Effects of Natural Gas Pipeline Condensate and Crude Oil Spills, and

Comments on Remediation, with emphasis on South Louisiana Salt Marshes:

A Review

by

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Executive Summary

The El Paso Energy Corporation owns a producing plant in Cocodrie, Louisiana, located in an extensive set of salt marshes. This ecosystem is known to be at risk from hydrocarbon introduction. This report reviews some of the natural ecological processes at work within a salt marsh as they relate to a spill of natural gas condensate - a mixture of aliphatic hydrocarbons, n-hexane, benzene, toluene, and xylene. It also reviews the environmental impacts of some of the components of natural gas condensate as well as related compounds (crude oil, higher molecular weight hydrocarbons, polycyclic aromatic hydrocarons - PAHs, linear alkyl-benzenes - LABs, etc.) on salt marsh ecosystems in southern Louisiana and elsewhere in the world. The behavior and persistence of these compounds once they have entered the environment is also considered.

N-alkanes are a natural component of estuarine waters and sediments in salt marshes. They are removed naturally through evaporation and bacterial degradation and their concentrations vary significantly by season. Odd numbered alkanes (a molecule with an an odd number of carbons) associated with chlorophyll-a in the water may be indicative of a biological origin - rather than that of an anthropogenic contaminant. Gaseous hydrocarbons are also produced naturally by bacteria in the sediment. The redox (oxidation-reduction) condition of the sediment strongly influences both the amount and composition of hydrocarbons produced there.

Introduced hydrocarbons are known to persist for years - up to decades, particularly higher molecular weight compounds. Lower molecular weight (LMW) hydrocarbons are lost quickly from the environment via evaporation (< 24 hrs). Both physical and

biological processes are responsible for the rapid loss of LMW hydrocarbons over longer periods of time.

Planktonic estuarine bacteria can oxidize toluene in estuarine water. Approximately 7% of the planktonic bacterial community are responsible for this. It is an induced response in the existing natural bacterial community and is not due to bacterial population increases.

PAHs are long-lived in cold estuaries; this is partially because they have certain bonds which are chemically inert. They are less easily mobilized than polychlorinated biphyenyls (PCBs). PCBs and can be metabolized into forms which are carcinogenic. Crude oil and PAHs are not harmful to marsh grasses and sediments in salt marshes at low concentrations. If heavier physical coverage of a plant occurs, however, photosynthesis within the plant ceases, the roots become contaminated, and the plant dies. Mortality of the root system removes the possibility of regeneration from the previous adult population. PAHs accumulate in much higher concentrations in the sediment than in the water column.

The benthic meiofaunal community (microscopic invertebrates living in the sediment) is also negatively affected by PAHs. Experiments examining the introduction of sub-lethal doses of PAHs into microcosms have demonstrated that they depress the physiological condition and activity of microalgae. They also change the structure of the benthic community, resulting in a higher representation of the more robust nematodes (minute infaunal worms). Benthic copepods (microscopic crustaceans which live on the bottom) show a gender-specific response to PAHs, which could produce secondary effects for fish with gender-specific food preferences. Because recovery of the benthic meiofaunal community is rapid, the Louisiana microbial/meiofaunal community may be genetically adapted to elevated PAH levels. After experimental exposure to PAHs, killifish were observed to exhibit stress and an "alarm response", but they acclimated within a short period of time.

The saltmarsh microbial community varies in its ability to process PAHs depending upon salinity (which varies greatly in Cocodrie). The history of the region with respect to exposure to petroleum hydrocarbons, however, is very important. Where exposure has occurred before, the microbial community may be adapted to degrade the compounds more effectively at the salinities found naturally in that region.

Linear alkyl-benzenes (LABs) can be preserved in sediments for decades. They biodegrade in the sediment but change isomeric composition, becoming more persistent with time. A model has been developed to predict this isomeric shift.

LMW hydrocarbons vary in their solubility in estuarine water. Aromatic compounds found in natural gas condensate are much more soluble than alkanes - by orders of magnitude. Addition of these lower weight compounds to the environment at low concentrations actually stimulates plant growth and activity. Higher concentrations, however, yield a negative effect. The effects of these LMW compounds on the marsh plant and microbial communities are non-linear with respect to concentration.

No. 2 fuel oil is very detrimental to marsh plants and has faster, more severe, and longerterm effects on *Spartina alterniflora* (marsh grass) than crude or heavy fuel oils. Marsh grass is increasingly sensitive to this petroleum product with increased exposure. Contamination of the upper plant travels to the roots, killing them and thwarts regeneration. Organic soils characteristic of saltmarshes are more prone to penetration of hydrocarbons than mineral soils. Spills of No. 2 fuel oil in Gulf salt marshes may have a generally lower impact on the ecosystem than spills in the northern temperate. This oil does not accumulate in Gulf sediments with time due to microbial breakdown.

In the 220,000 ton Amoco Cadiz spill, 30-35% of the crude oil was aromatics, and 3% was benzene. The spill caused tremendous mortality in the coastal marine community, affecting all trophic levels. Those invertebrates which were not killed via toxicity were rendered "comatose" and were lost from the community. The benthic community was significantly altered, rapidly changing to one characteristic of a highly polluted environment. The benthic community was also severely affected for hundreds of miles.

This type of crude oil also has a negative impact on bacterial populations which are responsible for methanogenesis, sulfate reduction, and evolution of CO₂. Crude oil does not affect sulfate reduction by bacteria in salt marshes. Lighter hydrocarbons do not affect the first two processes at low doses. Microbial communities have the capacity to adapt to hydrocarbon exposure, especially in sediments overlying oil- or gas-producing areas with a history of exposure (natural or anthropogenic).

Benzene and toluene can inhibit anaerobic processes (those occurring in the absence of oxygen in the sediment). Benzene and naphthalene can inhibit or stimulate methanogenesis in saltmarsh sediments, depending upon concentration. A spill of benzene could produce a population of small blue crabs with slow growth rates, and this could be very important in a southern Louisiana salt marsh. Benzene binds easily to colloids in high salinity estuaries - more easily than in terrestrial or freshwater environments, particularly in salt marshes where there are high concentrations of organic

matter. Benzene has negative effects on striped bass, accumulating in various tissues, including the liver, and causing a decrease in physiological and reproductive state, and an increase in parasite infection rate. Striped bass exhibit a physiological stress response to the compound, but none of these effects are not long-term.

With respect to mitigation, oil-degrading bacteria, commercial fertilizers, and oxidizers do not have a negative impact on southern Louisiana saltmarsh communities. The addition of fertilizer and fertilizer with oxidizers promotes growth of naturally occurring petroleum-degrading bacterial populations, and thus petroleum degradation, but only for alkanes. This clean-up technique may be particularly appropriate for application to condensate spills. With respect to PAHs, once weathering has occurred, there is little which can be done to hasten the natural, slow degradation of the petroleum in a salt marsh. Crude oil has been shown in some cases to have no effect on saltmarsh microfauna or meiofauna, indicating that this commnity may be well adapted to live in the presence of petroleum hydrocarbons. This indicates that if a spill is small, the best response might be no clean up action at all. In the event of a spill of the highly toxic No. 2 fuel oil, as much of the material should be removed as quickly as possible. A high degree of mortality of *Spartina alterniflora* may be expected, but dead roots and stems should not be removed to help decrease wave action, stabilize the sediment, and promote the entrainment and germination of new Spartina seeds and seedlings. Transplantation of *Spartina* into affected areas is also recommended.

With respect to crude oil spills, use of dispersants should be avoided, as they increase the toxicity of the oil. Physical collection of the oil and contaminated sediment is recommended. Steam cleaning of hard substrata should be avoided, as this greatly delays

natural recolonization processes. In southern Louisiana saltmarshes, because of potential flooding, delayed flushing of estuarine waters, and retention of the condensate, local meteorological and oceanographic conditions should be reviewed and taken into account immediately upon learning of a spill and incorporated into clean-up plans.

Data on natural levels of alkanes in the sediments and water column around the saltmarsh at risk of contamination should be in-hand from any area used for extraction. In the event of a condensate spill, the pristane/phytane ratio should be monitored to help determine whether observed concentrations are of biogenic or anthropogenic origin; a ratio of > 1 is indicative of a natural biogenic origin.

There is limited information available on the effects of a gas condensate or its components on a sub-tropical saltmarsh ecosystem. The responses of the fauna and flora, however, will vary depending upon the specific compound, its solubility in seawater, concentration, sorptive characteristics, the organics in the sediment, season, water temperature, salinity, wind velocity, community composition, degree of wind and wave exposure, and history of the site with respect to exposure to petroleum hydrocarbons. Initial concentrations are important to predictions of spill effects, but longer-term effects are dependent upon final chemical composition and concentrations in the sediment and water.

Cocodrie's saltmarshes, extant in this region, are the areas most sensitive to a condensate spill. A major problem is the propensity for these compounds to bind with marsh sediments in the water column and on the bottom. Sedimentation rates in Cocodrie are high, and this enhances pollutant concentration in bottom sediments. On the other hand, Cocodrie has been exposed to petroleum hydrocarbons - both naturally and

anthropogenically, for long periods of time, and it is probable that microbial populations capable of biodegrading these compounds already exist here.

Introduction

The El Paso Energy producing plant is located in a set of salt marshes extending for tens to hundreds of square miles near Cocodrie, Louisiana. Due to stringent US Environmental Protection Agency (EPA) and Department of Energy (DOE) regulations, and strict company safety policies and procedures, the probability of a spill of natural gas condensate is low. Nonetheless, in the event that such did occur, it is important to have some understanding of this saltmarsh ecosystem, the natural ecological processes which occur there, and the potential environmental impacts that a spill might have on it.

El Paso Energy has identified the following compounds as being included in their distillate:

- . a mixture of aliphatic hydrocarbons
- . n-hexane
- . benzene
- . toluene
- . xylene.

This report summarizes information available on the effects of these compounds on salt marsh ecosystems, but also those of related compounds such as polycyclic aromatic hydrocarbons (PAHs) and linear alkylbenzenes (LABs) which may have similar impacts. Some comments will also be made regarding the results of research on mitigation measures in the event of a spill.

Background Information

The Cocodrie Saltmarshes

Cocodrie is located on the Terrebonne Bay estuary ($29^{\circ}15'$ N, $91^{\circ}21'$ W). It is a highly productive salt marsh, dominated by the common marsh grass *Spartina alterniflora*, and its salinity ranges from 4 - 26 % (Carman et al., in press). Sediments in the area have a median grain size of 38 µm, with a composition of 41% silt and 17% clay (Chandler and Fleeger, 1983). The organic carbon content is ~2.5% and the ambient PAH concentrations are 0.24 ppm (Carman et al., 1995).

General background information regarding the Cocodrie area may be found in Barrett (1971) and Reed (1995). They include seasonal and annual variation in salinity, water temperature, dissolved oxygen, and turbidity. Information may also be found on nutrient concentrations such as nitrate, nitrite, inorganic phosphate, total phosphorus, as well as information on variation in tides, barometric pressure, rainfall, and wind speed and direction. Data on air temperature, precipitation, and stages and discharges of principal rivers in the region are also given. Key information regarding the effects of prevailing winds and rainfall on tides, flooding, and flushing of this estuary may be found in Prager (1992) and Inoue and Wiseman (in press).

The tidal range there is ~0.3m. The wind, however, generally over-rides the tides, causing changes in sea level of 3 to 5 times this amount. This can result in flooding several times per year. A southeast wind can force bay water up into the estuaries and prohibit the bayous and marshes from draining for up to 7 days (Prager, 1992; Inoue and Wiseman, in press; pers. comm.). After events such as this, an additional three days may be required for the bayous and marsh areas to drain into the bays and the Gulf of Mexico.

This slow flushing can be an important confounding factor should a spill of natural gas condensate occur in Cocodrie. Up-to-date local meteorological and oceanographic conditions should, upon learning of a spill in this region, be immediately reviewed and incorporated into any clean-up plans being made at that time.

Natural Seasonal Variation in n-alkanes in a Salt Marsh

N-alkanes occur naturally in salt marshes. It is therefore important to understand their nature and behavior before comparing possible changes in their distribution and abundance due to a condensate spill bearing n-alkanes.

N-alkanes are water soluble, but this solubility varies seasonally, depending upon temperature, evaporation rate, bacterial degradation, adsorption onto sediment, and photochemical oxidation. Such seasonal variations were assessed in an Iraqian freshwater marsh by Al-Saad and Ak.Al-Timari (1993).

Higher concentrations of n-alkanes were observed in the Iraqian marshes in the winter $(6.20 \ \mu g \ l^{-1})$ than in the summer $(0.14 \ \mu g \ l^{-1})$, indicating that breakdown rate is lower in the winter (Tables 1-4). A regular distribution pattern of odd and even-numbered carbon compounds (C13 to C34) was observed and found to be generally of biological origin. Dissolved n-alkanes were represented by C14 to C23 compounds, predominantly even in number, with a peak abundance at C17 (Figs. 1 and 2). They were also represented by those >C23, predominantly odd in number. Bedair and Al-Saad (1992) claim that the even numbered carbon compounds may be the result of microbial activity, while the higher weight odd ones may be derived from natural break-down products of terrestrial higher plants (Wakeham and Farrington, 1980).

Diatoms are abundant in marsh sediments (Al-Saadi et al., 1981; Al-Zubaidi, 1985) and also produce a range of n-alkanes from C15 to C31, with a predominance of even numbered compounds (Saliot, 1981). Algae contain n-alkanes lower than C21, such as C15, C17, and C19 (Goutx and Saliot, 1980). Bacteria produce C20 and C28 alkanes, with a peak at C23 (Grimalt et al., 1985). Higher plants produce C25-C31 alkanes, with a predominance of odd-numbered ones (Kennicutt et al., 1987). It is likely that aquatic plants are the main source of naturally produced n-alkanes in the water (Bedair and Al-Saad, 1992). The dominance of odd-numbered n-alkanes associated with high chlorophyll-*a* concentrations could be indicative of a biogenic origin of n-alkanes in the water (Al-Saad and Ak.Al-Timari, 1993; also see Al-Saad and Bedair, 1989).

Pristane and phytane were the most common isoprenoids in the Iraqian marsh, and were most likely derived from aquatic organisms or chlorophyll-a. The pristane/phytane ratio was >1, indicating a biogenic origin (Shaw et al., 1985). In the event of a spill, the pristane/phytane ratio should be monitored to help determine whether the observed concentrations are of biogenic origin or a result of anthropogenic introduction.

In order to assess the effects of any increase in the concentration of n-alkanes in a marsh, it would be necessary to have data in-hand on the natural background levels of n-alkanes in the sediments and water column. In addition, it would appear that any spill of natural gas condensate in such a marsh would have to add different and more toxic compounds or add them in such a way as to raise the concentrations considerably to produce a significant environmental impact on the community. This would be due to the presence of such compounds in the natural environment and the obvious adaptations of the organisms occurring within the benthic community to accommodate them.

DouAbul and Al-Saad (1985) and Gordan et al. (1978) have determined that temperature is the most important factor determining the removal of hydrocarbons from the water through increased evaporation rates and enhanced bacterial degradation (Cundell and Traxler, 1973; also see Shamshoom et al., 1990). In the Iraqian marsh, air temperatures varied from $< 0^{\circ}$ C ($< 32^{\circ}$ F) in the winter to 45° C (113° F) in the summer. Water temperatures varied between 12.4°C (54.3° F) in January and 33.9° C (93.0° F) in August (Al-Saadi and Al-Mousawi, 1988).

Photo-oxidation reduces alkane abundance during the summer. Water flow will also influence alkane abundance, as, in the Iraqian system, where water flow is highest in June and minimal in January. The amount of suspended matter in the water column will also influence the amount of n-alkanes in the system because of its sorption activity. This varies with season, being minimal during the summer (Al-Hilli, 1977), presumably due to low wind velocities, low surge, and low resultant resuspension of bottom sediments.

Influence of Marsh Soil Redox Condition on Natural Gaseous Hydrocarbon Production

As mentioned earlier, some lighter hydrocarbons present in natural gas condensate are produced naturally by marsh sediments. This production is influenced by the redox (oxidation-reduction) conditions of the sediment.

Methane (and other gasses) are produced in wetlands by soil bacteria (Cicerone and Oremland, 1989; Pullman and Meyer, 1992). Restricted aeration is known to be the most important factor governing the metabolic rate of these bacteria and hydrocarbon production in soils (Van Cleemput et al., 1983). Devai and DeLaune (1996) conducted a study on a freshwater Mississippi River Deltaic marsh (St. Charles Parish, LA) examining this problem. They experimentally controlled the redox levels at certain experimental sites to range between 550 and -170 mV. They found that as soil redox potential was decreased, the production of hydrocarbon gases such as methane by bacteria increased (Fig. 3); as did that of ethane, propane, butane (Fig. 4); and ethylene, propylene, and isobutane (Fig. 5). Methane production was maximized at -100 mV; that is, methanogenic bacteria are only active under strongly reducing conditions (Shutz et al., 1989). Isobutane was only produced at redox levels below 0 mV. Significant amount of both methane and non-methane hydrocarbons were found to be produced by these soils. Under aerobic conditions (> 430 mV), production of LMW hydrocarbons production be ranked as follows: methane > propylene > propane > butane > ethylene > ethane > isobutane (Fig. 6). Under anaerobic conditions (-170 mV), methane accounts for 99.4% of the hydrocarbon production.

Persistence of Hydrocarbons in a Salt Marsh

Macko et al. (1981) demonstrated experimentally in Harbor Island, Texas that low molecular weight alkanes and more volatile aromatics are lost rapidly from a Gulf Coast salt marsh. An oil spill occurred there and the pollutants persisted for at least two years. The pollutants included saturated hydrocarbons, isoprenoids, parent and substituted benzenes, naphthalenes, and phenanthrenes. Compounds $< C_{13}$ were lost within 24 hrs, as was naphthalene, methyl-naphthalenes, and the substituted benzenes. Compounds $<C_{20}$, longer-chain hydrocarbons, were gone within one month. After six months, it was difficult to detect any compounds below C15. The higher weight alkanes (> C22) and alkyl-substituted naphthalenes and phenanthrenes persisted longer, eventually decreasing after 24 months to background levels.

It is believed that the factors responsible for the rapid loss of these lower molecular weight hydrocarbons were (Gibson, 1977; Macko et al., 1981):

- . dissolution
- . diffusion
- . evaporation
- . adsorption into the sediments
- . microbial biodegradation
- . photo-oxidation, and
- . transport of material away from the spill site by winds, rainfall (drainage), and currents.

Concentration of hydrocarbons (an indicator of total lipids in the sediment) decreased logarithmically with time (Fig. 7). The early rapid loss was believed to be due to physical processes; the later slower loss was believed to be due to biological ones (microbial degradation).

Harris et al. (1984) modeled the dissipation of a spill containing three compounds benzene, naphthalene, and benzo(a)pyrene - in the southwest of the UK. The half-lives of these compounds were calculated under different insolation and wind-speed conditions, characteristic of different seasons. Interestingly enough, benzene and naphthalene exhibited shortest half-lives during the mid-winter due to high winds; therefore, their longest half-lives were during the mid-summer (Table 5). Variation in the concentration of particle-adsorbed benzene was influenced by overall particle distribution. Benzo(a)pyrene exhibited the opposite pattern, with its highest half-life occurring during the mid-winter, and lowest during the mid-summer. This difference was due to rapid photo-oxidation in the summer and slow photo-oxidation in the winter. The loss or biodegradation of closely related yet different compounds were affected differentially by different environmental factors.

Toluene Oxidation by Planktonic Estuarine Bacteria

Toluene exhibits a wide range of oxidation rates in estuarine water (Button et al., 1981), and terpenes have short turnover times in seawater (Button, 1984). In addition, direct counts of bacteria do not always correlate directly with toluene uptake rates (Meyer-Reil, 1978; Button and Robertson, 1985).

When planktonic bacteria from an Alaskan estuary were exposed to toluene, their metabolism increased immediately (Fig. 8). This response could be accounted for by ~7% of the planktonic bacterial community (Button and Robertson, 1985).

Increases in toluene metabolism and mineralization are dependent upon both the duration of exposure to the bacteria and the concentration of toluene in the water (Figs. 9 and 10; Table 6). The rate of metabolic increase reaches a maximum after 48 hrs, and is not a function of increase in the bacterial populations. The metabolic increase is induced, as has been found in other systems (Claus and Walker, 1964; Connors and Barnsley, 1980), and induction is often enhanced by the availability of amino acids for enzyme production (Wiseman, 1975).

The concentrations of toluene in estuarine water and seawater are sufficient to induce its metabolism by bacteria, and this was effected by a major portion of the bacterial community (in the Alaskan study area; Button and Robertson, 1985).

Effects of Related Compounds on Salt Marsh Communities - PAHs and LABs

Although El Paso Energy has not specifically listed polycyclic aromatic hydrocarbons (PAHs) as part of their condensate, they are closely related to cyclic aromatic hydrocarbons produced in the natural gas distillation process. It would therefore be prudent to review some of their environmental or health effects.

Distribution of PAHs in a Northern Temperate Estuary

PAHs can be long-lived in estuarine sediments. In the Scheldt estuary in the SW Netherlands, the PAH assemblage was very uniform between several salt marshes, despite the fact that the recent maximum input of PAHs to the areas was two to three times lower than in the mid-1960s (Van Zoest and Van Eck, 1993). The source of the compounds was traced to the combustion of coal in the area.

The Scheldt estuary study indicates that processes which mobilize PCBs are not as effective on PAHs. The reasons suggested for this are (a) PAHs have bonds which are chemically inert (Readman et al., 1987); or (b) microbial degradation may be expected to act compound-selectively and may not be important in cold-water estuaries.

Some of the member compounds of PAHs can be metabolized to form carcinogenic products (Newman, 1976; TRW Systems and Energy, Inc., 1976; Wijayaratne and Means, 1984).

Crude oil can have a negative effect on common grasses known to be critical to the biological and physical integrity of the salt marsh. It is known that concentrations of 4 - $32 \ 1 \ m^{-2}$ do not usually cause catastrophic damage to the standing crop of *S. alterniflora* (e.g. Hershner and Lake, 1980; Ferrell et al., 1984; see Li et al., 1990). Pezeshki and DeLaune (1993) have shown that when crude oil coats the entire leaf of *Juncus roemerianus* and *Spartina alterniflora*, photosynthetic activity ceases. Oil coats the stomatal openings on the leaf, creating a seal and preventing gas exchange. Partial leaf exposure results in a decrease in photosynthesis (Fig. 11). Photosynthetic activity in *J. roemerianus* was less sensitive to oil than in *S. alterniflora* (71-94% of normal, and 53-80%, respectively). After four weeks, net photosynthesis began to show improvement, and, after five weeks, growth and shoot production was normal in both species. No mortality was observed. It would appear that crude oil has a negative impact on marsh grass, but the effects are temporary.

It has been claimed that if a spill occurs in a salt marsh, the sediments may remain contaminated for years. This is because of low tidal flushing rates, slow biodegradation of complex hydrocarbons (Kennish, 1992), and slow loss into the overlying waters (Means and McMillin, 1993). Because of their relative insolubility, PAHs readily adsorb onto sediment particles and become concentrated in sediments at levels well above those found in the water column (MacLeod et al., 1981; Mueller et al., 1982; Olsen et al., 1982). Benthic sediments tend to adsorb and hold more PAHs than suspended sediments (Kirso et al., 1990; Baker et al., 1991), which can be a problem under spill conditions. Studies by Fleeger and Chandler (1983), DeLaune et al. (1984), and Smith et al. (1984) have indicated that the meiofaunal community structure (microscopic benthic invertebrates associated with sediment) is relatively insensitive to petroleum hydrocarbons. This, however, may be due to rapid post-event recolonization rates by the meiofauna (Decker and Fleeger, 1984; Spies et al., 1988; Warwick et al., 1988).

Some studies have used laboratory microcosms to demonstrate the effects of PAHs on benthic salt marsh communities. One study (Carman et al., 1995) conducted with sediments and organisms derived from Port Fourchon, Louisiana, attempted to predict subtle long-term ecological effects on the benthos, among organisms, and between trophic (feeding) levels. Effects on bacteria were also examined.

Surprisingly, concentrations of a mixture of PAHs ranging from 0.3 to 3 mg kg⁻¹ dry sediment did not affect bacterial activity, physiological condition, or abundance. Microalgal activity and physiological condition, on the other hand, were affected. Incorporation of ¹⁴C-bicarbonate into microalgal phospholipid was significantly increased under high PAH concentrations, indicating that photosynthetic rate was increased (Fig. 12). This was a delayed effect, however, for it did not occur until Day 14 in the experiment. The phospholipid/neutral-lipid ratio of incorporation of ¹⁴C-bicarbonate under high PAH concentrations, indicating that algal physiology was significantly affected. A greater amount of carbon was being allocated to membrane synthesis and cell division or growth. Algal biomass as measured by chlorophyll-*a* concentrations was not affected.

The hydrocarbons did not affect grazing activity or physiological condition in meiobenthic copepods (microscopic crustaceans living in the sediment). Community structure did change, however, as nematodes, known to be resistant to environmental perturbations, became disproportionately abundant under high PAH conditions (Fig. 13). This indicates an adaptation on the part of these animals to the disturbance and supports the premise that they are good indicators of environmental impact. Meiofaunal copepods are more sensitive to petroleum contaminants than other meiofauna, such as nematodes (Heip et al., 1988; Warwick et al., 1988; Sandulli and de Nicola-Guidici, 1989; Carman and Todaro, 1996).

Abundances of copepods, copepod nauplii, and total meiofauna were not significantly affected by the presence of PAH (Fig. 13). The nauplius (juvenile) to adult ratio in copepods, however, increased under high PAH concentrations (Fig. 14). In general, sublethal effects were not prominent. With these organisms, a spill resulting in concentrations of 0.3 to 3 mg kg⁻¹ PAH in the sediments would not constitute a significant perturbation to the benthic community, although most significant effects were seen at highest PAH concentrations.

This study showed that the microbial/meiofaunal community of salt marsh sediments the basis of the salt marsh food web - may be adapted to elevated PAH levels (Carman et al., 1995), as has also been proposed by Baker and Griffiths (1984). This is not unlikely, for it is also known that one species of freshwater oligochaete adapted to increased cadmium concentrations in as few as one to four generations (Klerks and Levinton, 1989). Petroleum hydrocarbons are known to affect life-history parameters (Bridges et al., 1994) and immune systems (Tahir et al., 1993) of benthic organisms and can change the structure of entire benthic communities (Nance, 1991). Fang (1990) and Carman et al. (1995) have pointed out that Louisiana's coast has been exposed to petroleum production for decades and that the Louisiana benthic marsh community has probably evolved a resistance to elevated levels of petroleum hydrocarbons.

In a later, more detailed microcosm study using sediment from Pass Fourchon, Louisiana, Carman and Todaro (1996) showed that high doses of PAH-contaminated sediment (27 mg PAH kg⁻¹ dry sediment) collected near a "produced-water" discharge site (Means and McMillin, 1993) elicited avoidance behavior in certain species of meiofauna. For example, in one copepod species, *Pseudostenhelia wellsi*, the authors believe that the nauplii migrated down below the top 1 cm of sediment to avoid the contaminants.

The effects observed were species-specific. *Enhydrosoma* sp., another meiobenthic copepod, exhibited a delayed increase in abundance. It appeared to be uniquely suited to exploit oil-contaminated sediments after a short period of weathering. The copepod *Pseudostenhelia wellsi* and their nauplii (juveniles) exhibited an avoidance response, particularly in the first 1/2 day. Other copepods exhibited a similar response, but less so, detectable only through increases in the nauplius/copepod ratio. There were gradual population decreases with time due to mortality, particularly at high doses (Figs. 15 and 16). Few if any diurnal effects were observed. Some gender-specific effects were noted, with the males being particularly sensitive to hydrocarbons (Fig. 17).

These effects are important because the meiofauna serve as a food source for fish in the region, and could affect fish production. Even the gender-specific effects on copepods are important because they can effect success of reproduction in the population. More pertinent to commercial impacts, fish prefer male copepods as a food source (McCall, 1992). In this experiment, reproduction continued normally. Some species were slow to respond to contamination; one species did not exhibit any negative effects until the last day of the experiment (28th day).

In Carman and Todaro's (1996) study using Pass Fourchon, LA sediment, they also demonstrated significant effects of PAHs on copepod populations, sexes, and developmental stages. Nematode/*Pseudostenhelia wellsi* (a benthic meiofaunal copepod) ratios increased with high doses of PAH (Fig. 18), as did the proportion of females of two other copepod species during the last two weeks of the experiment. They found that relatively low-level PAH contamination caused subtle ecological effects on the meiobenthic community not previously suspected. These effects would not have been detected if only higher taxa were examined.

In another microcosm experiment, Decker and Fleeger (1984) studied the effects of crude oil on meiofauna colonization of salt marsh sediments characteristic of Bayou Ferblanc, Leeville, Louisiana. In 60 days, no taxon had recolonized to the levels present in the surrounding marsh sediments. Copepods only recovered to 70%; nematodes to 40%. Total meiofauna recovered by 45%. Oiling also suppressed polychaete colonization. Although nematode densities were suppressed in the high oil treatments, no delay in recolonization occurred (Fig. 19). *Enhydrosoma woodini*, a meiobenthic copepod, exhibited a similar response. It first decreased in number but was the first to re-colonize. At the end of the experiment, its numbers increased to significantly higher levels than under non-oiled conditions. This indicates that it may be an opportunistic species, well adapted to the presence of these hydrocarbons. Species diversity was low for the first month under oiled conditions. All taxa other than nematodes, polychaetes, and the copepod *E. woodini* were unaffected.

Few species were able to colonize the oiled sediments, and different concentrations of oil caused different benthic communities to develop.

In general, hydrocarbon contamination has a negative effect on benthic polychaetes (Rossi et al., 1976; Carr and Reish, 1977; Neff and Anderson, 1981). There are certain polychaetes, however, which respond positively to this type of perturbation, such as Stresblospio benedicti and Capitella sp. I (Grassle and Grassle, 1974). Bridges et al. (1994) conducted a study examining the effects of hydrocarbon-amended sediments on growth, maturity, fecundity and reproduction of these two opportunistic infaunal polychaetes. The hydrocarbon used was No. 2 fuel oil at concentrations of 110 and 270 ppm (wet wt.). In S. benedicti, growth and size was reduced (Fig. 20) and age of first reproduction was delayed (Fig. 21). Fecundity was not affected. Capitella sp. I, however, responded positively to the addition of hydrocarbons via more rapid growth, younger age of first reproduction, larger size, and higher fecundity. This flexibility in growth and development may be a fundamental characteristic of species adapted for quick responses to temporal variation in the environment (Grassle and Grassle, 1977, 1978; Bridges et al., 1994). Population size also increased. Streblospio experienced increases in its population as well, indicating that it is capable of tolerating conditions of hydrocarbon contamination while other members of the benthic community are not. These effects indicate a change in infaunal community structure for areas affected by a spill.

Effects of PAHs on the Benthic Microbial Community of a Salt Marsh

The Cocodrie area experiences broad shifts in salinity - from 4 to 26 %. Salinity can affect the rate at which the microbial community breaks down petroleum hydrocarbons. It is therefore important to review some of these effects so that the duration of impacts might be better predicted.

Kerr and Capone (1988) studied the effect of salinity on microbial mineralization of two PAHs - naphthalene and anthracene - in estuarine sediments of the lower Hudson River, a northern temperate estuary. Rates of naphthalene mineralization (conversion of PAHs to CO₂) in surface sediments of the Hudson (salinity = 2 - 27 %) ranged from 0.011 to 1.5 nmol cm⁻³ day⁻¹ (turnover time $[T_n] = 60 - 2040$ days), with no discernible gradient along the estuary. At another study site, the microbial communities were found to be adapted to a wide range of salinities (5 - 23 %) and mineralization rates did not vary significantly with salinity. In two upstream freshwater marshes, natural rates of naphthalene mineralization ranged from 0.007 to 0.15 nmol cm⁻³ day⁻¹ (T_n = 14 - 368 days). Another freshwater marsh exhibited quite high natural rates of 66 nmol cm⁻³ day⁻¹ (T_n = 40 days). As salinity was increased experimentally, the rate of mineralization of naphthalene and anthracene decreased (Fig. 22).

In areas of chronic petroleum pollution (780 - 1600 nmol cm-3 day-1; $T_n = 5-6$ days) and stable salinity (27 %)(00), high rates of naphthalene mineralization were observed. These rates decreased for both naphthalene and anthracene when salinity was experimentally decreased (Fig. 23). The most pristine site yielded the lowest natural naphthalene mineralization rates - 0.003 - 0.004 nmol cm-3 day-1 ($T_n = 714 - 833$ days).

These results indicate that the effect of salinity on the rate of mineralization of PAHs is dependent upon the natural range of salinity occurring in that area (Kerr and Capone, 1988). The microbial community is adapted to survive, grow, and function under that specific salinity range, be it wide or narrow. A strong positive correlation also exists between PAH concentration and mineralization rate of naphthalene. The key factor affecting rate of PAH mineralization in all environments examined was the extent of chronic pollutant loading, not variations in salinity.

The fate of PAHs released into the environment has also been studied and summarized by DeLaune et al. (1990). Microbial degradation is a major process by which petroleum hydrocarbons are removed from benthic sediments. Two major factors controlling the rate of microbial breakdown of hydrocarbons in sediment-water systems are pH and redox conditions within the sediment (Howard and Banerjee, 1984). Most hydrocarbons degrade more rapidly under high redox (aerobic) conditions. Another factor which may affect rates of microbial degradation, aside from temperature and salinity reviewed above, is nutrient availability. For example, Bakker (1977) has shown that the absence of NO3⁻ may limit the biodegradation of some petroleum hydrocarbon compounds. Some compounds, due to slow degradation in anaerobic sediments, may persist for decades.

The fate of any petroleum product entering Louisiana's estuaries will be influenced by the depth to which they are buried by marsh sediments and the depth of the corresponding oxidized surface layer in that area. The hydrocarbons enter the sediment via the aerobic surface layer which was ~5 mm deep in DeLaune et al.'s (1990) study. The hydrocarbons are buried by deposition of new sediments, and become part of the reduced layer. In some Louisiana coastal wetlands, it has been shown via 137Cs dating that sedimentation rates are as high as 1 cm yr⁻¹ (DeLaune et al., 1978). Under these conditions, petroleum products are easily locked into sediments where there are anaerobic conditions and microbial breakdown is slow.

In the Barataria basin, the highest mineralization rates (conversion of petroleum products to CO₂) observed occurred at pH 8.0, with the lowest at 5.0 (DeLaune et al., 1980; Hambrick et al., 1980). Where oxygen was less available in the sediment, mineralization rates were slower (Fig. 20). This concurs with the results of Zobell (1946) and Floodgate (1984).

Effect of PAHs on Fundulus heteroclitus (killifish)

In fish, cytochrome P-450 enzymes catalyze the transformation of compounds in fish produced either by the fish itself or by other organisms hosted by the fish (parasites, symbionts, commensals, etc; Klotz et al., 1986; Snowberger and Stegeman, 1987). Their production can be an indicator of physiological stress from a pollutant and can be induced by PAH (Statham et al., 1978; Melancon and Lech, 1983; Gooch et al., 1989). Elevated levels of these cytochromes have been found in fish from contaminated areas (Bend et al., 1978; Stegeman et al. 1981; Foureman et al., 1983).

Elskus and Stegeman (1989) worked on the effects of PAHs, among other contaminants, on *Fundulus heteroclitus* (killifish) in Rhode Island. They found that microsomal protein, total spectral cytochrome P-450, and cytochrome b5 content did not differ significantly between their PAH-contaminated and reference areas - the Seekonk River and the Succotash Salt Marsh, respectively. Levels of the immuno-detectable homologue of P450E, a component of the PAH-inducible P-450, were higher in Seekonk River fish. PAH levels were 60 times higher in Seekonk River sediment than in the reference salt marsh. The relative degree of contamination in the sediments is mirrored in the fish's tissues. PAH content was actually low in the tissue, as low as 0.1% of the sediment concentration. The relative tissue concentrations between the two sites were different, however, being 4-fold higher in the Seekonk fish. This indicates that PAHs are rapidly metabolized by these fish and teleosts in general. Levels of P-450E and ethoxyresorufin O-de-ethylase (EROD) activity, but not aldrin epoxidase (AE) activity, are indicative of relative tissue PAH concentrations within *Fundulus*. The livers of fish from the contaminated site also exhibited hypertrophy, indicating possible altered liver function. Anthracene concentrations were found to be 449.4 (\pm 92.5) ng g⁻¹ dry wt. in Seekonk River sediment and 1.4 (\pm 0.9) ng g⁻¹ in fish tissue. In the Succotash Salt Marsh (reference area), concentrations of anthracene were 13.4 (\pm 20.3) ng g⁻¹ in the sediment and 0.3 (\pm 0.05) ng g⁻¹in the fish. The occurrence of lower-weight PAHs (three fused rings or less) in the fish most likely indicated either lower rates of metabolism or selective uptake of these compounds (Elskus and Stegeman, 1989). In 1981, Varanasi and Gmur demonstrated that benzo(a)pyrene (a five-ring compound) was metabolized faster than naphthalene (a two-ring aromatic compound) in English sole liver exposed to contaminated sediment. The higher molecular weight PAHs therefore appear to be metabolized faster than the lower-molecular weight ones, leaving concentrations of the latter relatively higher in the tissues.

Related Compounds: Linear Alkylbenzenes (LABs) in Estuarine Sediments

Linear alkylbenzene (LAB) is the term commonly given to a secondary phenyl alkane with a side chain, varying from 110-114 carbon atoms (Raymundo and Preston, 1992). They are generally used as precursors for anionic surfactants for commercial detergents or are used themselves as such. They may be preserved in sediments for decades (Eganhouse et al, 1983; Ishiwatari et al., 1983; Eganhouse, 1986). Differential solubility may also account for loss of shorter chain LABs in the sediments (see Murray et al., 1987; Sutton and Calder, 1975).

As LABs are exposed in the environment for longer periods of time, they lose their external isomers (specifically the ones in which the phenyl group is substituted near the terminal carbon of the alkyl chain - 2-phenylalkane) relative to the internal ones (Eganhouse et al., 1983a; also see Murray et al., 1987; Ishiwatari et al., 1983; Bayona et al., 1986; Takada and Ishiwatari, 1991). This may be due to selective biodegradation of

external vs. internal isomers and may act as an indicator of the extent of microbial degradation of LABs (Bayona et al., 1986).

An index of internal to external isomer abundances has been developed by Takada and Ishiwatari (1987) to describe this level of degradation. Phenyldodecane has been used predominantly for the development of this indicator because of its dominance in the samples and the clear peaks yielded in analysis by gas chromatography:

$$(4-C_{12}AB + C-C_{12}AB + 2-C_{12}AB + 5-C_{12}AB)$$

Internal/External (I/E) = $(4-C_{12}AB + 3-C_{12}AB + 2-C_{12}AB)$

Use of the Internal/External (I/E) index in the Thames did not assist in detecting increased amounts of LAB degradation with depth. It did, however, indicate that LAB concentrations decreased with depth, most likely indicating an increase in recent input of these contaminants (Fig. 25).

Eganhouse et al. (1983) suggests that LABs experience most of their degradation during settlement of the particles in the water column, requiring on the order of days to weeks. They believe that after deposition, only a small amount of change occurs. Raymundo and Preston (1992), however, found that this was not the case and observed that bacterial degradation was responsible for changes in LAB composition with time in the Thames River.

Preston and Raymundo (1993) have found that in contaminated sediments, grain size is a poor predictor of contaminant concentration. This is because hydrocarbon concentration is much more tightly linked to the organic carbon and lipid content of the sediment (Fig. 26). It is possible that the LABs may already be incorporated within the particles, either

within the interstices or within the associated organic matter. It may also become associated with the particle fraction via adsorption onto the surface. Unsulphonated LABs are relatively non-polar, and increasing chain length has little effect on their affinity for particulate matter.

Preston and Raymundo (1993) derived a model by which to describe the relationship between LAB concentration, associated sediment, and sediment properties. If the predominant mechanism of contamination is one of incorporation of LAB within the bulk particle, the following relationship is predicted:

 $K_1 [lipid]/\alpha \implies K_2 \{ [F_2 X \{ ([lipid]/\alpha F_1)^{2/3} \}$ and $\Sigma LAB = \sim K_1 [lipid]/\alpha.$

If the principal incorporation mechanism is one of surface adsorption, the following applies:

 $K_1 [lipid]/\alpha \iff K_2 \{ [F_2 X \{ ([lipid]/\alpha F_1)^{2/3} \}$ and

$$\Sigma LAB = ~ K_2 \{F_2 X ([lipid]/\alpha F_1)^{2/3} \}$$

where

$$\begin{split} \Sigma LAB &= [LAB]_b + [LAB]_s = \text{ total LAB concentration} \\ b &= \text{bulk-associated LAB} \\ s &= \text{surface associated LAB} \\ K_1 &= \text{constant} \\ F_1 &= \text{form factor related to particle shape} \\ \alpha &= \text{proportionality constant} \\ [lipid] &= \alpha [F_1 X r^3] \\ r &= \text{nominal particle radius.} \end{split}$$

A plot of log (Σ LAB) was predicted to yield a straight line, and this is what was produced.

In the Mersey Estuary, U.K., LAB concentrations are high and there is evidence for microbial degradation.

Effects of Lower Molecular Weight Hydrocarbons on Salt Marsh Plants and the Microbial Community

Li et al. (1990) conducted a 2-yr. microcosm study in South Carolina examining the effects of chronic sub-lethal exposure of a mixture of lower molecular weight hydrocarbons on plant growth and the microbial community in salt marshes. They concentrated on the marsh grass *Spartina alterniflora*. The hydrocarbon mixture was comprised of hexane, heptane, octane, nonane, benzene, toluene, ethylbenzene, and xylene - compounds readily found in natural gas condensate. These compounds are also known to comprise approximately 15% of crude oil (Nelson-Smith, 1972; Table 7). This subset of compounds would have comparatively high solubility in water and would most likely represent the suite of hydrocarbons that marshes would be exposed to when subjected to chronic pollution from refineries or tankers.

In the earlier study, Freedman (1989) showed that the aromatics are much more soluble in water than alkanes. The solubility of C₁ - C₄ alkane gases range from 24 - 62 g m⁻³ in freshwater; the light liquids C₅ - C₉ are 0.05 - 39 g m⁻³; C₁₀-C₁₇ are 1 - 2 x 10⁻⁴ g m⁻³; C₁₆-C₂₅ are 6 x 10⁻⁴ to 10⁻⁸ g m⁻³. The solubility of benzene is 1,730 g m⁻³, toluene is 515 g m⁻³, and naphthalene is 31 g m⁻³ (Parker et al, 1971; Clark and MacLeod, 1977).

Li et al.'s (1990) exposure treatments were 3.33 and 33.3 g C m⁻² day⁻¹ (vs. a control) for 10 months. This is the equivalent of an addition of 4.68 ml hydrocarbon m⁻² day⁻¹. The salinity used was 15 $^{\circ}$ /oo - within the salinity range characteristic of Cocodrie, Louisiana.

In the lower concentration treatment, plant biomass (Fig. 27) and total respiration rates increased significantly. These effects persisted for 7 months after hydrocarbon addition ceased. This concurs with the studies of Baker (1971). In the higher exposure treatment, however, growth, total sediment organic matter (Fig. 28), and macro-organic matter (Fig. 29) declined significantly. Likewise, with respect to microbial activity, CO₂ production, methanogenesis, N₂ fixation, and denitrification (Fig. 30) were stimulated in the lower concentration treatment but inhibited under higher concentrations. This concurred with the findings of Hershner and Lake (1980) and Thomson and Webb (1984).

Both plant and microbial populations exhibited a non-linear response to chronic hydrocarbon exposure, with stimulation of activity at lower concentrations and inhibition at higher ones. It appears that the microbes were carbon-limited, and the plants may have experienced increased growth due to enhanced nitrogen fixation or mineralization by bacteria. Accumulation of hydrocarbons added to marsh sediments daily for 9 months most likely did not occur due to evaporation and active mineralization or biodegradation of the compounds by microbial activity.

Bossert and Bartha (1984) demonstrated that oil and various oil fractions can be broken down by bacteria at rates of 0.05 to 0.11 g hydrocarbon kg⁻¹ soil day⁻¹. These rates greatly exceed (by 5-10 fold) rates necessary to biodegrade the hydrocarbons introduced in Li et al.'s (1990) experiment. The latter results suggest that the salt marsh ecosystem is quite resilient to pollution of LMW hydrocarbons at low levels. Spartina alterniflora is a primary component of saltmarsh ecosystems throughout the US. Numerous marsh species, both plant and animal, are dependent upon it for refuge, habitat, food, etc. (Zimmerman and Minello, 1984a,b). Some studies have shown that No. 2 fuel oil has a generally faster, more severe, and long-term impact on *S. alterniflora* than crude or heavy fuel oils.

Webb and Alexander (1991), on the other hand, conducted a one-year field study on the effects of a simulated spill of No. 2 fuel oil on *Spartina alterniflora* in a Texas salt marsh. They found that, as the degree of coverage of the plants by No. 2 fuel oil increased, initial damage to the plants increased, and regrowth from the roots decreased. There was evidence of translocation of oil components from the foliage to the root system, possibly affecting regeneration. When the plants were completely covered by oil, the above-ground foliage died and regrowth from the roots did not occur. If oil was applied only to the sediment, stem density declined slightly, apparently by penetration of the oil and its contact with the roots.

Scifres (1980) has shown that No. 2 fuel oil can act like an active foliage herbicide, killing vegetation upon contact and inhibiting regeneration. This was exemplified by the Bouchard Barge spill of No. 2 fuel oil in a a temperate salt marsh in Massachusetts, where little re-establishment of *S. alterniflora* by seedlings or rhizome growth occurred within a three-year period (Hampson and Moul, 1978). This was due to the highly toxic, oxidized oils and aromatics which stop respiration and cause widespread injury and death (Baker, 1970). It was aggravated by higher wave energy, permitted by the loss of *Spartina* stems on the marsh, inhibiting the establishment of new *Spartina* seedlings (Knutson et al., 1981). In addition, the organic soils which characterize salt marshes are

more prone to penetration of hydrocarbons (Hampson and Moul, 1978; Burns and Teal, 1971) than mineral soils (Webb et al., 1985).

Analysis of hydrocarbon in the sediment demonstrated that oil did not accumulate in the sediment with time. This is consistent with the studies of Li et al. (1991) for lighter hydrocarbons. Some oil did accumulate, however, in the roots and rhizomes. Regrowth from neighboring roots occurred, indicating that residual oil toxicity in the sediments was low.

It would appear that the impact of a spill of No. 2 fuel oil has a lesser and shorter-term impact on a salt marsh ecosystem in the Gulf than in the northern temperate.

General Effects of a Crude Oil Spill: The Amoco Cadiz

Southward (1978) reports that the wreck of the Amoco Cadiz (1978) represented the fourth large tanker spill of crude oil to occur on the Brittany coast of northern France in 11 years. It was preceded by the Torrey Canyon in 1967, the Boehlen in 1976, and the Olympic Bravery in 1976. All of these had massive impacts on the biota of the region. 120,000 tons of Iranian crude and 100,000 tons of light Arabian crude were introduced into the environment. It was estimated that 30-35% of the oil was comprised of aromatics; 3% or 6,600 tons was benzene.

The oil was highly toxic to the local marine life. There was not enough time for the lighter, more toxic components to evaporate because of the formation of water-in-oil emulsions (commonly referred to as "mousse"). Shore fishes, including wrasses, and benthic intertidal invertebrates, such as snails and limpets were killed, as were most of the algae. Many vagile (mobile) intertidal fauna became "comatose" and "lost their grip" on

the substratum, being swept away. The suite of grazing herbivores was almost completely eliminated from the community. This was followed by a "green flush" or growth of ephemeral algae, including *Enteromorpha* and *Ulva*, which is characteristic of the gross imbalance in ecosystems in highly polluted areas (Southward and Southward, 1978). ≥ 35 species of seabirds were affected, particularly the auks and cormorants. Oysters and other shellfish were also killed. Effects in the sediment were traced as far as 150 km away, including mortality of cockles (*Cardium*), heart-urchins (*Echinocardium*), and razor-shells. The infaunal, soft bottom polychaete *Arenicola marina* (the lugworm) appeared to survive well.

Effects of Crude Oil Spills on Bottom Sediments

Three of the processes for which anaerobic salt marsh microbes and other marine bacteria are responsible are methanogenesis (production of CH4), sulfate reduction (reduction of SO4 to SO2), and evolution of CO2 (an indicator of community metabolism). Organic contaminants can affect the rates of these processes.

It is believed that oil spills inhibit the growth of certain naturally occurring groups of bacteria and cause an increase in oil-degrading bacteria (Colwell and Walker, 1977). Growth in proteolytic, lipolytic, and chitonolytic bacteria (those which break down proteins, lipids, and chitin, respectively) was inhibited in a saltmarsh where crude or No. 2 fuel oil was spilled (Walker et al., 1975). On the other hand, nitrogen fixing bacteria were not inhibited by weathered crude oil or by various alkanes in arctic sediments (Knowles and Wishart, 1977). DeLaune et al. (1979) also found that crude oil spills did not affect sulfate reduction by bacteria in salt marshes.

Winfrey et al. (1982) studied the effects of the crude oil spill of the Amoco Cadiz along the Brittany coast of France. They examined methane-producing and sulphate-reducing bacteria in intertidal sediments. They found that an acute oil spill had few residual effects on pore-water chemistry, methane production, rate of sulfate reduction, and other similar microbial processes after one year.

Benzene and toluene, lighter hydrocarbons, are known to have inhibitory effects on anaerobic processes in sediments (Robertson et al., 1973). The addition of 0.05% of these compounds to sediments in oiled and unoiled marsh sites, however, had no effect on sulphate reduction or methane production (Table 8). This is reasonable, since this process appears to be naturally active in sediments overlying oil producing areas (Bailey et al., 1973; Zobell, 1958). The addition of these compounds did, however, reduce by 91-92% the rate of oxidation of [2-14C]-acetate to $14CO_2$ at the more pristine unoiled site (Table 9). This is particularly important if temperatures are low and evaporation rates are slowed (Atlas and Bartha, 1973), because the persistence of low molecular weight components such as benzene and toluene may result in the inhibition of acetate turnover and a possible inhibition of anaerobic decomposition processes (Winfrey et al., 1982).

There was a correlation between inhibition of methane production and sulfate reduction, and weathering. That is, a compound added to sediment recently which had been previously exposed to Amoco Cadiz crude oil, had less of an effect on microbial processes than when added to more pristine soil. This indicates that indigenous populations may have been replaced by oil toxicity-resistant ones in the spill area, and that the micro-organisms may be adapted to the presence of oil or selected to become oil resistant (Winfrey et al., 1982). The n-alkanes (e.g. C-24) were found to be less volatile than the low molecular weight compounds and remained in the short-term weathered oil for longer periods of time (on the order of hours to days). Toluene and various other volatile aromatic compounds such as benzene were significantly reduced due to evaporation after 8 hrs, and were almost gone after 48 hrs.

Effects of Benzene and Related Compounds on Salt Marshes

Effects of Benzene on Microbial Processes in Salt Marsh Sediments

Experiments performed by Kiene and Capone (1984) have demonstrated that the addition of 1,000 μ g g⁻¹ (dry weight sediment) of benzene had no significant effect on the rates of methane production, sulfate reduction, or CO₂ production in the sediments of a northern temperate, New York salt marsh. These processes affect nutrient regeneration and carbon mineralization within the marsh (Kiene and Capone, 1984); these are key ecological processes sustaining this ecosystem. Inhibition of sulfate reduction is a key process in the anaerobic metabolism of estuarine sediments.

The amounts added experimentally are much higher than those found naturally in many coastal sediments. Natural concentrations of benzene are usually less than 10 μ g g⁻¹, even in sediments considered polluted (Bagg et al., 1981; MacLeod et al., 1981), although naphthalene concentrations are known to exceed this in polluted areas (Bopp et al., 1982; Knap et al., 1982). Similar concentrations of the related compound naphthalene, however, at first inhibit methanogenesis and then stimulate it with time. Naphthalene also inhibits sulfate reduction; this may produce increased rates of methane production. 1000 μ g g⁻¹ naphthalene in some cases inhibit CO₂ evolution as well, although this effect was not consistent between experiments.

CO₂ evolution is considered to be the least sensitive indicator of environmental stress resulting from hydrocarbon pollution (Kiene and Capone, 1984). Methanogenesis appears to be the most sensitive indicator.

Except in highly polluted sediments, methanogenesis, sulfate reduction, and CO₂ evolution would not be affected by the presence of benzene and naphthalene at the low concentrations frequently found in the environment, irrespective of source.

Effects of Benzene on Blue Crabs

Blue crabs (*Callinectes sapidus*) represent a major natural resource in Louisiana. The area around Terrebonne Bay is heavily fished for these crabs - commercially and for recreation - and a spill could affect production of the marsh and bay for an extended period of time - up to decades. It is therefore important to have some understanding of the potential effects of a condensate spill on this fishery.

Cantelmo et al. (1981) performed a study examining the effects of benzene on molting and limb regeneration on juvenile blue crabs. This was done in a northern temperate estuary - Raritan Bay, NJ. "Blue-water" concentrations of benzene in the Atlantic Ocean, offshore of Georgia, have been measured at 1-3 ppb. Inshore and in estuaries, levels of 1-100 ppb have been measured (Whipple et al. 1981).

Cantelmo et al. (1981) performed experiments using concentrations of 1 ppm, which are considered to be sublethal to the crabs. They found that the benzene evaporated at an almost constant rate of 0.1 ppm/h, after which time it stabilized at 0.26-0.30 ppm after 6 hrs, or 260-300 ppb (Fig. 31).

They found that the length of the inter-molt cycle increased in the benzene treatment (Fig. 32), and the growth increment at ecdysis (new size after molting) also decreased (Fig. 33). Treated crabs also took longer to develop pigments in their limb buds. This growth stunting was most pronounced in the smaller juvenile crabs (Fig. 34). All of these responses indicate that growth had slowed significantly. Benzene has important physiological effects on blue crabs which appear to manifest themselves through the neuroendocrine system and metabolism. A spill of benzene into a salt marsh could produce a population of small blue crabs that grow slowly.

Sorption Properties of Benzene and Related Compounds on Estuarine Colloids

Exposure of marsh plants and animals to PAHs and other hydrocarbons is largely controlled by the partitioning behaviour of the compounds onto bottom and suspended sediments. The sorption and desorption equilibria of these compounds are important in determining their fate, transport, and toxicity within the marsh ecosystem. They may also affect the rate at which the microbial community transforms these compounds to other smaller, less harmful ones (Wijayaratne and Means, 1984).

The equilibrium sorption isotherm for benzene, a compound commonly found in natural gas condensate, is linear (Fig. 35; Wijayaratne and Means, 1984). The lack of deviation from linearity suggests that sorption of PAHs onto these estuarine colloids is result of partitioning within the sorptive capacity of the colloids.

The solubility of benzene in marshwater is 1,400 μ l l⁻¹ at a salinity of 25 % on and a temperature of 16% (Benville and Korn, 1977); it is 820.0 ppm (Wijayaratne and Means, 1984) at 18.8 % o. This is much higher than that for naphthalene or anthracene (31.7 and

0.073 ppm, respectively; Wijayaratne and Means, 1984). The Freundlich partition coefficient of benzene is 920 - much lower than that for naphthalene and anthracene (4,060 and 510,000, respectively) and an order of magnitude higher than that reported for soil/pond sediment systems (Karickhoff et al., 1979).

Hydrophobic compounds such as benzene and related compounds are more strongly bound to organic colloidal material in higher salinity estuaries than in soil on land or in freshwater environments. This relationship is altered by variations in pH. If the natural pH of 8.01 is decreased to 5.0, sorption of anthracene decreases (Fig. 36). This indicates that experimentation regarding effects of the compounds of interest must be conducted under ambient conditions to yield reliable results for that environment (Wijayaratne and Means, 1984).

PAH phase partitioning can be predicted in estuarine waters. The K_{OC} values for natural colloids are highly correlated with PAH solubilities. This then allows one to predict PAH phase partitioning in natural waters by using this statistical relationship (Fig. 37).

Effects of Benzene on Striped Bass

Although striped bass (*Morone saxatilis*) are not generally found in the estuaries of southern Louisiana, several closely related, commercially important species are. These include the white bass, a smaller related bass, and a smaller pseudo-striped bass, all of the genus *Morone*. It is therefore worthwhile briefly reviewing some impacts of condensate compounds on striped bass.

In the San Francisco Bay area, two-thirds of the striped bass were found to contain benzene in their liver tissue in concentrations up to ~4 ppm (1978 study; Whipple et al., 1983). The fish were in poor physiological and reproductive condition, were heavily parasitized, and exhibited rare responses to the parasites, such as open wounds which exposed their internal organs (Moser et al., 1985).

0.1 ppm benzene in estuarine water is considered to be a high environmental concentration (Korn et al., 1977; Whipple et al., 1981; MacFarlane and Benville, 1986).
1.0 ppm benzene is characteristic of water in the vicinity of a petroleum spill (Hirsch et al., 1987). The 96 hr LC50 for striped bass is 10.9 ppm benzene (Meyerhoff, 1975).

MacFarlane and Benville (1986) performed laboratory experiments where they exposed striped bass to sub-lethal concentrations of benzene (0.1 and 1.0 ppm) in estuarine water for ≤ 21 days. They found the compound concentrated in the blood (Fig. 38) and liver (Fig. 39) tissues of the animal up to 20 times the concentrations of experimental exposure. The liver did not accumulate unmetabolized benzene above concentrations found in the blood. Stress responses were moderate, and the fish returned to normal levels after 7 days.

Plasma cortisol concentration (a primary stress response; Selye, 1950) and indicators of energy mobilization in the blood (a secondary response) were not proportional to benzene exposure or accumulation levels. They increased 2- to 3-fold after 8 hrs and then returned to control levels after 48 hrs (Fig. 40).

In the initial 4 hrs to 7 days, concentrations of glucose, lactate, H⁺, protein, and triglycerides became elevated. Protein and triglycerides rapidly returned to normal. Benzene appears to be metabolized rapidly by the fish within the hepatic (liver) tissue,

stored in the gall bladder, and excreted in the feces and urine (Korn et al., 1976, 1977; Roubal et al., 1977; Mac Farlane and Benville, 1986).

With respect to behavioral responses, the experimental fish swam more continuously and appeared to be more restless for the first 4 days. This response was stronger in the 1.0 ppm benzene treatment. The behavior lulled between 4 and 7 days, and hyperactivity returned again after this time, particularly in the higher benzene concentration treatment. Feeding rates were lower in those fish exposed to benzene, particularly those in the higher concentration treatments.

Fish can sense benzene in the water (Babcock, 1987), and exposure to the compound causes stress responses in striped bass similar to an "alarm reaction." Physiologically, it appears that this fish responds to benzene exposure with mild but acute stress followed by acclimation. These researchers did not, however, investigate the effects of benzene on reproduction, which would be crucial to determining whether striped bass populations would be affected in the longer term over generations.

Comments on Mitigation

Walsh et al. (1995) have assessed different techniques for using bioremediation in oil spill cleanups in Louisiana coastal wetlands. Through microcosm studies, they found that neither commercial oil-degrading bacteria nor commercial fertilizer imposed negative impacts on the marsh ecosystem. Naturally occurring petroleum-degrading bacterial populations have been found to be significantly higher three weeks after being treated with fertilizer and oxidizer, which induces higher levels of petroleum degradation, but only for the alkanes. After 12 weeks, the microbial populations which were monitored decreased in size again and biodegradation rates for aromatic hydrocarbons fell to almost

immeasurable levels. This clean-up technique may be more applicable to natural gas condensate spills than oil spills.

Herbert et al. (1995) examined the relationship between the bioavailability of PAHs in marsh sediments and its effect on bioremediation. In particular, he examined effects on highly weathered petroleum in the field and in the laboratory. He found that there was no significant difference between the types of nutrient added, presence of surfactants, and inoculation with microorganisms vs. the controls. He concluded that *in situ* biodegradation of specific organic compounds present in the petroleum, including PAHs, was limited by low compound bioavailability. That is, once weathering has occurred, there is little that can be done to enhance biodegradation rates.

Experiments have been performed where salt marshes dominated by *Spartina alterniflora* have been exposed to crude oil and compared to non-oiled reference plots (DeLaune et al., 1979; Smith et al., 1984). There was no oil-induced mortality of microfauna or meiofauna, suggesting that the community was well adapted to the presence of these hydrocarbons and that, should there be a spill, the best response might be no cleanup action at all (DeLaune et al., 1990).

In other experiments dealing with No. 2 fuel oil, *Spartina alterniflora* was found to be highly negatively impacted. It is recommended that, due to the massive mortality of the salt marsh grass, as much of the contaminant be removed from the site as quickly as possible. Long-term effects on regrowth can be evaluated within days or weeks through the obvious immediate effects of the oil. Symptoms include the absence of green color and lack of shoot regrowth. The stems of dead plants should not be removed but should be permitted to stand in place - to dampen wave action (Webb and Alexander, 1991). Plants should be transplanted into the area (Lewis, 1982), because natural regrowth from

rhizomes and regeneration from seedlings may not occur (Webb and Alexander, 1991). The sediments should also be analyzed for concentration of hydrocarbons to determine whether the levels present would inhibit establishment of new plants.

With respect to crude oils, Southward (1978) recommends against the use of dispersants due to their high toxicity (even at concentrations of 1 o/oo). Some dispersants actually increase the toxicity of the oil. Physical removal and collection of the oil is recommended. This includes

- . concentration of the petroleum into ploughed furrows
- . pumping into settlement tanks on higher ground and then trucking it to waste oil recovery depots
- . using simple absorbents such as straw or mop-like structures to collect and concentrate the oil, and/or
- . removing the sediment and replacing it with clean sediment.

Steam cleaning of intertidal rocks was used in the Amoco Cadiz spill. This, however, has since been found to be highly destructive to the environment, delaying recolonization by the natural fauna and flora for long periods of time. It is no longer recommended.

General Remarks

Salt marshes exhibit a variety of responses to the introduction of petroleum hydrocarbons. The data available on the effects of specific compounds on the marsh environment are patchy, particularly when one considers only those compounds found in natural gas condensate. We know, however, that the responses of different components of the benthic salt marsh communities, both plants and animals, vary with respect to the following:

- . the type of compound to which they are exposed (low or high molecular weight hydrocarbon)
- . the extent of exposure
- . the solubility of the compound in estuarine water of variable salinity
- . the concentration of the contaminant, particularly where non-linear responses are known to occur
- . the concentration of organic matter in the sediments
- . the sorptive characteristics of the compound and the sediments
- . water temperature
- . salinity of the water
- . salinity range of the environment
- . wind velocity and direction (affecting evaporation rates and rates of flushing for the estuary)
- . community composition and structure
- . history of the site with respect to exposure to petroleum hydrocarbons.

The size of a spill required to produce those effects detailed in this report is difficult to predict, for the effects are firstly dependent upon concentration of the contaminants in the water and sediments and all of the physical, meteorological, and chemical factors which can influence those concentrations. The predictions will be derived from the final concentrations reached in the estuarine waters and particularly in the sediments.

Those areas in the Cocodrie region most sensitive to a condensate spill are the saltmarshes themselves; unfortunately, most of the Cocodrie area is saltmarsh. A small spill of natural gas condensate into open waters such as Terrebonne Bay would have a low impact, particularly if cleaned up quickly. One of the major problems here, however, is that, although the compounds found in the condensate evaporate rapidly (and more

rapidly in the winter), they also bind rapidly to sediment particles, particularly those coated with organic matter. The suspended sediment particles rapidly sink to the bottom and join those already initially contaminated by the hydrocarbons. It is generally through the sediment that most of the continuing damage to the marsh community is done. Cocodrie is an extremely well developed salt marsh ecosystem, and the process of sedimentation here is rapid.

One consoling point is that there is a high probability that the microbial community here is already adapted to biodegrade hydrocarbons rapidly in the environment. The area has had oil and gas reserves beneath its sediments for tens to hundreds of millions of years. These compounds have been released by humans into the local environment for over 50 years. As noted above, adaptation to environmental perturbations over evolutionary time (which can be short for microbes and some small invertebrates) can be very rapid in the marine environment.

There is no simple answer regarding the effects of a natural gas condensate spill on a salt marsh. Nevertheless, the studies reviewed here can help us to predict certain aspects of environmental impact, given the conditions at the time and with specific knowledge of the site in question.

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Figure Legends

- Fig. 1. Seasonal variation of n-alkanes in the marshes of Iraq (from Al-Saad and Ak.Al-Timari, 1993, reprinted with permission).
- Fig. 2. Chromatograms of n-alkanes in dissolved water from the marshes of Iraq (from Al-Saad and Ak.Al-Timari, 1993, reprinted with permission).
- Fig. 3. Production of methane from freshwater marsh sediment in microcosm experiments as influenced by soil redox potential (from Devai and DeLaune, 1996, with permission).
- Fig. 4. Production of ethane, propane, and butane from freshwater marsh sediment in microcosm experiments as influenced by soil redox potential (from Devai and DeLaune, 1996, with permission).
- Fig. 5. Production of ethylene, propylene, and isobutane from freshwater marsh sediment in microcosm experiments as influenced by soil redox potential (from Devai and DeLaune, 1996, with permission).
- Fig. 6. The composition (section) and quantities (bars) of light hydrocarbons produced by freshwater marsh sediment under oxidized (+ 430 mV) and reduced (- 170 mV) soil conditions (from Devai and DeLaune, 1996, with permission).
- Fig. 7. Concentration of toluene:methanol extracts of sediments versus time from spill of crude oil in Harbor Island, Texas. LM = Laguna Madre (from Macko et al., 1981, reprinted with permission).
- Fig. 8. ¹⁴C-toluene metabolism to ¹⁴CO₂ in fresh surface seawater from Resurrection Bay near Seward, Alaska. Triangle = 12 June, 1983, 2 μg toluene l⁻¹; open circle = 22 June 1983, 0.9 μg toluene l⁻¹; closed circle = 12 June 1983, 2 μg toluene l⁻¹ filtered (0.2 μm) control (from Button and Robertson, 1985, with permission).

- Fig. 9. Toluene metabolism to ¹⁴CO₂ 10 hrs. after addition of ¹⁴C-substrate following 10 to 120 hrs of exposure to (open triangle) 0.0, (closed triangle) 0.7, (open circle) 7.5, (closed circle) 14.2, and (open square) 142 μg ³H-toluene 1⁻¹. (Closed square) = control, 0.9 mg ¹⁴C-toluene 1⁻¹ added to freshly collected seawater. Inset shows typical time course for ¹⁴CO₂ production from 2 μg toluene 1⁻¹ following 48 hrs of exposure to inducing toluene (from Button and Robertson, 1985, with permission).
- Fig. 10. Rate of toluene metabolism vs. inducing toluene concentration and exposure time to substrate (from Button and Robertson, 1985, with permission).
- Fig. 11. Changes in net photosynthesis in *Juncus roemerianus* (open circles) and *Spartina alterniflora* (closed circles) to crude oil treatment. Arrow on the upper left hand section represents the treatment initiation. Each point is the mean for 50-70 measurements. Bars represent ± 1 SE. (from Pezeshki and DeLaune, 1993, reprinted with permission).
- Fig. 12 Measurements of algal activity and biomass. (A) Synthesis of phospholipids from ¹⁴C-bicarbonate; (B) synthesis of storage (neutral) lipids from ¹⁴C-bicarbonate; (C) physiological condition based on the ratio of ¹⁴C-bicarbonate incorporation into phospholipids an neutral lipids; (D) chl a concentration expressed as µg chl a in the top 1 cm of core with 2.3 cm2 surface area. C1 is the control microcosm to which no sediments were added. C2 is the control microcosm to which uncontaminated sediments were added. 'Low', 'Medium', and 'High' are microcosms to which sediment with various levels of PAH contamination were added. Values are averages for four replicates.

- Fig. 13. Abundances of major meiofaunal groups. (A) Total meiofauna; (B) total copepods (includes adults and juveniles); (C) copepod nauplii; (D) nematodes. See Fig. 1 for further details (from Carman et al., 1995, with permission).
- Fig. 14. Relative abundances of meiofaunal groups. (A) The nauplius/copepod ratio of abundance; (B) the nematode/copepod ratio of abundance. See Fig. 1 for further details (from Carman et al., 1995, with permission).
- Fig. 15. Abundances of total copepodites and *Enhydrosoma* sp. copepodites. Values are means plus 1 S.D. (controls, n=2; Low-PAH, Medium-PAH, and High-PAH treatments, n=4). Open bars represent combined controls; diagonally hatched bars represent Low-PAH; cross-hatched bars, Medium-PAH; black bars, High-PAH. Asterisks indicate individual days on which the effect of PAH was significant (from Carman and Todaro, 1996, with permission).
- Fig. 16. Abundances of Pseudostenhelia wellsi: total copepods, males, females, copepodites, and nauplii. Symbols as in Fig. 17. Asterisks indicate individual days on which the effect of PAH was significant (from Carman and Todaro, 1996, with permission).
- Fig. 17. Abundances of *Coullana* sp. total males and females. Symbols as in Fig.
 17. Asterisks indicate individual days on which the effect of PAH was significant (from Carman and Todaro, 1996, with permission).
- Fig. 18. Proportions of adults of total copepods, and of *P. wellsi* and Coullana sp. that were females. Symbols as in Fig. 17. Asterisks indicate individual days on which the effect of PAH was significant (from Carman and Todaro, 1996, with permission).

- Fig. 19. Upper graph: Mean densities of nematodes for each treatment and day after introducing crude oil. Means sharing the same letter(s) across treatments for each day are not significantly different (Duncan's Multiple Range Test; a = 0.05). Lower graph: Mean densities expressed per 10 cm² (from Decker and Fleeger, 1984, with permission).
- Fig. 20. Growth trajectories for (A) S. benedicti and (B) Capitella sp. I.
 Trajectories represent best fits to data using "proc-nlin" in SAS (version 5.18). Asterisk indicates age and corresponding size at first reproduction (from Bridges et al., 1994, with permission).
- Fig. 21. Treatment differences in age at first reproduction for (A) S. benedicti and (B) Capitella sp. I. Letters above each column denote those treatment means which were significantly different. Mean (+ SE) for each treatment appears above each column. Error bars represent the standard error. MMO = marsh mud only. HYDRO = hydrocarbon (from Bridges et al., 1994, with permission).
- Fig. 22. The effect of salinity on the mineralization of naphthalene and anthracene in Iona Marsh sediments (Hudson River, New York), and naphthalene mineralization in Tivoli Bay sediments (from Kerr and Capone, 1988).
- Fig. 23. The effect of salinity on the mineralization of naphthalene and anthracene in sediments from Port Jefferson Harbor, New York (from Kerr and Capone, 1988).
- Fig. 24. Degradation rates of ¹⁴C-labeled octadecane and naphthalene in oxidized and reduced sediment from the Leeville, Louisiana oil field area (from DeLaune et al., 1980; c.f. DeLaune et al., 1990, with permission).

- Fig. 25. Depth profiles of linear alkyl-benzene (LAB) concentrations (normalized to organic carbon) in five sediment cores taken from the Humber and Wash regions of England (from Raymundo and Preston, 1992, reprinted with permission).
- Fig. 26. Linear alkyl-benzene (LAB) concentration as a function of sedimentary organic-carbon (a) and lipid (b) contents. Data shown for Widnes (x), Eastham (*), and Speke (open square), England (from Preston and Raymundo, 1993, reprinted with permission).
- Fig. 27. Biomass of Spartina alterniflora (mean ± 1 SE, n = 5) derived from nondestructive measurements of stem height for control microcosms (open circles) and microcosms treated daily with hydrocarbons at rates equivalent to 3.33 g C m⁻² day⁻¹ (1X) (closed circles), and 33.3 g C m⁻² day⁻¹ (triangles). Arrows indicate when the hydrocarbon additions began and ended (from Li et al., 1990, reprinted with permission).
- Fig. 28. Total organic matter in the microcosm sediments (means ± 1 SE) as a percentage of dry sediment weight by depth and by treatment at the conclusion of the study. (Open circle) = control, (closed circle) = 1X treatment, (triangle) = 10X treatment (from Li et al., 1990, reprinted with permission).
- Fig. 29. Total macro-organic matter in the microcosm sediments (means \pm 1 SE) as a percentage of the total sediment dry weight by depth and by treatment at the conclusion of the study. Open circle) = control, (closed circle) = 1X treatment, (triangle) = 10X treatment (from Li et al., 1990, reprinted with permission).

- Fig. 30. Denitrification rates (means ± 1 SE) by treatment over time. Arrows indicate when the hydrocarbon additions began and ended. Open circle) = control, (closed circle) = 1X treatment, (triangle) = 10X treatment (from Li et al., 1990, reprinted with permission).
- Fig. 31. Measured concentration of benzene in a nominal 1 ppm solution as a function of time (from Cantelmo et al., 1981, with permission).
- Fig. 32. Mean number of days to molt in the blue crab, Callinectes sapidus, in control and benzene-exposed animals. Bar indicates ± 1 SEM (from Cantelmo et al., 1981, with permission).
- Fig. 33. Comparison of days to death (solid line) and final R-value (dashed line) in two groups of control and benzene-treated blue crabs (*Callinectes sapidus*), designated long-lived and short-lived (from Cantelmo et al., 1981, with permission).
- Fig. 34. Increase in carapace width at molt for large and small size-classes of blue crabs (*Callinectes sapidus*), under control and benzene-treated conditions.
 Bar indicates ± 1 SE (from Cantelmo et al., 1981, with permission).
- Fig. 35. Equilibrium adsorption isotherm at 20°C of benzene on estuarine colloids (from Wijayaratne and Means, 1984, with permission).
- Fig. 36. Effects of changes in pH on the K_{α} values of anthracene (from Wijayaratne and Means, 1984, with permission).
- Fig. 37. Relationship between $\log K_{0\alpha}$ and $\log(S)$ (water solubility) for benzene, naphthalene, and anthracene (top plot) on estuarine colloidal matter and for 22 compounds on riverine sediments (bottom plot; from Means et al., 1982; c.f. Wijayaratne and Means, 1984, with permission).
- Fig. 38. Benzene concentrations in exposure tanks and the blood of striped bass,
 Morone saxatilis. Targeted benzene concentrations in water were 0.1 and
 1.0 ppm for low and high exposures, respectively. Values represent means

 \pm 1 SE; where error bars are not shown, SE was less than size of symbol (from MacFarlane and Benville, 1986, with permission).

- Fig. 39. Accumulation of benzene in the liver of striped bass, Morone saxatilis. Targeted benzene-exposure concentrations are indicated on the graph. Values represent means ± 1 SE (from MacFarlane and Benville, 1986, with permission).
- Fig. 40. Plasma cortisol concentrations in benzene-exposed and control striped bass, *Morone saxatilis*. Values represent means ± 1 SE. Asterisks denote values significantly different from same-time control values (Duncan's Multiple Range Test: * p < 0.05; ** p < 0.01; from MacFarlane and Benville, 1986, with permission).

Table Legends

- Table 1. Concentrations of dissolved n-alkanes (μg l⁻¹) in water marshes during the winter in the northern Tyrrhenian Sea (from Al-Saad and Ak.Al-Timari, 1993, reprinted with permission).
- Table 2. Concentrations of dissolved n-alkanes (μg l⁻¹) in water marshes during the spring in the northern Tyrrhenian Sea (from Al-Saad and Ak.Al-Timari, 1993, reprinted with permission).
- Table 3. Concentrations of dissolved n-alkanes (μg l⁻¹) in water marshes during the summer in the northern Tyrrhenian Sea (from Al-Saad and Ak.Al-Timari, 1993, reprinted with permission).
- Table 4.Seasonal variations of the total dissolved n-alkanes in water marshesassociated with the northern Tyrrhenian Sea (from Al-Saad and Ak.Al-Timari, 1993, reprinted with permission).
- Table 5. Half-lives (in days) of representative aromatic hydrocarbons subject to the levels of insolation and wind speeds incorporated in the model for different seasons of the year in southwest England. Values have been calculated assuming a salinity of 20% oo, with a suspended particulate load of 50 ppm (from Harris et al., 1984, reprinted with permission).
- Table 6. Rates of ¹⁴CO₂ production (ng l⁻¹ h⁻¹) with respect to both inducing toluene concentration and exposure time. Rates are normalized to a toluene concentration 2 μg l⁻¹ used in the incubation period following induction (from Button and Robertson, 1985, with permission).
- Table 7. Components of the mixture of light hydrocarbons (including hexane, heptane, octane, nonane, benzene, toluene, ethylbenzene, and xylene) which were added daily to experimental cores in Li et al.'s (1990)

experiment. See table for details; (from Li et al., 1990, reprinted with permission).

- Table 8. Effect of hydrocarbons on sulphate reduction in marsh sediments. (a) Sediment samples were collected in April, 1980, 25 months after the spill.
 (b) Rates are the mean of three replicates and expressed as μmoles SO4=-S ml⁻¹ day⁻¹ (from Winfrey et al., 1982, reprinted with permission).
- Table 9.Effect of hydrocarbons on the metabolism of [2-14C]-acetate to 14CO2 in
sediments (from Winfrey et al., 1982, with permission).

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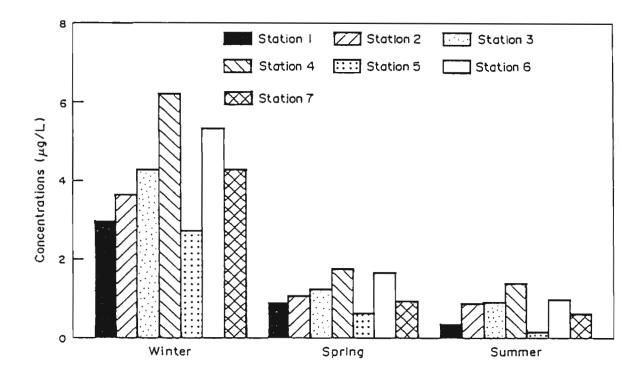
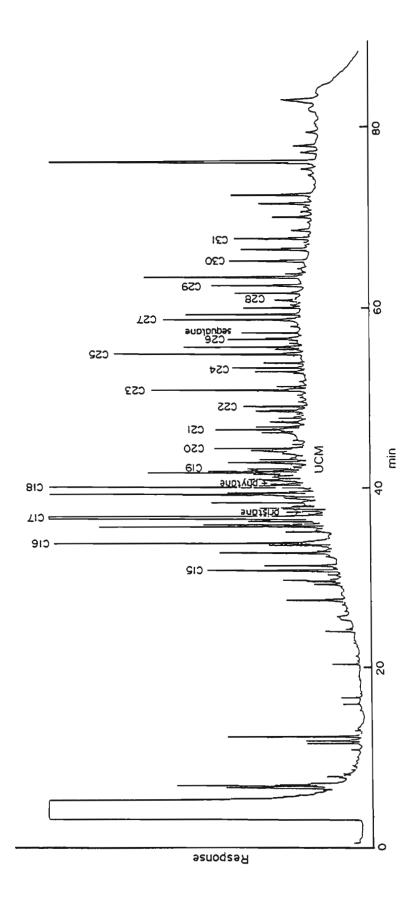
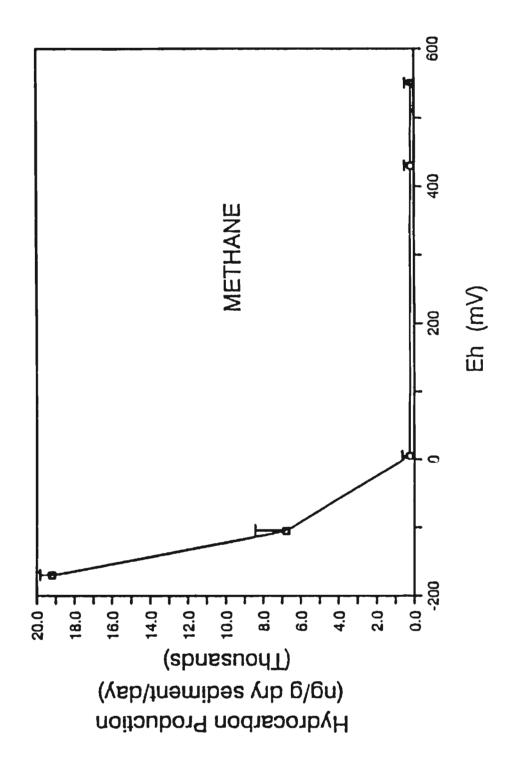
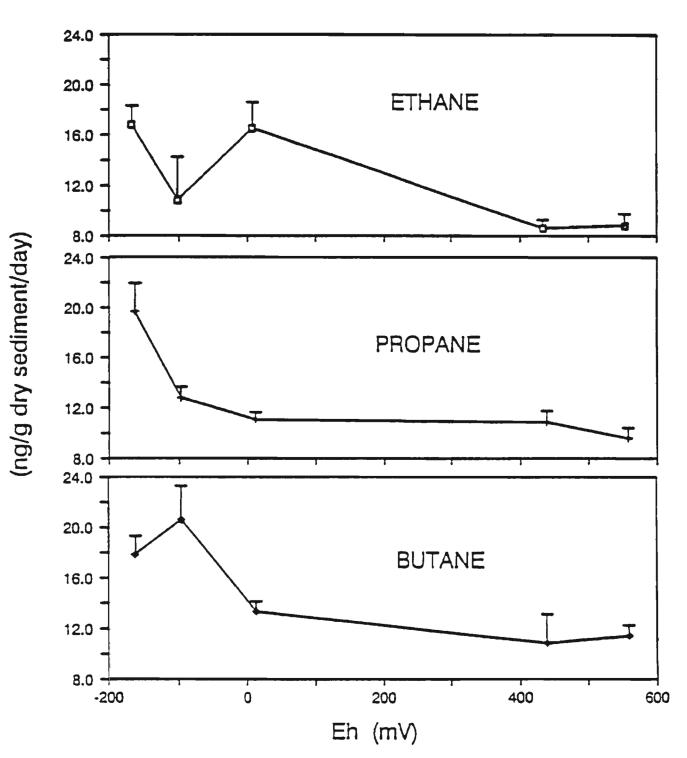


Figure 1.









Hydrocarbon Production

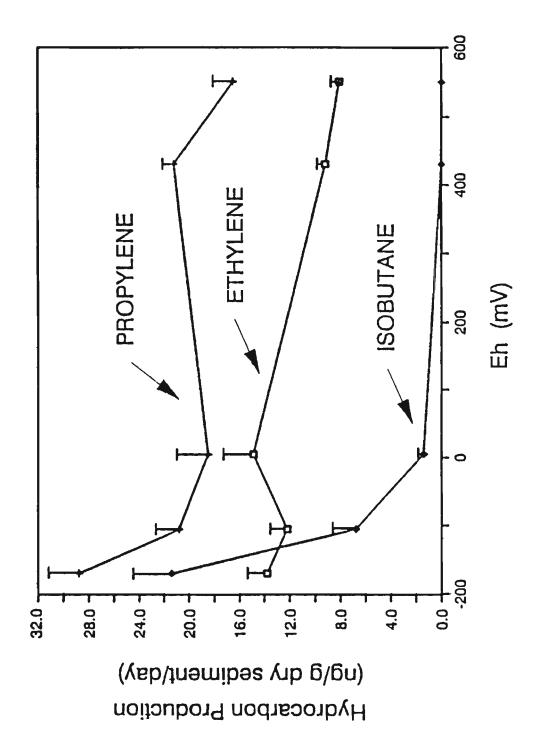


Figure 5.

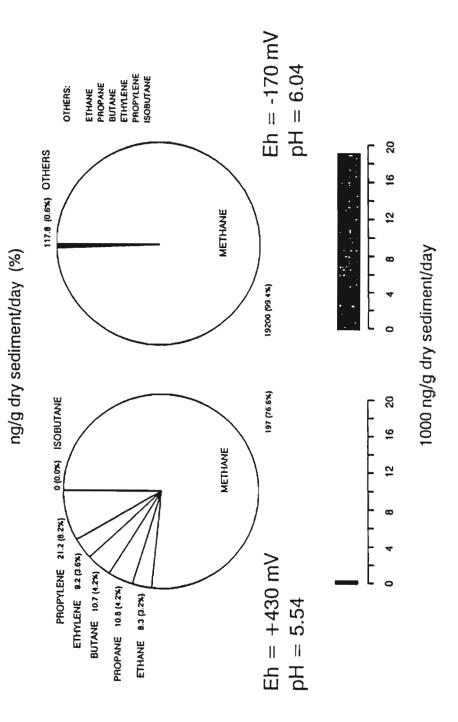
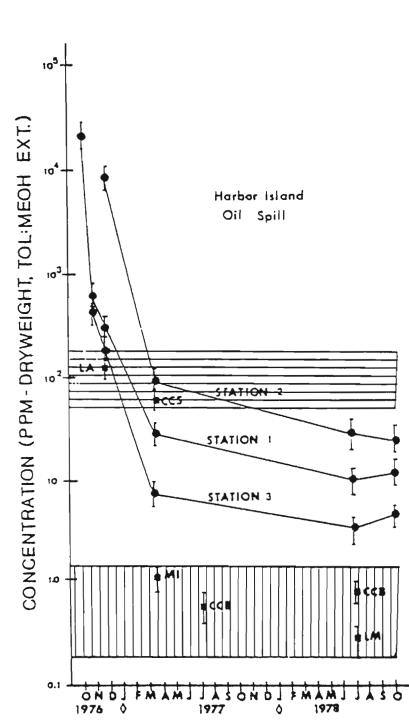


Figure 6.



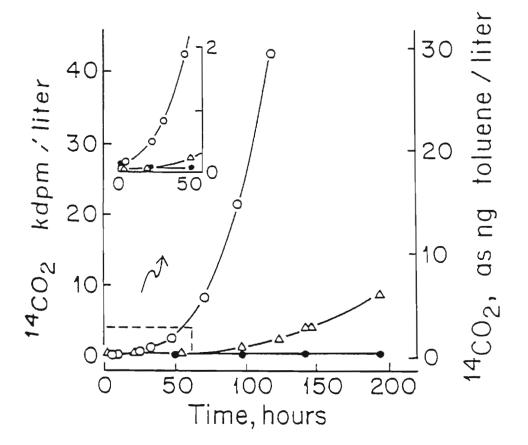
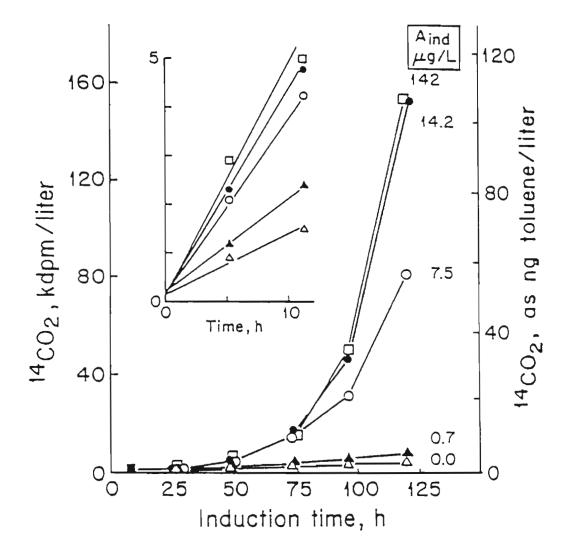


Figure 8.







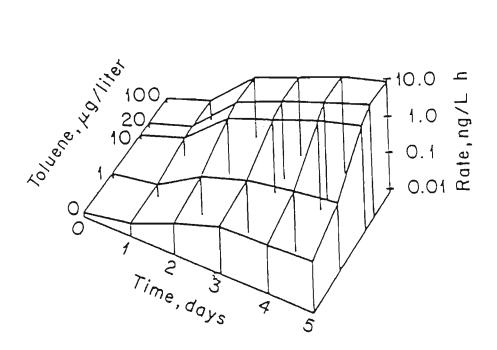
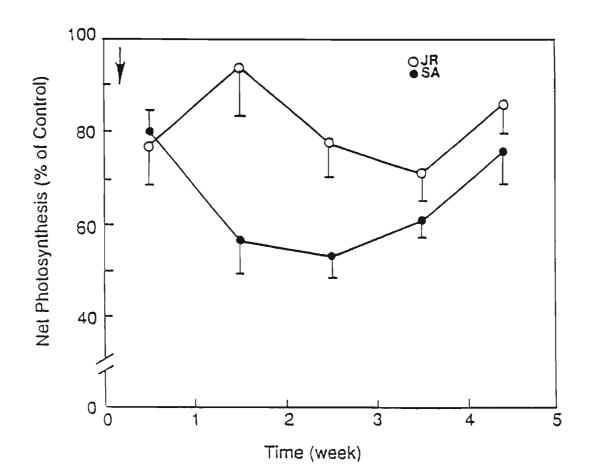
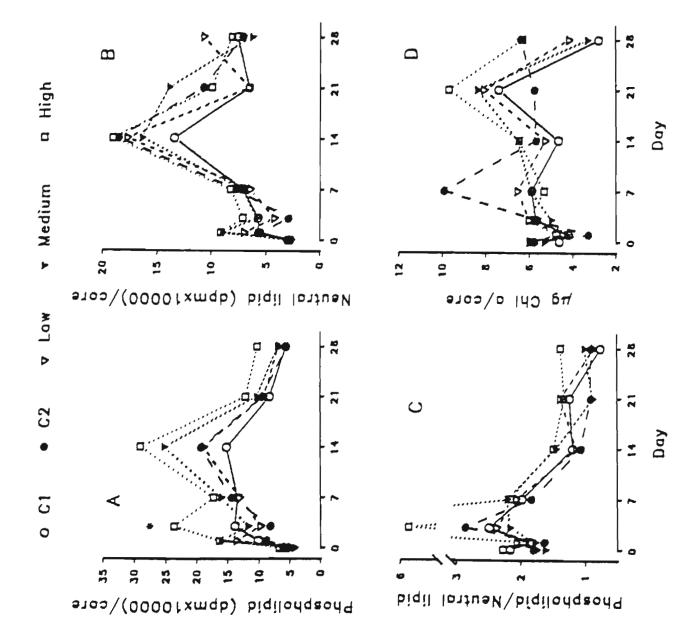


Figure 11.







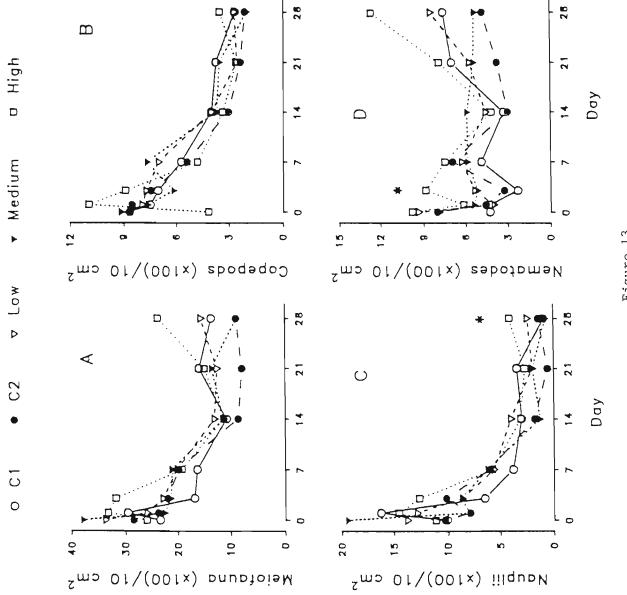


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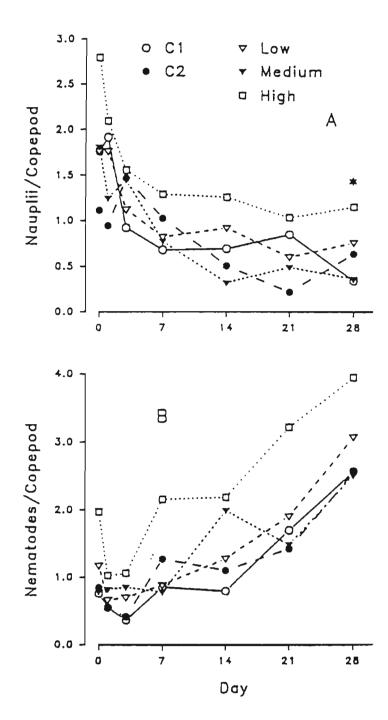


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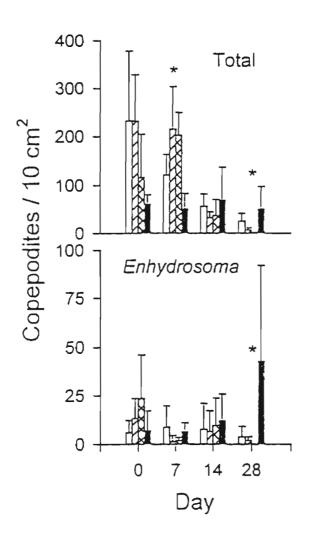
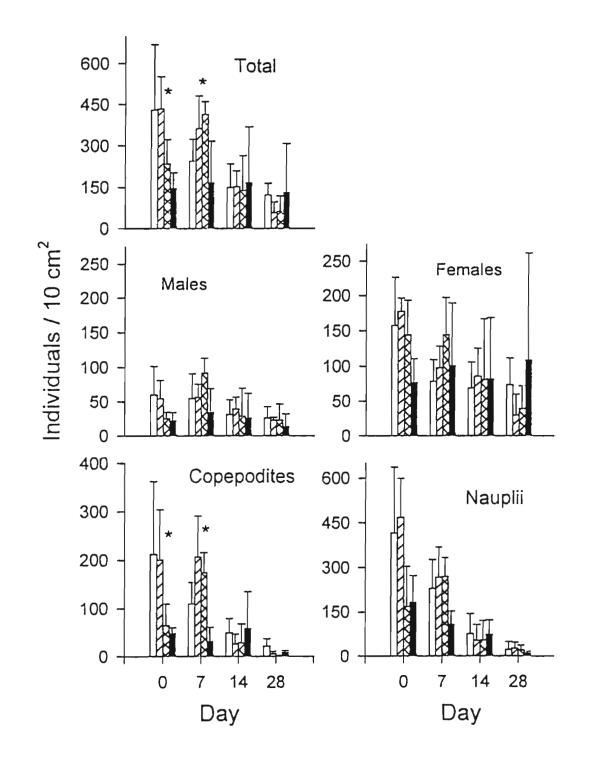


Figure 15.

Figure 16.





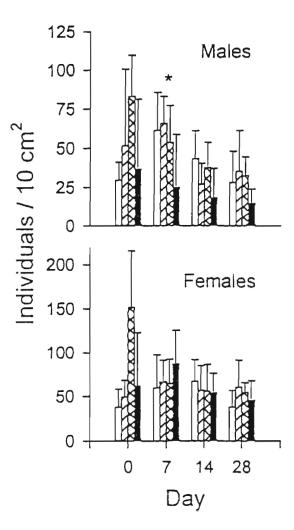
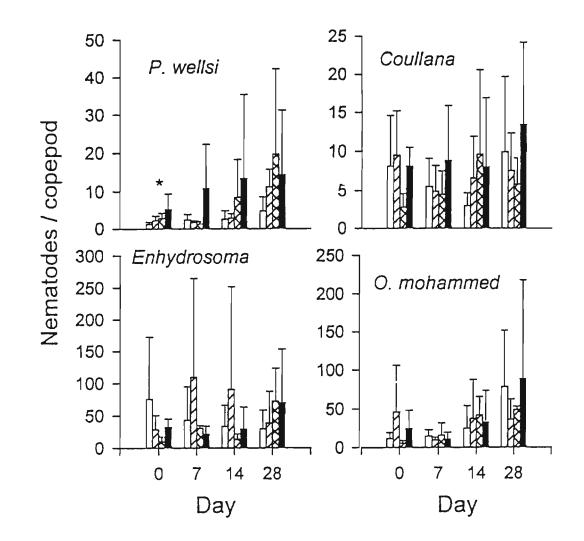
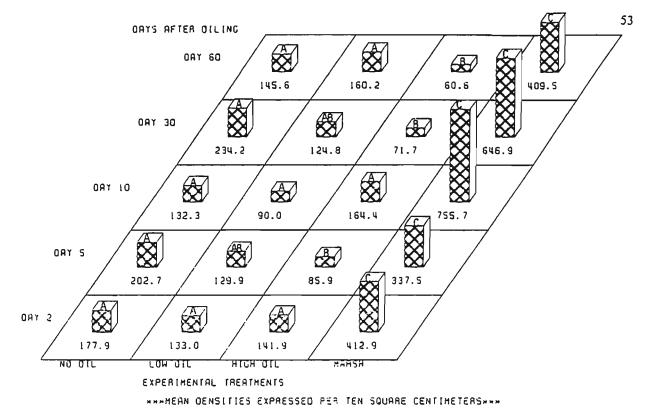
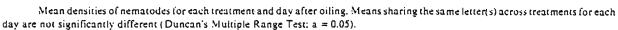


Figure 18.







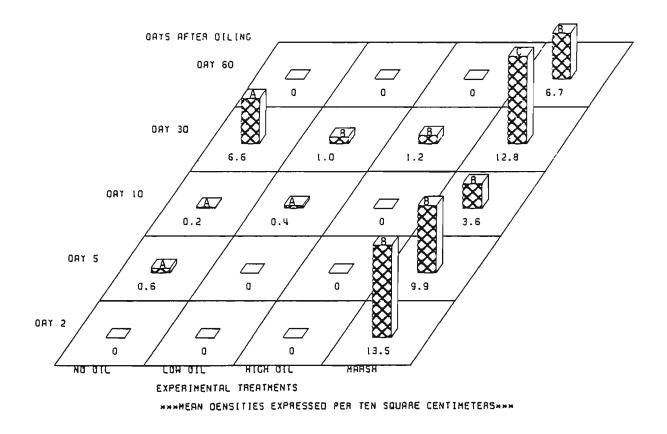
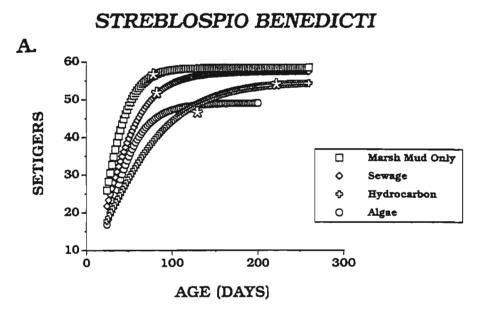
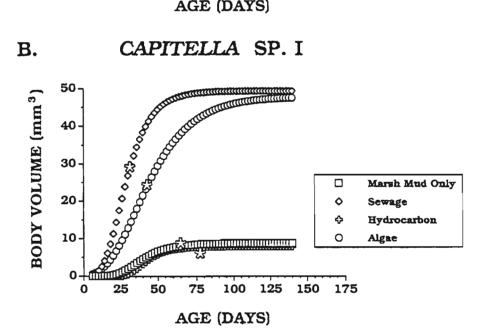


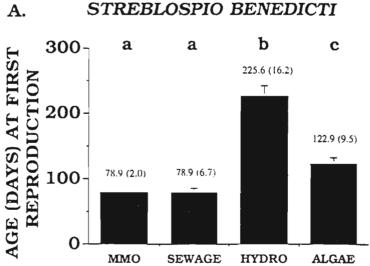
Figure 19.

Figure 20.









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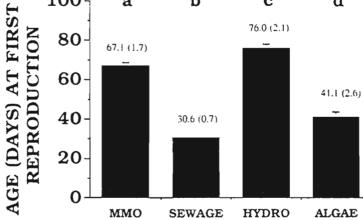
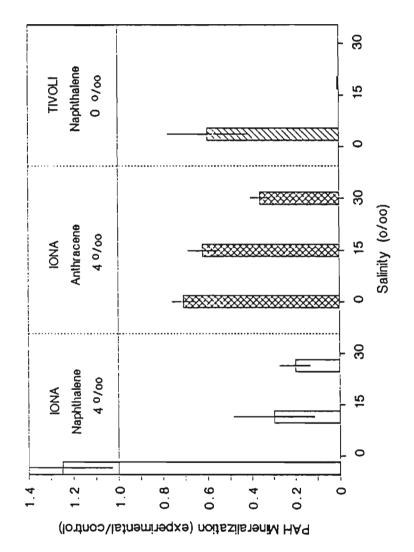


Figure 21.



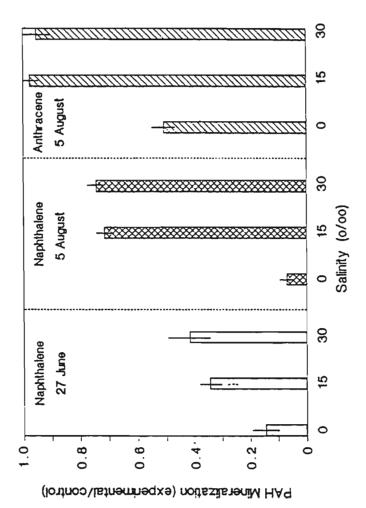
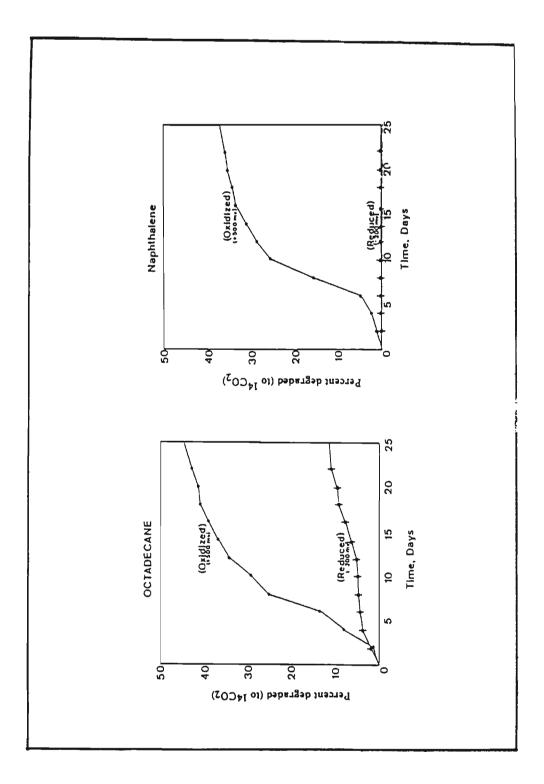


Figure 23.





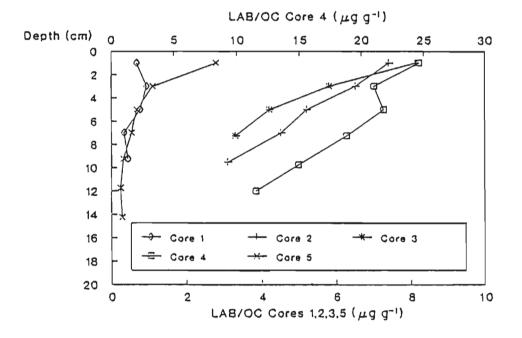
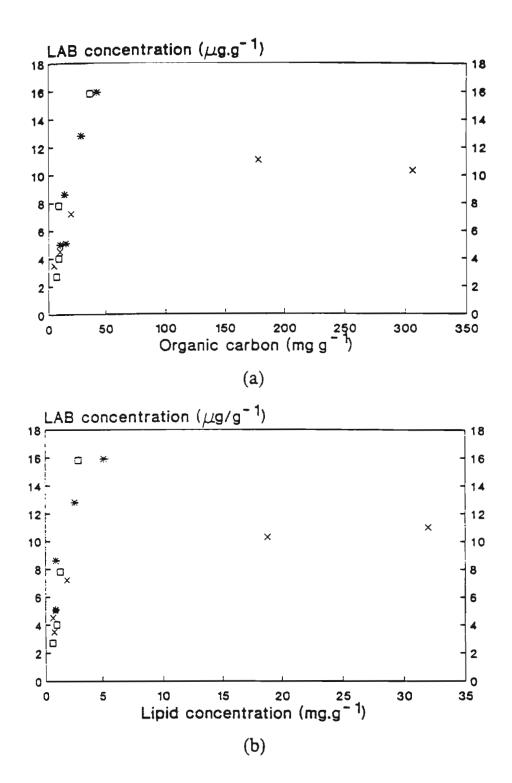
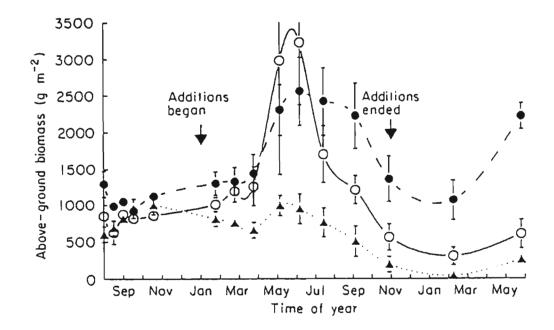


Figure 26.







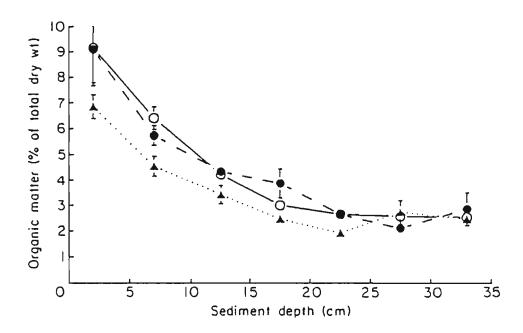


Figure 28.

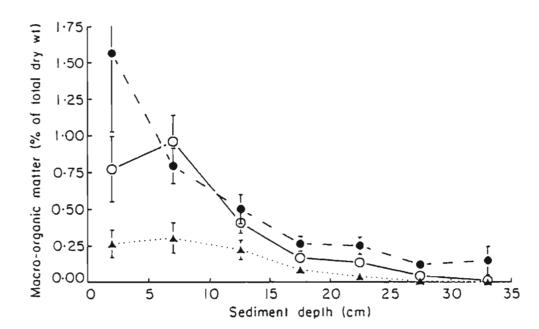
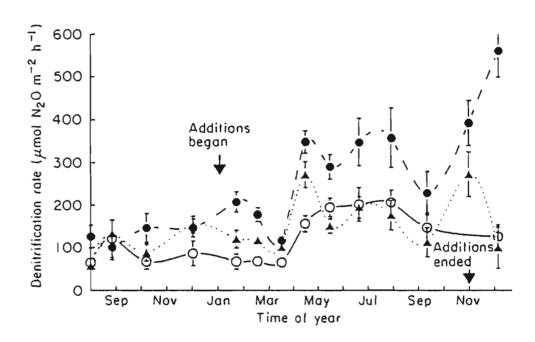
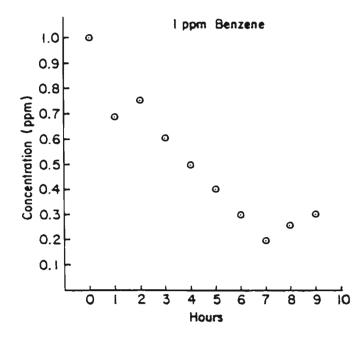


Figure 29.

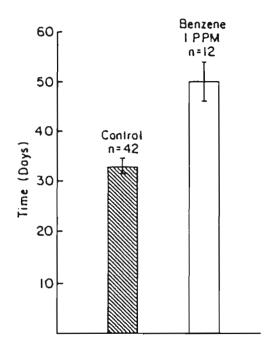












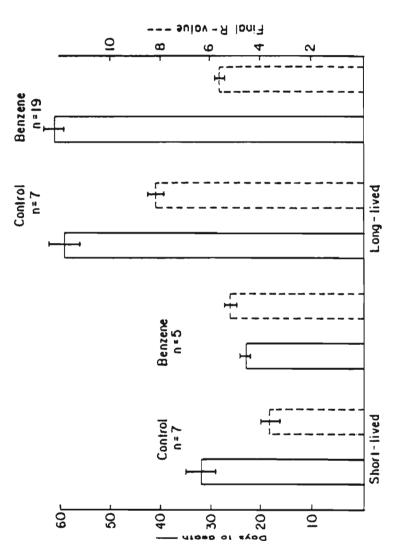
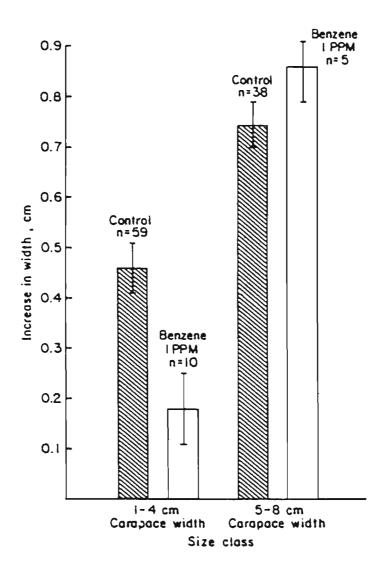
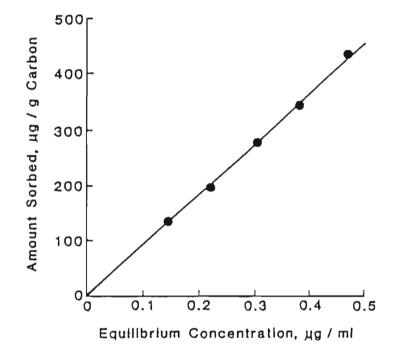


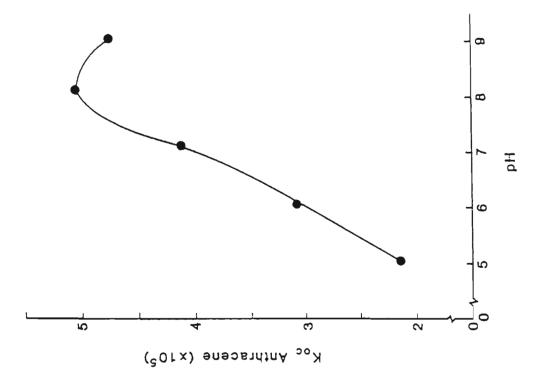
Figure 33.

Figure 34.









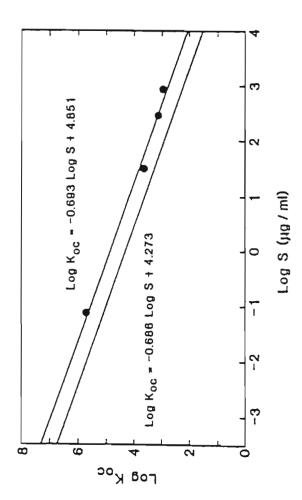


Figure 37.

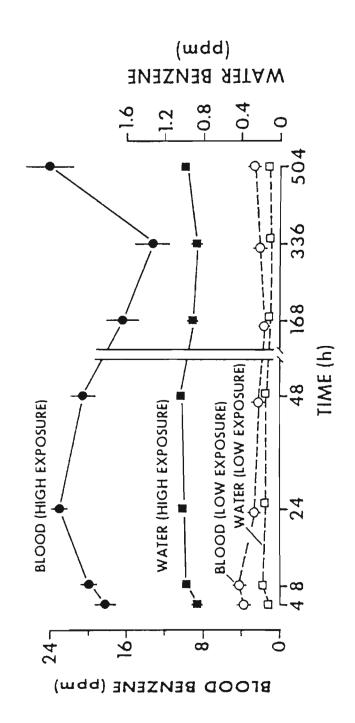


Figure 38.

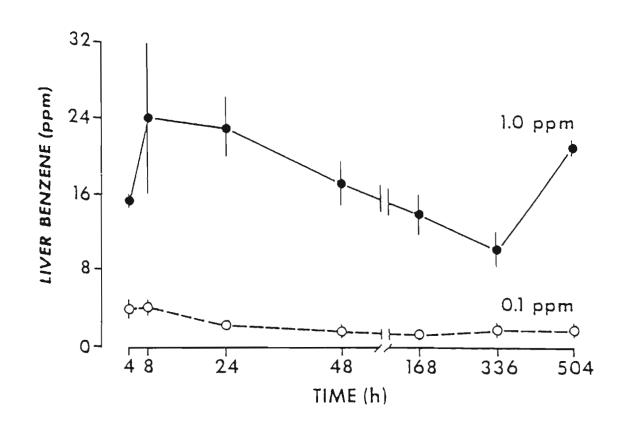


Figure 39.



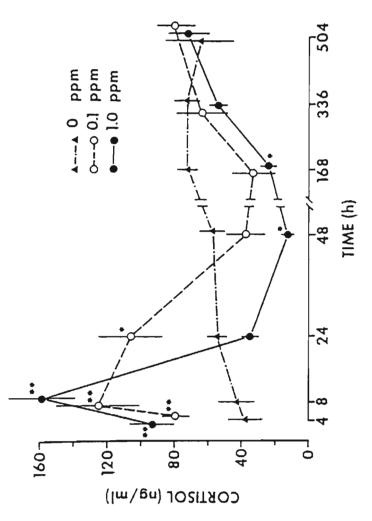


Table l

Concentrations of dissolved n-alkanes (µg 1⁻¹) in water marshes during winter.

Carbon number	1	2	3	Station 4	5	6	7
C13	0.04	0.04	0.04	0.06	0.02	0.03	0.03
C14	0.05	0.06	0.08	0.09	0.03	().()7	0.06
C15	0.08	0.07	0.09	0.20	0.02	0.08	0.08
C16	0.06	0.08	0.10	0.19	().()4	0.06	().07
C17	0.30	0.29	0.32	0.43	0.29	0.41	0.38
C18	0.10	0.19	0.25	0.31	0.13	0.38	0.21
C19	0.24	0.27	0.32	0.38	().27	0.39	0.30
C20	0.18	0.19	0.20	0.23	0.08	0.28	0.11
C21	0.26	0.25	0.28	0.38	0.20	0.28	0.23
C22	0.17	0.15	0.19	0.26	0.19	0.21	0.19
C23	0.19	0.25	0.23	0.26	0.10	0.23	0.29
C24	0.15	0.10	0.17	().30	().()9	0.26	0.18
C25	0.25	0.29	0.14	0.34	0.24	0.36	0.26
C26	0.13	0.23	0.13	0.28	0.09	0.24	0.20
C27	0.18	0.29	0.38	0.42	0.28	0.34	0.36
C28	0.09	0.18	0.24	0.28	0.10	0.29	0.16
C29	0.18	0.25	0.26	0.48	0.16	0.30	0.29
C30	0.09	0.09	0.15	0.29	0.09	0.28	0.23
C31	0.07	0.13	0.19	0.39	0.10	0.29	0.20
C32	0.06	0.07	0.21	0.25	0.08	0.16	0.10
C33	0.05	0.09	0.10	0.20	0.07	0.18	0.16
C34	0.03	0.08	0.19	0.18	0.05	0.20	0.19

Table 2.

				-5.			
Carbon	1	2	3	Station	5	6	7
number	1	<i></i>	د	+	5	0	/
C13	0.05	0.03	0.02	0.03	0.01	0.02	0.01
C14	0.06	0.02	0.03	0.04	0.01	0.03	0.02
C15	0.05	0.02	0.04	0.05	0.02	0.01	0.05
C16	0.03	0.02	0.01	0.04	0.01	0.04	0.04
C17	0.09	0.19	0.11	0.18	0.07	0.18	0.09
C18	0.03	0.05	0.02	0.09	0.03	0.07	0.05
C19	0.08	0.08	0.09	0.10	0.05	0.10	0.07
C20	0.04	0.06	0.06	0.08	0.02	0.08	0.03
C21	0.05	0.05	0.07	0.19	0.06	0.14	0.06
C22	0.02	0.06	0.05	0.09	0.03	0.09	0.05
C23	0.02	0.07	0.09	0.09	0.04	0.08	0.04
C24	0.06	0.03	0.03	0.08	0.01	0.08	0.03
C25	0.09	0.04	0.10	0.16	0.02	0.09	0.01
C26	0.01	0.03	0.07	0.05	0.03	0.07	0.04
C27	0.04	0.09	0.13	0.13	0.06	0.09	0.09
C28	0.04	0.05	0.08	0.07	0.04	0.08	0.06
C29	0.04	0.08	0.09	0.09	0.02	0.13	0.08
C30	0.03	0.05	0.06	0.05	0.01	0.06	0.04
C31	0.02	0.04	0.04	0.06	0.03	0.08	0.03
C32	0.02	0.06	0.02	0.03	0.02	0.06	0.04
C33	0.01	0.03	0.01	0.04	0.01	0.03	0.02
C34	0.01	0.01	0.02	0.02	0.02	0.04	0.01

Concentrations of dissolved n-alkanes ($\mu g l^{-1}$) in water marshes during spring.

Ta	ble	3.

			sumr		j in wate		s dunne
Carbon				Station			
number	τ	2	3	4	5	б	7
C13	_	0.01	0.01	0.02	-	0.01	-
C14	-	0.02	0.03	0.03	-	0.02	-
C15	0.01	0.02	0.04	0.03	-	0.03	0.10
C16	0.01	0.01	0.03	0.02	-	0.03	0.03
C17	0.04	0.10	0.09	0.15	0.02	0.10	0.14
C18	0.01	()_()4	0.09	0.09	0.01	().()9	0.02
C19	0.02	0.05	0.06	0.06	0.02	0.07	0.01
C20	0.02	0.03	0.09	0.08	-	0.04	0.10
C21	0.03	0.05	0.07	0.06	0.02	0.10	0.03
C22	0.03	0.02	0.05	0.07	0.01	0.08	0.01
C23	0.02	0.06	0.03	0.06	-	0.03	0.04
C24	0.01	0.04	0.02	0.05	0.01	().()4	0.01
C25	0.03	0.07	0.09	0.09	0.01	0.03	0.02
C26	0.01	0.02	().07	0.07	0.01	0.02	0.0 l
C27	0.05	0.18	0.06	0.09	0.02	0.13	0.03
C28	0.02	0.02	0.04	0.05	0.01	0.03	0.02
C29	0.01	0.08	0.02	0.13	-	0.05	0.04
C30	0.01	0.03	0.03	0.10	-	0.05	0.01
C31	-	0.01	0.02	0.09	-	0.03	-
C32	-	0.02	0.01	0.08	-	0.02	-

Concentrations of dissolved n-alkanes (µg l⁻¹) in water marshes during

- = Not detected.

	Tc	Total n-alkanes (µg l ⁻¹)	(hg l-1)
Station	Winter*	Springi	Summer
number	January	March	July
	2.95	0.89	0.33
2	3.64	1.06	0.88
S	4.26	1.24	0.92
1	6.20	1.76	1.42
5	2.72	0.62	0.14
6	5.32	1.65	1.00
7	4.28	0.96	0.62
* Water temp.*	= 12°C. † Water to	emp. = 20°C.	* Water temp. = 12° C. † Water temp. = 20° C. ‡ Water temp. = 24° C.

Seasonal variations of the total dissolved n-alkanes in water marshes.

Table 4.

Benzo(α)pyrene	1.3	0.9	1.4	6.6
Naphthalene	6.0	9.3	7.8	4.9
Benzene	4.5	7.6	5.9	3.4
	XC		xou	

S
Table

Vernal Equinox

Midsummer

Autumnal Equind

Midwinter

Table	6
-------	---

Inducing toluene conc.	To	oluene	exposi	ıre dur	ation (h)
(µg l ⁻¹)	5	24	48	72	96	120
0.0	-	0.02	0.08	0.15	0.20	0.25
0.7	_	0.03	0.14	0.23	0.36	0.56
7.5	-	0.04	0.26	0.91	2.05	6.20
14.2		0.03	0.28	1.12	2.99	10.37
142.0	-	0.04	0.30	1.04	3.29	9.50
0.9ª	0.04	-	-	-	-	-

* Inducing substrate was ¹⁴C-toluene

T	at	1	e	7	•

		ge of total me in	Hydrocarbons add	led at the $1 \times rate$
Component	crude oil†	this study‡	(µl core ⁻¹ day ⁻¹)	(g C m - 2 day - 1)
Hexane	1.8	15.8	16	0.40
Heptane	2.3	20.2	21	1.10
Octane	1.9	16.7	17	0-46
Nonane	1.8	15-8	16	0.45
Decane	l · 8	I 5·8	16	0.45
Benzene	0.2	1.7	2	0.06
Toluene	0-5	4-4	5	0.14
Ethylbenzene	0.5	l·7	2	0.06
Xylene	0.9	7.9	8	0.21
Total	11.4	100	103	3-33

Components of the hydrocarbon mixture added daily* to each core

* For the basic 1× treatment, 3.6 ml of a stock solution containing a pure hydrocarbon mixture in the proportions given above was added to a flask and diluted to a final volume of 1.75 litre with equal amounts of sea water and distilled water. From this, 50 ml (or 103 μ l of hydrocarbons) was added daily to each of the five microcosms. The procedure for the 10× treatment was the same except that 36 ml of the stock hydrocarbon mixture was used to make 1.75 litre of solution.

† From Nelson-Smith 1972.

‡ Hydrocarbon components used in this study were maintained at the same ratio relative to one another as they are in crude oil.

EFFECT OF H	IYDROCAF	EFFECT OF HYDROCARBONS ON SULPHATE REDUCTION IN MARSH SEDIMENTS ^a	REDUCTION	I IN MARSH	SEDIMENTS ^a	
Addition		Ile Grande Unoiled			Ile Grande Oiled	
	Rate ^b	Rate ^b Per cent of control	d	Rate ^b	Per cent of control	d
Control	2.27	100		10.1	100	I
10% fresh oil	1-67	73	0.95	0.85	84	0.08
10% 8 h weathered oil	1.57	11	0.16	0.87	86	0.11
10% 48 h weathered oil	1.82	82	0.97	1-07	106	0.48
005 % toluene	1.42	64	0·10	1.1.1	110	0·23
005 % benzene	1.32	59	0·09	I · I 3	112	0.15

Table 8.

Addition	¹⁴ CO ₂ ^b	Ile Grande Unoiled ¹⁴ CO ₂ ^b Per cent of control	d	¹⁴ <i>CO</i> ² ^b	Ile Grande Oiled Per cent of control	d
Control	358	100		244	100	
resh oil		5	0.00	17	7	< 0.001
8h weathered oil	44	12	0.01	99	27	< 0.001
48 h weathered oil		24	0.01	119	49	< 0.001
Toluene	29	8	0.002	86	35	< 0.001
Benzene	31	6	0.002	65	27	< 0.001

Table 9.