Evaluation Of Habitat Response To *In Situ* Burning As A Method Of Oil Removal Phase III — *Sagittaria Lancifolia* Fresh Marsh Field Study

C.W. Lindau, Ph.D.

R.D. DeLaune, Ph.D.

A. Jugsujinda, Ph.D.

R.N. Millward, Ph.D.

Nuclear Science Center

Wetland Biogeochemistry Institute

Louisiana State University

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Evaluation of Habitat Responses to *In Situ* Burning as a Method of Oil Removal Phase III - *Sagittaria lancifolia* Fresh Marsh Field Study

Abstract

A field study was conducted to evaluate habitat responses to burning as a method of oil removal following a simulated oil spill. Two *in situ* burns were conducted in a *Sagittaria lancifolia* fresh marsh habitat located south of Houma, Louisiana in the Pointe Au Chien Wildlife Management Area in Terrebonne Basin. Experimental treatments included: (1) control, (2) oiling, and (3) oiling plus burning. South Louisiana Crude was applied to replicated oiled and oiled plus burned treatment plots (2.4 m x 2.4 m) at a rate of 2 L m^{-2}. A propane torch was used to ignite the crude oil. The first burn was conducted August 14, 1996, and the second burn was initiated on April 4, 1997. Leaf regeneration, above ground biomass production, plant height, and gross carbon fixation of *Sagittaria lancifolia* were monitored over post-burn evaluation periods.

Sagittaria lancifolia responses to oiling and oiling plus burning were monitored for 53 weeks after the August 14, 1996 burn. At the conclusion of the field study, the average green leaf count in oiled and oiled plus burned treatment subplots was approximately 59% and 76% higher than the average control plot's live leaf count, respectively. Fifty-two weeks after burning, the maximum height of *Sagittaria lancifolia* in oiled subplots was about 5% less than plant heights measured in control plots (80 cm). Maximum plant heights recorded in oiled and burned subplots were approximately 4% higher than control plot *Sagittaria lancifolia* heights. After the August 14, 1996 post-treatment burn cycle, *Sagittaria lancifolia* gross carbon fixation rates in oiled and oiled plus burned plots (about 0.83 g CO_2-C fixed m^{-2} h^{-1}) were about 7% higher than control plant carbon fixation rates. Above ground *Sagittaria lancifolia* biomass production was measured in 1 m^2 subplots 34 and 53 weeks after the first burn. Biomass measured in oiled and oiled plus burned plots at 34 weeks was about 26% and 56% greater than *Sagittaria lancifolia* biomass clipped from control plots (236 g m^{-2}). At 53 weeks, biomass in oiled and oiled plus burned treatment subplots was nearly equal and averaged approximately 18% more compared to a recorded control plot biomass average of 429 g m^{-2}.

Sagittaria lancifolia responses to oiling and oiling plus burning were monitored for 19 weeks after the April 4, 1997 simulated oil burn. Total green leaf production in oiled and oiled plus burned plots was approximately 44% and 53% higher than an average control plot's green leaf count of 15.3 green leaves per chamber. Nineteen weeks after burning, maximum heights of *Sagittaria lancifolia* measured in control, oiled and oiled plus

burned subplots were about equal and averaged 79 cm over all treatment replications. Fixation of CO_2-C measured in oiled and oiled plus burned subplots was about 92% and 110% of the control plot *Sagittaria lancifolia* carbon fixation rate of 0.78 CO_2-C m^{-2} h^{-1}, respectively. Biomass production at 20 weeks after the second burn (April 4, 1997) was reduced in the oiled and oiled plus burned treatments. Biomass cut from 1 m^2 subplots was approximately 43% lower than the control plot above ground biomass average of 432 g m^{-2}.

1.0 Introduction

1.1 Background

Coastal wetlands bordering the northern Gulf of Mexico account for 58% of all the coastal wetlands in the United States (Alexander *et al.* 1986). These coastal areas support wildlife that is heavily dependent upon production of estuarine flora and fauna. In many areas of this region, considerable quantities of petroleum are transported, received, and stored on a regular basis. An oil spill associated with oil and gas production has the potential to reduce productivity of the area. The adverse effects of oil spills on marsh vegetation could have widespread repercussions on the food web for the entire estuarine ecosystem. Studies by Webb *et al.* (1981) and Hershner and Moore (1977) suggest that the effects of oil on vegetation and the subsequent rate of recovery depend on many factors, including oil type and concentration, the extent of coverage and the timing of the spill. These effects may also be species specific (Hershner and Moore, 1977; Webb *et al.* 1981, 1985).

1.2 Effects of Oil Spills on Vegetation

Data in the literature show great variations in responses of macrophytes to oil hydrocarbons (Crapp, 1971; American Petroleum Institute, 1985; Mendelssohn *et al.* 1995). This reflects several factors including the differences in species susceptibility to oil compounds, the types of oil used, the experimental conditions, and the stage of growth for a given species during its life cycle. The toxicity of crude oil is primarily due to the lower boiling point of volatile aromatic hydrocarbons. Oil type and the degree of weathering largely determine the toxicity levels (Crapp, 1971).

Exposure to oil may adversely affect marsh vegetation (Cowell, 1969; Holt *et al.* 1978; de la Cruz *et al.* 1981; Pezeshki and DeLaune, 1993). An oil spill severely reduced growth in a Texas salt marsh (Holt *et al.* 1978). Exposure to crude oil at 1.5 L m^{-2} caused the death of *Juncus roemerianus* with recovery reported within three years (de la Cruz *et al.* 1981). Petroleum hydrocarbons at 2 L m^{-2} adversely affected gas exchange functions of *Juncus roemerianus* and *S. alterniflora* under laboratory conditions (Pezeshki and DeLaune, 1993). Net photosynthesis decreased in both species shortly after treatment

initiation, remaining within 71% and 94% of control in *J. roemerianus* and within 53% and 80% of control plants in *S. alterniflora*. However, no lethal effects were observed. Under field conditions, plants are likely to recover once residual oil is removed by rainfall or tidal action. Growth responses, including growth of new shoots and overall plant health, show adverse effects of oil application in several species. However, these effects are likely to be short-term (Sjotun and Lein, 1993). In previous studies (DeLaune *et al.* 1979, 1984), experimental application of oil to a Louisiana salt marsh caused no reduction in biomass production as measured by above ground biomass at the end of the second growing season. Application of 0.25 L m^{-2} of crude oil to *S. alterniflora* salt marsh caused little damage to the existing stocks and to the regeneration of new plants. DeLaune *et al.* (1979) observed no significant changes in regeneration of new shoots or above ground biomass of *S. alterniflora.* The plants were examined four and 16 months after adding oil under field conditions at the rate of 1, 2, 3, 4 and 8 L m^{-2}. Various studies have indicated that biomass in *S. alterniflora* is not sensitive to crude oil at levels of 32 L m^{-2} (DeLaune *et al.* 1979, 1984; Smith *et al.* 1981, 1984).

1.3 Effects of Oil Spills on Microbial Biomass

In general, microbial biomass increases initially after an oil spill in marsh sediments. Studies have documented changes in microbial wetland populations in response to oil impacts (e.g., Kator and Herwig, 1977; Hood *et al.* 1975). These responses were generally increases in total microbial populations and increases in the ratio of hydrocarbon degraders to total heterotrophs. However, little is understood on how these changes in microbial numbers affect the turnover of oil components and the length of time for remediation of wetland systems.

The rate and extent of microbial degradation of petroleum hydrocarbons is largely determined by environmental conditions. These conditions include temperature (Bartholomew and Pfaender, 1983), salinity (Bourquin and Pryzybyszewski, 1977), Eh (Hambrick *et al.* 1980; Pardue *et al.* 1988), pH (DeLaune *et al.* 1981), and the oxygen and nutrient status of the environment (Cooney, 1984). Biodegradation of petroleum hydrocarbons is primarily an aerobic process, requiring the presence of molecular oxygen (high Eh). Several novel microbial processes have been identified that degrade oil components under anaerobic conditions (e.g., degradation of BTEX), that degrade oil components under anaerobic conditions (e.g., degradation of BTEX compounds) (Hutchins *et al.* 1991), and that degrade naphthalene under denitrifying conditions (Milhelcic and Luthy, 1988a, b). It is likely that other anaerobic processes will be determined, however, aerobic processes act on a broader spectrum of compounds and are more rapid and complete (e.g., mineralization to CO_2 and H_2O).

1.4 Vegetative Response to Burning

Fires in wetland habitats occur naturally (Wilbur and Christensen, 1983; Davison and Bratton, 1988) and as a marsh management tool, fire is used extensively in North America (Kirby *et al.* 1988). Periodic or annual burning is also used as a marsh management tool in Louisiana. In areas of intense management such as state refuges, as much as 80% of the marsh is burned annually as compared to the estimated 25 to 30% of marsh burned in other areas (Feijtel *et al.* 1985). After a fire, there is an increase in species richness in marsh habitats (Davison and Bratton, 1988). Burning changes the relative importance of species in *S. cynosuroides* and in *Juncus* marshes (Hackney and de la Cruz, 1983). In other marsh systems, no change in species composition was found due to fire (Vogl, 1973, Van Arman and Goodrick 1979). Shifts in the relative importance of species were also reported after a fire in Florida marshes (Schmalzer *et al.* 1991).

The post-fire recovery of productivity is dependent on many factors including species present at the time of burning and the growing season. Significant increases in regenerating culms, plant gas exchange, and above ground production were found in annually burned *S. pectinata* as compared to biennially burned natural tallgrass in Kansas (Johnson and Knapp, 1993). Similar findings are reported in the coastal marsh species *S. bakeri* in Florida (Schmalzer *et al.* 1991). Live biomass in burned *S. bakeri* marsh did not recover to preburn levels in one year (Schmalzer *et al.* 1991). Total biomass in *Cladium jamaicense* was only 38% of unburned stands within 18 months after burning (Steward and Ornes, 1975). On the other hand, productivity in a *S. cynosuroides* marsh was enhanced by burning (Hackney and de la Cruz, 1983). In other marsh habitats, there are reports of little change in productivity or reduced production due to burning (Smith and Kadlec, 1985a,b; Turner, 1987). The recovery appears to be rapid in marshes that die back annually. For example, burned *Panicum hemitomon* marshes produced greater live biomass within six months of burning as compared to unburned stands (Vogl, 1973; van Arman and Goodrick 1979).

Grass burning stimulates new growth of above ground biomass, but the effects on root biomass in most cases are unknown. The effects on root biomass, however, may be significant to marsh ecology because roots make up 90 to 95% of most organic peat soils. Although the root contribution to the soil organic matter is significant, few researchers have examined the below ground biomass responses to burning. Plant health, growth, and productivity help sustain organic matter necessary for peat accumulation, which in turn, maintains the intertidal marsh surface (DeLaune *et al.* 1983). Marsh surfaces developed in sediment deficient habitats remain intertidal primarily as a result of plant growth, organic detritus accumulation, and limited mineral sediment deposition (DeLaune *et al.* 1983). Marsh burning reduces the organic source, which may indirectly affect marsh aggradation in areas experiencing aggradation deficits. Burning during dry periods can also cause damage to plant root systems, and thus may accelerate marsh deterioration in unstable coastal areas (Hoffpauer, 1968). While burning may be an acceptable practice in stable coastal marsh regions, in other areas where large aggradation deficits exist, marsh burning may reduce the source of organics below the critical level needed for maintaining a viable marsh.

1.5 Burning as a Method of Oil Removal

Burning involves igniting oil affected areas, sometimes with the addition of chemical agents such as Knotax and primers such as gasoline and kerosene (Freiberger and Byers, 1971). To increase effectiveness, combustion promoters such as wicking agents, thermal insulators, and volatility modifiers may also be used (Energetex Engineering, 1979). A successful burning operation may leave a thin, viscous film ranging between 0.5 to 1.2 mm in thickness on the surface (American Petroleum Institute, 1985).

The technique of burning is controversial at best. It has been considered inefficient in certain habitats (Ford, 1970; Der and Ghormley, 1975; Logan *et al.* 1975), and one of the most effective cleanup techniques available in other habitats (Vandermeulen and Ross, 1977). For instance, in an oil affected *Spartina* marsh in Texas, burning partially removed oil from vegetation, with some heavily oiled vegetation and residue remaining on unburned portions of stems. Within six months, burned vegetation had recovered, while heavily oiled unburned marshes showed only moderate recovery (Holt *et al.* 1978).

1.6 Rationale and Significance

The susceptibility of individual wetland habitats to an oil spill, and the proper cleanup method to use for habitat recovery has been a topic of interest to various agencies. The potential impact of burning as a method of oil removal includes disturbance and death of biota from the direct effects of burning as well as the potentially toxic effects of residual compounds. These compounds include unburned oil and burned oil products that may penetrate into the lower sediment where degradation is slow and the potential for rerelease and continued harm to biota is great. These residual materials may have lethal or sublethal effects on various organisms. Marsh burning also results in temporary loss of cover, loss of detrital materials important to the food web, and loss of other functions such as feeding and resting areas for birds and other wildlife species. In marsh habitats, the technique results in loss of vegetation and other organisms through direct heat effects. Uncontrolled burning may have adverse effects on adjacent marshes not directly affected by oil, and may cause slow recovery or elimination of certain species. However, the effects of surface burns on oiled marsh biota have not been studied in detail.

While the existing work does address some oil effects on vegetation, it does not address the combined oiling and burning effects. We recently completed two oil related research projects (Pezeshki and DeLaune, 1993; DeLaune *et al.* 1994). These studies, however, addressed macrophyte responses to oiling and did not address oiling and burning. In addition, very little is known about the behavior and fate of hydrocarbon compounds (or residual compounds left by burning) in various marsh systems. Using a

multidisciplinary approach, we attempted to address not only the effects of such stressors on biota but also the movement and degradation of hydrocarbon compounds (with or without burning) in various habitats. Several questions need to be answered including: What are the specific effects of oil spills on biota in each marsh type (salt and fresh)? What are the consequences of burning for marsh biota? What percentages of carbohydrate components are removed by burning? What happens to the specific residual carbohydrate compounds that are byproducts of burning? What are the degradation rates of these compounds?

While the proposed project has not answered all of these questions, it does provide insights into various effects of oil spills and burning. This research offers immediate benefits to Louisiana's coastal/interior wetlands. The data allow evaluation of burning as a method of oil removal in specific habitats in Louisiana wetland habitats, an area of concern listed in the RFP for OSRADP. In particular, the data provide information about the environmental consequences and the effectiveness of *in situ* burning in a salt marsh (second year) and in a fresh marsh (third year). The recovery of various habitats, and the post-treatment lethal/sublethal effects of burned and unburned oil residual components have been investigated. Based on this information, the feasibility of burning as a method of oil removal in various marsh habitats has been evaluated and its effectiveness quantified, allowing us to make recommendations about the feasibility of this technique for dealing with oil spills in U.S. Gulf Coast marsh habitats.

1.7 Hypotheses

I. Burning of oiled marsh can be used as a remediation technique in selected marsh habitats of the U.S. Gulf Coast.

II. Burning impact is short-term (one to two years). Marsh recovery following oil spill is enhanced by oil removal effects of burning.

1.8 Objectives

Our study allows quantification of oil spill and burning impacts on several species representing salt marsh, brackish, and freshwater (greenhouse studies—Year 1), a field *in situ* study in a salt marsh habitat (Year 2), and a field *in situ* study in fresh marsh habit (Year 3). Specific study objectives are to evaluate:

1. the effects of oiling and burning on flora in selected marsh habitats<
2. the mechanisms of such impacts on marsh macrophytes
3. the impact of oiling and burning on flora recovery during the post-treatment period
4. the percentage removal of oil compounds by fire and the degradation of various residual compounds that are byproducts of burning (Phase I)

2.0 Methodology

2.1 Methods

The study was conducted in three phases. Phase I of the study was conducted in the greenhouse and laboratory (Year 1). Phase II and III were conducted in the field and were designed to complement and reconcile the laboratory experiments in Year 1. The work plan and time schedule for Phase III are shown in Table 2.1.

2.1.1 Laboratory and Greenhouse Studies (Phase I —Year 1) Completed (1994/1995)

South Louisiana "sweet" crude (SLC), which is enriched in light aromatic hydrocarbons, paraffins, and olefins, was used in the greenhouse studies. The SLC is moderately toxic to various organisms and is degraded by indigenous microflora. Sediment cores, 30 cm deep, and 15 cm in diameter containing *Spartina alterniflora/S. patens* (representing salt marsh/brackish habitat) and *Panicum hemitomon/Sagittaria lancifolia* (representing freshwater habitat), were collected from Louisiana marshes. The cores were transferred to a greenhouse for the study. Replicated cores were randomly assigned to main treatments of (1) oiling; (2) oiling plus burning; (3) no oiling or burning, plants clipped at marsh surface; and (4) a control (no oiling or burning).

Cores containing plants were placed in large containers 75 cm deep that were partially filled with water from the field site where the plants were collected. Oil was added to these containers at 2 L m^{-2}. The water level was raised slowly to mimic high tide conditions until reaching 25 cm above the soil surface of each pot. After eight hours, the water was released slowly to mimic low tide conditions by removing the rubber stoppers and allowing the water level to fall to the soil surface. In this manner, plants were coated with oil in a way that mimicked the rise and falling of tides in the field. The pots designated for burning were burned by ignition. The plants were monitored continuously for the entire period of post-treatment evaluations. Sediment/plant cores were kept waterlogged and/or a two to three inch floodwater layer was maintained. Fresh water was added to the pots daily to maintain constant salinity levels and to compensate for erosion. The soil was flushed each month and water from the respective field sites was used to fill the pots.

The study was conducted in a greenhouse, which allowed observation and measurements of plant responses to oiling, oiling plus burning, and control treatments. The experiment employed a randomized block design with a factorial treatment arrangement of four replications.

2.1.2 Field Studies (Phase II and III)

Based on the greenhouse experiments, a salt marsh study area was established in the Terrebonne Basin's Pointe Au Chien Wildlife Management Area (Phase II—1995/1996). An additional *in situ* burn study was conducted in a fresh marsh during Phase III (1996/1997).

Table 2.1 Work plan and time schedule of fresh marsh field burn study to be conducted in Year 3.

	1996						1997					
	J	A	S	O	N	D	J	F	M	A	M	J
FIELD STUDIES (fresh marsh)												
- site selection	X											
- obtain permit	X											
- plot layout	X											
- oiling/burning		X								X		
- plant growth	X	X	X	X	X	X		X	X			X
- regeneration	X	X	X	X	X	X		X	X			X
- biomass	X	X	X	X	X	X		X	X			X
- carbon/nitrogen flux	X	X	X	X	X	X		X	X			X
- data analyses, report writing									X	X	X	X

Each plot was 2.4m x 2.4m. Enclosures were made of plywood and aluminum sheets installed to a depth of 15 cm to minimize oil leaks. Oil was slowly applied at 2 L m^{-2} slowly to the surface of the marsh in each plot. Seventy-two hours after completing the treatments, designated plots were burned following a procedure described in detail by Breuel (1981) and the American Petroleum Institute (1985 Manual). The remaining floating oil from oiled unburned plots was collected for proper disposal. The retainers were removed to allow normal water exchange between each plot and the surrounding areas.

The experiment employed a randomized block field design with a factorial treatment arrangement of four replications. Two burn times (spring and fall), two oiling levels

(oiled and unoiled), and two burning levels (burned and unburned) were used. Data analysis was conducted using the Statistical Analysis System (SAS). The package is available to researchers through the Louisiana State University Network Computer Systems.

Proposed measurements include those outlined below. Field measurements were taken about one week, two weeks, one month, two months, four months, and eight months after each burn cycle (see Work Plan/Time Schedule).

2.1.2.1 Site Selection and Burn Permit

The Pointe Au Chien Wildlife Management Area South of Houma, Louisiana was selected as the site for conducting the salt marsh and fresh marsh *in situ* burn studies. The management area was ideal for the proposed studies because it had limited public access. In addition, roads leading to the marsh site allowed us to transport plot construction material during the post-monitoring phases of the studies. Use of the roads kept marsh damage to a minimum.

A Louisiana coastal use permit for the salt marsh study received final approval from the Coastal Management Division of the Department of Natural Resources on June 19, 1995. The approved coastal use permit (C.U.P. No. P950232) is in the possession of the principal investigator (C.W. Lindau). Additional approvals from the Louisiana Department of Health and Hospitals and Department of the Army, Corps of Engineers (New Orleans District) were received and are in the possession of the principal investigator.

A second coastal use permit was applied for on October 17, 1995 to conduct the fresh marsh study during the third year of our project (96/97). The permit was approved on December 27, 1995 (P950232 revised) and is in the possession of the Principal Investigator. Additional approvals from the Department of Wildlife and Fisheries, Department of Health and Hospitals and Department of the Army were also received.

2.1.3 Proposed Measurements for Phase I, II and III

Various measurements outlined in this proposal were conducted during Phases I, II and III as applicable. These methodologies and the necessary instrumentation are presently available at LSU's Wetland Biogeochemistry Institute and the Nuclear Science Center.

2.1.3.1 Plant Growth and Regeneration

Culm density (vegetative and reproductive) was recorded for each plot/treatment throughout the study.

To assess changes in carbon flux and net photosynthesis from plant/soil systems, light and dark chambers were placed over each sub-plot in the field (Smith *et al.* 1981). Light chambers constructed from 3 mm clear Plexiglass with 0.366 m^2 in cross-sectioned area and internal volume of 281 liters, were used for measurements. Chambers similar in dimension insulated with Styrofoam (2 cm thick) and covered with a reflective space blanket were used for dark CO_2 flux measurements (respiration). Method and calculations were according to Smith *et al.* (1981).

Measurements were conducted using portable infra-red gas analyzers (ADC, model A120 and PP systems, Model CIRAS-1). These techniques have been previously used for evaluation of plant responses both in the laboratory and in the field (Pezeshki and DeLaune, 1988; Pezeshki *et al.* 1989). Implementation of these methods provided useful information about seasonal patterns of plant gas exchange for each study habitat.

2.1.3.1.1 Biomass

Changes in above ground biomass were determined in Phase I, II and III experiments. In the greenhouse, replicated pots were harvested for assessing the above ground biomass component. In the field, the above ground biomass was measured by cutting the vegetation at sediment level using a 1.00 m^2 quadrat in randomly selected sub plots. The subplots were carefully marked in each area to avoid resampling of the same quadrat. The above ground materials were cut at sediment level and sealed in plastic bags. In the laboratory, biomass was separated into live and dead fractions and dried to a constant weight.

2.1.3.1.2 Plant Community Structure

The plant composition, structure, and density were determined in study plots in the field. In addition, plots were photographed for a visual record of change. The procedure was described in detail by Kadlec and Wentz (1974) and Smith and Kadlec (1985 a, b).

2.1.3.2 Degradation and Compositional Changes in Hydrocarbon Components

Following burning, two degradation mechanisms were quantified: (1) loss of oil components during burning, and (2) potential for microbial degradation by wetland

microbiota following burning. To assess loss mechanisms from burning, soil cores (10 cm x 15 cm deep) were taken from each plot immediately following treatment to determine the crude oil fraction remaining in the sediment. Cores were extruded and vertically sectioned into 4 cm increments according to DeLaune *et al.* (1983). The core sections were dried at 28 ^{o}C and ground to pass through a 25-mesh sieve. Soil was extracted using supercritical fluid extraction with a suitable modifier. The extract was fractionated on activated alumina. Hydrocarbon fractions were analyzed with GC-MS using a modification of EPA Method 8720. A mass balance approach was used in all studies.

To assess loss from microbial degradation, identical cores were removed from each plot at one, four and eight weeks following treatment. Cores were extracted and analyzed as described above. Loss of oil during this period was primarily due to microbial degradation plus abiotic processes such as volatilization. Loss rates were correlated with measurements of microbial biomass, as described above.

2.1.3.2.1 Oil Extraction and GC-MS Techniques

Sediment samples (about 4 g) with and without oil (South Louisiana Crude and Arabian Crude oil), burned and unburned, were extracted for hydrocarbons using a modified extraction procedure similar to Koques *et al.* (1994). Sediment/oil samples were transferred to Teflon centrifuge tubes for hydrocarbon extraction. The extracting solution was a 1:1 mixture of hexane and acetone. Twenty ml of the extracting solution were added to the Teflon tubes, and the tubes were shaken for 12 hours. After shaking, the Teflon tubes were centrifuged at 10,000 rpm for 13 minutes at laboratory temperature. The hexane/acetone solvent was decanted at the top, and anhydrous sodium sulfate was added to remove trace amounts of water. The solvent mixture was evaporated to 10 ml using ultra high pure nitrogen gas. The solvent/hydrocarbon mixture was then diluted 100 times to permit GC-MS analysis. From the diluted sample, 1 ml was added to GC-MS reduced volume vials and 0.04 ml of internal standard were added to each vial before GC-MS instrumental analysis.

Hydrocarbon analyses were performed on a gas chromatograph (Hewlett Packard 5890 Series II plus) equipped with an automatic sampler and a HP-5 high resolution capillary column (30 m, 0.25 mm film thickness, 0.25 mm i.d.). The capillary column was directly interfaced to a quadruple mass spectrometer (Hewlett Packard 5972 Mass Selective Detector). The carrier gas was ultra high pure helium. The flow rate was 1.0 ml/min, the injector temperature was 300 ^{o}C, and the column temperature was programmed from 50 ^{o}C to 310 ^{o}C at 8 ^{o}C/min rate with an initial 3.0 minute delay and 15 minute hold at the end of the run. The interface to the mass selective detector was maintained at 28 ^{o}C. Sample and standard injections were made using a Hewlett Packard 7673 automatic liquid sampler into a splitless injection port.

A Hewlett Packard Vectra 486/66 XM computer system and Hewlett Packard 61034C software for the MS chem station (DOS series) were used to collect and analyze data. Hydrocarbon peaks were identified using the G1033A NIST PBM Library software.

3.0 Results and Discussion: Phase III—Fresh Marsh

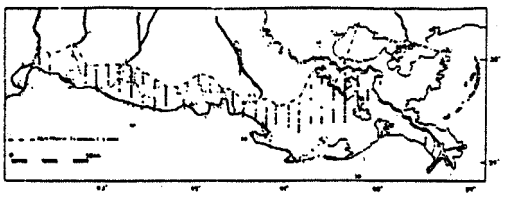
3.1 Plant Species

Sagittaria lancifolia (Bulltongue) is a perennial herb found in fresh and brackish marshes (Figure 3.1). Plants produce lanceolate blades two to three feet tall and white flowers arranged in whorls. *Sagittaria lancifolia* plants grow in dense stands, which makes the species a major contributor to marsh building and aquatic food chains.

3.2 Field Design

Plywood plot enclosures (2.4 m x 2.4 m) were constructed and installed in the fresh marsh prior to each burn cycle. Metal sheeting was used to line the burn treatment plot enclosures. Treatments consisted of: (1) control—no oiling or burning, (2) oiling, and (3) oiling plus burning. Oil treatments received 2 L m^{-1} of South Louisiana Crude (Figure 3.2). Treatments were replicated four times for statistical analysis. A 15 to 20 cm floodwater layer above the fresh marsh sediment surface was established before the burn plots were ignited. A propane torch was used to ignite the South Louisiana Crude.

Two burns were initiated in the *Sagittaria lancifolia* marsh. The late summer/fall burn was conducted on August 14, 1996 and the spring burn was initiated on April 4, 1997.



Sagittaria lancifolia L.
(Sagittaria falcata)
Alismataceae
Bulltongue

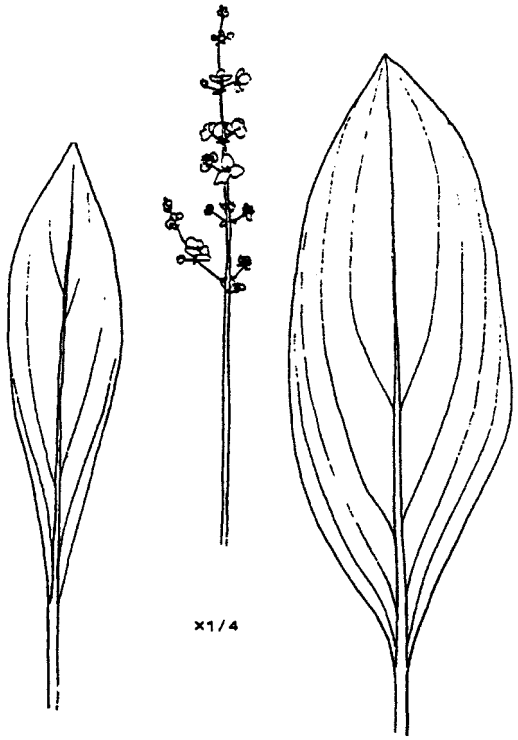


Figure 3.1 *Description of Sagittaria lancifolia herb. (from R.H. Chabreck and R.E. Condrey, Common Vascular Plants of the Louisiana Marsh, Sea Grant Publication No. LSU-T-79-003, Center for Wetland Resources)*

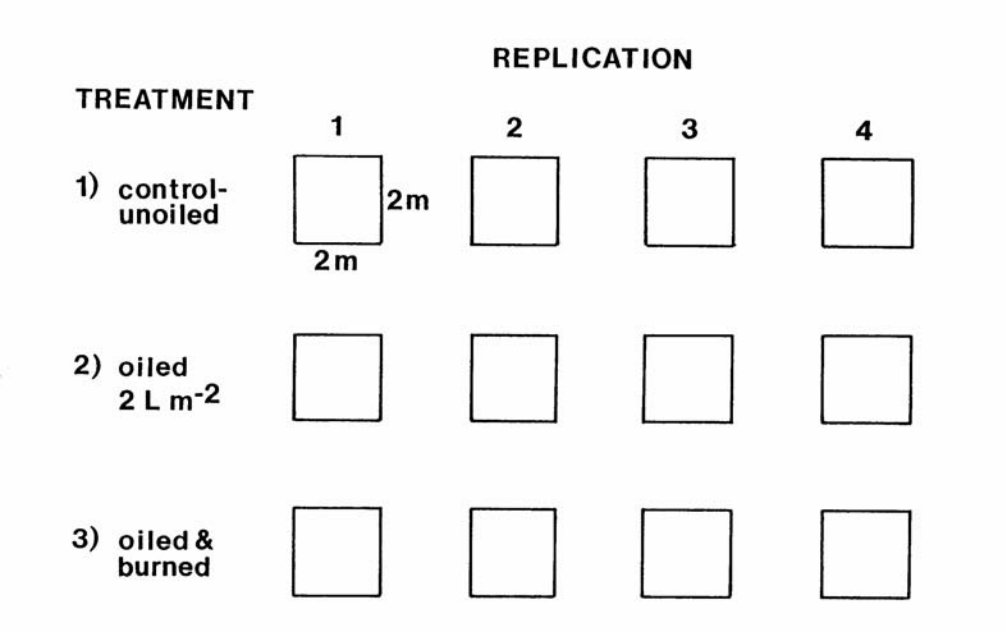


Figure 3.2 *Field plot experimental design for the fresh marsh burn (phase III).*

3.3 Results From First *In Situ* Burn

3.3.1 Plant Regeneration

Plant regeneration capacity (number of live plants and green leaves) of control, oiled, and oiled plus burned plots for the fall burn (August 14, 1996) are shown in Tables 3.1 and 3.2 for the *Sagittaria lancifolia* fresh marsh site. The number of live plants and green leaves was evaluated at one, five, nine, 36, 39, 42, 44, 48, and 52 weeks after oiling and burning. Measurements were not taken from week 10 to 35 due to salt water intrusion by an offshore tropical depression and plant die back during the winter months. Table 3.1 displays the number of new or live *Sagittaria lancifolia* plants measured on each sampling date for each of the three treatments. Individual and average values (3 to 4 replications) are given for each treatment. For the control treatments, the number of live plants per plot (30 cm x 30 cm) ranged from 2.8 (week 9) to a high of 5.3 live plants per chamber at week 1. Over the 52 week sampling time, the average number of live *Sagittaria lancifolia* plants was approximately 4.2 per chamber replication. Application of South Louisiana Crude to designated oil treatment plots did not appear to have a significant effect on the number of live plants compared to the controls. In the oiled plots, the number of live plants ranged from 3.8 (week 9) to 6.3 recorded 48 and 52 weeks after oiling and burning. The average number (over 52 weeks) of live plants in the oiled plots was 4.9. Very little difference in live plant count was observed for the oiled plus burned treatment. One week after the burn, 3.5 live plants per chamber were measured and plant count steadily increased to 6.7 *Sagittaria lancifolia* plants per plot at 52 weeks. An

average of 5.0 live plants per chamber for the oiled plus burned plots was calculated for the 52 week study period.

Table 3.2 summarizes the number of green *Sagittaria lancifolia* leaves counted after the August 14, 1996 burn. Green leaf count indicated the effects of oiling and burning on plant health. The number of green leaves per chamber was lowest in the control plots. Average green leaf count ranged from a low of 4.0 (week 9) to a high of 15.7 per plot measured at 52 weeks. The leaf count averaged 12.4 over nine sampling intervals. Green leaves per replication increased for the oiled treatments. The number of green leaves steadily increased from 8.0 (week 1) to 25.0 per plot at 52 weeks. An average of 16.2 green leaves per sampling date was observed, which was about four green leaves per chamber higher than the control average (12.4). Low leaf count (8.0) observed at Week 1 may have been due to the toxic effect of oil on the plant, but by Week 5 and for the remainder of the study, oil appeared to enhance green leaf production. The greatest number of green leaves per chamber was observed for the oiled plus burned treatment after Week 5 (Table 3.2). The number of *Sagittaria lancifolia* green leaves per plot, post-burn, ranged from a low of 4.0 (Week 1) to a maximum average of 28.0 recorded at 48 weeks. The 52 week average green leaf count was 19.5 compared to 16.2 for the oiled treatment and 12.4 for control plots.

As these data show, burning South Louisiana Crude appeared to enhance green leaf regeneration. This may have been due to the release of nutrients during burning and the elimination of competing species, which was observed after burning. The *Sagittaria lancifolia* roots were protected from burning oil by a 15 to 20 cm flood water layer.

Table 3.1 Number of live *Sagittaria lancifolia* plants after the August 14, 1996 burn

Treatment	Rep.	Live Plants per Chamber									
		weeks:	1	5	9	36	39	42	44	48	52
	1		5	5	2	6	5	5	4	5	5
	2		5	3	2	4	4	3	3	4	5
Control	3		6	5	4	4	2	3	3	5	6
	4		6	4	3	---	---	---	---	---	---
	Avg.		**5.5**	**4.2**	**2.8**	**4.7**	**3.7**	**3.7**	**3.3**	**4.7**	**5.3**
	1		6	7	5	4	5	5	6	5	6
	2		4	5	4	6	4	4	7	8	8
Oil	3		5	3	2	5	3	3	3	6	5
	4		5	4	4	---	---	---	---	---	---
	Avg.		**5.0**	**4.7**	**3.8**	**5.0**	**4.0**	**4.0**	**5.3**	**6.3**	**6.3**
	1		3	3	4	4	3	3	3	6	5
	2		6	4	5	6	4	5	8	7	9
Oil + Burn	3		3	4	5	5	7	7	8	9	6
	4		2	---	3	---	---	---	---	---	---
	Avg.		**3.5**	**3.7**	**4.2**	**5.0**	**4.7**	**5.0**	**6.3**	**6.3**	**6.7**

Table 3.2 Number of green *Sagittaria lancifolia* leaves after the August 14, 1996 burn

Treatment	Rep.	Green Leaves per Chamber									
		weeks:	1	5	9	36	39	42	44	48	52
	1		18	14	5	14	11	15	17	18	17
	2		7	8	4	10	11	12	16	15	14
Control	3		20	15	4	8	8	11	12	13	16
	4		19	12	3	---	---	---	---	---	---
	Avg.		**16.0**	**12.2**	**4.0**	**10.7**	**10.0**	**12.7**	**15.0**	**15.3**	**15.7**
	1		7	23	14	9	16	21	28	27	24
	2		9	18	10	12	9	15	26	25	28
Oil	3		6	8	4	13	9	15	17	22	23
	4		10	14	8	---	---	---	---	---	---
	Avg.		**8.0**	**15.7**	**9.0**	**11.3**	**11.3**	**17.0**	**23.7**	**24.7**	**25.0**
	1		3	11	9	18	9	12	11	21	17
	2		7	21	20	20	1	27	29	30	31
Oil + Burn	3		3	12	15	14	25	46	38	33	35
	4		3	---	7	---	---	---	---	---	---
	Avg.		**4.0**	**14.7**	**12.8**	**17.3**	**17.0**	**28.3**	**26.0**	**28.0**	**27.7**

3.3.2 Plant Height

Maximum height of *Sagittaria lancifolia* was monitored over a 52 week period after the first burn (8/14/96) for three treatments (Table 3.3). Maximum plant height (cm) of *Sagittaria lancifolia* plants in control plots remained fairly constant over nine sampling intervals, and average values ranged from 59.0 (Week 9) to 79.7 cm measured (Week 52). Averaged over nine sample intervals, maximum plant height in control plots was 72.3 cm. Measured plant heights in oiled treatment plots were less than control plant heights. Plant heights measured in oiled plots ranged from an average of 59.0 cm recorded at nine weeks to 77.0 cm at 48 weeks. An average value of 67.0 cm was calculated over nine measurement intervals (one, five, nine, 36, 39, 42, 44, 48, and 52 weeks). Maximum plant height of *Sagittaria lancifolia* measured in oiled plus burned plots steadily increased over the study period. One week after the burn, plant height averaged 23.7 cm and at 52 weeks averaged 82.6 cm. Averaged over the study period, maximum height of *Sagittaria lancifolia* was 67.3 cm measured in oiled plus burned replicated plots. At the conclusion of the monitoring phase (52 weeks) plant heights measured in oiled and oiled plus burned plots were about 97% and 101% of control plot plant heights, respectively.

3.3.3 Carbon Fixation

Gross carbon fixation for control, oiled, and oiled plus burned treatments was measured in the fresh marsh experimental plots three, five, nine, 36, 39, 42, 44, 48, and 52 weeks after the August 14, 1996 burn. Carbon fixation measurements and average values are tabulated in Table 3.4 and expressed in g CO_2-C fixed m^{-2} h^{-1}. Carbon fixation in control plots varied over nine sampling periods and ranged from an average low of 0.39 g CO_2-C m^{-2} h^{-1} (39 weeks) to a high of 0.96 g CO_2-C m^{-2} h^{-1} recorded nine weeks after the burn. Over the 52 week study period, carbon fixation in control plots averaged 0.70 g CO_2-C m^{-2} h^{-1}. Oiling of plots had little effect on *Sagittaria lancifolia* carbon fixation rates (Table 3.4). Gross carbon fixation in oil only treatment plots ranged from 0.40 g CO_2-C m^{-2} h^{-1} (Week 39) to a high of 1.10 g CO_2-C m^{-2} h^{-1} (Weeks 3 and 5). Carbon fixation in oiled plots was 0.80 g CO_2-C m^{-2} h^{-1} averaged over eight measurements. Carbon fixation rates measured in oiled plus burned plots were about the same as those observed for control and oiled only treatment plots. Gross carbon fixation in burned plots varied from a low of 0.42 g CO_2-C m^{-2} h^{-1} measured at Week 39 to a high of 1.24 g CO_2-C m^{-2} h^{-1} recorded at Week 36. Over eight sampling dates, *Sagittaria lancifolia* gross carbon fixation averaged 0.76 g CO_2-C m^{-2} h^{-1} in oiled plus burned plots. At the conclusion of the study, carbon fixation measured in oiled and oiled plus burned plots was approximately 7% higher compared to *Sagittaria lancifolia* control plot fixation values.

Table 3.3 Maximum height of *Sagittaria lancifolia* after the August 14, 1996 burn

Treatment	**Rep.**	**Plant Height per Chamber (cm)**									
		weeks:	1	5	9	36	39	42	44	48	52
	1		74	77	73	67	67	65	80	81	79
	2		68	75	70	86	73	80	80	77	82
Control	3		70	70	47	60	60	56	76	80	78
	4		80	83	46	---	---	---	---	---	---
	Avg.		**73.0**	**76.2**	**59.0**	**71.0**	**66.7**	**67.0**	**78.7**	**79.3**	**79.7**
	1		55	60	54	65	55	60	70	76	73
	2		76	75	76	60	65	60	71	80	76
Oil	3		68	80	60	64	70	56	66	75	79
	4		63	70	46	---	---	---	---	---	---
	Avg.		**65.5**	**71.2**	**59.0**	**63.0**	**63.3**	**58.7**	**69.0**	**77.0**	**76.0**
	1		25	88	64	81	65	74	78	82	84
	2		20	67	67	78	70	70	70	79	81
Oil + Burn	3		33	70	54	80	60	64	70	80	83
	4		17	---	46	---	---	---	---	---	---
	Avg.		**23.7**	**75.0**	**57.7**	**79.7**	**65.0**	**69.3**	**72.7**	**80.3**	**82.6**

Table 3.4 Carbon fixation of *Sagittaria lancifolia* after the August 14, 1996 burn

Treatment	Rep.	Gross Fixation (g CO_2-C m^{-2} h^{-1})									
		weeks:	3	5	9	36	39	42	44	48	52
	1		1.25	1.33	0.75	0.78	0.43	0.75	0.84	0.71	0.83
	2		0.91	0.82	1.43	1.08	0.40	0.78	0.82	0.83	0.80
Control	3		0.86	0.65	0.68	0.48	0.34	0.51	0.39	0.60	0.71
	4		0.46	0.45	0.99	---	---	---	---	---	---
	Avg.		**0.87**	**0.81**	**0.96**	**0.78**	**0.39**	**0.68**	**0.68**	**0.71**	**0.78**
	1		1.77	1.33	0.60	1.01	0.47	0.78	---	0.83	0.91
	2		1.24	1.34	0.72	0.62	0.33	1.00	---	0.67	0.82
Oil	3		0.52	0.80	0.71	0.80	0.38	0.52	---	0.90	0.75
	4		0.85	0.91	0.36	---	---	---	---	---	---
	Avg.		**1.10**	**1.10**	**0.60**	**0.81**	**0.40**	**0.76**	**---**	**0.80**	**0.83**
	1		0.29	0.91	0.62	1.04	0.47	0.11	---	0.59	0.79
	2		0.91	1.33	1.08	1.47	0.51	0.67	---	0.85	0.80
Oil + Burn	3		0.66	1.07	0.76	1.19	0.28	0.72	---	0.67	0.93
	4		0.38	0.72	0.77	---	---	---	---	---	---
	Avg.		**0.56**	**1.01**	**0.81**	**1.24**	**0.42**	**0.50**	**---**	**0.70**	**0.84**

3.3.4 Biomass Production

Live above ground *Sagittaria lancifolia* biomass produced in control, oiled, and oiled plus burned plots was cut and measured 34 and 53 weeks after the August 14, 1996 burn. Biomass cut from 1 m^2 subplots is tabulated in Table 3.5, and average values represent a mean of four replication per treatment.

Thirty-four weeks after oiling and burning, biomass cut and collected from the control plots ranged in value from 196 to 302 g m^{-2} and averaged 236 g m^{-2} for four replications. Live biomass removed from oiled treatment plots varied from a low of 246 g m^{-2} to a high of 401 g m^{-2} recorded in one plot and averaged 297 g m^{-2} (Table 3.5). The greatest live *Sagittaria lancifolia* biomass was measured in oiled plus burned treatment plots. Biomass production in burned plots ranged from 264 to 572 g m^{-2} and over four replications averaged 367 g m^{-2}. Compared to control plot values, *Sagittaria lancifolia* biomass harvested (at 34 weeks) in oiled plots was approximately 26% higher and 56% higher in oiled plus burned treatment plots.

Sagittaria lancifolia above ground biomass was harvested and measured a second time at the conclusion of the study. Fifty-three weeks after the August 14, 1996 burn, subplots were cut again. Total biomass collected at the second cutting was higher for all treatments compared to first cutting biomass values. At 53 weeks, control plot above ground biomass varied from 282 to 554 g m^{-2} and averaged 429 g m^{-2} (Table 3.5). Biomass removed from oiled treatment plots was higher and varied over a wide range (289 to 726 g m^{-2}) with an average of 510 g m^{-2}. *Sagittaria lancifolia* harvested from oiled plus burned plots was similar to biomass cut from oiled plots, but production was more than control plot values. Production of biomass in oiled plus burned plots ranged from a low of 392 g m^{-2} to a maximum of 605 g m^{-2} cut from four oiled plus burned plots. Total *Sagittaria lancifolia* above ground biomass cut in the oiled plots represents a 19% increase compared to control plots at 53 weeks. Total oiled plus burned above ground plant biomass increased approximately 18% above control biomass values. Oiling and burning appears to have a positive effect on *Sagittaria lancifolia* biomass production in the fresh marsh test sites.

Table 3.5 Biomass production of *Sagittaria lancifolia* after the August 14, 1996 burn

Treatment	**Rep.**	**Biomass (g m^{-2})**		
		Weeks:	34	53
	1		237	554
	2		302	282
Control	3		210	460
	4		196	420
	Avg.		**236**	**429**
	1		279	289
	2		401	699
Oil	3		263	726
	4		246	325
	Avg.		**297**	**510**
	1		320	392
	2		264	588
Oil + Burn	3		572	605
	4		312	444
	Avg.		**367**	**507**

3.4 Results From Second *In Situ* Burn

3.4.1 Plant Regeneration

The number of live plants and total green leaf regeneration of control, oiled, and oiled plus burned treatments after the April 4, 1997 spring burn are tabulated in Tables 3.6 and 3.7. Plant regeneration was evaluated at three, six, nine, 11, 15, and 19 months after the second *in situ* burn. Individual and average values (three replications) are given for each treatment at each sampling interval. Live *Sagittaria lancifolia* plants recorded in control plots (no oil or burn) remained fairly constant over the 19 week sampling interval. Average values ranged from 3.3 (Week 11) to 4.7 (Weeks 3 and 19) plants per chamber with a 4.1 plants per plot average over six sampling times. Oiled treatment plant averages were higher than control values and varied between 3.7 (Week 3) and 6.7 (Week 15) plants per plot. Averaged over 19 weeks, plant count in oiled plots was 5.4 plants per chamber base. Low plant count at Week 3 (3.7) may have been partially due to the short-term effect of applied oil. *Sagittaria lancifolia* plant count in oiled plus burned plots was similar to the oiled treatment. After the burn, plant count ranged from 4.7 (Week 9) to 6.3 live plants per chamber recorded at Week 19, with an average of 5.5 plants per plot over the 19 week sampling period. Based on live plant count, it would appear that oiling and burning enhanced *Sagittaria lancifolia* regeneration compared to control plots (Table 3.6).

Table 3.7 tabulates the total number of green *Sagittaria lancifolia* leaves counted in treatment plots after the April 4, 1997 burn. Green leaf count, over time, is an indication both general plant health and the effects of oiling and burning on marsh plants. The number of green leaves in *Sagittaria lancifolia* control plots ranged from 10.0 (Week 6) to 16.3 leaves per plot recorded on Week 15. Averaged over 19 weeks, the control plot average was 13.3 green leaves per chamber. An increase in green leaf production was observed in oiled and oiled plus burned plots over six sampling times (Table 3.7). Average values varied from 8.3 (Week 6) to 23.0 (Week 11) with a 19 week average of 16.7 green leaves per plot for the oiled treatment. Green leaf regeneration started out slow in oiled plus burned plots (6.0 leaves per plots at Week 3), but steadily increased to 25.3 leaves per chamber at Week 11. The oiled plus burned green leaf average (over 19 weeks) of 18.5 was the highest of any treatment investigated. Nineteen weeks after the second burn cycle, the green leaf counts in oiled and oiled plus burned plots were about 44% and 52% greater than the control plot leaf count, respectively. Burning of applied South Louisiana Crude appeared to enhance green leaf production in the *Sagittaria lancifolia* fresh marsh experimental plots. This plot response was observed for both the August 14, 1996 and April 4, 1997 post-burn monitoring periods. The same effect was observed for the oiled treatments, but to a lesser degree (Tables 3.2 and 3.7).

3.4.2 Plant Height

Sagittaria lancifolia maximum plant heights were measured for 19 weeks after the second burn. Heights were measured and recorded for each treatment replication (3) at three, six, nine, 11, 15 and 19 weeks. Individual and treatment height average values are listed in Table 3.8. Over the study period, control plot height ranged from a low of 66.7 cm (Week 6) to a high of 78.7 cm measured at Weeks 11, 15 and 19 with a study period average of 73.5 cm. A reduction in plant height was observed in oiled treatment plots. After oiling, plant height averages varied from 60.7 cm (Week 9) to 79.3 cm measured at Week 19. The 19 week average plant height in oiled plots was 67.6 cm. Plant height measurements in oiled plus burned plots were similar and below control plot values (Table 3.8). *Sagittaria lancifolia* maximum height, after the April 4, 1997 burn, ranged from 58.7 cm (Week 3) to 80.0 cm (Week 19) with a 19 week average of 66.4 cm. At the conclusion of the 19 week monitoring period, plant heights measured in oiled and oiled plus burned plots were approximately 1% to 2% higher than control plot plant heights.

Table 3.6 Number of live plants of *Sagittaria lancifolia* after the April 4, 1997 burn

Treatment	**Rep.**	**Live Plants per Chamber**						
		Weeks:	3	6	9	11	15	19
	1		6	5	5	4	5	5
Control	2		4	4	3	3	4	5
	3		4	2	3	3	4	4
	Avg.		**4.7**	**3.7**	**3.7**	**3.3**	**4.3**	**4.7**
	1		5	8	2	5	6	6
Oil	2		2	6	5	5	6	6
	3		4	2	7	7	8	7
	Avg.		**3.7**	**5.3**	**4.7**	**5.7**	**6.7**	**6.3**
	1		6	5	5	4	5	6
Oil + Burn	2		6	6	3	6	6	6
	3		5	6	6	5	6	7
	Avg.		**5.7**	**5.7**	**4.7**	**5.0**	**5.7**	**6.3**

Table 3.7 Number of green leaves of *Sagittaria lancifolia* after the April 4, 1997 burn

Treatment	**Rep.**	**Green Leaves per Chamber**						
		Weeks:	3	6	9	11	15	19
	1		14	11	15	17	17	16
Control	2		10	11	12	16	17	17
	3		8	8	11	12	15	13
	Avg.		**10.7**	**10.0**	**12.7**	**15.0**	**16.3**	**15.3**
	1		9	9	10	23	22	21
Oil	2		9	9	18	20	19	20
	3		8	7	18	26	27	25
	Avg.		**8.7**	**8.3**	**15.3**	**23.0**	**22.7**	**22.0**
	1		5	7	21	19	20	23
Oil + Burn	2		6	15	14	24	23	21
	3		7	16	22	33	31	26
	Avg.		**6.0**	**12.7**	**19.0**	**25.3**	**24.7**	**23.3**

Table 3.8 Maximum height of *Sagittaria lancifolia* after the April 4, 1997 burn

Treatment	Rep.	Plant Height per Chamber (cm)						
		Weeks:	3	6	9	11	15	19
	1		67	67	65	80	83	81
Control	2		86	73	80	80	79	80
	3		60	60	56	76	74	75
	Avg.		**71.0**	**66.7**	**67.0**	**78.7**	**78.7**	**78.7**
	1		60	58	60	60	76	77
Oil	2		68	70	60	70	80	78
	3		65	60	62	54	75	83
	Avg.		**64.3**	**62.7**	**60.7**	**61.3**	**77.0**	**79.3**
	1		68	62	65	65	80	83
Oil + Burn	2		50	55	58	60	81	80
	3		58	60	55	64	74	77
	Avg.		**58.7**	**59.0**	**59.3**	**63.0**	**78.3**	**80.0**

3.4.3 Carbon Fixation

Gross CO_2-C fixation for control, oiled, and oiled plus burned treatments was measured at three, six, nine, 11, 15 and 19 weeks after the second burn (April 4, 1997). Gross fixation in g CO_2-C m^{-2} h^{-1} is tabulated in Table 3.9 with individual and treatment averages displayed.

Control plot (no oil or burn) gross carbon fixation values ranged from a low of 0.39 g CO_2-C m^{-2} h^{-1} to a high of 0.78 g CO_2-C m^{-2} h^{-1} measured at Weeks 3 and 19. Averaged over six sampling dates, control plot carbon fixation was 0.67 g CO_2-C m^{-2} h^{-1}. Carbon

fixation was reduced at three weeks (0.10 g CO_2-C m^{-2} h^{-1}) in oiled treatment plots compared to control plot fixation averages but rapidly increased to 0.75 g CO_2-C m^{-2} h^{-1} at nine weeks (Table 3.9). Gross carbon fixation measured in *Sagittaria lancifolia* oiled treatment plots averaged 0.55 g CO_2-C m^{-2} h^{-1} over 19 months. Burning initially lowered carbon fixation to 0.2 g CO_2-C m^{-2} h^{-1} measured three weeks after the burn, but fixation increased to 1.02g CO_2-C m^{-2} h^{-1} at nine weeks. Over the 19 week sampling period, average carbon fixation in burned *Sagittaria lancifolia* plots was 0.65 g CO_2-C m^{-2} h^{-1}. At the conclusion of the study (19 weeks) carbon fixation measured in oiled and oiled plus burned plots was approximately 92% and 110% of recorded control plot fixation values, respectively.

3.4.4 Biomass Production

Above ground *Sagittaria lancifolia* biomass was cut and collected from 1 m^2 subplots of each treatment replication 20 weeks after the April 4, 1997 burn. Individual plots and average plot biomass values for each treatment are shown in Table 3.10. Live biomass cut from *Sagittaria lancifolia* control plots ranged from 282 g m^{-2} to 554 g m^{-2}. Over three replications, average plot biomass weight was 432 g m^{-2}. Biomass production in oiled treatment plots was much less and varied from 196 g m^{-2} to 227 g m^{-2} with a replication average of 244 g m^{-2}. Above ground *Sagittaria lancifolia* biomass clipped from oiled plus burned plots ranged in value from 234 g m^{-2} to 275 g m^{-2} and averaged 250 g m^{-2} at 20 weeks after the April 4, 1997 burn (Table 3.10). Biomass cut from oiled and oiled plus burned treatment plots was approximately 56% and 58% lower than the control plot biomass average, respectively.

Table 3.9 Carbon fixation of *Sagittaria lancifolia* after the April 4, 1997 burn

Treatment	**Rep.**	**Gross Fixation (g CO_2-C m^{-2} h^{-1})**						
		Weeks:	3	6	9	11	15	19
	1		0.78	0.43	0.75	0.84	0.71	0.83
Control	2		1.08	0.40	0.78	0.82	0.83	0.80
	3		0.48	0.34	0.51	0.39	0.60	0.71
	Avg.		**0.78**	**0.39**	**0.68**	**0.68**	**0.71**	**0.78**
	1		-0.77	0.52	0.65	0.63	0.60	0.67
Oil	2		0.56	0.49	0.94	0.75	0.71	0.73
	3		0.51	0.27	0.65	0.47	0.77	0.75
	Avg.		**0.10**	**0.42**	**0.75**	**0.62**	**0.69**	**0.72**
	1		0.15	0.49	1.27	0.59	0.64	0.90
Oil + Burn	2		0.33	0.25	1.10	0.72	0.81	0.83
	3		0.31	0.31	0.67	0.73	0.69	0.85
	Avg.		**0.26**	**0.35**	**1.02**	**0.68**	**0.71**	**0.86**

Table 3.10 Biomass production of *Sagittaria lancifolia* after the April 4, 1997 burn

Treatment	**Rep.**	**Biomass (g m^{-2}) cut at 20 weeks**
	1	554.1
Control	2	281.6
	3	460.2
	Avg.	**432.0**
	1	257.2
Oil	2	277.2
	3	196.3
	Avg.	**243.6**
	1	239.3
Oil + Burn	2	234.1
	3	275.1
	Avg.	**249.5**

4.0 Bibliography

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Appendix A

The Impact of Oiling and Post-Oil Burning as a Remediation Technique on Salt Marsh Meiofauna, with Particular Reference to the Nematoda

by Rod N. Millward

Abstract

The study investigates the medium to long-term effect of experimental oiling and burning of *Spartina* marsh plots upon the meiofauna, and in particular, the nematode community. Four plot treatments were investigated: plots treated with crude oil and left for one year, plots oiled and burned and left for one year, plots oiled and burned and left for six months, and control plots. Results suggest a decrease in the number of meiofauna taxa, and significant changes in the nematode community up to six months after oiling and burning. However, after one year, both the oiled and oiled and burned plots were no different than the control plots. The mechanism of this impact is unclear. It might be due to direct hydrocarbon toxicity, stresses associated with organic enrichment, indirect effects on microflora/fauna, predators, or the *Spartina* beds themselves. The study shows no ameliorative effect of burning upon the meiofauna or nematoda after one year, compared to control plots or plots exposed to oil and not burned.

1.0 Introduction

Meiofauna are generally described as benthic fauna that pass through a 1 mm sieve and are collected upon a 43 μm (0.043 mm) sieve (Higgins & Thiel, 1988). The meiofaunal taxa are employed to asses the impact of potential pollutants and disturbance on marine systems. Because of the ease with which they can be sampled, their inherent diversity, range of sensitivities and benthic life history, meiofauna taxa are good subjects for local impact assessment studies (Moore and Bett, 1989; Coull and Chandler, 1992). In addition, recent advances in multivariate analyses have provided powerful tools for investigating structure and hypothesis testing with such benthic data sets (Clarke, 1993).

The oiling and post-burning of experimental *Spartina* plots presented an opportunity to study the medium to long-term effects upon the meiofauna community, and nematode community in particular. Samples were taken from plots that had been: (1) oiled with SLC and left for approximately one year, (2) oiled, burned and left for one year, (3) oiled, burned and left for six months, and (4) unoiled and unburned.

Although the impact of remediative burning following oil spills on *Spartina* marsh meiofauna is not known, some studies have addressed the impact of oil on marine and brackish salt marsh meiofauna. In summarizing these studies, Coull and Chandler (1992) have suggested that results are oftern contradictory and depend upon pollutant type, sediment characteristics, meiofaunal composition, and the degree of taxonomic rigor employed. However, when oil contamination has had a significant effect on the meiofauna, short-term decrease in diversity and richness is observed in both the nematode and copepod communities [the two most common meiofaunal taxa, Coull and Chandler (1992)]. Longer-term studies suggest that recovery of nematode density and richness following a spill takes a few months (DeWilde and Kuipers, 1977) to several years (Boucher, 1985). Copepod communities tend to recover much more slowly (Coull and Chandler, 1992). In one of the few studies of oil effects on subtropical salt marshes, Fleeger and Chandler (1983) performed a short-term field based oiling experiment upon a Louisiana *Spartina* marsh and described a number of subtle effects upon the meiofauna. Nematode densities significantly increased after five days, while densities of copepods increased after 30 days following oil contamination. However, closer examination of the copepod community revealed that this "recovery" was mainly due to a disproportionate increase in one copepod species, while rarer species were eliminated from oiled plots. It is conceivable that such impacts might be due to a direct toxicity challenge upon the local community, selecting for one polycyclic aromatic hydrocarbon (PAH) tolerant species. It has also been suggested that these might be indirect effects of PAH contamination, either by a toxicological reduction in meiofauna predators, by microbial or algal enhancement following organic enrichment (Carmen *et al.* in press), or by competitive release (Carman and Todaro, 1996).

Fleeger and Chandler (1983) terminated their study after 144 days, by which time the copepod community density was significantly lower than control plots, but of a higher species richness. The present study addresses the longer term impact of oiling upon the meiofuna, and in particular the Nematoda, using site treatments similar to those used by Fleeger and Chandler (1983). In addition, the study examines how burning oiled *Spartina* affects the marsh meiofauna community.

2.0 Methods

The meiofaunal community, and in particular the nematoda, were investigated in four experimental treatments during November 1996. Each treatment consisted of two sediment core (26.5 mm internal diameter) samples collected from three replicate enclosures. The four treatments investigated were:

- Treatment W - enclosures oiled one year previously
- Treatment X - enclosures oiled one year previously and burned for oil removal
- Treatment Y - enclosures oiled six months previously and burned for oil removal
- Treatment Z - control enclosures unoiled and unburned

Each core was taken to a depth of >10 cm and preserved on site in 10% formalin solution. Due to the high density of fibrous *Spartina* material in the cores, the meiofauna were extracted from the samples using a variation of the sieving methodology outlined by Pfannkuche and Thiel (1988). The preserved sample was placed on a 500 μm sieve and the plant material teased apart with forceps. The dissected material was washed with approximately 5 l of deionized water and then poured through a 45 μm sieve. The material retained on the sieve was preserved in 10% formalin solution containing rose bengal protein stain. This dissecting and washing process was repeated three times for each core. Due to the high content of fine, fibrous plant material in each sample, the samples were split into quarters using a plankton wheel splitter, and the total number of individuals from each meiofauna taxon were noted. In addition, a minimum of 110 nematodes were randomly picked from each sample and mounted for identification to genus using an Olympus BX50 binocular microscope at x 1000 with phase contrast interference.

2.1 Total Meiofauna

The major meiofaunal taxa data were used to calculate Shannon-Wiener's H' and Pielou's Evenness Index J'. These indicies, the total number of nematodes, the total number of copepods, and the number of meiofauna taxa encountered, were tested for significant difference using Analysis of Variance (ANOVA). In addition, the meiofauna community was investigated for differences between treatments in community

composition using the PRIMER (Plymouth Routines In Multivariate Ecological Research, Clarke, 1993) suite of programs. Since the taxal abundances differed by two orders of magnitude, the replicate data were transformed to the fourth root, and were used to construct a triangular similarity matrix using the Bray-Curtis similarity measure and group-average sorting. The resulting coordinates were used to construct a two dimensional representation of the data using the Multidimensional Scaling (MDS) technique, enabling a visual investigation of the associations between groups.

2.2 Nematode Community

The nematode species data were used to calculate the total number of nematode species as expressed by Shannon-Wiener's H' and Pielou's Evenness Index J'. Significant differences between treatments was investigated using ANOVA. The nematode species data were further investigated using the multivariate PRIMER suite of programs. PRIMER transformed data to the fourth root to construct a two dimensional MDS plot of similarities in nematode species composition between site replicates. Significance of groupings revealed by MDS were tested using the *a posteriori* Analysis of Similarity (ANOSIM) permutation test within the PRIMER suite (Clarke, 1993). The contributions of individual nematode species to the average dissimilarities between these groups were studied using the Similarities Percentages (SIMPER) procedure (Clarke, 1993).

3.0 Results

3.1 Analysis of Total Meiofauna Community

During the study, 14 animal types were enumerated from 13 taxal groups. The groups encountered are listed in Table 1, in descending order of abundance.

Table 1 Animal Types Enumerated from Taxal Groups

Ranked Taxa			
1	Nematoda	8	Ciliata
2	Ostracoda	9	Halacaridae
3	Oligochaeta	10	Turbellaria
4	Copepoda	11	Insecta pupae
5	Rotifera	12	Bivalvia
6	Copepod Nauplii	13	Cnidaria
7	Polychaeta	14	Tardigrada

The mean values and standard deviation for each univariate index for the meiofaunal taxa are presented in Table 2, together with the significance of variance by ANOVA.

Table 2 Univariate Indices Calculated from Major Meiofaunal Taxa Data

Treatment	Nematodes 0 per cm^2	Copepods 0 per cm^2	Taxa 0 per cm^2	Shannon-Wiener H'	Pielou's Evenness
W	92.7 ± 87.6	1.29 ± 1.35	5.00 ± 0.82	0.42 ± 0.25	0.26 ± 0.16
X	100.5 ± 87.1	1.24 ± 0.76	6.50 ± 1.92	0.45 ± 0.10	0.25 ± 0.12
Y	60.9 ± 59.4	0.49 ± 0.25	4.25 ± 1.50	0.18 ± 0.30	0.14 ± 0.06
Z	72.1 ± 40.0	39.4 ± 31.7	6.00 ± 1.58	0.45 ± 0.08	0.26 ± 0.11
P*	0.838	0.407	0.193	0.154	0.378

* Results of one way analysis of variance (ANOVA)

The statistical comparison of samples by treatment reveals no significant differences between treatment groups for any index. However, a low power of analysis suggests that this lack of significance should be viewed with some caution. More samples are currently being analyzed to address this issue. In the meantime, the data do suggest that treatment Y (enclosures oiled and burned six months prior to sampling) supports lower numbers of taxa, lower values of Shannon-Wiener's diversity, and possibly lower taxal evenness than all other treatment types. Figure 1 shows the taxal fauna isolated from each treatment, and demonstrates how treatment Y differs from all other treatments by the absence of many of the rarer taxa found in samples collected from other treatments.

A graphical representation of similarities between major taxa replicates by MDS are presented in Figure 2 (stress = 0.15). The arrangements of replicates reveals no definable structuring with respect to treatment, and hence no *a posteriori* tests were performed.

3.2 Analysis of the Nematode Community

The study revealed a total of 18 species of nematode representing 17 genera. The mean values and standard deviations for each univariate index for the nematode community are presented in Table 3, together with the significance of variance by ANOVA. The table reveals no significant differences in nematode community structure between the four treatment types.

Table 3 Statistical Analysis of Nematode Community

Treatment	SpeciesNumber 0 per cm^2	Shannon-Wiener H'	Pielou's Evenness	Simpson's λ
W	0.50 ± 0.05	1.60 ± 0.21	0.67 ± 0.10	0.32 ± 0.11
X	0.57 ± 0.08	1.72 ± 0.18	0.68 ± 0.07	0.28 ± 0.06
Y	0.44 ± 0.07	1.51 ± 0.12	0.67 ± 0.04	0.29 ± 0.04
Z	0.47 ± 0.02	1.45 ± 0.10	0.62 ± 0.04	0.37 ± 0.06
P value*	0.117	0.237	0.629	0.368

*Results of one way analysis of variance (ANOVA)

A graphical representation of similarities between nematode replicates by MDS is presented in Figure 3 (stress = 0.15). Figure 3 reveals a distinct grouping of replicates with respect to treatment: the replicates from treatment Y are removed from all other replicates, and these replicates appear contiguous. ANOSIM confirmed that the two groupings revealed by MDS (Treatment Y; Treatments W, X, and Z) are significantly different (R = 0.899, P= 0.001), while SIMPER revealed that over 40% of the difference between these groups was due to only four species (Table 4).

Table 4 Ranking of Nematode Species Contributing Highest Dissimilarity to MDS groups, using SIMPER

Species	**% Abundance in Treatment Y (0)**	**% Abundance in Treatments W, X, and Z (0)**	**Percentage Dissimilarity between Groups**	**Cumulative %**
Paracanthoncus sp.	36.5	3.9	13.5	13.5
Terschellingia sp.	0.9	8.8	9.3	22.8
Daptonema sp 1	0.6	6.9	8.8	31.6
Daptonema sp 2	0.0	1.9	8.7	40.3

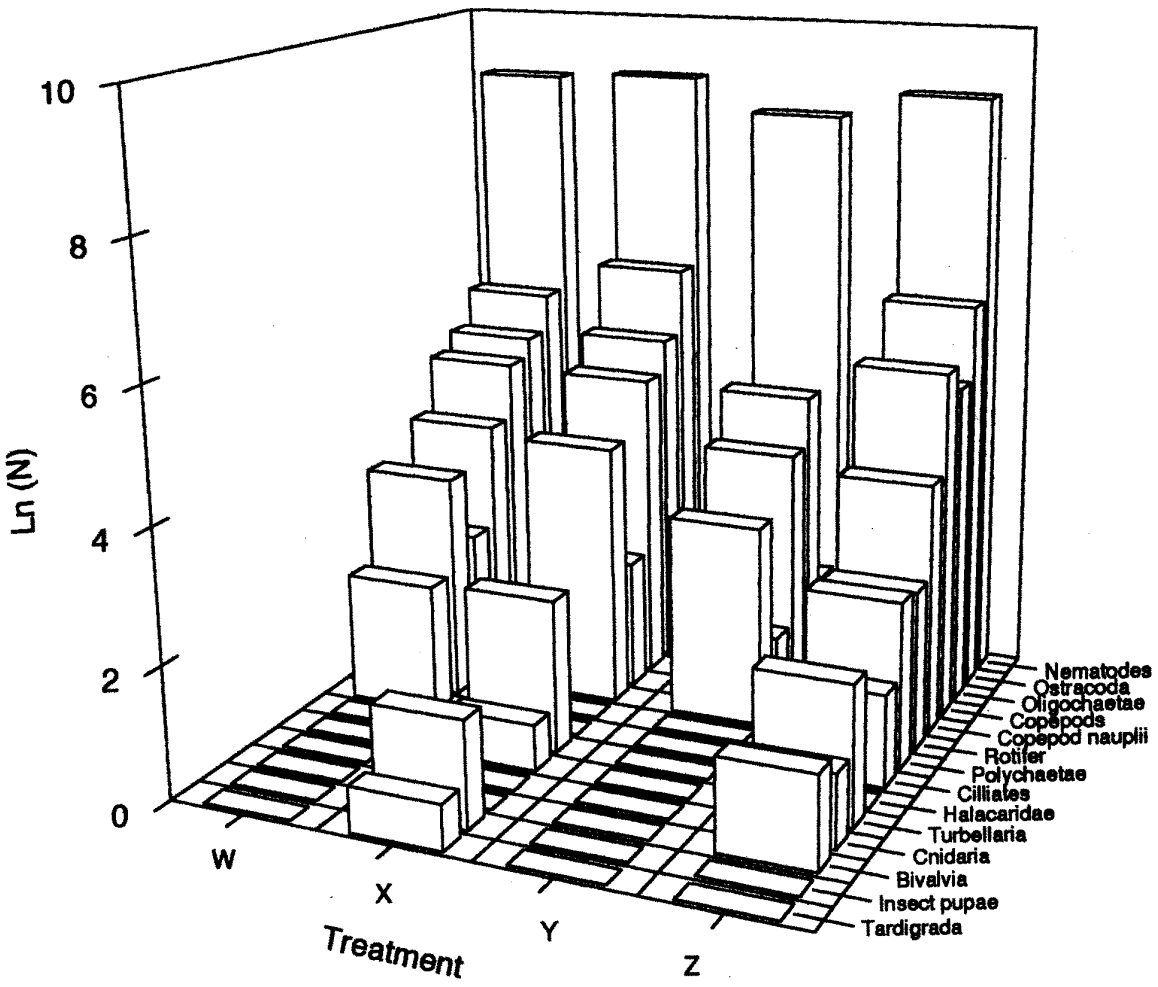

Figure 1 *Taxa arranged by treatment type.*

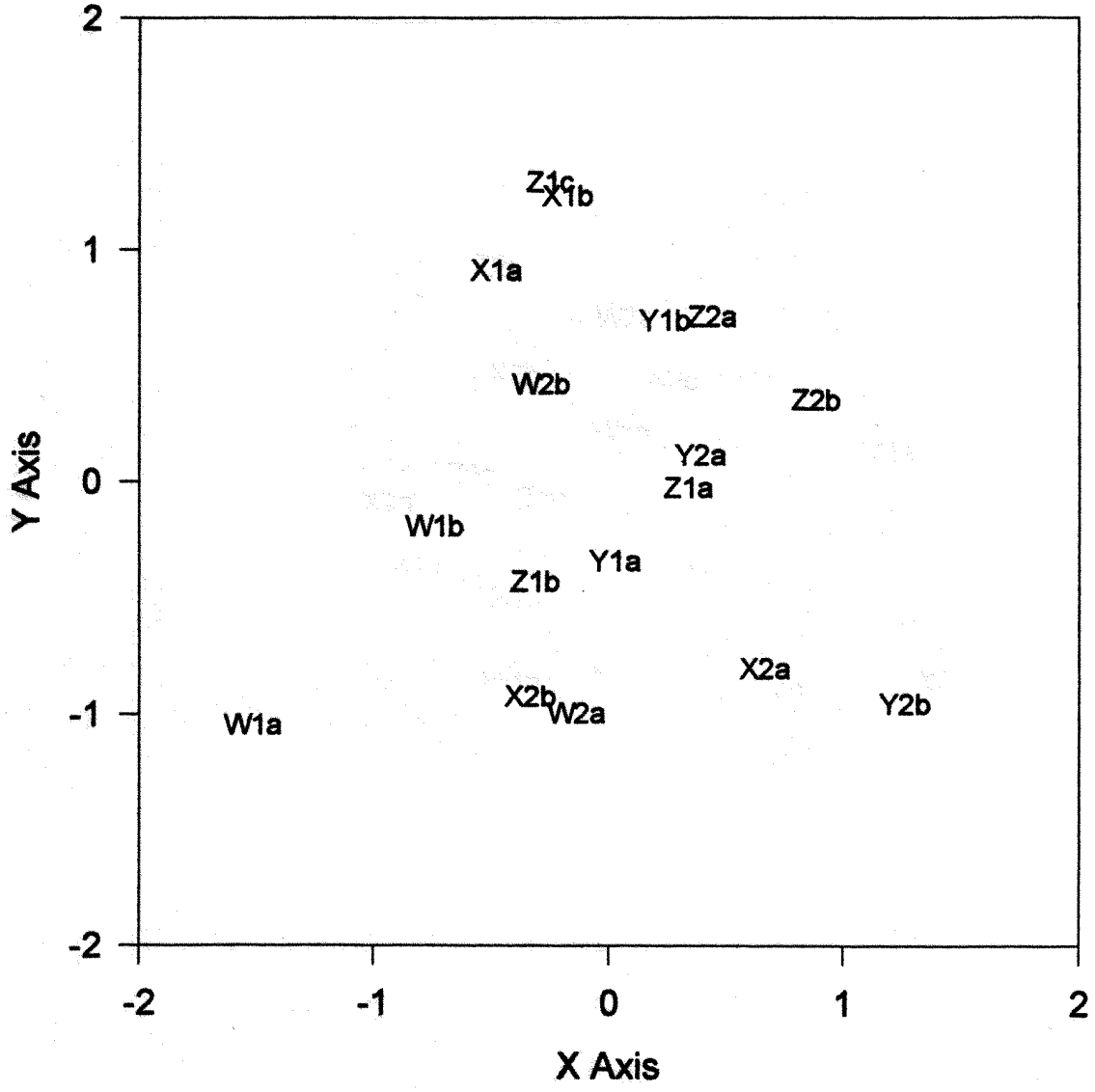

Figure 2 *Multidimensional scaling plot, major meiofaunal taxa from Houma sites.*

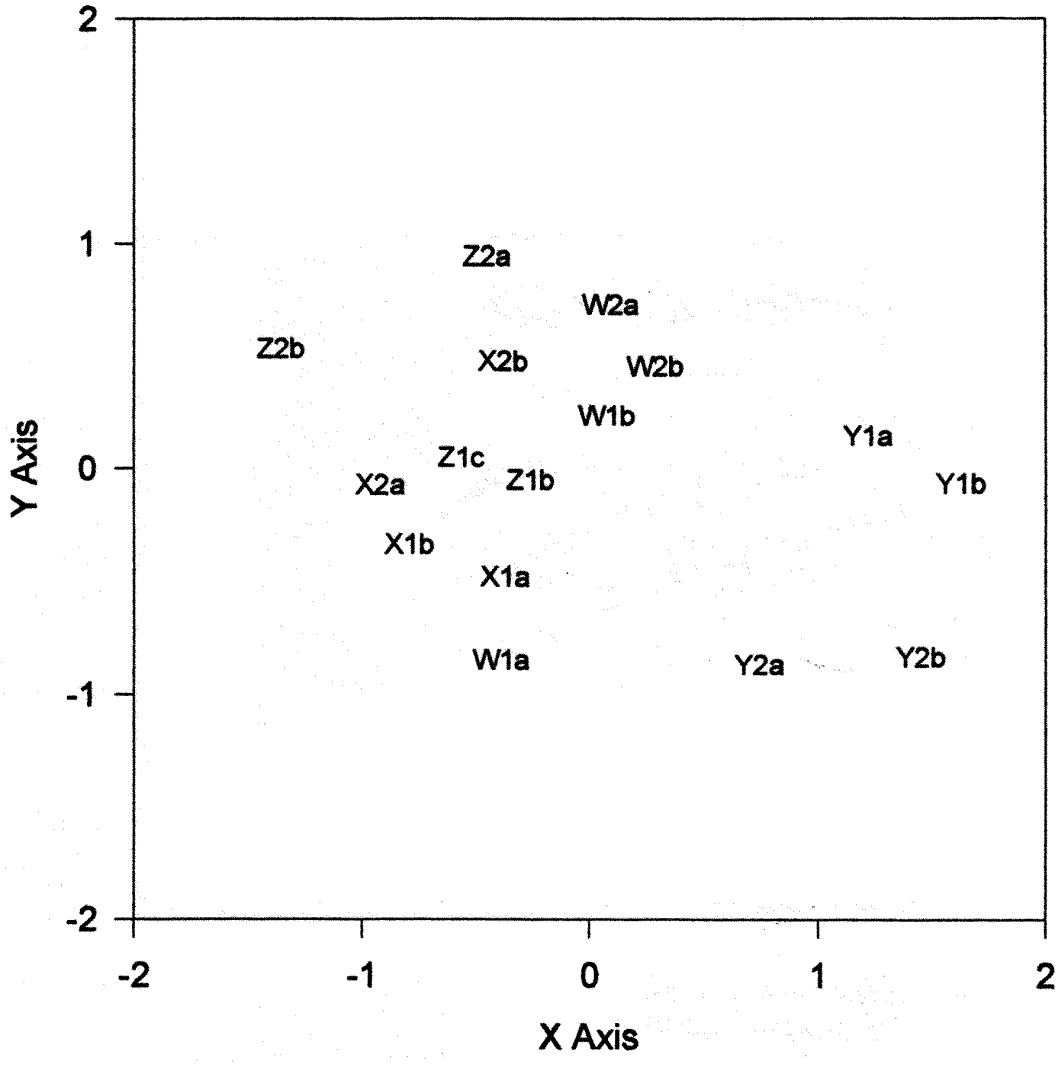

Figure 3 *Multidimensional scaling plot, Nematode community comparisons, Houma sites.*

4.0 Discussion

While the study of the meiofauna taxa revealed no significant differences between treatments, the data from the plots oiled and burned six months previously did show the absence of many less common taxa (polychaetes, cilliates, halacarids, tubellarians, cnidarians, bivalves, insects, and tardigrades). These treatment plots also yielded approximately 30% of the copepod density found in all other plots, although again this distinction was not significant. Such trends have been previously reported from studies of

oil impacts in field conditions, which described short-term decreases in meiofauna taxa (e.g. Elmgrem *et al.* 1980; Grassle *et al.* 1980; Oviatt *et al.* 1982) and copepod density (e.g. Grassle *et al.* 1980). However, the present data reveal that plots exposed to oil one year previously along with plots oiled and burned one year previously both yielded a meiofauna taxal diversity and number of copepods that were similar to the control plots. This suggests that any impact following treatment was undetectable one year after exposure. Similar recoveries following significant impact have also been described previously (Elmgren and Frithson, 1982; Renaud-Mornant and Gourbault, 1980), although recovery times do seem to vary between studies. The lack of distinction between plots exposed to oil one year previously and plots oiled and burned one year previously suggest that the ameliorative burning of oil contaminated plots had no significant effect upon the meiofauna within this time period.

Univariate analysis of the nematode communities revealed no significant differences in richness or diversity between treatments. However, the plots oiled and burned six months previously did yield fewer numbers of species than all other treatment plots. The MDS plot revealed two significantly dissimilar groupings of nematode species than all other treatment plots. The MDS plot revealed two significantly dissimilar groupings of nematode species due to treatment, with the six month oiled and burned plots distinct from a group comprised of plots exposed to oil one year previously, plots oiled and burned one year previously, and the control plots. While many nematode species contributed to this distinction, the herbivorous *Paracanthoncus* sp. was the most significant source of dissimilarity, contributing 13.5% to the total dissimilarity. This species was found to be highly abundant (36.5% mean abundance) in the six month oiled and burned plots but rare (3.9%) in all other plots. Other major contributors were the selective bacterivore *Terschellingia* sp. (9.3% dissimilarity) and two species of the herbivorous *Daptonema* sp. (each contributing 8.8% and 8.7% dissimilarity), which were all rare to absent in the six month oiled and burned plots.

As with many toxicological studies, it is hard to attribute these shifts in community composition and structure to the direct impact of oiling (Gray, 1979). While it is conceivable that these observations describe the local extinction of species sensitive to oil contamination, anoxia, H_2S elevation, other effects of hydrocarbon elevation, or a concomitant increase in more tolerant species, it is possible that any change in the nematode community is an indirect result of the contamination. There are a number of possible indirect mechanisms that might account for the observed distinction. Petroleum hydrocarbons have a dual character as contaminants, presenting the biota with organic enrichment as well as a toxicological challenge (Peterson *et al.* 1996). It has also been suggested that this organic enrichment might stimulate a response within the nematode community due to an increase in bacteria and/or algal density (Fleeger and Chandler, 1983). Alternatively, Fleeger and Chandler (1983) have suggested that changes in meiofauna densities in oiled salt marshes might be caused by a reduction in meiofauna predation pressure, for while oiling does not seem to influence the abundancy of many salt marsh predators (Lee *et al.* 1981, Fleeger and Chandler, 1983), oil has been shown to impair predation in Louisiana salt marshes (Gregg *et al*. 1997). It is also likely that the observed differences in the nematode communities might be due to changes in local

habitat resulting from the direct impact of oiling and burning on the *Spartina* beds. Oiling and burning of *Spartina* results in a decrease in the standing crop for one growing season (DeLaune, pers. com.), and these plots were clearly denuded of vegetation during sample collection. It is therefore conceivable that such a dramatic impact on the flora and physical habitat would have a short-term influence upon the meiofauna, at least until the *Spartina* returned to its pre-treatment density.

5.0 References

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