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Report Number 070

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THE USE OF CHEMICAL DISPERSANTS IN SALT MARSHES

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

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Résumé

Nous avons exposé dans ce rapport les résultats d'une étude d'impact sur l'environnement et de l'efficacité de la dispersion du pétrole répandu dans un marais salant situé sur la côte Nord-Ouest de l'Atlantique.

Les impacts du pétrole, du dispersant et du mélange pétrole-dispersant, sur les plantes vasculaires, les communautés algues-bactéries, les propriétés des sédiments et les transformations chimiques du pétrole dans les sédiments ont été examinés. Des expériences ont été réalisées, en parallèle, sur le terrain et dans une serre. L'étude de terrain a eu lieu sur la plage Conrad de l'Anse de Petpeswick, Nouvelle-Ecosse ($44^{o}42'N$, $63^{o}11O$).

L'étude a été répétée dans des stations établies dans 3 zones à végétation distincte, dénommées zone du bord de l'anse, zone moyenne du marais et zone supérieure du marais.

L'herbe à liens, Spartina alterniflora était dominante dans la zone du bord de l'anse et la zone moyenne du marais alors que l'espèce voisine, S. patens, dominait dans la zone supérieure.

Dans les expériences en serre, des repliquats d'échantillons de chacune de ces zones de végétation ont été récoltés dans le marais salant et cultivés en serre. Dans les expériences de terrain et en serre, un plan d'échantillonnage stratifié du hasard a été utilisé. Des témoins et des échantillons traités par le pétrole, le dispersant et le mélange pétrole-dispersant ont été inclus dans ce plan.

Le pétrole et le dispersant ont été tous les deux répandus à l'aide d'un pulvérisateur portatif après que la végétation du marais ait été mouillée par l'eau de mer. Les effets expérimentaux ont été observés au niveau de la communauté et quand cela était possible, au niveau de l'espèce ou de la cellulle. Les hypothèses developpées pour les différents paramètres ont été testées par des analyses de variance à deux entrées et le test de rangs multiples de Student-Newman-Keuls.

Les paramètres physiques comprenaient la température du sol et les observations sur sa structure. Les résultats indiquent que dans toutes les zones de végétation, aucun des paramètres étudiés ne fut affecté par les différents traitements.

Les données concernant la chimie des sols étaient très variables, ce qui a rendu leur interprétation difficile. Cette variabilité a été attribuée à la distribution en tâches de la végétation et des détritus qui pourrait affecter la rétention du pétrole dans les sédiments. Les observations visuelles ont suggéré que l'application du dispersant n'éliminait qu'une faible fraction du pétrole de la vegétation. Les concentrations de pétrole dans le sol étaient comparables dans les quadrats et les microcosmes traités avec le pétrole et le mélange pétrole-dispersant, ce qui suggère que le dispersant affecte peu les mouvements du pétrole dans les sédiments.

Les paramètres étudiés pour les plantes vasculaires comprenaient: la taille des plantes, la densité de la tige, le recouvrement spécifique, l'anatomie des feuilles et comme variable physiologique: des mesures de la fluorescence. Les mesures de taille des plantes ont indiqué que le traitement par le pétrole avait relativement peu d'impact sur la taille moyenne dans toutes les zones de végétation. Seul le traitement par le dispersant a causé d'importantes réductions de la taille moyenne des plantes, suivi de près par l'effet du traitement par le mélange pétrole-dispersant. Les fluctuations rapides de la taille moyenne des plantes semblaient être dues à une mortalité plus élevée des petites plantes.

Les données de croissance provenant des expériences en serre, ont indiqué que la pétrole provoquait les plus faibles réductions de taille dans toutes les zones de végétation alors que le dispersant causait les réductions de tailles les plus fortes. Les taux de croissance des plantes traitées avec le mélange pétrole-dispersant étaient généralement plus élevés que les taux auxquels on s'attendait. Il est possible que les mares de pétrole entourant les tissus mérismatiques à la base des plantes aient protégé ces tissus du dispersant relativement plus toxique. Les taux de croissance des plantes traitées avec le mélange pétrole-dispersant semblaient redevenir normaux à la fin de la période de croissance, cependant, les grandes augmentations de la mortalité des plantes associées avec ce traitement vont à l'encontre de cet avantage.

La densité des tiges des plantes était le plus affectée par le traitement pétrole-dispersant et le moins affectée par le traitement avec le pétrole seul dans la zone moyenne du marais et du bord de l'anse. Les toxicités relatives du traitement avec le dispersant étaient différentes dans les expériences sur le terrain et en serre. Le dispersant était moins toxique dans les expériences de terrain qu'en serre. Dans la zone supérieure du marais, le pétrole a induit les plus larges réductions de la densite de la tige tandis que le traitement avec le dispersant a induit les plus faibles réductions. La zone moyenne du marais était extrêmement sensible à tous les traitements avec le pétrole et/ou le dispersant avec des réductions de

90% de la densité de la tige associées avec tous les traitements durant les expériences rélisées sur le terrain et en serre.

La production aérienne de biomasse était significativement réduite par les traitements avec le dispersant et le mélange pétrole-dispersant; cependant, les traitements avec le pétrole n'ont pas produit de réduction significative. Les traitements avec le dispersant et le mélange pétrole-dispersant avaient généralement des impacts similaires, cependant, le mélange pétrole-dispersant était légèrement moins toxique dans les expériences réalisées en serre que sur le terrain. Dans la zone moyenne du marais, tous les traitements ont provoqué des réductions significatives de la biomasse aérienne. Dans la zone supérieure du marais, tous les traitements avec le pétrole et/ou le dispersant avaient un impact identique et causaient une faible réduction de la biomasse aérienne. En somme, les traitements avec le pétrole et/ou le dispersant ont produit l'impact maximum dans la zone moyenne du marais et l'impact minimum dans la zone supérieure du marais.

La couverture de Spartina dans la zone du bord de l'anse et la zone moyenne du marais était le moins réduite par le traitement avec le pétrole et le plus réduite par le traitement avec le mélange pétrole-dispersant. Dans l'expérience en serre, les impacts dus au traitement avec le dispersant et le mélange pétrole-dispersant étaient équivalents, tandis que dans les expériences de terrain, le traitement avec le mélange pétrole-dispersant était nettement plus toxique. Ceci pourrait être lié à une interaction inhibitrice entre le pétrole et le dispersant apparue uniquement dans l'expérience réalisée en serre. Dans la zone supérieure du marais, tous les traitements semblaient avoir des impacts de même intensité et ont causé une faible réduction du taux de recouvrement de Spartina. La zone moyenne du marais était la plus sensible aux traitements tandis que la zone supérieure du marais l'était le moins. Salicornia europea était l'unique autre espèce trouvée régulièrement sur les stations étudiées. Son abondance ne fut pas affectée par les différents traitements.

L'examen microscopique des feuilles de Spartina a montré que les mêmes symptômes se sont développés pour tous les traitements avec le pétrole et/ou le dispersant. Le seul symptôme perceptible était la perte de chloroplastes avec le temps. Les taux auquels cette chlorose avait lieu, variaient avec les traitements, les taux les plus rapides étant associés soit avec le dispersant soit avec le mélange pétrole-dispersant et le taux le plus bas étant associé au traitement avec le pétrole.

Les résultats de la fluorométrie ont indiqué qu'une fluorescence variable était un bon prédicteur des réponses à long terme des plantes soumises aux

stress des différents traitements. Les valeurs prises à intervalles de 100 secondes sont un indicateur plus sensible du stress subi par les plantes que les différentes la variation du maximum de fluorescence. Les premières ont indiqué un stress dans la période d'application du traitement d'une journée alors que ces dernières n'ont varié que quand les plantes étaient sur le point de mourir. Le dispersant seul induisait le stress le plus intense dans toutes les zones de végétation. Le traitement avec le mélange pétrole-dispersant produisait généralement moins de stress que le traitement avec le dispersant. Le pétrole avait été mis en contact avec les plantes avant le dispersant et a peut-être agit comme une barrière réduisant l'entrée du dispersant dans les tissus des plantes. Les traitements avec le pétrole ont causé relativement peu de stress dans toutes les zones sauf dans la zone moyenne du marais. Cette zone était la plus sensible au traitement avec le pétrole et/ou le dispersant et la zone supérieure du marais était la moins sensible.

Les paramètres reliés aux algues ou aux bactéries comprenaient des estimations du degré de recouvrement d'algues ou de bactéries, des mesures de l'activité hétérotrophique et des taux de fixation d'azote. L'abondance des communautés dans le tapis d'algues, celle des bactéries hétérotrophiques et celle des bactéries roses photosynthétiques ont été temporairement réduites par les traitements avec le pétrole et le mélange pétrole-dispersant, mais n'étaient pas affectées ou étaient même stimulées par le traitement avec le dispersant. Les traitements avec le pétrole ont conduit éventuellement à l'augmentation de l'activité des bactéries hétérotrophiques, ce probablement dû surtout à la prolifération des espéces dégradant le pétrole, cependant d'autres espéces comme les bactéries oxydant le sulfure semblaient aussi avoir une réponse positive.

La fixation de l'azote n'était pas affectée par les traitements avec le pétrole et/ou le dispersant dans la zone du bord de l'anse et la zone moyenne du marais. Les apparences suggèrent que ce déclin était causé par du à des altérations du micro-habitat microbial plutôt qu'à une toxicité directe.

En général, les résultats de cette étude suggèrent que la zone de végétation du bord de l'anse et la zone supérieure du marais sont relativement tolérantes aux phénomènes de déversement de pétrole et peuvent probablement s'auto-épurer naturellement. la zone moyenne du marais est, cependant, très sensible, à la fois au dispersant et au pétrole; des mesures de nettoyage alternatives, comme un écoulement vers l'extérieur à faible débit, devrait être utilisées dans des régions du marais où une végétation de ce type domine.

Les résultats des études réalisées en serre et sur le terrain étaient généralement comparables. Les microcosmes des serres étaient faciles à entrenir et pas chers. Leur proximité du laboratoire a permi d'effectuer des observations plus fréquentes et plus détaillées que sur le terrain. Ces facteurs suggèrent que les microcosmes constituent une méthode efficace d'étude des effets du pétrole et des dispersants sur la végétation des marais salants et des communautés microbiennes. Les résultats de cete étude indiquent que la dispersion du pétrole déversé dans les marais salants, en utilisant la technique étudiée ici, n'est pas une mesure viable d'élimination des déversements de pétrole. On a montré que le dispersant (Corexit 9527) est innefficace pour éliminer le pétrole sur la végétation et qu'il est plus toxique pour les communautés de plantes vasculaires que le pétrole seul. D'autres types de dispersant ou d'autres techniques d'application pourraient produire de meilleurs résultats et devraient être recherchés.

SUMMARY

This report documents the results of a study of the environmental impacts and efficacy of dispersing stranded oil in a north-western Atlantic coast salt marsh. The impacts of oil, dispersant, and oil plus dispersant treatments on vascular plants, algal-bacterial communities, sediment properties, and the chemical fate of the oil in the sediment were examined. Parallel experiments were conducted in both the field and in a greenhouse. The field study was conducted at Conrods Beach on Petpeswick Inlet, Nova Scotia ($44^{o}42'N$, $63^{o}11'W$). Replicate study plots were established in three distinct vegetation zones termed creek edge, midmarsh and high marsh zones. Creek edge and midmarsh zones were dominated by the marsh cordgrass Spartina alterniflora while the high marsh zone was dominated by a closely related species S. patens. In the greenhouse experiment, replicate samples of each of these vegetation zones were removed from the salt marsh and cultivated in a greenhouse. In both field and greenhouse experiments a stratified random design was used. Control, oil, dispersant, and oil plus dispersant treatments were incorporated into the design. Both oil and dispersant were applied by back-pack sprayer following wetting of the salt marsh vegetation with seawater. Experimental effects were observed at the community level and where possible, at the species and cellular levels. Hypotheses developed for the various parameters were tested using two-way analysis of variance and the Student-Newman-Keuls multiple range test.

Physical parameters studied included soil temperature and structure observations. Results indicated that neither was affected by the various treatments in any of the vegetation zones.

Soil chemistry data were highly variable, making interpretation extremely difficult. This variability was attributable to the patchy distribution of vegetation and detritus which may intercept oil, and the variable structure of the sediments which may affect retention of oil in the sediments. Visual observations suggested that little oil was removed from the vegetation by the dispersant applications. Concentrations of oil in the soil were similar in oil and oil plus dispersant treated quadrats and microcosms suggesting that the dispersant had little effect on the movement of oil through the sediments.

Vascular plant parameters included plant height, stem density, species cover, leaf anatomy, and physiological variable fluorescence measurements. Plant height data indicated that the oil treatments had relatively little impact on average plant height in all vegetation zones. The dispersant only treatment caused the largest reductions in average plant height,

closely followed by the oil plus dispersant treatment. Rapid fluctuations in average plant height appeared to be caused by higher mortality among small plants.

Growth rate data from the greenhouse experiment indicated that oil was responsible for the smallest reductions in plant growth rates in all vegetation zones while the dispersant caused the largest reductions. Growth rates for oil plus dispersant treated plants were generally higher than expected. Pooling of oil around the meristematic tissues at the base of the plant may have protected these tissues from the relatively more toxic dispersant. Growth rates for oil plus dispersant treated plants appeared to recover late in the growing season, however, large increases in plant mortality associated with this treatment countered this benefit.

Plant stem density was affected most by the oil plus dispersant treatment and least by the oil treatment in the midmarsh, and creek edge zones. The relative toxicity of the dispersant treatment varied between the field and greenhouse experiments. Dispersant was less toxic in the field experiments than in the greenhouse experiments. In the high marsh, oil caused the largest reductions in stem density while the dispersant treatment caused the smallest reductions. The midmarsh zone was extremely sensitive to all oil and/or dispersant treatments with 90% reductions in stem density associated with all of the treatments in both the field and greenhouse experiments.

Above-ground biomass production was significantly reduced by the dispersant and oil plus dispersant treatments, however, oil treatments caused no significant reductions. The dispersant and oil plus dispersant treatments were generally equal in their impact, however, the oil plus dispersant treatment was somewhat less toxic in the greenhouse experiment than in the field experiment. In the midmarsh zone all treatments caused significant reductions in above-ground biomass. In the high marsh zone, all oil and/or dispersant treatments were approximately equal in their impact and caused relatively little reduction in above-ground biomass. Overall the oil and/or dispersant treatments had the most impact in the midmarsh zone and the least impact in the high marsh zone.

Spartina cover in the creek edge and midmarsh zones was reduced least by the oil treatment and most by the oil plus dispersant treatment. In the greenhouse experiment the impacts of the dispersant and the oil plus dispersant treatments were equivalent, while in the field experiment the oil plus dispersant treatment was clearly more toxic. This appeared to be related to an inhibitory interaction between oil and dispersant toxicity which appeared only in the greenhouse experiment. In the high marsh zone

all treatments were approximately equal in impact and caused little reduction in Spartina cover. The midmarsh zone was most susceptible to the treatments while the high marsh was least susceptable. Salicornia europaea was the only other species which occurred regularly in the study plots. Its abundance was not affected by the various treatments.

Microscopic examination of Spartina leaves indicated that the same symptoms developed in all the oil and/or dispersant treatments. The only noticeable symptom was the loss of chloroplasts over time. Treatments varied in the rates at which this chlorosis proceeded, with the fastest rates associated with either the dispersant or oil plus dispersant treatment and the slowest rate associated with the oil treatment.

Fluorometry results indicated that variable fluorescence was a good predictor of longer term response of plants to treatment induced stress. One hundred second difference values were a more sensitive indicator of plant stress than peak variable fluorescence. The former indicated stress within one day of treatment application while the latter indicated changes only when the plants were near death. Dispersant alone induced the most stress in all vegetation zones. The oil plus dispersant treatments generally induced less stress than the dispersant treatment. Oil was applied to the plants before the dispersant and may have acted as a barrier to entry of the dispersant into plant tissues. Oil treatments caused relatively little stress in all but the midmarsh zone. The midmarsh zone was most sensitive to the oil and/or dispersant treatments and the high marsh zone was least sensitive.

Algal and bacterial parameters included estimates of algal and bacterial cover, measurements of heterotrophic activity and nitrogen fixation rates. Algal mat communities, heterotrophic bacteria and pink photosynthetic bacteria were temporarily reduced in abundance by oil and oil plus dispersant treatments, but were unaffected or were stimulated by the dispersant treatment. Oil treatments eventually led to increased activity of heterotrophic bacteria, probably largely through the proliferation of oil degrading species, however, other species such as sulfur oxidizing bacteria also seemed to respond positively.

Nitrogen fixation was unaffected by the oil and/or dispersant treatments in the creek edge and high marsh zones, however, all treatments reduced nitrogen fixation in the midmarsh zone. Evidence suggests that this decline was caused by alterations of the microbial micro-habitat rather than by direct toxicity.

In general, results form this study suggest that the creek edge and high

marsh vegetation zones are relatively tolerant of single oil spill events and can probably be left to cleanse themselves naturally. The midmarsh zone, however, was very sensitive to both oil and dispersant and alternative clean up measures such as low pressure flushing ought to be utilized in areas of marshes dominated by vegetation of this type.

The results from the greenhouse and field studies were generally very similar. The greenhouse microcosms were inexpensive and easy to maintain. The close proximity of the microcosms to the laboratory allowed more frequent and more detailed observations than were possible in the field. These factors suggest that microcosms provide an efficient method of studying the effects of oil and dispersant on salt marsh vegetation and microbial communities.

The results of this study indicate that dispersion of stranded oil in salt marshes using the application technique studied here, is not a viable oil spill clean-up measure. The dispersant (Corexit 9527) appeared to be ineffective in removing the oil from the vegetation and proved to be more toxic to the vascular plant communities than the oil alone. Other dispersant formulations or application techniques might provide better results, and should be investigated.

INTRODUCTION

Salt marshes are one of the most valuable of coastal ecosystems. As intertidal ecosystems, they provide a high yield of plant material which may be transported by tidal action into coastal waters, where the detritus fuels marine food chains including those leading to commercial fisheries. Salt marshes also serve as nursery areas for larvae and other juveniles of many coastal species. These environments provide protection against coastal erosion. Salt marshes are low energy environments, subject to little turbulent mixing and as such are particularly vulnerable to oil pollution. Floating oil is readily trapped by salt marsh vegetation and sediments, and may remain in the marsh for long periods of time. This persistence is exacerbated by the anaerobic conditions of salt marsh sediments which slows the rate of biodegradation of the oil.

The vulnerability of salt marshes to oil pollution emphasizes the need to develop ecologically-sound oil spill clean up procedures which can be safely used in these ecosystems. Burning, mowing, plowing, and removal of contaminated sod have proven to be ineffective or destructive methods of cleansing oiled salt marshes (Vandermeulen and Jotcham, 1986). Chemical dispersants may provide an alternative method.

Relatively few studies have investigated the effects of dispersed oil on the vegetation of salt marsh ecosystems. Delaune et al (1984) conducted a major study of the effects of oil and dispersed oil on a salt marsh in Louisiana. In their study, dispersants were applied three ways: mixed with water, after oiling of plots; mixed with water and applied alone; and applied to foliage prior to oiling. The oil applications did not reduce Spartina alterniflora productivity in the marsh nor did the dispersant applications. Oil concentration in the sediments was not significantly reduced by the dispersant treatments. A continuation of this study (Smith et al, 1984) demonstrated that there was no long term inhibitory or stimulatory effects of the oil or oil plus dispersant on the growth of Spartina alterniflora.

Baker et al. (1984), reported on a major study of oil and dispersants for a variety of intertidal habitats. Treatments consisted of oil, dispersant, oil plus dispersant, and pre-mixed oil plus dispersant. The dispersants used were BP1100WD, BP1100X, Corexit 8667, and Corexit 7664. Results from

the salt marsh study sites demonstrated a one to two year reduction in the stem density of Spartina anglica associated with both the oil and dispersed oil. Short term loss of Salicornia europaea was also found with both treatments, with recovery after two years. Heavier oils were found to be more toxic than lighter ones, probably killing plants by direct smothering.

Traditionally, the use of chemical dispersants has not been favoured as a countermeasure technique for oil spills in saltmarshes because of concerns that the oil/dispersant mixture or the chemical dispersant alone might be harmful to marsh biota, and that the chemical dispersant would enhance the penetration of the oil into the fine sediments of the marsh (Baker 1975) where it would remain for many years. Recent experiences, however, have suggested that the use of chemical dispersants in saltmarshes may present certain advantages in accelerating their recovery. Experience with oiling of fine to very fine (silt to mud) sediments, such as are found in typical saltmarsh systems, have shown that crude oils and other similarly viscous products treated with dispersants do not readily penetrate into the fine sediments (Vandermeulen 1981). Similar studies with dispersed oil over coarser sediments, in 3.5 m water, have shown little incorporation of such dispersed oil into sediments (Page et al. 1983). Of more direct interest are studies of the biological recovery of oiled marshes that were treated with dispersant. Unpublished reports of follow-up visits to one such oiled marsh (first visited and studied by Vandermeulen and Ross in 1977) indicated that the vegetation of the marsh recovered to normal levels within five years of the spill.

Based on these results, the use of dispersant to clean up oiled saltmarshes may be environmentally advantageous and merits investigation. Considering the virtual absence of acceptable alternatives for dealing with the problem, attempts to expand our understanding in this area can only improve our capability to clean up and protect these very sensitive coastal ecosystems.

This study investigated the impacts of oil dispersion on the macrophytes, microphytes and heterotrophs of a salt marsh ecosystem in Nova Scotia. In addition the chemical fate of the oil and the influence of oil and/or dispersant treatments on physical properties of the sediments were determined. Where possible, experiments were duplicated in both the laboratory where environmental control was possible and in the field where conditions were most realistic.

In order to determine ecosystem level impacts of the oil and/or dispersant treatments this study was conducted at different levels of biological organization. Treatment impacts were investigated at the community level and where possible, at the species, and cellular levels to establish a fully integrated and comprehensive approach to evaluating the biological effects of the treatments.

MATERIALS AND METHODS

FIELD EXPERIMENT

Description of Study Site

The field study site was located at Conrods Beach on Petpeswick Inlet ($44^{o}42'N$, $63^{o}11'W$) (Figure 1). The Conrods Beach salt marsh has developed behind a barrier dune system, and is drained by a single channel which penetrates the dune system. The vegetation of the salt marsh is divisible into three distinct zones, which will be referred to as creek edge, midmarsh, and high marsh zones.

The creek edge zone was dominated by a lush growth of Spartina alterniflora. Small quantities of Salicornia europaea, Plantago juncoides, Suaeda maritima, and Atriplex patula were also present in this zone. The creek edge zone was usually restricted to within two metres of drainage channels in the marsh. The lush growth of Spartina alterniflora was attributable to the relatively well drained and well aerated sediments of this zone.

The midmarsh zone was dominated by a short ecotype of Spartina alterniflora, with lower abundances of Spartina patens, Salicornia europaea, Suaeda martima, Triglochin elata, Limonium nashii, and Plantago juncoides. This was the most extensive of the three zones in the Conrods Beach salt marsh and was generally found in poorly drained areas in the marsh interior. The stunted growth of Spartina alterniflora in this zone was attributable to the poor aeration of the sediments in these areas.

Spartina patens was the dominant species of the high marsh zone. Small quantities of Spartina alterniflora, Salicornia europaea, Triglochin elata, Suaeda maritima, and Glaux maritima were also found in association with S. patens. The high marsh zone was located on slightly elevated, better drained and aerated soils of the marsh.

Experimental Design

Twelve, 0.5 m x 4.0 m ($2 \cdot m^{-2}$) plots were established at random locations in the marsh in each of the three vegetation zones, and four treatments (control, oil, dispersant, and oil + dispersant) were randomly assigned to each zone (Figure 2). 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 other plots. Nondestructive sampling was conducted within

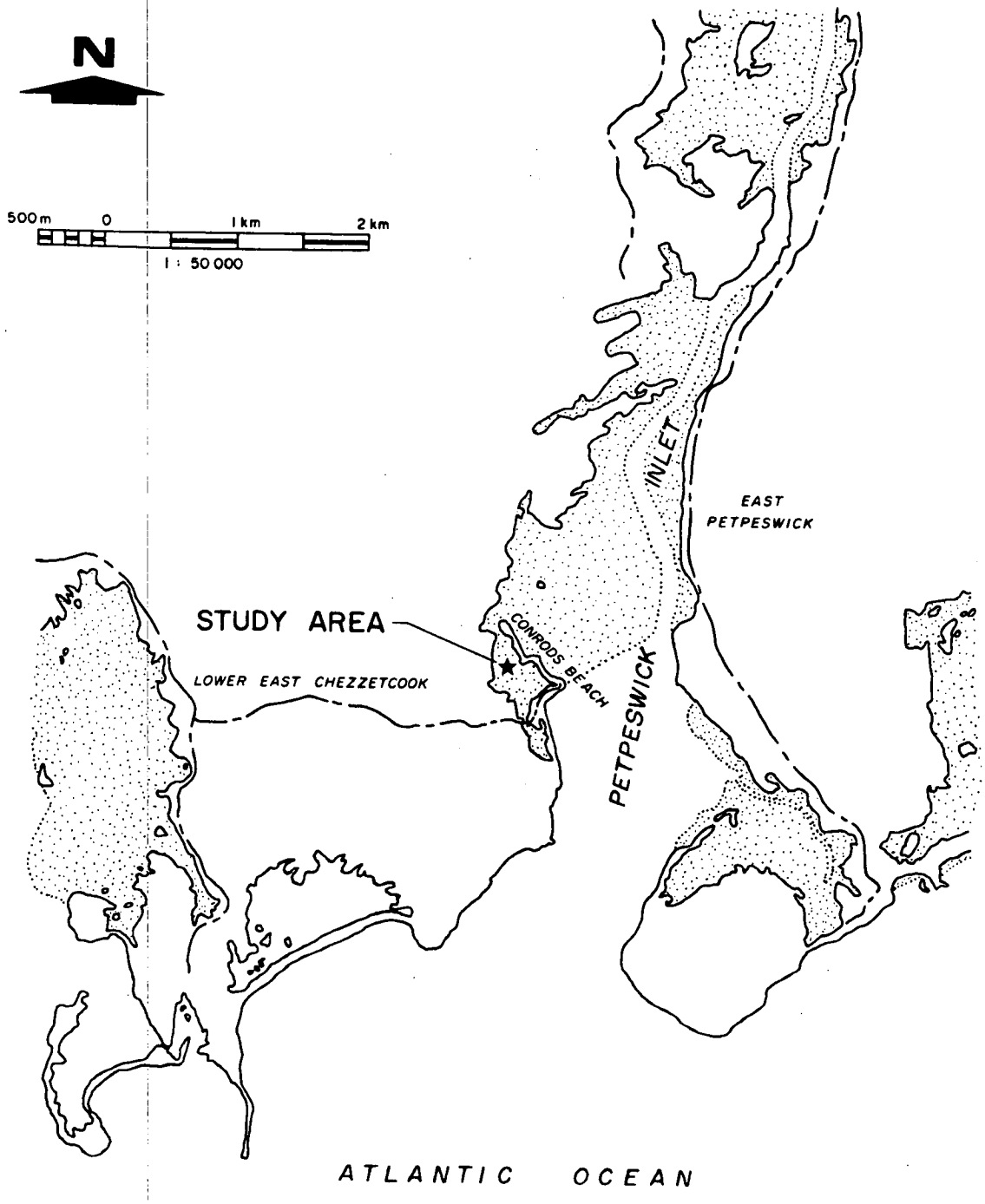

Figure 1: Map showing the location of Petpeswick Inlet and the Conrods Beach salt marsh.

the interior of the plots while destructive sampling was done in a $0.25 \cdot m^{-2}$ strip surrounding each plot which received the same treatment as the plot interior. Each quadrat was surrounded by a wall of oil sorbent material to prevent the escape of oil from the quadrat. Quadrats were also covered with garden netting to exclude wildlife. Field measurements commenced on May 9 (day 1) and treatments were applied on July 7 (day 60).

Oil and Dispersant Applications

Alberta sweet blend crude oil, weathered by evaporation and stirring for 24 hours to 15-25% loss by weight, was used for all experiments. In the field experiments, 2.25 liters of weathered oil per plot were applied from a back-pack sprayer approximately two hours after the onset of the ebb period yielding a nominal 0.5 mm oil slick. The tide did not rise high enough to cover the marsh surface with water on the day of treatment applications, therefore, each plot was sprayed with seawater prior to treatment. 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 the dispersant treatment, 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 liters of the dispersant:seawater mixture was applied to each plot from a back-pack 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.

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

Physical and Chemical Parameters

Tidal Analysis - Tidal data were collected over a two week period in order to determine the relative degree of submergence of the quadrats and the elevations of the vegetation groups. An Aanderaa water level recorder (WLR-5) was installed in the main channel, near the center of the test site area (Figure 2). This tide gauge measured the pressure at 5 minute intervals from August 8 to 22. Pressure values were converted into depth values by correcting for atmospheric pressure (pressure data obtained at Shearwater airport, meteorology center of Environment Canada). Water density was approximated at 1.025 g/cc.

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

The elevations of the plots relative to the water level recorder were determined using a theodolite. The elevations of the ends and middle portion of each plot were measured and averaged to give the mean elevation for each plot. The level of the water in the tidal creek was measured at regular intervals for comparison with the results of the water level recorder.

Soil Profile Descriptions - Soil profile descriptions were recorded for all cores taken for chemical analysis. On each sampling date, in each vegetation zone, three cores were taken from each of the oil and oil plus dispersant plots and a single core was taken from each of the control and dispersant plots. At each sampling site, a core 6 cm wide by 20 cm long was removed with a modified bulb planter. Each core was split longitudinally and the thicknesses of distinctive sediment layers and presence of oxidizing and reducing zones were recorded on a standard core diagram. The presence of bacterial zones, algal mat communities, the colour of roots, and the degree of root mat development were also recorded for each core. Cores were taken on five dates between early July and early September.

Soil Temperature - Soil temperature was taken at 5 cm depth in the center of each 2 m^2 plot on 3 dates during the growing season, using a Fisher Accumet Model 640A mini pH and temperature meter. Mean soil temperature values were calculated for each treatment in each vegetation zone on each sampling date.

Soil Chemistry - On 5 dates, using the cores collected above for soil profile descriptions, a 50 g sample was removed from each core and was extracted 3 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 sulfate, copper and activated florisil. Total oil and grease values were determined with UV fluorescence analysis, scanning from 200 to 300 nm. Chrysene equivalents per dry weight (UV fluorescence at 256 mm) were converted to ppm of oil/dry weight of sediment using fresh and weathered oil samples as standards.

Selected sample extracts were analysed by glass capillary gas chromatography (GC^2) using a flame ionization detector (F.I.D.). Relative concentrations of the n-chain hydrocarbon components were determined from the chromatograms. Identifications of the peaks were made using glass capillary gas chromatography mass spectrometry (GC^2MS). Procedures followed those of Geiger and Schaffner (1978).

Vascular Plant Parameters

Plant Height - Within each plot, 40 Spartina shoots were systematically selected at 10 cm intervals and their heights were measured. Plant height was measured as the distance from the tallest point of the plant to ground level. Measurements were taken on eight occassions during the growing season.

Stem Density - Within each plot, 12 miniquadrats were systematically positioned at 30 cm intervals. In Spartina alterniflora dominated marsh zones (creek edge and midmarsh zones), $0.01 \cdot m^{-2}$ quadrats were used while in the S. patens dominated zone (high marsh zone), $0.006 \cdot m^{-2}$ quadrats were used. All shoots in each quadrat and all flowering shoots in each plot were counted and means and standard deviations were calculated for each treatment in each vegetation zone on each sampling date. Measurements were taken on four occassions during the growing season.

Biomass - Biomass harvesting was conducted in the first week of September. A $0.09 \cdot m^{-2}$ quadrat was systematically positioned in one end of each plot and all aboveground living biomass in the quadrat was harvested, sorted by species, dried at 80° C in a convection oven and weighed on an analytical balance. Detrital biomass was harvested, dried and weighed but was not sorted by species. All flowering stems in each plot were harvested. Reproductive portions were removed from vegetative portions and weighed separately. Mean above-ground standing crops for living plants, detritus and reproductive tissues were calculated for each treatment in each vegetation zone.

Species Cover - Percent cover for each species of vascular plant was estimated in each plot. Cover values were estimated relative to soil surface area rather than to other species in the quadrat; therefore, total cover could be higher than 100% when species overlapped. Mean cover values were calculated for each species in each treatment in all vegetation zones for 3 sampling dates.

Leaf Anatomy - Two leaves were randomly selected from each plot. These leaves were pooled by treatment within each zone and three were randomly chosen from each treatment for observation. Radial cross-sections were taken from areas on each leaf exhibiting different symptoms of the treatments, and were examined under a microscope at 200x. Observations included the abundance, relative sizes, and coloration of chloroplasts, measurements of cuticle thickness and the presence or absence of plasmolysis.

Microbial Populations

Algal and Bacterial Mat Cover - Color characteristics were used to describe the surface distributions of algal mats (green), sulfate-reducing bacteria (black) sulfur-oxidizing bacteria (white) and photosynthetic bacteria (pink). Percent cover values of algal mat, sulfate-reducing bacteria, sulfur oxidizing bacteria and photosynthetic bacteria were estimated in each plot. Mean cover values were calculated for all categories for each treatment in all vegetation zones on two sampling dates.

GREENHOUSE EXPERIMENT

Description of the Greenhouse

The greenhouse experiment was conducted in the Dalhousie University greenhouse which is equipped with running seawater. Freshwater was supplied by an overhead sprinkler system. Air temperature was maintained above 10°C at all times but was not held at a constant level.

Experimental Design

Eighty-four experimental marsh "plugs" (33 cm diameter x 20 cm depth; 28 per marsh zone) were removed from the Conrods Beach salt marsh and transported to the greenhouse where they were maintained in plastic buckets fitted with flushing ports (Figure 3). Creek edge plugs (microcosms) were flooded with seawater daily and after standing two hours, the seawater was allowed to drain. Midmarsh plugs (microcosms) were flooded daily, and after standing for two hours, were allowed to drain to the sediment surface only, simulating the poorly drained condition of the midmarsh zone. High marsh samples were flooded in the same manner, but for only three consecutive days per two week period, and were allowed to drain completely through top and bottom drainage ports. All microcosms were briefly sprinkled daily with fresh water to simulate rainfall in the field. Laboratory measurements commenced on May 9 (day 1) and the randomly assigned treatments were applied on June 20 (Day 43).

Oil and Dispersant Applications

Oil and dispersant application procedures for the greenhouse study were similar to those used in the field except that the amounts of oil and dispersant were scaled down. The 0.5 mm nominal oil slick was simulated by the application of 400 ml of weathered crude oil per bucket. Four hundred ml of 10% (V:V) dispersant/seawater solution was applied for the dispersant treatments. Microcosms receiving the oil plus dispersant treatment were

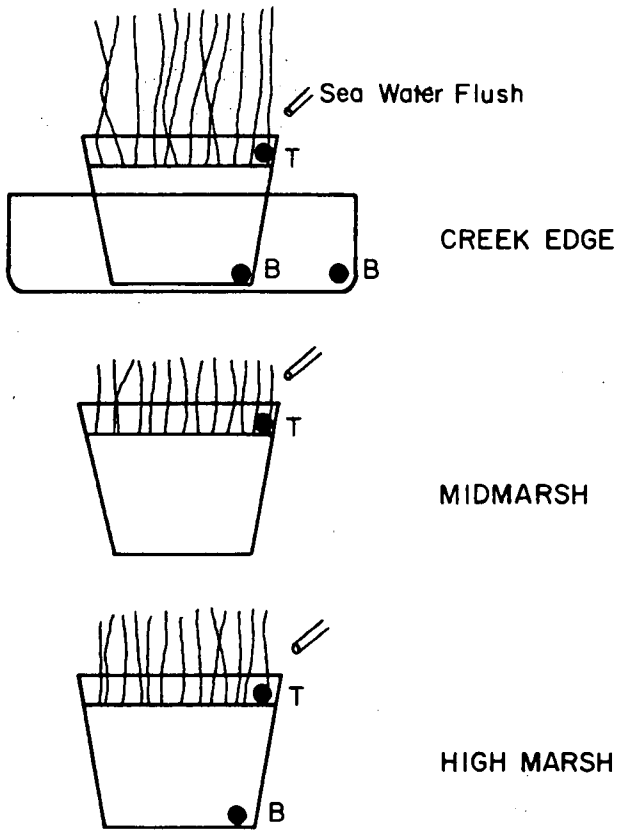

Figure 3: Systems used for maintenance of experimental salt marsh microcosms. Drainage ports are indicated, where present, at top (T) and bottom (B).

first sprayed with 400 ml of weathered crude oil per bucket, followed six hours later by 400 ml of the 10% dispersant/sea water solution.

Physical and Chemical Parameters

Soil Profile - Methods were identical to those used in the field experiment except only one core per treatment was taken on each sampling date.

Soil Chemistry - Analytical techniques for greenhouse soil samples were identical to those used in the field study. The only difference was the number of samples taken per sampling date. In the laboratory study, only one sample was taken for each treatment in each vegetation zone.

Vascular Plant Parameters

Plant Height - In each microcosm, 10 plants were systematically selected at 3 cm intervals along a central transect. These plants were tagged and height measurements were made at regular intervals through the growing season as per the field methods. In instances where a tagged plant had died, its tag was shifted to the nearest plant. Measurements were taken on 9 dates through the growing season. Mean plant height was calculated for each treatment, in each vegetation zone on each sampling date. Growth rates were calculated by subtracting the height of a plant on a particular date by its height on the previous sampling date, then dividing by the number of days elapsed between sampling dates. Growth rates were calculated for the 10 original tagged plants for the interval between each sampling date from day 35 to day 117 for all treatments in all vegetation zones.

Stem Density - A single quadrat was positioned in the center of each microcosm. A $0.01 \cdot m^{-2}$ quadrat was used in the creek edge and midmarsh microcosms while a $0.006 \cdot m^{-2}$ quadrat was used in the high marsh microcosms. All shoots within each quadrat and all flowering shoots in each microcosm were counted. Measurements were taken on six dates. Mean stem density was calculated for each treatment in each vegetation zone on each sampling date.

Biomass - Biomass harvesting was conducted in the first week of September. All above-ground biomass in each microcosm was harvested, sorted by species, dried at $80^{o}C$ in a convection oven and weighed on an analytical balance. Detrital biomass was harvested in only the creek edge and high marsh microcosms. All flowering stems in each microcosm were harvested. Reproductive portions were removed from vegetative portions and were weighed separately. Mean above-ground standing crops for living plants,

detritus, and reproductive tissues were calculated for each treatment in each vegetation zone.

Species Cover - The methods followed those for the field experiment except that cover estimates were made on two occassions.

Leaf Anatomy - Methods followed those for the field experiment.

Fluorometry - An explanation of the principle of plant fluorescence induction measurements is presented in Appendix 1. Two plants were randomly selected from each microcosm for fluorometric analysis. Oiled leaves were rejected if selected because of possible interference of light transmission or fluorescence by the oil film. The upper-most fully expanded leaf was removed. All of the leaves from a particular vegetation zone were placed on a pad of water soaked absorbent paper and were covered with a layer of opaque plastic. These leaves were allowed to equilibrate in the dark for one hour. Working illumination was provided by a 6 volt lantern with its lens covered with a green filter. Fluorometric measurements were made with a Brancker Research Ltd. (Ottawa) model SF-20 portable fluorometer. The fluorescence induction curve was monitored for 10 seconds for one of the leaves in each pair while the second was monitored for 100 seconds. Initial, peak, transient peak, and final readings were recorded (Figure 4). Values used in analysis included the peak variable fluorescence (peak reading minus initial reading) and the 100 second difference value (value at 100 seconds minus initial value).

Microbial Population Parameters

Algal and Bacterial Mat Cover - Color characteristics were used to describe the surface distributions of algal mat (green), sulfate-reducing bacteria (black), sulfur-oxidizing bacteria (white) and photosynthetic bacteria (pink). Percent cover values of algal mat, sulfate-reducing bacteria, sulfur oxidizing bacteria and photosynthetic bacteria were estimated in each microcosm. Mean cover values were calculated for all categories for each treatment in the three vegetation zones on two sampling dates.

Heterotrophic Activity - A sample of salt marsh sod (30 cm diameter, 30 cm deep), was taken from the midmarsh vegetation zone. The above-ground vegetation was removed and the sod was cut into pieces such that no root fragments were longer than 2 cm. This material was thoroughly mixed and twenty, 500 g samples were placed in 1 liter mason jars along with 200 ml of seawater. Samples designated for oil or oil plus dispersant treatments had 12.25 ml of Alberta sweet blend crude oil poured onto the surface of the seawater in the mason jars. The seawater was then removed by siphon to

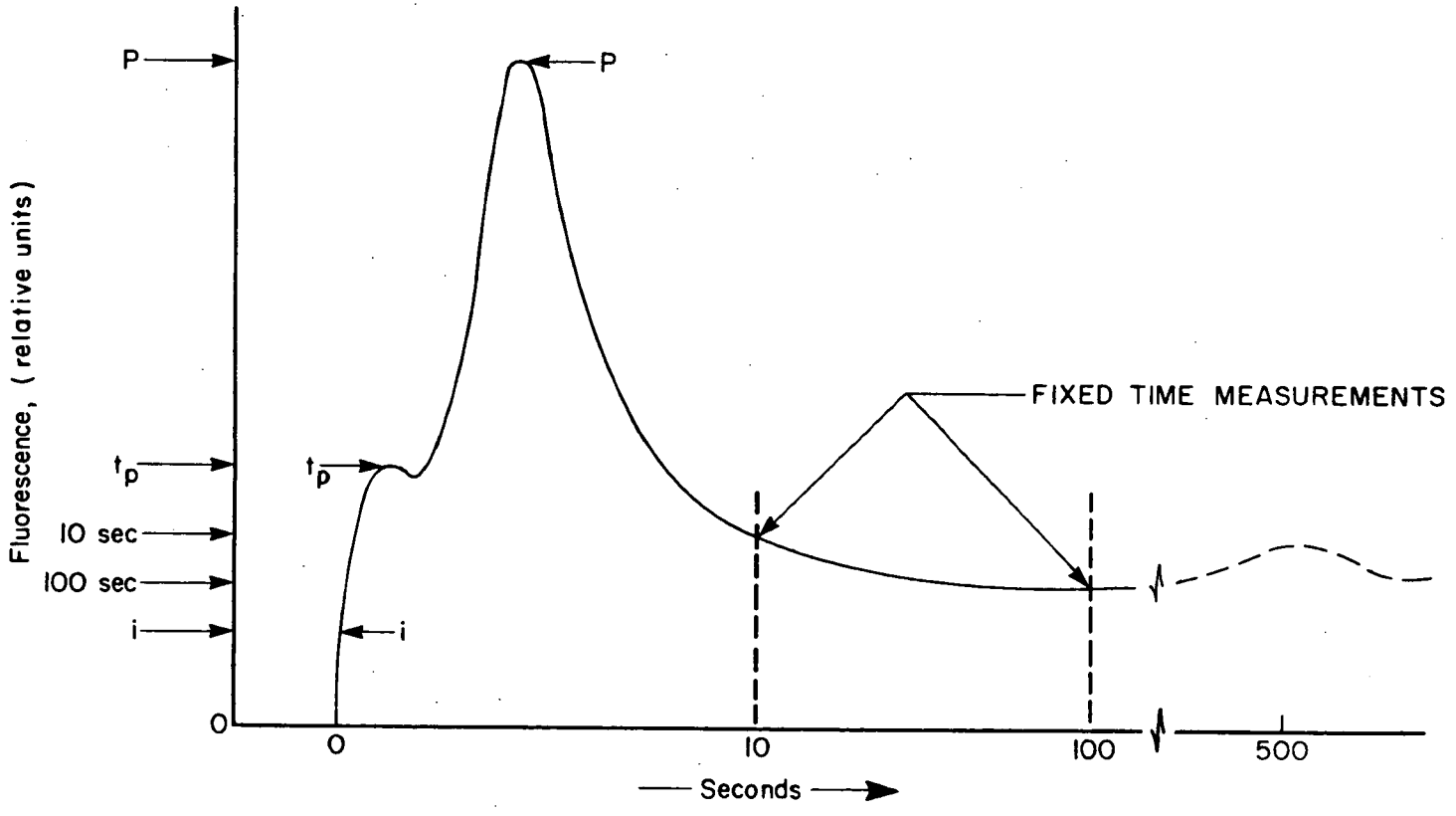

Figure 4: Diagramatic representation of the fluorescence induction curve for Spartina alterniflora. Arrows indicate recorded fluorescence values. The initial value (i) is the "fixed fluorescence". Fluorescence above this level is "variable fluorescence" which is sensitive to the physiological status of the photosynthetic system. We refer to (P-i) as "peak fluorescence" and 100 second values minus initial values as the "100 second difference values". +p refers to a transient peak.

allow the oil to settle on the sediment surfaces as evenly as possible. Dispersant and oil plus dispersant treated samples had 12.25 ml of a 10% solution of corexit 9527 and seawater sprayed onto the sediment surface with a plant mister. For the oil plus dispersant treatment, the application of the dispersant solution was delayed for 6 hrs to simulate the procedures used in the field. Heterotrophic activity (carbon dioxide production) was monitored 1 and 8 days prior to treatment applications and 4, 7, 13, 26 and 35 days after application. Seawater in the mason jars was replaced each week. Prior to analysis, the bottles were sealed with serum stoppers. Samples were taken at one and four hour intervals and analysed for carbon dioxide using an infra-red gas analyser.

Nitrogenase Activity and Composition of the Mud Surface Microbial Community - The procedures used for measuring soil surface nitrogenase activity (nitrogen fixation) by the acetyelene reduction technique follow those of Patriquin and McClung (1978). Three plugs taken from each bucket with a sharpened cork borer of 0.5 mm diameter to a depth of 1 cm were extruded into a 50 ml flask. Two milliliters of seawater were added, the flasks were closed with serum stoppers, and 3 ml acetylene was added. Flasks were incubated under 6000 lux for 12 hours, and then in the dark for a further 12 hours. Gas samples for analysis of ethylene were taken at 12 and 24 hours. Analysis of nitrogenase activity was conducted on four dates.

Forceps were used to remove a small sample of the sediment surface from a random location in each of the buckets from which cores had been removed. Samples from each vegetation zone were pooled by treatment, thoroughly mixed and then examined under a microscope. The microorganisms observed were classified on the basis of morphological features and the relative abundance of each group (rare, uncommon, common and abundant), was recorded. One sample from each treatment in each zone was examined immediately following each assay of nitrogenase activity.

Statistical Analyses

Statistical analyses were conducted on plant height, stem density, total above-ground biomass, reproductive biomass, Spartina cover, fluorometry, algal cover, heterotrophic activity, and nitrogenase activity data in both the field and greenhouse experiments. Prior to analysis the data were tested for normality and homoscedasticity. In instances where these conditions were not met, the data were transformed. Data analysis was conducted using the SPSS statistical package (Nie et al. 1975).

The first step of data anlaysis consisted of a preliminary analysis of variance which treated replicate as a factor. This analysis consisted of a

three-way analysis of variance with blocked design and no replication. The purpose of this analysis was to verify that true replication had been achieved by testing whether replicate as a factor had a significant effect.

The second step in the statistical analysis was the use of one-way analysis of variance to test for treatment effects among the four levels of the treatment factor (control, oil, dispersant, and oil plus dispersant). In instances where the means of the four levels were significantly different, the Student-Newman-Keuls multiple range test was used to determine which pairs of treatments were significantly different.

Finally, the data were subjected to a two-way factorial analysis of variance, the results of which indicated which factors (oil and dispersant) had significant effects and whether there was any interaction between these factors.

RESULTS AND DISCUSSION

PHYSICAL AND CHEMICAL EFFECTS

Field Experiment

Tidal Analysis - A comparison of the tidal readings recorded by water level recorder and theodolite are presented in Figure 5. The good correlation between the two methods (r=0.98) indicates that the data from both methods are compatible, therefore, comparisons of tidal height and plot elevation are valid.

Figure 6 shows the cumulative frequency distribution of immersion time at different elevations. The mean elevations of the plots in the three vegetation zones are located on the diagram. As shown on Figure 6, test sites located in the creek edge zone were covered with water 10 to 25% of the time. Test sites located in the high marsh zone were covered with water less than 15% of the time, and, in some cases, less than 5% of the time, during the two week period of observations. The midmarsh zone had the narrowest range of elevations and was immersed between 5 and 12% of the time. The results indicated that boundaries between the three vegetation zones were not related to submergence periods, since the three types of vegetation were found at the same levels in the marsh. All sampling sites were located in a narrow band of elevations ranging from 67 to 96 cm above the tide gauge pressure sensor. The highest level of the tide was 113 cm which was 17 cm above the level of the highest quadrat and 46 cm above th lowest quadrat. In both cases, most of the mature vegetation was partly exposed to air during the highest tide.

To illustrate this situation, data from Figure 6 was plotted in Figure 7, with elevation data grouped according to a semi-diurnal tidal cycle. The frequency of submergence periods represent the number of tidal cycles during which the quadrats were submerged instead of the amount of time that they were submerged. According to this figure, the plots were inundated by the rising tide during 35 to 95% of the tidal cycles. If oil was present in the water, those sites would likely be in contact with oil. This confirms that the selection of test sites was representative of an area highly susceptible to oil spills.

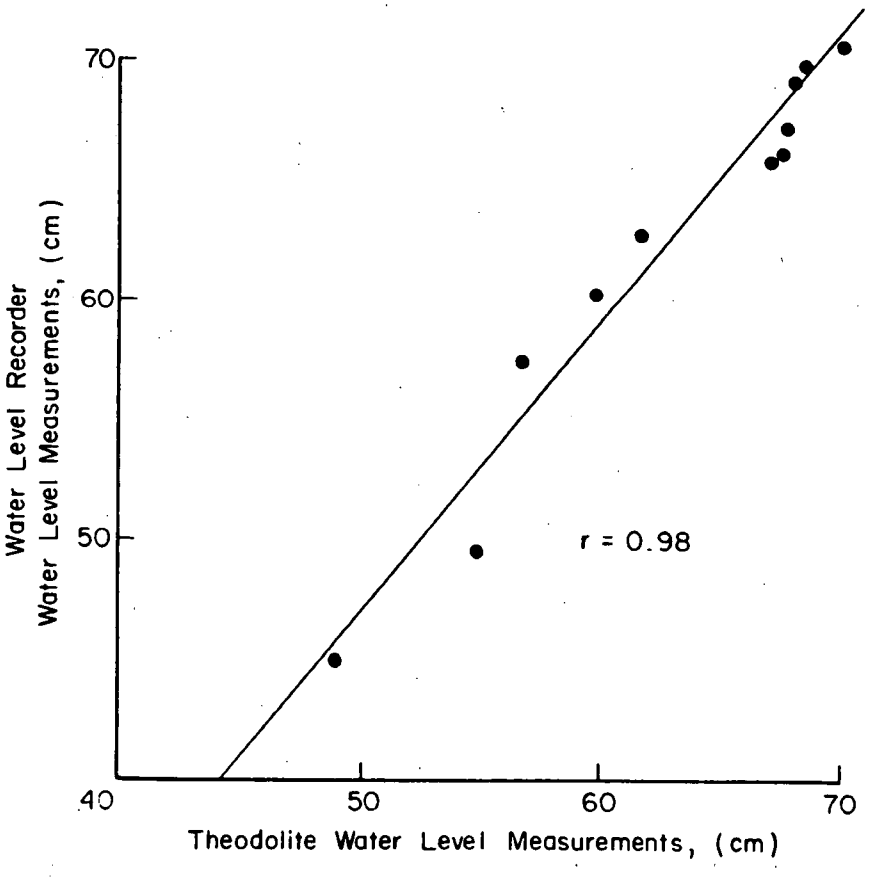

Figure 5: Comparison of theodolite and water level recorder measurements of tide height.

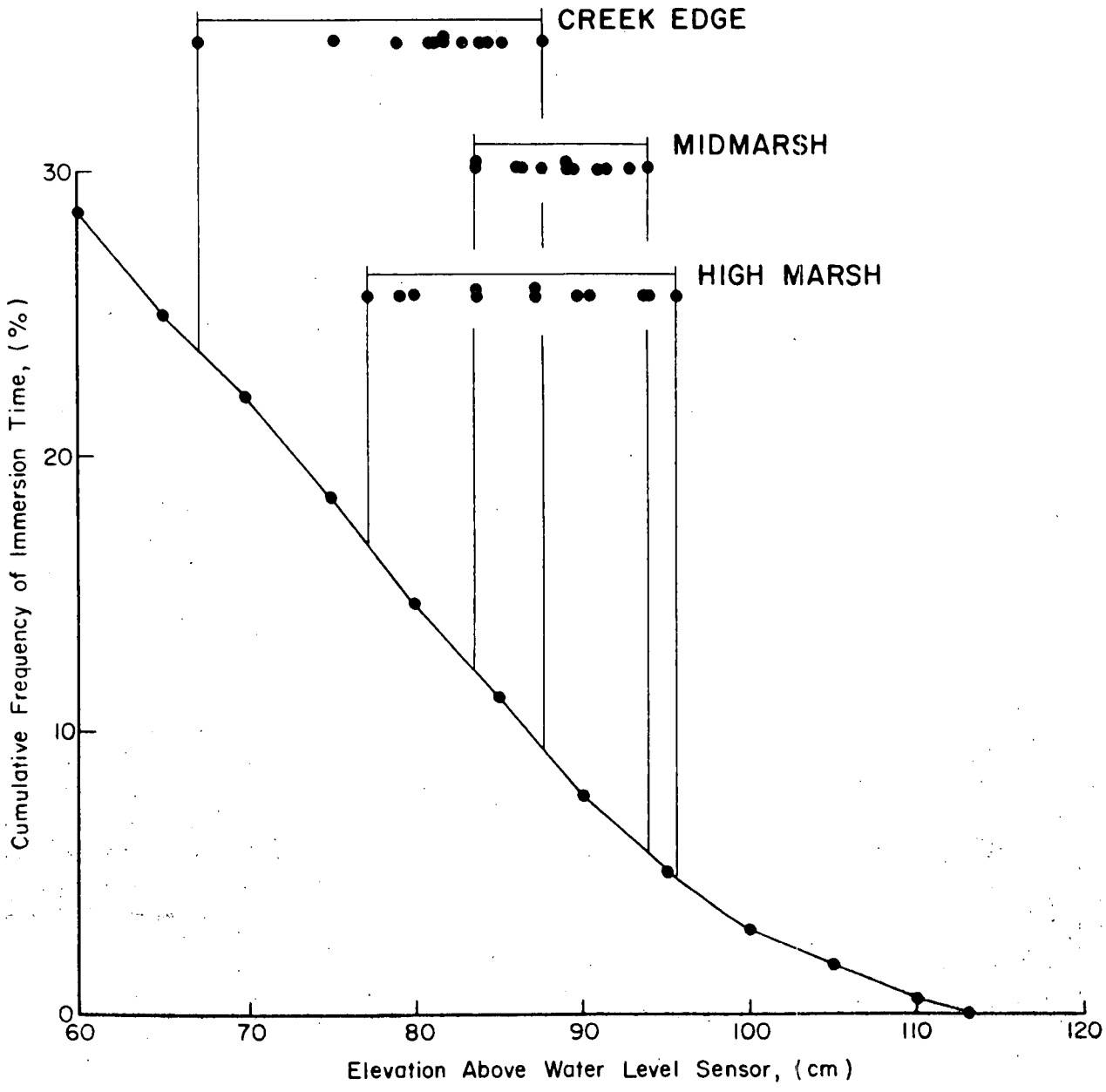

Figure 6: Cumulative frequency of immersion time versus elevation.

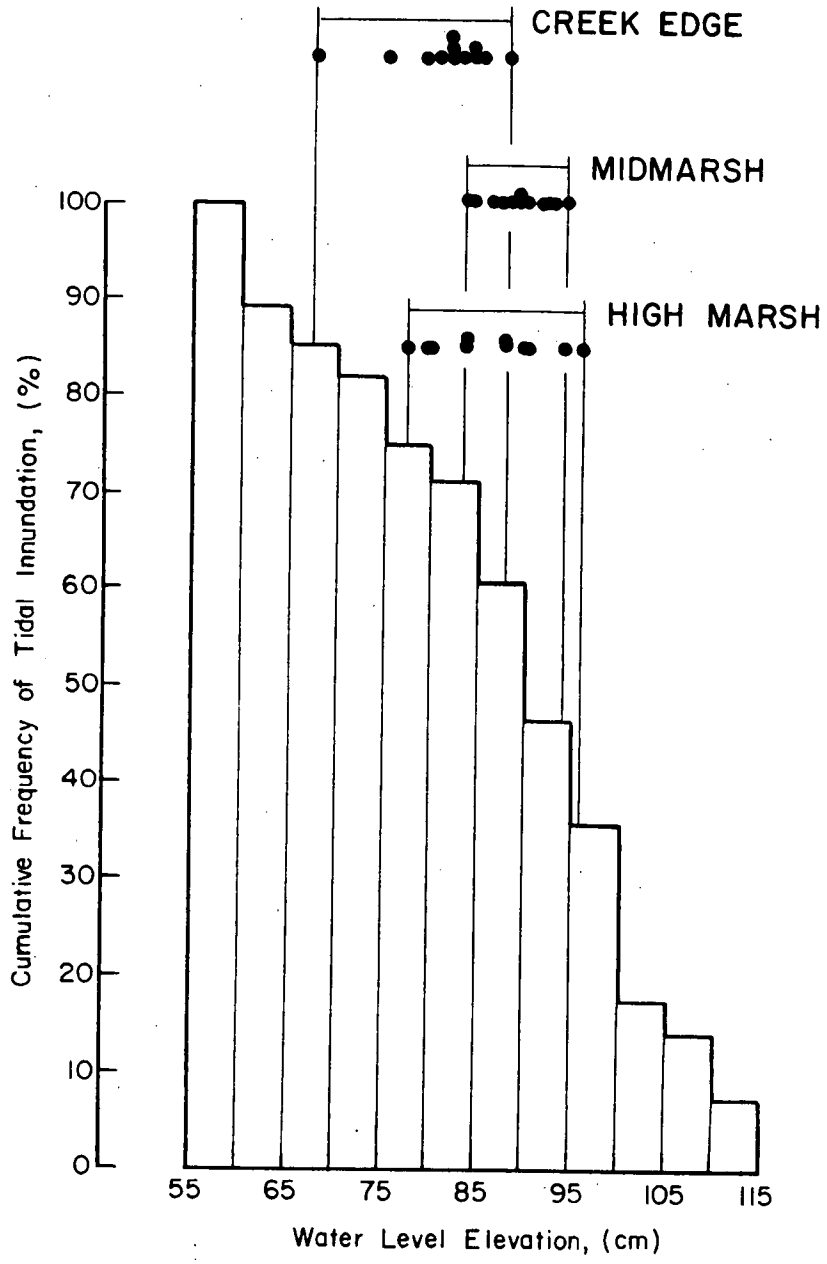

Figure 7: Histogram illustrating the proportion of tidal cycles during which tidal waters reached or surpassed specified elevations.

Soil Profile Descriptions - The oil and/or dispersant treatments could alter the appearance of the sediments by changing the patterns of soil aeration. The death of the vegetation would disrupt plant-mediated soil aeration (via lacunae) and the deposition of a layer of low viscosity weathered oil over the sediment surface could create a barrier to diffusion of oxygen into the sediments. Decreased soil aeration could result in the development or expansion of reducing zones (black in colour) and changes in root coloration (they are black if the roots are not aerating the rhizosphere and brown or white if they are aerating the rhizosphere). Disruption of soil aeration would be expected to be most severe in the waterlogged midmarsh zone where Spartina alterniflora is an important factor in soil aeration. The vegetation of this zone was also the most sensitive to the oil and/or dispersant treatments.

Soil aeration was found to be highly variable in spatial distribution, consequently, temporal changes in soil aeration associated with the treatments were difficult to distinguish. Table 1 presents the thickness of the reducing zones in quadrats receiving the various oil and/or dispersant treatments. Average reducing zone thicknesses in the oil and oil plus dispersant treated quadrats appeared to increase after treatment applications, however, the results were highly variable.

Root coloration did not appear to be affected by the treatments. Living roots ranged from white to light tan in colour regardless of treatment.

Root development differed between vegetation zones and was independent of treatment. The high marsh and midmarsh zones had well developed networks of fine roots, while the root systems of the creek edge zone were characterized by well developed rhizomes but poorly developed mats of fine roots. Aerated microzones were evident in the fine root mats of the midmarsh and high marsh quadrats but were generally absent in the creek edge quadrats where fine roots were relatively scarce. No apparent declines in the extent of these zones were associated with any of the oil and/or dispersant treatments.

Soil Temperature - The oil and/or dispersant treatments had little effect on the soil temperature of the three vegetation zones (Figure 8). For the high marsh and creek edge zones, the relationships of temperatures in the various treatments were similar before and after treatment applications. In the midmarsh zone, oil and/or dispersant treated quadrats were on average 1.1^{o}C (7%) warmer than the control. Albedo may have been decreased by the high mortality of vegetation for all treatments in this zone resulting in a small increase in soil temperature. Soil temperature differences between vegetation zones, however, were larger than soil

Table 1 - Average thickness of reducing zones in oil and/or dispersant treated creek edge, midmarsh, and high marsh field plots on five sampling dates. Treatments were applied on day 60.

Zone	Treatment	Average Reducing Zone Thickness (cm (1 standard deviation))				
		July 9 Day 62	July 24 Day 77	Aug. 6 Day 90	Aug. 21 Day 105	Sept. 15 Day 130
Creek Edge	Control	-	2.0	0.5	0	0
	Oil	0	0.3(0.6)	0.8(1.4)	1.4(2.3)	0
	Dispersant	0	-	0	0	0
	Oil plus Dispersant	0.1(0.1)	2.0(3.5)	1.2(1.3)	2.0(1.7)	1.7(1.8)
Midmarsh	Control	-	0	1.5	2.0	0.5
	Oil	0.6(0.8)	1.8(1.4)	0.7(0.6)	1.7(0.6)	0.8(0.8)
	Dispersant	-	2.0	1.0	4.0	1.0
	Oil plus Dispersant	0.2(0.3)	0.8(1.0)	0.7(0.8)	0.6(0.8)	2.2(2.6)
High Marsh	Control	0	0	0	0	0
	Oil	0.5(0.5)	1.2(0.8)	0	1.8(1.6)	3.0(3.0)
	Dispersant	-	0	0	-	1.0
	Oil plus Dispersant	0	1.2(2.0)	0.3(0.6)	1.5(2.6)	1.7(2.5)

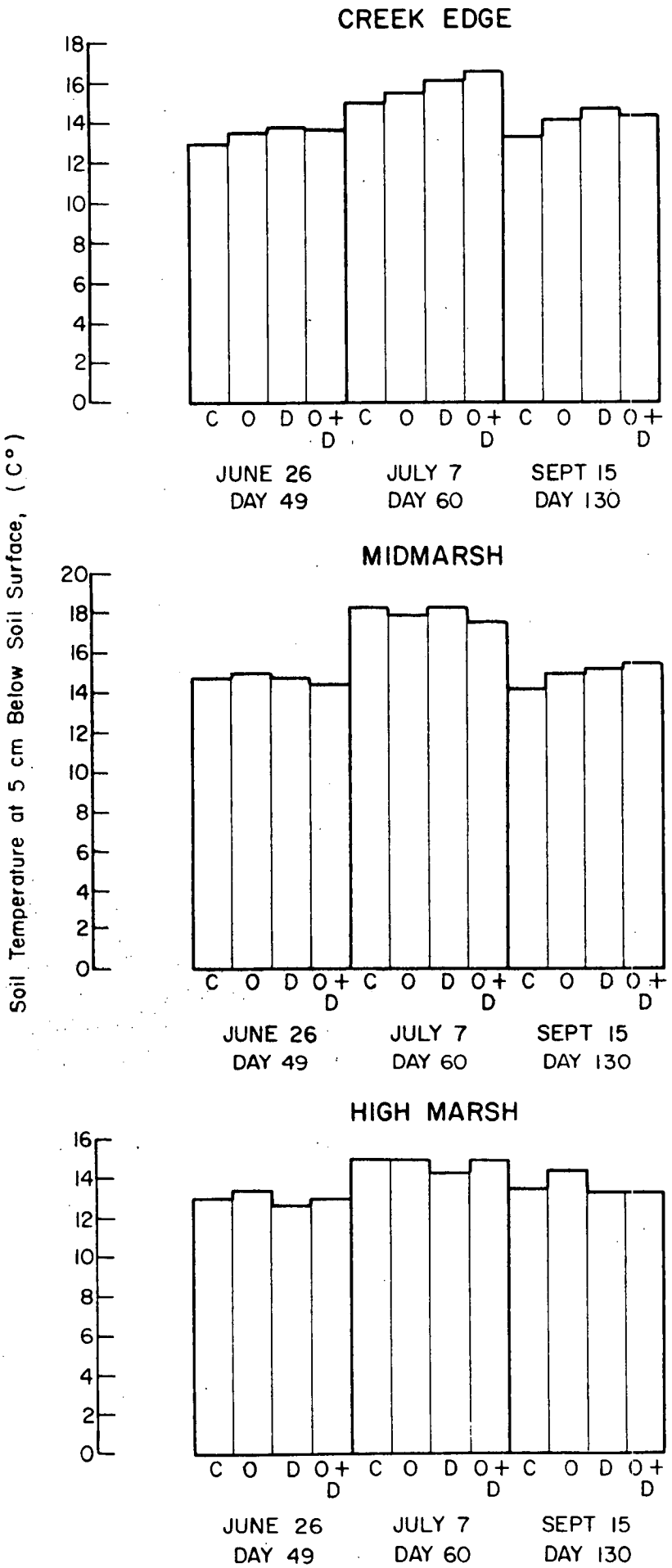

Figure 8: Histogram of mean soil temperatures in the control, oil and/or dispersant treated plots in the creek edge, midmarsh, and high marsh vegetation zones on three sampling dates.

temperature differences between treatments within a particular vegetation zone. The midmarsh zone was consistantly warmer than the creek edge and high marsh zones which had similar soil temperatures. This is probably attributable to the relatively low vegetation cover in the midmarsh zone which would reduce the albedo of this zone.

Soil Chemistry - Based on visual observations of both the field and laboratory experiments, the dispersant was not effective in removing oil from the test plots. There are several possible explanations for this: 1) the oil may have been too weathered for the dispersant to penetrate and mix with it, 2) the tidal flushing may not have been energetic enough to disperse the oil, or 3) the dispersant may not have contacted the oil.

The oil, as applied to the test plots, was artificially weathered to about 15% weight loss. Based on results from other studies with this oil (Bobra and Chung, 1986) this translates into a viscosity of 50 cP at 15°C, certainly within the range of viscosity considered to be dispersible. The oil is also known to be easily dispersible in standard laboratory effectiveness tests using Corexit 9527.

Without additional tests (i.e. plots with premixed oil and dispersant), it was impossible to determine whether or not the reason for the apparent ineffectiveness of the dispersant was because of lack of mixing energy or inefficient application. Recent dispersant effectiveness tets in shallow sloughs in Alberta during low winds indicated that dispersants can be effective in dispersing a significant portion of oil on water under very low energy conditions (Quaife et al. 1986). The oil in this study was applied to the plots at low tide and much of the oil was retained by sediment and foliage to the point that it was not floated by the incoming tide but remained submerged. It is thus not surprising that the dispersant was ineffective.

The dispersant/water mixture was applied as a mist from backpack sprayers from above the marsh grass. It is possible that, although this resulted in an even application of dispersant over the test plot (as evidenced by the consistency of the impact data), the dispersant ended up on the leaves rather than the oil. Any coalesced drops of dispersant would tend to run down the stems of the plants to the sediment, thus missing any oil on surrounding surfaces.

In summary, although it is clear in this study that the dispersant was ineffective in removing oil from the saltmarsh test plots, it is not certain whether this means that dispersants are inherently ineffective in saltmarsh environments or that the method of application prevented the

dispersant from reaching and mixing with the oil. Similarly, Baker et al. (1984) noted that the dispersant BP 1100 WD failed to effectively remove oil from salt marsh vegetation. Further experiments, including premixed oil and dispersant, dispersant applied to oil slicks on water in a saltmarsh on an incoming tide and the use of low presure water flushing to assist dispersion, need to be conducted before the efficacy of dispersant use in saltmarshes can be definitively evaluated.

The oil, as applied to the test plots, had been artificially weathered by exposure to air and sunlight in open containers for 24 h. Weathering was assisted by regular mixing. This resulted in about 15% weight loss. Figure 9 shows the subsequent evaporative loss of the oil on test plots, both in the laboratory and in the field, determined by comparing gas chromatographic (GC) traces of samples with those of a laboratory weathered standard. As shown, the oil evaporated an additional 15% by weight during the first day, after which the evaporation rate was very slow. The scatter of the data points is most likely related to sampling different areas, with different oil thicknesses, in each test plot. The extraction/GC procedure produces an error of about 3% as indicated by the replicate creek edge data points.

Figure 10 shows the results of oil-in-sediment samples taken from both the field and laboratory test plots throughout the study. The data scatter is considerable; this is most likely related to variation in sampling of the sediments. Statistical analysis of replicate samples shows that the average reproducibility of the extraction/ultra-violet (UV) fluorescence analysis is $\pm$ 20%. Conversion of the UV results from ppm chrysene to ppm oil is accurate to within 10%.

The chrysene detection technique (UV fluorescence at 256 nm) would not detect the presence of dispersant, thus no information is available on the penetration or residence time of dispersant in the sediments. The dispersant-only test plot data are shown only as a measure of the repeatability of the extraction/analysis technique, compared to the control plot data.

The scatter in the data is so severe that no conclusions can be drawn regarding differences between plots in different marsh locations, differences between oil alone and oil and dispersant plots or differences between field and laboratory results. All that can be concluded is that those plots that had oil applied to them had significant amounts of oil remaining on and in the sediment at the end of the experiment. Deluane et al. (1984), investigated the concentration of hydrocarbons in a Louisiana salt marsh subjected to oil and oil plus dispersant treatments.

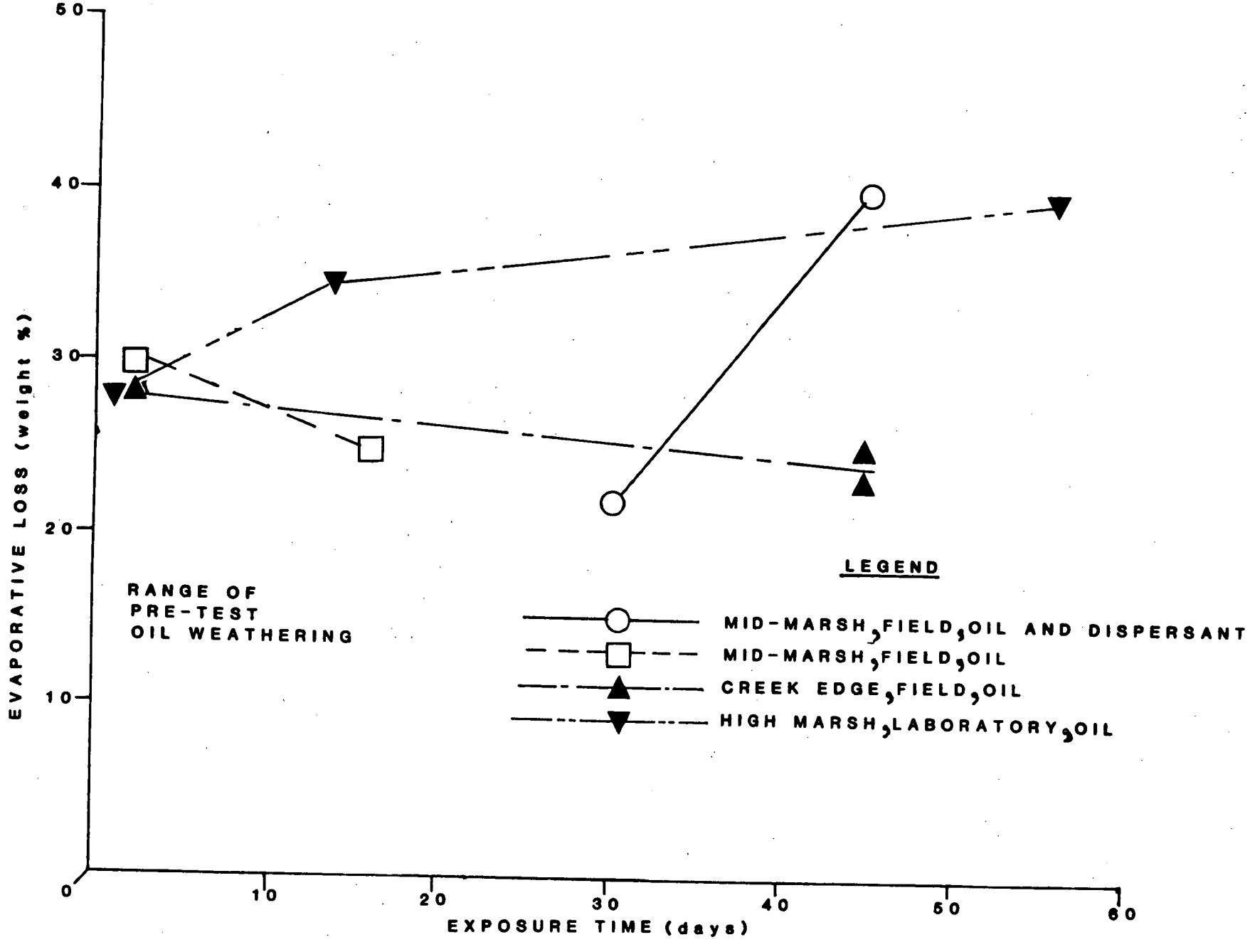

FIGURE 9 - OIL EVAPORATION

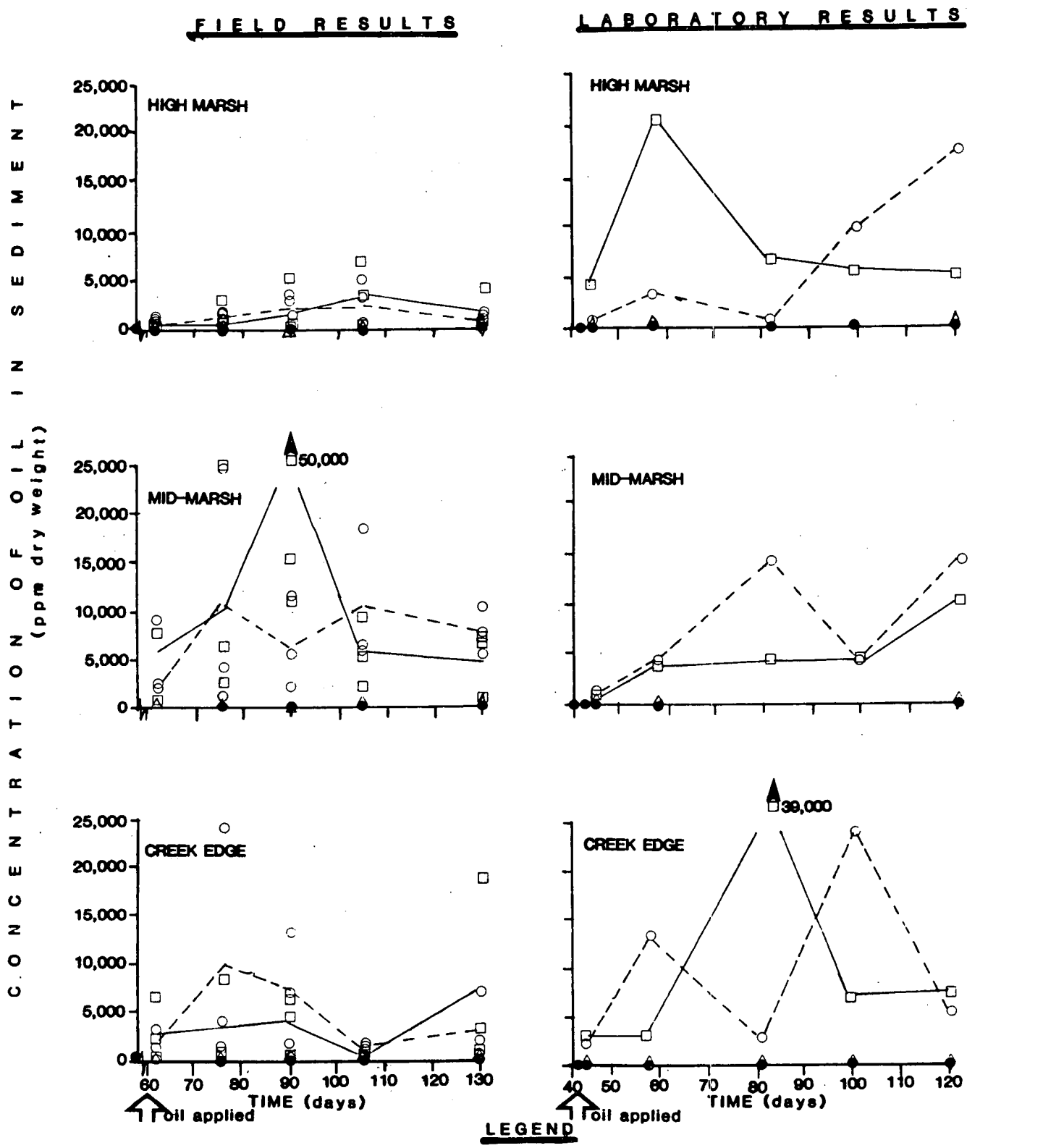

FIGURE 10 - OIL IN SEDIMENT RESULTS

Hydrocarbon concentrations were similar in both treatments. Based on visual observations of the test plots and cores in this study, the oil was contained in the upper few millimetres of sediment and little sediment penetration occurred.

The data suggest that oil-in-sediment concentrations initially increased. Higher concentrations of oil in the sediment may have been caused by the movement of oil from the foliage down the stems to the sediment surface. It also seemed that, in the field, oil-in-sediment concentrations were lower in the high marsh zone than in other areas. Unfortunately, the scatter in the data does not permit a verification of this result.

Greenhouse Experiment

Soil Profile Descriptions - Soil profiles were found to be spatially highly variable in the greenhouse study as in the field study, consequently, temporal changes were difficult to document. Observations revealed a general increase in the volume of anaerobic sediment (as indicated by an increase in grey or black sediment) for the poorly drained midmarsh zone and to a lesser extent the high marsh zone. All treatments (including the control) were similarly affected, suggesting that this was a normal seasonal phenomenon induced by oxygen depletion through heterotrophic activity. Soil profiles for the well drained creek edge zone did not appear to change over time.

Table 2 presents data for the thickness of reducing layers (black) over time for creek edge, midmarsh, and high marsh microcosms receiving the various oil and/or dispersant treatments. Reducing layers were generally restricted to the poorly drained midmarsh zone where they tended to increase through the summer. Similar impacts were noted for all treatments indicating a normal seasonal change.

Extensive colonies of green and pink photosynthetic bacteria were found on the outer surfaces of the marsh plugs. These bacteria were restricted to the midmarsh microcosms because of their preference for anaerobic conditions. All treatments (including the control) had extensive colonies of these bacteria which remained relatively stable through the summer, suggesting that the oil and/or dispersant treatments had no long term effect on them.

Root coloration was not affected by any of the treatments. Root morphology was found to vary between vegetation zones but not between treatments. The midmarsh and high marsh microcosms had well developed fine root systems, while, the creek edge microcosms had poorly developed fine root systems but

Table 2 - Thickness of reducing zones in oil and/or dispersant treated creek edge, midmarsh, and high marsh greenhouse microcosms on four sampling dates. Treatments were applied on day 43.

Zone	Treatment	Reducing Zone Thickness			
		June 21 Day 44	July 28 Day 81	Aug. 15 Day 99	Sept. 5 Day 120
Creek Edge	Control	5.0	0	0	0
	Oil	0	0	0	0
	Dispersant	0	0	0	0
	Oil+Dispersant	0	0	0	0
Midmarsh	Control	0	0	0	3.0
	Oil	0	0	2.0	0
	Dispersant	0	0	1.5	1.0
	Oil+Dispersant	0	1	0	2.5
High Marsh	Control	0	0	0	0
	Oil	0	0	0	0
	Dispersant	0	0	0	0
	Oil+Dispersant	0	0	0	0

large rhizomes. Aerated microzones were common around the fine roots of the midmarsh and high marsh microcosms. No apparent decline in the extent of these zones was noted after treatment application. Aerated microzones were generally not observed in the creek edge microcosms because of the scarcity of fine roots.

Comparison of Physical and Chemical Effects Noted in the Field and Greenhouse Experiments

Although soil profile data were highly variable, the general trends noted in the field and greenhouse studies were very similar. Sediment chemistry data were also highly variable in both experiments. No differences in the retention of oil in the sediments or the effectiveness of the dispersant could be discovered in either the field or greenhouse experiments.

IMPACTS ON VASCULAR PLANTS

Field Experiment

General Observation - Logistical constraints prevented the production of frequent and detailed observations of symptoms in the field experiment. Available observations revealed that the symptoms of oil and/or dispersant toxicity and the rates at which these symptoms progressed were very similar to those observed in the greenhouse experiment. These observations can be found in the general observations section for the greenhouse experiment.

Plant Height - The null hypotheses tested in this experiment were:

- H_1: Oil has no effect on Spartina alterniflora or S. patens height growth.
- H_2: The Dispersant has no effect on Spartina alterniflora or S. patens height growth.
- H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting height growth of Spartina alterniflora or S. patens.

These hypotheses were tested in each of the three vegetation zones. Results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are presented in Appendix 2.

In the creek edge zone, plant heights did not differ significanly between plots before treatment applications (Figure 11, Appendix 2). Fifteen days after treatment applications (day 75), plants in the dispersant and oil

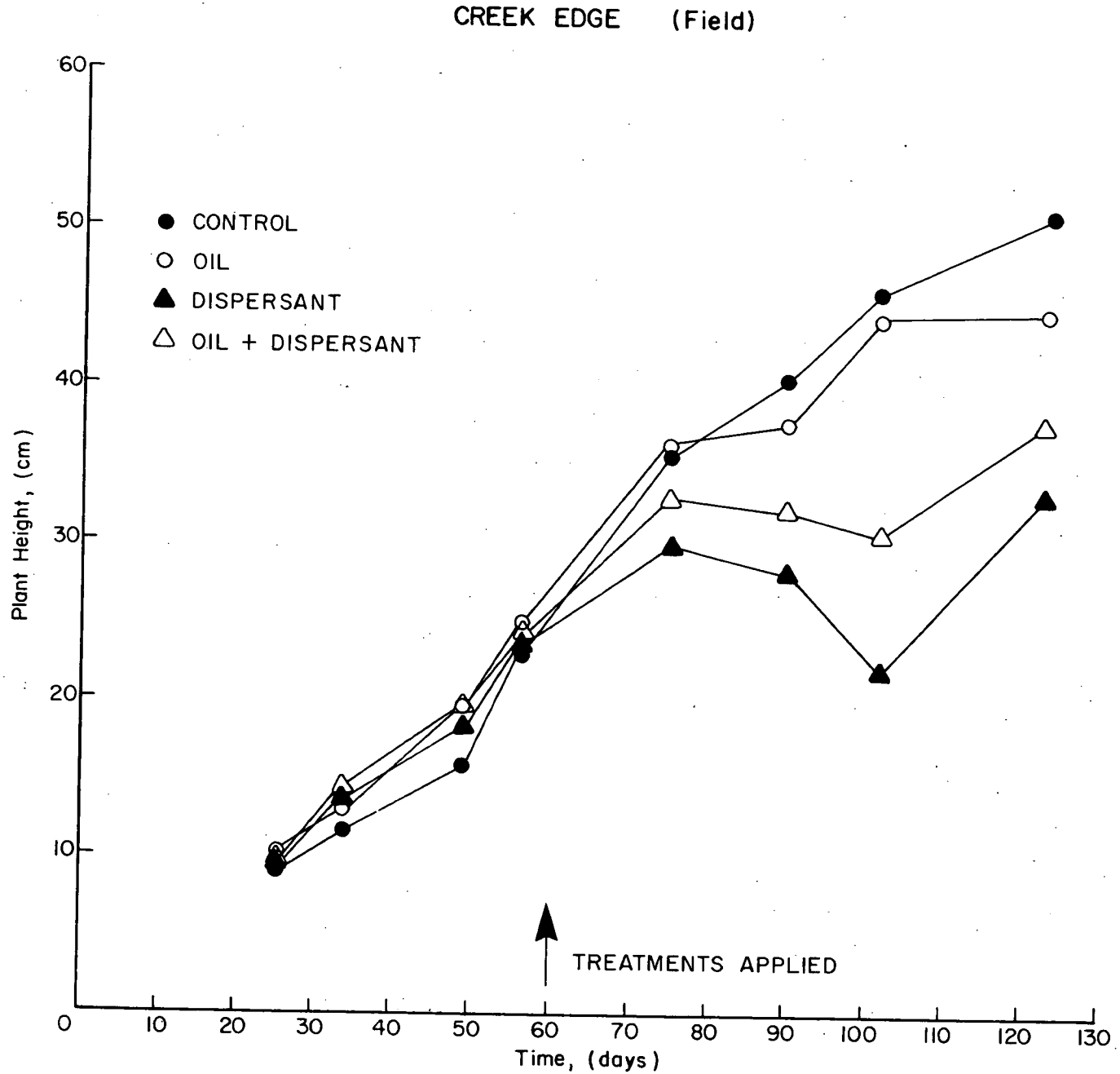

Figure 11: Mean Spartina shoot height for the creek edge zone versus time. Data are from the field study

plus dispersant treated plots were significantly shorter than control plants ($\alpha = 0.05$, df=2) and remained significantly shorter than controls for the duration of the experiment. The dispersant treatment caused larger reductions in mean height than the oil plus dispersant treatment. The oil treatment had relatively little long term effect on plant height, although 29 and 63 days after treatment (days 89 and 123) oiled plants were significantly shorter than the controls ($\alpha = 0.05$, df=2). These results suggest that the null hypotheses H_1 and H_2 were not true. Plant height in the dispersant and oil plus dispersant treated plots appeared to recover between 42 and 63 days after treatment (days 102 and 123). This trend was probably caused by selective mortality of short plants which would increase mean plant height although growth rates of surviving plants probably did not increase. Short plants would be at a disadvantage in competition for light and nutrients and would therefore suffer more stress than the dominant plants. Higher mortality rates would be expected for smaller plants.

Two way analyses of variance indicated a significant reduction ($\alpha = 0.01$ on days 89 and 102, $\alpha = 0.05$ on day 123, df=2) in toxicity (as indicated by reduced plant height) when oil and dispersant were applied together. This inhibitory interaction was noted on all sampling dates following treatment application indicating that hypothesis H_3 was not true. The oil coating on the plants may have neutralized dispersant contacting the plant or it may have plugged or induced closure of the stomata preventing the more toxic dispersant from entering the plant.

In the midmarsh zone, no significant differences ($\alpha = 0.05$, df=2) in pretreatment plant heights were noted (Figure 12, Appendix 2). Significant reductions ($\alpha = 0.05$, df=2) in plant height were not noted in the zone until 29 days after treatment (day 89). This was unexpected since heavy mortality and expression of severe stress symptoms were noted in all oil and/or dispersant treatments within one week of treatment. Rapid reduction in stem density of small plants may have maintained mean plant height at an artificially high level during this period. Dispersant treated plants were signficantly shorter ($\alpha = 0.05$, df=2) than controls from 29 days after treatment (day 89) until the end of the experiment. This treatment was associated with the greatest reductions in stem density. The oil plus dispersant treated plants were significantly shorter ($\alpha = 0.05$, df=2) than control plants 29 and 42 days after treatment (days 89 and 102) but were not significantly different from the controls 63 days after the treatment (day 123). Stunting associated with this treatment was significantly less ($\alpha = 0.05$, df=2) than that caused by the dispersant. Mean plant heights in the dispersant and oil plus dispersant treated plots appeared to recover on the last sampling date, however, this was probably the result of high

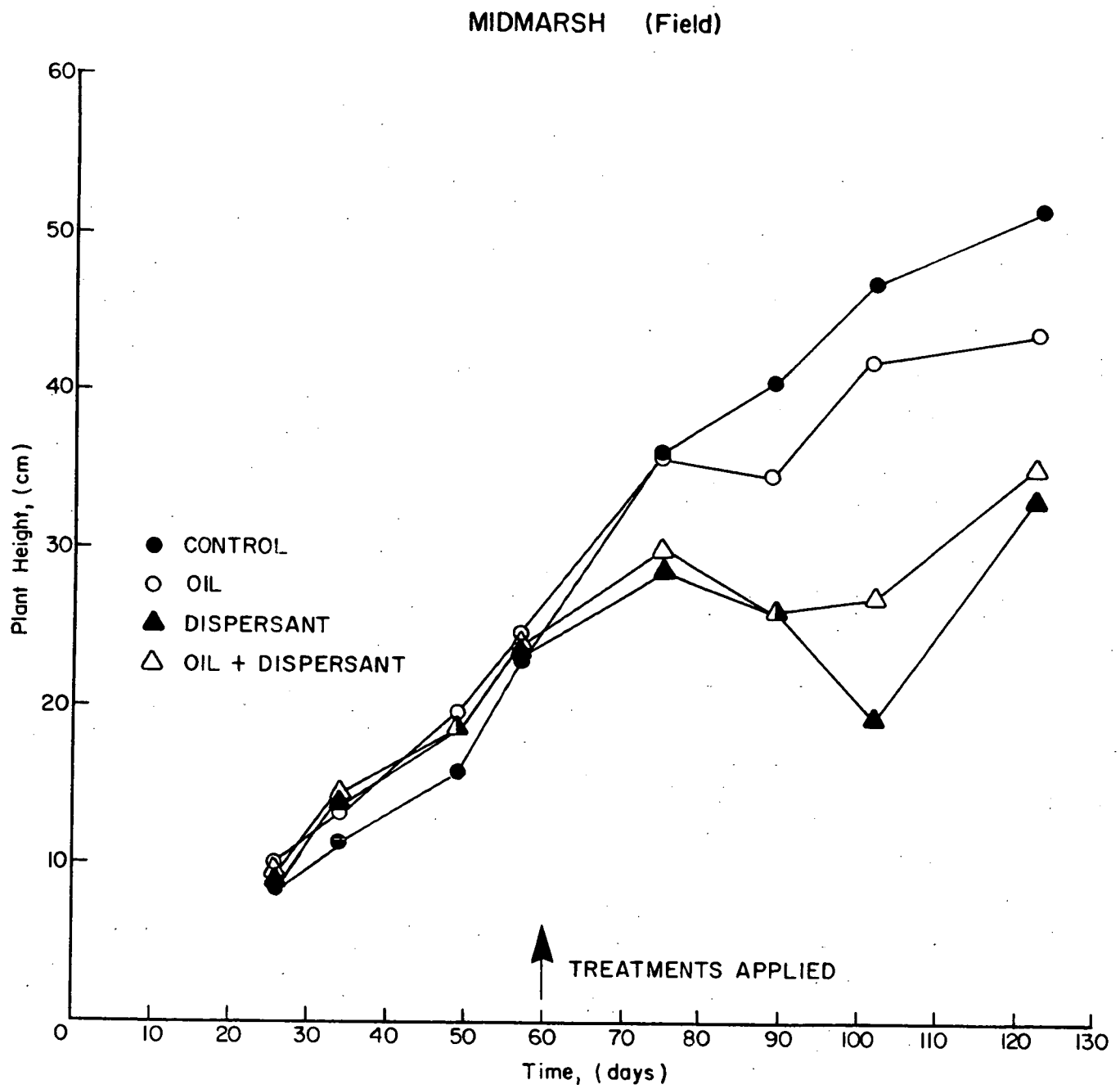

Figure 12: Mean Spartina shoot height for the midmarsh zone versus time. Data are from the field study.

mortality of small plants. Oil treated plants were significantly taller (α = 0.05, df=2) than either dispersant or oil plus dispersant treated plants, but were significantly shorter (α= 0.05, df=2) than control plants on days 89 and 123 (29 and 63 days after treatment). These results suggest that the null hypotheses H_1 and H_2 were not true.

Two way analysis of variance results indicated significant reductions in toxicity when oil and dispersant were combined. Significant inhibitory interactions between treatments were noted on days 75 (α= 0.05, df=2) and 102 (α= 0.05, df=2 (15 and 42 days after treatment) but not on days 89 and 123 (29 and 63 days after treatment). The oil coating on oil plus dispersant treated plants may have neutralized the dispersant or prevented its entry into the interstitial spaces of the plants via the stomata. Based on these results, the null hypothesis H_3 was rejected.

In the high marsh plots, pretreatment plant heights were not significantly different (α=0.05, df=2) on days 26, 34, and 49, however, on day 57 plants in the quadrats slated for oil treatment were significantly shorter (α = 0.05, df=2) than the controls (Figure 13, Appendix 2). Mean height values were signficantly reduced (α= 0.05, df=2) by the oil and dispersant treatments on all posttreatment sampling dates and both treatments caused similar reductions in shoot height. Mean plant heights for the oil plus dispersant treated plots were similar to those of the dispersant and oil treated plants on days 75 and 89 (15 and 29 days after treatment), however, by day 102 mean plant heights for the oil plus dispersant treated plants had risen to levels similar to the control where it remained until the end of the experiment. This sudden increase was probably caused by high mortality of small plants.

Two way analysis of variance results indicated a significant inhibitory interactions between oil and dispersant toxicity (α= 0.05, df=2) on all post treatment sampling dates. Neutralization of the dispersant or prevention of its entry into the plants as outlined in the creek edge and high marsh zones probably account for this inhibitory interaction. Based on these results, the null hypothesis H_3 was rejected.

In summary, these data suggest that the midmarsh zone was the most sensitive to the oil and/or dispersant treatments while the high marsh zone was the least sensitive of the three zones. The dispersant treatment was associated with the largest reductions in mean plant height in all vegetation zones, while the oil treatment generally had the least impact.

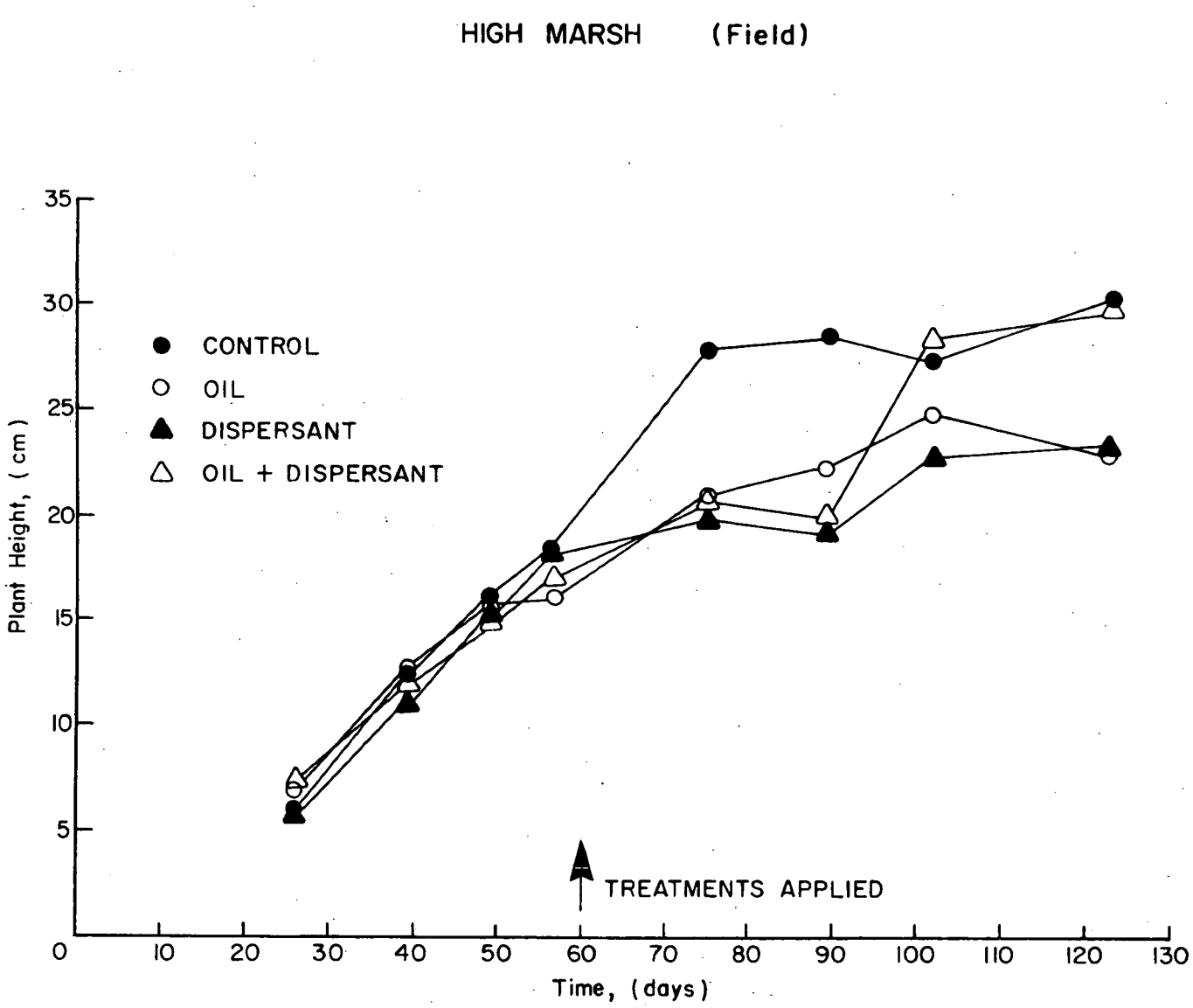

Figure 13: Mean Spartina shoot height for the high marsh zone versus time. Data are from the field study.

Dispersant toxicity was significantly inhibited by the oil in all vegetation zones. Large increases in mean height associated with the dispersant and oil plus dispersant treatments appear to be caused by high mortality of small plants and do not indicate a recovery of growth rates.

Stem Density - The null hypotheses tested in this experiment were:

H_1: Oil has no effect on Spartina alterniflora or S. patens stem density.

H_2: The Dispersant has no effect on Spartina alterniflora or S. patens stem density.

H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting Spartina alterniflora or S. patens stem density.

These hypotheses were tested in each of the three vegetation zones. Results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 3.

Stem density in the field varied substantially between plots, making evaluation of trends difficult in some instances. In the creek edge zone, pretreatment stem densities for the plots assigned to the dispersant treatments were signficiantly lower (α = 0.05, df=2) than all other treatments on both day 26 and 49 (Figure 14, Appendix 3). On days 89 and 123 (19 and 63 days after treatment), all oil and/or dispersant treatments had significantly lower stem densities (α= 0.05, df=2) than the control with oil plus dispersant being the most toxic treatment, therefore the null hypotheses H_1 and H_2 were rejected. The stem density trend over time for the dispersant treated quadrats, however, was almost identical to that of the control, suggesting that stem densities may not have been affected by the dispersant treatment. Two-way analysis of variance results indicated a significant synergistic enhancement of toxicity when oil and dispersant were combined. On the basis of these data hypothesis H_3 was rejected.

Significant differences between pretreatment stem density values (α= 0.05, df=2) were also encountered in the midmarsh zone (Figure 15, Appendix 3). On day 26, there were no significant differences between treatments, however, on day 49, plots assigned to the dispersant and oil plus dispersant treatments had signficantly lower (α = 0.05, df=2) stem densities than the control. Stem densities in all oil and/or dispersant treated plots declined rapidly after treatment application. Oil plus dispersant was the most toxic treatment reducing stem density by over 99% within 29 days of treatment. Stem densities in all oil and/or dispersant

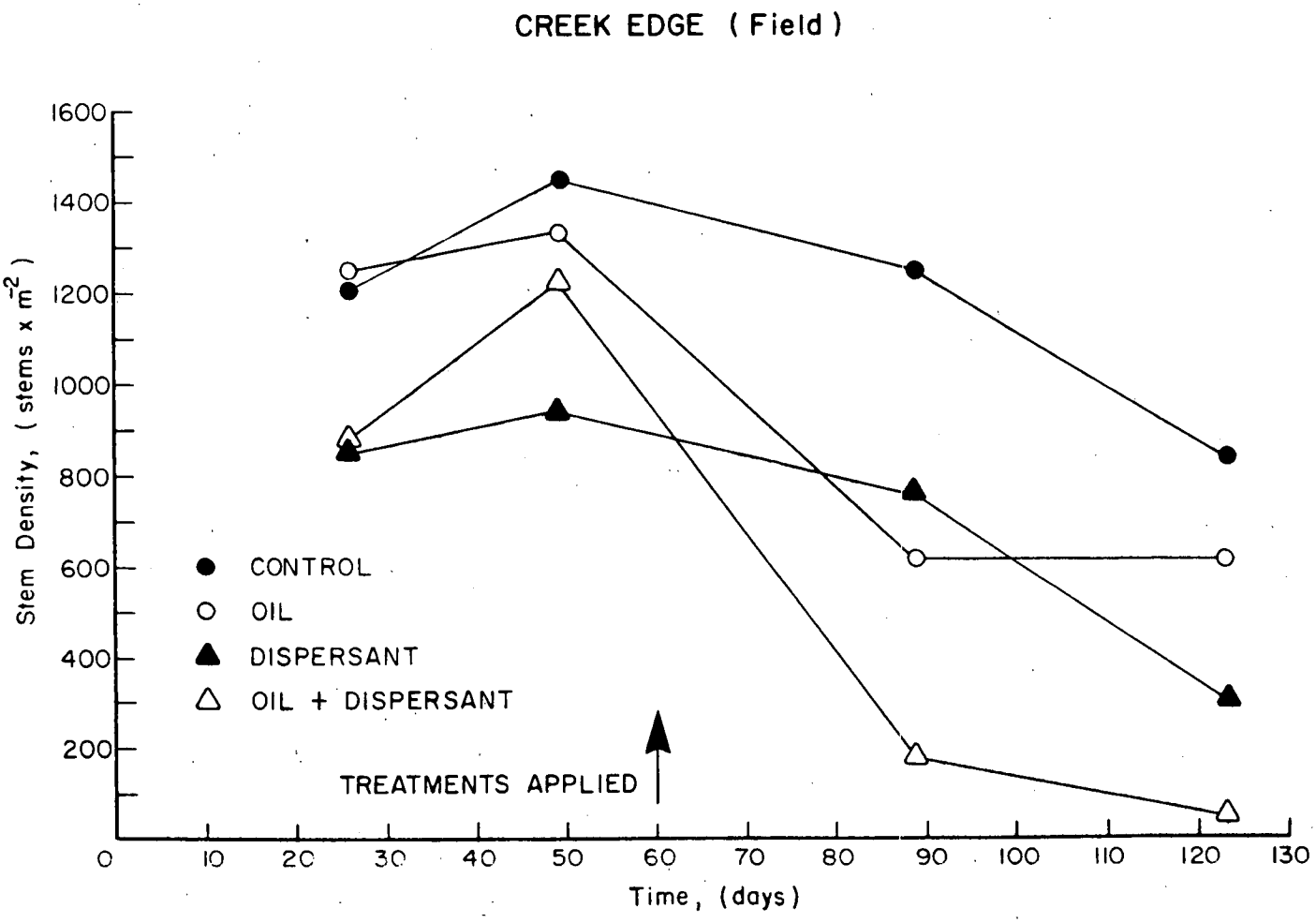

Figure 14: Mean stem density of Spartina alterniflora versus time. Data for control, oil and/or dispersant treated field plots from the creek edge zone are presented. Treatments were applied on day 60.

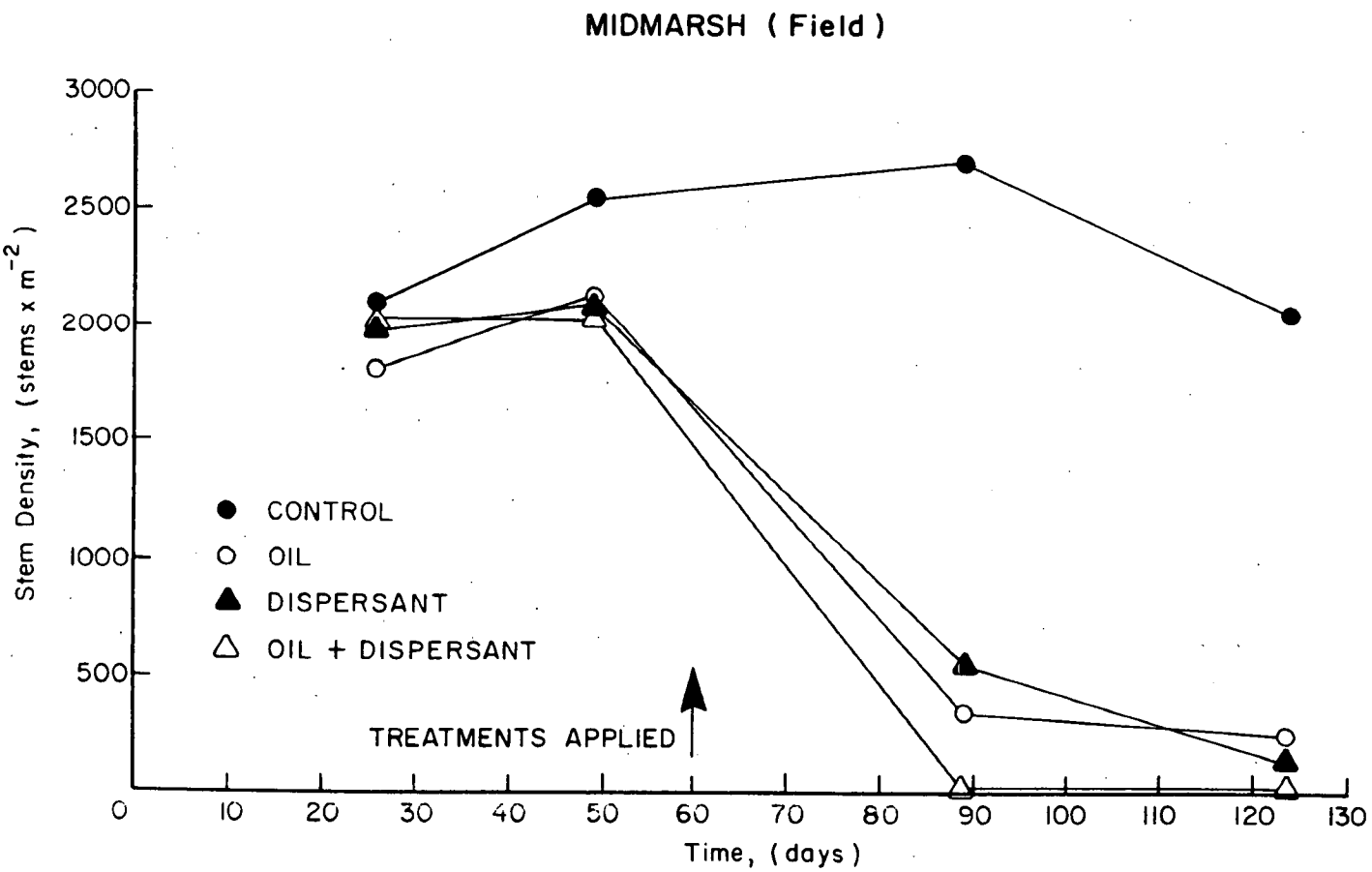

Figure 15: Mean stem density of Spartina alterniflora versus time. Data for control, oil and/or dispersant treated field plots from the midmarsh zone are presented. Treatments were applied on day 60.

treatments were significantly reduced (α = 0.05, df=2) on all sampling dates following treatment applications, therefore, the null hypotheses H_1 and H_2 were rejected. Two-way analysis of variance indicated a synergistic enhancement of toxicity when oil and dispersant were combined. This interaction was significant (α = 0.05, df=2 on day 89; α = 0.01, df=2 on day 123) on all sampling dates following treatment, consequently, H_3 was rejected.

In the high marsh zone (Figure 16, Appendix 3), plots assigned to the oil treatments had stem densities significantly higher (α = 0.05, df=2) than other plots on day 26, however, by day 49 this difference had disappeared. The application of oil and/or dispersant significantly reduced (α = 0.05, df=2) stem density for the rest of the growing season, indicating that the null hypotheses H_1 and H_2 were not true. The oil treatment caused the largest reductions. Dispersant and oil plus dispersant induced similar reductions in stem density. Two-way analysis of variance indicated that oil toxicity was significantly inhibited (α = 0.01, df=2) by the dispersant for all sampling dates following treatment application. The oil appeared to be more toxic to Spartina patens than S. alterniflora, while the dispersant was less toxic to S. patens than S. alterniflora. The dispersant application may have been successful in removing some of the oil from the plants, thereby reducing oil toxicity. Based on these results, the null hypothesis H_3 was rejected. A slight recovery in stem density was noted for all oil and/or dispersant treatments 63 days after treatment (day 123).

In summary, the midmarsh zone was the most sensitive plant community with the oil and/or dispersant treatments causing heavy mortality. The high marsh zone was least sensitive zone with the creek edge zone intermediate in sensitivity.

The various treatments differed in relative toxicity between vegetation zones. Vegetation zones dominated by Spartina alterniflora (creek edge and midmarsh) were impacted the most by the oil plus dispersant. Oil and dispersant treatments had similar effects. There was a significant synergistic enhancement of toxicity when oil and dispersant were applied together. The S. patens dominated zone (high marsh) was affected most by the oil treatment. Dispersant and oil plus dispersant treatments caused similar reductions in stem density. The dispersant significantly inhibited the toxicity of oil in this zone.

Flowering shoot density was severely affected in all zones by all treatments (Table 3). In the midmarsh and creek edge quadrats, flowering shoot density was reduced the most by the oil plus dispersant and least by

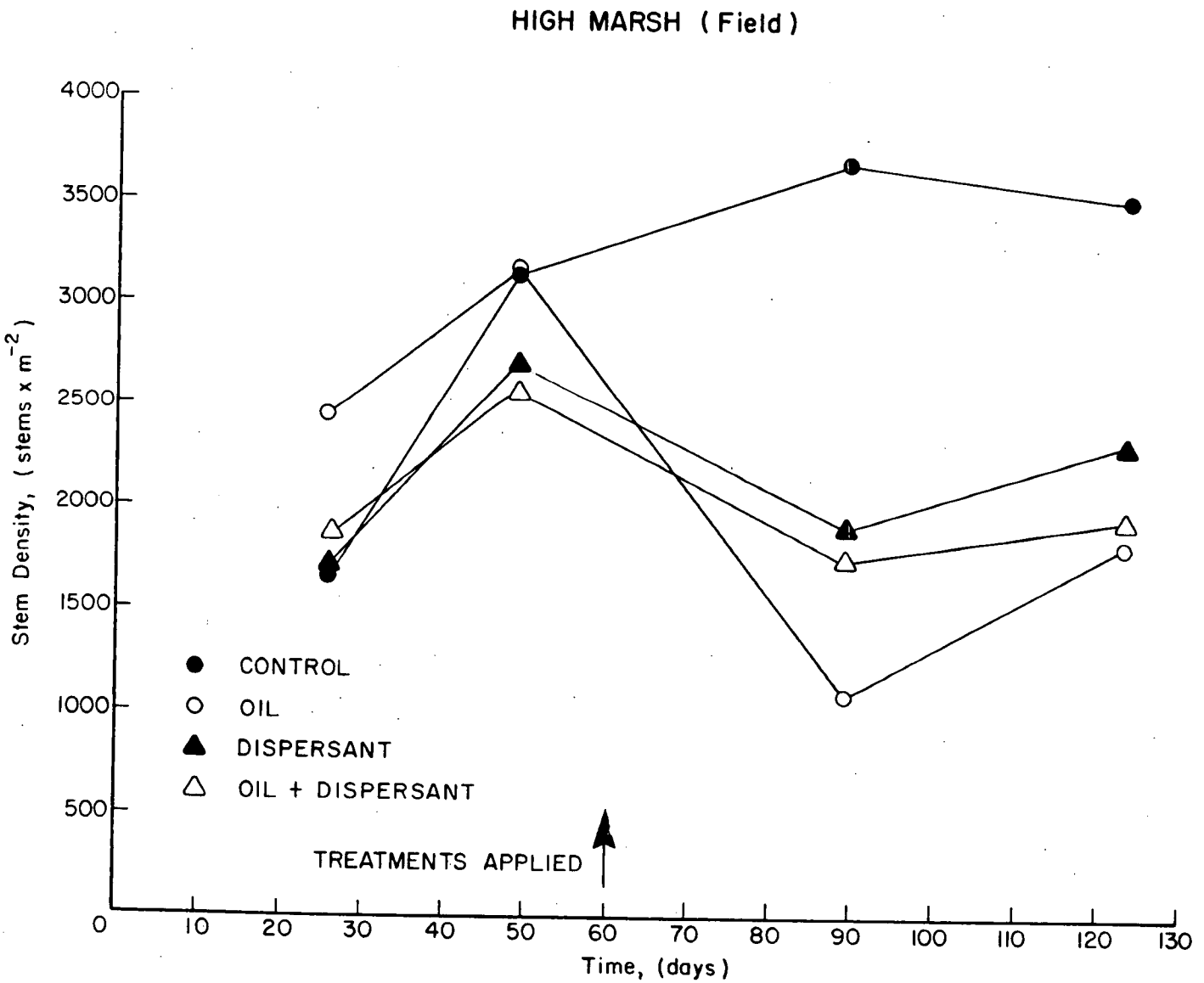

Figure 16: Mean stem density of Spartina alterniflora versus time. Data for control, oil and/or dispersant treated field plots from the high marsh zone are presented. Treatments were applied on day 60.

Table 3 - Stem density of flowering shoots (stems$\cdot m^{-2}$) in field plots 59 days after application of oil and/or dispersant to salt marsh vegetation.

	Control	Oil	Dispersant	Oil plus Dispersant
Creek Edge	36.2(58.8)	0.5(0.9)	1.7(2.9)	0
Mid Marsh	7.0(6.1)	1.0(0.9)	0	0
Upper Marsh	51.8(41.3)	18.5(2.2)	0	0.2(0.3)

the dispersant. In the midmarsh and high marsh zones, both dispersant and oil plus dispersant had the most impact while oil had the least impact.

Biomass - The null hypotheses tested in this experiment were:

H_1: Oil has no effect on total above-ground biomass production.

H_2: Dispersant has no effect on total above-ground biomass production.

H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting total above-ground biomass production.

These hypotheses were tested in each of the three vegetation zones. Results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 4.

Results of the above-ground biomass harvest indicated that the midmarsh zone was very sensitive to all of the treatments. All oil and/or dispersant treatments significantly reduced ($\alpha = 0.05$, df=2) above-ground biomass in this zone (Figure 17, Appendix 4). The creek edge zone was intermediate in its sensitivity to the treatments. The dispersant and oil plus dispersant treatments caused significant reductions ($\alpha = 0.05$, df=2) in above-ground biomass while the above-ground biomass of oil treated plots was not significantly different from the control quadrats. The high marsh zone was the least sensitive to the treatments. In this zone, only the dispersant treated plots had significantly reduced ($\alpha = 0.05$, df=2) above-ground biomass. Based on these results, the null hypothesis H_1 was retained in the creek edge and high marsh zones but was rejected in the midmarsh zone. The null hypothesis H_2 was rejected in all of the vegetation zones. No significant interactions between oil and dispersant were noted in any of the vegetation zones, therefore, the null hypothesis H_3 was retained in all three vegetation zones.

Reproductive biomass was greatly reduced by all oil and/or dispersant treatments (Table 4, Appendix 4). The distribution of reproductive plants was very patchy, making the data highly variable, consequently, the reductions in reproductive biomass were not significant.

Species Cover - The null hypotheses tested in this experiment were:

H_1: Oil has no effect on *Spartina alterniflora* or *S. patens* cover.

H_2: Dispersant has no effect on *Spartina alterniflora* or *S. patens* cover.

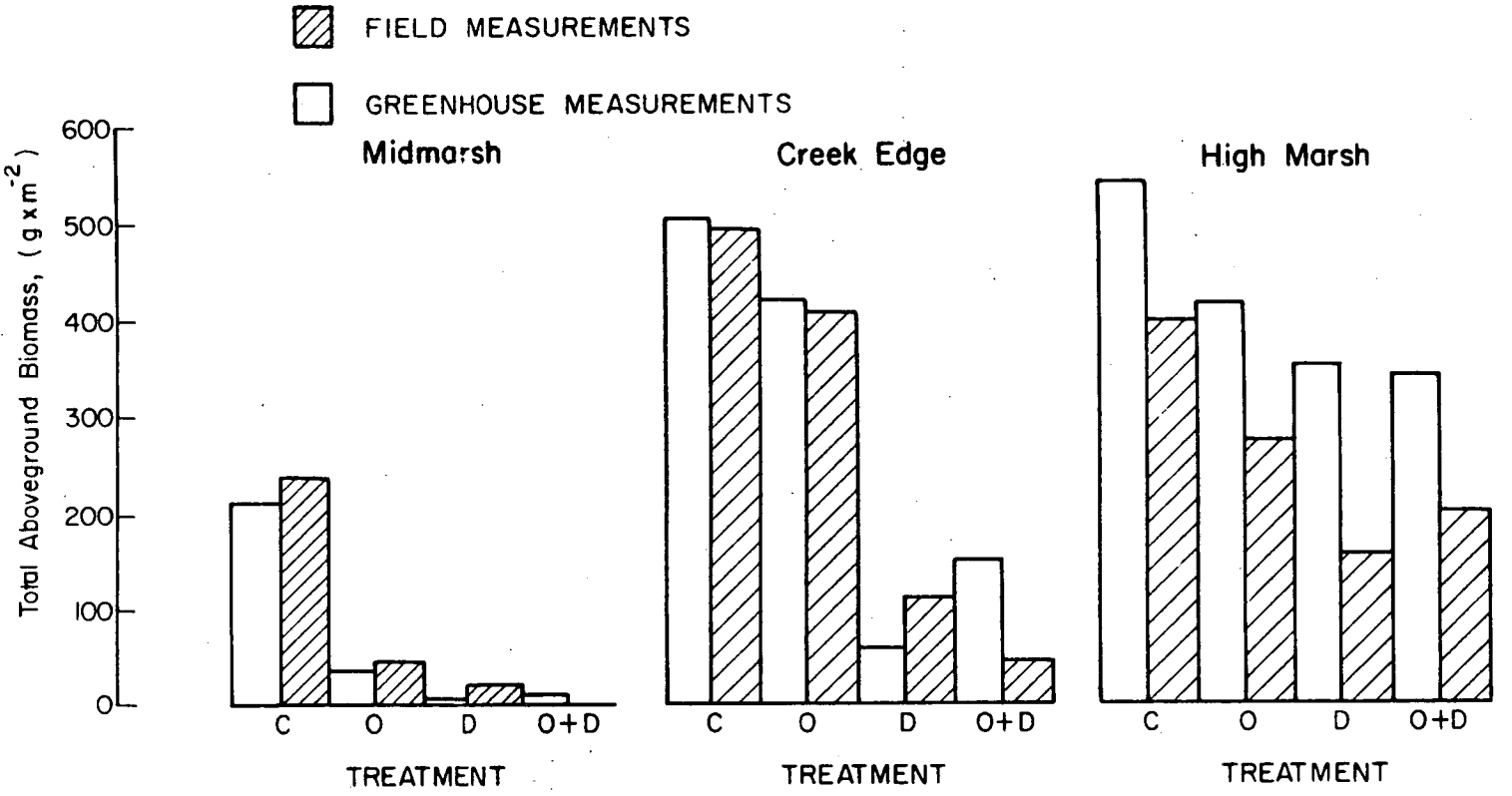

Figure 17: Biomass harvested from salt marsh plots and microcosms 119 days after initiation of the experiment (65 days after treatment application in the field, 77 days after treatment application in the greenhouse).

Table 4 - Reproductive biomass (g/m^2) in field plots 119 days after application of oil and/or dispersant to salt marsh vegetation.

	Control	Oil	Dispersant	Oil plus Dispersant
Reproductive Biomass				
Creek Edge	2.884(4.804)	0.033(0.056)	0.134(0.231)	0
Mid Marsh	0.095(0.076)	0.007(0.023)	0	0
Upper Marsh	1.241(1.232)	0.306(0.142)	0	0.002(0.003)

H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting S. alterniflora or S. patens cover.

These hypotheses were tested in each of the three vegetation zones. Results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 5.

Spartina alterniflora or S. patens on average composed over 95% of total species cover in the three vegetation zones, consequently, statistical analysis was restricted to these species. In the creek edge zone, there were no significant pretreatment differences between S. alterniflora cover of plots assigned to the various treatments (Figure 18, Appendix 5). Fifteen days after treatment (day 75) the average cover values of all oil and/or dispersant treated plots were significantly reduced ($\alpha = 0.05$, df=2) with oiled plots affected the least and oil plus dispersant treated quadrats affected the most. S. alterniflora cover values for the dispersant and oil plus dispersant treatments continued to decline until 42 days after treatment (day 102), however, the cover values of oiled plots remained stable. S. alterniflora cover values for all oil and/or dispersant treatments were significantly lower than the control at this time. These results suggest that the null hypotheses H_1 and H_2 should be rejected. No significant treatment interactions were noted. The null hypothesis H_3 was therefore retained.

In the midmarsh zone, there were no significant pretreatment differences in S. alterniflora cover values between plots (Figure 19, Appendix 5). The application of treatments caused significant reductions ($\alpha = 0.05$, df=2) the cover values of all oil and/or dispersant treated quadrats on all posttreatment sampling dates. Fifteen days after treatment (day 75), oil plus dispersant treated plots had the largest reductions in cover values. Oil and dispersant treated plots were equally affected. S. alterniflora cover values for all oil and/or dispersant treated plots continued to decline until 42 days after treatment (day 102), with extremely large reductions associated with all of them. Based on these results, the null hypotheses H_1 and H_2 were rejected. Two way analysis of variance results for day 102, indicated a significant synergistic enhancement of toxicity when oil and dispersant were applied together. The null hypothesis H_3 was rejected on the basis of these results.

The oil and/or dispersant treatments had no effect on S. patens cover values in the high marsh (Figure 20, Appendix 5). Cover values for all oil and/or dispersant treatments continued to rise over time and none were

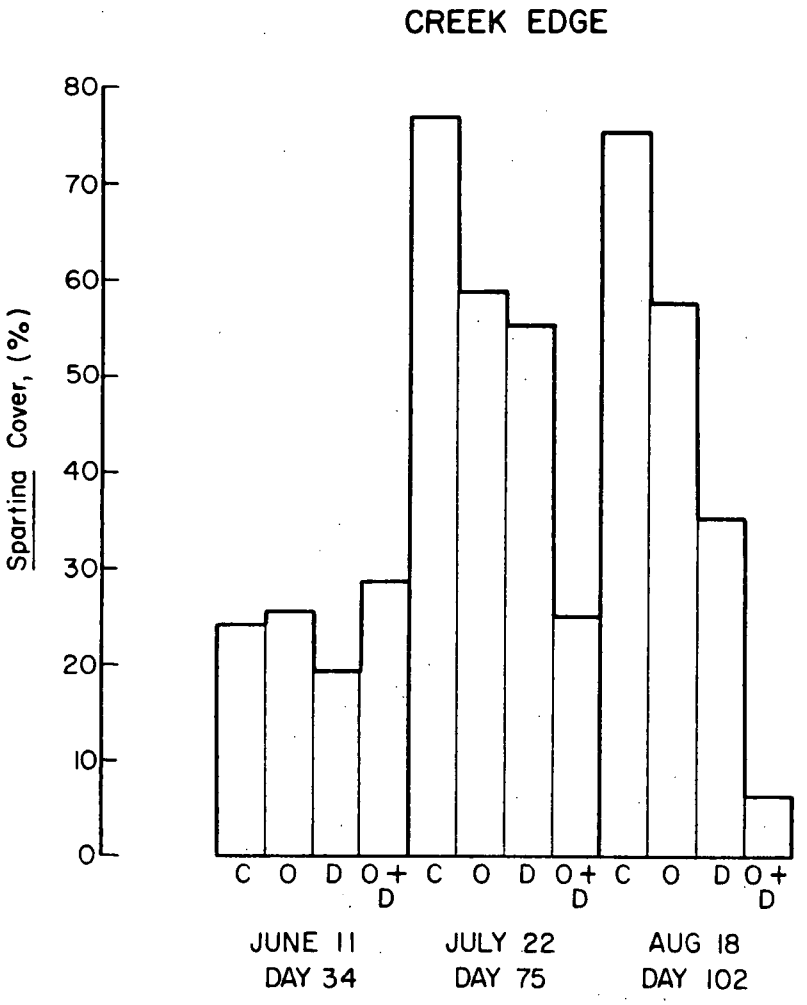

Figure 18: Histogram of mean Spartina alterniflora cover in oil and/or dispersant treated plots from the creek edge zone. Data are from the field study. Treatments were applied on day 60.

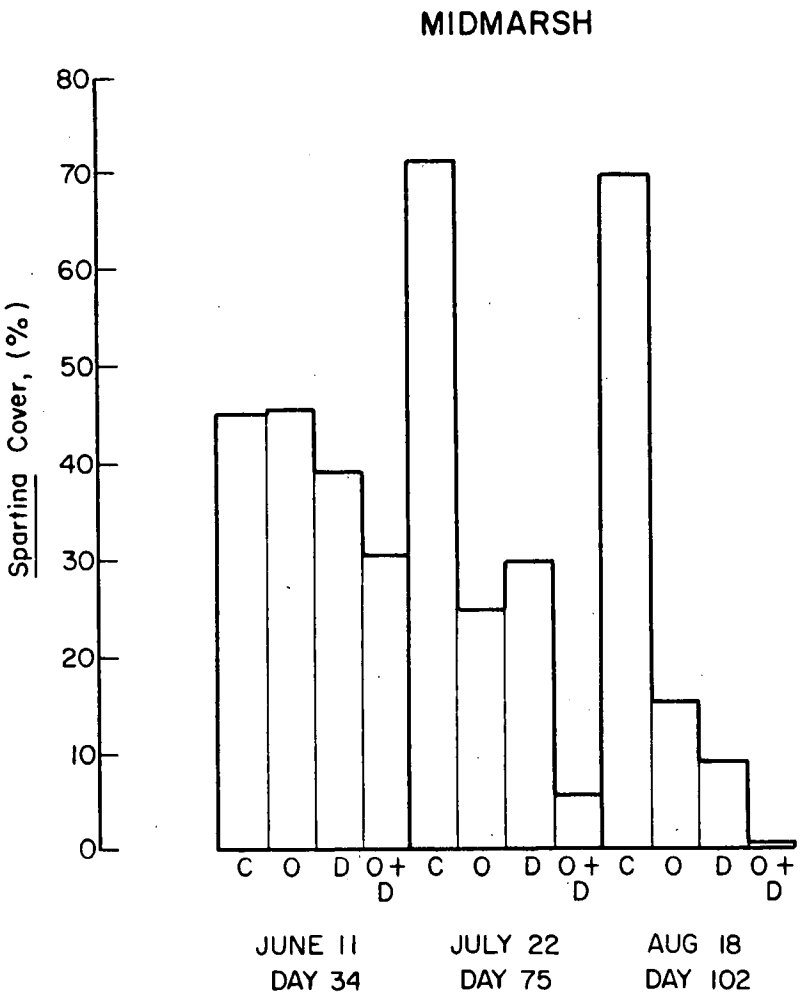

Figure 19: Histogram of mean Spartina alterniflora cover in oil and/or dispersant treated plots from the midmarsh zone. Data are from the field study. Treatments were applied on day 60.

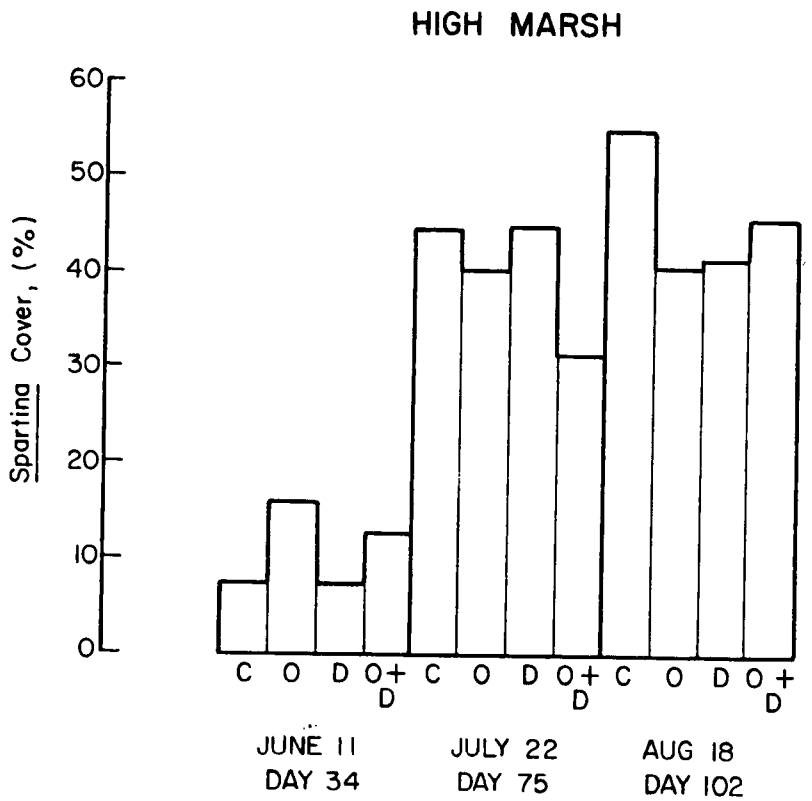

Figure 20: Histogram of mean Spartina alterniflora cover in oil and/or dispersant treated plots from the high marsh zone. Data are from the field study. Treatments were applied on day 60.

significantly different from control values. The null hupotheses H_1 and H_2 were threfore retained. Significant treatment interactions were not found, therefore H_3 was retained.

In summary, the impact of the oil and/or dispersant treatments on Spartina cover was highest in the midmarsh zone and lowest in the high marsh zone. In the creek edge and midmarsh zones, the oil plus dispersant treatment had the most impact on Spartina cover, while oil had the least. In the high marsh zone, there was very little difference between treatments. Significant interactions between oil and dispersant treatments were found only in the midmarsh zone where a synergistic enhancement of toxicity was noted.

Spartina patens cover values in the midmarsh zone tended to increase over time while S. alterniflora cover values declined. In the oil plus dispersant treated plots S. alterniflora was replaced as the dominant species by S. patens. Inspection of the S. patens shoots revealed very few symptoms of oil or dispersant toxicity while the surrounding S. alterniflora shoots exhibited severe stress. The survival of this species outside of its optimal growing conditions while exposed to the toxic effects of oil and/or dispersant treatments suggests that S. patens is particularly tolerant of oil and dispersant.

Species other than Spartina alterniflora and S. patens on average comprised less than 3% cover in all zones (Tables 5-7). Of these species only Salicornia europaea occurred regularly. Salicornia cover responded differently in the various vegetation zones. In the creek edge zone, Salicornia cover values declined in the oil and oil plus dispersant treated plots and remained stable in the dispersant treated plots. Salicornia cover values, however, also declined in the control plots making it impossible to determine whether natural mortality or oil and/or dispersant toxicity was responsible for the declines in the treated plots. Decreases in control plot Salicornia cover were also noted in the high marsh zone. Salicornia cover in the high marsh oil and/or dispersant treated plots appeared unaffected by the treatments. In the midmarsh zone, Salicornia cover remained stable in all treatments. The overall impression from these results is that Salicornia cover was not affected by the treatments. These results are contrary to the results of Baker et al. (1984) who found Salicornia europea to be sensitive to both oil and dispersant.

Leaf Anatomy - The development of microscopic symptoms appeared to be identical for all species and for all treatments. As the conditions of plants deteriorated, the number of chloroplasts within the outer bundle sheath and mesophyll cells declined and eventually disappeared.

Table 5 - Species composition of control, oil and/or dispersant treated field plots in the creek edge zone on three sampling dates. Treatments were applied on day 60.

Species	Treatment	Average Cover (1 Standard Deviation)		
		June 11 Day 34	July 22 Day 75	Aug. 18 Day 102
Spartina alterniflora	Control	24.2(3.8)	77.1(6.9)	75.4(7.4)
	Oil	25.4(6.4)	58.0(7.3)	57.5(8.8)
	Dispersant	18.9(3.0)	55.4(11.4)	35.4(8.9)
	Oil+Dispersant	28.1(8.0)	24.6(1.9)	6.6(4.4)
Spartina patens	Control	0	0	0
	Oil	0.1(0.2)	0	0.4(0.8)
	Dispersant	0	0	0.2(0.3)
	Oil+Dispersant	0	0	0.1(0.1)
Salicornia europaea	Control	0.4(0.4)	0.2(0.2)	0
	Oil	0.1(0.1)	0	0
	Dispersant	0.2(0.2)	0.3(0.5)	0.2(0.3)
	Oil+Dispersant	0.3(0.3)	0.1(0.2)	0.1(0.1)
Suaeda maritima	Control	0	0	0
	Oil	0	0	0
	Dispersant	0.1(0.1)	0	0
	Oil+Dispersant	0.1(0.2)	0	0
Plantago juncoides	Control	0	0	0
	Oil	0	0	0
	Dispersant	0.7(1.2)	2.1(3.6)	2.4(4.2)
	Oil+Dispersant	0	0	0
Atriplex patula	Control	0	0	0
	Oil	0	0	0
	Dispersant	0.1(0.1)	0	0
	Oil+Dispersant	0	0	0

Table 6 - Species composition of control, oil and/or dispersant treated field plots in the midmarsh zone on three sampling dates. Treatments were applied on day 60.

Species	Treatment	Average Cover (1 Standard Deviation)		
		June 11 Day 34	July 22 Day 75	Aug. 18 Day 102
Spartina alterniflora	Control	45.2(21.4)	71.3(9.9)	69.6(11.8)
	Oil	45.8(8.0)	25.0(11.9)	15.4(12.1)
	Dispersant	39.3(22.5)	29.8(14.8)	8.6(8.9)
	Oil+Dispersant	30.8(5.8)	5.8(4.0)	0.4(0.2)
Spartina patens	Control	0.2(0.3)	0.4(0.8)	1.8(3.2)
	Oil	0	0	0.5(0.8)
	Dispersant	0	0	0.6(0.5)
	Oil+Dispersant	0	0	0.7(0.7)
Salicornia europaea	Control	0.2(0.1)	0.1(0.2)	0.4(0.1)
	Oil	0.2(0.2)	0.2(0.3)	0.2(0.2)
	Dispersant	0.3(0.3)	0.3(0.2)	0.3(0.3)
	Oil+Dispersant	0.5(0.4)	0.3(0.2)	0.2(0.2)
Suaeda maritima	Control	0	0	0.1(0.1)
	Oil	0.1(0.1)	0	0
	Dispersant	0.1(0.2)	0	0
	Oil+Dispersant	0	0	0
Triglochin elata	Control	0.3(0.3)	0	0.2(0.3)
	Oil	0.1(0.1)	0	0.1(0.1)
	Dispersant	0	0	0
	Oil+Dispersant	0	0	0
Plantago juncoides	Control	0	0	0
	Oil	0	0	0
	Dispersant	0	0	0.1(0.2)
	Oil+Dispersant	0	0	0
Limonium nashii	Control	0	0	0
	Oil	0	0.1(0.1)	0
	Dispersant	0	0	0
	Oil+Dispersant	0	0	0

Accompanying this was the appearance of orange granules in these cells. These may have been the remains of chloroplasts or residual photosynthetic pigments such as carotene. Concommitant to the progression of these microscopic symptoms, was the development of chlorosis at the macroscopic level. Plasmolysis or erosion of the cuticle were not observed in any of the samples.

There are several ways in which oil can affect plants (Baker 1970, 1971a). It can act physically by blocking stomata, thereby interfering with gas exchange and transpiration. Oil, which enters the plant can damage cell membranes resulting in ion "leakage" and osmoregulatory problems (Hutchinson, 1979). Evidence of osmoregulatory damage (overactive salt glands) were noted for oiled plants in the midmarsh zone. Disruption of membranes allows oil to enter cells where it can damage chloroplasts, thereby reducing photosynthetic rates. The evidence presented here suggests that chloroplasts were probably damaged by the oil treatment. Very little is known of the mode of dispersant toxicity in plants. Animal research indicates that dispersants may have their initial effect upon the outer cell membrane, solubilizing the lipid components of membranes and lowering cell surface tension (Wells, 1984). The sites of toxicity for the oil and dispersant are similar and may explain the similarity of microscopic symptoms observed in this study.

Greenhouse Experiment

General Observations - General observations regarding plant health were made at frequent intervals in the greenhouse. The trends noted in these observations were corroborated by less frequent observations in the field.

Plant stress symptoms were generally similar in all three vegetation zones, with the single exception that the symptoms progressed at different rates in different vegetation zones. Symptoms progressed fastest in the midmarsh zone and slowest in the high marsh zone. In the oil treatments in all vegetation zones, oiled portions of leaves became chlorotic within three days. Chlorosis was initially restricted to oiled portions, and unoiled tissues remained apparently healthy. Seven days after oiling, plants in the midmarsh samples became encrusted with salt, suggesting disruption of osmoregulatory functions. This symptom was restricted to the midmarsh samples. With time, chlorosis and eventual death of oiled tissues progressed up the stems and plant mortality continued to increase with time. Twenty days after oiling, 47% of plants in the midmarsh were judged to be dead, while in the creek edge and high marsh zones, only 5% of plants had died. After 68 days, mortality had increased to 79% in the midmarsh, 34% in the high marsh and 23% in the creek edge.

Dispersant-treated plants became slightly chlorotic (yellow) over the entire plant within three days of application. Within five days, the leaf tips had become severely chlorotic (white), and this chlorosis proceeded down the leaves over the following days, moving fastest in the midmarsh samples and slowest in the high marsh samples. Chlorotic areas died within a few days, and by day 20, over 87% of the plants had died in the midmarsh, 20% in the creek edge and 8% in the high marsh.

Combined oil plus dispersant application resulted in a combination of the symptoms described for plants treated with oil alone or dispersant alone. In all zones, symptoms were evident within three days after treatment. Oiled portions of leaves became chlorotic and eventually affected tissues died, while unoiled portions became slightly chlorotic as in dispersant treated samples. Eventually, the leaves became chlorotic at the tips and died back. Mortality after 20 days was similar to that of dispersant alone treatments with 90% in the midmarsh samples, 27% in the creek edge and 11% in the high marsh.

Two kinds of deformities of plant morphology were observed in high marsh S. patens, treated with either oil or with the oil plus dispersant mixture. The first was the occurrence of wavy ridges on the adaxial leaf surfaces. The second abnormality was more obvious, and was found in approximately 10% of the plants. In these, a U-shaped crook or loop developed in the stems, usually occurring immediately above the oiled portions of the plants. Microscopic comparison of deformed and normal stems revealed that the inner layers of leaves were almost fully developed in the deformed stems. (A grass shoot consists of concentric layers of leaves in various stages of development.) All layers in the deformed plants appeared to be developmentally mature while in the normal stems the inner layers were much less developed than the outer layers. A component of the oil may mimic or interfere with the production or reception of plant growth hormones, thereby causing these deformities. Boney (1974) noted that certain polynuclear aromatic hydrocarbons appeared to be able to affect growth form in macroalgae, possibly through altering normal apical growth of the plants.

Plant Height - The null hypotheses tested in this experiment were:

H_1: Oil has no effect on Spartina alterniflora or S. patens height growth.

H_2: Dispersant has no effect on Spartina alterniflora or S. patens height growth.

Table 7 - Species composition of control, oil and/or dispersant treated field plots in the high marsh zone on three sampling dates. Treatments were applied on day 60.

Species	Treatment	Average Cover (1 Standard Deviation)		
		June 11 Day 34	July 22 Day 75	Aug. 18 Day 102
Spartina patens	Control	7.6(1.2)	44.2(0.7)	54.2(1.4)
	Oil	16.2(14.5)	40.0(21.4)	40.4(9.1)
	Dispersant	6.9(2.7)	44.6(13.7)	41.3(5.8)
	Oil+Dispersant	12.5(10.2)	31.3(11.5)	45.4(10.7)
Spartina alterniflora	Control	0	0.2(0.3)	0.3(0.5)
	Oil	0	0	0
	Dispersant	0	0	0.1(0.2)
	Oil+Dispersant	0.5(0.6)	1.7(2.7)	0.7(0.7)
Salicornia europaea	Control	0.2(0.3)	0.1(0.2)	0
	Oil	0.7(0.3)	0.6(0.4)	0.4(0.4)
	Dispersant	0.2(0.3)	0.4(0.3)	0.3(0.4)
	Oil+Dispersant	0	0	0
Triglochin elata	Control	0	0	0
	Oil	0.2(0.2)	0	0
	Dispersant	0.1(0.1)	0	0
	Oil+Dispersant	0	0	0
Suaeda maritima	Control	0	0	0
	Oil	0.1(0.1)	0.1(0.1)	0
	Dispersant	0	0	0
	Oil+Dispersant	0	0	0
Atriplex patula	Control	0	0	0
	Oil	0	0.1(0.1)	0.1(0.1)
	Dispersant	0	0	0
	Oil+Dispersant	0	0	0
Plantago juncoides	Control	0	0	0
	Oil	0	0	0
	Dispersant	0	0	0
	Oil+Dispersant	0.1(0.2)	0.1(0.2)	0

Table 7 (continued)

Glaux maritima	Control	0	0	0
	Oil	0	0.1(0.1)	0.2(0.3)
	Dispersant	0	0	0
	Oil+Dispersant	0	0	0
Limonium nashii	Control	0	0	0
	Oil	0	0.1(0.1)	0
	Dispersant	0	0	0
	Oil+Dispersant	0	0	0

H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting S. alterniflora or S. patens height growth.

These hypotheses were tested in each of the three vegetation zones. Results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are summarized in Appendices 6 and 7.

There were significant differences in the pretreatment plant heights among treatments in the creek edge zone (Figure 21, Appendix 6). Plants slated for the oil plus dispersant treatment were significantly taller (α= 0.05, df=4) than plants slated for oil and dispersant treatments on all pretreatment sampling dates. Plants slated for the dispersant treatment were significantly shorter (α= 0.05, df=4) than the control plants on all pretreatment sampling dates except day 27. After treatment applications all oil and/or dispersant treatments suffered similar reductions in height growth for a period of at least 25 days, however, because of the pretreatment differences in plant height, only the oil and dispersant treated plants were significantly shorter (α= 0.05, df=4) than the control plants. Between days 68 and 82, however, there was a rapid increase in mean height in all oil and/or dispersant treatments. At this point, only the dispersant treated plants were significanly shorter (α= 0.05, df=4) than the controls. These increases were probably attributable to higher mortality of small plants. Mean heights for dispersant and oil plus dispersant treated plants remained high between days 82 and 91, then declined as larger plants eventually succumbed and were replaced by new shoots. Mean height for oiled plants, however, continued to rise through the experiment and was not significantly different from the control on days 82, 91 and 117.

In the midmarsh zone, the oil treatment had no significant effect on plant height (Figure 22, Appendix 6). The oil plus dispersant treatment had no apparent impact on average plant height until day 68 (25 days after treatment), after which average plant height was significantly reduced (α= 0.05, df=4) for the rest of the experiment. The dispersant treatment was associated with significant reductions (α= 0.05, df=4) in plant height from day 41 (2 days before treatment application) to the end of the experiment. Contrary to other parameters investigated, the oil and/or dispersant treatments appeared to have less impact in the midmarsh zone than in the creek edge zone. Ironically, this discrepancy is probably attributable to the heavy mortality associated with the oil and/or dispersant treatments in the midmarsh zone. Mortality of tagged plants 12 days after treatment (day 55) were 18% for oiled plants, 70% for plants

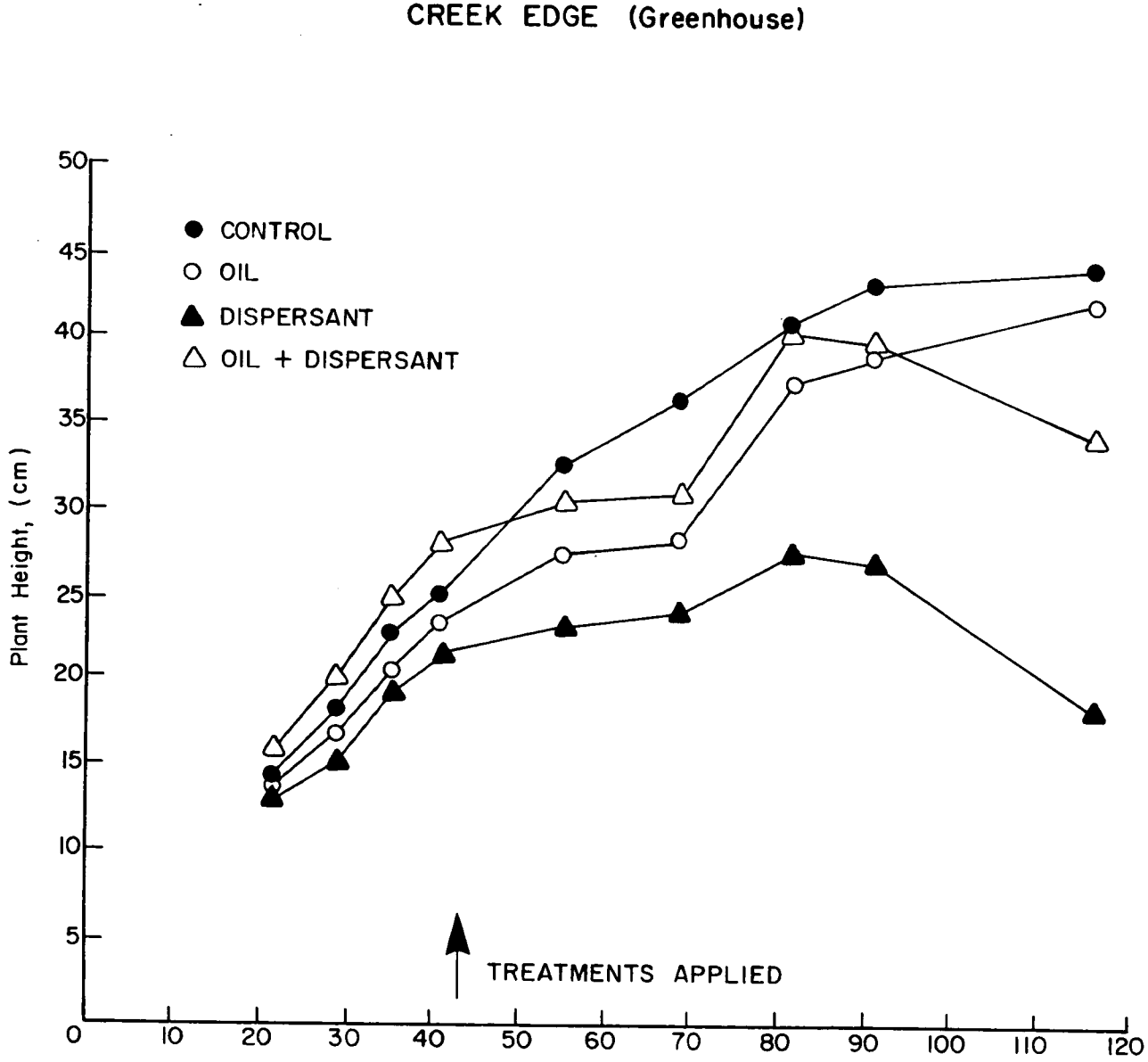

Figure 21: Mean Spartina alterniflora shoot height for the creek edge zone versus time. Data are from the greenhouse study.

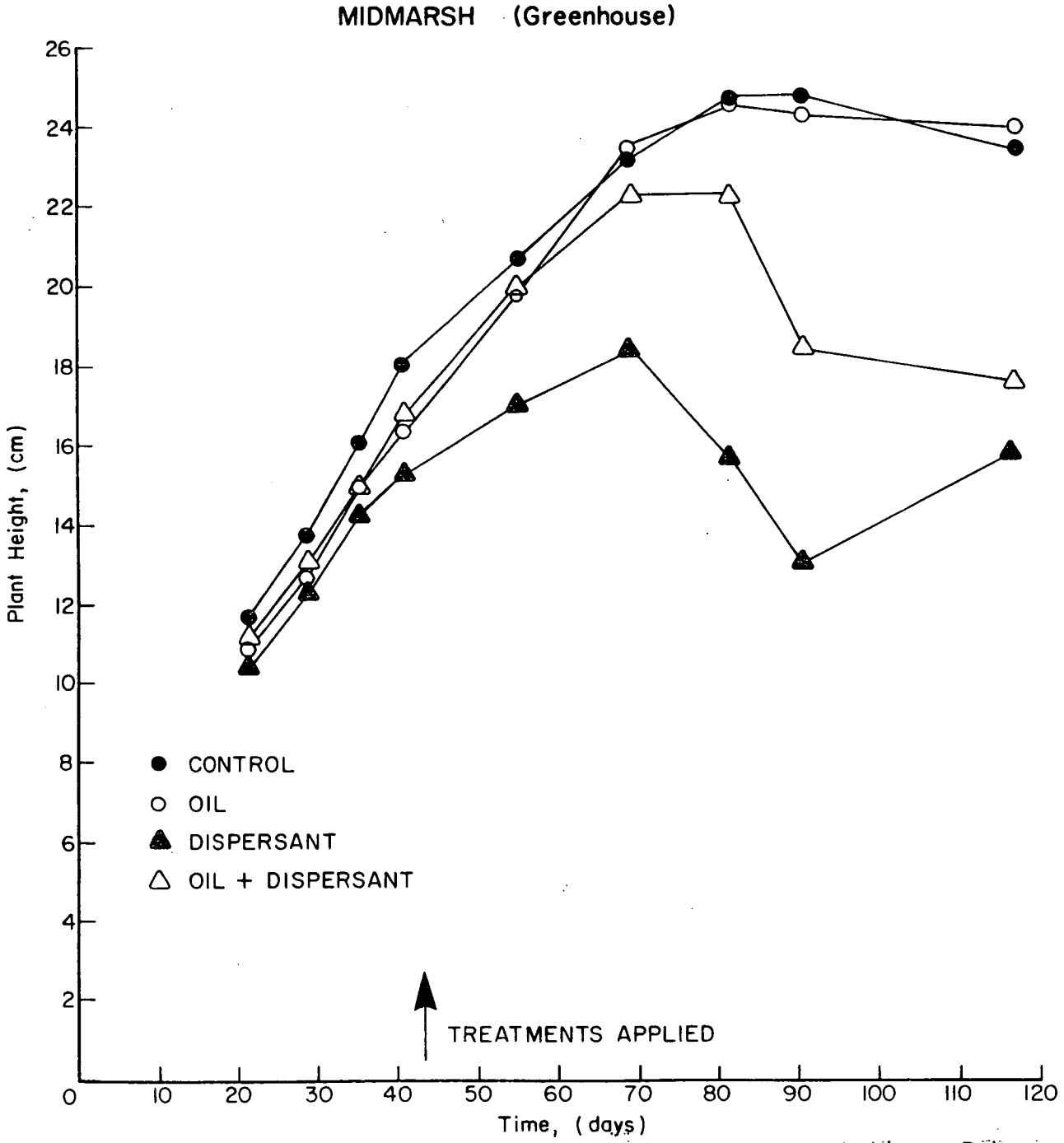

Figure 22: Mean Spartina alterniflora shoot height for the midmarsh zone versus time. Data are from the greenhouse study.

receiving the dispersant treatment and 60% for plants treated with oil plus dispersant (Table 8). Mortality of the oil treated plants was relatively light initially, however, small plants tended to die first, thereby increasing the average height of surviving plants which compensated for the significant reduction (α = 0.05, df=4) of height growth associated with this treatment. The rapid loss of most of the plants in the oil and/or dispersant treatments restricted the selection of replacement plants to those located at the outside edges of the microcosms. These plants were exposed to less oil and/or dispersant and were therefore less impacted than the majority of plants in the microcosms. These plants were able to maintain (at least temporarily) growth rates similar to the controls. Eventually, these plants were affected by the oil and/or dispersant and their growth rates declined. In addition, small numbers of new shoots emerged and their heights tended to further reduce average plant height as occurred in the dispersant and oil plus dispersant treated microcosms after day 68.

Pretreatment mean plant heights for high marsh microcosms slated for the dispersant treatment were significatnly taller (α= 0.05, df=4) than all other treatments (Figure 23, Appendix 6). The control, oil and oil plus dispersant treatments, however, were not significantly different from each other. Twelve days after treatment applications (day 55), all treatments caused reductions in plant height, however, only the oil treatment caused a significant reduction (α=0.05, df=4) in plant height. Within 25 days of treatment application (day 68), all treatments had caused significant reductions (α=0.05, df=4) in plant height. Mean plant height for oiled plants recovered to control values within 39 days of treatment (day 82) and remained at control levels for the rest of the experiment. Between 25 and 48 days after treatment (days 68 and 91), dispersant treated plants remained shorter than control plants. They were significantly shorter (α0.05, df=4), however, only on days 68 and 82. Seventy-four days after treatment applications, the mean height of dispersant treated plants was similar to that of the mean height of control plants. The mean height of oil plus dispersant treated plants remained lower than control plants through the post-treatment portion of the experiment. This reduction was significant (α=0.05, df=4) on all sampling dates except day 117.

Rapid fluctuations in the mean heights of oil and/or dispersant treated plants were not noted in the high marsh zone, probably as a result of the relatively low plant mortality rates associated with this zone. Large modifications of size structure caused by size dependent mortality (as noted in the creek edge and midmarsh zones) were not found in the high marsh zone.

Table 8: Percentage of original tagged plants alive on each sampling date. Treatments were applied on day 43.

Treatment	Percent Surviving (1 standard deviation)					
	Day 41	Day 55	Day 68	Day 82	Day 91	Day 117
			Creek Edge Zone			
Control	100(0)	96(6)	94(6)	86(9)	86(9)	76(18)
Oil	100(0)	88(13)	72(16)	54(15)	52(11)	30(10)
Dispersant	100(0)	88(13)	80(10)	58(21)	54(15)	2(5)
Oil plus Dispersant	100(0)	66(18)	52(24)	36(11)	32(8)	12(5)
			Midmarsh Zone			
Control	100(0)	92(5)	88(5)	84(9)	80(12)	66(20)
Oil	100(0)	82(19)	30(7)	20(10)	16(13)	12(13)
Dispersant	98(5)	30(42)	16(31)	2(4)	0(0)	0(0)
Oil plus Dispersant	100(0)	40(28)	0(0)	0(0)	0(0)	0(0)
			Highmarsh Zone			
Control	100(0)	100(0)	98(5)	96(9)	92(8)	82(13)
Oil	100(0)	98(5)	78(22)	70(24)	64(21)	58(22)
Dispersant	100(0)	96(6)	90(7)	88(8)	76(21)	46(25)
Oil plus Dispersant	96(6)	84(9)	66(6)	58(11)	52(11)	40(16)

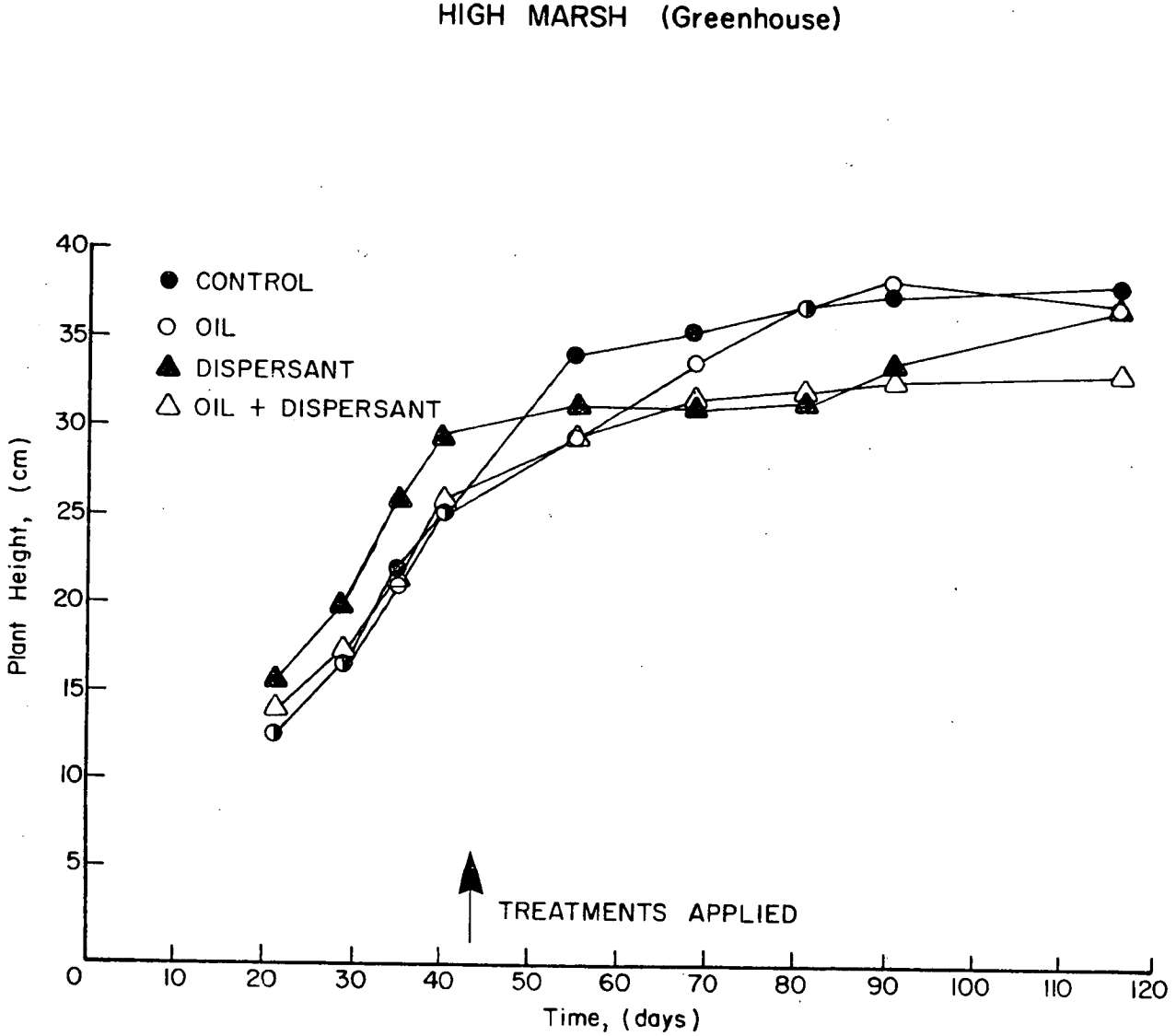

Figure 23: Mean Spartina patens shoot height for the high marsh zone versus time. Data are from the greenhouse study.

The use of tagged plants allowed the determination of plant growth rates. Figure 24 (Appendix 7) presents growth rate data for control, oil and/or dispersant treated plants in the creek edge zones over the course of the experiment. Growth rates were significantly reduced (α = 0.05, df=4) by all treatments for approximately 40 days after treatment (day 43 to day 82). During this period dispersant reduced growth the most and oil the least. Between 39 and 48 days after treatment (days 82-91) only dispersant treated plants had significantly reduced (α=0.05, df=4) growth rates, while 48 to 74 days after treatment (days 91-117) dispersant treated plants grew significantly slower than controls while oil plus dispersant treated plants grew significantly faster. Two-way analysis of variance indicated that oil significantly inhibited (α = 0.01-0.05, df=4) dispersant toxicity through the experiment. The meristematic tissues of grasses are located at ground level. Oil deposits on the soil surface may have shielded the meristematic tissues from the relatively more toxic dispersants allowing these plants to maintain higher growth rates. Growth rates of oil plus dispersant treated plants increased between 39 and 74 days after treatment (days 82-117) and were significantly higher (α = 0.05, df=4) than controls during the last sampling period indicating an apparent amelioration of toxic effects. As Table 8 indicates, however, the oil plus dispersant treatment was responsible for the largest mortality rates among the tagged plants, negating the long term beneficial effect it had on growth rates.

These data indicate that both oil and dispersant were responsible for reductions in Spartina alterniflora height growth in the creek edge zone, therefore the null hypotheses H_1 and H_2 were rejected. There were significant inhibitory interactions between the oil and dispersant, therefore the null hypothesis H_3 was retained.

In the midmarsh zone (Figure 25, Appendix 7), significant reductions (α= 0.05, df=4) of growth rate were noted for the oil and dispersant treatments during the first 12 days after treatment (days 43 - 55). During this period, the dispersant treatment caused the largest reduction in growth rate while the oil plus dispersant treatment caused the least reduction in growth rate.

As in the creek edge zone, height growth benefits associated with the oil plus dispersant treatment were outweighed by the high mortality rates associated with this treatment. All tagged plants in the oil plus dispersant treated microcosms were dead within 25 days of treatment ((day 68) Table 8). Two-way analysis of variance indicated a significant (α= 0.01, df=4) inhibitory interaction between the oil and dispersant. Oil may have coated the meristematic tissues, protecting them from the relatively more toxic dispersant. The fact that growth rates for oil plus dispersant

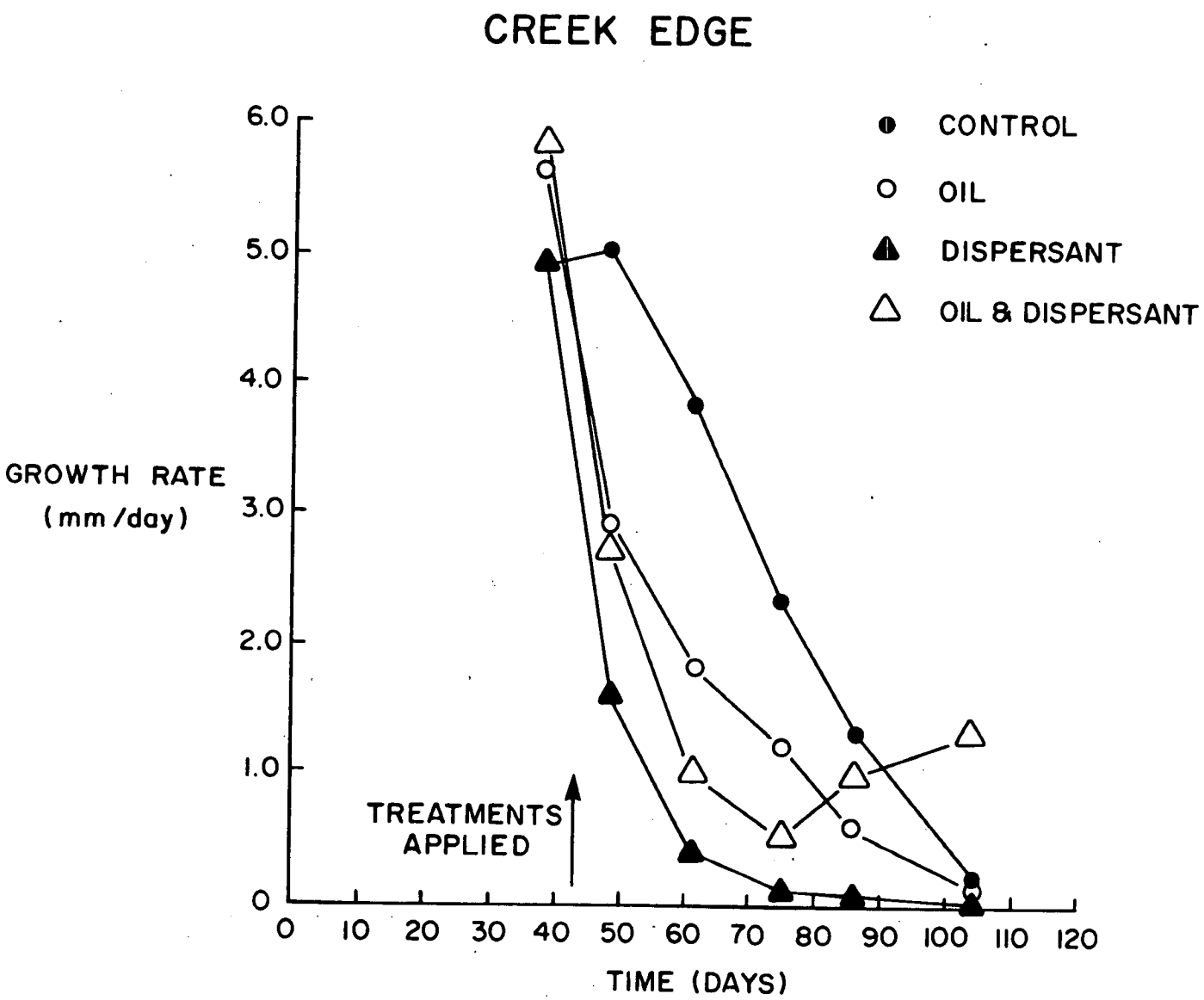

Figure 24: Mean growth rate of control, oil and/or dispersant treated plants from the creek edge microcosms versus time.

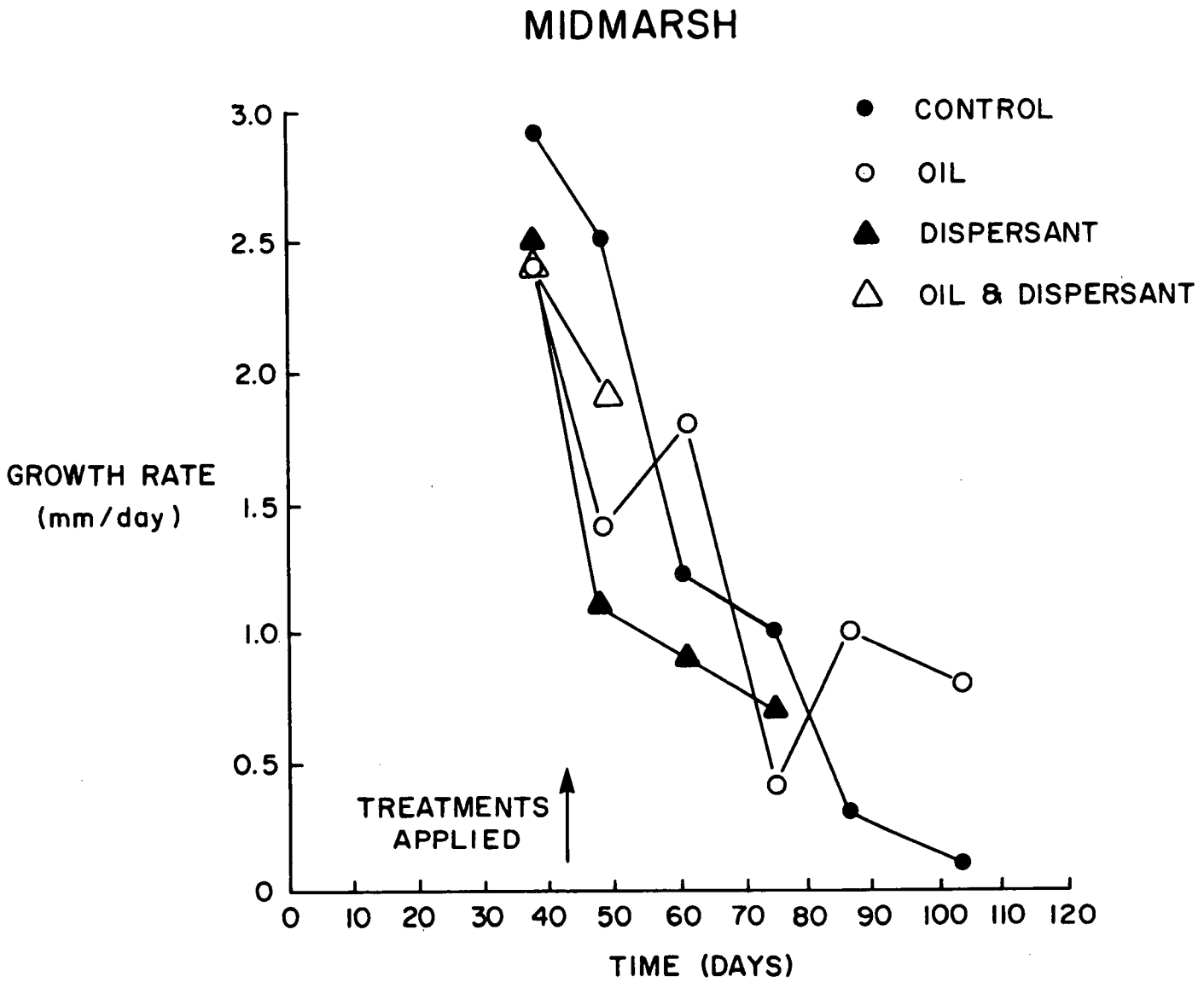

Figure 25: Mean growth rates of control, oil and/or dispersant treated plants from the midmarsh microcosms versus time. No data were available for oil plus dispersant treated plants beyond day 55 and beyond day 82 for dispersant treated plants as a result of mortality of all tagged plants.

treated plants were higher than the growth rates of oiled plants may be attributable to the erratic fluctuations of growth rates exhibited by oiled plants. Growth rates for dispersant treated plants were consistantly lower than those of controls, however they were significantly lower (α= 0.05, df=4) only between 0 and 12 days after treatment (days 43 and 55) and 25 to 39 days after treatment (days 68 to 82). All tagged dispersant treated plants had died within 39 days of treatment ((day 82) Table 8). Growth rates for oiled plants were highly variable and were significantly different from the controls only within 12 days of treatment (days 43 to 55).

Growth rate data indicate that the oil treatment had a significant acute impact on plant height growth, however, both average plant height and growth rate data suggest that no long term effects occurred. Based on these results, the null hypothesis H_1 was retained. Both average plant height and growth rate data indicate long term impacts on Spartina alterniflora growth associated with the dispersant treatment. The null hypothesis H_2 was, therefore, rejected. Significant inhibitory interactions between oil and dispersant were noted in both the average height and growth rate data indicating that the null hypothesis H_3 should be rejected.

In the high marsh zone, the oil treatments had the least impact on growth rates (Figure 26, Appendix 7). Oil treatments significantly reduced (α= 0.05, df=4) growth rates during the first 12 days following treatment applications, however, no significant differences were found in the growth rates of oiled and control plants on any other sampling date. The dispersant treatment caused the largest reductions in growth rates. Significant growth rate reductions (α= 0.05, df=4) associated with the dispersant treatment persisted for approximately 40 days after treatment application (days 43 to 82). After this period growth rates of dispersant treated plants recovered to control levels. Growth rates for oil plus dispersant treated plants were generally intermediate between the growth rates of oil and dispersant treated plants during the first 40 days following treatment, then quickly recovered and had the highest growth rates by the end of the experiment. Significant reductions (α= 0.05, df=4) in growth rate associated with the oil plus dispersant treatment were noted during the first 25 days following treatment applications. During the last sampling period (days 91 to 117), growth rates for oil plus dispersant treated plants were significantly higher than all other treatments. Two-way analysis of variance indicated a significant inhibitory interaction (α= 0.01, df=4) between the oil and dispersant during the first 12 days following treatment (days 43 to 55) and the last 26 days of the experiment (days 91 to 117). As in the creek edge and

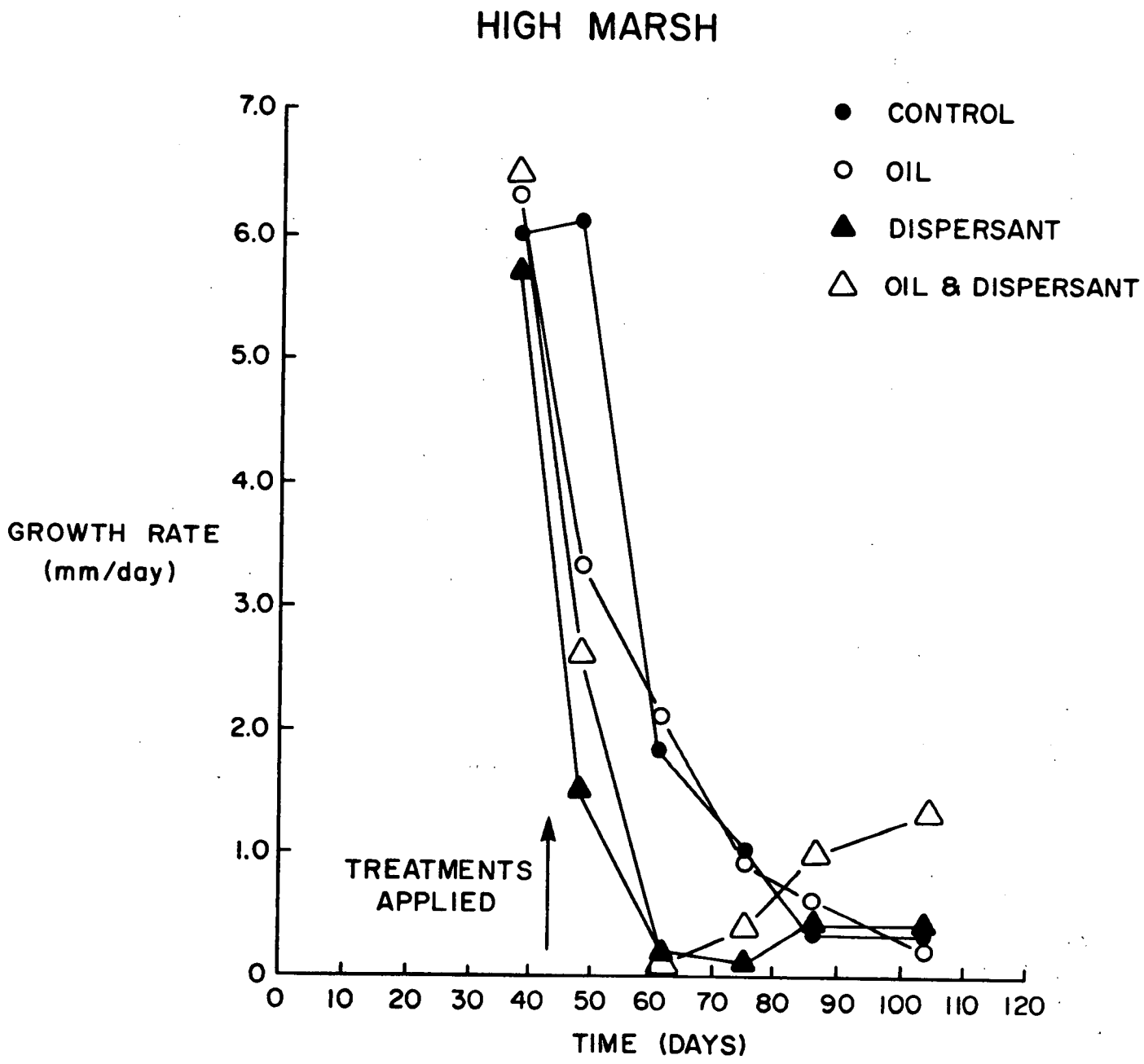

Figure 26: Mean growth rates of control, oil and/or dispersant treated plants from the high marsh microcosms versus time.

midmarsh zones, a layer of oil deposited around the meristematic tissues may have protected them from the relatively more toxic dispersant. The late increase in growth rates associated with the oil plus dispersant treatment may indicate an amelioration of toxicity. This benefit was outweighed by the increased mortality associated with this treatment (Table 8).

The results of both the average plant height and growth rate data indicate a temporary reduction in plant growth in the oiled microcosms followed by a rapid recovery to control levels. These results indicate that the null hypothesis H_1 should be retained. The data suggests long term impacts on plant growth for dispersant treated plants, therefore, the null hypothesis H_2 was rejected. The presence of significant inhibitory interactions indicated that the null hypothesis H_3 should be rejected.

Stem Density - The null hypotheses tested in this experiment were:

H_1: Oil has no effect on Spartina alterniflora or S. patens stem density.

H_2: Dispersant has no effect on Spartina alterniflora or S. patens stem density.

H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting S. alterniflora or S. patens stem density.

These hypotheses were tested in each of the three vegetation zones. Results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 8.

Oiling of creek edge vegetation caused no significant reductions in stem density through the growing season (Figure 27, Appendix 8). The dispersant treatment caused a progressive reduction in stem density which was significantly lower ($\alpha = 0.05$, df=4) than the control only on the last sampling date (day 106). The oil plus dispersant treatment also resulted in a progressive decline in stem density, however, stem density was not significantly different from the control at any time. For both of these treatments, stem density did not begin to decline until at least two weeks after treatment application. The rate of stem density decline was much faster in the dispersant and oil plus dispersant treated microcosms than in the control or oil treated microcosms. Initial stem density in the dispersant and oil plus dispersant microcosms were higher than those of the control and oil microcosms, thereby masking the different rates of stem density reduction. Based on these results, the null hypothesis H_1 was

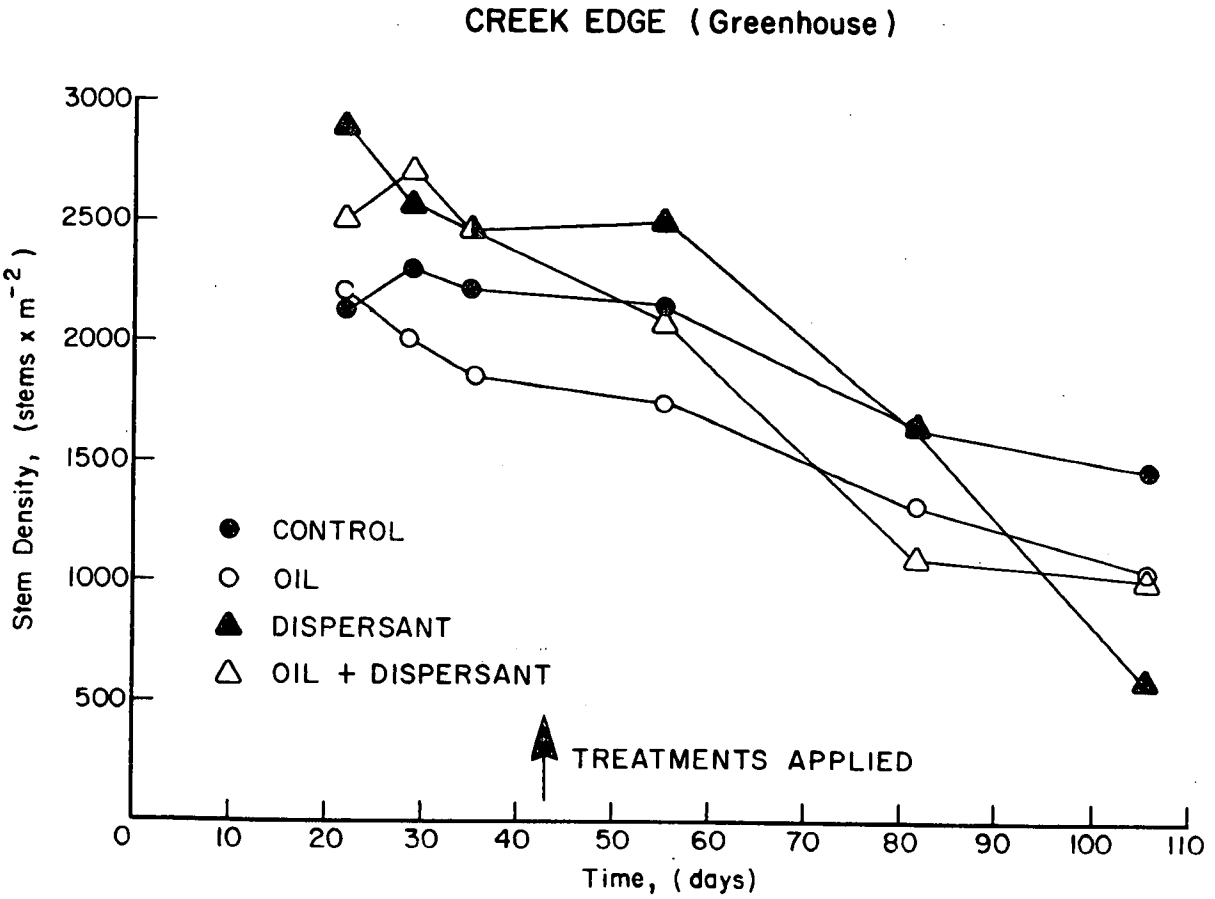

Figure 27: Mean stem density of Spartina alterniflora versus time. Data from control, oil and/or dispersant treated creek edge zone greenhouse microcosms are presented. Treatments were applied on day 43.

retained while H_2 was rejected. No significant interactions were noted between the oil and dispersant treatments. The null hypothesis H_3 was therefore rejected.

In the midmarsh zone all oil and/or dispersant treatments caused significant reductions ($\alpha = 0.05$, df=4) in stem density from 39 days after treatment applications (day 82) to the end of the sampling period (day 106) (Figure 28, Appendix 8). Dispersant and oil plus dispersant almost eliminated Spartina alterniflora from the midmarsh microcosms. Oil caused only slightly less mortality of stems. Mortality in the dispersant treated microcosms was evident within one week of treatment application, however, in the oil and oil plus dispersant microcosms it was delayed at least two weeks. Oil appeared to temporarily inhibit the toxic effects of the dispersant, probably by forming a film over the plants which neutralized dispersant contacting the plant surface or reducing the uptake of the dispersant by plugging or stimulating the closure of stomata. Based on these results, the null hypotheses H_1 and H_2 were rejected. Significant treatment interactions were not observed in the midmarsh zone. The null hypothesis H_3 was therefore retained.

Stem density in the high marsh zone was affected most by the oil and oil plus dispersant treatments (Figure 29, Appendix 8). Stem densities for quadrats receiving these treatments were significantly lower ($\alpha = 0.05$, df=4) than the controls on the last two sampling dates (days 82 and 106), although there was a small recovery of stem density between days 82 and 106. Stem density reductions were not evident until at least two weeks after treatment applications. The dispersant treatment had no significant impact on stem density. Based on these results, the null hypothesis H_1 was rejected while H_2 was retained. Two-way analysis of variance results for the high marsh zone revealed no significant interactions between the oil and dispersant, therefore, the null hypothesis H_3 was retained.

Comparison of the three vegetation zones revealed that the midmarsh zone was the most sensitive to the oil and/or dispersant treatments. The creek edge appeared to be the most tolerant zone, however, when the differences in pretreatment stem density were taken into consideration, the treatment impacts on this zone and the high marsh zone were approximately equal.

Treatments varied in toxicity between vegetation zones. In Spartina alterniflora dominated zones (creek edge, and midmarsh) stem density was most reduced by either the dispersant or oil plus dispersant treatments which were approximately equal in impact. Oil had the least impact although it was responsible for substantial reductions in stem density in the midmarsh zone. In the Spartina patens dominated zone (high marsh), oil

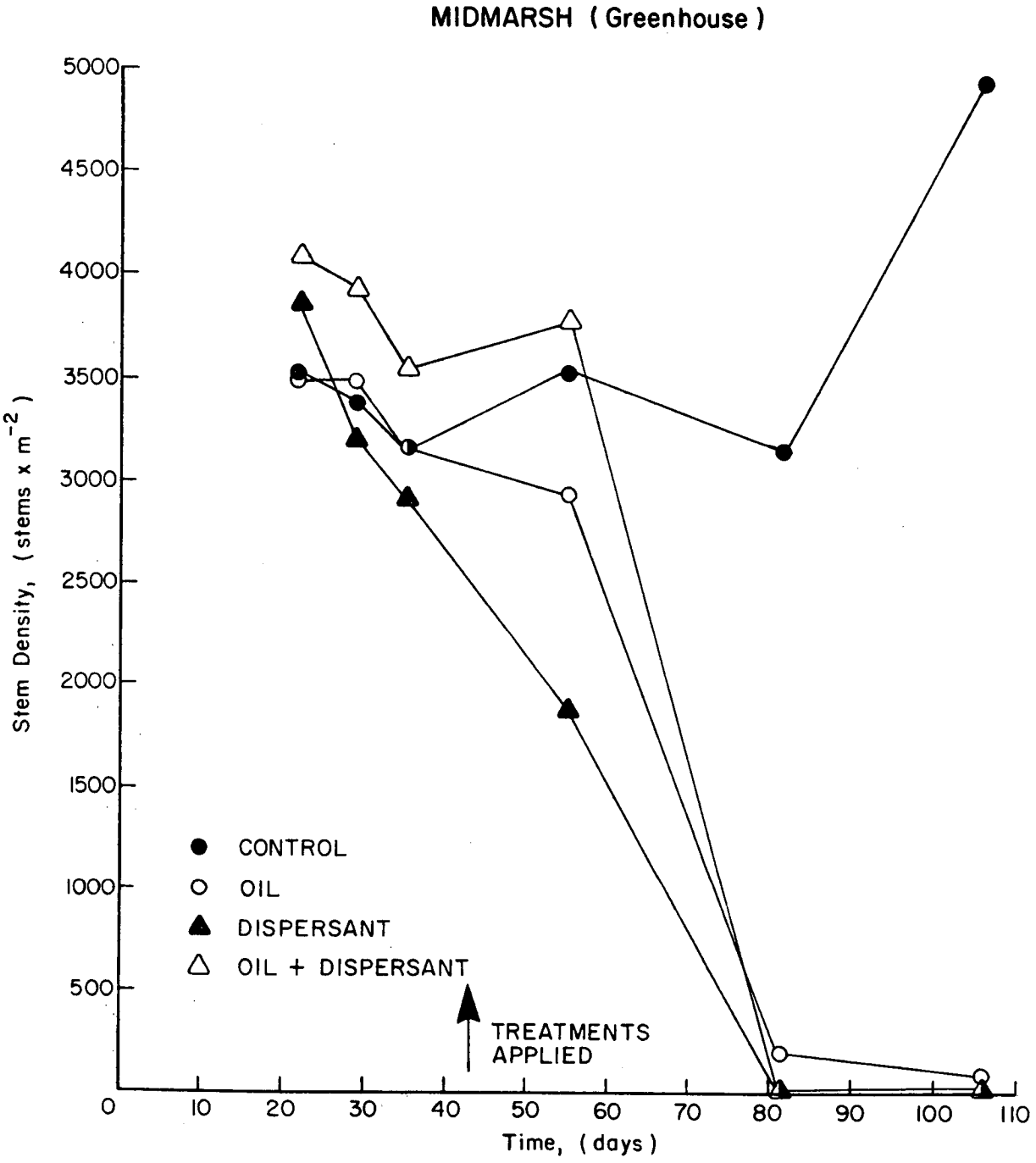

Figure 28: Mean stem density of Spartina alterniflora versus time. Data from control, oil and/or dispersant treated midmarsh zone greenhouse microcosms are presented. Treatments were applied on day 43.

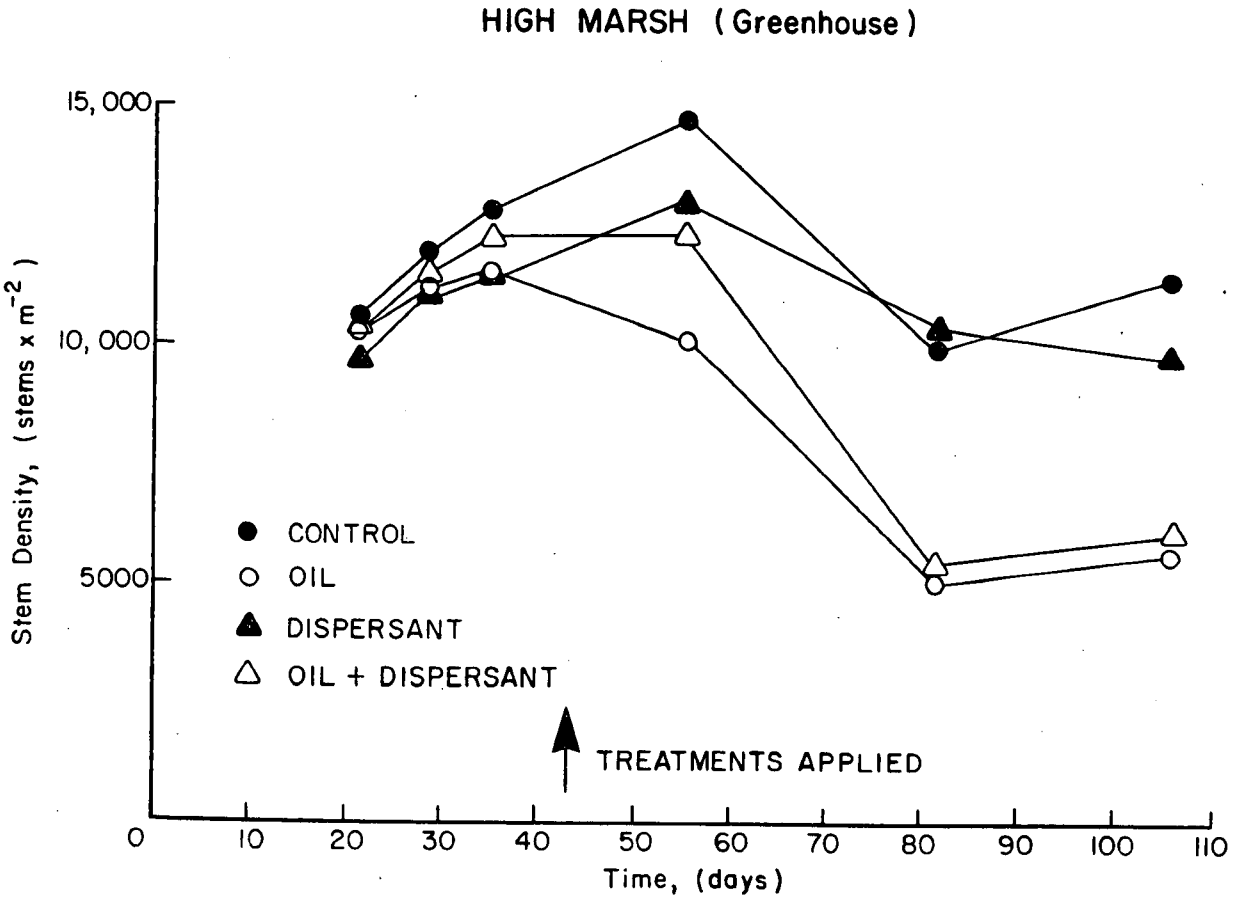

Figure 29: Mean stem density of Spartina alterniflora versus time. Data from control, oil and/or dispersant treated high marsh zone greenhouse microcosms are presented. Treatments were applied on day 43.

and oil plus dispersant had the most impact while the dispersant had no effect.

Flowering shoot density was extremely low and patchy in the greenhouse experiment because of the small size of the microcosms. Consequently, these results have been eliminated from the analysis.

Biomass - The null hypotheses tested in this experiment were:

H_1: Oil has no effect on total above-ground biomass production.

H_2: Dispersant has no effect on total above-ground biomass production.

H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting total above-ground biomass production.

These hypotheses were tested in each of the three vegetation zones. Results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 9.

Figure 20 and Appendix 9 presents the results of the above-ground biomass harvest for the greenhouse experiment. The midmarsh zone was severely impacted by all of the oil and/or dispersant treatments. All treatments caused significant reductions ($\alpha = 0.05$, df=4) in above-ground biomass. Average above-ground living biomass in the oil, dispersant and oil plus dispersant treated microcosms were 13, 2, and 4% of the average for the control microcosms. The null hypotheses H_1 and H_2 were rejected because of these results. In the creek edge zone treatment impacts were intermediate in intensity. Average above-ground biomass for oiled microcosms was 85% of the control (not significantly different) while average above-ground biomass in the dispersant and oil plus dispersant treated microcosms were only 12 and 29% of average control biomass (significantly lower than the control microcosms ($\alpha = 0.05$, df=4)). The null hypothesis H_1 was retained and H_2 was rejected based on these results. The high marsh zone was impacted the least by the oil and/or dispersant treatments. Average above-ground biomass for oil, dispersant and oil plus dispersant treated microcosms were 76, 65 and 63% of the control values. All three treatments were significantly lower than the control. Although the high marsh zone was affected the least, the fact that these relatively small reductions were significant forced the rejection of the null hypotheses H_1 and H_2. No significant interactions were noted between oil and dispersant in the creek edge or high marsh zones, therefore the null hypothesis H_3 was retained in each zone. There was a significant interaction ($\alpha = 0.01$, df=4) in the

midmarsh zone which caused the null hypothesis H_3 to be rejected. Oil appeared to inhibit the toxicity of the dispersant, possibly by forming a layer over the surface of the plant which excluded the more toxic dispersant.

Flowering plants were rare in the greenhouse experiment because of the relatively small size of the microcosms. These data are not included in the data analysis.

Species Cover - The null hypotheses tested in this experiment were:

H_1: Oil has no effect on Spartina alterniflora or S. patens cover.

H_2: Dispersant has no effect on Spartina alterniflora or S. patens cover.

H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting Spartina alterniflora or S. patens cover.

These hypotheses were tested in each of the three vegetation zones. Results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 10.

Figure 30 illustrates the impacts of the oil and/or dispersant treatments on the percent cover of Spartina alterniflora or S. patens in the creek edge, midmarsh, and high marsh zones. S. alterniflora cover in the creek edge zone was reduced significantly ($\alpha = 0.05$, df=4) by all oil and/or dispersant treatments. S. alterniflora cover values in the dispersant and oil plus dispersant treated microcosms were most reduced while S. alterniflora cover in the oiled microcosms was least reduced. The null hypotheses, H_1 and H_2 were rejected on the basis of these results. There was a significant inhibition ($\alpha = 0.05$, df=4) of dispersant toxicity in the oil plus dispersant treated microcosms forcing the rejection of the null hypothesis H_3. The oil film coating the plants may have neutralized the more toxic dispersant as it contacted the plant.

In the midmarsh all oil and/or dispersant treatments induced significant reductions ($\alpha = 0.05$, df=4) in S. alterniflora cover. The dispersant and oil plus dispersant treated microcosms were affected equally and most severely while the oiled microcosms suffered somewhat smaller reductions. The null hypotheses H_1 and H_2 were therefore rejected. As was noted in the creek edge zone, there was a significant inhibition ($\alpha = 0.01$, df=4) of dispersant toxicity by the oil in the oil plus dispersant treatment, causing the null hypothesis H_3 to be rejected.

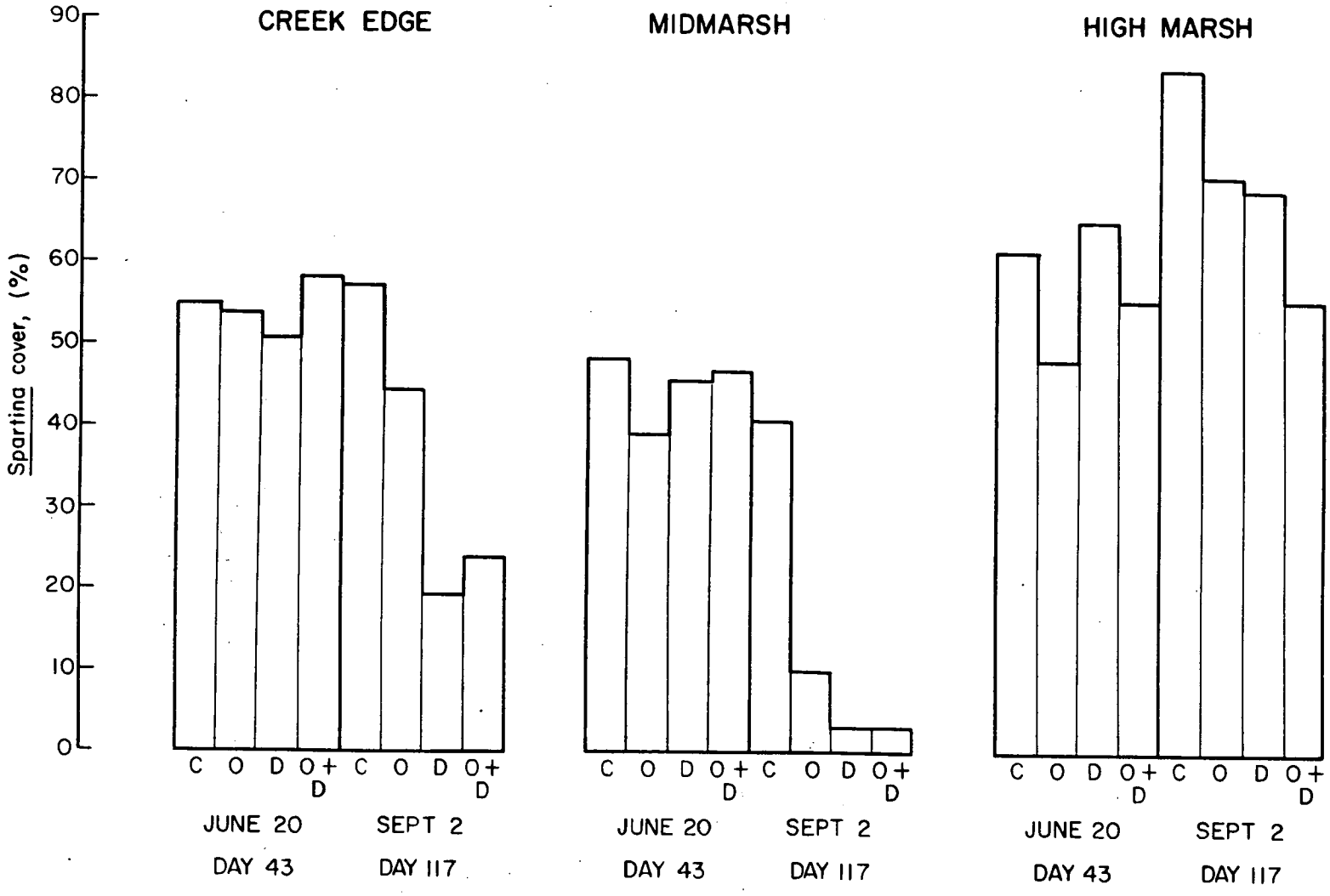

Figure 30: Histogram of mean Spartina alterniflora or S. patens cover in oil and/or dispersant treated creek edge, midmarsh, and high marsh zone microcosms. Data are from the greenhouse study.

In the high marsh zone, S. patens cover was slightly though not significantly reduced by all of the oil and/or dispersant treatments. Cover in the oil plus dispersant treated microcosms was decreased the most, while oil and dispersant treated microcosms experienced similar reductions. No significant interactions were noted between the oil and dispersant. These results justified the retention of the null hypotheses H_1, H_2 and H_3.

In summary, Spartina alterniflora cover was most affected by the oil and/or dispersant treatments in the midmarsh zone and least affected in the high marsh zone. Responses to the treatments were similar in the creek edge and midmarsh zones, with oil having the least impact and dispersant and oil plus dispersant treatments having greater impacts. Dispersant and oil plus dispersant treatments resulted in similar reductions in S. alterniflora cover. The oil significantly inhibited the toxicity of the dispersant in both the creek edge and midmarsh zones. In the high marsh zone, oil and dispersant caused similar reductions in Spartina patens cover with oil plus dispersant treatments causing somewhat greater mortality, however, these reductions were insignificant.

Species other than Spartina alterniflora and S. patens generally composed less than 3% of total cover in any of the vegetation zones (Tables 9-11). Trends in abundance were noted for several of these species. Salicornia europaea declined in cover in all vegetation zones and under all treatment regimes. This species is an annual and had probably reached the end of the vegetative phase of its life cycle by day 117 (September 2), thereby accounting for its reduction in salicornia europaea cover in all treatments. The same trend was noted for another annual, Atriplex patula, however, this species was not found in any of the control pots so it is impossible to determine whether this trend was caused by oil and/or dispersant toxicity or by natural senescence.

Leaf Anatomy - The microscopic symptoms observed in the greenhouse experiment were identical to those of the field experiment.

Fluorometry - The null hypotheses tested in this experiment were:

H_1: Oil has no effect on peak variable fluorescence of Spartina alterniflora or S. patens.

H_2: Dispersant has no effect on peak variable fluorescence of Spartina alterniflora or S. patens

H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting peak variable fluorescence of Spartina alterniflora or S. patens.

Table 9 - Species composition of control, oil and/or dispersant treated greenhouse microcosms in the creek edge zone on two sampling dates. Treatments were applied on day 43.

Species	Treatment	Average Cover (1 Standard Deviation)	
		June 20 Day 43	Sept. 2 Day 117
Spartina alterniflora	Control	55.0(17.3)	57.0(5.7)
	Oil	54.0(12.4)	44.0(9.6)
	Dispersant	51.0(11.9)	19.0(4.2)
	Oil+Dispersant	58.0(9.1)	24.0(13.9)
Salicornia europaea	Control	1.1(0.9)	0.3(0.4)
	Oil	1.7(2.4)	0
	Dispersant	1.2(1.3)	0
	Oil+Dispersant	0.4(0.2)	0
Atriplex patula	Control	0	0
	Oil	0	0
	Dispersant	0.5(0.9)	0
	Oil+Dispersant	0.4(0.4)	0

Table 10 - Species composition of control, oil and/or dispersant treated greenhouse microcosms in the midmarsh zone on two sampling dates. Treatments were applied on day 43.

Species	Treatment	Average Cover (1 Standard Deviation)	
		June 20 Day 43	Sept. 2 Day 117
Spartina alterniflora	Control	48.6(11.6)	41.0(4.2)
	Oil	39.4(7.2)	10.0(3.5)
	Dispersant	46.4(12.6)	3.0(4.5)
	Oil+Dispersant	47.0(2.7)	3.0(4.5)
Spartina patens	Control	0	0.4(0.9)
	Oil	0	0
	Dispersant	0	0
	Oil+Dispersant	0	0
Salicornia europaea	Control	0.3(0.4)	0
	Oil	0	0
	Dispersant	0.1(0.2)	0
	Oil+Dispersant	0	0
Plantago juncoides	Control	1.0(2.2)	0
	Oil	0	0
	Dispersant	0	0
	Oil+Dispersant	0	0

Table 11 - Species composition of control, oil and/or dispersant treated greenhouse microcosms in the high marsh zone on two sampling dates. Treatments were applied on day 43.

Species	Treatment	Average Cover (1 Standard Deviation)	
		June 20 Day 43	Sept. 2 Day 117
Spartina patens	Control	62.0(9.1)	84.0(4.2)
	Oil	48.0(11.5)	71.0(2.2)
	Dispersant	66.0(10.8)	69.0(16.7)
	Oil+Dispersant	56.0(16.0)	56.2(28.1)
Spartina alterniflora	Control	0.2(0.4)	0
	Oil	0	0
	Dispersant	0	0
	Oil+Dispersant	0.2(0.4)	0
Salicornia europaea	Control	0.2(0.4)	0
	Oil	0	0
	Dispersant	1.0(1.7)	0
	Oil+Dispersant	0.2(0.4)	0
Plantago juncoides	Control	3.4(6.5)	7.0(11.0)
	Oil	9.4(19.9)	0.1(0.2)
	Dispersant	2.4(4.3)	4.0(8.9)
	Oil+Dispersant	0	0
Triglochin elata	Control	1.0(2.2)	0
	Oil	0	0
	Dispersant	0	0
	Oil+Dispersant	0	0
Atriplex patula	Control	0	0
	Oil	0	0
	Dispersant	0.4(0.9)	0
	Oil+Dispersant	0	0

H_4: Oil has no effect on 100 second difference values of Spartina alterniflora or S. patens.

H_5: Dispersant has no effect on 100 second difference values of Spartina alterniflora or S. patens.

H_6: There is no interaction (synergistic or inhibitory) between the factors, oil and dispersant affecting 100 second differences values of Spartina alterniflora or S. patens.

These hypotheses were tested in each of the three vegetation zones. Results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 11.

Peak variable fluorescence values for the four treatments are plotted for the creek edge, midmarsh and high marsh zones in Figures 31-33. Control treatments for all of the zones exhibited an increase in peak fluorescence through the growing season, although peak fluorescence for the two Spartina alterniflora zones (creek edge and midmarsh) began to level off between day 69 (middle of July) and day 95 (middle of August).

In the creek edge zone, values for oil treated quadrats were not significantly different from control values throughout the experiment (Figure 31, Appendix 11), except on the day after the treatments were applied (day 43) when they were significantly lower ($\alpha = 0.05$, df=4) than the control. The dispersant and oil plus dispersant treatments deviated significantly ($\alpha = 0.05$, df=4) from the control only on the last day of observation. The Student-Newman-Keuls procedure indicated that all treatments except the control and oil treatments were significantly different from each other on day 96. Based on these results, the null hypothesis H_1 was retained. The null hypothesis H_2, however, was rejected. Although significant reductions were noted on only one day, trends in the data suggest a gradual reduction of peak variable fluorescence for the dispersant and oil plus dispersant treatments. Two way analysis of variance results indicated a significant inhibitory interaction ($\alpha = 0.05$, df=4) between oil and dispersant on day 44, however, this interaction did not persist. The null hypothesis H_3 was therefore retained.

In the midmarsh zone, peak fluorescence values for the oil and/or dispersant treatments (Figure 32, Appendix 11) were generally much lower than control values with the exceptions of the dispersant treatment on day 69 and the oil treatment on day 83. The data, however, were highly variable resulting in inconsistencies in the trends for the statistics. Student-Newman-Keuls procedures indicated that peak fluorescence values for the oil treated pots were significantly higher than those of the oil plus

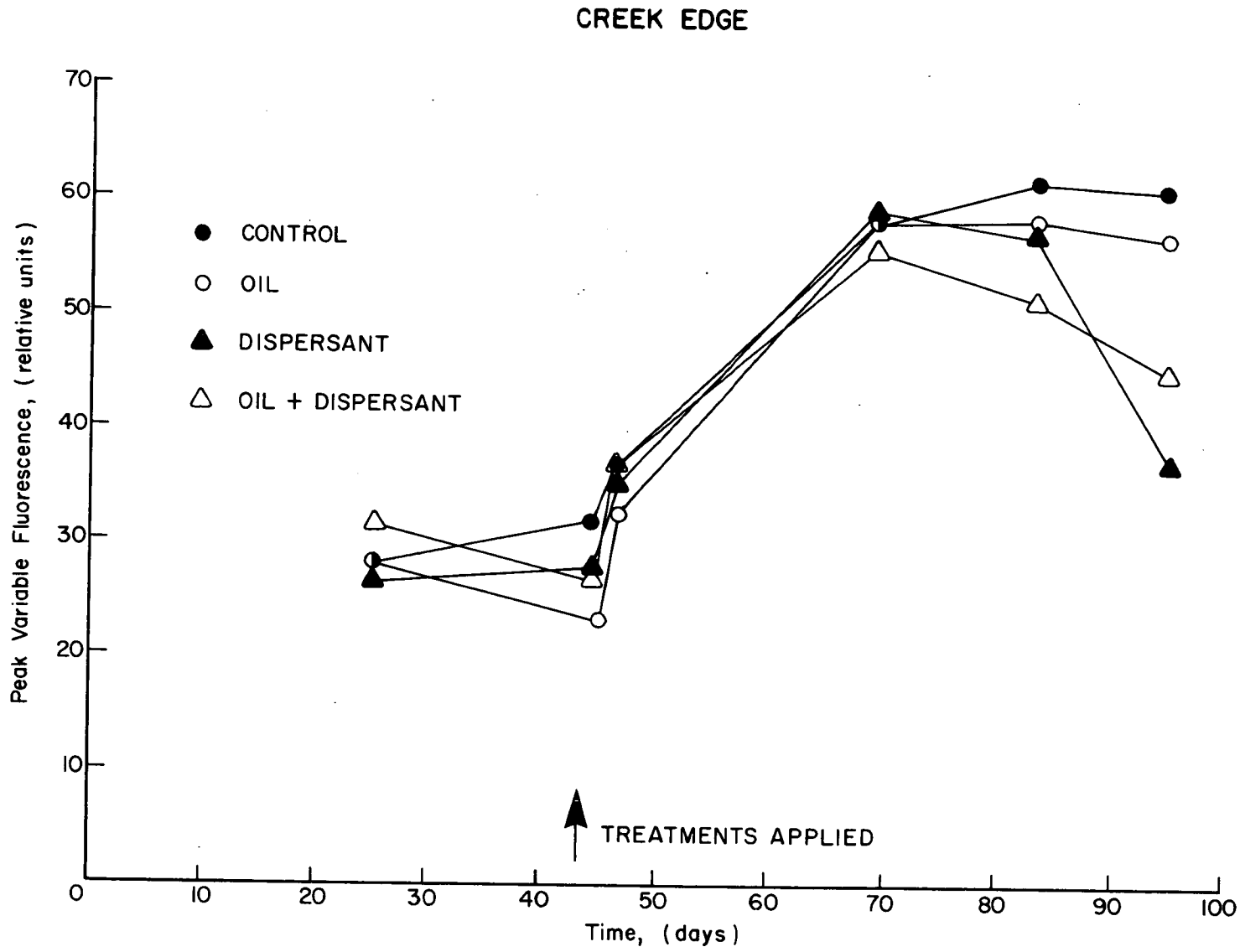

Figure 31: Plot of peak variable fluorescence values versus time for control, oil and/or dispersant treated plants from the creek edge microcosms on six sampling dates. Treatments were applied on day 43.

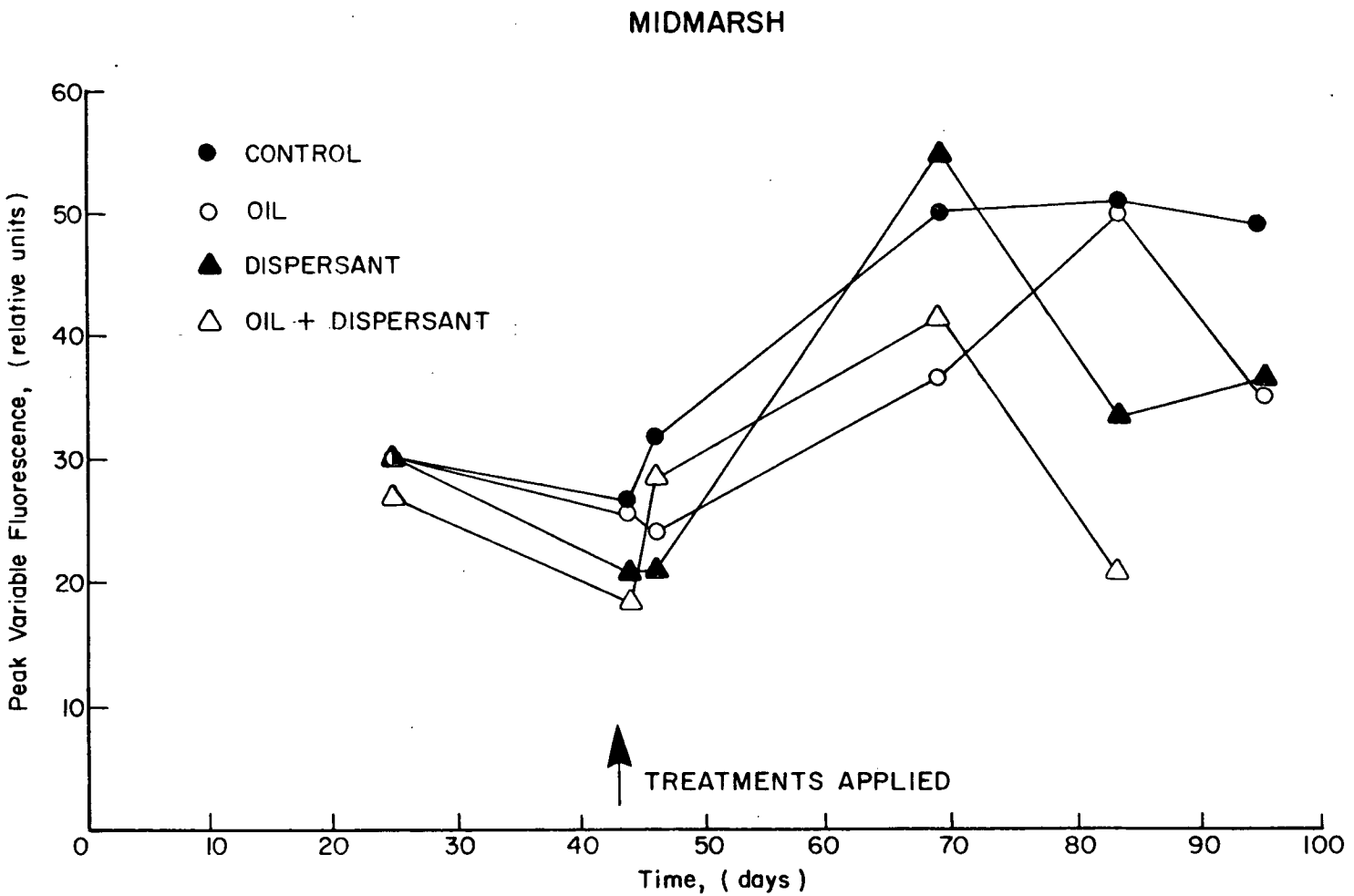

Figure 32: Plot of peak variable fluorescence values versus time for control, oil and/or dispersant treated plants from the midmarsh microcosms on six sampling dates. Treatments were applied on day 43. Data for oil plus dispersant treated plants were unavailable on day 95.

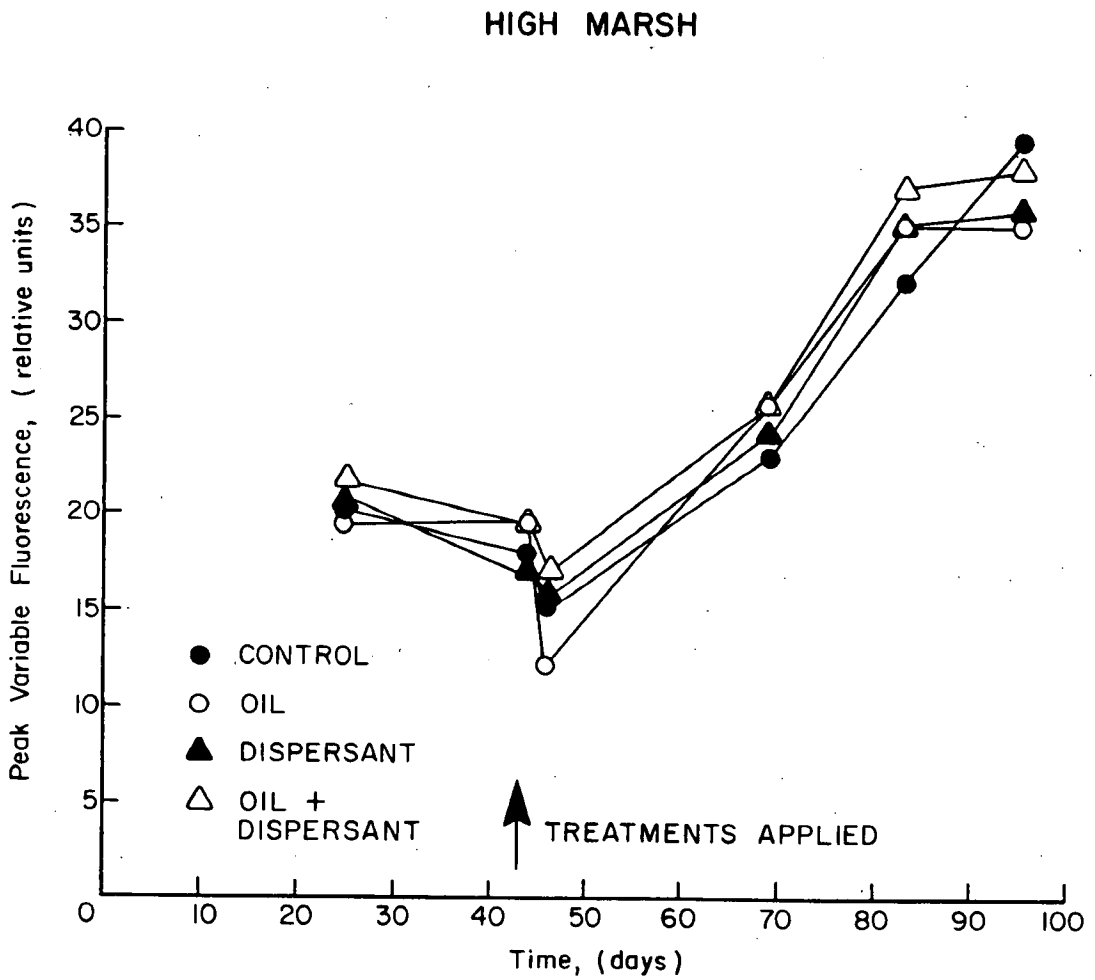

Figure 33: Plot of peak variable fluorescence values versus time for control, oil and/or dispersant treated plants from the high marsh microcosms on six sampling dates. Treatments were applied on day 43.

dispersant treated pots on day 44 (α= 0.05, df=4) while on day 46 the only significant difference was between the control and dispersant pots (α = 0.05, df=4). On day 69, peak fluorescence values for the oiled pots were significantly lower (α= 0.05, df=4) than both the control and dispersant pots. Peak variable fluorescence for the oil plus dispersant treated pots was significantly lower (α= 0.05, df=4) than those of all other treatments on day 83. Lack of data for the oil plus dispersant treated plots prevented statistical analysis of the fluorometry results of day 95. Although significant reductions in peak variable fluoresance associated with the various treatments were not consistent, the overall trend suggests that the treatments produced a considerable impact. Therefore the null hypotheses H_1 and H_2 were rejected. Results of the two-way analysis of variance indicated a significant inhibitory interaction (α= 0.05, df=4) between oil and dispersant on day 46 which did not persist. The lack of persistance of the interaction suggested that it may have been an anomally possibly caused by sampling problems. The null hypothesis H_3 was therefore retained.

The unusually high peak fluorescence readings for the dispersant treated microcosms on day 69 and the oil treated microcosms on day 83 correspond to periods following large reductions in stem density. Surviving plants were generally concentrated around the edges of the microcosms where exposure to the oil or dispersant was lowest. Selection of leaves for analyses was restricted to these relatively healthy plants, consequently, an increase in peak fluorescence might be expected at this time. The decline in peak fluorescence following these peaks corresponded to a gradual deterioration of the condition of these surviving plants.

In the high marsh zone, peak fluorescence values for the oil and/or dispersant treatments were not significantly different from those of the control (Figure 33, Appendix 11) at any time. No significant interactions between oil and dispersant were noted. Based on these results, the null hypotheses H_1, H_2 and H_3 were retained.

One hundred second difference values for the four treatments are plotted against time for the creek edge, midmarsh, and high marsh zones (Figures 34-36). In the creek edge zone, (Figure 34, Appendix 11), 100 second difference values for oil treated microcosms were not significantly different from control values on any sampling date. One hundred second difference values for dispersant and oil plus dispersant treatments declined within one day of treatment applications and remained low, however, these reductions were statistically significant (α= 0.05, df=4) only on days 69, 83 and 95. These results indicated that the null hypothesis H_4 was true while H_5 was false. No significant interactions

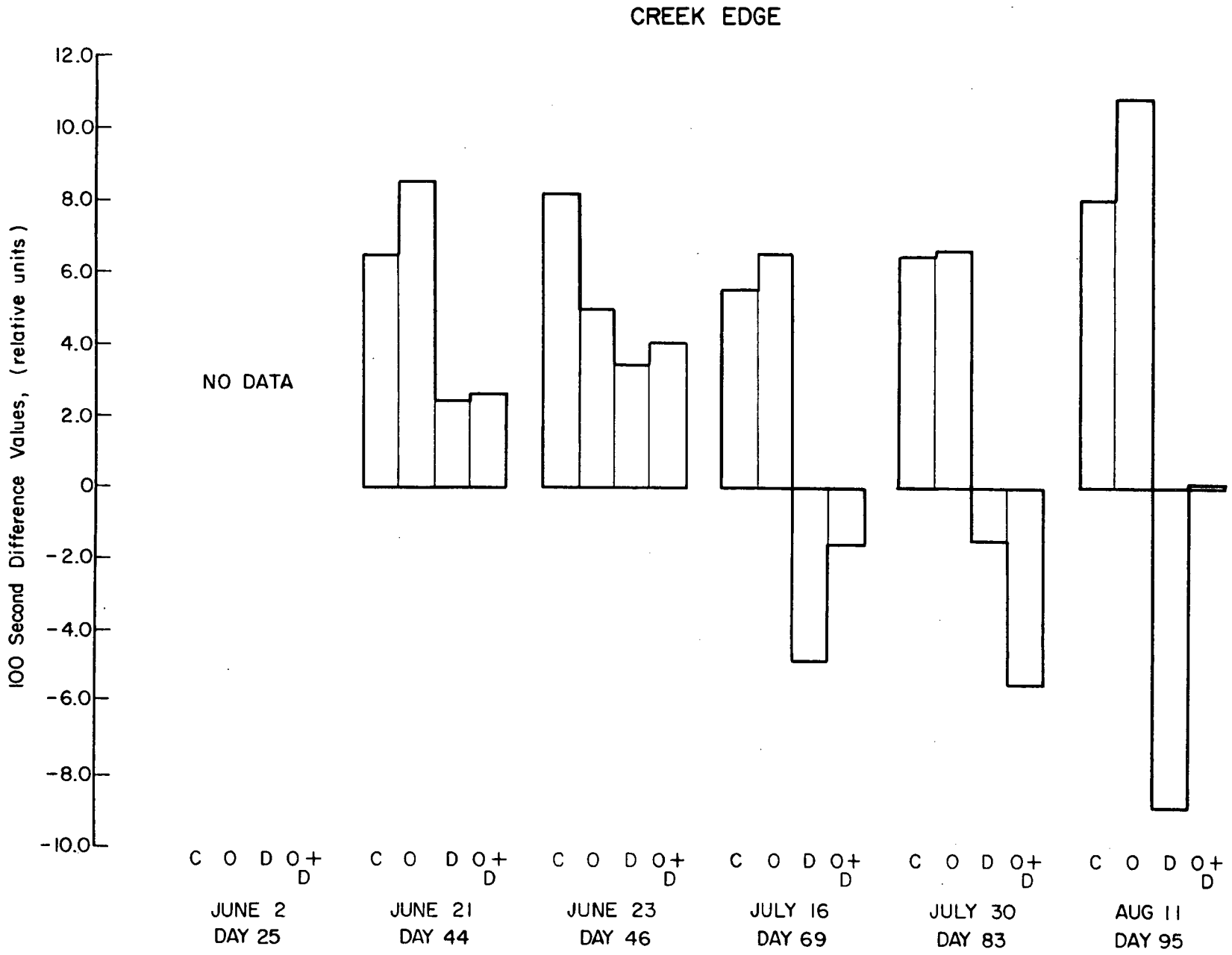

Figure 34: Histogram of one hundred second difference values for control, oil and/or dispersant treated plants from the creek edge microcosms on five sampling dates. Treatments were applied on day 43. Pretreatment data were unavailable on day 25.

were noted between the oil and dispersant suggesting that the null hypothesis H_6 was true.

In the midmarsh zone (Figure 35, Appendix 11), all oil and/or dispersant treatments resulted in declines in 100 second difference values. Values for the dispersant and oil plus dispersant treated plants declined within one day of teatment application (day 44). Two way analysis of variance results indicated a significant reduction (α= 0.01, df=4) in 100 second difference values for the dispersant treatment on this day, however, multiple range tests indicated no significant reductions. Oil treated plants exhibited no reductions in 100 second difference values at this time. On day 46, values for all treatments were reduced relative to the control, however, only the dispersant and oil plus dispersant treated plants exhibited significant reductions (α= 0.05, df=4). By day 69, all oil and/or dispersant treatments had significantly reduced (α= 0.05, df=4) 100 second difference values. Based on these results, the null hypotheses H_4 and H_5 were rejected. Analysis of variance results for day 69 indicated a significant inhibitory interaction (α= 0.01, df=4) between oil and dispersant which was also evident on day 83. The null hypothesis H_6 was rejected as a consequence of these results.

One hundred second difference values for oil treated microcosms had begun to recover by day 83 and were no longer significantly different from the control, while the dispersant and oil plus dispersant treated plants still had significantly reduced values (α= 0.05, df=4). Data for day 95 indicated continued recovery of 100 second difference values for oil treated plants and the beginning of a recovery phase for dispersant treated plants. Statistical analyses were not performed on the data for this day since data for oil plus dispersant treated plants were unavailable. The onset of recovery phases was associated with a large reduction in stem density. Selection of plants was thus restricted to relatively healthy plants concentrated around the edges of the microcosms. This suggests that the apparent recovery of oil and dispersant treated plants is erroneous.

One hundred second difference values for the high marsh zone were relatively unaffected by the treatments (Figure 36, Appendix 11). Values for dispersant and oil plus dispersant treatments were significantly reduced (α= 0.05, df=4) one day after treatment applications but recovered to control levels within three days. On day 46 oil and dispersant treatments had 100 second difference values higher than the control, and on day 69, dispersant and oil plus dispersant treated plants had elevated values, however, these fluctuations were not statistically significant. On days 83 and 106, there were no differences between treatments. The null hypotheses H_4 and H_5 were threfore retained. No significant interactions

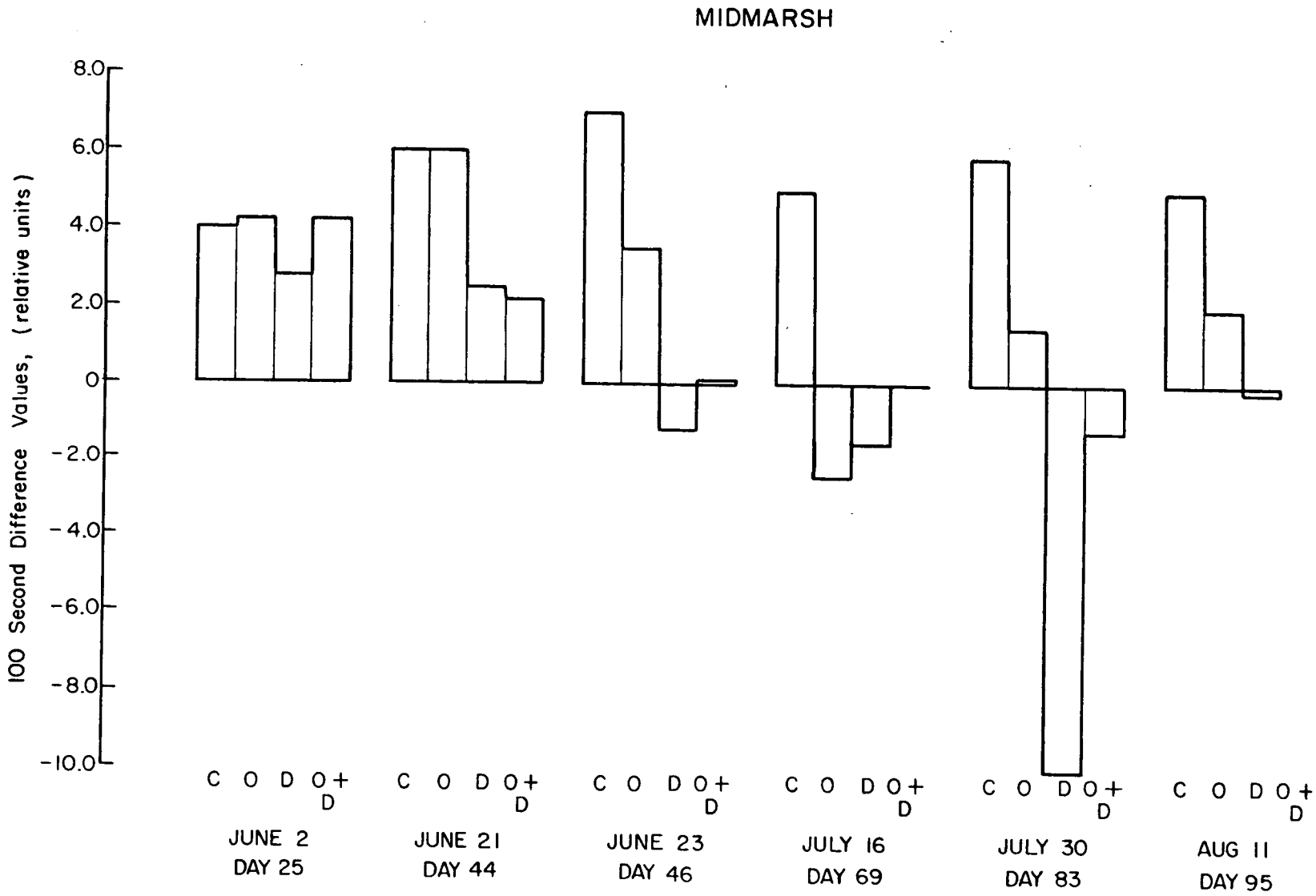

Figure 35: Histogram of one hundred second difference values for control, oil and/or dispersant treated plants from the midmarsh microcosms on five sampling dates. Treatments were applied on day 43.

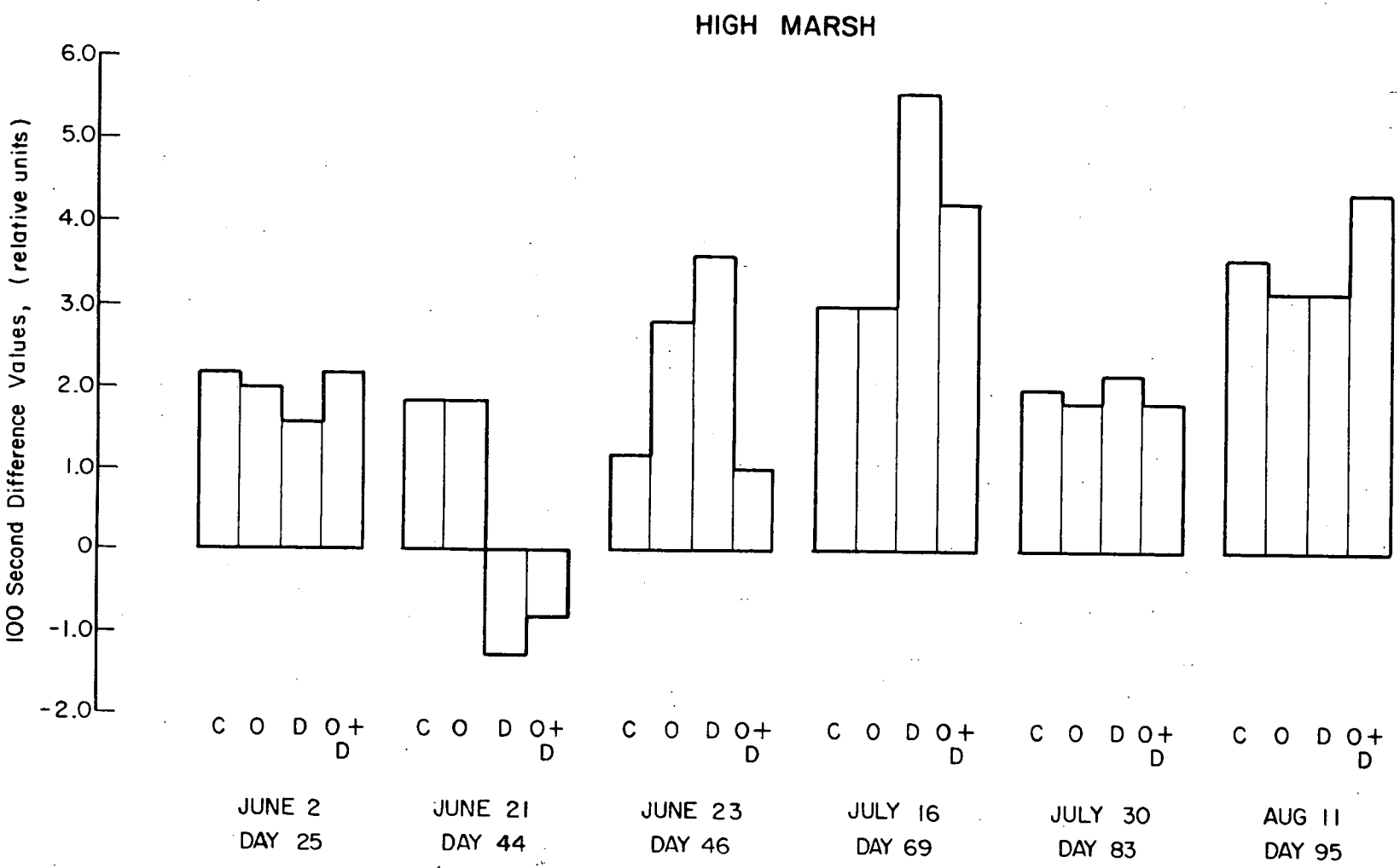

Figure 36: Histogram of one hundred second difference values for control, oil and/or dispersant treated plants from the high marsh microcosms on six sampling dates. Treatments were applied on day 43.

were noted between the oil and dispersant treatments; therefore, the null hypothesis H_6 was also retained.

Fluorescence induction measurements provide evidence of damage to the photosynthetic systems of Spartina alterniflora and S. patens in all three vegetation zones. Plant stress was apparent within one day of treatment applications, two days before any visible signs of stress were noticed. Fluorescence effects varied between treatments within zones in the same way that other symptoms varied, providing a useful forecasting method for other symptoms. Damage to photosynthetic systems was heaviest in the midmarsh zone and lightest in the high marsh zones. In the creek edge and midmarsh zones, plant stress lasted for the duration of the experiment while in the high marsh zone it was temporary. These data suggest that S. patens is more tolerant of oil and/or dispersant than S. alterniflora.

Dispersant alone was the most toxic treatment in all vegetation zones. The oil plus dispersant treatments generally induced less stress than the dispersant treatment. Oil was applied to these plants before the dispersant and may have acted as a barrier to entry of the dispersant into plant tissues, however, this inhibitory interaction was significant only in the midmarsh microcosms. Oil treatments caused relatively little stress in all but the midmarsh zone.

The 100 second difference values were the most sensitive measure of toxic effects, indicating plant stress within one day of treatment application. Peak fluorescence was much less sensitive, reliably indicating stress only near the time of death of the plants when visible symptoms were apparent.

The fluorescence effects observed consisted of reduced peak fluorescence and a faster return to initial values. These are suggestive of damage to the light receptor system.

Comparison of Vascular Plant Effects Noted in the Field and Greenhouse Experiments

The results of the field and greenhouse experiments involving vascular plants were generally very similar. Table 12 summarizes the results of each of the field and greenhouse experiments conducted on the vascular plants. Treatments are ranked in increasing magnitude of the parameter studied and the greater than, less than, or equal to signs indicate the relationships between treatments. There were only minor differences between field and greenhouse results for the plant height, Spartina cover and leaf anatomy experiments. The largest differences between field and greenhouse results were found in the stem density experiment. The

Table 12 - Summary table comparing results from the field and greenhouse experiments on vascular plants. Treatments and zones are arranged in descending order of impact. Signs indicate impact relationships between treatments or zones. C=creek edge; M=midmarsh; H=high marsh; C=control; O=oil; D=dispersant; O+D=oil+dispersant.

		Field Experiment		Greenhouse Experiment	
Parameter	Zone	Order of Impact	Overall Impact	Order of Impact	Overall Impact
Plant Height	C	D>O+D>O=C	M>C>H	D>O+D>O=C	M>C>H
	M	D>O+D>O=C		D>O+D>O=C	
	H	D=O+D=O=C		D=O+D=O=C	
Plant Density	C	O+D>D=O>C	M>C>H	O+D=D>O=C	M>H=C
	M	O+D>D=O>C		O+D=D=O>C	
	H	O>O+D=D>C		O=O+D>D=C	
Aboveground Biomass	C	O+D=D>O=C	M>C>H	O+D=D>O=C	M>C>H
	M	O+D=D>O>C		O+D=D=O>C	
	H	D=O+D=O=C		O+D=D=O>C	
Spartina Cover	C	O+D>D>O>C	M>C>H	D=O+D>O>C	M>C>H
	M	O+D=D=O>C		O+D=D=O>C	
	H	O=D=O+D>C		O+D=D=O>C	
Leaf Anatomy		Symptoms similar in all zones and between all oil and/or dispersant treatments.		Symptoms similar in all zones and between all oil and/or dispersant treatments.	

greenhouse experiment indicated that the creek edge and high marsh zones were equally susceptible to the oil and/or dispersant treatments while the field results agreed with all other parameters by indicating that the high marsh zone was the least susceptible. Field and greenhouse results for the high marsh zone indicated that oil was marginally more toxic than oil + dispersant and that the dispersant had the least impact. Field and greenhouse results differed somewhat in that dispersant was relatively more toxic in the field experiment than in the greenhouse experiment. Larger differences were noted between the field and greenhouse results for the creek edge zone. Large reductions in stem density were associated with the oil plus dispersant treatments in the field, however, reductions associated with oil plus dispersant treatments in the greenhouse were insignificant. A major reason for the relatively poor correspondence between the creek edge field and greenhouse results was the high variability of stem density between plots or microcosms which may have masked treatment related trends and altered the interaction of the treatments with the plants.

Stem density was consistently higher in the green house study than in the field. Greenhouse creek edge, midmarsh, and high marsh stem density values for controls were on average 1.7, 1.5 and 4.0 times higher than those in the field study. In the greenhouse experiment detritus was removed for use in another experiment. This decreased the shading of the sediment surface (particularly in the high marsh zone which maintains a very large detrital standing crop) and increased the survival rates of new tillers or stimulated their production.

Above-ground biomass values for various treatments in the creek and midmarsh vegetation zones were very similar, however, substantial differences were noted between the field and greenhouse experiments in the high marsh zone. Above-ground biomass was consistently higher in the greenhouse experiment than in the field experiment. This is attributable to the stimulation of tiller production associated with the removal of detritus in the green house high marsh microcosms.

In the field experiment only the dispersant treatment caused significant reductions in biomass, while in the field experiment, all treatments caused a small but significant reduction in biomass. Relative reductions of above-ground biomass were actually higher in the field experiment than in the greenhouse experiment. In the field experiment treated plots produced between 39 and 70% of control biomass, while in the greenhouse experiment, they produced between 63 and 76%. The biomass reductions in the greenhouse study were significant because of the unusually low variances associated with the means of the various treatments.

Impacts on Microbial Populations

Field Experiment

Algal and Bacterial Cover - Statistical analysis was restricted to the algal mat component since it was generally the most abundant microbial type. The null hypotheses to be tested were:

H_1: Oil has no effect on algal cover.
H_2: Dispersant has no effect on algal cover.
H_3: There is no interaction (synergistic or inhibitory) between the factors, oil and dispersant affecting algal cover.

These hypotheses were tested in each of the three vegetation zones. The results of the Student-Newman-Keuls multiple range tests and the two-way analyses of variance procedures are summarized in Appendix 12.

The distributions of the four microbial zones noted in the field study were very patchy which made interpretation of the data difficult. In the creek edge zone (Table 13, Appendix 12), algal mat, sulfur-oxidizing bacteria and sulfate reducing bacteria communities were noted, however, only the first two types occurred consistently. Between days 75 and 125 (15-65 days after treatment application), algal cover remained relatively stable in the control and oil plus dispersant treatments, increased greatly in the dispersant treated plots and disappeared in the oiled plots. On day 75 there was no significant difference between algal cover on any of the treatments, however, on day 125 algal cover on the oiled plots was significantly lower ($\alpha = 0.05$, df=2) than the control while algal cover in the dispersant and oil plus dispersant treated plots was signficantly higher ($\alpha = 0.05$, df=2) than the control, suggesting that the oil treatment was relatively more toxic than the dispersant treatment. No significant interactions were noted between the oil and dispersant treatments; therefore, the null hypothesis H_3 was retained. Based on these results, the null hypothesis H_1 was rejected. Although a significant change in algal mat cover was associated with the dispersant treatment (consequently H_2 must be rejected), it is unclear whether this was directly or indirectly attributable to the dispersant. The increase in algal cover in the dispersant treated plots was probably caused by the increased availability of light and nutrients associated with the decline of the macrophyte over story.

Sulfur-oxidizing bacteria declined between days 75 and 125 in all treatments suggesting that normal seasonal abundance patterns were not affected by the treatments. The high cover value for the oil treated plots

Table 13 - Algal and bacterial cover in the creek edge field plots on two sampling dates. Treatments were applied on day 60.

Treatment	Algal and Bacterial Types	Average Cover (1 Standard Deviation)	
		July 22 Day 75	Sept. 10 Day 125
Control	Algal Mat	0.03(0.06)	0.3(0.6)
	Sulfur-Oxidizing Bacteria	1.7(2.9)	0.7(1.2)
	Sulfate-Reducing Bacteria	0	1.0(1.7)
Oil	Algal Mat	1.3(2.2)	0
	Sulfur-Oxidizing Bacteria	17.1(14.6)	3.3(5.8)
Dispersant	Algal Mat	2.3(3.9	36.7(30.6)
Oil plus	Algal Mat	3.9(4.3)	5.3(4.5)
Dispersant	Sulfur-Oxidizing Bacteria	1.3(2.2)	0

on day 75 suggests that the oil may have directly (through direct contribution of sulfur compounds) or indirectly (through alteration of microbial metabolism), provided a source of hydrogen sulfide.

In the midmarsh zone (Table 14, Appendix 12), all four microbial types were observed, however, only the algal mat and sulfur-oxidizing bacteria were found consistently. The algal cover in the control, oil, and dispersant plots remained relatively stable over time. Algal mat cover in the oil plus dispersant treated plots increased between days 75 and 125. There was no significant difference between the algal covers of any of the treatments on day 75 or 125. These data suggest that the algal mat in the midmarsh zone was relatively tolerant of all treatments. Therefore, the null hypotheses H_1 and H_2 were retained. The increase in algal mat cover in the oil plus dispersant treated plots may have been caused by the increased availability of light and nutrients associated with the decline of the macrophyte over story. No significant interactions were noted between the oil and dispersant, therefore, the null hypothesis H_3 was retained

The sulfur-oxidizing bacteria in the midmarsh zone responded in the same manner as that in the creek edge zone. The cover of this community declined in all treatments suggesting that normal seasonal patterns of abundance were not affected by the treatments, however, cover in the oil and oil plus dispersant treatments were unusually high suggesting direct or indirect stimulation of this community by the oil applications.

Only the algae and sulfur-oxidizing bacteria were found in the high marsh zone (Table 15, Appendix 12). Algal cover disappeared in the control and dispersant treated quadrats between days 75 and 125 while in the oiled plots, it increased. Although this increase was large, the oiled plots did not have significantly higher cover on day 125 as a result of the large variance associated with the cover in this treatment. No significant difference was found between treatments on day 75. The increase in algal cover may be related to the large reduction of stem density in the oiled plots which would make light and nutrients available to the algal mat. The results suggest that the treatments had little direct effect on algal cover in the high marsh zones, therefore, the null hypotheses H_1 and H_2 were retained. No significant interaction was noted between the oil and dispersant. Based on this result, the null hypthosis H_3 was retained.

The cover of sulfur-oxidizing bacteria in the high marsh zone responded to time and treatments in the same manner as it did in the creek edge and midmarsh zones.

Table 14 - Algal and bacterial cover in the midmarsh field plots on two sampling dates. Treatments were applied on day 60.

Treatment	Algal and Bacterial Types	Average Cover (1 Standard Deviation)	
		July 22 Day 75	Sept. 10 Day 125
Control	Algal Mat	29.2(33.8)	17.3(28.3)
	Sulfur-Oxidizing Bacteria	1.7(2.9)	0
Oil	Algal Mat	12.1(11.3)	18.3(2.9)
	Sulfur-Oxidizing Bacteria	20.4(32.2)	0
	Sulfate-Reducing Bacteria	0	1.7(2.9)
Dispersant	Algal Mat	1.7(2.9)	2.0(2.6)
	Sulfur-Oxidizing Bacteria	2.9(5.1)	0
Oil plus Dispersant	Algal Mat	8.0(1.4)	45.0(8.7)
	Sulfur-Oxidizing Bacteria	25.0(10.9)	0
	Photosynthetic Bacteria	1.7(2.9)	0

Table 15 - Algal and bacterial cover in the high marsh field plots on two sampling dates. Treatments were applied on day 60.

Treatment	Algal and Bacterial Types	Average Cover (1 Standard Deviation)	
		July 22 Day 75	Sept. 10 Day 125
Control	Algal Mat	0.8(1.4)	0
	Sulfur-Oxidizing Bacteria	0.4(0.8)	0
Oil	Algal Mat	0.2(0.3)	25.0(35.0)
	Sulfur-Oxidizing Bacteria	3.9(5.3)	0
Dispersant	Algal Mat	1.2(1.3)	0
Oil plus Dispersant	Sulfur-Oxidizing Bacteria	0.03(0.06)	0

Greenhouse Experiment

Algal and Bacterial Cover - As in the field experiment, statistical testing was conducted on only the algal cover data. the null hypotheses tested in this experiment were:

H_1: Oil has no effect on algal cover.
H_2: Dispersant has not effect on algal cover.
H_3: There is no interaction (synergistic or inhibitory) between the factors, oil and dispersant affecting algal cover.

These hypotheses were tested on the creek edge, midmarsh and high marsh microcosms. The results of the Student-Newman-Keuls multiple range tests and two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 13.

In the creek edge zone (Table 16, Appendix 13), only algae and sulfate-reducing bacteria were noted. The algal cover of the control and oil plus dispersant treatments remained relatively stable from day 39 to day 106. In the oiled microcosms algal cover was reduced on day 67 (24 days after oiling) but returned to the pretreatment level by day 106. Algal cover in dispersant treated microcosms increased through the growing season. No significant differences in algal cover between treatments were found on days 39 and 67. On day 106, the dispersant treated plots had significantly higher ($\alpha = 0.05$, df=4) cover than the control. These results suggest that the oil and/or dispersant treatments had little direct effect on algal cover. The reduction in algal cover in the oiled plots was temporary and statistically insignificant, therefore, the null hypothesis H_1 was retained. The significant increase in algal mat cover associated with the dispersant treatment suggests that the null hypothesis H_2 should be rejected. It is not clear, however, whether or not the dispersant directly affected the algae. It is likely that the dispersant indirectly stimulated algal mat development through the destruction of the macrophyte canopy which would increase the availability of light and nutrients to the algae. Significant interactions between the oil and dispersant were not observed. The null hypothesis H_3 was therefore, retained.

Sulfate-reducing bacteria were not observed in the microcosms until day 106. Similar cover values were observed in the control, oil and/or dispersant treatments suggesting that these treatments had no impact on sulfate-reducing bacteria. The development of reducing zones late in the summer (day 106) would be expected since peak heterotrophic activity at this time would bring anaerobic zones closer to the surface.

Table 16 - Algal and bacterial cover in the creek edge microcosms on three sampling dates. Treatments were applied on day 43.

Treatment	Algal and Bacterial Types	Average Cover (1 Standard Deviation)		
		June 16 Day 39	July 14 Day 67	Aug. 22 Day 106
Control	Algal Mat	6(9)	15(21)	11(15)
	Sulfur-Oxidizing Bacteria	0	0	3(7)
Oil	Algal Mat	20(21)	2(3)	16(20)
	Sulfur-Oxidizing Bacteria	0	0	1(2)
Dispersant	Algal Mat	18(16)	26(34)	50(32)
	Sulfate-reducing Bacteria	0	0	2(3)
Oil plus Dispersant	Algal Mat	10(12)	8(13)	15(7)

All four algal and/or bacterial types were observed in the midmarsh zone (Table 17, Appendix 13). The algal mat community was particularly well developed in this zone. In the control and dispersant treated microcosms algal cover remained relatively stable over time, while in the oil and oil plus dispersant treated microcosms it was significantly reduced (α= 0.05, df=4) on day 67 but recovered to the control level by day 106. There were no significant differences between treatments on days 39 and 106. This evidence suggests that the oil treatments caused an acute reduction in algal cover (probably by smothering of the algae) which quickly dissipated. The null hypothesis H_1 was rejected. The null hypothesis H_2, however was retained. No significant interactions were noted between the oil and dispersant, therefore, the null hypothesis H_3 was retained.

Cover values for sulfur reducing and sulfur oxidizing bacteria were reduced somewhat by the dispersant treatment and remained low through the experiment. Cover values in the other treatments, however, remained stable, suggesting that oil and dispersed oil had no affect on the sulfate reducing and sulfur oxidizing bacterial types.

Photosynthetic bacteria were found in all oil and/or dispersant treated microcosms following the application of treatments. Cover of this type peaked 24 days after treatment applications (day 67) then declined to low levels by day 106. Cover values were highest in the dispersant treated pots on day 67. Spartina cover was reduced by approximately 90% in these microcosms at this time, resulting in increased availability of light and nutrients to the pink photosynthetic bacteria. Expansion of the algal mat eventually replaced or obscured the photosynthetic bacteria.

Algae were the only microbial type observed in the high marsh zone (Table 18, Appendix 13). Algal cover in this zone generally increased over time in all treatments. In the oil and oil plus dispersant treated microcosms, a reduction in algal cover was noted; however, it was not statistically significant and cover values returned to the level of the control by day 106. No significant differences in algal cover between treatments were found on any of the sampling dates, nor were significant interactions noted between the treatments. Therefore, the null hypotheses H_1, H_2 and H_3 were retained.

In summary, algal cover data for all three vegetation zones exhibited the same trend. Microcosms receiving oil or oil plus dispersant treatments experienced a decline in algal cover followed by a fairly rapid recovery. A similar trend was noted by Baker (1971b). The dispersant treatment

Table 17 - Algal and bacterial cover in the midmarsh microcosms on three sampling dates. Treatments were applied on day 43.

Treatment	Algal and Bacterial Types	Average Cover (1 Standard Deviation)		
		June 16 Day 39	July 14 Day 67	Aug. 22 Day 106
Control	Algal Mat	96(6)	99(2)	94(6)
	Sulfate-Reducing Bacteria	2(5)	1(1)	4(6)
	Sulfur-Oxidizing Bacteria	2(5)	1(1)	2(3)
	Photosynthetic Bacteria	0	0	0
Oil	Algal Mat	88(18)	6(8)	69(44)
	Sulfate-Reducing Bacteria	10(17)	5(3)	8(15)
	Sulfur-Oxidizing Bacteria	2(5)	1(1)	4(6)
	Photosynthetic Bacteria	0	1(1)	1(1)
Dispersant	Algal Mat	78(18)	68(29)	99(1)
	Sulfate-Reducing Bacteria	10(7)	2(2)	1(1)
	Sulfur-Oxidizing Bacteria	12(13)	2(1)	1(1)
	Photosynthetic Bacteria	0	27(29)	0
Oil plus Dispersant	Algal Mat	96(9)	11(14)	88(6)
	Sulfate-Reducing Bacteria	2(5)	5(11)	5(6)
	Sulfur-Oxidizing Bacteria	2(5)	6(7)	6(5)
	Photosynthetic Bacteria	0	4(2)	1(1)

Table 18 - Algal and bacterial cover in the high marsh microcosms on three sampling dates. Treatments were applied on day 43.

Treatment	Algal and Bacterial Types	Average Cover (1 Standard Deviation)		
		June 16 Day 39	July 14 Day 67	Aug. 22 Day 106
Control	Algal Mat	6(13)	8(18)	16(36)
Oil	Algal Mat	4(9)	1(2)	14(31)
Dispersant	Algal Mat	0	0	8(12)
Oil plus Dispersant	Algal Mat	2(5)	0	8(13)

appeared to have little impact on the algal community. The oil and/or dispersant treatments also had little long-term impact on the other microbial zones.

Heterotrophic Activity - The null hypotheses tested in this experiment were:

- H_1: Oil has no effect on hetrotrophic activity in salt marsh sediments.
- H_2: Dispersant has no effect on heterotropic activity in salt marsh sediments.
- H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting heterotrophic activity in salt marsh sediments.

These hypotheses were tested for sediments from the midmarsh zone. The results of the Student-Newman-Keuls multiple range tests and the two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 14.

Data on hetrotrophic activity (measured by carbon dioxide evolution) are presented in Figure 37, Appendix 14). Pre-treatment heterotrophic activity rates were similar for all treatments. The oil treatment significantly reduced heterotrophic activity on days 96 and 99 (4 and 7 days after treatment applications). Heterotrophic activity returned to normal 13 days after oiling (day 105) and was significantly higher (α= 0.05, df=4) than the controls 26 and 35 days after oiling (days 118 and 127). Based on these results, the null hypothesis H_1 was rejected.

The dispersant treatment had no significant impact on heterotrophic activity four days after treatment application (day 96), however, 7, 13 and 26 days after treatment (days 99, 105 and 118), heterotrophic activity was significantly higher (α= 0.05, df=4) than control values. Heterotrophic activity returned to control levels within 35 days of treatment application (day 127). Based on these results the null hypothesis H_2 was rejected.

The oil plus dispersant treatment resulted in heterotrophic activity which was intermediate between the oil and dispersant treatments. Four days after treatment (day 96), heterotrophic activity was significantly reduced (α= 0.05, df=4) relative to the control and was very similar to the oil treatment. Heterotrophic activity for the oil plus dispersant treatment was not significantly different from the control 7, 13 and 26 days after treatment applications (days 99, 105 and 118). Thirty-five days after treatment applications (day 127), heterotrophic activity was significantly

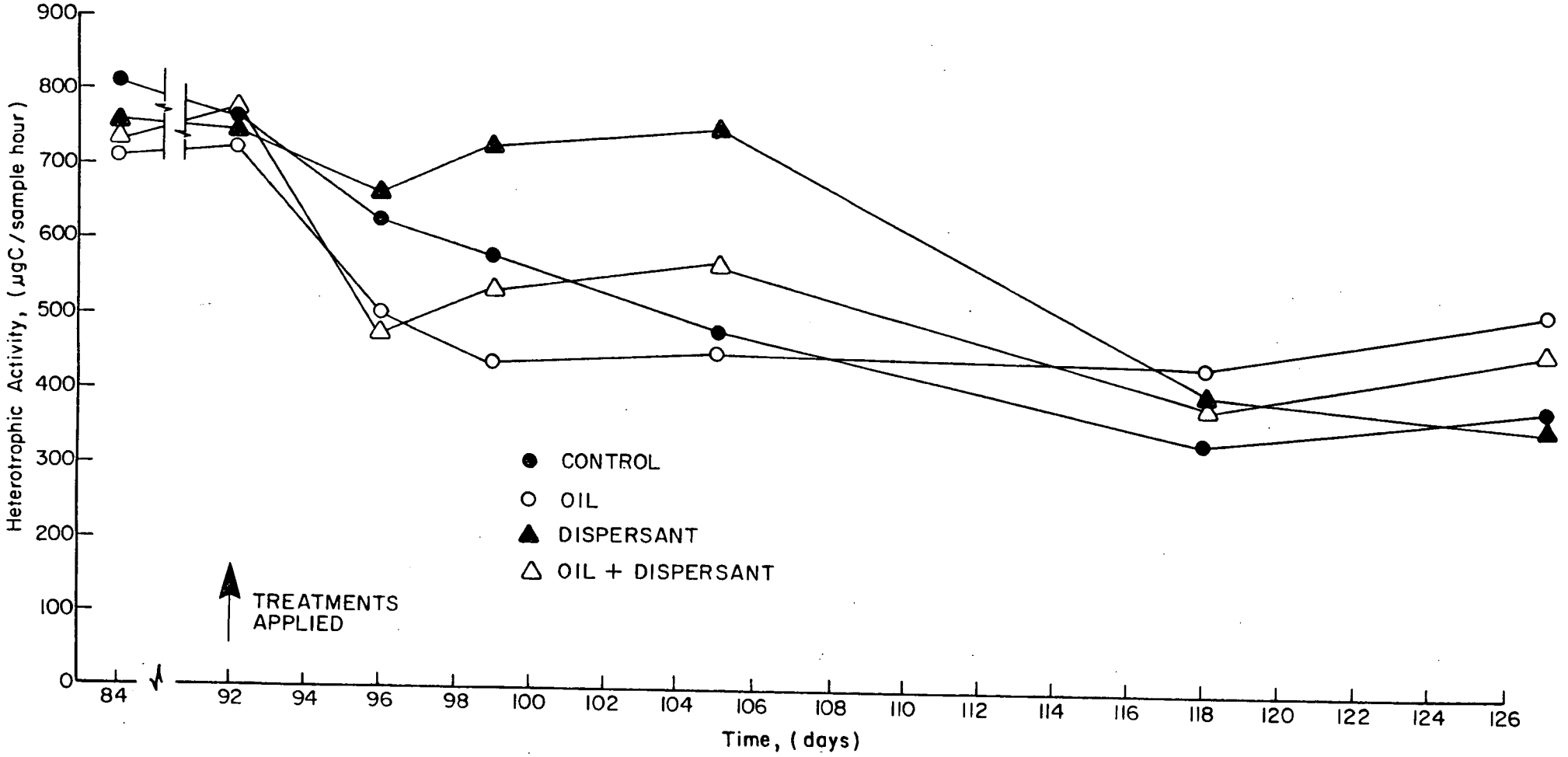

Figure 37: Plot of heterotrophic activity versus time for control, oil and/or dispersant treated midmarsh sediments. Treatments were applied on day 92.

higher (α= 0.05, df=4) in the oil plus dispersant treated microcosms than in the controls. Significant interaction of oil and dispersant (α= 0.01, df=4) was noted on only one sampling date (day 118). The combination of oil and dispersant produced a synergistic enhancement of toxicity. These results would suggest that the null hypothesis H_3 should be rejected, however, it should be remembered that the interaction was only temporary.

The oil and oil plus dispersant treatments evoked a two phase response from the heterotrophic bacteria. Initially, heterotrophic activity was reduced, presumably by the toxic effects of short chain hydrocarbons in the oil or by smothering of bacterial colonies. This was followed by a significant increase in heterotrophic activity relative to the controls. This increase may be associated with the weathering of short chain hydrocarbons or the colonization of the oil by bacteria capable of using it as a carbon source. A temporary reduction in heterotrophic activity was not noted for the dispersant treatments, instead, a significant flush of heterotrophic activity lasting approximately 27 days was observed. Jenkinson (1966) noted a similar increase in heterotrophic activity (carbon dioxide evolution) after the fumigation of soils, which was attributed to the death of microbial cells followed by accelerated growth of microbes growing on nutrients released by the killed cells. These effects were transient as in this case.

The results for the oil and oil plus dispersant treatments noted here are similar to those obtained by Dutka and Kwan (1984) for heterotrophic bacteria in freshwater ponds. They found that bacterial populations were reduced for approximately one month after oiling, however, within 84 days the oil treated ponds had higher bacterial populations relative to the control pond. Dispersed oil in their study had a stimulatory affect on heterotrophic activity which lasted approximately one month. This response is more similar to the dispersant treatment in this study than the oil plus dispersant treatment. This may be the result of differences in oil and dispersant application ratios. Dutka and Kwan (1984) used a 5:1 oil:dispersant ratio while a 10:1 ratio was used in this study. Dispersant effects would be expected to be more prominent in their oil plus dispersant treatment than in ours, which was the case. Increases in bacterial populations in Dutka and Kwans' (1984) study were attributed to increased carbon availability or elimination of predators.

Nitrogenase Activity and Composition of the Mud Surface Microbial Community - The null hypotheses tested in this experiment were:

H_1: Oil has no effect on nitrogenase activity.
H_2: Dispersant has no effect on nitrogenase activity.

H_3: There is no interaction (synergistic or inhibitory) between the factors oil and dispersant, affecting nitrogenase activity.

These hypotheses were tested in each of the three vegetation zones. The results of the Student-Newman-Keuls multiple range tests and the two-way analysis of variance procedures used to test these hypotheses are summarized in Appendix 15.

Nitrogenase activity (acetylene reduction) was highest in the midmarsh zone (Tables 19-21), ranging from 2 to 17 times higher than creek edge rates and 2 to 81 times higher than high marsh rates when control values from each zone were compared. Within treatment variation was very high for all vegetation zones. Nitrogenase activity for light and dark incubations within treatments were generally similar in all vegetation zones. Some exceptions were noted in the creek edge and high marsh zones.

Light and dark nitrogenase activity for the oil and oil plus dispersant treatments in the creek edge zone remained stable throughout the experiment (Table 19, Appendix 15). Nitrogenase activity for the dispersant treatment was significantly depressed ($\alpha = 0.05$, df=4) three days after treatment applications, then increased until the end of the experiment. No significant differences between treatments were noted on other sampling dates. Based on these results, the null hypothesis H_1 was retained while H_2 was rejected. The transient nature of the dispersant impact, however, suggests that in the long term dispersant toxicity is minimal. No significant interactions were noted between the oil and dispersant, therefore, the null hypothesis H_3 was retained.

In the midmarsh zone, oil and/or dispersant treatments had no significant impact on light or dark nitrogenase activity 3 days after treatment applications (Table 20, Appendix 15), however, on days 70 and 119 (27 and 76 days after treatment), nitrogenase activity for these treatments were substantially reduced in comparison to the control. This reduction was not significant on day 70 but was significant ($\alpha = 0.05$, df=4) for all oil and/or dispersant treatments on day 119. The null hypotheses H_1 and H_2, were therefore rejected. The fact tha 1) the decline in nitrogenase activity was delayed for a number of days and 2) the degree of reduction was similar in all oil and/or dispersant treatments suggest that this decline may have been caused by secondary habitat modifications rather than direct toxicity. The rapid deterioration of vegetation in the oil and/or dispersant treated midmarsh microcosms may have modified, light regimes, nutrient status, or soil aeration, thereby affecting rates of nitrogen fixation. No significant interactions between the oil and dispersant were noted, therefore, the null hypothesis H_3 was retained.

Table 19 - Acetylene reduction rates in the midmarsh microcosms on four sampling dates. Light and dark incubation data are presented. Treatments were applied on day 43.

Treatment		Average Acetylene Reduction Activity (nanomoles C_2H_4 produced/cm^3/hr (1 standard deviation))			
		June 4 Day 27	June 23 Day 46	July 17 Day 70	Sept. 4 Day 119
Control	Light	2.34(1.95)	3.72(2.48)	-	1.14(1.09)
	Dark	2.39(1.96)	4.39(3.03)	4.06(3.90)	1.85(2.01)
Oil	Light	3.24(2.45)	3.44(2.43)	-	0.11(0.01)
	Dark	3.72(3.13)	5.36(3.92)	1.50(1.53)	0.18(0.16)
Dispersant	Light	5.23(2.96)	5.00(4.12)	-	0.29(0.19)
	Dark	4.94(3.21)	4.70(3.41)	1.89(1.10)	0.40(0.41)
Oil plus Dispersant	Light	4.49(3.43)	8.32(5.74)	-	0.14(0.14)
	Dark	4.74(3.08)	8.33(6.68)	0.62(0.54)	0.21(0.31)

Table 20 - Acetylene reduction rates in the creek edge microcosms on four sampling dates. Light and dark incubation data are presented. Treatments were applied on day 43.

Treatment		Average Acetylene Reduction Activity (nanomoles C_2H_4 produced/cm^3/hr (1 standard deviation))			
		June 4 Day 27	June 23 Day 46	July 17 Day 70	Sept. 4 Day 119
Control	Light	0.21(0.18)	1.88(3.82)	-	0.15(0.18)
	Dark	0.28(0.30)	0.51(0.55)	0.24(0.25)	0.15(0.17)
Oil	Light	0.17(0.15)	0.22(0.14)	-	0.19(0.19)
	Dark	0.27(0.40)	0.26(0.26)	0.22(0.19)	0.24(0.21)
Dispersant	Light	0.21(0.29)	0.06(0.08)	-	0.43(0.60)
	Dark	0.17(0.23)	0.01(0)	0.16(0.17)	0.35(0.50)
Oil plus Dispersant	Light	0.13(0.17)	0.65(0.98)	-	0.04(0.02)
	Dark	0.10(0.12)	0.03(0.02)	0.06(0.03)	0.04(0.01)

In general, nitrogenase activity in oil and/or dispersant treated microcosms of the high marsh zone were not significantly different from the controls (Table 21, Appendix 15). There were no significant differences between treatments on any sampling date for the light incubations. For the dark incubations, nitrogenase activity was significantly elevated (α = 0.05, df=4) in the oiled microcosms three days after treatment application (day 46). Although the mean nitrogenase activity rate for the oil plus dispersant treated microcosms on day 119 was much lower than the control, high variance of the data prevented it from being significantly different. Based on these results, the null hypotheses H_1 and H_2 were retained. No significant interactions were noted between the oil and dispersant, therefore, the null hypothesis H_3 was also retained.

Nitrogenase activity did not appear to be directly affected by the oil and/or dispersant treatments in any of the vegetation zones. Although significant reductions in nitrogenase activity were associated with all oil and/or dispersant treatments in the midmarsh zone, the delay in the reduction suggests that secondary environmental changes not direct toxicity were responsible. Similarly, Thomson and Webb (1984) found no reduction in nitrogenase activity following chronic exposure of salt marsh mud to oil.

Light and dark activities were of approximately equal magnitude. Heterocystous blue-green algae were absent in the creek edge and high marsh zones and were uncommon in the midmarsh zone (Tables 22-24). Non-heterocystous blue-green algae were very common in all vegetation zones as were sulfur bacteria in the midmarsh zone. These observations are similar to those of Patriquin and McClung (1978) for another Petpeswick salt marsh, and suggest that nonheterocystous blue-green algae and photosynthetic bacteria are the primary agents of nitrogen fixation.

All three vegetation zones were dominated by the same microbial types (Tables 22-24). The main differences lay in the abundances of the various types. Algae, blue-green algae photosynthetic bacteria and sulfur-oxidizing bacteria were much more common in the midmarsh zone than in either the creek edge or high marsh zones. The large microbial population of the midmarsh zone corresponds to the high rates of nitrogen fixation noted in this zone. The abundance of the various microbial types was little affected by the oil and/or dispersant treatments. No changes were evident in the creek edge microcosms (Table 22). In the midmarsh microcosms there was a reduction in the abundance of a thick grey nonheterocystous blue-green algae in all oil and/or dispersant treatments and an increase in the abundance of sulfur bacteria associated with all oil and/or dispersant treatments (Table 23). In the high marsh microcosms

Table 21 - Acetylene reduction rates in the high marsh microcosms on four sampling dates. Light and dark incubation data are presented. Treatments were applied on day 43.

Treatment		Average Acetylene Reduction Acivity (nanomoles C_2H_4 produced/cm^3/hr (1 standard deviation))			
		June 4 Day 27	June 23 Day 46	July 17 Day 70	Sept. 4 Day 119
Control	Light	0.05(0.04)	0.09(0.13)	-	0.28(0.27)
	Dark	0.06(0.07)	0.05(0.10)	0.07(0.06)	0.97(1.25)
Oil	Light	0.11(0.06)	0.27(0.20)	-	0.81(1.27)
	Dark	0.12(0.10)	0.25(0.20)	0.18(0.19)	1.29(2.07)
Dispersant	Light	0.07(0.04)	0.08(0.06)	-	0.31(0.23)
	Dark	0.19(0.26)	0.03(0.02)	0.08(0.14)	0.64(0.81)
Oil plus Dispersant	Light	0.10(0.09)	0.05(0.05)	-	0.09(0.06)
	Dark	0.23(0.22)	0.05(0.03)	0.09(0.15)	0.10(0.12)

Table 22 - Abundance of various algal, blue-green algal, and bacterial types in control, oil and/or dispersant treated creek edge microcosms on four sampling dates. Treatments were applied on day 43. 0=absent, 1=rare, 2=uncommon, 3=common, 4=abundant.

	Abundance															
	Control				Oil				Dispersant				Oil+Dispersant			
Morphological	Day				Day				Day				Day			
Classifications	31	57	74	119	31	57	74	119	31	57	74	119	31	57	74	119
ALGAE																
Thin Unbranched Filamentous Green Algae	3	3	3	3	4	3	3	4	3	4	2	4	2	2	3	3
Large Unbranched Filamentous Green Algae	0	0	2	2	0	1	0	0	0	2	0	0	0	0	0	0
Large Branched Filamentous Green Algae sp #1	1	0	0	0	0	0	0	0	1	0	2	3	0	0	0	0
Large Branched Filamentous Green Algae sp #2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Large Aseptate Branched Filamentous Green Algae	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Unbranched Filamentous Brown Algae sp#1	0	0	0	1	2	0	0	0	2	0	0	0	2	0	0	0
Unbranched Filamentous Brown Algae sp#2	0	1	0	0	0	0	0	2	1	0	2	0	0	0	0	0
Spherical Colonies of Green Algae	0	4	1	0	0	0	0	0	0	1	0	0	0	1	0	0
Spherical Unicellular Green Algae	2	2	1	3	2	1	2	2	2	1	1	2	1	1	2	2
Diatoms	4	3	3	3	3	3	3	4	4	3	3	4	4	2	3	4
BLUE-GREEN ALGAE																
Thick Nonheterocystous Blue-green Algae	2	2	2	2	1	2	2	1	2	2	2	3	2	2	2	3
Thin Nonheterocystous Blue-green Algae	0	0	2	2	2	0	1	3	0	0	2	3	2	0	0	2
Thick Greyish Non-heterocystous Blue-green Algae	0	1	0	2	0	0	0	0	0	0	2	2	0	0	0	0
Spiral Nonheterocystous Blue-green Algae	0	1	2	0	0	4	3	0	0	0	0	1	0	0	0	1
Coccoid Nonheterocystous Blue-green Algae	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0
BACTERIA																
Pink Photosynthetic Bacteria	0	0	0	2	0	0	0	3	0	0	0	2	0	0	0	2

Table 23 - Abundance of various algal, blue-green algal, and bacterial types in control, oil and/or dispersant treated midmarsh microcosms on four sampling dates. Treatments were applied on day 43. 0=absent, 1=rare, 2=uncommon, 3=common, 4=abundant.

	Abundance															
	Control				Oil				Dispersant				Oil+Dispersant			
Morphological	Day				Day				Day				Day			
Classifications	31	57	74	119	31	57	74	119	31	57	74	119	31	57	74	119
ALGAE																
Thin Unbranched Filamentous Green Algae sp #1	4	3	4	3	4	4	2	0	4	3	3	3	3	4	0	0
Thin Unbranched Filamentous Green Algae sp #2	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0
Thick Unbranched Filamentous Green Algae	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Large Branched Filamentous Green Algae	0	2	3	0	0	0	2	0	0	0	0	0	3	0	0	0
Unbranched Filamentous Brown Algae sp #1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Unbranched Filamentous Brown Algae sp#2	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0
Branched Filamentous Brown Algae	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Spherical Colonies of Green Algae	4	4	2	2	3	2	1	1	3	2	1	3	1	2	2	0
Thallus-like Colonies of Green Algae	0	2	2	0	2	0	0	0	0	0	0	0	0	0	0	0
Diatoms	4	3	3	3	4	1	3	4	4	2	2	4	4	1	3	4
BLUE-GREEN ALGAE																
Thick Nonheterocystous Blue-green Algae	3	3	2	0	3	1	2	3	3	4	4	4	3	3	3	3
Thick Greyish Non-heterocystous Blue-green Algae	0	3	4	4	0	2	3	2	0	1	1	3	0	0	0	3
Thin Nonheterocystous Blue-green Algae	0	1	0	4	0	1	0	2	2	1	0	0	2	1	0	4
Spiral Nonheterocystous Blue-green Algae	2	3	3	2	1	3	4	4	2	0	1	4	0	0	2	3
Thin Heterocystous Blue-green Algae	0	0	2	2	1	0	2	0	1	1	0	0	0	0	0	0
BACTERIA																
Sulfur Bacteria	0	2	0	0	1	3	4	3	3	3	4	3	4	2	3	3
Pink Photosynthetic Bacteria	0	0	0	4	0	0	0	3	0	1	4	4	0	0	0	3

Table 24 - Abundance of various algal, blue-green algal, and bacterial types in control, oil and/or dispersant treated high marsh microcosms on four sampling dates. Treatments were applied on day 43. 0=absent, 1=rare, 2=uncommon, 3=common, 4=abundant.

	Abundance															
	Control				Oil				Dispersant				Oil+Dispersant			
Morphological	Day				Day				Day				Day			
Classifications	31	57	74	119	31	57	74	119	31	57	74	119	31	57	74	119
ALGAE																
Unbranched Filamentous Green Algae	1	0	3	0	0	0	1	2	0	0	0	3	0	2	0	0
Large Branched Filamentous Green Algae sp #1	0	0	3	3	2	0	0	1	1	0	2	0	2	0	0	0
Large Branched Filamentous Green Algae sp #2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Large Aseptate Branched Filamentous Green Algae	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unbranched Filamentous Brown Algae sp #1	3	0	0	0	3	1	0	0	3	0	0	0	2	0	0	0
Unbranched Filamentous Brown Algae sp#2	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Branched Filamentous Brown Algae	0	0	0	0	0	0	1	2	0	0	1	0	0	0	1	0
Spherical Colonies of Green Algae	0	1	0	0	2	2	1	0	1	3	0	0	0	1	0	0
Spherical Unicellular Green Algae	3	0	0	2	2	0	2	3	3	0	2	2	2	0	3	2
Diatoms	4	3	3	4	3	3	3	4	4	3	3	4	4	2	3	4
BLUE-GREEN ALGAE																
Thick Nonheterocystous Blue-green Algae	2	1	2	2	3	1	3	2	2	2	0	1	3	0	2	3
Thick Greyish Non-heterocystous Blue-green Algae	0	0	3	4	0	0	2	1	0	0	2	1	0	0	1	0
Thin Nonheterocystous Blue-green Algae	2	3	3	4	1	0	3	3	0	0	2	2	3	0	0	2
Spiral Nonheterocystous Blue-green Algae	12	2	1	2	0	0	2	0	0	0	0	0	0	0	0	0
BACTERIA																
Pink Photosynthetic Bacteria	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2

there were reductions in the abundances of a thin nonheterocystous blue-green algae and a spiral shaped non- heterocystous blue-green algae associated with all oil and/or dispersant treatments (Table 24).

Comparison of Microbial Effects Noted in the Field and Greenhouse Experiments

Algal and bacterial cover was the only microbial parameter studied in both the field and greenhouse experiments. There was relatively poor correspondence between the field and greenhouse results. This was probably caused by a number of factors:

1) Sampling intensity was insufficient to compensate for the extreme patchiness of these communities.
2) Sampling frequency was insufficient to intercept the short term population fluctuations of which these communities are capable.
3) Estimation of algal and bacterial cover in the field was extremely difficult because of the large differences in light levels below and above the macrophyte canopy; resulting in increased error of estimation.

Trends of algal and bacterial cover in the greenhouse experiment were more consistant than those of the field experiment suggesting less masking of treatment effects by sampling error. Although bottle effects associated with the microcosms may have altered microbial community structure, we consider the results from the greenhouse study to be more representative of oil and/or dispersant impacts than the field study.

CONCLUSIONS

Elevation data indicated that the field plots were located in a very narrow range of elevations and were innundated only 5 to 25% of the time. There was much overlap in the range of elevations inhabited by each vegetation zone, suggesting that the factors characterizing boundaries of the zones were related to drainage rather than submergence.

Soil aeration was not noticeably altered by the oil and/or dispersant treatments. Reducing zones (indicative of anaerobic conditions) generally increased in thickness throughout the growing season, however, this trend was also noted in control quadrats and microcosms indicating that this was a normal seasonal phenomenon. Root coloration and the volume of aerated microzone around fine roots were not altered by any of the treatments.

Soil temperature was not significantly altered by the oil and/or dispersant treatments. Soil temperature differences between vegetation zones were much larger than temperature differences between treatments within a particular vegetation zone.

Visual observations in both the field and laboratory experiments indicated that the dispersant was not effective in removing oil form the vegetation. Oil concentrations in the sediments were highly variable reflecting the spatial heterogeneity of the sediment as revealed in the soil profile descriptions. In general, oil concentrations were high in both oil and oil plus dispersant treated plots, suggesting that the dispersant had little effect on the movement of oil through the sediments. The data, however, are too variable to confirm this result definitively.

Plant height data indicated that the oil treatments had relatively little impact on average plant height in all vegetation zones. The dispersant only treatment caused the largest reductions in average plant height, closely followed by the oil plus dispersant treatment. Rapid fluctuations in average plant height appeared to be caused by higher mortality among small plants. The data suggest that the midmarsh zone is the most sensitive to all treatments while the high marsh is the least sensitive. In a similar study conducted by Baker et al. (1984), Spartina anglica height was not significantly affected by any of the oil and/or dispersant treatments. The dispersant used in their study (BP1100WD) appeared to be much less toxic than the Corexit 9527 used in this study and may account for their lack of impact.

Growth rate data from the greenhouse experiment indicated that oil was responsible for the smallest reductions in plant growth rates in all vegetation zones while the dispersant caused the largest reductions. Growth rates for oil plus dispersant treated plants were generally higher than expected. Pooling of oil around the meristematic tissues at the base of the plant may have protected these tissues from the relatively more toxic dispersant. Growth rates for oil plus dispersant treated plants appeared to recover late in the growing season, however, large increases in plant mortality associated with this treatment countered this benefit.

Plant stem density was affected most by the oil plus dispersant treatment and least by the oil treatment in the midmarsh, and creek edge zones. The relative toxicity of the dispersant treatment varied between the field and greenhouse experiments. Dispersant was less toxic in the field experiments than in the greenhouse experiments. In the high marsh, oil caused the largest reduction in stem density while the dispersant treatment caused the smallest reductions. The midmarsh zone was extremely sensitive to all oil

and/or dispersant treatments with more than 90% reductions in stem density associated with all of the treatments in both the field and greenhouse experiments. Baker et al. (1984) noted a similar trend for Spartina anglica communities in Great Britain treated with oil, dispersant and oil followed by dispersant.

In the creek edge zone, oil had no significant impact on above-ground biomass production. The dispersant and oil plus dispersant treatments were generally equal in their impact, however, the oil plus dispersant treatment was somewhat less toxic in the greenhouse experiment than in the field experiment. Delaune et al. (1984) noted a similar trend for Spartnia alterniflora in Louisiana. Oil alone did not significantly reduce above ground biomass; however, when oil and dispersant were applied together (without flushing) a significant reduction in biomass was noted. In the midmarsh zone all treatments caused significant reductions in above-ground biomass. In the high marsh zone all oil and/or dispersant treatments were approximately equal in their impact and caused relatively little reduction in above ground biomass. Overall the oil and/or dispersant treatments had the most impact in the midmarsh zone and the least impact in the high marsh zone.

Spartina cover values in the creek edge and midmarsh zones was reduced least by the oil treatments and most by the oil plus dispersant treatment. In the greenhouse experiment the impacts of the dispersant and the oil plus dispersant treatments were equivalent, while in the field experiment the oil plus dispersant treatment was clearly more toxic. This appeared to be related to an inhibitory interaction between oil and dispersant toxicity which appeared only in the greenhouse experiment. In the high marsh zone all treatments were approximately equal in impact and caused little reduction in Spartina cover. The midmarsh zone was most susceptable to the treatments while the high marsh was least susceptable. Salicornia europaea was the only other species which occurred regularly in the study plots. Its abundance was not affected by the various treatments.

Microscopic examination of Spartina leaves indicated that the same symptoms developed in all the oil and/or dispersant treatments. The only noticeable symptom was the loss of chloroplasts over time. Treatments varied in the rates at which this chlorosis proceeded, with the fastest rates associated with either the dispersant or oil + dispersant treatment and the slowest rate associated with the oil treatment.

Fluorometry results indicated that variable fluorescence was a good predictor of long term response of the plants to treatment induced stress. One hundred second difference values were a more sensitive indicator of

plant stress than peak variable fluorescence. The former indicated stress within one day of treatment application while the latter indicated changes only when the plants were near death. Dispersant alone induced the most stress in all vegetation zones. The oil plus dispersant treatments generally induced less stress than the dispersant treatment. Oil was applied to the plants before the dispersant and may have acted as a barrier to entry of the dispersant into plant tissues. Oil treatments caused relatively little stress in all but the midmarsh zone. The midmarsh zone was most sensitive to the oil and/or dispersant and the high marsh zone was least sensitive.

Algal mats, heterotrophic bacteria and pink photosynthetic bacteria appeared to be temporarily reduced in abundance by treatments incorporating oil but were unaffected or stimulated by the dispersant treatments. Oil treatments eventually increased the abundance of heterotrophic bacteria, probably largely through the proliferation of oil degrading species, however, other species such as sulfur-oxidizing bacteria also seemed to benefit.

Nitrogen fixation was unaffected by the oil and/or dispersant treatments in the creek edge and high marsh zones, however, all treatments reduced nitrogen fixation in the midmarsh zone. Evidence suggests that this decline was probably caused by alterations in microbial microhabitat rather than by direct toxicity. Nitrogen fixation rates were highest in the midmarsh zone. Results of light and dark incubations and microscopic observation of the algal mat suggest that nonheterocystous blue-green algae and photosynthetic bacteria are the primary agents of nitrogen fixation.

It is obvious that dispersant applied to saltmarshes has deleterious effects on the vegetation, more so than oil alone (at least initially). Unfortunately, the results of the experiments did not show whether or not dispersants could accelerate the natural cleansing of oil from salt marshes and, without long term monitoring, whether or not dispersants retard the recovery of oiled saltmarshes.

Until further research is undertaken to assess the efficacy of dispersant use in saltmarshes (including spray application to slicks entering marshes, premixed oil and dispersant test plots and dispersant use in conjunction with low pressure water flushing), the use of dispersants to cleanse oiled saltmarshes cannot be recommended.

This study has also demonstrated that sensitivity to oil and dispersant varies greatly among plant communities. Variations in the sensitivities of species to oil have been noted by a number of authors (Baker 1971b, Cowell

1969, Cowell and Baker 1969). Specific differences in susceptability to dispersants have been demonstrated by Baker et al (1984). This fact demonstrates the need to tailor oil spill response planning to the floristic composition of the threatened marsh; a view which is shared by Roberts and Robertson (1986). Results from this study suggest that the creek edge and high marsh vegetations are relatively tolerant of single oil spill events and can probably be left to cleanse themselves naturally. The midmarsh zone, however, was very sensitive to both oil and dispersant and alternative clean up measures such as low pressure flushing ought to be utilized in areas of marshes dominated by this community.

Another factor which merits consideration in oil spill response planning for salt marshes is the variation of plant sensitivity within a species which is attributable to differences in pre-oiling plant stress. This study demonstrated that stunted Spartina alterniflora in the water logged midmarsh zone was far more sensitive to all of the oil and/or dispersant treatments than tall S. alterniflora in the well drained creek edge zone.

APPENDIX I: PRINCIPLES OF FLUORESCENCE INDUCTION MEASUREMENTS

Light absorbed by the chloropyll-a reaction center of photosystem II (the oxygen evolving part of the photosynthetic system) excites ground state electrons in the chlorophyll molecule. An excited electron very quickly (within ca 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 reemitted 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 concommittant release of free oxygen). The primary electron acceptor in the meantime loses the electron to an electron carrier which loses it ot another carrier and so on and through photosystem I, until finally an electron is passed to NADP. The electron carrying-NADP then moves out of the internal membranes of the choroplast where the 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 reemitted 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 light intensties, 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 electron 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 center cannot pass on its excited electrons, the excited electrons drop back to their unexicted ground state and fluorescence rises. It reaches a peak value within about 3 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, therefore,

fluorescence begins to decline. Within about 10 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 aand begin to regulate the 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 measureable characteristics (Smilie & 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, and a slow decline from the peak could be due to damage further downstream.

To our knowledge, this technique has not been previously used in studies of oil and/or dispersant damage to plants. We had several objectives in applying it in this study:

i) We wished to determine if any of the treatments affected the photosynthetic system, and how such effects varied between treatments and zones.

ii) By comparing the fluorescence induction parameters with other criteria of stress (growth rates, flowering, etc.) measured at different times, we wished to determine if this method could be used to forecast stress effects that would otherwise be apparent only after longer intervals.

iii) Assuming that changes in the chlorophyll induction curve would be one

of the first responses to stress, we wished to use the method to indicate how quickly after applying oil and/or dispersant, the plants begin to be stressed.

APPENDIX 2: SUMMARY TABLES OF STATISTICS FOR THE FIELD PLANT HEIGHT DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D) and oil plus dispersant (O+D) treatments on five sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, two degrees of freedom) between means are indicated by asterisks. Data were transformed by ln (x =median + 1). Treatments were applied on day 60.

	Creek Edge	Midmarsh	High Marsh
Day 57	No significant differences between treatments	No significant differences between treatments	C O D O+D C O * D * O+D
Day 75	C O D O+D C O D * * O+D * * *	No significant differences between treatments	C O D O+D C O * D * * O+D *
Day 89	C O D O+D C O * D * * O+D * * *	C O D O+D C O * D * * O+D * *	C O D O+D C O * D * * O+D * *
Day 102	C O D O+D C O D * * O+D * * *	C O D O+D C O D * * O+D * * *	C O D O+D C O * D * O+D * *
Day 123	C O D O+D C O * D * * O+D * *	C O D O+D C O * D * * O+D *	C O D O+D C O * D * O+D * *

APPENDIX 2 (Continued)

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by ln (x ÷median +1). Treatments were applied on day 60.

* = significant at the 0.05 level of significance, two degrees of freedom.

** = significant at the 0.01 level of significance, two degrees of freedom.

F-ratios

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 57	4.61*	0.29	13.0 **
Day 75	11.8 **	0.41	16.2 **
Day 89	1.53	6.16*	11.0 **
Day 102	9.08**	0.09	3.15
Day 123	0.35	11.0 **	0.095
Dispersant			
Day 57	0.08	0.04	0.76
Day 75	70.5**	0.41	70.0 **
Day 89	125 **	6.16*	59.2 **
Day 102	224 **	0.09	0.37
Day 123	47.7**	11.00**	0.01
Two-way Interaction			
Oil and Dispersant			
Day 57	0.75	1.20	0.10
Day 75	7.04**	4.42*	33.5 **
Day 89	17.9 **	0.64	19.9 **
Day 102	25.1 **	10.4 **	22.7 **
Day 123	6.22 *	1.91	79.8 **

APPENDIX 3: SUMMARY TABLES OF STATISTICS FOR THE FIELD STEM DENSITY DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D) and oil plus dispersant (O+D) treatments on four sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, two degrees of freedom) between means are indicated by asterisks. Data were transformed by ln (x + 1). Treatments were applied on day 60.

	Creek Edge				Midmarsh				High Marsh			
	C	O	D	O+D	No significant differences between treatments				C	O	D	O+D
C												
O												
D	*	*								*		
O+D			*									
Day 26												
	C	O	D	O+D	C	O	D	O+D	No significant differences between treatments			
C					C							
O					O							
D	*	*			D	*						
O+D			*		O+D	*						
Day 49												
	C	O	D	O+D	C	O	D	O+D	C	O	D	O+D
C					C				C			
O	*				O	*			O	*		
D	*				D	*			D	*	*	
O+D	*	*	*		O+D	*	*	*	O+D	*	*	
Day 89												
	C	O	D	O+D	C	O	D	O+D	C	O	D	O+D
C					C				C			
O	*				O	*			O	*		
D	*				D	*			D	*		
O+D	*	*	*		O+D	*	*	*	O+D	*		
Day 123												

APPENDIX 3 (Continued)

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by ln (x + 1). Treatments were applied on day 60.

* = significant at the 0.05 level of significance, two degrees of freedom.

** = significant at the 0.01 level of significance, two degrees of freedom.

	F-ratios		
Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 26	2.78	0.01	6.82**
Day 49	0.82	1.34	2.22
Day 89	134 **	209 **	39.6 **
Day 123	46.0 **	208 **	17.4 **
Dispersant			
Day 26	18.1 **	0.34	4.51*
Day 49	14.0 **	7.23**	1.50
Day 89	81.5 **	152 **	0.25
Day 123	195 **	283 **	2.05
Two-way Interaction Oil and Dispersant			
Day 26	3.58	0.62	0.05
Day 49	9.07**	2.21	0.19
Day 89	13.6 **	6.71*	36.4 **
Day 123	14.2 **	54.2 **	10.5 **

APPENDIX 4: SUMMARY TABLES OF STATISTICS FOR THE FIELD BIOMASS DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D) and oil plus dispersant (O+D) treatments on five sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, two degrees of freedom) between means are indicated by asterisks. Data were transformed by square root.

Total Above-Ground Biomass

Creek Edge

	C	O	D	O+D
C				
O				
D	*	*		
O+D	*	*		

Midmarsh

	C	O	D	O+D
C				
O	*			
D	*			
O+D	*	*		

High Marsh

	C	O	D	O+D
C				
O				
D	*			
O+D				

Day 124

Reproductive Biomass

Creed Edge	Midmarsh	High Marsh
No significant differences between treatments	No significant differences between treatments	No significant differences between treatments

Day 124

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by square root. Treatments were applied on day 60.

* = significant at the 0.05 level of significance, two degrees of freedom.

** = significant at the 0.01 level of significance, two degrees of freedom.

Total Above Ground Biomass
F-ratios

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 124	3.46	27.7 **	0.13
Dispersant			
Day 124	41.6 **	49.0 **	8.59*
Two-way Interaction			
Oil and Dispersant			
Day 124	1.03	4.97	2.72

Reproductive Biomass

Two-way analysis of variance was not conducted on the field reproductive biomass data.

APPENDIX 5: SUMMARY TABLES OF STATISTICS FOR THE FIELD SPARTINA COVER DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments on three sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, two degrees of freedom) between means are indicated by asterisks. Data were untransformed. Treatments were applied on day 60.

	Creek Edge	Midmarsh	High Marsh
Day 34	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 75	C O D O+D C O * D * O+D * * *	C O D O+D C O * D * O+D *	No significant differences between treatments
Day 102	C O D O+D C O * D * * O+D * * *	C O D O+D C O * D * O+D *	No significant differences between treatments

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were untransformed. Treatments were applied on day 60.

* = significant at the 0.05 level of significance, two degrees of freedom.

** = significant at the 0.01 level of significance, two degrees of freedom.

F-ratios

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 34	2.47	0.56	1.72
Day 75	32.2 **	32.3 **	1.20
Day 102	28.5 **	33.2 **	1.16
Dispersant			
Day 34	0.27	0.67	0.17
Day 75	39.5**	24.3 **	0.27
Day 102	108 **	48.9 **	0.86
Two-way Interaction Oil and Dispersant			
Day 34	2.15	0.04	0.08
Day 75	1.78	3.25	0.36
Day 102	1.49	18.33**	4.33

APPENDIX 6: SUMMARY TABLES OF STATISTICS FOR THE GREENHOUSE PLANT HEIGHT DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments on nine sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, four degrees of freedom) between means are indicated by asterisks. Data were untransformed. Treatments were applied on day 43.

	Creek Edge				Midmarsh	High Marsh			
	C	O	D	O+D		C	O	D	O+D
C					No significant	C			
Day 22 O					differences	O			
D					between	D	*	*	
O+D	*	*			treatments	O+D			
	C	O	D	O+D		C	O	D	O+D
C					No significant	C			
Day 28 O					differences	O			
D	*				between	D	*	*	
O+D		*	*		treatments	O+D		*	
	C	O	D	O+D		C	O	D	O+D
C					No significant	C			
Day 35 O					differences	O			
D	*				between	D	*	*	
O+D		*	*		treatments	O+D		*	
	C	O	D	O+D	C O D O+D	C	O	D	O+D
C					C	C			
Day 41 O					O	O			
D	*				D *	D	*	*	
O+D		*	*		O+D	O+D		*	
	C	O	D	O+D	C O D O+D	C	O	D	O+D
C					C	C			
Day 55 O	*				O	O	*		
D	*	*			D *	D			
O+D			*		O+D	O+D			

APPENDIX 6 (Continued)

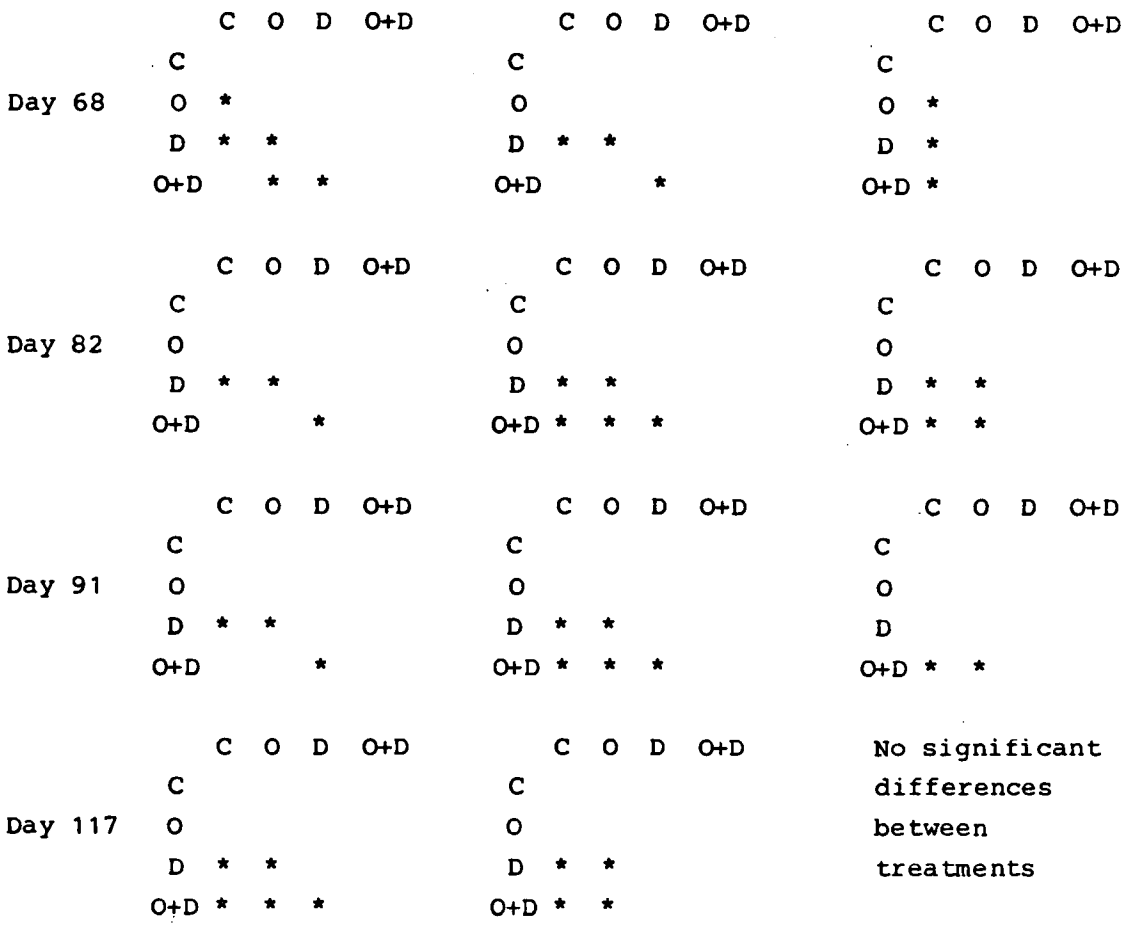

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were untransformed. Treatments were applied on day 43.

* = significant at the 0.05 level of significance, four degrees of freedom.

** = significant at the 0.01 level of significance, four degrees of freedom.

	F-ratios		
Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 22	3.31	0.09	1.25
Day 28	6.71**	0.14	2.49
Day 35	4.83*	0.01	6.81**
Day 41	5.15*	0.02	3.17
Day 55	1.11	0.50	5.22*
Day 68	3.79	2.43	5.53*
Day 82	9.41**	1.26	0.44
Day 91	7.40**	1.52	0.06
Day 117	14.7 **	1.18	0.43
Dispersant			
Day 22	0.79	0.76	7.04**
Day 28	0.01	1.69	5.04*
Day 35	0.00	2.03	4.17*
Day 41	0.14	2.41	5.46*
Day 55	6.23*	6.69**	1.67
Day 68	5.74**	12.3 **	7.16**
Day 82	12.1 **	39.5 **	13.9 **
Day 91	21.5 **	51.0 **	12.5 **
Day 117	54.1 **	19.1 **	2.35

APPENDIX 6 (Continued)

Two-way Interaction			
Oil and Dispersant			
Day 22	15.1 **	2.03	1.26
Day 28	27.0 **	3.16	2.05
Day 35	21.7 **	2.67	4.28*
Day 41	16.5 **	4.53*	3.64
Day 55	27.9 **	5.79*	1.38
Day 68	34.8 **	2.06	0.82
Day 82	35.6 **	3.58	0.00
Day 91	30.9 **	5.30*	0.53
Day 117	20.8 **	1.17	0.29

APPENDIX 7: SUMMARY TABLES OF STATISTICS FOR THE GREENHOUSE SPARTINA GROWTH RATE DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments during six sampling periods in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, four degrees of freedom) between means are indicated by asterisks. Data were untransformed. Treatments were applied on day 43.

	Creek Edge	Midmarsh	High Marsh
Day 35-41	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 41-55	C O D O+D C O * D * * O+D * *	C O D O+D C O * D * O+D	C O D O+D C O * D * * O+D * *
Day 55-68	C O D O+D C O * D * * O+D *	No significant differences between treatments	C O D O+D C O D * * O+D * *
Day 68-82	C O D O+D C O * D * * O+D *	C O D O+D C O D * * O+D	C O D O+D C O D * * O+D
Day 82-91	C O D O+D C O D * O+D	Insufficient data for analysis	No significant differences between treatments

APPENDIX 7 (Continued)

		C	O	D	O+D
Day 91-117	C				
	O				
	D	*	*		
	O+D	*	*	*	

Insufficient data for analysis

	C	O	D	O+D
C				
O				
D				
O+D	*	*	*	

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were untransformed. Treatments were applied on day 43.

* = significant at the 0.05 level of significance, four degrees of freedom.

** = significant at the 0.01 level of significance, four degrees of freedom.

	F-ratios		
Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Days 35-41	2.52	0.21	1.29
Days 41-55	6.47**	9.88**	8.98**
Days 55-68	6.57**	1.32	0.29
Days 68-82	1.96	2.99	0.10
Days 82-91	0.01	-	2.51
Days 91-117	0	-	1.47
Dispersant			
Days 35-41	0.29	0.57	0.01
Days 41-55	38.3 **	0.54	74.6 **
Days 55-68	47.0 **	0.27	47.6 **
Days 68-82	23.3 **	0.07	11.1 **
Days 82-91	4.04*	-	0.59
Days 91-117	8.73**	-	6.79**
Two-way Interaction			
Oil and Dispersant			
Days 35-41	0.02	0.34	0.18
Days 41-55	24.5 **	12.7 **	40.6 **
Days 55-68	15.1 **	-	0.33
Days 68-82	4.57*	-	0.54
Days 82-91	6.80**	-	0.27
Days 91-117	2.68	-	7.00**

APPENDIX 8: SUMMARY TABLES OF STATISTICS FOR THE GREENHOUSE STEM DENSITY DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D) and oil plus dispersant (O+D) treatments on six sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, four degrees of freedom) between means are indicated by asterisks. Data were transformed by ln (x + 1). Treatments were applied on day 43.

	Creek Edge	Midmarsh	High Marsh
Day 22	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 28	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 35	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 55	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 82	No significant differences between treatments	C O D O+D C O * D * * O+D * *	C O D O+D C O * D * O+D * *
Day 106	No significant differences between treatments	C O D O+D C O * D * O+D *	No significant differences between treatments

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by ln (x + 1). Treatments were applied on day 43.

* = significant at the 0.05 level of significance, four degrees of freedom.

** = significant at the 0.01 level of significance, four degrees of freedom.

F-ratios

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 22	0.03	0.07	0.00
Day 28	0.02	0.22	0.03
Day 35	0.26	0.21	0.03
Day 55	0.74	1.30	3.29
Day 82	2.09	197 **	17.0 **
Day 106	0.02	130 **	8.62**
Dispersant			
Day 22	0.95	0.30	0.11
Day 28	1.58	0.00	0.00
Day 35	1.32	0.00	0.00
Day 55	0.46	2.05	0.27
Day 82	0.85	688 **	0.15
Day 106	6.46*	172 **	0.09
Two-way Interaction Oil and Dispersant			
Day 22	0.15	0.01	0.22
Day 28	0.23	0.21	0.25
Day 35	0.23	0.29	0.76
Day 55	0.00	4.55*	2.27
Day 82	0.51	197 **	0.11
Day 106	2.00	111 **	0.28

APPENDIX 9: SUMMARY TABLES OF STATISTICS FOR THE GREENHOUSE BIOMASS DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, four degrees of freedom) between means are indicated by asterisks. Data were transformed by square root.

Creek Edge

Day 118	C	O	D	O+D
C				
O				
D	*	*		
O+D	*	*		

Midmarsh

	C	O	D	O+D
C				
O	*			
D	*	*		
O+D	*	*		

High Marsh

	C	O	D	O+D
C				
O	*			
D	*			
O+D	*			

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by square root. Treatments were applied on day 43.

* = significant at the 0.05 level of significance, four degrees of freedom.

** = significant at the 0.01 level of significance, four degrees of freedom.

	F-ratios		
Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 118	0.28	16.2 **	4.53*
Dispersant			
Day 118	33.8 **	65.7 **	19.5 **
Two-way Interaction			
Oil and Dispersant			
Day 118	2.35	23.7 **	3.25

APPENDIX 10: SUMMARY TABLES OF STATISTICS FOR THE GREENHOUSE SPARTINA COVER DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments on two sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, four degrees of freedom) between means are indicated by asterisks. Data were untransformed. Treatments were applied on day 43.

	Creek Edge	Midmarsh	High Marsh
Day 43	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments

Day 117

	C	O	D	O+D
C				
O	*			
D	*	*		
O+D	*	*		

	C	O	D	O+D
C				
O	*			
D	*	*		
O+D	*	*		

	C	O	D	O+D
C				
O	*			
D	*			
O+D	*			

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were untransformed. Treatments were applied on day 60.

* = significant at the 0.05 level of significance, four degrees of freedom.

** = significant at the 0.01 level of significance, four degrees of freedom.

	F-ratios		
Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 43	0.27	1.05	4.90*
Day 117	0.96	68.6 **	3.16
Dispersant			
Day 43	0.00	0.41	1.23
Day 117	50.2 **	145 **	5.23*
Two-way Interaction Oil and Dispersant			
Day 43	0.47	1.36	0.14
Day 117	4.84*	68.6 **	2.32

APPENDIX 11: SUMMARY TABLES OF STATISTICS FOR THE FLUOROMETRY DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments on six sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, four degrees of freedom) between means are indicated by asterisks. Data were transformed by ln (x + 1). Treatments were applied on day 43.

Peak Variable Fluorescence

	Creek Edge	Midmarsh	High Marsh
Day 25	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 44	C O D O+D C O * D O+D	C O D O+D C O D O+D *	No significant differences between treatments
Day 46	No significant differences between treatments	C O D O+D C O D * O+D	No significant differences between treatments
Day 69	No significant differences between treatments	C O D O+D C O * D * O+D	No significant differences between treatments
Day 83	No significant differences between treatments	C O D O+D C O D O+D * * *	No significant differences between treatments

APPENDIX 11 Table 1 (Continued)

	Creek Edge	Midmarsh	High Marsh
Day 95	C O D O+D C O D * * O+D * * *	No significant differences between treatments	No significant differences between treatments

One-Hundred Second Difference Values

	Creek Edge	Midmarsh	High Marsh
Day 25	No Data	No significant differences between treatments	No significant differences between treatments
Day 44	No significant differences between treatments	No significant differences between treatments	C O D O+D C O D * * O+D * *
Day 46	No significant differences between treatments	C O D O+D C O D * * O+D * *	No significant differences between treatments
Day 69	C O D O+D C O D * * O+D * *	C O D O+D C O * D * O+D *	No significant differences between treatments
Day 83	C O D O+D C O D * * O+D * *	C O D O+D C O D * * O+D * *	No significant differences between treatments

APPENDIX 11 Table 1 (Continued)

		Creek Edge				Midmarsh	High Marsh
		C	O	D	O+D		
	C					No significant differences between treatments	No significant differences between treatments
Day 95	O						
	D	*	*				
	O+D	*	*			Oil plus dispersant data unavailable	

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by square root. Treatments were applied on day 43.

* = significant at the 0.05 level of significance, four degrees of freedom.

** = significant at the 0.01 level of significance, four degrees of freedom.

Peak Variable Fluorescence

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 25	1.66	0.75	0.01
Day 44	11.2 **	0.35	1.21
Day 46	0.33	0.05	0.41
Day 69	0.30	10.6 **	1.17
Day 83	0.56	2.27	1.14
Day 95	0.99	2.53	0.08
Dispersant			
Day 25	0.64	0.69	1.33
Day 44	0.01	9.05**	0.31
Day 46	0.33	2.12	3.45
Day 69	0.07	1.91	0.04
Day 83	1.43	14.09**	1.70
Day 95	32.7 **	1.67	0.08
Two-way Interaction			
Oil and Dispersant			
Day 25	0.82	0.49	0.56
Day 44	6.48*	0.26	0.53
Day 46	1.21	7.02*	1.16
Day 69	0.22	0.04	0.05
Day 83	0.03	2.47	0.02
Day 95	4.02	-	0.82

APPENDIX 11 (Continued)

One-Hundred Second Difference Values

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 25	-	1.32	0.36
Day 44	0.31	0.10	0.17
Day 46	0.92	0.19	0.02
Day 69	0.28	2.70	0.00
Day 83	0.29	0.18	0.06
Day 95	2.80	1.03	0.77
Dispersant			
Day 25	-	1.59	0.10
Day 44	3.23	8.70**	39.3 **
Day 46	6.92*	20.1 **	0.52
Day 69	16.9 **	4.12	0.46
Day 83	20.4 **	18.7 **	0.01
Day 95	29.8 **	4.12	0.19
Two-way Interaction			
Oil and Dispersant			
Day 25	-	0.59	0.26
Day 44	0.06	0.08	0.70
Day 46	0.57	1.19	0.05
Day 69	0.08	9.52**	0.03
Day 83	0.00	11.4 **	0.00
Day 95	1.05	-	0.24

APPENDIX 12: SUMMARY TABLES OF STATISTICS FOR THE FIELD ALGAL COVER DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments on two sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, two degrees of freedom) between means are indicated by asterisks. Data were transformed by ln (x + 1). Treatments were applied on day 60.

	Creek Edge	Midmarsh	High Marsh
Day 75	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments

Day 125

	C	O	D	O+D
C				
O				
D	*	*		
O+D	*	*	*	

	C	O	D	O+D
C				
O				
D				
O+D			*	

	C	O	D	O+D
C				
O				
D				
O+D				*

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by ln (x + 1). Treatments were applied on day 60.

* = significant at the 0.05 level of significance, two degrees of freedom.

** = significant at the 0.01 level of significance, two degrees of freedom.

F-ratios

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 75	1.21	0.25	2.68
Day 125	6.53**	10.9 *	3.27
Dispersant			
Day 75	2.01	2.33	0.03
Day 125	38.2 **	0.00	3.27
Two-way Interaction			
Oil and Dispersant			
Day 75	0.02	3.32	0.41
Day 125	3.82	1.76	3.27

APPENDIX 13: SUMMARY TABLES OF STATISTICS FOR THE GREENHOUSE ALGAL COVER DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments on three sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, four degrees of freedom) between means are indicated by asterisks. Data were untransformed. Treatments were applied on day 43.

	Creek Edge	Midmarsh	High Marsh
Day 39	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 67	No significant differences between treatments	C O D O+D C O * D * O+D * *	No significant differences between treatments
Day 106	C O D O+D C O D * O+D	No significant differences between treatments	No significant differences between treatments

APPENDIX 13 (Continued)

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by ln (x + 1). Treatments were applied on day 43.

* = significant at the 0.05 level of significance, four degrees of freedom.

** = significant at the 0.01 level of significance, four degrees of freedom.

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 39	0.03	0.62	0.15
Day 67	2.82	49.9 **	0.32
Day 106	0.18	1.53	0.01
Dispersant			
Day 34	0.35	0.67	0.62
Day 67	1.54	0.00	4.15
Day 106	6.46*	1.19	0.31
Two-way Interaction			
Oil and Dispersant			
Day 39	1.43	4.59*	0.29
Day 67	0.23	1.64	0.32
Day 106	3.36	1.04	0.02

APPENDIX 14: SUMMARY TABLES OF STATISTICS FOR THE HETEROTROPHIC ACTIVITY DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments on seven sampling dates in the midmarsh zone. Significant differences (0.05 level of significance, four degrees of freedom) between means are indicated by asterisks. Data were transformed by ln (x + 1). Treatments were applied on day 92.

Midmarsh

Day 34 — No significant differences between treatments

Day 92 — No significant differences between treatments

Day 96

	C	O	D	O+D
C				
O	*			
D		*		
O+D	*		*	

Day 99

	C	O	D	O+D
C				
O	*			
D		*		
O+D			*	

Day 105

	C	O	D	O+D
C				
O				
D	*	*		
O+D			*	

Day 118

	C	O	D	O+D
C				
O	*			
D	*			
O+D				

Day 127

	C	O	D	O+D
C				
O	*			
D		*		
O+D	*		*	

APPENDIX 14 (Continued)

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by ln (x + 1). Treatments were applied on day 92.

* = significant at the 0.05 level of significance, four degrees of freedom.

** = significant at the 0.01 level of significance, four degrees of freedom.

	F-ratios
Main Effects	Midmarsh
Oil	
Day 84	1.03
Day 92	0.04
Day 96	18.9 **
Day 99	17.0 **
Day 105	5.30*
Day 118	5.61*
Day 127	25.9 **
Dispersant	
Day 84	0.25
Day 92	0.01
Day 96	0.01
Day 99	7.15*
Day 105	17.5 **
Day 118	0.07**
Day 127	3.92
Two-way Interaction	
Oil and Dispersant	
Day 84	0.47
Day 92	0.25
Day 96	0.72
Day 99	0.02
Day 105	1.96
Day 118	11.4 **
Day 127	0.21

APPENDIX 15: SUMMARY TABLES OF STATISTICS FOR THE NITROGENASE ACTIVITY DATA

Table 1

Matricies of the Student-Newman-Keuls multiple range test results for the control (C), oil (O), dispersant (D), and oil plus dispersant (O+D) treatments on four sampling dates in the creek edge, midmarsh and high marsh zones. Significant differences (0.05 level of significance, four degrees of freedom) between means are indicated by asterisks. Data were transformed by ln (x + 0.05). Treatments were applied on day 43.

Light Incubation

	Creek Edge	Midmarsh	High Marsh
Day 27	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 46	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 70	No data	No data	No data
Day 119	No significant differences between treatments	C O D O+D C O * D * O+D *	No significant differences between treatments

Dark Incubation

	Creek Edge	Midmarsh	High Marsh
Day 27	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments

APPENDIX 15 (Continued)

Day 46	No significant differences between treatments	No significant differences between treatments	C O D O+D C O * D O+D
Day 70	No significant differences between treatments	No significant differences between treatments	No significant differences between treatments
Day 119	No significant differences between treatments	C O D O+D C O * D * O+D *	No significant differences between treatments

Table 2

Two-way analysis of variance results. F-ratios refer to the variance accounted for by the oil and dispersant and the interaction between these factors. Data were transformed by ln (x + 0.05). Treatments were applied on day 43.

* = significant at the 0.05 level of significance, four degrees of freedom.

** = significant at the 0.01 level of significance, four degrees of freedem.

Light Incubation

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 27	0.22	0.00	3.33
Day 46	0.06	0.42	0.85
Day 70	-	-	-
Day 119	0.63	8.73**	0.10
Dispersant			
Day 27	0.15	2.21	0.01
Day 46	0.80	1.75	0.82
Day 70	-	-	-
Day 119	0.32	2.48	2.06
Two-way Interaction Oil and Dispersant			
Day 27	0.00	0.51	0.63
Day 46	1.14	0.50	1.40
Day 70	-	-	-
Day 119	1.83	2.92	2.84

Dark Incubation

Main Effects	Creek Edge	Midmarsh	High Marsh
Oil			
Day 27	0.16	0.14	1.05
Day 46	0.05	1.18	5.45*
Day 70	0.20	3.54	0.90
Day 119	0.32	6.54*	1.49

APPENDIX 15 (Continued)

Dispersant			
Day 27	0.88	1.79	2.43
Day 46	7.05*	0.23	1.78
Day 70	1.53	0.97	1.30
Day 119	0.72	2.66	5.44*
Two-way Interaction			
Oil and Dispersant			
Day 27	0.00	0.28	0.06
Day 46	0.30	0.27	2.37
Day 70	0.49	0.11	0.17
Day 119	2.42	2.62	1.80

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