of chrysene equivalents (UV fluorescence at 256 nm).

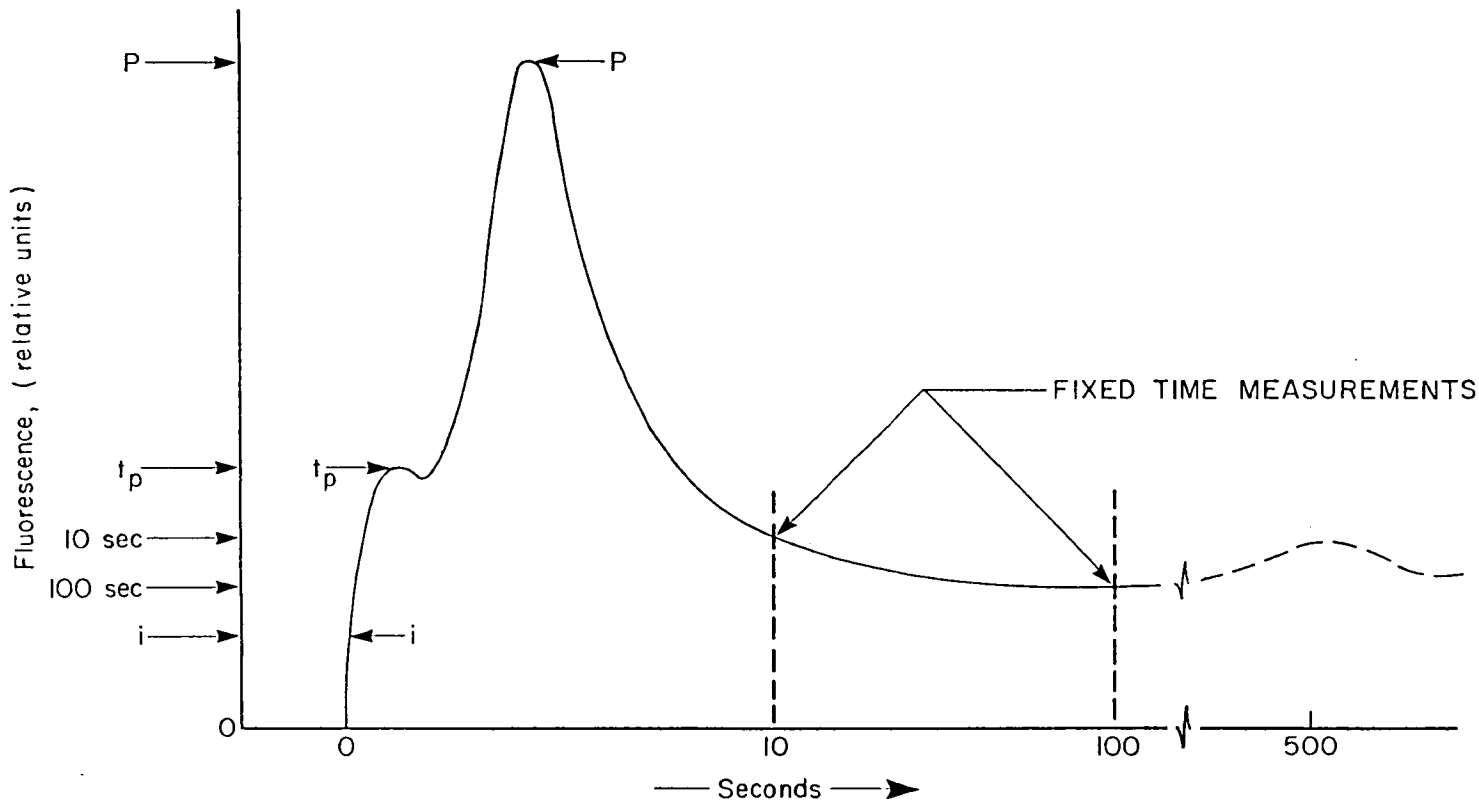

Figure 3. Diagrammatic representation of the fluorescence induction curve for Spartina alterniflora. Arrows indicate recorded fluorescence values. The initial value (i) is the "fixed fluorescence". Fluorescence above this level is "variable fluorescence" which is sensitive to the physiological status of the photosynthetic system. "(P-i)" is referred to as "peak fluorescence" and 100-second values minus initial values are referred to as "100-second difference values". "'p" refers to a transient peak.

Selected sample extracts were analyzed by glass capillary gas chromatography using a flame ionization detector. Relative concentrations of the n-alkanes were determined from the chromatograms. Identifications of the peaks were made using glass capillary gas chromatography mass spectrometry. Procedures followed those of Geiger and Schaffner (1978).

2.3 Data Analysis

All variables were examined for normality and homoscedasticity (equality of variances). Various transformations (e.g. square root, logarithmic, and inverse) were made as suggested by the relationships between means and variances. Where transformations failed to reduce heteroscedasticity and non-normality, variables were analyzed with the realization that resulting probability values would not be exact.

Data were analyzed using one-way analysis of variance (ANOVA) in each zone, with experimental treatment as the factor. [A 2-way ANOVA was inappropriate because different variables were measured in each zone (e.g. Spartina alterniflora in the mid-marsh and Spartina patens in the high-marsh).] Although there were three replicate plots per treatment, some variables, such as plant height and stem density, were subsampled within plots. Whether subsampled or not, all samples were treated as simple and random (e.g., for plant height, treatment sample sizes were 3 x 40 = 120 and for biomass, 3 x 1 = 3). Differences between controls and treatments were examined using a priori contrasts. All analyses were performed using SPSS programs (SPSS Inc., 1986).

Temporal data series (1986, 1987, 1988, and 1990) resulting from this and earlier studies (Lane et al., 1987; Crowell and Lane, 1988) were examined graphically but should be regarded cautiously for among-year comparisons. Field observations indicated that the use of different field personnel and lack of statistical controls for procedural effects such as trampling and destructive sampling may have influenced among-year variability. Additionally, 1988 data, collected by inexperienced personnel (students), may not be directly comparable to those of other years.

3.0 Results and Discussion

Plant Height

Significant differences in average Spartina height between control plots and treatments were observed in both 1988 and 1990 (Figure 4a; also Appendix B). In 1988, creek-edge Spartina shoots averaged 10.2 cm taller in dispersant-treated plots than controls ($P < 0.001$). In 1990, this difference increased to 12.6 cm, and oil+dispersant-treated plants averaged 9.8 cm taller than controls (both $P < 0.001$). There was no significant difference between oil and control plots in 1988, but in 1990, oiled plants averaged 4.5 cm shorter than controls ($P = 0.043$). In the mid-marsh, oiled plants averaged 7.6 cm shorter, and dispersant-treated

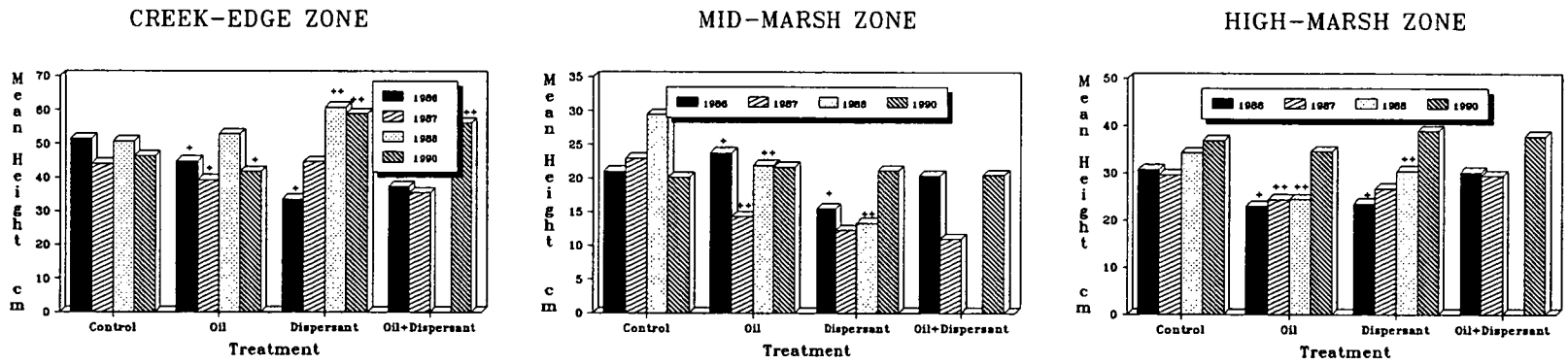

Figure 4a. Mean *Spartina* Height*

*No data for 1988 'Oil+Dispersant'

\+ Difference between Control and Treatment in corresponding year significant at 0.05 > P > 0.01

++ Difference between Control and Treatment in corresponding year significant at P < 0.01

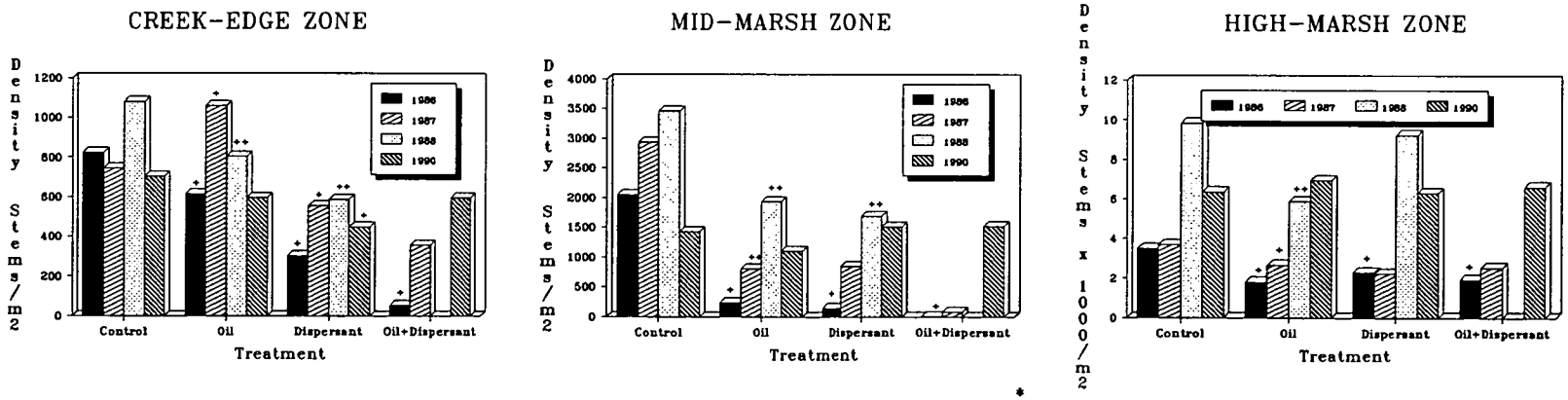

Figure 4b. *Spartina* Density*

plants 16.2 cm shorter, than controls in 1988 (both $P < 0.001$). In 1990, there were no significant differences between controls and treatments. In the high-marsh zone, oiled plants averaged 9.9 cm shorter, and dispersant-treated plants 3.9 cm shorter, than controls in 1988 (both $P < 0.001$). In 1990, oiled plants remained marginally shorter (2.3 cm; $P = 0.076$) than controls. Because of the large sample size and small potential for measurement error, height was a more sensitive indicator of differences between treatments than biomass or cover (see below).

Over the entire study period (Figure 4a), control plant height values remained relatively stable in the creek-edge and high-marsh zones but varied in the mid-marsh. In the creek-edge, dispersant and oil+dispersant treatments showed the largest initial impacts but recovered relatively rapidly to pre-treatment levels. All mid-marsh treatment effects persisted through 1988, with dispersant having the greatest impact. The 1990 mid-marsh values were remarkably similar among controls and treatments. In the high-marsh zone, both controls and treatments increased through the study period. Treatment effects ranged from below detectability for oil+dispersant to relatively small but persistent negative effects for oil. Treated areas appeared to recover by 1990.

Plant Density

In the creek-edge, Spartina density was lower on oil- and dispersant-treated plots than controls in 1988 (by 270 and 490 stems/m^2, respectively; $P = 0.001$ and $P < 0.001$ respectively; Appendix C). By 1990, only dispersant plots had significantly fewer (250 stems/m^2 fewer; $P = 0.019$) stems than control plots. In the mid-marsh, oil plots had 1530 fewer stems/m^2 than controls in 1988 ($P = 0.005$) while dispersant plots had 1770 fewer stems/m^2 ($P = 0.001$). In 1990, there were no differences between treatments and controls. In the high-marsh, oil plots had 3950 fewer stems/m^2 than controls in 1988 ($P < 0.001$); no differences were observed in 1990.

Compared with previous years (Figure 4b), control plot densities peaked in 1988 and fell sharply in 1990 in all zones, suggesting that a procedural bias influenced Spartina densities on the study plots. As stated in Section 2, 1988 data were collected by inexperienced personnel and may have been overestimated. In the creek-edge, only dispersant plots failed to recover to control levels by 1990; in the mid-marsh, neither oil nor dispersant plots recovered. A sharp decline in mid-marsh control densities between 1988 and 1990 was probably caused by treatment-unrelated algal mat and sod deposition (which occurred on two of the three control replicates but on no treatment plots). In the high-marsh, no easily explained pattern was observed. Treatment plots had lower densities than controls in the first two years (1986 and 1987); in 1988, all plots increased substantially (by greater than 100%), oiled plots least. In 1990, all plots had similar densities which were considerably higher than 1986 control levels. This again suggests a procedural bias, perhaps related to sampling intensity (which decreased over the study period), or an unexpected interaction between the experiment and natural saltmarsh dynamics.

Biomass

In the creek-edge zone, total live Spartina biomass (Figure 5a; also Appendix D) was similar among controls and treatments in 1988 but marginally greater in dispersant plots in 1990 ($P = 0.091$). Total detrital biomass (Figure 5b; also Appendix D) did not differ between controls and treatments in 1988 or 1990. Total reproductive biomass of Spartina (Figure 6a; also Appendix D) in 1988 was significantly lower in oil, and higher in dispersant plots, than controls (by 1.8 g/m^2 [$P = 0.041$] and 12.6 g/m^2 [$P = 0.013$] respectively). In 1990, although there were greater differences in reproductive biomass than those observed in 1988, none was statistically significant.

In the mid-marsh, total live Spartina biomass was lower in dispersant plots than controls in 1988 (by 204 g/m^2; $P = 0.052$). In 1990, there were no differences in live biomass but detrital biomass was significantly lower in oil+dispersant plots than controls (by 245 g/m^2; $P = 0.013$). Oil plots had marginally greater reproductive biomass of Spartina than controls in 1988 ($P = 0.096$); there were no differences in 1990.

In the high-marsh zone, total live Spartina biomass was significantly less in dispersant plots than controls in 1988 (by 265 g/m^2; $P = 0.009$). Total detrital biomass was marginally lower in oil- and dispersant-treated plots than controls in 1988 ($P = 0.085$ and 0.069 respectively). No differences were observed in 1990 in either total live or detrital biomass. No differences in reproductive biomass were observed between controls and treatments in 1988, but in 1990, oil+dispersant plots had marginally lower reproductive biomass than controls ($P = 0.058$).

Over the entire study period, live Spartina biomass (Figure 5a) gradually increased after initial treatment impact for most treatment-zone combinations. In control plots, however, temporal patterns were variable and differed among zones, suggesting that variability in control biomasses was not simply due to procedural bias. In all zones in 1990, dispersant plots had greater (but not significantly so) biomasses than controls or other treatments.

Detrital biomass (Figure 5b) was high in the first growing season after treatment, probably because of high plant mortality, and was low in the second growing season because of a shortage of live plant material and subsequent detritus production. In the third growing season, as biomass production increased, so did detritus. Detrital biomass declined more gradually on high-marsh plots following treatment, probably because of lower rates of decomposition (see Lane et al., 1987). Detritus in mid-marsh control plots increased steadily throughout the study period, mirroring the trend in live biomass. The reasons for this are unclear but may again be related to recovery from procedural damage as sampling intensity decreased.

Spartina reproductive biomass (Figure 6a) increased on all creek-edge treatment plots in 1987, especially on dispersant-treated plots. In 1988, reproductive biomass declined on all plots, especially on dispersant-treated plots. In 1990, both dispersant and oil+dispersant

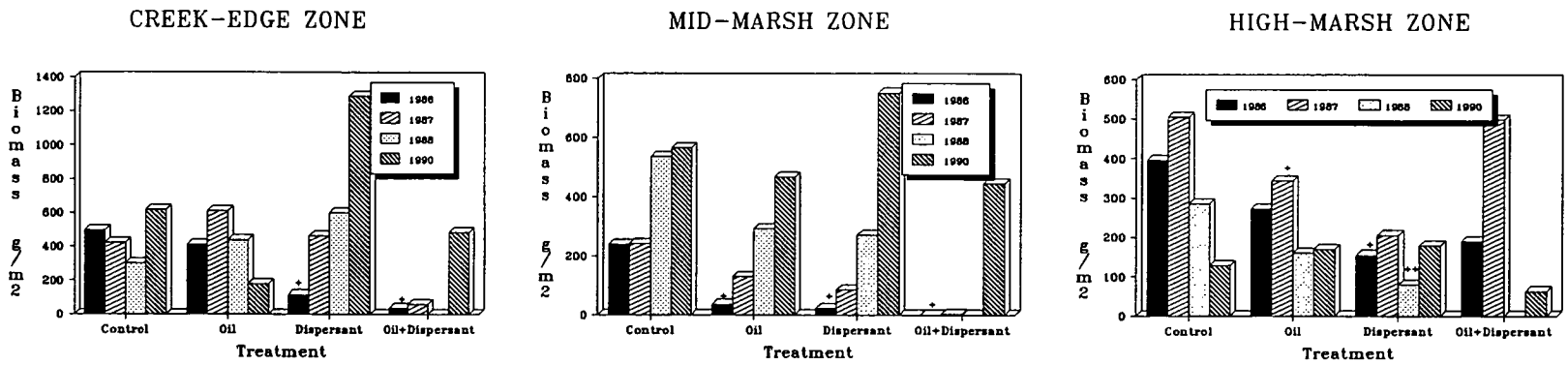

Figure 5a. Biomass of Live *Spartina*[*]

[*] No data for 1988 'Oil+Dispersant'

[+] Difference between Control and Treatment in correspondingyears significant at 0.05 > P > 0.01

[++] Difference between Control and Treatment in correspondingyears significant at P < 0.01

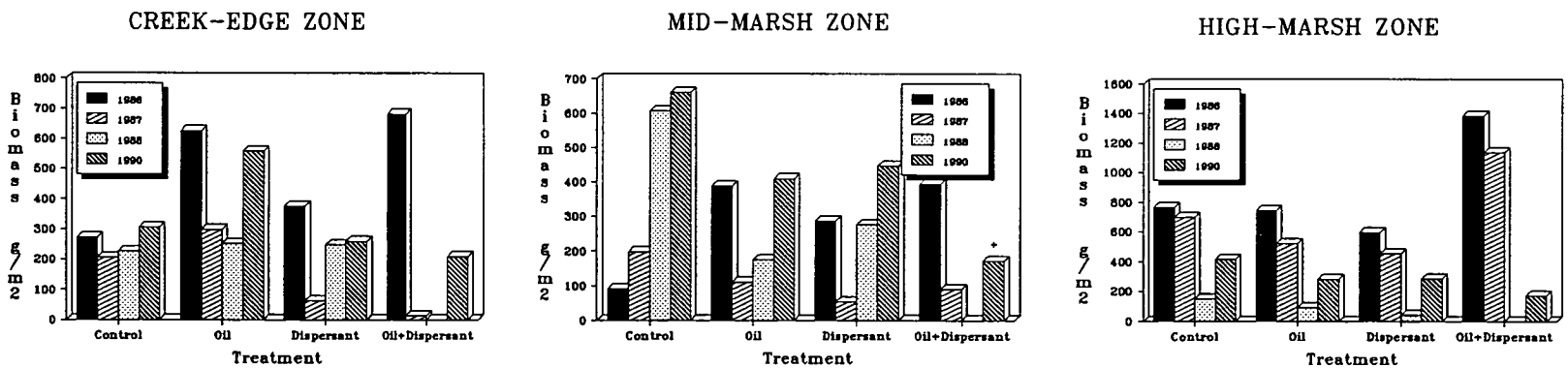

Figure 5b. Detrital Biomass[*]

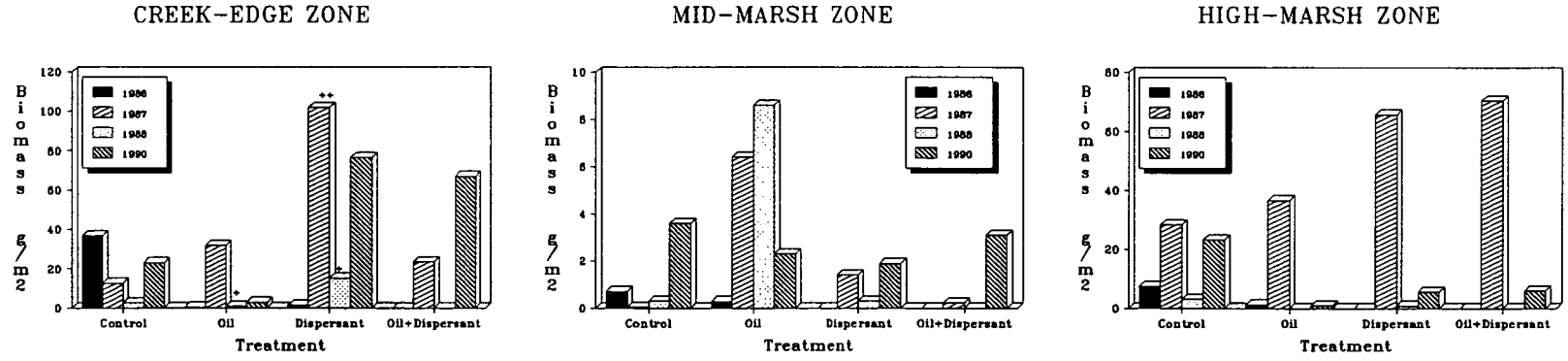

Figure 6a. *Spartina* Reproductive Stem Biomass*

*No data for 1988 'Oil+Dispersant'

1No data for 1987 'Oil+Dispersant'

2No data for 1987 'Dispersant'

+Difference between Control and Treatment in corresponding years significant at 0.05 > P > 0.01

++Difference between Control and Treatment in corresponding years significant at P < 0.01

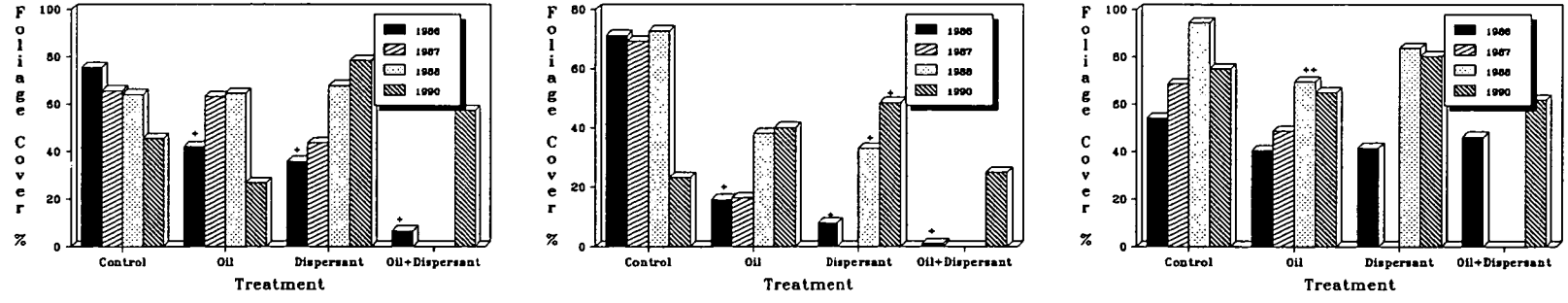

Figure 6b. Foliage Cover of *Spartina* spp.*1

plots again had relatively high reproductive biomasses. In the mid-marsh, reproductive biomass increased sharply on oil plots in 1987 and 1988 but the response to other treatments was less dramatic. In 1990, reproductive biomass declined on oil plots to levels similar to controls. In the high-marsh zone, a sharp increase in reproductive biomass on all treated plots in 1987 was followed by an equally sharp decrease in 1988; control plots remained relatively stable.

The observed pattern of change in reproductive biomass differed from that of vegetative biomass. Rather than a gradual increase after the initial treatment impact, there was a burst of reproductive output far surpassing that observed on control plots. This burst was usually short-lived (a single growing season) but continued for two seasons on mid-marsh oil plots. This pattern is consistent with a trade-off between photosynthetic/structural and reproductive investment. At low densities, resources may permit a high reproductive output but at high densities, it may be necessary to invest more heavily in photosynthetic and/or nutrient-gathering tissue simply to shade or sequester nutrients from other individuals.

Cover

In the creek-edge, Spartina alterniflora cover (Figure 6b; also Table 1) did not differ between controls and treatments in 1988, but was marginally lower on oil plots in 1990 ($P = 0.086$, 45% control vs. 26% oil). Density of flowering Spartina stems (Table 1) was significantly higher on dispersant plots than controls in 1988 (48 stems/1.5m^2 for dispersant vs. 10 stems/1.5m^2 for control; $P = 0.033$). In 1990, although greater differences were observed, none was statistically significant.

In the mid-marsh, Spartina alterniflora cover was significantly lower on dispersant plots than controls in 1988 (25% dispersant vs. 70% control; $P = 0.013$) but was marginally higher in 1990 ($P = 0.053$; Table 1). There were no differences in flowering stem density between controls and treatments in either 1988 or 1990.

In the high-marsh zone, Spartina patens had significantly less cover on oil plots and marginally less cover on dispersant plots than controls in 1988 (69% oil vs. 94% control [$P = 0.004$] and 83% dispersant vs. 94% control [$P = 0.081$] respectively; Table 1). There were no differences between controls and treatments in 1990. Density of flowering stems was significantly lower on oil+dispersant plots than controls in 1990 (28 stems/1.5 m^2 oil+dispersant vs. 270 stems/1.5m^2 control; $P = 0.044$).

In general, there was a fairly rapid recovery in the creek-edge and high-marsh zones, and a slow and as yet incomplete recovery in the mid-marsh (Figure 6b). In 1990, no mid-marsh treatment plots had recovered to pre-treatment control levels, but 1990 control levels had declined to less than 30% of their initial values, again suggesting a procedural bias or unexpected interaction with ongoing saltmarsh processes.

Table 1. Cover and flowering stem density of Spartina in saltmarsh plots two and four years after treatment with oil and/or dispersant. "-" indicates no data.

	Spartina alterniflora Cover (%)		Spartina patens Cover (%)		Density of Flowering Spartina Stems (no./1.5 m^2)	
	1988 Mean (SE)	1990 Mean (SE)	1988 Mean (SE)	1990 Mean (SE)	1988 Mean (SE)	1990 Mean (SE)
Creek-edge						
Control (n=3)	64.0 (2.1)	45.0 (13.2)	0.0 (0.0)	0.3 (0.3)	10.0 (2.1)	74.0 (71.5)
Oil (n=3)	63.7 (4.7)	26.0 (9.5)*	0.7 (0.7)	0.7 (0.7)	29.0 (10.4)	11.3 (9.0)
Dispersant (n=3)	67.7 (5.9)	76.7 (8.8)	0.0 (0.0)	1.7 (1.7)	44.7 (11.3)**	149.3 (55.0)
Oil+Dispersant (n=3)	- -	56.7 (23.2)	- -	0.7 (0.7)	- -	86.0 (42.8)
Mid-marsh						
Control (n=3)	69.7 (6.7)	18.3 (13.3)	3.0 (3.0)	5.0 (5.0)	0.7 (0.7)	19.3 (9.8)
Oil (n=3)	37.3 (13.3)	40.0 (5.0)	0.7 (0.7)	0.0 (0.0)	54.3 (22.3)	16.7 (7.9)
Dispersant (n=3)	24.7 (4.9)**	43.3 (3.3)*	8.3 (5.2)	2.3 (1.5)	29.7 (7.5)	20.5 (0.5;n=2)
Oil+Dispersant (n=3)	- -	18.3 (4.4)	- -	6.7 (6.7)	- -	52.7 (36.8)
High-marsh						
Control (n=3)	0.7 (0.7)	0.0 (0.0)	93.7 (0.7)	75.0 (2.9)	76.0 (44.2)	270.3(112.6)
Oil (n=3)	0.0 (0.0)	0.0 (0.0)	69.3 (5.9)**	65.0 (16.1)	4.0 (0.0)	5.7 (2.9)
Dispersant (n=3)	0.3 (0.3)	0.2 (0.2)	83.3 (0.9)*	80.0 (10.4)	137.3(105.0)	56.3 (29.7)
Oil+Dispersant (n=3)	- -	0.0 (0.0)	- -	61.7 (15.9)	- -	28.0 (17.0;n=2)**

* Difference from control significant at $0.1 > P > 0.05$
** Difference from control significant at $0.05 > P > 0.01$
*** Difference from control significant at $P < 0.01$

Fluorometry (1990 only)

In the creek-edge zone (Table 2), differences were observed between control and oil+dispersant-treated plants in both peak and peak-minus-initial fluorescence (P = 0.026 and 0.089 respectively). In the mid-marsh, control and dispersant-treated plants differed marginally in their peak fluorescence (P = 0.071) while control and oil+dispersant-treated plants differed significantly in final-minus-initial fluorescence. In the high-marsh zone, oil+dispersant-treated plants differed significantly from controls in final-minus-initial fluorescence values (P = 0.038).

The biological significance of these results is difficult to assess. In earlier studies, differences in fluorescence due to experimental treatment provided the first signs of plant stress but by the second growing season, few differences remained between controls and treatments, at least in the creek-edge and high-marsh zones (Crowell and Lane, 1988). Data from the present study are suggestive of lingering effects of oil+dispersant in all zones. If so, the effects probably result from physiological stresses associated with vegetation recovery (e.g. light and nutrient competition and competition-related thinning and successional processes) rather than residual toxicity.

Soil Chemistry

Of 72 soil cores examined for hydrocarbons, all but one were below the detection limit of 5 ppb. One creek-edge, oil-plot sample showed weak evidence (5 ppb) of hydrocarbons, these suggesting highly-aged and weathered oil.

Procedural Effects and Experimental Interaction with Natural Dynamics

Qualitative observations in 1990 suggested that a number of processes including trampling, ice-scour, deposition of sod and tidally-transported algal mats, and erosion, were interacting to affect the growth of vegetation in the experimental plots. The small number of replicates rendered the experiment sensitive to such confounding influences. In general, plots affected by such perturbations were easily identified; sampling was therefore conducted, as far as possible, in such a way as to avoid areas of the plot that had obviously been affected by 'demonic' intrusion. Trampling damage, however, was difficult to correct for in sampling because nearly all mid-marsh plots showed signs of it, and it was frequently so extensive as to affect the entire plot. Although care was taken during sampling to avoid stepping in the plots, damage to peripheral vegetation was sometimes sufficient to expose the entire plot to further erosion, ice scour, and pooling. Where Spartina cover was disturbed, Salicornia and Suaeda tended to colonize, a result similarly found by Bertness (1991) in a New England saltmarsh.

Table 2. Fluorescence induction response (relative units) of Spartina in salt marsh plots 4 years after treatment with oil and/or dispersant.

	Peak Fluorescence **Mean (SE)**	Peak minus Initial Fluorescence **Mean (SE)**	Final minus Initial Fluorescence **Mean (SE)**
Creek-edge			
Control (n=9)	165.6 (5.6)	27.3 (1.6)	9.9 (2.3)
Oil (n=9)	168.8 (4.2)	27.7 (1.2)	6.6 (0.9)
Dispersant (n=9)	174.1 (2.8)	26.6 (1.9)	6.9 (0.9)
Oil+Dispersant (n=9)	180.3 (4.7)**	31.6 (2.0)*	7.1 (0.7)
Mid-marsh			
Control (n=9)	146.7 (4.0)	27.3 (1.9)	7.1 (1.1)
Oil (n=9)	152.9 (9.3)	27.0 (2.7)	5.8 (1.5)
Dispersant (n=9)	162.1 (10.6)*	29.8 (2.7)	4.7 (0.8)
Oil+Dispersant (n=9)	132.2 (13.3)	22.9 (3.6)	3.4 (1.2)**
High-marsh			
Control (n=9)	88.9 (7.4)	15.8 (3.2)	2.0 (0.8)
Oil (n=9)	80.7 (4.6)	11.3 (1.5)	0.7 (0.4)
Dispersant (n=9)	77.9 (4.7)	10.6 (1.8)	0.6 (0.3)
Oil+Dispersant (n=9)	83.0 (7.0)	12.6 (2.2)	0.6 (0.4)**

* Difference from control significant at $0.1 > P > 0.05$
** Difference from control significant at $0.05 > P > 0.01$
*** Difference from control significant at $P < 0.01$

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Appendix A. Explanation of the Principles of Plant Fluorescence Induction Measurements.

Light absorbed by the chlorophyll-a reaction centre of photosystem II (the oxygen-evolving part of the photosynthetic system) excites ground state electrons in the chlorophyll molecule. An excited electron quickly (within approximately 10^{-10} milliseconds loses some of its excitation energy, which is dissipated as heat. This less excited, lower energy state may then jump to a primary electron acceptor, or it may return to its ground state. When it does the latter, its energy is dissipated as fluorescent light re-emitted from the molecule, or as heat. If the electron is passed to the primary electron acceptor, the lost electron is replaced by an electron taken from water (with concomitant release of free oxygen). The primary electron acceptor in the meantime loses the electron to an electron carrier which loses it to another carrier and so on through photosystem I, until finally an electron is passed to NADP. The electron-carrying NADP then moves out of the internal membranes of the chloroplast where chlorophyll and carriers are located, into the stroma where it participates in the reactions which convert carbon dioxide to sugar.

Because some of the excited electron energy is always lost as heat, and because there is an inverse relationship between the wavelength of light and its energy, the wavelength of light re-emitted as fluorescence is longer than that of the light that was absorbed. The fluorescence wavelength is characteristic of the molecule: for chlorophyll-a under physiological conditions, there is a main band maximum at 685 nm. To measure fluorescence, the sample is subjected to light of lower intensity, and light emission at 685 nm is monitored.

What is termed "variable fluorescence" can be thought of as an overflow phenomenon; when there is more light absorbed than can be immediately processed via the chemical route, part or most of the excited energy is dissipated as fluorescence.

When the plant is first exposed to light, the initial electron acceptor and the downstream carriers are empty handed and can readily receive electrons from chlorophyll. As they become saturated with electrons, their ability to process more electrons decreases, the chlorophyll reaction centre cannot pass its excited electrons, the excited electrons drop back to their unexcited ground state and fluorescence rises. It reaches a peak value within about three seconds of the light being turned on. During this period, the rest of the electron carrier system and the Calvin cycle become activated and begin to accept electrons from the upstream carriers and the primary electron acceptor. More electrons can be processed and therefore fluorescence begins to decline. Within about ten seconds, a steady state in the processing system is established, and there is a relatively low, steady level of fluorescence (Figure 3).

The pattern of fluorescence change to this point is called the fluorescence induction curve. Over longer periods (minutes), there may be another, slower increase in fluorescence and a new plateau in fluorescence reached as processes further downstream from the initial events become saturated and begin to regulate upstream flow.

Fluorescence induction is a sensitive probe for photosynthesis over very short periods, and responds to any changes affecting photosystem II activity. Thus, it has been very important in elucidation of photosynthetic mechanisms (Papageorgiou, 1975). More recently, it has been used in studies investigating the effects of various types of stress on plants. Many types of stress - water, heat, excess light, salt, chilling, freezing - result in changes in fluorescence induction characteristics long before there are changes in other measurable characteristics (Smilie and Hetherington, 1983). There appear to be no studies on stress to foliage that have indicated effects on other processes without there being effects on fluorescence induction.

Changes in fluorescence curves brought about by stress can be interpreted to provide presumptive evidence about the way in which the stress is affecting photosynthesis. For example, a reduced peak in fluorescence could indicate damage to the light receptor system; increased peak fluorescence could be due to damage on the carrier side of PS II close to the reaction centre; a slow decline from the peak could be due to damage further downstream.

Appendix B. Heights of Spartina shoots in saltmarsh plots two and four years after experimental treatment with oil and/or dispersant. "-" indicates no data.

	Spartina Height (cm)	
	1988 **Mean (SE)**	1990 **Mean (SE)**
Creek-edge (S. alterniflora)		
Control (n=120)	50.7 (1.3)	46.3 (1.9)
Oil (n=120)	52.8 (1.3)	41.8 (2.0;n=119)**
Dispersant (n=120)	60.9 (1.2)***	58.9 (0.8)***
Oil+Dispersant (n=120)	- -	56.1 (1.4)***
Mid-marsh (S. alterniflora)		
Control (n=120)	29.5 (0.8)	20.1 (0.9)
Oil (n=120)	21.9 (0.7)***	21.7 (0.9)
Dispersant (n=120)	13.3 (0.6)***	21.2 (0.9)
Oil+Dispersant (n=120)	- -	20.5 (0.8)
High-marsh (S. patens)		
Control (n=120)	34.3 (0.6)	36.9 (0.7)
Oil (n=120)	24.4 (0.7)***	34.6 (1.0)*
Dispersant (n=120)	30.4 (1.0)***	38.9 (0.8)
Oil+Dispersant (n=120)	- -	37.8 (1.1)

* Difference from control significant at $0.1 > P > 0.05$
** Difference from control significant at $0.05 > P > 0.01$
*** Difference from control significant at $P < 0.01$

Appendix C. Density of Spartina shoots in saltmarsh plots two and four years after experimental treatment with oil and/or dispersant. "-" indicates no data.

	Spartina Density (stems/m^2)	
	1988 **Mean (SE)**	1990 **Mean (SE)**
Creek-edge		
Control (n=36)	1080 (65)	700 (90)
Oil (n=36)	810 (65)***	590 (99)
Dispersant (n=36)	590 (43)***	450 (35)**
Oil+Dispersant (n=36)	- -	590 (38)
Mid-marsh		
Control (n=36)	3460 (575)	1430 (245)
Oil (n=36)	1930 (253)***	1100 (120)
Dispersant (n=36)	1690 (172)***	1510 (167)
Oil+Dispersant (n=36)	- -	1310 (266)
High-marsh		
Control (n=36)	9780 (537)	6330 (254)
Oil (n=36)	5830 (564)***	6920 (501)
Dispersant (n=36)	9190 (958)	6280 (375)
Oil+Dispersant (n=36)	- -	6580 (667)

* Difference from control significant at $0.1 > P > 0.05$
** Difference from control significant at $0.05 > P > 0.01$
*** Difference from control significant at $P < 0.01$

Appendix D. Biomass in saltmarsh plots two and four years after experimental treatment with oil and/or dispersant. "-" indicates no data.

	Total Reproductive Biomass (g/m^2)		Total Live Spartina Biomass (g/m^2)	
	1988 Mean (SE)	1990 Mean (SE)	1988 Mean (SE)	1990 Mean (SE)
Creek-edge				
Control (n=3)	2.6 (2.6)	23.1 (12.7)	303.3 (51.0)	618.9 (345.6)
Oil (n=3)	0.8 (0.8)**	2.9 (16.2)	438.9 (120.0)	178.9 (70.1)
Dispersant (n=3)	15.2 (3.6)**	76.6 (41.2)	600.0 (143.3)	1287.8 (504.4)*
Oil+Dispersant (n=3)	- -	67.0 (36.8)	- -	484.4 (110.6)
Mid-marsh				
Control (n=3)	0.3 (0.3)	3.6 (2.0)	284.4 (83.2)	128.9 (73.1)
Oil (n=3)	8.6 (5.8)*	2.3 (1.0)	160.0 (54.2)	170.0 (74.7)
Dispersant (n=3)	0.3 (0.3)	1.9 (0.8)	80.0 (30.3)*	178.9 (20.3)
Oil+Dispersant (n=3)	- -	3.1 (1.4)	- -	63.3 (25.2)
High-marsh				
Control (n=3)	3.0 (1.9)	23.3 (10.1)	535.6 (28.0)	565.6 (60.4)
Oil (n=3)	0.0 (0.0)	1.0 (0.3)	291.1 (76.6)	465.6 (104.1)
Dispersant (n=3)	0.8 (0.8)	5.6 (3.0)	271.1 (22.0)***	750.0 (234.4)
Oil+Dispersant (n=3)	- -	6.0 (3.3)*	- -	445.6 (258.9)

* Difference from control significant at $0.1 > P > 0.05$
** Difference from control significant at $0.05 > P > 0.01$
*** Difference from control significant at $P < 0.01$

Appendix D. (continued)

	Total Detrital Biomass (g/m^2)	
	1988 Mean (SE)	1990 Mean (SE)
Creek-edge		
Control (n=3)	226.7 (77.7)	305.6 (251.1)
Oil (n=3)	252.2 (99.1)	558.9 (133.3)
Dispersant (n=3)	247.8 (116.7)	258.9 (44.4)
Oil+Dispersant (n=3)	- -	208.9 (116.7)
Mid-marsh		
Control (n=3)	148.9 (71.3)	416.7 (45.9)
Oil (n=3)	90.0 (36.3)	282.2 (62.6)
Dispersant (n=3)	41.1 (10.9)	284.4 (57.2)
Oil+Dispersant (n=3)	- -	172.2 (49.2)**
High-marsh		
Control (n=3)	605.6 (133.3)	660.0 (85.4)
Oil (n=3)	175.6 (34.7)*	410.0 (136.7)
Dispersant (n=3)	275.6 (120.0)*	446.7 (140.0)
Oil+Dispersant (n=3)	- -	712.2 (246.7)

* Difference from control significant at $0.1 > P > 0.05$
** Difference from control significant at $0.05 > P > 0.01$
*** Difference from control significant at $P < 0.01$

This publication is printed on paper containing recovered waste.