

GC
9.1
B93
1975

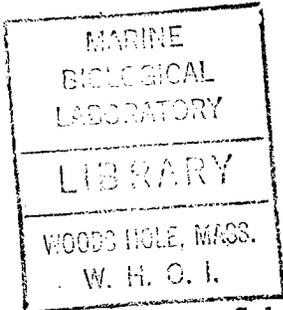
DISTRIBUTION OF HYDROCARBONS IN A SALT MARSH ECOSYSTEM
AFTER AN OIL SPILL AND PHYSIOLOGICAL CHANGES IN MARSH
ANIMALS FROM THE POLLUTED ENVIRONMENT

by

Kathryn A. Burns

B.S., Michigan State University

(1970)



Submitted in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy at the

Massachusetts Institute of Technology

and the

Woods Hole Oceanographic Institution

June, 1975

Author:
Massachusetts Institute of Technology/Woods Hole
Oceanographic Institution Joint Program in Biological
Oceanography, June, 1975.

Certified by:
Thesis Supervisor

Accepted by:
Chairman, Joint Program in Biological Oceanography,
Massachusetts Institute of Technology/Woods Hole
Oceanography Institution.

Distribution of Hydrocarbons in a Salt Marsh Ecosystem after an Oil
Spill and Physiological Changes in Marsh Animals from the Polluted
Environment

by

Kathryn A. Burns

Submitted to the Massachusetts Institute of Technology/ Woods
Hole Oceanographic Institution Joint Program in Biological
Oceanography of the degree of Doctor of Philosophy, June, 1975.

ABSTRACT

The studies described in this thesis were designed to answer several problems relating to the recovery of a salt marsh heavily polluted by an accidental spill of Number 2 fuel oil. Field and laboratory studies were conducted for 5 years comparing the oiled Wild Harbor Marsh with the unoiled Sippewissett Marsh, both on Buzzards Bay in Massachusetts. The data contributes information 1) on the incorporation of oil into the sediments and organisms at the oiled marsh, 2) on the residence times of certain components of the oil in the marsh ecosystem, 3) on changes in chemical composition of the oil with time due to physical and chemical weathering processes and biochemical degradation of hydrocarbons, 4) on the effects of oiled sediments on the population distribution, behavior, and survival of the intertidal fiddler crab, Uca pugnax, 5) on the relatively small ability of Uca to metabolize hydrocarbons, 6) on the presence of an inducible in vitro microsomal mixed function oxidase (MFO) enzyme system in the marsh minnow, Fundulus heteroclitus, 7) on the presence of high MFO rates in field populations of Fundulus exposed to hydrocarbon pollution, and 8) for the synthesis into a discussion of some of the physiological reasons for the relative sensitivity of marsh animals to oil pollution and their relative ability to adapt to an oil polluted environment.

Thesis Supervisor:

John M. Teal

Senior Scientist

Woods Hole Oceanographic Institution

TABLE OF CONTENTS

	Page
Abstract	2
Acknowledgements	4
Introduction	5
Chapter 1 "Hydrocarbons in the Salt Marsh Ecosystem up to Five years after an Oil Spill at West Falmouth, Massachusetts" (co-author: John M. Teal)	12
Chapter 2 "Long Term Effects of Hydrocarbon Contamination of a Salt Marsh on Populations of the Fiddler Crab, <u>Uca pugnax</u> : a Summary"	38
Chapter 3 "Hydrocarbon Metabolism in the Intertidal Fiddler Crab, <u>Uca pugnax</u> "	43
Chapter 4 "Microsomal Mixed Function Oxidases in an Estuarine Fish, <u>Fundulus heteroclitus</u> , and their Induction as a Result of Environmental Contamination".	61
Chapter 5 "Physiological Adaptations of Marsh Animals to a Hydrocarbon Polluted Environment: a Discussion"	80
References	92
Appendix: Bibliography of other papers published during doctoral program	101

ACKNOWLEDGEMENTS

Special thanks are due to my thesis advisors John M. Teal and George R. Harvey for advice and encouragement throughout my doctoral program. Thanks are also extended to all others on the staff of the Woods Hole Oceanographic Institution and the Massachusetts Institute of Technology who aided me with particular problems.

Support for my doctoral work was provided by The Bureau of Commercial Fisheries Fish and Wildlife grant no. 14-17-0007-1128 (G), The Jesse-Smith Noyes Foundation, National Science Foundation Doctoral Dissertation Improvement grant no. GA 40987, and the Woods Hole Oceanographic Institution Education Program.

INTRODUCTION

The discharge of oil into the marine environment was estimated in 2 1973 by the National Academy of Sciences to be about 6 million metric tons per year (N.A.S., 1975). About one third of this input was attributed to losses during transportation operations. [With increasing world wide demand for petroleum products, large increases in marine transportation and the resulting marine oil pollution are predicted 1 (Mostert, 1974). Coastal oil spills have received the most attention] but oil residues are common even on the open sea (Horn, et al., 1970; Morris, 1971).

[The ultimate fate of this petroleum spilled in the marine environment is 1) evaporation and decomposition in the atmosphere, 2) dispersal in the water column, 3) incorporation into sediments, and 4) oxidation 3. by chemical or biological means. The relative rates of these dissipation processes are unknown but depend on weather conditions, environmental parameters, and the nature of the pollutants.]

[The effects of accidental oil spills on the marine biota can be divided into 1) initial impact and 2) population and community recovery. Several extensive reviews of the literature on effects of oil on individual organisms have been reported (e.g., N.A.S., 1975; Moore, et al., 4 1974; Nelson-Smith, 1973). Moore, et al. categorized the effects of oil on individual organisms as: 1) lethal toxic effects due primarily to soluble aromatic hydrocarbons; 2) sub-lethal toxic effects from soluble aromatics; 3) coating of birds, mammals and inter and subtidal

sessile species with oil; 4) alterations of substrates by oil, which makes habitats uninhabitable for normally found species; and 5) incorporation of hydrocarbons into organism tissues causing tainting or accumulation of potential carcinogens. Oils and petroleum products have been shown to effect reproductive, growth, metabolic, behavioral, and histological changes in many marine organisms (N.A.S., 1975).

[Hydrocarbons are readily accumulated in the tissues of marine 5 animals either by assimilation of contaminated food or by direct absorption from water through respiratory and other body surfaces (Reinert, 1969; Zitko and Carson, 1970; Hamelink, et al., 1971; Lee, et al., 1972a, b; Burns and Teal, 1973). Because of the high lipid solubility of hydrocarbons and low water solubility, marine organisms would be expected to retain substantial quantities of hydrocarbons in their lipid stores unless they have some physiological mechanism for actively clearing their body tissues.] Experiments with oysters showed initial rate of uptake of petroleum hydrocarbons depends on the concentration in the water medium. Amounts accumulated in 49 days of exposure to oil in solution were related to the amounts of body lipids in the animals. When placed in clean seawater oysters discharged 90% of incorporated hydrocarbons in 2 weeks. The other 10% was retained in body tissues with no apparent decrease in body burden for at least 30 days (Stegeman and Teal, 1973). Similar uptake, discharge and retention of some fractions was observed in mussels and fish using pure compounds (Lee, et al., 1972a,b).

Many questions arise about 1) the residence time of petroleum hydrocarbons in the marine environment and in tissues of marine organisms, 2) processes of dissipation and degradation, 3) the physiological effects 6.

of oil incorporated into tissues and the marine environment on individual organisms, populations and communities (Nelson-Smith, 1973).

[In September, 1969, the oil barge "Florida" ran aground off Little Island in Buzzards Bay near West Falmouth, Massachusetts. An estimated 700,000 liters of Number 2 fuel oil leaked into adjacent near-shore and marsh areas killing most marine life in the heavily oiled areas. Sanders, et al. (1972) surveyed the benthic near-shore populations eight days later and reported 95% of the bottom animals dead or dying. Similar toxic effects were seen on beaches and marshes where windrows of dead organisms accumulated immediately after the spill (Sanders, 1973). Krebs (1973) noted behavioral disruptions of fiddler crabs surviving the initial spill. Oil was absorbed into Bay and marsh sediments having long term effects on the survival of marine organisms (Blumer and Sass, 1972; Sanders, et al., 1972; Michael, et al., 1975; Krebs, et al., 1975). Heavily oiled areas of marsh showed a great reduction in higher plants, macrofauna, and algal photosynthesis by 1970 (Teal, personal communication)]

I undertook the studies described in this thesis to answer several problems related to the recovery of the salt marsh from this pollution. Studies were conducted at the Wild Harbor Marsh (referred to in this manuscript as West Falmouth). Control samples were collected at the Great Sippewissett Marsh which opens onto Buzzards Bay 6-1/2 Km south of Wild Harbor (see Map). Sippewissett was unaffected by the oil spill and was free of Number 2 fuel oil contamination as shown by analyses of sediments and organisms. My data contributes information on 1) the incorporation of oil into sediments and organisms, 2) residence times of oil in the marsh system, 3) changes in chemical composition of the oil with time in certain components of the ecosystem, 4) the effects of

oiled sediments on the distribution of fiddler crabs, 5) the ability of marsh animals to metabolize hydrocarbons, and 6) physiological reasons for the relative sensitivity of marsh animals to oil pollution and their relative ability to adapt to an oil polluted environment.

Chapter 1 describes the chemical aspects of monitoring the incorporation of oil into the marsh ecosystem. Surface sediments and deep mud cores were analyzed showing the absorption of oil into the marsh muds. Oil content of surface muds correlated with inability of plants and animals to survive in heavily polluted areas. Composition of oil in surface sediments changed with time demonstrating weathering processes. Residence times of various classes of hydrocarbons in a Northern salt marsh were estimated as <4 years for paraffins and >5 years for high boiling aromatics and naphthenes. Thus organisms, especially those burrowing into oiled sediments, had continuous exposure to oil all five years studied. Oil was absorbed into all plants and animals analyzed a year after the spill. Analyses in subsequent years showed the continued uptake of petroleum into tissues of animals recolonizing the area.

Studies were begun to examine the effects of this chronic exposure on the population structure and physiology of two marsh animals showing different abilities to tolerate the pollution. Crustaceans were very sensitive to oil as shown by observations by Sanders, et al. (1972) on amphiscid amphipods and by Krebs (1973) on fiddler crabs. Uca pugnax, the mud fiddler, was chosen for study because of its sensitivity to the pollution and its abundance in the marsh fauna. Fish appeared less sensitive to the pollution and the marsh minnow, Fundulus heteroclitus, was chosen for comparison. The major aims of the studies described in

Chapters 2 to 5 were 1) to identify some physiological reasons for the difference in tolerance to oil pollution in these two species and 2) to determine if these animals could adapt to oil in their environment by changing their behavior, physiology, or genetics to tolerate higher levels of oil than a non-exposed population.

Chapter 2 is a summary of work done in cooperation with Charles T. Krebs of the Boston University Marine Program on the "Long term effects of hydrocarbon contamination of a salt marsh on populations of the fiddler crab, Uca pugnax". We correlated distributions of Uca with oil content of the marsh surface muds. We noted reductions in field populations, a shift in age and sex structure toward adult males (that immigrated from surrounding unoiled areas), high juvenile mortality, and locomotor impairment in adults in oiled areas.

Chapter 3 describes my work on hydrocarbon metabolism in Uca. Aldrin epoxidation was used as in the in vitro assay to establish the presence of the microsomal mixed function oxidase (MFO) system in Uca. Rates were slow, comparable to insecticide-sensitive insects and freshwater crayfish. No difference in rates of metabolism could be detected in crabs from clean or polluted marshes. In vivo naphthalene oxidation rates were measured and used to calculate a clearance time based on the aromatic hydrocarbon content of Uca collected at the oil polluted marsh.

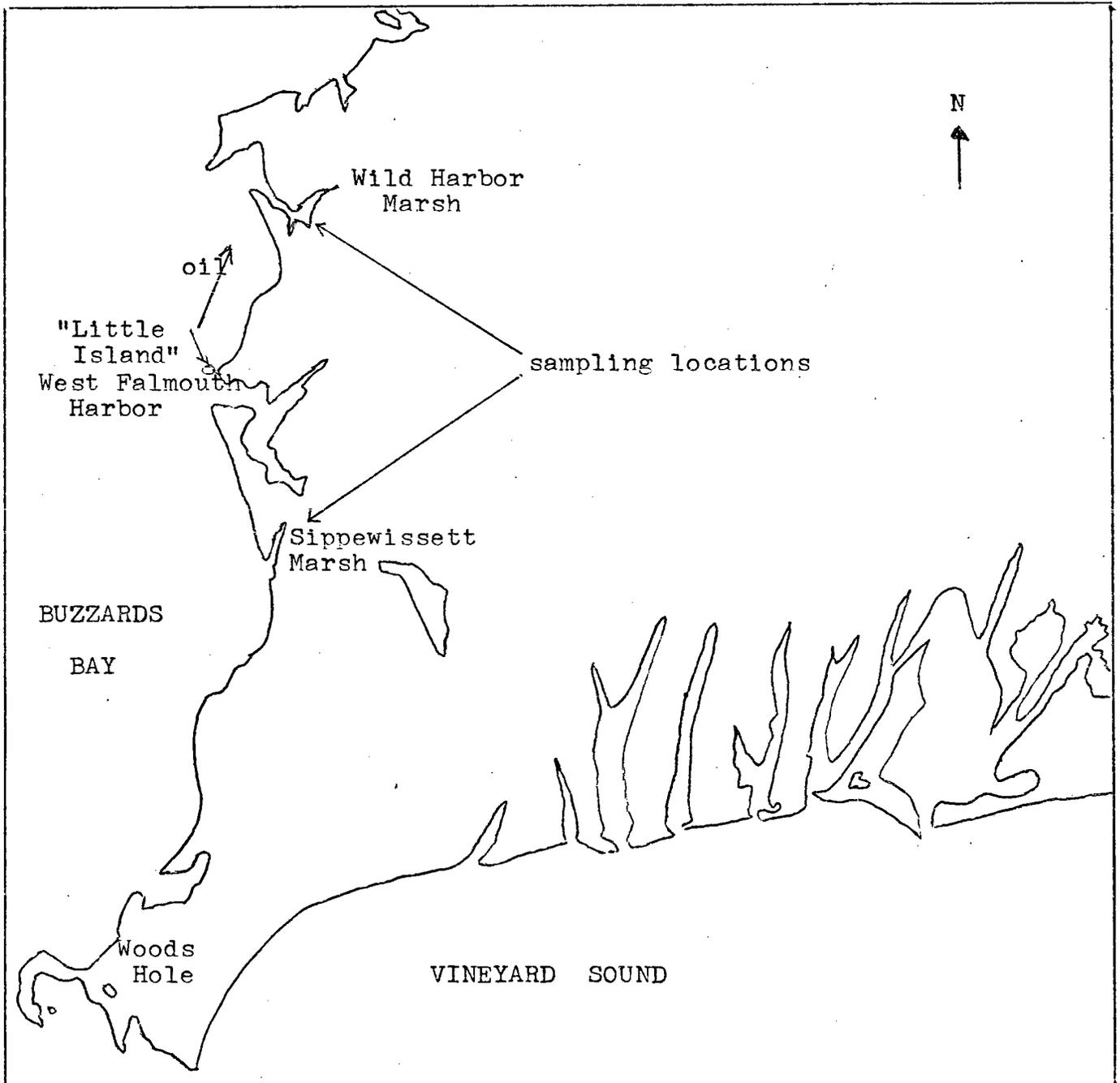
Chapter 4 describes the "Microsomal mixed function oxidases in an estuarine fish, Fundulus heteroclitus, and their induction as a result of environmental contamination". Aldrin epoxidation rates and cytochrome P-450 levels were used to describe the MFO system in Fundulus. I characterized the subcellular distribution of oxidase activity and some enzyme parameters. A laboratory experiment with phenylbutazone

showed the system was inducible. Measurements of several populations showed higher enzyme levels in fish living in hydrocarbon contaminated areas compared to clean marshes.

Chapter 5 is a synthesis of available data into a discussion on the "Physiological adaptations of marsh animals to a hydrocarbon polluted environment".

Chapters are written as discrete papers, each with introduction and discussion. Chapter 4 is in press in Comparative Biochemistry and Physiology.

SOUTHWESTERN CAPE COD, MASSACHUSETTS



The oil barge "Florida" ran aground off West Falmouth, Massachusetts in September, 1969. About 700,000 liters of Number 2 fuel oil leaked into Buzzards Bay and Wild Harbor Marsh.

CHAPTER I

"HYDROCARBONS IN THE SALT MARSH ECOSYSTEM UP TO FIVE
YEARS AFTER AN OIL SPILL AT WEST FALMOUTH,
MASSACHUSETTS"

ABSTRACT

As part of a study of the effects of Number 2 fuel oil on a salt marsh heavily oiled in the 1969 West Falmouth oil spill, marsh surface sediments, deep mud cores, plants and animals were analyzed for hydrocarbon content. Amounts of oil in surface sediments agreed well with observations on plant growth and animal recolonization. Oil penetrated deep into the anoxic marsh muds where degradation is extremely slow. N-alkanes were degraded in surface muds in 3 1/2 years; branched chain alkanes within 4 years after the spill. We predict about 30% of the oil absorbed into marsh sediments will have a residence time of over 5 years. Surface muds showed toxicity to plants and animals for at least 5 years.

All plants and animals analyzed incorporated oil into their tissues. No biomagnification was apparent in the aquatic food chain, but analyses of seagull tissues showed high levels of hydrocarbons and a tendency toward selective uptake of hydrocarbons into brain tissue. Analyses of animals collected in 1973 and 1974 suggested species differences in amount and types of hydrocarbons retained in body tissues.

Introduction

The oil barge "Florida" ran aground in Buzzards Bay September, 1969, spilling an estimated 700,000 liters of Number 2 fuel oil. The immediate kill of marine and marsh life was studied by Blumer et al (1970a) who reported 95% of the benthic animals in heavily oiled areas dead or dying eight days after the spill. Windrows of dead organisms accumulated on beaches and marshes (Sanders, 1973). Long term effects of the oil polluted environment on the recolonization of killed areas have been studied by Sanders et al (1972), Michael et al (1975), and Krebs et al (1975). Heavily oiled areas were not yet fully recolonized up to five years after the original spill.

Blumer et al (1970b) reported absorption of petroleum hydrocarbons in Buzzards Bay sediments and shellfish surviving the spill. Blumer's subsequent analyses showed little reduction in the amount or types of contaminating hydrocarbons several months after the spill. Biochemical degradation had the effect of slowly decreasing the concentration of straight chain and branched chain hydrocarbons relative to the concentration of cyclic and aromatic compounds. [The water removed some of the lower boiling aromatics by dissolution. Periodic sampling indicated surface sediments and organisms suffered continued contamination by fresh undegraded oil released from subsurface sediments. The oil gradually spread along the sea bottom affecting larger areas than had the original spill (Blumer et al, 1970a)]

We measured the incorporation of oil hydrocarbons into the salt marsh ecosystem. Marsh surface sediments, deep mud cores, animals, and plants at West Falmouth (Wild Harbor Marsh) were analyzed one year after

the spill and up to five years later. Preliminary findings were reported in Burns and Teal (1971). Control sediments and organisms were collected at Sippewissett Marsh which opens onto Buzzards Bay 6 1/2 km south of the Wild Harbor Marsh and which was unaffected by the spill. This report contributes information on the residence time of oil pollution in a New England salt marsh, patterns of degradation and dissipation in the environment, and fate of hydrocarbons in animal and plant tissues.

Methods

Samples

Marsh organisms were collected in summer 1970, washed with tap water, packaged in glass jars with aluminum foil lined caps, and frozen at -30° C until analyzed. Surface sediments were collected with a piston core made of 4.5 cm diameter stainless steel. Cores were extruded onto solvent-rinsed aluminum foil, cut to 5 cm in depth, packaged in glass jars and frozen. Deep mud cores, taken with a 6.5 cm diameter plexiglass piston core, were extruded onto solvent-rinsed foil, wrapped and frozen. Subsequent collections were made as needed. All sampling jars and utensils were rinsed with redistilled acetone and pentane before each use.

Hydrocarbon extraction

Marsh animals were thawed, rinsed with ethanol to remove surface contamination, shells removed, wet tissue weighed, cut into small pieces, and placed in the cellulose thimble of a Soxhlet extractor.

Larger animals such as fish had stomach and gut removed and were ground with sodium sulfate (precombusted at 500° C) in a blender. Plants and surface sediments were cut into small pieces. Deep mud cores were partially defrosted, cut into 5 cm lengths, outside layers (which had come in contact with the corer) removed, and cut into small pieces for extraction. In all cases the Soxhlet apparatus was pre-extracted with methanol. After 24 hours the methanol was changed, samples were placed in the thimbles and extracted for 48 hours. Water samples were extracted three times with pentane in a separatory funnel. Methanol extracts were partitioned into pentane. All samples had lipid weights determined gravimetrically and were saponified. Sediment extracts were percolated through an activated copper column to remove sulphur compounds. Hydrocarbons were separated from other non-saponifiable lipids by column chromatography on a 10 ml silica gel/ 10 ml alumina column (5% deactivated with water). Hydrocarbons were eluted from the column with 80 ml pentane, concentrated on a rotary evaporator and transferred to a glass vial with Teflon lined cap. After evaporating the pentane, hydrocarbons were taken up in 0.5 ml carbon disulfide or hexane, and analyzed quantitatively and qualitatively by flame ionization gas chromatography (GLC). Aliquots of most samples were weighed on a Cahn electrobalance to give a gravimetric estimate of hydrocarbons in the samples. Procedures and percent recovery were detailed in Farrington et al (1973, 1974). This procedure recovered Number 2 fuel oil from spiked samples with 78 to 80% efficiency. The gas chromatograph was a Hewlett Packard 5700 equipped with either a 50 ft SCOT OVI01 stainless steel column

(Perkin Elmer Co.) or a 50 ft PLOT Apiezon L steel column (Blumer, 1973) with helium as the carrier gas. Oven temperatures were programmed 100° to 280° C at 4°/ min. All solvents were reagent grade and were redistilled in all glass stills. All glassware and equipment was soap and water washed, distilled water rinsed, and solvent rinsed before use. Blank runs were performed by refluxing clean thimbles and extracting the methanol as a sample. Blank values were negligible.

Aromatic hydrocarbon determinations

To quantify the aromatic fraction some hydrocarbon extracts were separated by chromatography on a silica gel/ alumina column. Saturated hydrocarbons were eluted with 1 volume pentane. The aromatic fraction was eluted with 1 volume 10% benzene/pentane plus 1 volume 20% benzene/pentane. Amounts in both fractions were quantified by weighing aliquots on a Cahn electrobalance and by gas chromatography.

One aromatic fraction (from fiddler crabs) was analyzed on a Finnigan 1015C gas chromatograph-mass spectrometer. We used the "Standard method of test for hydrocarbon types in middle distillates by mass spectrometry" (ASTM, 1967) to identify mass fragments from general classes of aromatic hydrocarbons in the low voltage spectra. This method is not quantitative but is useful for determining the presence of series of mass fragments associated with different classes of hydrocarbons.

Quantification of petroleum derived hydrocarbons

Gas chromatographic analyses of uncontaminated marsh sediments, animals, and plants were used to identify probable recently biosynthesized (biogenic) hydrocarbons in the marsh ecosystem. Hydrocarbon

peaks were quantified by comparison to peak areas generated by external standards in the I2 to 30 carbon n-paraffin retention index range. Retention indices of peaks were calculated by co-injection of sample and standards. Biogenic hydrocarbons were identified in the gas chromatograms of West Falmouth samples when they were visible over the background of petroleum contamination. These were subtracted from the calculated total hydrocarbons in the C12 to C30 range to give a value of oil hydrocarbons per gram wet weight of sample.

Blumer's data indicated that biochemical degradation had the effect of selectively removing first the straight chain hydrocarbons and then the branched chains in relation to the unresolved cyclic and aromatic compounds in the chromatograms. As indicators of the relative degree of degradation between samples, we measured the ratio of unbranched to isoprenoid hydrocarbons by computing n-C17/pristane or n-C18/phytane ratio (whichever had the best resolution and least interference from biogenic hydrocarbons in the chromatograms) and the ratio of phytane to unresolved background at the same retention index.

Results

Surface sediments

There was an obvious correlation between the amounts of oil in the sediments and plant growth in 1971 (Table I). In area I, a Spartina patens marsh, there was 2.7 mg oil per gram wet mud and no living higher plants. Sample 2 with one third the amount of oil was from an area in which S. patens had been killed but Salicornia seeds germinated and grew. Two originally unoiled areas, one in S. patens marsh (Sample 3)

and one in S. alterniflora marsh (Sample 4) had less than 30 µg hydrocarbons per gram mud but which in 1971, clearly showed traces of fuel oil. Another series of four samples taken within a radius of 1 meter in the heavily oiled area in December, 1972 contained from 2.2 to 1.1 mg oil/gm mud. At one of these areas there was 1.6 mg/gm and Salicornia and S. patens had grown to a limited extent during the previous summer. Water samples taken the same day as the later mud samples contained 3.0 ng oil/gm in water at high tide immediately above the mud and 1.0 ng oil/gm in water from the adjacent creek.

The composition of hydrocarbons in surface muds changed with time (Table 2). From 1971 to 1972 there was a gradual decrease in unbranched/isoprenoid ratio but no change in phytane/background ratio. There was also an increase in percent aromatic hydrocarbons. By May, 1973 (3 1/2 years after the oil spill) most of the straight chain compounds were gone and the branched chains were decreasing. In November, 1973 GLC's of hydrocarbons from the marsh muds showed most of the straight and branched chain peaks had disappeared leaving the unresolved envelope. The % aromatics subsequently decreased (Figure 1).

Deep mud cores

A deep mud core was taken in May, 1971 from the completely dead S. patens area. There was 4.3 mg oil/gm mud at the surface (Table 3). Oil had penetrated to at least 70 cm where the series of peaks from plant waxes equaled those of the fuel oil in intensity. Below this depth the plant waxes dominated the spectra. The concentration of oil in the sediment decreased exponentially with depth. The unbranched/isoprenoid ratio increased with depth. The small amount of fuel oil

present at 115 cm did not follow this pattern but was relatively degraded.

A similar distribution was seen in 1972 although amounts were lower. In the 1973 core no oil was observed below 20 cm.

Plants

The green algae, Enteromorpha clathrata was one of the most highly contaminated marsh organisms analyzed (Table 4). The chromatogram showed relatively undegraded fuel oil with boiling range identical to the spilled oil. The red algae, Polysiphonia fibrillosa, contained much less oil. From cursory observation, the green algae appeared far more abundant on the W. Falmouth Marsh than the red. Spartina alterniflora and Salicornia spp. took up about the same amount of fuel oil per gram tissue. These two higher plants and algae provide the bulk of plant material for detritus feeders.

Animals

The GLC's from all Wild Harbor animals analyzed in 1970 showed hydrocarbons of the same retention range as those from fuel oil. They were completely different than the hydrocarbons in the control samples. The oil in the Wild Harbor animals had lower n-alkane/isoprenoid ratio than that in the surface muds in 1971.

Fundulus showed great differences in the amount and composition of incorporated hydrocarbons between 1970 and 1974. By 1974, hydrocarbon amounts in the West Falmouth fish were indistinguishable from controls. Most of those remaining in the fish were probably biogenics. The small amount of petroleum in the Fundulus tissues in 1974 bore little resemb-

lence to oil hydrocarbons present in surface sediments. Uca showed no significant reduction in the amount of oil in their body tissues over the 4 years studied. Composition was similar to the hydrocarbons present in the surface muds. Mass spectral data showed naphthalenes with 4 and 5 carbon substituents were present in the crab tissues 4 years after the original spill. Other aromatics may have been present as indicated by their mass fragments but could not be resolved from the complex mixture. The sum of the ion currents of mass fragments common to aromatics (ASTM, 1967) accounted for 80% of the total ion current of the 10% plus 20% benzene/pentane fraction in Uca tissues.

Herring gulls, Larus argentatus, represented the highest level of the food chain we analyzed. It seemed likely that because of the mobility of the gulls and their habit of feeding in places likely to be contaminated by oil (eg. behind ships and in garbage dumps) that an uncontaminated gull would be impossible to find. But individual gulls do have definite feeding areas and we thought that those feeding in the West Falmouth area would show a different pattern of pollution (reflecting the Number 2 fuel oil) than gulls feeding in other areas. The idea was supported by the observation that the gulls feeding on the animals killed just after the oil spill were all immatures. These would be the individuals more likely to be in an unfavorable social position in the regular feeding grounds and to be less selective in their food (supposing that heavily oiled animals were not a preferred gull food). We found no dead gulls in the West Falmouth area so we killed one immature that fed in the contaminated

area and one adult from the Weepeeket nesting colony, about 15 km southwest, but still in Buzzards Bay.

The muscle of the Weepeeket gull contained few hydrocarbons boiling below nonadecane (C19) (Figure 3). There were three groups of peaks resolved between C19 and C25. The brain of the Weepeeket gull contained hydrocarbons very similar to those of its muscle. The resolved peaks in the C19 to C25 range were also found in the West Falmouth gull and were presumed to be biogenic in origin. The muscle of the West Falmouth gull showed the whole spectrum of fuel oil hydrocarbons plus the biogenic hydrocarbons. The brain of this animal showed a large high boiling unresolved envelope. An ultra-violet scan of this extract showed absorption spectrum characteristics of single ring aromatics. Any biogenic hydrocarbons present were completely masked by this material.

Discussion

The spilled fuel oil settled onto the Wild Harbor Marsh in a band a few meters wide at the lowest tidal elevations next to the marsh creeks. Behind this, at higher elevations, was a band of marsh untouched by the spill. When we made our first analyses, 15 months after the spill, there was an obvious correlation between the amount of oil in the sediments and the condition of the marsh vegetation. At the highest levels, from 1 to 3 mg oil/ gm mud, all plants were dead. At levels below about 1 mg/gm the plants had been killed but regrowth of Salicornia had begun. By this time there was a spread of oil into the originally unsoiled areas. The amounts were only in the

few tens of parts per million and the low ratio of unbranched/iso-prenoids was characteristic of degraded oil. Presumably this oil had been on the topmost surface of the nearby marsh and was transported on surface particles and dissolved in the water as the tide flooded first the oiled and then the unoiled areas.

Two years after the spill the oil was fairly evenly spread over the affected marsh as indicated by the GLC analyses. There was about a million fold difference in concentration between the water and the mud. Water movement was presumably the mechanism by which oil was distributed. Regrowth of plants had begun by this time but was very uneven over the oiled area. We did not have enough or sufficiently detailed analyses to investigate the patchiness of the recovery process.

As seen in the tables, gravimetric analyses compared fairly closely to GLC calculated amounts in surface sediments where biogenic hydrocarbons were hidden by the large amounts of oil present. Difficulties arise with the methods when amounts of oil approach the biogenics in concentration. Our GLC determinations measure only the hydrocarbons in the C12 to C31 n-paraffin boiling range and are subject to error by loss of some of the low boiling compounds (<C14). The underestimate of total hydrocarbons by the GLC method may be due to the lack of detection of the high boiling compounds (retention indices greater than C31), evaporation of low boilers, or error in placing the column bleed signal with samples of very low hydrocarbon concentrations. We feel that use of the GLC calculations and subtraction of the biogenic hydrocarbons is a more

accurate estimate of the amount of fuel oil in our samples than a total weight determination which includes the biogenics and hydrocarbons higher boiling than the fuel oil.

The change in composition of the oil with time is consistent with observations by Blumer and Sass (1972) on Wild Harbor River and Buzzards Bay sediments. Microbial degradation and solution occurred simultaneously but at rates which gave the following picture: The first observed changes were the disappearances of the normal alkanes followed by the branched alkanes. This was seen in the decreasing ratio of n-alkane/isoprenoid and by inspection of the GLC's. Next we saw a decrease in the isoprenoids as indicated by the phytane/background ratio decrease. In the Wild Harbor Marsh sediments these changes took place in about 4 years with the n-alkanes almost completely gone in 3 1/2 years. During this time, the percent aromatic content of the oil in the sediments increased. After the alkanes were gone we saw a decrease in the % aromatic fraction of the oil. These results are consistent with culture experiments in which microbes isolated from coastal water and sediments oxidized straight and branched chains first when grown on crude oils (Atlas and Bartha, 1972; Ahearn and Meyers, 1973). Aromatics are more resistant to metabolism while the cycloparaffins (naphthenes) are metabolized only very slowly if at all. Previous field observations and laboratory experiments indicated low molecular weight aromatics are more water soluble than other classes of hydrocarbons in oil (Boyland and Tripp, 1971; Blumer and Sass, 1972). Thus, we expect the longest lasting residue in the marsh will consist of the higher

molecular weight aromatics, naphthenoaromatics, and naphthenes.

Our analyses showed the spilled oil was roughly 18% n-alkanes, 8% branched alkanes including the isoprenoids, and 42% aromatics. ¹³ The remaining 32% was presumed to be naphthenes. These estimates were similar to those by Schrader et al (1974) who summarized the composition of several oils. We estimate that about 1/3 of the oil absorbed by the marsh sediment will have a residence time greater than five years.

Vertical distribution of the oil was even more patchy than horizontal distribution at the surface. Our 1971 sample showed ¹³ undegraded oil to a depth of 70 cm (Table 3). The fuel oil at 120 cm in that core was more degraded and may have reached that depth from the surface through a channel in the mud. The shallower depths showed an exponential decrease of progressively less degraded oil indicating the oil was reaching depth by a process of diffusion or small scale mixing. The increase in branched/isoprenoid ratio with depth indicates degradation of the oil ceased once it diffused below the surface. The samples from subsequent years did not show oil as deep as the first core, but this may have resulted from inhomogeneities in distribution. All the samples below the surface after 1971 contained only degraded oil. Oil degradation in anoxic environments is very slow (Davis, 1968). Since salt marsh muds are completely anoxic and highly reduced, the oil in the deeper marsh sediments must have been exchanging with that at the surface. It is also possible (as indicated by the 1973 core) that it was decreasing in amount. Recolonization of the marsh by burrowing animals speeds the

exchange and dissipation at depth by opening channels for exchange of oil between sediments and tidal waters. In return, the speeded exchange permits the recolonization of dead areas by marsh organisms, even though the original colonists are killed in the process of burrowing into the highly polluted muds.

Krebs et al (1975) followed the return of the fiddler crab, Uca pugnax, populations to the marsh in 1972 and 1973. They showed a correlation between the size and structure of the population and amount of oil in surface sediments. Not all areas were fully recolonized four years after the spill. We did not have analyses of the residual oil after five years but some areas were still devoid of higher plants and macrofauna. Thus, there was still toxicity in surface muds despite substantial weathering and degradation of the oil.

Our analyses show no indication of a food chain magnification among any of the aquatic marsh organisms. Mussels feed on detritus derived partly from algae and partly from marsh grasses. They had oil levels intermediate to those food sources. Eels feed on Fundulus but had average oil levels in their tissues below those in their food. The only animal we analyzed that showed more oil than its food was the herring gull which contained twice as much as mussels.

There is a difference in amount of oil to which these various animals would be exposed. Fiddler crabs eat mud, detritus, and algae and so would be exposed to oil levels in their food comparable to that in the mud (about 2 mg/gm). Mussels eating detritus and algae were exposed to somewhere between 10 and a few hundred ppm in their

food. Both were continuously exposed to the approximately 2 ppb oil in the water. Gulls were exposed to the tens or hundreds of ppm in their food but do not have gills in contact with the water. All the animals contained, at least in the first year, a relatively degraded oil which could have entered their tissues either from the water or surface muds.

These results are consistent with the hypothesis that animals absorb oil both from water and food, lose it by equilibration with water passing over their gills, excrete it after metabolizing it, and/or store it in their body lipids. Fiddler crabs probably obtain most of their oil by feeding, but lose most through discharge across their gills. Mussels were exposed to approximately equal amounts of oil in their food and respiratory water. The same would be true for the fish. In both cases equilibration with water would initially be the most important process determining body burdens. Gulls obtain oil only from their food and the body level is determined by a balance between intake and excretion. 15

The herring gull also showed evidence of a partitioning within the body where only selected classes of hydrocarbons (certain aromatics) passed the blood-brain barrier. These are generally the most toxic fraction of oils. Selective concentration of high boiling substituted aromatics should have implications on the mechanisms of toxicity of oil in birds.

The initial picture of absorption of oil changed in subsequent years as shown by comparing oil analyses in Fundulus and Uca. Both absorbed large amounts of oil in 1969-1970. During the following years they were continuously exposed to the same oil, though more degraded with time. By 1974 Fundulus no longer retained large amounts 16 →

of oil in the body. They could, of course, leave the marsh at every low tide for the cleaner waters of the Bay. But studies of Fundulus behavior indicate they are very territorial and move very little from their home streams (Lotrich, 1974; Wright; 1972, Butner and Brattstrom, 1960). Fundulus also developed high levels of hydrocarbon metabolizing enzymes compared to control fish (Burns, 1975a), which indicated they were actively clearing their tissues of incorporated hydrocarbons by physiological adaptations (Burns, 1975d).

Uca showed no significant difference in amount of body hydrocarbons over the four years. This amount probably represented the maximum concentration they could build up internally and still survive. Population data showed Uca living in the oiled areas were mostly adult males that immigrated from the surrounding areas (Krebs et al, 1975). There was no indication they ever developed behavioral or physiological mechanisms for significantly adapting to oil in their environment (Burns, 1975b,d).

Since the fuel oil spilled onto the marsh, a number of changes in distribution of its component parts have occurred. The oil in the marsh sediments became relatively enriched in aromatics over the first three to four years. Animal responses to the oil and its changes varied from simple uptake without discrimination against various fractions as shown by Uca, uptake and selective concentration of certain fractions as shown by the herring gull brain, to discrimination against the pollutant hydrocarbons as shown by Fundulus after the first year. Since Fundulus initially showed uptake without discrimination but later accumulation of mostly biogenic hydrocarbons,

we believe its later response represents selective metabolism and excretion of the pollutants rather than lack of absorption. Obviously the marsh is recovering from the spill, though recovery was not yet complete after five years. Traces of the more persistent components of the oil, the naphthenes and heavier aromatics, should be present in the marsh ecosystem for many years to come.

Acknowledgements

We wish to extend thanks to the following people for help with specific aspects of this work: Manfred Erhardt performed the U.V. analysis on the gull brain. Nelson Frew analyzed the Uca extract on the mass spectrometer. David Masch obtained the seagulls. Christopher Van Raalte identified the algae. George Harvey. John Farrington, and Frank Carey criticized the manuscript.

TABLE I

Oil hydrocarbons in surface muds at Wild Harbor (West Falmouth) salt marsh. Amounts were estimated by subtracting biogenic hydrocarbons from GLC's. (No gravimetric determinations were available for 1971 samples.)

Sample	Oil Hydrocarbons ($\mu\text{g}/\text{gm}$)	<u>unbranched</u> <u>isoprenoid</u>	<u>phytane</u> <u>background</u>
Wild Harbor Surface Muds Jan, 1971	I. 2,700	I.7	I.I
	2. 880	I.2	I.2
	3. 28	0.8	0.8
	4. II	0.9	I.I

TABLE 2

Change in composition of oil hydrocarbons in Wild Harbor Marsh surface sediments with time. Both GLC determinations and gravimetric determinations are given for comparison. *probable error in GLC due to small aliquot used in the determination ($< 0.5 \mu\text{l}$). No biogenic hydrocarbons were visible in the GLC's. N.D. Not Determined.

Sample	gravimetric total ($\mu\text{g}/\text{gm}$)	GLC calculated ($\mu\text{g}/\text{gm}$)	<u>unbranched</u> <u>isoprenoid</u>	<u>phytane</u> <u>background</u>	percent aromatics
No. 2 Fuel oil			2.7	1.5	43%
W.H. Surface Muds					
Jan., 1971	--	2,700	1.7	1.1	27%
July, 1972	2,045	1,960	0.6	1.2	36%
July, 1972	1,189	911	0.4	1.5	N.D.
May, 1973	2,660	1,718*	0.1	0.7	N.D.
Nov., 1973	2,460	2,438	0.0	0.1	35%

TABLE 3

Concentrations of oil hydrocarbons in deep mud cores from Wild Harbor salt marsh 1971 to 1973. Both GLC calculated amounts and gravimetric determinations are given for the 1972 and 1973 data.

cm depth	1971		1972		1973	
	GLC calculated (ug/gm) oil biogenic	unbranched isoprenoid	GLC calculated (ug/gm) oil biogenic	gravim. total (ug/gm)	GLC calculated (ug/gm) oil biogenic	gravim. total (ug/gm)
0-5	4,307	0.7	734	989	I,718*	2,660
10-15					I03	269
25-30	72	I.4	I5.3	I8.5	-	I4.3
45-50	I5	I.8	I.0	5.5	-	7.6
67-72	2	I.4	0.4	9.1	-	2.0
85-90	-	-	8.0	I2.3	0.8	4.1
I15- I20	I.5	I.I				I5.4
						0.03
						0.56
						-
						-
						-
						-
						-

* probable error in GLC amount due to small aliquot used in the determination (< 0.5ul).

TABLE 4

Oil content of hydrocarbon extracts of plant and animal tissues at the West Falmouth (Wild Harbor) marsh in 1970. Amounts of oil were estimated by subtracting biogenics from the GLC determinations.

Organism	Date & Location of collection	Oil Hydrocarbons ($\mu\text{g}/\text{gm}$)	$\frac{\text{n-C17}}{\text{pristane}}$
A. Plants			
	Wild Harbor		
green algae	Aug. 1970		
<u>Enteromorpha clathrata</u>		429	1.06
red algae	"		
<u>Polysiphonia fibrillosa</u>		6.3	8.25
<u>Salicornia spp.</u>	"	13.2	1.44
<u>Spartina alterniflora</u>	"	15.2	3.67
B. Animals			
ribbed mussels	"		
<u>Modiolus demissus</u>		218	0.58
minnows	"		
<u>Fundulus heteroclitus</u>		75	0.65
eel liver	"		
<u>Anguilla rostrata</u>		85	0.87
eel muscle	"		
<u>Anguilla rostrata</u>		23	0.54
fiddler crabs	"		
<u>Uca pugnax</u>		280	0.33

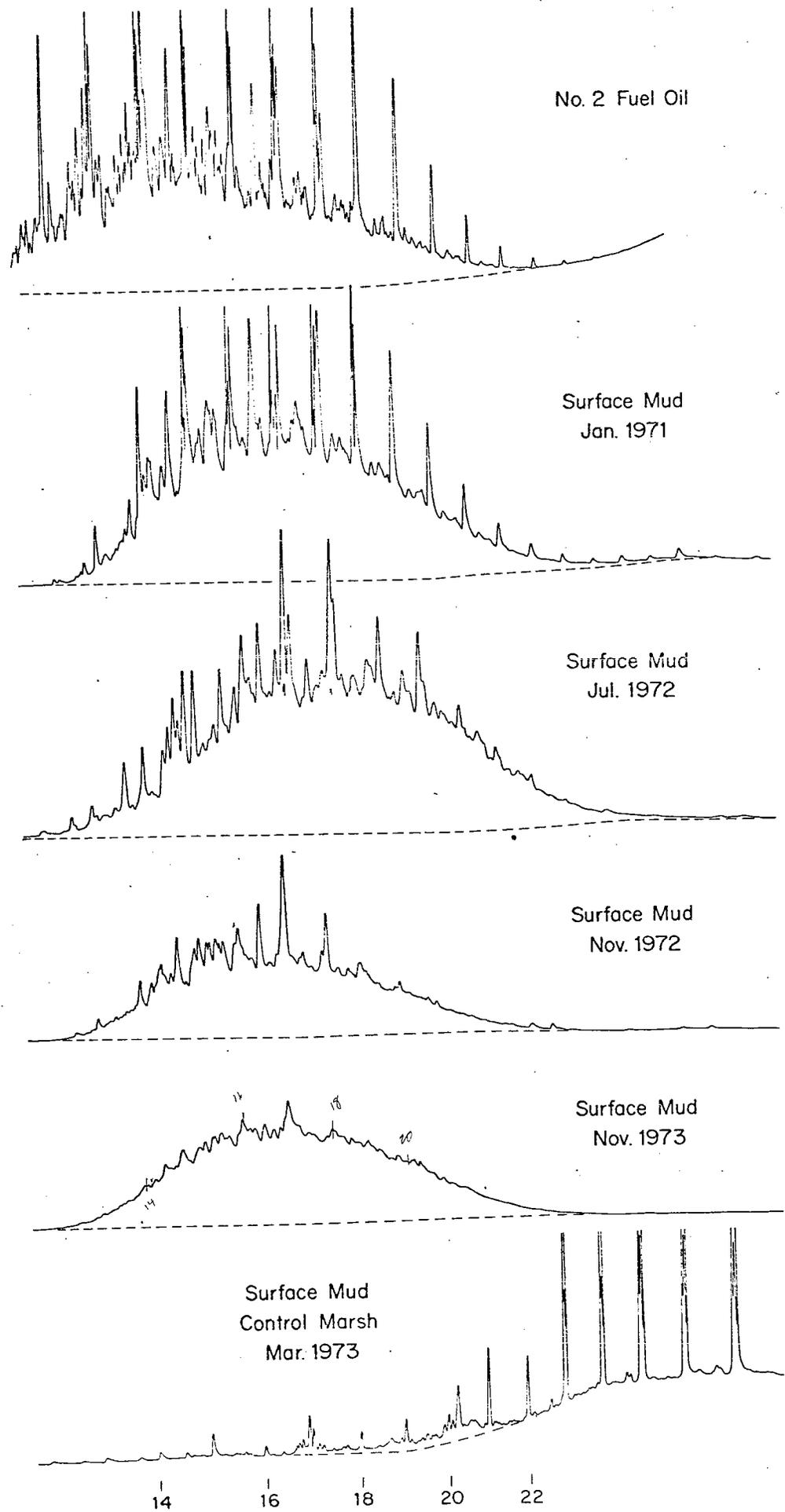
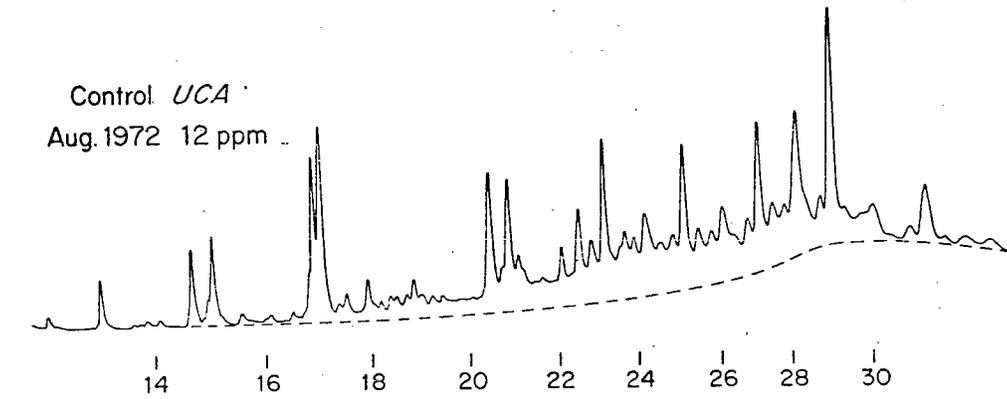
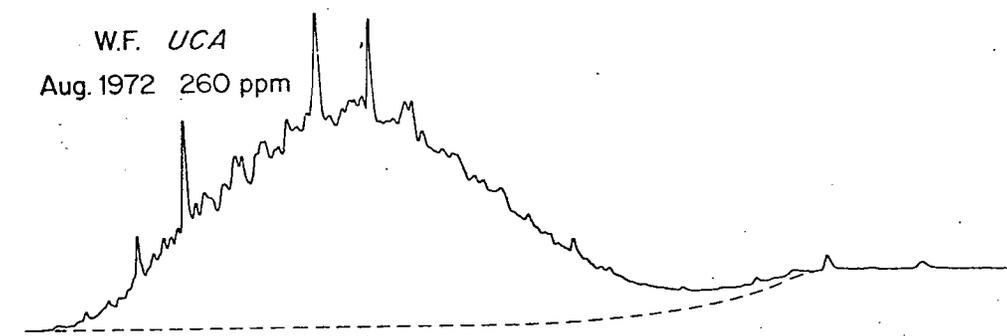
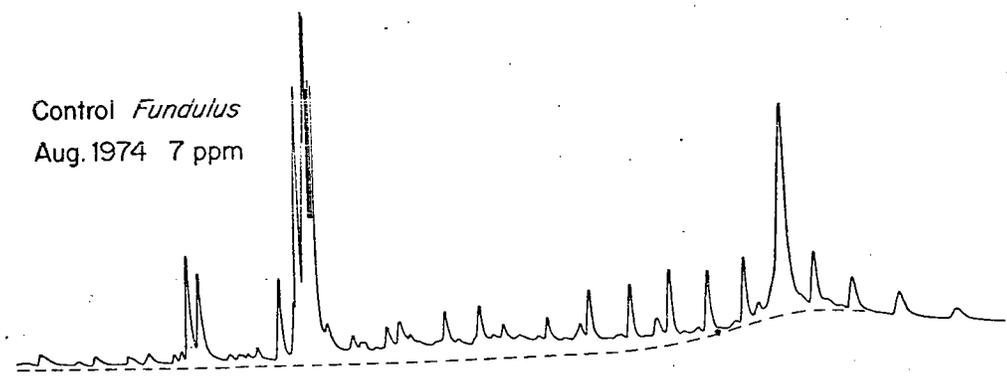
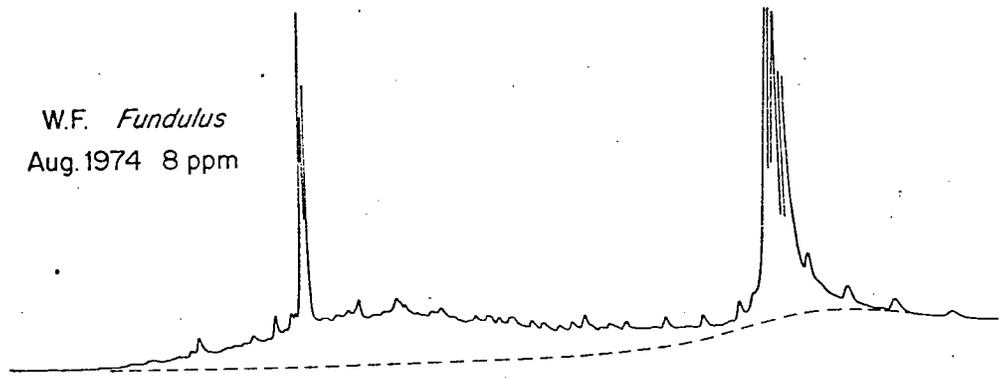
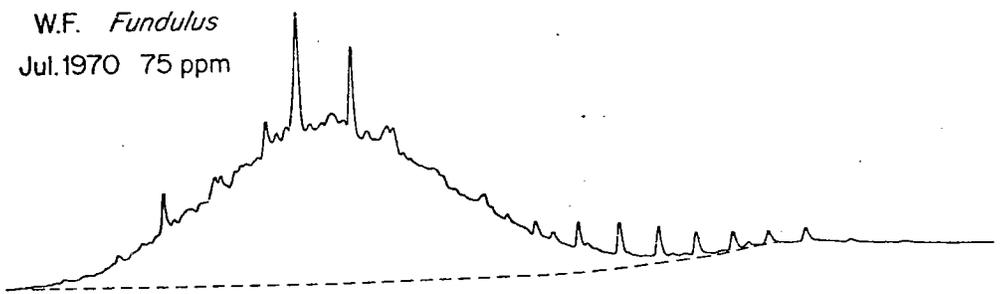


FIGURE 1



14 16 18 20 22 24 26 28 30

FIGURE 2

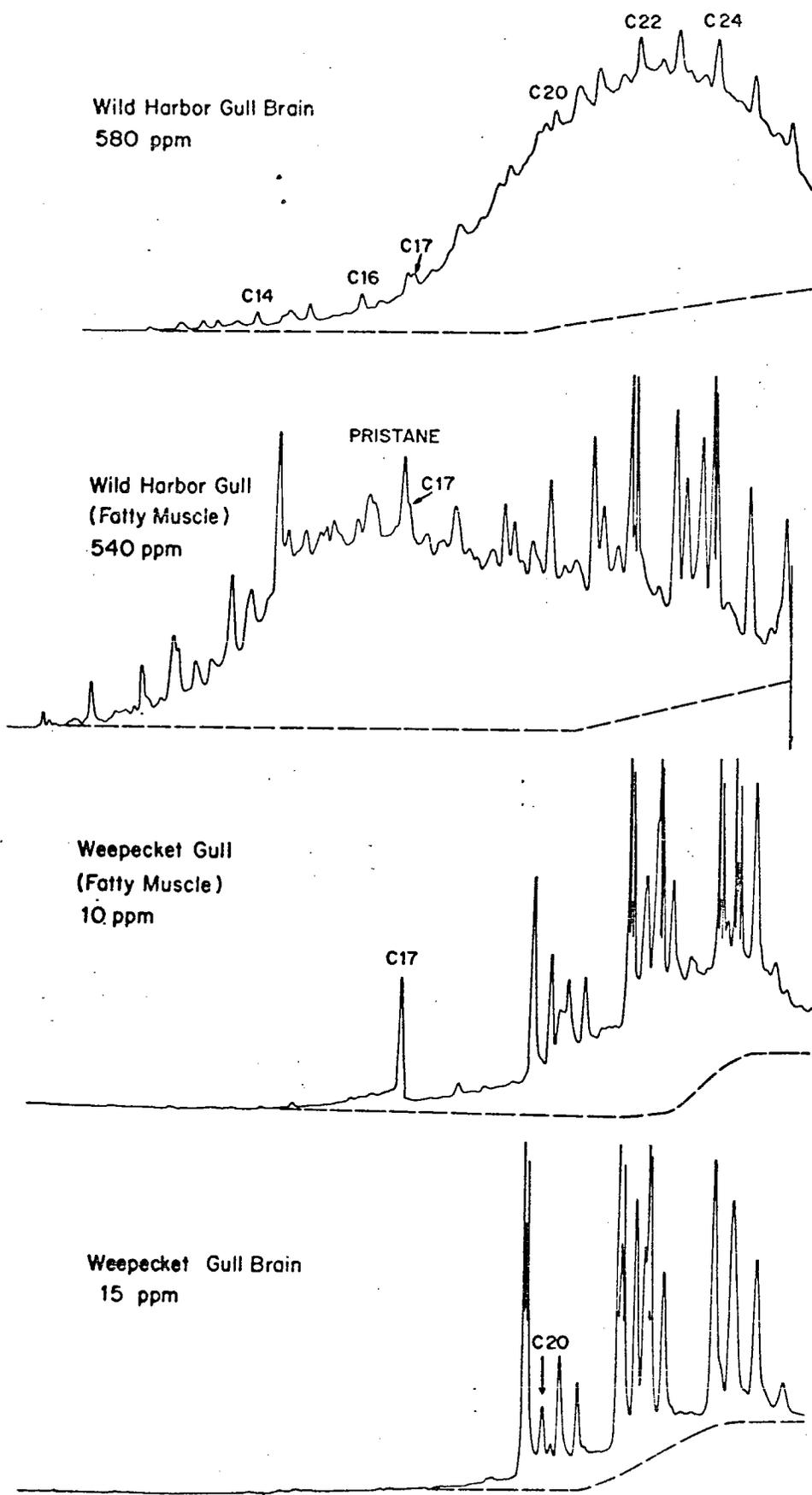


FIGURE 3

FIGURE CAPTIONS

Figure 1. Gas chromatograms of hydrocarbon extracts from fuel oil and marsh surface muds. Temperature was programmed 100 to 280°C. Dashed lines are signal level due to column bleed as determined by blank runs. Carbon chain lengths of standard paraffins are shown at bottom. Top to bottom: Number 2 fuel oil (42% aromatics), W. Falmouth surface mud Jan., 1971 (27% aromatics), same July, 1972 (36% aromatics), same Nov., 1972 (43% aromatics) , same Nov., 1973 (35% aromatics), Surface mud control marsh March, 1973.

Figure 2. Gas chromatograms of hydrocarbon extracts from marsh minnows, Fundulus heteroclitus, and fiddler crabs, Uca pugnax.

Figure 3. Gas chromatograms of hydrocarbon extracts from seagull, Larus argentatus, tissues.

CHAPTER 2

"THE LONG TERM EFFECTS OF HYDROCARBON CONTAMINATION OF
A SALT MARSH ON POPULATIONS OF THE FIDDLER
CRAB, UCA PUGNAX: A SUMMARY"

Following the 1969 West Falmouth oil spill, fiddler crab populations in the Wild Harbor marsh estuary (West Falmouth, Massachusetts) were exposed to Number 2 fuel oil contamination. Observations immediately after the spill showed large numbers of moribund and dead fiddler crabs throughout the heavily oiled marsh regions. There was evidence that many of the surviving crabs had moved from the heavily oiled banks and were burrowing in the sandier drier areas above the line of oil contamination. Crabs remaining in the oiled areas responded lethargically and displayed aberrant behavior. Symptoms were similar to those induced in Uca by exposure to chlorinated hydrocarbon insecticides (Krebs, et al., 1974; Odum, et al., 1969). Analyses of crab tissues showed Uca at Wild Harbor incorporated up to 280 parts per million hydrocarbons (wet weight basis) with 20% aromatic content or up to 56 ppm aromatics. Krebs' and Odum's analyses showed 300 ppb Dieldrin (wet weight) and 800 ppb total DDT residue (dry weight) respectively. Thus, weathered mixtures of petroleum hydrocarbons produced similar behavioral aberrations as chlorinated hydrocarbons but in concentrations 100 to 200 times greater. However, this data does not eliminate the possibility that one or a few petroleum hydrocarbons within the mixture in concentrations similar to those observed for chlorinated compounds produced these effects.

In the Spring of 1972 stations were established in areas of the marsh that hydrocarbon analyses had shown were lightly, moderately and heavily contaminated with oil. Stations were duplicates of four 1/4 m² plots marked horizontally along the creek banks. All stations were at the same average tide level to eliminate Uca population variations due to tidal height. Numbers of crabs per unit area were estimated by counting and measuring open crab burrows. This non-destructive sampling method was related to actual numbers of crabs by digging up selected quadrants

in control and oiled areas and sieving mud through a 1 mm mesh screen (Krebs, 1975). Quantitative measurements of population parameters were continued through the winter of 1973. The crab population reductions (compared to the uncontaminated Sippewissett Marsh) were dose dependent with populations in heavily oiled areas severely reduced or absent. Lightly oiled areas were less severely affected (Figures 1 and 2).

A major shift in sex and age distribution was observed in crabs surviving in the oiled areas. Numbers of immature individuals and females were greatly reduced compared to controls. In spite of substantial juvenile settlements in all marsh areas studied, only small numbers of immature crabs survived in oiled areas. Surviving crabs (mostly adult males that immigrated from surrounding unoiled areas) continued to show locomotor impairment as measured by a standard bioassay (Krebs, et al., 1974), and showed significant reduction in burrowing depth. Heavy over-winter mortality in oiled areas may have been partly related to this reduction in burrow depth.

We suggest that the toxicity of the oiled sediments to juvenile crabs and the impairment of locomotor ability and other behavior in adults accounts for the persistent reduction in fiddler crab populations observed at Wild Harbor at least four years after the original oil spill.

By comparing the hydrocarbon content of the sediments with the numbers of surviving crabs, we estimated levels of oil contamination which would affect Uca populations. Surface sediment hydrocarbon concentrations greater than about 1,000 to 2,000 ppm (wet weight) with aromatic content greater than 20% were toxic to adults. Concentrations of about 100 to 200 ppm were toxic to juveniles. These are rough estimates based on the sediment content of highly weathered Number 2 fuel oil.

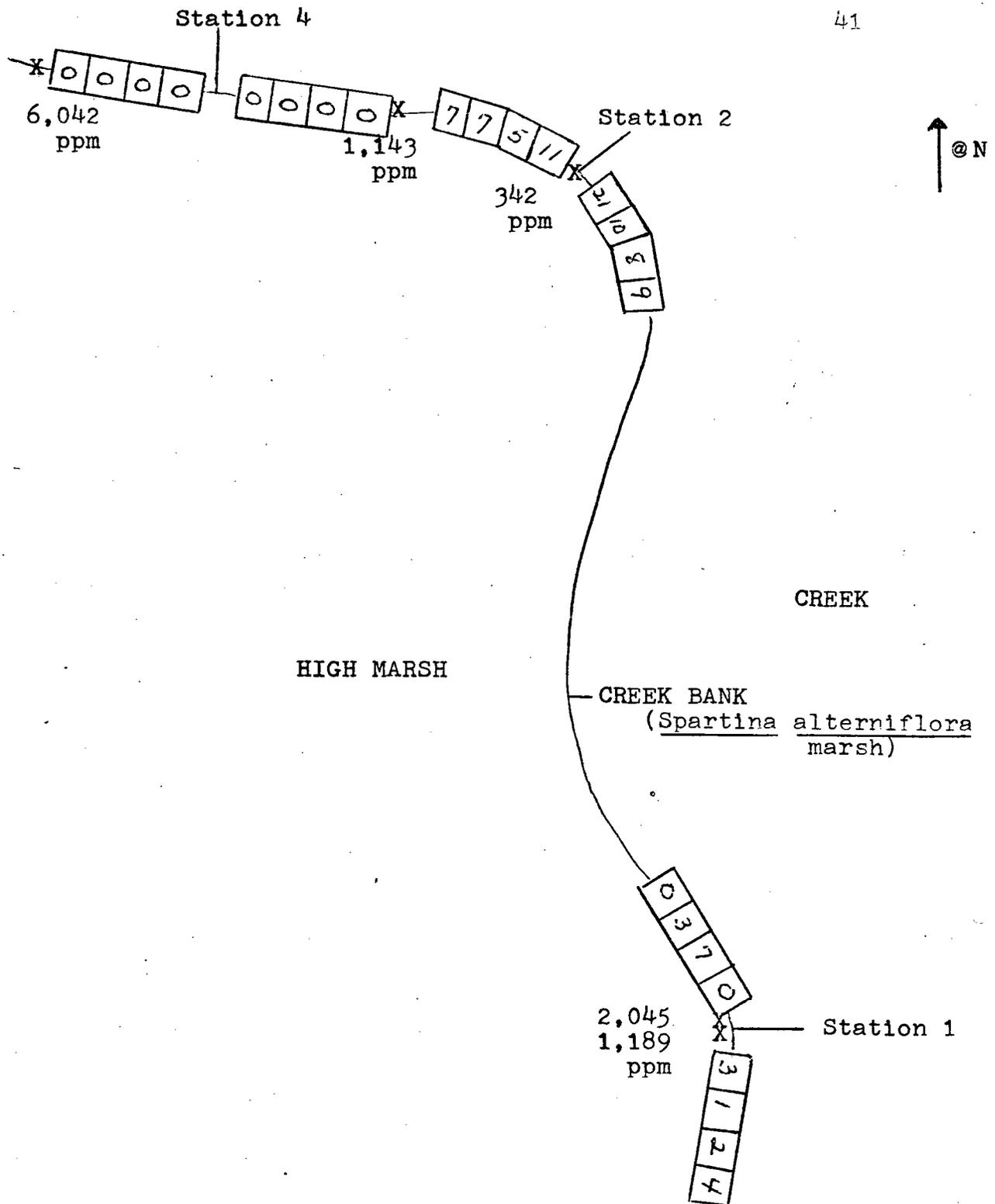


Figure 1. Detail of map of Wild Harbor Marsh showing stations used to study populations of *Uca pugnax* in July, 1972.

(Distances between stations were larger than drawn.)

X are sites of mud collection for hydrocarbon analyses. Total amounts of hydrocarbons are listed as parts per million ($\mu\text{g}/\text{gm}$ wet mud).

n are numbers of *Uca* per $\frac{1}{4}$ m² quadrant, estimated by counting open crab burrows.

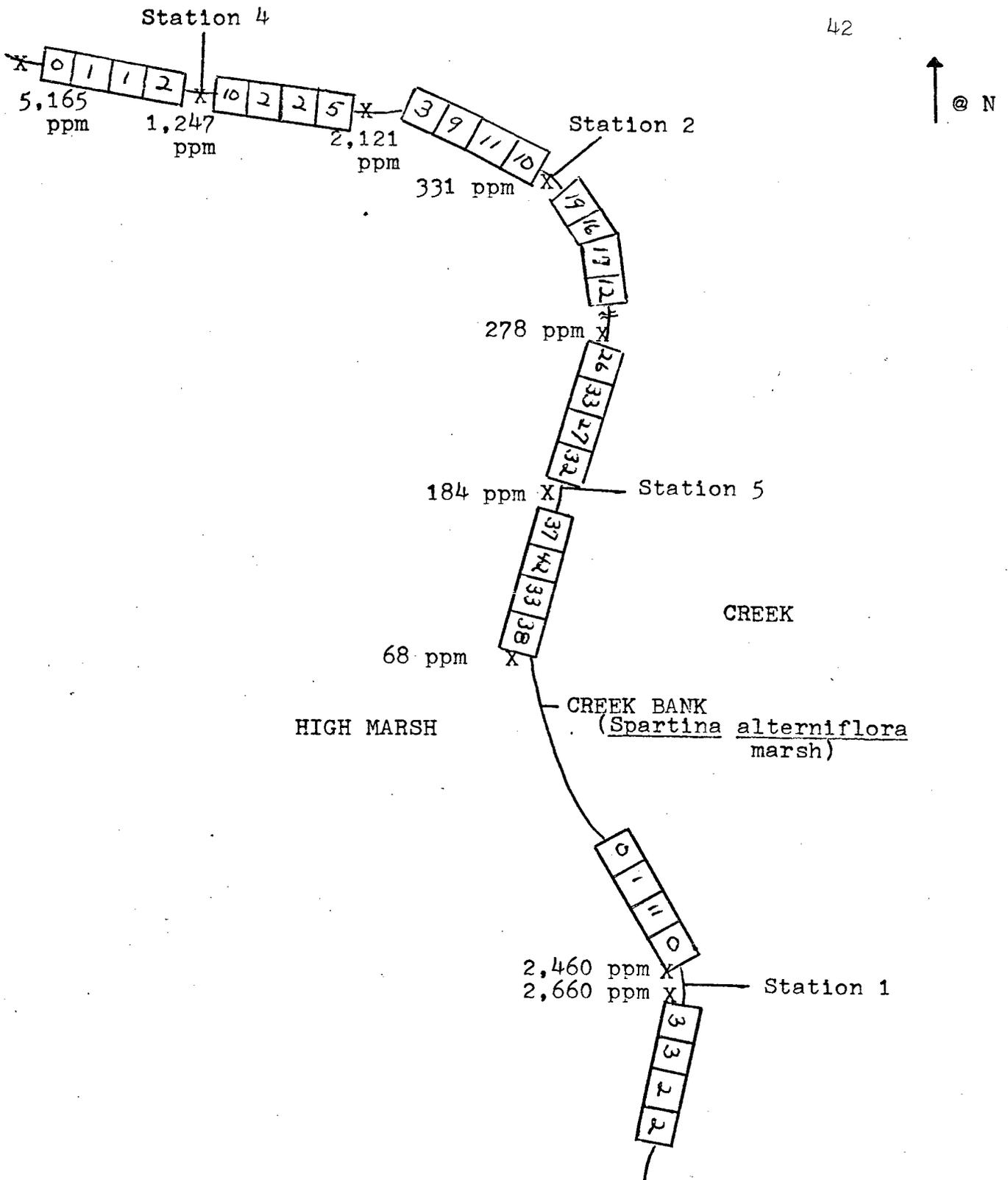


Figure 2. Detail of map of Wild Harbor Marsh showing stations used to study populations of *Uca pugnax* in July, 1973. (Distances between stations not drawn to scale.) X are sites of mud collection for hydrocarbon analyses. Total amounts of hydrocarbons are listed as parts per million ($\mu\text{g}/\text{gm}$ wet mud). \boxed{n} are numbers of *Uca* per $\frac{1}{4}$ m² quadrant, estimated by counting open crab burrows.

CHAPTER 3

"HYDROCARBON METABOLISM IN THE INTERTIDAL FIDDLER CRAB,

UCA PUGNAX"

ABSTRACT

The fiddler crab, *Uca pugnax*, was examined for its ability to metabolize foreign hydrocarbons. The microsomal mixed function oxidase system was identified in *Uca* tissues using Aldrin epoxidation rates as the assay. Rates were slow: 96 pmoles Dieldrin per mg microsomal protein in one hour in hepatopancreas, 438 pm $\text{mg}^{-1} \text{hr}^{-1}$ in gill, and 228 pm $\text{mg}^{-1} \text{hr}^{-1}$ in claw muscle microsomes. No difference in rates could be detected in animals living in clean areas or environments highly contaminated with foreign hydrocarbons. In vivo rates of naphthalene oxidation were measured and used to calculate a clearance time for *Uca* body tissues based on the hydrocarbon content of crabs collected from an oil polluted salt marsh.

INTRODUCTION

The accidental grounding of an oil barge in Buzzards Bay, Massachusetts in September, 1969 resulted in petroleum hydrocarbon contamination of near shore and marsh communities on the northeastern side of the Bay. Analyses of marsh sediments and organisms living in the contaminated area showed the presence of fuel oil hydrocarbons in all components of the marsh ecosystem a year after the spill (Burns and Teal, 1971). Subsequent analyses showed contamination of the anoxic marsh sediments continued at least five years later (Burns, 1975c). Population studies of near shore benthic fauna showed that crustaceans suffered the heaviest mortality rates from the oil and were the last group of animals

to return to the contaminated area (Sanders, et al., 1972). With this information as background, a study of the effects of this oil pollution on aspects of the physiology and population dynamics of Uca pugnax was undertaken. Population data will be presented separately (Krebs, et al., 1975) as will details of the hydrocarbon chemistry (Burns, 1975c).

The uptake of foreign hydrocarbons into body tissues presents organisms with physiological problems of metabolism, storage, and excretion. The metabolic enzymes responsible for the oxidation of foreign compounds, including hydrocarbons, are termed mixed function oxidases (MFO) and have been described in vitro in some mammals (Conney, 1967), insects (Casida, 1968), crayfish and other freshwater invertebrates (Khan, et al., 1972a,b), and both freshwater and marine fish (Burns, 1975a; Pedersen, et al., 1974). The oxidized products of the MFO system are more polar than the highly lipid soluble substrates and can be discharged from body tissues by diffusion across membranes or conjugation with serum components and excretion. High levels of these enzymes have been shown to confer resistance to insecticides and hydrocarbons in insects, freshwater fish, and mammals (Fukami, et al., 1969). Qualitative evidence for the presence of the MFO system in marine invertebrates was given by Corner, et al. (1973) who described the in vivo metabolism of naphthalene by spider crabs, Maia squinado.

I report here a study to determine: 1) if the marsh fiddler crab, Uca pugnax, had the in vitro and in vivo metabolic ability to oxidize foreign hydrocarbons; 2) if higher levels of these enzymes were induced or selected for in field populations of crabs living in the oil contaminated area; and 3) if this metabolic rate was fast enough to be an effective means of clearing the body tissues of oil hydrocarbons and thus

affect the survival of Uca in this heavily polluted environment.

MATERIALS AND METHODS

Animals

Fiddler crabs were collected at the oiled West Falmouth salt marsh and the clean Sippewissett marsh on Buzzards Bay for comparison. Animals used for hydrocarbon analysis were rinsed with ethanol to remove surface contamination and frozen at -20°C in solvent rinsed glass jars with aluminum foil lined caps. Live crabs for enzyme experiments (all adult males 15 to 23 mm carapace width) were held in fiberglass tanks with seawater running at ambient temperature and fed surface mud from the area they were collected.

Determination of Rates of Hydrocarbon Metabolism, *In Vitro*

The rate of Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo,exo-5,8-dimethanonaphthalene, Analabs, Inc.) epoxidation was measured to characterize the MFO system in vitro (Figure 2). Tissues were quickly removed from 15 to 30 crabs, pooled, and homogenized in 10 volumes cold 1.15% potassium chloride (KCl) in a glass tissue grinder. Homogenates were centrifuged 1,500 x g, 15 min, pellet discarded and supernatant recentrifuged 15,000 x g, 15 min. This post-mitochondrial supernatant was then spun 80,000 x g, 90 min to sediment the microsomes. The pellet surface was rinsed with 0.1 M Tris-HCl buffer, pH 7.4, resuspended to about 2 mg protein/ml in buffer, and used immediately. All operations were performed at 4°C . Protein concentrations were determined by the Lowry method (1951) using crystalline bovine serum albumin (Calbiochem) as standard. The assay conditions are described

in detail elsewhere (Burns, 1975a).

Thirty nmoles substrate were added to a 2 ml incubation mixture containing 0.231 μ M KCl, 5 mM nicotinamide, 5 mM magnesium chloride, 0.346 mM NADP (nicotinamide adenine dinucleotide phosphate), 3.42 mM glucose-6-phosphate (G6P), 0.54 units glucose-6-phosphate dehydrogenase (G6PDH) (Sigma Chemical Co.), 0.05 M Tris-HCl buffer pH 7.4, and 1 to 2 mg microsomal protein. Tubes were incubated with frequent shaking at 26°C for 1 hour. Reactions were stopped by capping and boiling 3 min. Aldrin and its epoxide Dieldrin were extracted with 15% ether in pentane, dried with sodium sulfate, concentrated, and measured by electron capture gas chromatography. Determinations were made in duplicate or triplicate as indicated by (n) in the tables. All values were corrected for zero-time blanks.

Determination of Rates of Hydrocarbon Metabolism, In Vivo

To determine the in vivo rate of naphthalene metabolism (a major component of the fuel oil spilled in Buzzards Bay) 16 adult male crabs were placed in a 2 liter Erlenmeyer flask containing 1 liter of 1-¹⁴C-naphthalene seawater solution at 0.25 parts per million. The solution was made by adding 160 μ l of 0.0125 M (2 mCi/mole) 1-¹⁴C-naphthalene (Amersham-Searle Co.) in ethanol to 1 liter glass fiber-filtered seawater. Two crabs were removed at time intervals of 0, 2, 4, 6, 8, 12, 24, and 48 hrs, rinsed in ethanol and frozen in liquid nitrogen. After thawing, viscera including hepatopancreas and gills were removed, pairs combined, and homogenized in 5 ml glass distilled water in a glass tissue grinder. Homogenates were then transferred to screw cap test tubes with Teflon lined caps, 0.5 ml concentrated HCl added, capped and boiled 10 min. (This procedure converts the 4 major expected metabolites to

1-naphthol, Booth and Boyland, 1958.) Homogenates were cooled and extracted with 3 aliquots of 10% ether in distilled pentane to a total solvent volume of 10 ml. Extracts were dried and filtered by running through a Pasteur pipette plugged with cotton and filled with dry Na_2SO_4 . Extracts were then evaporated to near dryness with N_2 gas and spotted on the bottom of 2 cm by 20 cm aluminum thin layer plates coated with activated alumina (Eastman Co.). Plates were developed in benzene and the naphthalene bands (top 3 cm) and 1-naphthol bands (bottom 3 cm) scraped into separate scintillation vials containing 15 ml Brays solution. Vials were counted in a Packard Tri Carb counter. All values were blank corrected and quantified by comparison to external standards.

Hydrocarbon Analyses

Crabs frozen for hydrocarbon analysis were thawed, weighed, ground with Na_2SO_4 in a Virtis homogenizer, and placed in cellulose extraction thimbles in a Soxhlet extractor. Hydrocarbons were extracted by refluxing with methanol for 48 hours. Extracts were then partitioned into pentane, lipid weight determined gravimetrically, saponified, and fats removed by column chromatography on a 10 ml silica gel/10 ml alumina column. Hydrocarbons were eluted from the column with 80 ml pentane, concentrated on a rotary evaporator and transferred to a glass vial with Teflon lined cap. After evaporating the pentane, the hydrocarbons were taken up in 0.5 ml hexane, weighed, and analyzed qualitatively and quantitatively by flame ionization gas chromatography. Procedures and recovery rates are described in detail in Farrington, *et al.* (1973, 1974) except the gas chromatograph used was a Hewlett Packard 5700 equipped with a 50 ft. SCOT OV-101 steel column (Perkin Elmer Co.) programmed from 100°C to 280°C at 4°/min. All solvents were reagent grade and were

redistilled in all glass stills. Glassware and equipment was solvent rinsed before use.

RESULTS

In Vitro MFO Activity

The subcellular distribution of Aldrin epoxidase activity in the claw muscle of Uca pugnax was determined by assaying the various fractions obtained when isolating the microsomes (Table 1). Isolating the microsomes increased specific activity 4 to 6 times over the nuclear and mitochondrial supernatants.

Table 2 shows the distribution of microsomal oxidase activity in various tissues of Uca. The green gland-eye stalk preparation showed the highest specific activity, and hepatopancreas had the lowest of the tissues surveyed. Claw muscle was used in most assays because of the higher total activity it contained.

Table 3 shows that bubbling the reaction mixture with carbon monoxide (CO) for 30 sec before adding the substrate reduced Dieldrin formation 75%. Bubbling with N₂ gas had no effect on Dieldrin formation. Leaving out the NADPH generating system resulted in no detectable conversion of Aldrin to Dieldrin.

Changing the pH of the assay system had no significant effect on the rate of Dieldrin production between pH 7.0 and 8.2. A pH of 8.6 resulted in no enzyme activity.

I could detect no differences in rates of Aldrin epoxidation in crabs from clean or oiled areas. Therefore I have combined all determinations to produce average values for the in vitro rate of Aldrin

epoxidation in U. pugnax. The following numbers are average values \pm the standard deviation of (n) determinations: 96 ± 36 (4) pmoles $\text{mg}^{-1} \text{hr}^{-1}$ for hepatopancreas microsomes, 438 ± 120 (4) pmoles $\text{mg}^{-1} \text{hr}^{-1}$ for gill microsomes, and 228 ± 108 (14) pmoles $\text{mg}^{-1} \text{hr}^{-1}$ for claw muscle microsomes.

Naphthalene Metabolism In Vivo

Results of the in vivo experiment are shown in Figure 1. Uca were able to oxidize naphthalene at about the rate of 10 pmoles/mm crab width per day. That is, a 15 mm crab could metabolize about 150 pmoles per day (10% of the absorbed radioactivity). Attempts to keep the crabs in 0.25 ppm naphthalene for more than 48 hours resulted in the death of the animals. No attempt was made to measure metabolic products in the seawater or $^{14}\text{CO}_2$ which would have been produced if further metabolism of the 1-naphthol compounds had occurred. This may have been a source of error but is probably negligible since the rates obtained compared closely with in vivo Aldrin metabolism rates obtained by Khan, et al. (1972b) for freshwater crayfish.

Hydrocarbon Analyses

Table 4 shows the results of analyses of crabs living in the oil contaminated area compared to clean areas. (See Blumer and Sass, 1972 for a detailed discussion on differentiating petroleum hydrocarbons from recently biosynthesized biogenic hydrocarbons.) Uca living in the contaminated sediments incorporated oil into their body tissues (Table 4). Salt marsh muds are anoxic and it is presumed that the oil will remain absorbed in them for many years due to slow degradation rates. Oil remained at high levels ($>2,500$ μg petroleum hydrocarbon per gm

TABLE 1

Subcellular distribution of in vitro Aldrin epoxidase activity in claw muscle fractions of U. pugnax.

Subcell Fraction	pmoles Dieldrin produced mg mic.protein hr \pm s.d.*	Total Activity (nm/hr)	% Total Activity
Nuclear supernatant 1,500 x g, 15 min	59 \pm 4.1 (3)	13.7	100
Mitochondrial sup. 15,000 x g, 15 min	37 \pm 13.7 (3)	7.1	52
Microsomal supernatant 80,000 x g, 90 min	not detected	--	--
Microsomes	232 \pm 86 (3)	7.1	52

* s.d. is the standard deviation of (n) determinations.

TABLE 2

Distribution of in vitro microsomal Aldrin epoxidase
in various tissues of U. pugnax.

Tissue	pmoles Dieldrin produced mg mic. protein hr \pm s.d.*	Total mg Protein	Total (nm/hr) Activity
Green gland-eye stalk microsomes	482 \pm 29 (2)	3.7	1.8
Gut microsomes	282 \pm 13 (2)	4.4	1.2
Gill microsomes	264 \pm 173 (2)	4.7	1.2
Claw muscle microsomes	250 \pm 115 (2)	14.4	3.6
Hepatopancreas microsomes	103 \pm 61 (2)	12.2	1.3

* s.d. is the standard deviation of (n) determinations.

TABLE 3

Effects of CO addition and NADPH generating system deletion on the in vitro rate of Aldrin epoxidation in claw muscle microsomes of U. pugnax.

Treatment	pmoles <u>Dieldrin produced</u> mg mic. protein hr \pm s.d.*
Whole reaction mixture	166 \pm 57 (3)
CO bubbled 30 sec	52 \pm 48 (3)
N ₂ bubbled 30 sec	203 \pm 13 (3)
No NADPH generating system	not detected

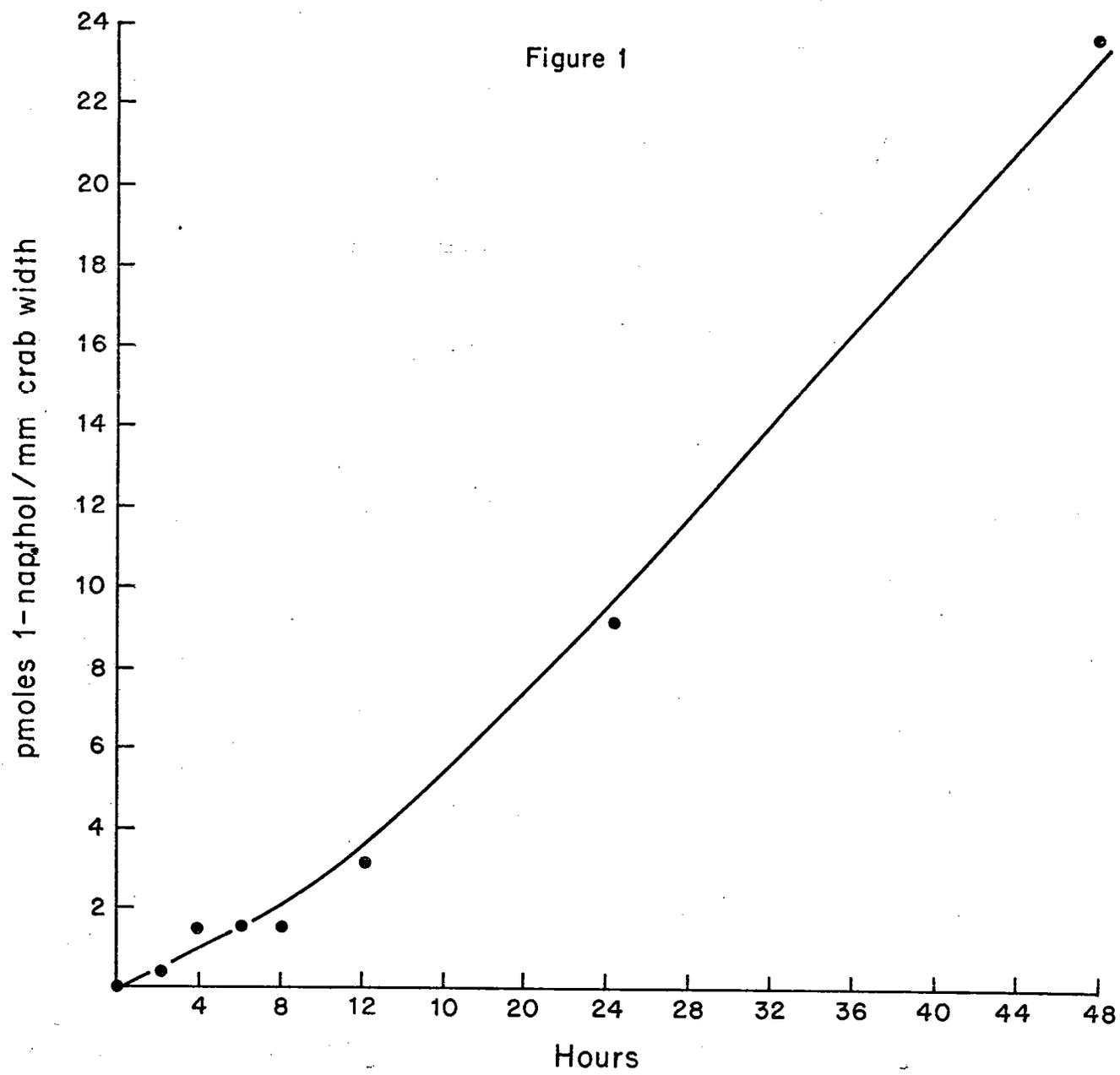
* s.d. is the standard deviation of (n) determinations.

TABLE 4

Hydrocarbon content of adult U. pugnax.

Listed in the table is the date and place of collection of animals, total body burden of μg hydrocarbons per gram wet weight, and a qualitative description of the composition of the hydrocarbons determined by column and gas chromatography.

Place of Collection of Animals	Date of Collection	Total Gravimetric Determination	% Aromatics	Qualitative Description
Sippewissett Marsh (clean area)	Aug., 1970	7.1 ppm		biogenic hydrocarbons consisting mostly of algae and plant hydrocarbons obtained by eating normal marsh detritus
	May, 1972	10.6 ppm		
	Aug., 1972	11.8 ppm		
West Falmouth Marsh (oiled area)	Aug., 1970	280 ppm	20%	all petroleum derived hydrocarbons. biogenics completely masked. aromatic hydrocarbon content 10 to 20%. " "
	May, 1972	203 ppm		
	Aug., 1972	259 ppm	18%	
	May, 1973	287 ppm		
	Aug., 1973	183 ppm	10%	



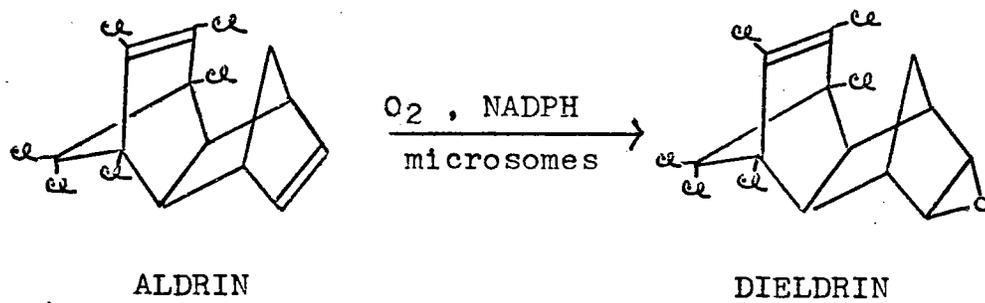


Figure 2

wet sediment) for at least 4 years (Burns, 1975c). Uca who burrow into these contaminated muds have had continuous exposure to these oil hydrocarbons. The total body burden appears fairly constant for the three years data.

DISCUSSION

In Vitro Aldrin Epoxidation

I have established the presence, in vitro, of the mixed function oxidase system in Uca pugnax. The system is microsomal, requires NADPH, and is inhibited by carbon monoxide suggesting the participation of a CO-binding component, probably cytochrome P-450. (Attempts to show the presence of cyt P-450 in suspensions of Uca microsomes by the method of Omura and Sato (1964) failed because of turbidity in the suspensions.) Rates of in vitro Aldrin epoxidation were similar to those reported by Khan, et al. (1972a,b) for freshwater invertebrates and by Khan and Terriere (1968) for insecticide-sensitive insects. These rates were one to three orders of magnitude slower than insecticide-resistant insects (Khan and Terriere, 1968), fish (Burns, 1975a), and mammals (Conney, 1967) respectively. Further support for low MFO rates in marine crustacea is given by Carlson (1973) and Pohl, et al. (1974). I was not able to detect differences in rates of oxidation from crabs living in the highly oil-polluted environment compared to clean areas. Nor was there any detectable difference in rates in Uca collected from a salt marsh contaminated with chlorinated hydrocarbon insecticides for at least five years (Krebs, et al., 1974). Since substantial juvenile settlement occurred at all these marshes all five years studied, natural

selection should have had time to operate. Thus, in contrast to fish from these areas (Burns, 1975a), Uca appeared unable to significantly raise their MFO activity to high levels in response to environmental pollution either through enzyme induction or genetic selection.

In Vivo Rates and Calculation of Clearance Time

In vivo rate of naphthalene oxidation (about 10% of that absorbed into body tissues in 24 hrs) in Uca was similar to the rate of Aldrin oxidation reported by Khan, et al. (1972b) for freshwater crayfish. Using the rate obtained from Uca as 10 pmoles/mm crab per day, I estimated how long it would take a 15 mm adult crab to clear its tissues of oil contamination by means of metabolism alone. I assumed optimum conditions for clearing, that is: that the animal is moved to a clean area and no further uptake of petroleum occurs; that as soon as the hydrocarbon is oxidized it is excreted; that substitution of the aromatic ring does not significantly alter the rate of oxidation; and that all other classes of hydrocarbons are oxidized faster than the aromatics. Detailed analyses of the crab tissue extracts showed the oil content of their tissues contained from 10 to 20% aromatic hydrocarbons.

Using a value of total body burden of hydrocarbons as 250 ppm, and aromatic content of 15%, a 1 gm, 15 mm crab would have a burden of 37.5 μ g aromatic hydrocarbons. At 10 pmoles/mm/day, and assuming an average molecular weight of 250, a 15 mm crab could oxidize 1.5×10^{-10} moles/day or 3.75×10^{-8} gm aromatic hydrocarbons per day. To oxidize 37.5×10^{-6} gm would take 1×10^3 days or about 3 years. Since Uca live a maximum of 3 to 4 years and a 15 mm crab is already 2 years old,

metabolism alone, even under these assumed ideal conditions would not be fast enough to clear these animals' body tissues of contaminating hydrocarbons.

In this chronically polluted situation where the crabs burrow into oiled sediments and eat oiled detritus off the marsh surface, uptake of hydrocarbons into body tissues occurs continuously. I gave evidence that uptake of foreign hydrocarbons occurs faster in crabs than they can be metabolized and excreted in the in vivo experiment. Uca metabolized only 10% of the absorbed naphthalene in 24 hours. Gas chromatograms showed the oil hydrocarbons in the crab body tissues were similar in composition to those in marsh surface muds. The petroleum content in Uca was not extensively modified by the crabs' own metabolic processes as were those in fish from this area (Burns, 1975c).

This minimal biochemical ability to metabolize foreign hydrocarbons coupled with Uca's life style of burrowing into sediments even when oiled and their detritus feeding habits account for the relative sensitivity of fiddler crabs to this type of pollution (Burns, 1975b). Similar behavioral and physiological mechanisms may account for the relative sensitivity of other marine crustacea to oil pollution.

SUMMARY AND CONCLUSIONS

As part of a study of the effects of oil pollution on a salt marsh community fiddler crabs, Uca pugnax, were examined for their ability to metabolize foreign hydrocarbons. By measuring Aldrin epoxidation rates in microsomes from various body tissues, it was determined that Uca do have the microsomal mixed function oxidase system described in other organisms. However, rates of oxidation were very slow, comparable to

rates in freshwater invertebrates and insecticide-susceptible strains of insects. No difference in rates was seen in animals living in highly contaminated areas compared to controls. Therefore, I concluded that unlike fish from the same areas, Uca did not have the physiological ability to significantly increase their rate of enzymatic oxidation of foreign hydrocarbons, either through enzyme induction or genetic selection.

A calculation of the time necessary for Uca to clear their body tissues of contaminating hydrocarbons through metabolism alone was made based on in vivo naphthalene oxidation rates and in situ hydrocarbon content of crabs living in the polluted area. Even under ideal conditions, metabolism alone would not be fast enough to clear body tissues within the life span of the crabs. That Uca do not have much biochemical ability to metabolize foreign hydrocarbons partially accounts for their relative sensitivity to this type of chemical pollution.

CHAPTER 4

"MICROSOMAL MIXED FUNCTION OXIDASES IN AN ESTUARINE FISH,
FUNDULUS HETEROCLITUS, AND THEIR INDUCTION AS A RESULT
OF ENVIRONMENTAL CONTAMINATION"

ABSTRACT

1. An hepatic microsomal mixed function oxidase (MFO) system is described in the estuarine fish, Fundulus heteroclitus.
2. In vitro Aldrin¹ epoxidation rates were measured and compared to those seen in freshwater fish.
3. The oxidases are microsomal, require NADPH², and were poisoned by carbon monoxide (CO) indicating the involvement of a CO-binding component.
4. Liver microsomal preparations contained the CO-binding cytochrome P-450.
5. High levels of these enzymes were induced both by a drug in the laboratory and by contamination of the aquatic environment by foreign hydrocarbons.

INTRODUCTION

The uptake of petroleum derived and chlorinated hydrocarbons into the tissues of fish through several routes of exposure is well documented (Lee, et al., 1972; Hamelink, et al., 1971; Burns and Teal, 1971). The accumulation and body burden of these compounds depends on their relative rates of uptake, metabolism and discharge.

¹Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo, exo-5,8-dimethano naphthalene) (Analabs Inc.).

²NADPH (reduced nicotinamide adenine dinucleotide phosphate).

In terrestrial and freshwater organisms metabolism occurs mainly through the mixed function oxidase (MFO) system to form oxidized metabolites. These more polar derivatives can then be discharged by diffusion across membranes or conjugated with serum components and excreted. The system is inducible in mammals and serves as a mechanism of detoxification for a variety of compounds.

The MFO system has been described in vitro in freshwater and anadromous fish (Stanton and Khan, 1974; Buhler and Rasmusson, 1968; Chan, et al., 1967) and several workers have shown foreign hydrocarbons are metabolized in vivo by both freshwater and marine fish (Lee, et al., 1972; Guarino, et al., 1971; Pritchard, et al., 1971). Great inter-species variation in MFO rates is noted by Adamson (1967).

I undertook to characterize in vitro the hepatic MFO system in the estuarine fish Fundulus heteroclitus and to show that the system is inducible on the individual level as a result of exposure to foreign compounds. My data also shows that high levels of these enzymes are induced in field populations of these fish from environments contaminated with chlorinated and petroleum derived hydrocarbons.

MATERIALS AND METHODS

Animals

Fundulus were collected by seining and trapping at salt marshes on Cape Cod, Massachusetts, June through October, 1974. Control animals came from areas presumed to be free of contamination by large amounts of foreign hydrocarbons (Cuttyhunk Island and Great Barnstable Marshes). Fish living in contaminated environments were taken from the site of the West Falmouth oil spill (Burns and Teal, 1971) and from areas of

Great Sippewissett Marsh experimentally treated with sludge fertilizer containing chlorinated pesticides (Krebs, et al., 1974). Fish were held in fiberglass tanks with seawater running at ambient temperature without food and assayed for enzyme activity within two days of capture. No distinction was made for sex but all animals were adults of 5-10 cm body length.

Fish livers were removed, weighed, pooled, and homogenized in 10 volumes of 1.15% potassium chloride (KCl) in a glass tissue grinder. Homogenates were centrifuged 1,500 x g for 15 min and the supernatant centrifuged 15,000 x g 15 min. This post-mitochondrial supernatant was then spun 90 min at 80,000 x g to sediment the microsomes. The pellet surface was rinsed with 0.1 M Tris HCl buffer pH 7.4, resuspended to about 2 mg protein per ml buffer, and used immediately. All operations were performed at 4°C and steps carefully timed to be consistent in each preparation since all enzyme activity was lost after overnight refrigeration or freezing in buffer. Protein concentrations were determined by the Lowry method (1951) using crystalline bovine serum albumin (Calbiochem) as standard.

Cytochrome P-450 Determination

Cytochrome P-450 was measured in the microsomal suspensions by the method of Omura and Sato (1964) or, when hemoglobin interfered with the spectra, by the method of Miyake, et al. (1974).

Aldrin Epoxidation Assay

Incubation mixtures contained 0.231 μ M KCl, 5 mM nicotinamide, 5 mM magnesium chloride, 0.346 mM NADP, 3.42 mM glucose-6-phosphate (G6P), 0.54 units glucose-6-phosphate dehydrogenase (G6PDH) (Sigma Chemical Co.),

0.05 M Tris HCl buffer pH 7.4, and 0.2 to 0.6 mg microsomal protein in a final volume of 2.0 ml. Thirty nmoles of Aldrin were added in 10 μ l ethanol and tubes were incubated in a water bath at 26°C for 15 min with frequent swirling. Reactions were stopped by capping and boiling for 3 min. Aldrin and its epoxide Dieldrin were extracted from the inactivated reaction mixture with 5 volumes (in three aliquots) of 10% ether in pentane. Each extract was run through a Pasteur pipette plugged with cotton and filled with 2 inches Florisil and 1/2 inch dry sodium sulfate, concentrated by boiling off the solvent in a glass concentrator tube, and analyzed by electron capture gas chromatography. Assays were quantified by comparison to external standard curves. Blanks were determined by adding substrate and boiling immediately. All values were corrected for zero time blanks and percent recovery during the experimental procedure. Determinations were performed in triplicate. Recovery yields for the entire procedure were 85% for both Aldrin and Dieldrin.

RESULTS

Enzyme Localization and Characterization

The subcellular distribution of Aldrin epoxidation activity was determined by assaying the various fractions obtained when isolating the microsomes. Table 1 shows that the microsomal fraction contained approximately 3 times the specific activity seen in the nuclear or mitochondrial supernatants.

Figures 1, 2, and 3 show the effect of varying amounts of enzyme, time of incubation at 26°C, and temperature of incubation, respectively. The rate of Dieldrin production under these conditions was linear up to 0.7 mg protein per assay, at least 15 min incubation, and optimum

TABLE 1

Subcellular distribution of Aldrin epoxidation activity
in liver fractions of Fundulus heteroclitus

Subcell Fraction	$\frac{\text{nmoles Dieldrin formed}}{\text{mg protein per min}} \pm \text{S.D.}^*$	Total mg Protein	Total Activity (nmoles/min)
Nuclear supernatant 1,500 x g, 15 min	0.10 \pm 0.009	185.2	18.2
Mitochondrial sup. 15,000 x g, 15 min	0.07 \pm 0.017	159.4	11.1
Microsomal sup. 80,000 x g, 90 min	not detected	122.8	n.d.
Microsomes	0.25 \pm 0.081	36.3	8.9

*S.D. is the standard deviation of 3 determinations.

between 26° and 37°C. The standard assay conditions used to compare rates of enzyme activity from various field populations were all in the linear range for Dieldrin production.

Table 2 shows the requirement for the NADPH generating system. Deletion of NADP, G6P, and G6PDH from the assay mixture resulted in no detectable conversion of Aldrin to Dieldrin. Bubbling the mixture for 30 sec with carbon monoxide (CO) reduced Dieldrin formation 98%, demonstrating the involvement of a CO-binding component in the fish MFO system. CO difference spectra of the microsomal suspensions showed the presence of a CO-binding cytochrome with maximum absorption at 450 m μ similar to mammalian systems (Figure 4).

Induction in the Laboratory

To determine if higher levels of these enzymes could be induced by exposure to a drug, groups of 10 Fundulus were placed in all glass, 10 gal. aquaria with clean seawater, 10 ppb (parts per billion) and 100 ppb phenylbutazone (sodium salt) in seawater. Fish were fed daily to satiation with commercial fish food and solutions were aerated and changed daily for 9 days. All fish appeared to lose weight but no difference was seen between control and experimental groups in either weight loss or mortality during the experiment. Fish were assayed for enzyme activity on day 10. Phenylbutazone increased rates of Aldrin epoxidation, levels of cytochrome P-450, and liver microsomal protein in the drugged fish compared to controls (Table 3).

Enzyme Induction in Field Populations

Comparisons of rates of Aldrin epoxidation and levels of cytochrome P-450 were made on Fundulus collected from clean and polluted marshes as

TABLE 2

Effects of CO addition and NADPH generating system
deletion on the rate of Aldrin epoxidation

Treatment	$\frac{\text{nmoles Dieldrin formed}}{\text{mg mic. protein per min}} \pm \text{s.d.}^*$
Complete Incubation Mixture	0.42 \pm 0.019
No NADPH generating system	not detected
CO bubbled 30 sec	0.01 \pm 0.003
N ₂ bubbled 30 sec	0.55 \pm 0.022

*s.d. is the standard deviation of 3 determinations.

TABLE 3

Aldrin epoxidation rates and cytochrome P-450 levels in fish maintained in clean seawater or seawater drugged with phenylbutazone

Treatment	$\frac{\text{nm Dieldrin formed}}{\text{mg mic.protein min}} \pm \text{S.D.}^*$	$\frac{\text{pm CytP-450}}{\text{mg mic.prot.}}$	$\frac{\text{mg liver prot.}}{\text{gm liver wt.}}$
Control fish kept in clean seawater	0.10 ± 0.003	9	4.3
10 ppb phenylbutazone in seawater	0.20 ± 0.008	27	6.4
100 ppb phenylbutazone in seawater	0.29 ± 0.025	97	8.7

* S.D. is the standard deviation of 3 determinations.

TABLE 4

Aldrin epoxidation rates and levels of cytochrome P-450 in populations of Fundulus collected from different environments

Environment Collected	$\frac{\text{nmoles Dieldrin formed}}{\text{mg mic. protein min.}} \pm \text{S.D.}^*$	$\frac{\text{pm Cyt P-450}}{\text{mg mic. prot.}}$
Oil Polluted Area	0.64 \pm 0.037	322
West Falmouth	0.54 \pm 0.075	437
	0.75 \pm 0.080	350
	0.55 \pm 0.022	342
Pesticide Contaminated Area	0.69 \pm 0.103	404
Sippewissett		
Clean Area	0.25 \pm 0.081	249
Cuttyhunk Island		
Clean Area	0.39 \pm 0.052	363
Barnstable Marsh		
Clean Area	0.39 \pm 0.018	251
Sippewissett		

* S.D. is the standard deviation of 3 determinations.

described above. Table 4 shows that higher levels of these enzymes occur in populations living in hydrocarbon contaminated environments. Rates of Aldrin epoxidation were about twice as high for fish from polluted areas as those from clean areas. Levels of cytochrome P-450 did not exhibit such a consistent difference suggesting this is not a good parameter with which to compare populations.

Figure 5 shows Lineweaver-Burke plots for two of these populations. The calculated Michaelis Constants (K_m) and maximum velocities (V_{max}) were 4.5×10^{-6} M and 0.26 nmole/mg min for the Cuttyhunk population living in a clean environment and 6.5×10^{-6} M and 0.93 nm/mg min for the West Falmouth population living in an oil polluted environment.

DISCUSSION

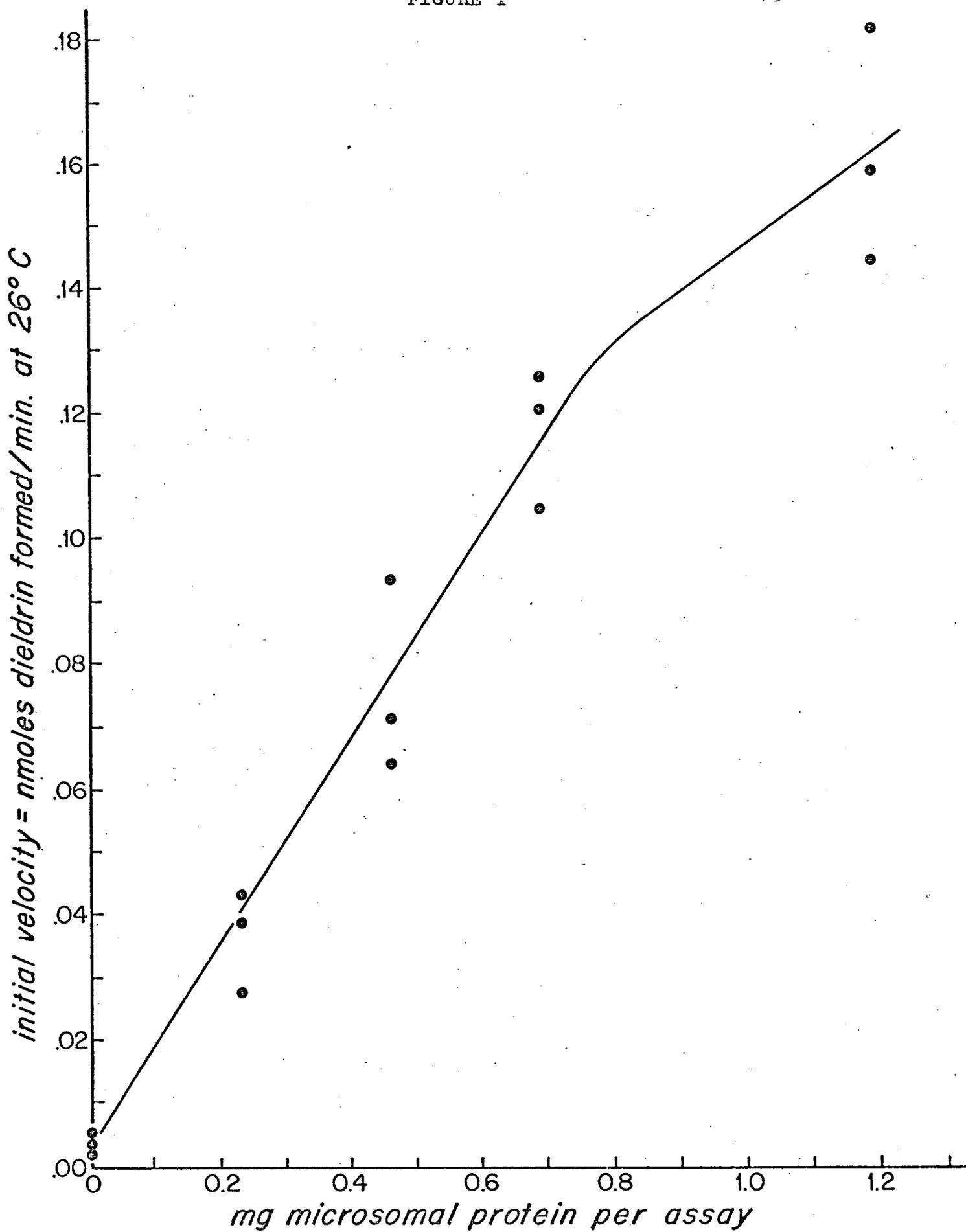
This investigation demonstrates the presence of the microsomal mixed function oxidase system in the liver of the estuarine fish, Fundulus heteroclitus. It is similar to the system studied in some mammals and freshwater fish. The oxidation of Aldrin by Fundulus microsomes required NADPH and a CO-binding component. The calculated Michaelis Constants and V_{max} are in the same order of magnitude as those reported by Stanton and Khan (1974) in freshwater fish. The larger temperature tolerance of Fundulus microsomal enzymes compared to trout MFO reported by Buhler and Rasmussen (1968) may be related to the eurythermal adaptation of this species and the wide temperature range it normally encounters in its environment.

The increase in specific activity of Aldrin epoxidation rates, levels of cytochrome P-450, and liver microsomal protein when exposed to phenylbutazone in the laboratory compared to controls indicates the

inducibility of this enzyme system. The small difference in K_m but large difference in V_{max} in the two field populations is consistent with the hypothesis that environmental contamination induces high levels of MFO in fish (Dixon and Webb, 1964).

Significant in vivo oxidation rates for foreign compounds have been reported (Guarino, et al., 1971; Pritchard, et al., 1971) and Lee, et al., (1972) showed that the rate of oxidation of benzopyrene was fast enough in fish liver to reach a steady state so that the amount of substrate entering the liver was balanced by the amount of metabolites leaving. Lee also noted rapid transport and excretion of metabolites in the urine of fish possibly as glutathione or glycoside conjugates. Thus, Fundulus and other fish may have an efficient mechanism of oxidizing and excreting transient, sublethal doses of several foreign compounds. But the induction of drug metabolizing enzymes does not insure the survival of fish in chronically or heavily polluted environments. The benefit of the MFO system to the organisms to survive in such areas depends on the relative toxicities of substrates and products, their relative rates of discharge, and many other physiological effects associated with the induction of MFO (Conney, 1967).

FIGURE 1



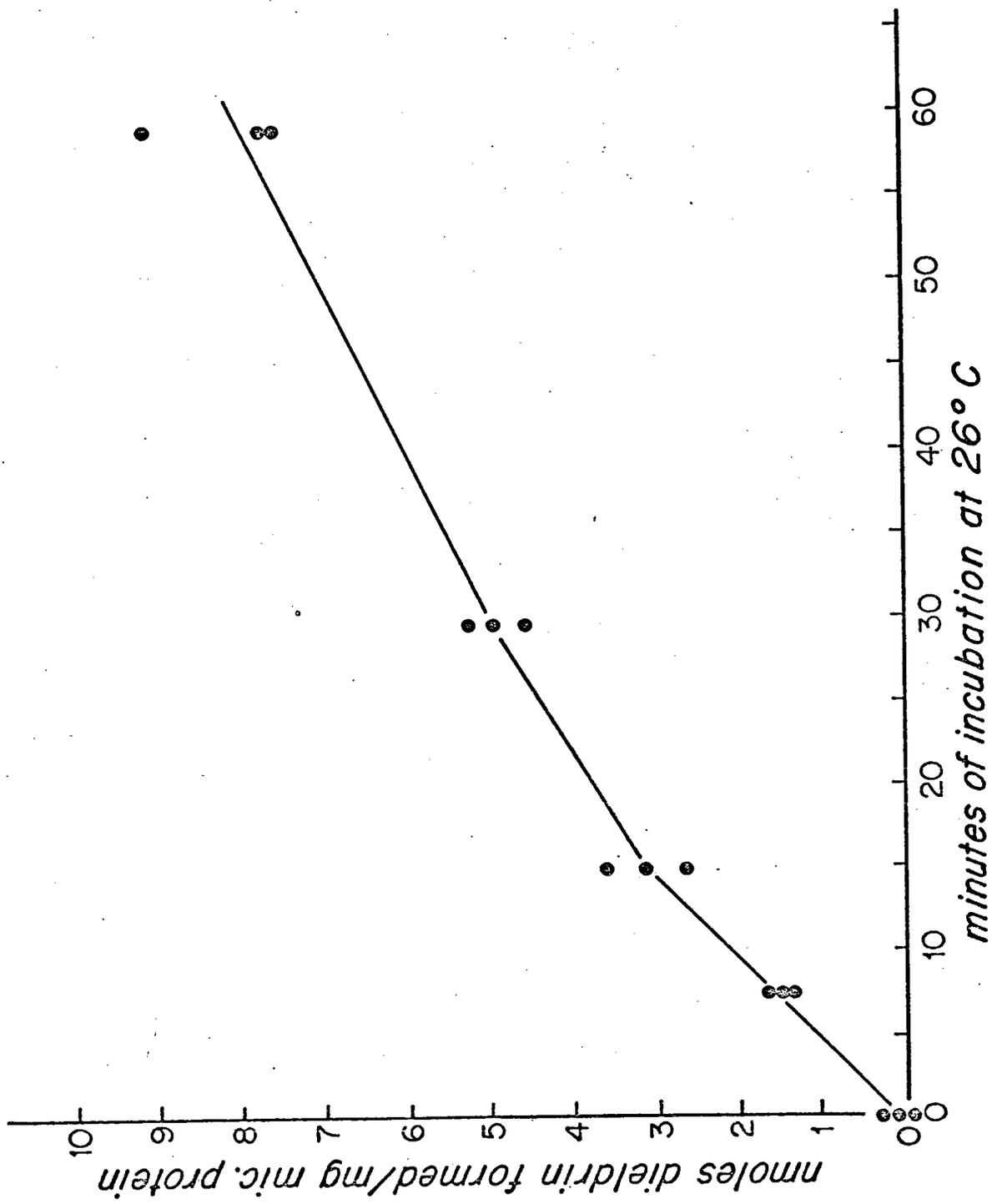


FIGURE 2

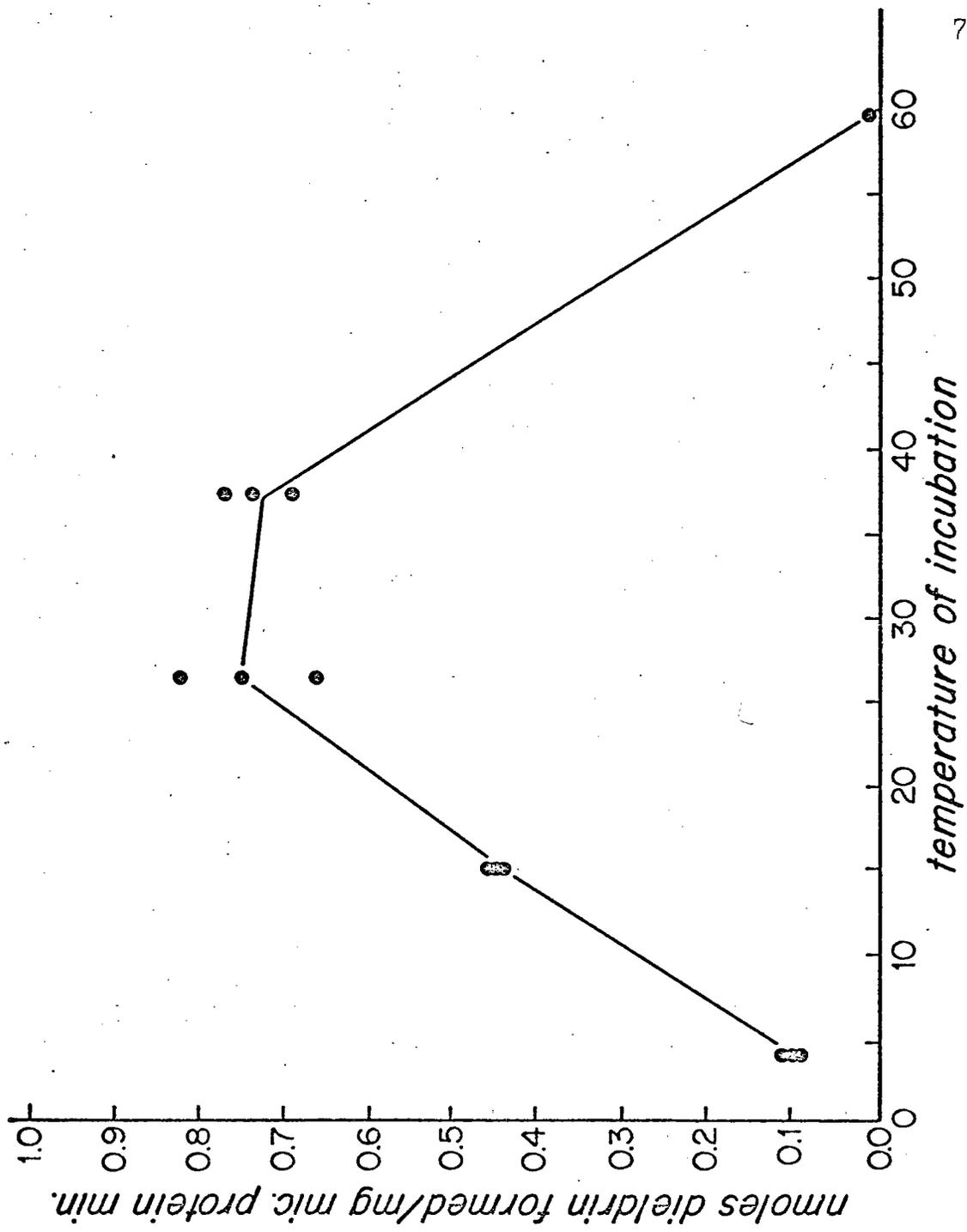


FIGURE 3

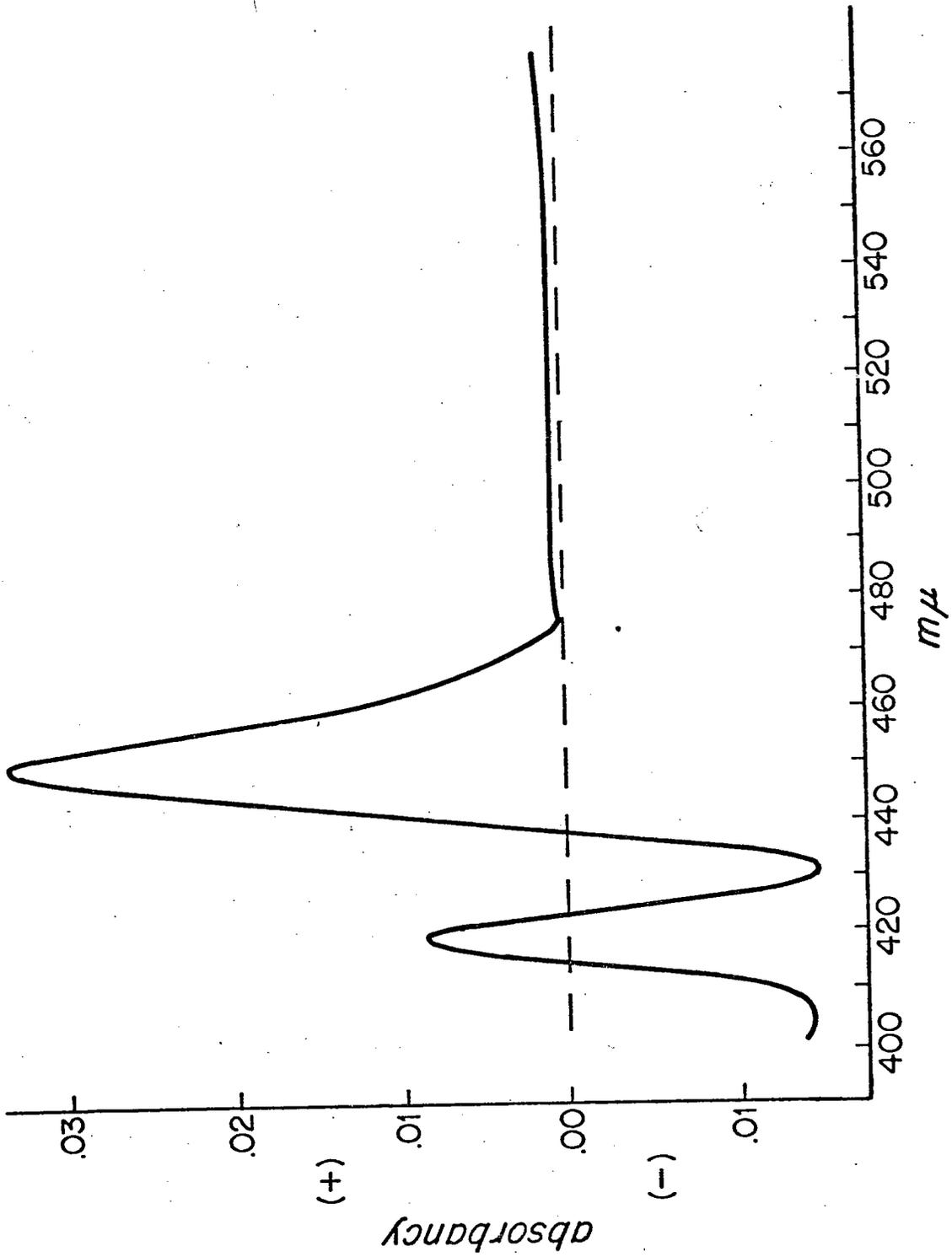


FIGURE 4

FIGURE 5

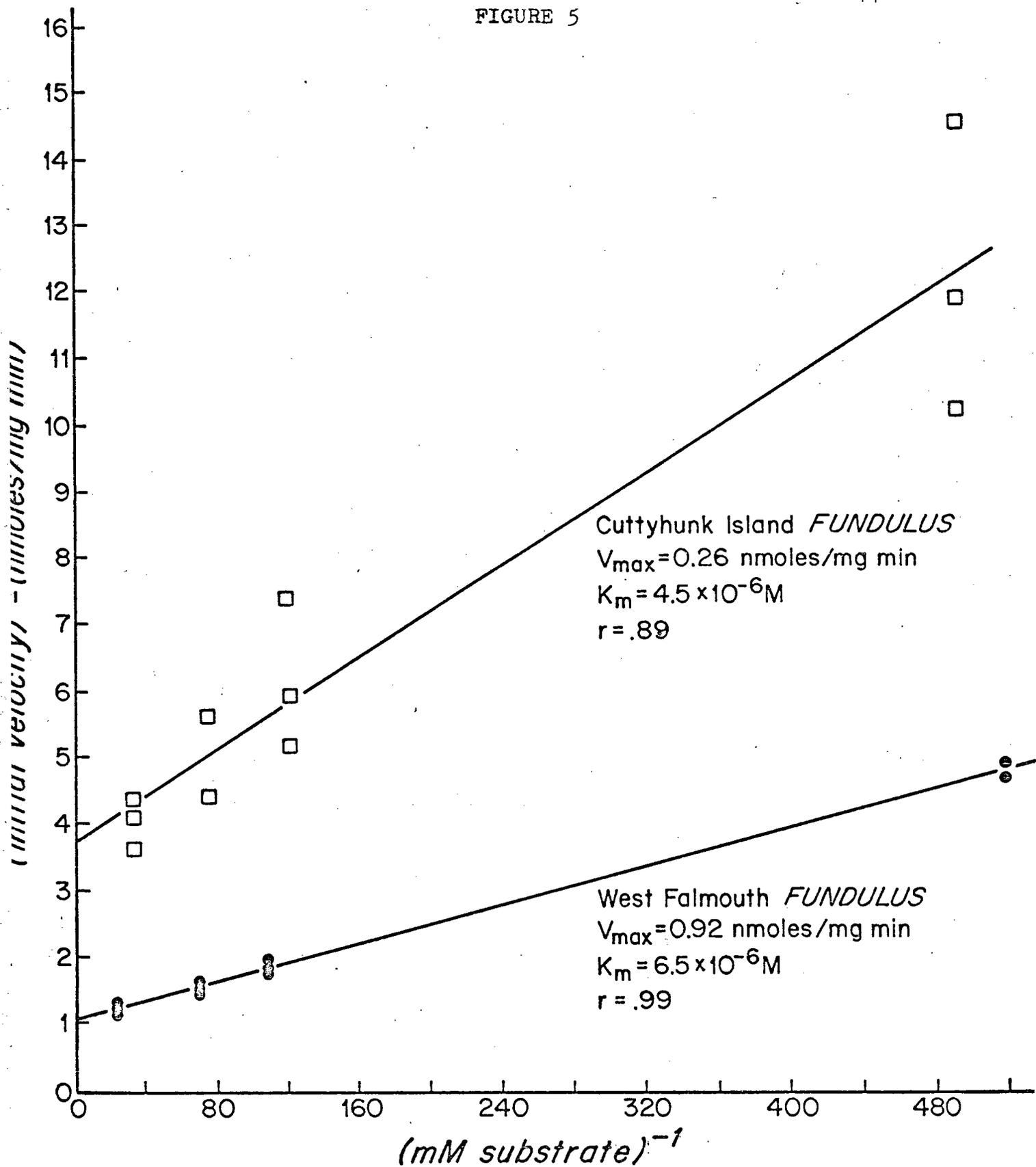


FIGURE CAPTIONS

- Fig. 1. Effect of amount of microsomal protein added per 2.0 ml assay on Aldrin epoxidation rate.
- Fig. 2. Epoxidation of Aldrin by Fundulus liver microsomes as a function of time.
- Fig. 3. Effect of temperature of incubation on the in vitro Aldrin epoxidation rate.
- Fig. 4. CO difference spectrum of liver microsomal suspension of F. heteroclitus. Microsomes were reduced with sodium dithionite and the baseline obtained. CO was bubbled through the sample cell 30 sec and the difference spectrum recorded. Protein concentration in this determination was 1.4 mg/ml.
- Fig. 5. (Substrate concentration)⁻¹ vs (initial velocity)⁻¹ for two populations of Fundulus. Km and Vmax values obtained by linear regression. γ is the regression constant. All conditions described in the assay procedure.

ACKNOWLEDGEMENTS

This research was supported by National Science Foundation Doctoral Dissertation Grant GA-40987, The Jesse Smith Noyes Foundation and the Woods Hole Oceanographic Institution. Drs. D. Sabo, G.R. Harbison, J. Stegeman, and J.M. Teal reviewed the manuscript. Contribution no. 3473 from the Woods Hole Oceanographic Institution.

CHAPTER 5

"PHYSIOLOGICAL ADAPTATIONS OF MARSH ANIMALS TO A
HYDROCARBON POLLUTED ENVIRONMENT: A DISCUSSION"

INTRODUCTION

The spill of about 175,000 gallons of Number 2 fuel oil near West Falmouth, Massachusetts in Buzzards Bay September, 1969, initially killed most marine life in heavily polluted areas. Sanders, et al. (1972) surveyed the benthic near-shore populations eight days later and reported 95% of the bottom animals dead or dying. Similar toxic effects were seen on beaches and marshes where windrows of dead organisms accumulated immediately after the spill (Sanders, 1973). Oil was absorbed into the sediments having long term effects on the survival of marine organisms (Blumer and Sass, 1972; Sanders, et al., 1972; Michael, et al., 1975; Krebs, et al., 1975). Heavily oiled areas of marsh showed a great reduction in higher plants, macrofauna, and algal photosynthesis during 1970 (Teal, personal communication).

The incorporation of petroleum hydrocarbons into the West Falmouth salt marsh ecosystem was monitored by analyzing surface sediments, deep mud cores, organisms surviving the spill, and organisms recolonizing the polluted area (Burns, 1975c). Heavily oiled areas showed the most complete kill of both plants and animals. Oil penetrated deep into the anoxic marsh muds where degradation is extremely slow. Analyses showed the persistence of oil in marsh sediments over the five years studied (Burns, 1975c). During this time animals had continuous exposure to oil hydrocarbons by eating oiled detritus and food organisms, by burrowing into the oiled muds, and by possible direct absorption from the water when oil was released by leeching from normal tidal flooding, burrowing,

and storm erosion of sediments. Analyses of animal tissues from the oiled areas in 1970 showed oil incorporation (Burns, 1975c). Analyses in subsequent years showed the continued uptake of petroleum into tissues of animals recolonizing the area.

Studies were begun to examine the effects of this chronic oil exposure on the population structure and physiology of two marsh animals showing different abilities to tolerate the pollution. Crustaceans were very sensitive to the oil as shown by observations by Sanders, et al. (1972) on amplicid amphipods and by Krebs (1973) on fiddler crabs. Uca pugnax, the mud fiddler, was chosen for study because of its sensitivity to the pollution and its abundance in the marsh fauna. Fish appeared less sensitive to the pollution and the marsh minnow, Fundulus heteroclitus, was chosen for comparison. The major aims of the studies described in this discussion were 1) to identify some physiological reasons for the difference in tolerance to oil pollution in these two species and 2) to determine if these animals could adapt to oil in their environment by changing their behavior, physiology, or genetics to tolerate higher levels of oil than a non-exposed population.

DISCUSSION OF DATA

Uca

In 1972 and 1973 population parameters of Uca inhabiting heavily oiled areas were compared to areas of the same marsh less heavily oiled and populations at the unaffected Sippewissett marsh further south on Buzzards Bay. Uca populations were very sensitive to the presence of oil (Krebs, et al., 1975). Populations were greatly reduced in oiled areas; sex and age structure was skewed toward adult males (that

19.

immigrated from surrounding unaffected areas); and settling juveniles showed low survival rates. Those crabs able to survive showed heavy winter mortality and reduced burrowing depth. Their escape responses were so slow as to be totally ineffective.

No quantitative measurements were made on the fish populations but they appeared much less affected than the crabs. Equal effort produced similar catches at West Falmouth and Sippewissett in 1973 and 1974 with no obvious differences in numbers, sex ratio, age structure, or behavior (Burns, unpublished).

I attempted to show adaptation of Uca to the oil polluted salt marsh in several ways. If adaptation had occurred, animals from the oiled areas should have been more tolerant to oil than animals from clean areas. Laboratory experiments were set up in which three groups of five adult male Uca (15) collected from Sippewissett and the same number from West Falmouth were fed detritus spiked with 100 parts per million Number 2 fuel oil. The effects of the ingested oil on the crabs' escape responses were measured by the technique of Krebs, et al. (1974). Ingestion of oil doubled escape response times in Uca with no significant difference between groups. Respiration measurements were made every two days during the experiments using polarographic oxygen electrodes (Kanwisher, 1959). All values were in the range reported for Uca by Teal and Carey (1969) with no consistent difference between groups. In another experiment three groups of 6 Uca from the two marshes were placed in three concentrations of fuel oil/seawater mixtures and the time for 50% mortality measured. No significant difference in tolerance was evident between crabs from the two marshes (Burns, unpublished).

Population data showed no significant ability of Uca to adapt to

high levels of oil in the marsh. Animal densities correlated directly with oil content of the muds. Recolonization of heavily oiled areas did not occur until leeching of oil from the sediments and bacterial decomposition reduced concentrations and changed chemical composition to tolerable limits. Hydrocarbon levels of about 100 to 200 $\mu\text{g}/\text{gm}$ wet mud were lethal to juvenile crabs and about 1,000 to 2,000 $\mu\text{g}/\text{gm}$ was lethal to immigrating adults. These estimates were based on weathered Number 2 fuel oil in marsh surface sediments (Krebs, et al., 1975).

I developed a more sensitive measure of the adaptability of marsh animals using biochemical methods. One mechanism of biochemical adaptation to this toxic environment would be the induction of high levels of detoxification or hydrocarbon metabolizing enzymes in animals to clear their bodies of incorporated oil. The system of microsomal enzymes termed mixed function oxidases (MFO), first described in mammals, is responsible for the oxidation and hydroxylation of steroids, drugs, and foreign compounds including hydrocarbons. The MFO system in general is capable of oxidizing a variety of substrates with cytochrome P-450 involved in the last step of the oxidation. I used Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,-endo,exo-5,8-dimethano-naphthalene) as the substrate because I needed the sensitivity of an electron capture detector and the low blank values obtained with this method to measure the slow rates of metabolism found in Uca. Use of this substrate as an indicator of the level of the MFO system is justified by the competitive inhibition seen in the in vitro oxidation of Aldrin by naphthalene (Khan, 1969), and cross resistance and cross induction phenomenon observed in other animals in response to Aldrin and other MFO substrates (Schonbrod, et al., 1965; Khan, et al., 1970). The oxidized products of the MFO system are more

polar than their highly lipid soluble substrates and can be discharged from body tissues by diffusion across cell membranes or by conjugation with serum components and excretion (Khan, et al., 1974). High levels of these enzymes have been shown to confer resistance to insecticides and hydrocarbons in insects, freshwater fish, and mammals (Fukami, et al., 1969).

I looked for hydrocarbon metabolizing ability in Uca and Fundulus to establish the presence and characteristics of the MFO system in these marine animals. If this system were operating as a mechanism of biochemical adaptation, animals living in the oil polluted area would show higher enzyme levels than controls, either through enzyme induction in individuals or through genetic selection of the population. The assay procedure and results are detailed in Burns (1975a,b).

In vitro rates of Aldrin metabolism in Uca were in the same range as those reported for freshwater crayfish and insecticide-susceptible insects (Table 1). No difference in MFO rates was seen when comparing crabs from clean or polluted areas. In spite of substantial juvenile settlement on the oiled marsh all five years (Krebs, et al., 1975) high MFO levels were not induced or selected for in Uca (Burns, 1975b). Other measures of a slow MFO rate in marine crustacea were given by Carlson (1973) and Pohl, et al. (1974).

Gas chromatographic analyses showed that hydrocarbons extracted from Uca collected in the oiled areas were very similar in composition to those of the surface muds on which these crabs feed (Burns, 1975c). No significant reduction in amount of incorporated hydrocarbons occurred in Uca tissues from 1970 to 1973. Thus it appeared that Uca did not have the ability to raise their MFO activity in response to the oil

polluted environment. They could not use the MFO system as an adaptive strategy to clear their body tissues of oil contamination.

Fundulus

A laboratory experiment with an enzyme inducing drug, phenylbutazone, showed that Fundulus had an inducible MFO system similar in its parameters to the enzymes described for mammals. Fundulus were able to oxidize Aldrin in vitro 100 times faster than Uca. Rates were similar to those seen in other fish and some insecticide-resistant insects, but 1/10th the rate in mammals (Table 1). Enzyme rates were twice as fast in fish living in hydrocarbon contaminated areas as compared to clean areas. Lineweaver-Burke plots showed a significant increase in maximum velocity of Aldrin oxidation but no significant increase in enzyme affinity for substrate. This is consistent with the hypothesis that induction had occurred in the West Falmouth population. (Burns, 1975a).

Hydrocarbon extracts of Fundulus tissues showed great differences in the composition of absorbed hydrocarbons between 1970 and 1974. The 1970 analysis reflected the whole composition of oil hydrocarbons to which the fish were exposed. In 1974 hydrocarbon content of Fundulus tissues was much lower than in 1970 and showed little resemblance to the composition they were exposed to either in the water column or detritus (Burns, 1975c). This indicated the MFO system in Fundulus liver in 1974 was operating fast enough to clear their body tissues of foreign hydrocarbons.

Further evidence that fish can use the hydrocarbon metabolizing system as part of an adaptive strategy to tolerate sublethal doses of oil pollution was provided by other observations. 1) Lee, et al. (1972) showed that in vivo rates of aromatic hydrocarbon oxidation in livers of marine fish were fast enough to reach a steady state where the amount of

substrate entering the liver (main site of oxidation) was balanced by the amount of metabolites leaving. Lee also noted rapid internal transport and excretion of hydrocarbon metabolites after fish were placed in clean seawater. 2) Studies by Sabo, et al. (1975) indicated increases in other cellular processes associated with induction of MFO in West Falmouth Fundulus had occurred by 1974. Electron micrographs showed proliferation of the rough endoplasmic reticulum in the livers of West Falmouth fish. (This would facilitate the increased protein synthesis used in MFO induction.) 3) Induction of MFO is thought to be specific. Only the enzymes involved in the oxidations and related systems are increased rather than a general increase in metabolic activity (Schimke, et al., 1968; Dehlingher and Schimke, 1972).

All this evidence indicates that at least some coastal fish can use the induction of high levels of mixed function oxidases and related metabolic changes as an adaptive strategy to clear their body tissues of some classes of incorporated hydrocarbons.

However, caution must be employed in attempting to generalize the effects of exposure to foreign hydrocarbons in the environment on marine fish. The survival of fish in a toxic environment shows great species variation depending on the physiology and behavior of the species and on the nature of the pollutant.

The metabolites of certain compounds are more toxic than the parent compounds. The intermediary oxides of polynuclear aromatic hydrocarbons are electrophilic and have been shown to bind to the proteins and nucleic acids of the cell before hydrolysis could occur (Jerina and Daly, 1974). The reaction of epoxides with nucleic acids is a proposed mechanism of carcinogenesis for some hydrocarbons. Synergistic action between

different hydrocarbons should be expected since induction of increased metabolism of one compound or class of compounds affects the rate of oxidation of others. Hormones are also substrates for the MFO system and prolonged induction may result in hormonal imbalance and reproductive failure (Conney, 1967). Increased oxidation rates of other steroids such as cholecalciferol (Vitamin D₃) may result in alterations of calcium metabolism (Villareate, et al., 1974). Thus, while the MFO system represents part of a potentially adaptive mechanism in some fish to clear their tissues of hydrocarbon pollution, detrimental physiological effects may result from prolonged induction of these enzymes.

CONCLUSIONS

Fiddler crabs were very sensitive to oil in their environment. Uca had no significant ability to change their physiology or behavior to adapt to toxic quantities of hydrocarbons and significantly raise their tolerance levels. Uca had low MFO rates and could not significantly increase them in response to oil pollution either through enzyme induction or genetic selection. This minimal biochemical adaptability and life style of burrowing into marsh muds, even when oil contaminated, accounts for the relative sensitivity of Uca to this type of chemical pollution. The crab populations at West Falmouth will remain disrupted and dependent on migration of new stocks from adjacent unoiled areas until natural processes reduce hydrocarbon content and composition of the salt marsh muds to crab-tolerable levels.

Fundulus were less sensitive to oil in their environment than Uca. Their MFO systems were operating fast enough by 1974 to clear their body tissues of contaminating hydrocarbons. Fast MFO rates are related

to high tolerance to hydrocarbons. Fundulus had raised their MFO levels in response to the oil polluted environment and therefore adapted.

IMPLICATIONS FOR OIL SPILLS

To predict the impact of oil pollution on an ecosystem, research should be directed at the most sensitive species selected for study for their importance in maintenance of the total system. Oil can disrupt the biology of organisms in many ways. Information on the sensitive species should include the effects of pollution on individual parameters such as behavior and physiology, as well as population parameters such as abundance, age and sex distributions, survival, and reproductive success.

Observations on Uca and Fundulus agree with the general trend summarized by Moore, et al. (1974) of fish being more tolerant of dissolved aromatic hydrocarbons than crustacea. I have provided evidence for some of the physiological reasons for the relative sensitivity and adaptability of salt marsh animals to oil pollution.

The West Falmouth oil spill data shows that some species are very sensitive to oil in their environment and are subject to high mortality over long time spans (more than five years in a northern salt marsh). Some are not significantly able to adapt to the presence of petroleum in the marine environment. Areas killed by this local disaster were dependent on migration of new stocks from unaffected areas for recovery. Thus, in order to insure the survival of sensitive species in spite of the projected increase in offshore oil drilling and transport, estuarine sanctuaries must be established as provided for in the Coastal Zone

Management Act of 1972. In addition, effective measures must be taken to exclude accidental petroleum contamination.

Acknowledgement

Thanks are extended to Taber Hand of Pomona College, Calif. for help with the respiration and behavior experiments.

TABLE 1

Rates of Aldrin oxidation by microsomes of various species

Species	Rate of Aldrin oxidation (pmoles/mg protein min.)	Reference
Cambarus (crayfish)* hepatopancreas	1.1	Khan, <u>et al.</u> , 1972
green gland	2.9	
Uca (fiddler crab) hepatopancreas	1.6	Burns, 1975b
claw muscle	3.8	
gills	7.3	
Musca domestica (housefly) DDT-susceptible	1.8	Khan & Terriere, 1968
DDT-resistant	14.3	
Heliothis zea (corn earworm)	200	Chandran & Khan, 1972
Stenotomus (coastal fish)	106	Burns, unpublished
Fundulus (marsh minnow) clean marsh	250-400	Burns, 1975a
hydrocarbon polluted	550-750	
Lepomis (sunfish) adult	440	Stanton & Khan, 1974
fry	1,450	
Mus muscalus (mouse)	3,450	"

* Data for crayfish was not expressed in specific activity by the authors, therefore to allow comparison, I assumed a content of microsomal protein per gram wet weight similar to Uca.

REFERENCES

- Ahearn, D.G. and S.P. Meyers. 1973. ed. The Microbial Degradation of Crude Oil Pollutants. La.State Un. publ. LSU-SG-73-01.
- Adamson, R.H. 1967. "Drug metabolism in marine vertebrates." Federation Proceedings (FASEB) 26(4), 1047-1055.
- American Society for Testing Materials (ASTM). 1967. "Standard method of test for hydrocarbon types in middle distillates by mass spectrometry." D2425-67.
- Blumer, M. 1973. "Preparation of porous layer open tubular columns by dynamic coating and rapid conditioning." Analytical Chemistry 45, 980-982.
- Blumer, M., J. Sass, G. Souza, H. Sanders, F. Grassle, and G. Hampson. 1970a. "The West Falmouth oil spill." Woods Hole Oceanographic Institution Technical Report 70-44. 32 pg. (unpublished manuscript).
- Blumer, M., G. Souza, and J. Sass. 1970b. "Hydrocarbon pollution of edible shellfish by an oil spill." Marine Biology 5, 195-202.
- Blumer, M. and J. Sass. 1972. "The West Falmouth oil spill II. Chemistry." Woods Hole Oceanographic Institution Technical Report 92-19. (unpublished manuscript) 60 pg.
- Booth, J. and E. Boyland. 1958. "Metabolism of polycyclic compounds 13: Enzymatic hydroxylation of naphthalene by rat liver microsomes." Biochemical Journal 70, 681-688.
- Buhler, D.R. and M.E. Rasmusson. 1968. "The oxidation of drugs by fishes." Comparative Biochemistry and Physiology 25, 223-239.
- Boyland, D.B. and B.W. Tripp. 1971. "Determination of Hydrocarbons in seawater extracts of crude oil and crude oil fractions." Nature 230, 44-47.

- Burns, K.A. and J.M. Teal. 1971. "Hydrocarbon incorporation into the salt marsh ecosystem after the West Falmouth oil spill." Woods Hole Oceanographic Institution Technical Report 70-69. 24 pg. (unpublished manuscript).
- Burns, K.A. and J.M. Teal. 1973. "Hydrocarbons in the pelagic Sargassum community." Deep Sea Research 20, 207-211.
- Burns, K.A. 1975a. "Microsomal mixed function oxidases in an estuarine fish, Fundulus heteroclitus, and their induction as a result of environmental contamination." Comparative Biochemistry and Physiology (in press).
- Burns, K.A. 1975b. "Hydrocarbon metabolism in the intertidal fiddler crab, Uca pugnax." (in preparation).
- Burns, K.A. 1975c. "Distribution of hydrocarbons in a salt marsh ecosystem after an oil spill and physiological changes in marsh animals from the polluted environment." Doctoral Dissertation. M.I.T./W.H.O.I.
- Burns, K.A. 1975d. "Physiological adaptations of marsh animals to a hydrocarbon polluted environment: A discussion." (in preparation).
- Butner, A. and B.H. Brattstrom. 1960. "Local movements in Menidia and Fundulus." Copeia 139-141.
- Carlson, G.P. 1973. "Comparison of the metabolism of Parathion by lobsters and rats." Bulletin of Environmental Contamination and Toxicology 9(5), 296-300.
- Casida, J.E. 1968. "Insect microsomes and insecticide chemical oxidations." In: Microsomes and Drug Oxidations (ed. by Gillette, Conney, Cosmides, Estabrook, Fouts, and Mannering), Academic Press, Inc.

- Chan, T.M., J.W. Gillett, and L.C. Terriere. 1967. "Interaction between microsomal electron transport systems of trout and male rat in cyclodiene epoxidation." *Comparative Biochemistry and Physiology* 20, 731-742.
- Chandran, S.R. and M.A.Q. Khan. 1972. "Microsomes: Mixed function oxidase in the mid-gut of the corn earworm." *Journal of Economic Entomology* 65(5), 1510-1512.
- Conney, A.H. 1967. "Pharmacological implications of microsomal enzyme induction." *Pharmacological Reviews* 19(3), 317-365.
- Corner, E.D.S., C.C. Kilvington, and S.C.M. O'Hara. 1973. "Qualitative studies on the metabolism of naphthalene in Maia squinado, (spider crabs)." *Journal of the Marine Biological Association of the United Kingdom* 54(4), 819-832.
- Davis, J.B. 1968. Petroleum Microbiology. American Elsevier Press, Inc.
- Dehlinger, P.J. and R.T. Schimke. 1972. "Effects of phenobarbital, 3-methylcholanthrene, and hematin on the synthesis of membrane components of rat liver microsomal membranes." *Journal of Biological Chemistry* 247(4), 1257-1264.
- Dixon, M. and E.C. Webb. 1964. Enzymes. Academic Press, Inc., New York.
- Farrington, J.W., J.M. Teal, J.G. Quinn, P.L. Parker, K. Winters, T.L. Wade, and K.A. Burns. 1974. "Analyses of hydrocarbons in marine organisms: Results of I.D.O.E. intercalibration exercises." *Proceedings of Marine Pollution Monitoring (Petroleum)-Symposium and Workshop May 13-17, 1974, Gaithersburg, Md., U.S.A.*

- Farrington, J.W., J.M. Teal, J.G. Quinn, T. Wade, and K.A. Burns. 1973. "Intercalibration of analyses of recently biosynthesized and petroleum hydrocarbons in marine lipids." *Bulletin of Environmental Contamination and Toxicology* 10(3), 129-136.
- Fukami, J.I., T. Shishido, K. Fukunaga, and J.E. Casida. 1969. "Oxidative metabolism of Rotenone in mammals, fish, and insects and its relation to selective toxicity." *Journal of Agriculture and Food Chemistry* 17(6), 1217-1226.
- Guarino, A.M., R.H. Janicki, and W.B. Kinter. 1971. "Distribution and metabolism of ¹⁴C-DDT in the eel (*Anguilla rostrata*) after six hours of uptake from ambient water." *Bulletin of the Mt. Desert Island Biological Laboratory* 11, 23-24.
- Hamelink, J.L., R.C. Waybrant, R.C. Ball. 1971. "A proposal: Exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments." *Transactions of the American Fisheries Society* 100, 207-214.
- Horn, M.H., J.M. Teal, R.H. Backus. 1970. "Petroleum lumps on the surface of the sea." *Science* 168, 245-246.
- Jerina, D.M. and J.W. Daly. 1974. "Arene oxides: A new aspect of drug metabolism." *Science* 185, 573-582.
- Kanwisher, J.W. 1959. "Polarographic oxygen electrode." *Limnology and Oceanography* 4, 210-217.
- Khan, M.A.Q. 1969. "Some biochemical characteristics of the cyclodiene epoxidase and its inheritance in the housefly." *Journal of Economic Entomology* 62(2), 388-392.
- Khan, M.A.Q. and L.C. Terriere. 1968. "DDT-dehydrochlorinase activity in housefly strains resistant to various groups of insecticides." *Journal of Economic Entomology* 61(3), 732-736.

- Khan, M.A.Q., J.L. Chang, D.J. Sutherland, J.D. Rosen, and A. Kamal. 1970. "Housefly microsomal oxidation of some foreign compounds." *Journal of Economic Entomology* 63(3), 1807-1813.
- Khan, M.A.Q., W. Coello, A.A. Khan, H. Pinto. 1972a. "Some characteristics of the microsomal mixed function oxidase in the freshwater crayfish, Cambarus." *Life Sciences* 11(2), 405-415.
- Khan, M.A.Q., A. Kamal, R.J. Wolin, J. Runnels. 1972b. "In vivo and in vitro epoxidation of Aldrin by aquatic food chain organisms." *Bulletin of Environmental Contamination and Toxicology* 8(4), 219-228.
- Khan, M.A.Q., R.H. Stanton, G. Reddy. 1974. "Detoxification of foreign chemicals by invertebrates." In: Survival in Toxic Environments (ed. by M.A.Q. Khan and J.P. Bederka) Academic Press Inc. 558 pg.
- Krebs, C.T. @1975. Doctoral Dissertation. Boston University Marine Program. Boston, Mass., U.S.A.
- Krebs, C.T. 1973. "Qualitative observations of the marsh fiddler crab, Uca pugnax, populations in Wild Harbor Marsh following the September, 1969 West Falmouth oil spill." National Academy of Sciences, Washington, D.C. (unpublished manuscript).
- Krebs, C.T., K.A. Burns, and J.M. Teal. 1975. "Long term effects of hydrocarbon contamination of a salt marsh on populations of the fiddler crab, Uca pugnax." Paper presented at the American Society for Limnology and Oceanography Meetings in Dalhousie, N.S., June, 1975.
- Krebs, C.T., I. Valiela, G.R. Harvey, and J.M. Teal. 1974. "Reduction of field populations of fiddler crabs, Uca pugnax, by uptake of chlorinated hydrocarbons from sewage sludge." *Marine Pollution Bulletin* 5(9), 140-142.

- Lee, R.F., R. Sauerheber, and A.A. Benson. 1972b. "Petroleum hydrocarbons: Uptake and discharge by the marine mussel, Mytilus edulis." Science 177, 344-346.
- Lee, R.F., R. Sauerheber, G.H. Dobbs. 1972a. "Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish." Marine Biology 17, 201-210.
- Lotrich, V.A. 1974. "Summer home range and movement of Fundulus heteroclitus in a tidal creek." Ecology (in press).
- Lowry, O.H., N.J. Rosenbrough, A.L. Farr, and R.J. Randall. 1951. "Protein measurements with the folin phenol reagent." Journal of Biological Chemistry 193, 265-275.
- Michael, A.D., C.R. Van Raalte, L.S. Brown. 1975. "Long term effects of an oil spill at Wild Harbor, Massachusetts." Proceedings of Joint Conference on Prevention and Control of Oil Spills, American Petroleum Institute.
- Miyake, Y., J.L. Gaylor, and H.P. Morris. 1974. "Abnormal microsomal cytochromes and electron transport in morris hepatomas." Journal of Biological Chemistry 249(6), 1980-1987.
- Moore, R.F., G.R. Chirlin, G.J. Puccia, B.P. Schrader. 1974. "Potential biological effects of hypothetical oil discharge in the Atlantic Coast and Gulf of Alaska." Report to the Council on Environmental Quality MITSG 74-19. 121 pg.
- Morris, B.F. 1971. "Petroleum: Tar quantities floating in the North-western Atlantic taken with a quantitative neuston net." Science 173, 430-432.
- Mostert, Noel. 1974. Supership. Alfred A. Knopf, Publisher. New York.

- National Academy of Sciences (N.A.S.). 1975. Petroleum in the Marine Environment. Report of Workshop on Inputs, Fates and Effects of Petroleum in the Marine Environment. May, 1973. 107 pg.
- Nelson-Smith, A. 1973. Oil Pollution and Marine Ecology. Plenum Press, New York. 260 pg.
- Odum, W.E., G.M. Woodwell, C.F. Wurster. 1969. "DDT residues absorbed from organic detritus by fiddler crabs." *Science* 164, 576-577.
- Omura, T. and R. Sato. 1964. "The carbon monoxide binding pigment of liver microsomes I and II." *Journal of Biological Chemistry* 239(2), 370-385.
- Pedersen, M.G., W.K. Hershberger, and M.R. Juchau. 1974. "Metabolism of 3,4-Benzpyrene in rainbow trout, Salmo gairdneri." *Bulletin of Environmental Contamination and Toxicology* 12(4), 481-486.
- Perry, J.J. and C.E. Cerniglia. 1973. "Studies on the degradation of petroleum by filamentous fungi." In: The Microbial Degradation of Oil Pollutants (ed. by D.G. Ahearn and S.P. Meyers) pg. 89-94. Louisiana State Univ. Publication no. LSU-SG-73-01.
- Pohl, R.J., J.R. Bend, A.M. Guarino, and J.R. Fouts. 1974. "Hepatic microsomal mixed function oxidase activity in several species from coastal Maine." *Drug Metabolism and Disposition* 2(6), 545-555.
- Pritchard, J.B., A.M. Guarino, and W.B. Kinter. 1971. "Metabolism of ¹⁴C-DDT by the winter flounder, Pseudopleuronectes americanus." *Bulletin of the Mt. Desert Island Biological Laboratory* 11, 81-83.
- Reinert, R.E. 1967. "The accumulation of Dieldrin in an alga, daphnia, guppy food chain." *Doctoral Dissertation*. University of Michigan. 76 pg.

- Sabo, D.J., J.J. Stegeman, and L.S. Gottlieb. 1975. "Petroleum hydrocarbon pollution and hepatic lipogenesis in the marine fish, Fundulus heteroclitus." Abstract in Federation Proceedings (FASEB).
- Sanders, H.L. 1973. "Some biological effects related to the West Falmouth oil spill." Background papers vol. 2 to Petroleum in the Marine Environment. National Academy of Sciences Report.
- Sanders, H.L., F. Grassle, G.R. Hampson. 1972. "The West Falmouth oil spill I. Biology." W.H.O.I. Technical Report 72-20. (unpublished manuscript) 23 pg.
- Schimke, R.T., R. Ganschow, D. Doyle, I. Arias. 1968. "Regulation of protein turnover in mammalian tissues." Federation Proceedings (FASEB) 27(5), 1223-1230.
- Schonbrod, R.D., W.W. Philleo, and L.C. Terriere. 1965. "Hydroxylation as a factor in resistance in houseflies and blowflies." Journal of Economic Entomology 58(1), 71-77.
- Schrader, B.P., S.F. Moore, B.A. Ackerman, A.D. Long, and L.H. Tower. 1974. "Potential biological effects of hypothetical oil spills occurring in the nearshore waters of Long Island's south shore." Final report to Nassau-Suffolk Regional Planning Board. 217 pg.
- Stanton, R.H. and M.A.Q. Khan. 1974. "Mixed function oxidase activity towards cyclodiene insecticides in bass and bluegill sunfish." Pesticide Biochemistry and Physiology 3(4), 351-357.
- Stegeman, J.J. and J.M. Teal. 1973. "Accumulation, release, and retention of petroleum hydrocarbons by the oyster, Crassostrea virginica." Marine Biology 22, 37-44.
- Teal, J.M. Woods Hole Oceanographic Institution (personal communication).

- Teal, J.M. and F.G. Carey. 1969. "Metabolism of marsh crabs under conditions of reduced O₂ pressure." *Physiological Zoology* 40, 83-91.
- Villareale, M., L.V. Gould, R.H. Wasserman, A. Barr, R.T. Chiroff, and W.H. Bergstrom. 1974. "Diphenylhydantoin: Effects on calcium metabolism in the chick." *Science* 183, 671-673.
- Wright, J. 1972. "Growth, mortality, production and consumption of a population of the salt marsh killifish, Fundulus heteroclitus." M.A. Thesis, Boston University, 46 pg.
- Zitko, V. and W.V. Carson. 1970. "The characterization of petroleum oils and their determination in the aquatic environment." *Fisheries Research Board of Canada Technical Report no. 217*. 29 pg.

APPENDIX

Bibliography of Other Papers Published During Doctoral Program

- K.A. Burns and J.M. Teal. 1971. "Hydrocarbon incorporation into the salt marsh ecosystem from the West Falmouth oil spill." W.H.O.I. Technical Report No. 71-69. 24 pg.
- K.L. Smith, Jr., K.A. Burns, and J.M. Teal. 1972. "In situ respiration of benthic communities in Castle Harbor, Bermuda." Marine Biology 12(3), 196-199.
- K.L. Smith, Jr., K.A. Burns, and E.J. Carpenter. 1973. "Respiration of the pelagic Sargassum community, a preliminary study." Deep Sea Research 20, 213-217.
- K.A. Burns and J.M. Teal. 1973. "Hydrocarbons in the pelagic Sargassum community." Deep Sea Research 20, 207-211.
- J.W. Farrington, J.M. Teal, J.G. Quinn, T. Wade, and K.A. Burns. 1973. "Intercalibration of analyses of recently biosynthesized hydrocarbons and petroleum hydrocarbons in marine lipids." Bulletin of Environmental Contamination and Toxicology 10(3), 129-136.
- J.W. Farrington, J.M. Teal, J.G. Quinn, P.L. Parker, K. Winters, T. Wade, and K.A. Burns. 1974. "Analyses of hydrocarbons in marine organisms: Results of I.D.O.E. intercalibration exercises." Proceedings of Marine Pollution Monitoring (Petroleum) Symposium and Workshop. May 13-17, 1974. Washington, D.C., sponsored by the Intergovernmental Oceanographic Commission, the World Meteorological Organization, and the U.S. Department of Commerce.

Biographical Information

I. Kathryn A. Burns

Box 628 Woods Hole, Mass 02543

Born: December 1, 1948 in Detroit, Michigan

Married; no children

II. Education:

1966-1970 Michigan State University. B.S. in
Biochemistry cum laude

Honors: member $\Lambda\Lambda\Delta$ womens honorary society
member $\beta\beta\beta$ biological honorary society
scholarships from the State of Michigan
N.S.F. summer fellowship in biochemistry

Employed part time as laboratory assistant for
Dr. Fritz Rottman, Dept. Biochemistry, M.S.U.

1970-1975 Massachusetts Institute of Technology/ Woods
Hole Oceanographic Institution Joint Program in
Biological Oceanography PhD. June, 1975

Advisors: Dr. John M. Teal, Dr. George Harvey, W.H.O.I.
and Dr. Phillip Robbins M.I.T.

Thesis work on the recovery of a salt marsh from the
effects of petroleum hydrocarbon pollution with
emphasis on sediment and organism hydrocarbon
chemistry, and metabolic biochemistry and physiol-
ogy of hydrocarbons in the marine intertidal fiddler
crab, *Uca pugnax*, and minnow, *Fundulus heteroclitus*.