from variations in oxygen isotope ratios with depth in the long ice core from Byrd Station (80°S, 120°W). Epstein *et al.* (*9*) and Johnsen *et al.* (*10*) have calculated tentative time scales in calendar years. Epstein *et al.*, by assuming that ice thickness, vertical strain rate, and accumulation rate have remained constant, have concluded that temperatures rose after a minimum 27,000 years ago, and then fell to a lower minimum 17,000 years ago. From 17,000 to 11,000 years ago, temperatures rose without interruption. They suggest that the date of 17,000 years ago is remarkably close to the culmination of the Late Wisconsin glaciation in North America. Johnsen *et al.* believe that the glaciological regime at Byrd Station is much too complicated for simplistic assumptions to be valid. They conclude that, with present knowledge, any suggested time scale for the Byrd core will be encumbered by considerable error. Tentatively, as the best approximation that is possible with present data, they suggest an oxygen isotope curve which is reproduced in part in Fig. 2. However, it is important to remember that the ^{14}C year and calendar year time scales are not equivalent, and that these two scales perhaps diverge greatly before 14,000 ^{14}C years B.P. (*11*).

Glacier variations in Chile and eastern North America imply a pronounced interstade centered at approximately 15,500 ^{14}C years B.P., preceded by a prolonged cold interval peaking at about 20,000 to 19,000 years B.P., and followed by a brief and final return to full-glacial cold peaking at approximately 14,500 to 14,000 years B.P. This finding strongly suggests a global temperature oscillation, but, before this sequence can be more confidently so described, the evidence for a New Zealand glacier readvance at about 16,000 years B.P. needs reexamination. An oscillation of temperature that might correspond to the North American Erie Interstade and to the Chilean Varas Interstade, and the following readvances, is not evident in the Byrd Station oxygen isotope ratio curve, which shows more resemblance to the New Zealand data. However, more information is needed on the relation of calendar years to ^{14}C years before significant comparisons can be made between the Byrd Station oxygen isotope curve and glacier fluctuations dated by ^{14}C.

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3 January 1972

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Oil Pollution: Persistence and Degradation of Spilled Fuel Oil

Abstract. *In September 1969, approximately 600 metric tons of number 2 fuel oil were spilled in Buzzards Bay, Massachusetts. Two years later, fuel oil hydrocarbons still persisted in the marsh and in offshore sediments. Hydrocarbon degradation is slow, especially below the immediate sediment surface and appears to proceed principally through microbial utilization of alkanes and through partial dissolution of the lower-boiling aromatic hydrocarbons. The boiling range of the spilled oil and the relative abundances of homologous hydrocarbons (for example, phytane and pristane) have been well preserved. The findings are in agreement with the known geochemical stability of hydrocarbons. Fuel oil is an appreciable fraction of whole crude oil. This fact suggests that oil products and crude oils have a considerable environmental persistence.*

Oil pollution of the sea and the public awareness of it have increased rapidly in recent years. In spite of this, little information exists on the persistence and long-term effect of hydrocarbon pollutants in the marine environment.

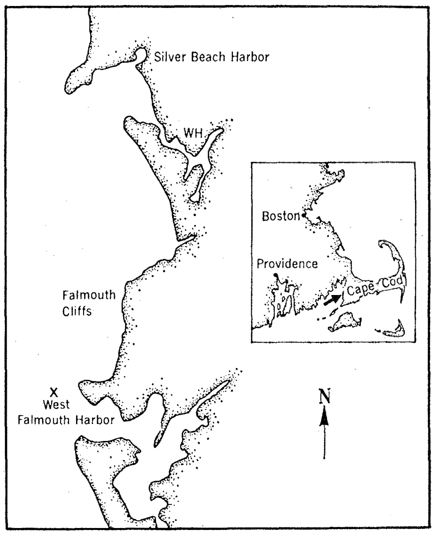

Fig. 1. Site of the September 1969 Buzzards Bay oil spill (×). Samples described here were taken at Silver Beach Harbor and Wild Harbor River (*WH*). For analyses from additional sites, see (*3*).

The fate of fuel oil from a spill in Buzzards Bay, off West Falmouth, Massachusetts, on 16 September 1969 (Fig. 1) has been under investigation for more than 2 years. The relatively small spill involved the discharge from a stranded barge of approximately 600 metric tons of number 2 fuel oil into the coastal waters and marshes. It led to persistent pollution of the sediments and of the fisheries resources (*1–3*).

Among the events that followed the 1969 spill, we can distinguish three distinct, although partly overlapping, series of events. Within the first few hours or days after the accident there was a heavy kill of organisms that came into contact with the oil; the effect extended over all phyla and over benthic and intertidal organisms (*2, 4*).

Next, within weeks or months after the spill, the oil spread to areas that had not been affected initially, and the kill extended, although in some cases more slowly than the spread of the oil, to outlying areas. For a considerable time after the spill, the oil prevented resettlement of the sediments by the original fauna. At present, degradation of the oil is evident; concurrently with the chemical changes in the oil, the immediate toxicity of the oil in the sediment has been reduced. This reduction in the toxicity has permitted resettlement of the polluted region, first by the most resistant faunal opportunists and later by a more varied and normal fauna (*4*).

Our analyses show the slow decrease of the oil content of the sediments from the high initial values in the marshes [Wild Harbor River (Fig. 2A), predominantly sand with clay layers] and offshore [station 31, Silver Beach Harbor (Fig. 2B), predominantly clay with sand; water depth, 1.5 m]. At both locations the oil content in the uppermost 2½ cm of sediment has remained well above the content of the indigenous sedimentary hydrocarbons, which average 5 to 7 mg per 100 g of dry sediment. The steep increase in the oil content at station 31 between December 1969 and March 1970 is attributed to the redistribution of oil from the same spill, since the gas chromatograms of the sediment extracts before and after this time agree in terms of boiling range, boiling point distribution, and characteristic detail and since no new spill occurred in that area. The degradation of the oil appears to involve both bacterial utilization and partial dissolution. Our experience with field samples confirms qualitatively those laboratory observations that indicated a decrease in the degradation rate from normal alkanes to iso- and cycloalkanes and to aromatic hydrocarbons. The ratio of *n*-heptadecane to pristane [n-C_{17}/pristane (Fig. 3, B and C)], is a sensitive indicator of incipient oil degradation (*2, 3*). It decreases more rapidly in the marshes and in lightly polluted offshore sediments than at the heavily polluted station 31; presumably, this reflects differences in the availability of oxygen. At station 31, the n-C_{17}/pristane ratio remained nearly constant for the first 3 months after the spill; a slight decrease in December 1969 was followed by a rise, which coincided with the steep rise in pollution level at that station in March 1970. A similar trend in the n-C_{17}/pristane ratio at more distant stations during the same period suggests the seaward spread of the fuel oil from the heavily polluted marshes and inshore regions during the first winter after the spill (*2, 3*).

The rate of bacterial degradation, even of the *n*-alkanes, is much lower in the environment than under laboratory conditions with the use of aeration, nutrients, agitation, and elevated temperatures. After 2 years small amounts of *n*-alkanes still persisted. This finding is not surprising, however, in view of the well-documented geochemical stability of sedimentary hydrocarbons (*5*). Branched and cyclic hydrocarbons are attacked even more slowly than the *n*-alkanes; after 2 years, isoprenoids (phytane, pristane, and the C_{18} homolog are evident) and alicyclic and aromatic hydrocarbons remained prominent in the polluted sediments.

Mass spectral analyses of the aromatic hydrocarbons at station 31 from March 1970 to April 1971 show a relative increase in the more highly substituted benzenes, naphthalenes, and tetrahydronaphthalenes at the expense of the lower homologs, especially of the most soluble compounds, such as naphthalene and the C_1 to C_3 alkylnaphthalenes. This result suggests that dissolution is more important than bacterial utilization of the aromatic hydrocarbons; the latter process would not show the same molecular weight dependence.

The ratio of saturated to aromatic hydrocarbons in the fuel oil in the sediments at station 31 remained nearly constant from March 1970 to April 1971. Thus the overall rate of depletion of the saturated and aromatic hydrocarbons is the same, even if there is preferred bacterial degradation or dissolution of some components within each group.

Analyses of a core from station 31 in September 1971 show the presence of fuel oil to a depth of 7.5 cm below

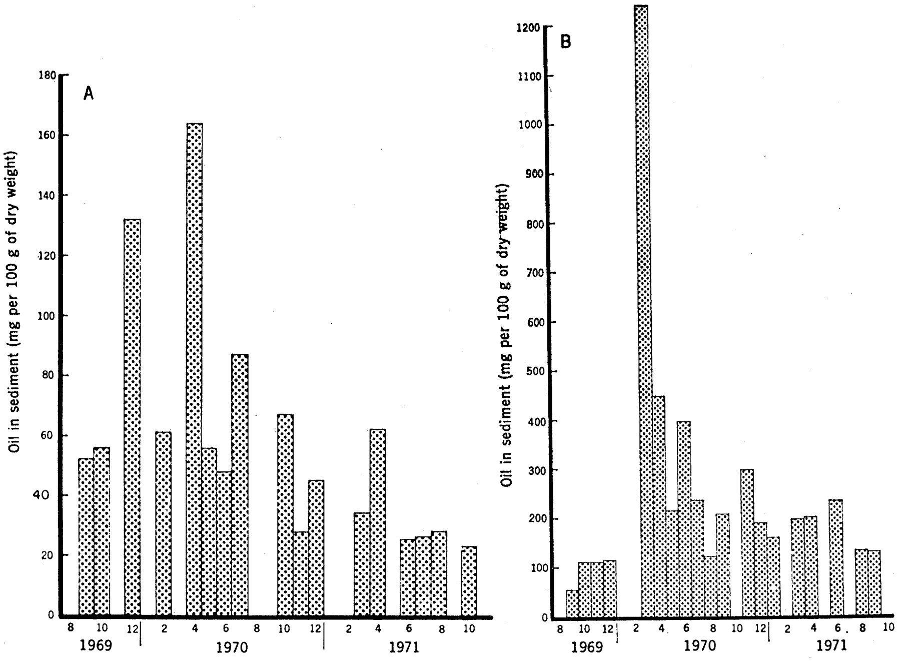

Fig. 2. Hydrocarbon content of marsh and offshore sediments, affected by the fuel oil spill in Buzzards Bay. (A) Wild Harbor River stations 2, 4, and 5 (average); (B) Silver Beach Harbor station 31. The weight of oil was determined according to the method of Blumer and Sass (*3*).

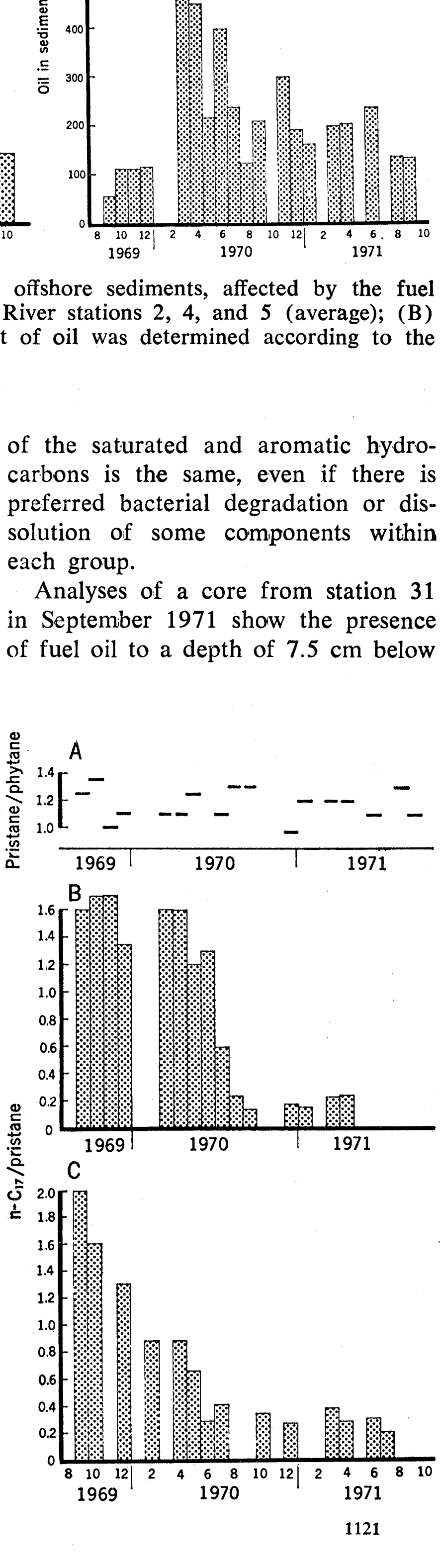

Fig. 3. The pristane/phytane ratio in sediments (A) at Silver Beach Harbor station 31 and the *n*-heptadecane/pristane ratio in sediments (B) at Silver Beach Harbor station 31 and (C) at Wild Harbor River station 4.

the sediment surface. On the basis of the n-C_{17}/pristane ratio, the oil below 2.5 cm is as fresh after 24 months as the oil at the surface was after 10 months (*3*). In the marshes penetration has been greater, extending to at least 60 cm, and bacterial degradation and dissolution are evident at that depth; this is probably the result of the greater permeability and aeration of the marsh sediments.

Some properties of the fuel oil have changed little, in spite of its gradual degradation. Initially the boiling range of the spilled oil extended from 170° to 370°C, and the normal alkanes ranged from decane to docosane. The boiling range of the fuel oil in the sediments is well preserved after 2 years; even at stations with low pollution level, C_{13} to C_{14} alkanes are still present. At station 31 and in the marshes dodecane can still be detected.

Adjacent members of the same or of closely related homologous series are affected by weathering to nearly the same degree; therefore, their concentration ratios change only slowly. Thus, the ratio of pristane to phytane at station 31 has remained 1.17 ± 0.09 during the 2-year period (Fig. 3A). This value differs characteristically from that encountered in unpolluted recent sediments (*6*). The pristane/phytane ratio and similar isomer ratios are distinguishing features that vary from one crude oil to another. That this ratio remains constant suggests that the identification of fossil fuel pollutants in the environment and their distinction from the natural hydrocarbon background may be possible over extended time periods.

Our continuing investigation will provide a framework for the consideration of the effect and fate of oil pollution in the coastal environment. Hydrocarbons in the boiling range of the oil spilled at West Falmouth are abundant in crude oil. Smith, in summarizing analyses of 6496 crude oils, showed that the "gas oil" content of the vast majority ranges from 20 to 35 percent, except for Tertiary oil, where higher values occur (*7*). Smith has defined gas oil as boiling between 200°C at 760 mm and 225°C at 40 mm (or 335°C at 760 mm). This range is comprised within that of this fuel oil (170° to 370°C, 760 mm). Thus, the "fuel oil" component, in the sense of the oil spilled at West Falmouth, constitutes one-quarter to one-third of the weight of a large majority of all crude oils. Therefore, we anticipate that the environmental effect and the persistence of a crude oil spill resembles that of a fuel oil spill qualitatively, and to a degree quantitatively. The presence in whole crude oils or residual oils of less rapidly degraded and less soluble hydrocarbons of higher molecular weight should lead to greater environmental persistence.

The preservation of hydrocarbons in marine sediments for geologically long time spans is one of the accepted key facts in the current thought on petroleum formation. Similarly, the uptake of dietary hydrocarbons in the food chain and their preservation in the lipids of organisms seem to be well documented. Our present findings do not contradict these background data.

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3 March 1972

Development of Cellular Dependence on Infective Organisms: Micrurgical Studies in Amoebas

Abstract. *Nuclei and cytoplasm were transferred between a normal strain and a variant strain of* Amoeba discoides *heavily infected with bacteria. After 5 years of infection, the infective bacteria that were initially harmful to the host cells became harmless, and the nucleus of the host cell became dependent on the infective organisms for its normal functions.*

A strain of *Amoeba discoides* became spontaneously infected with a large number of rod-shaped bacteria (60,000 to 150,000 per cell) in 1966 (*1*) and has been carried in culture since then. After 5 years of infection, the infective organisms that affected the host cells adversely became harmless. Furthermore, the results of micrurgical experiments show that the nucleus of the host cell has become dependent on the infective organisms to the extent that it cannot function normally in the absence of the infective organisms. This is apparently the first recorded instance of intracellular parasitic organisms being converted to true symbionts within an observable period of time.

When the infection was first found (*1*), the bacteria were harmful to amoebas in that the infected amoebas (i) grew slower, with a mean generation time of 5 days at 18°C as compared to 2 days for the normal amoebas, (ii) had an extremely low percentage of clone formation when isolated singly, (iii) were more sensitive to starvation, (iv) were smaller in size, and (v) were much more fragile. Also, the bacteria could infect normal amoebas under experimental conditions, and killed most of the newly infected cells. On the basis of these observations, we regarded the bacteria as parasitic.

Attempts to infect amoebas with several different kinds of bacilli normally in the amoeba culture solutions failed, since amoebas usually digest foreign objects introduced into their cytoplasm (*2*). The infective bacteria would not grow outside the amoebas under any conditions tried, a result indicating that they were obligatory parasites. Within the past 5 years, the adverse effect of the infective bacteria has disappeared and at present the infected strain of amoebas grows normally while carrying the same number of bacteria within the cytoplasm as before. Examinations by light and electron microscopy indicate that the bacteria living in the amoebas are the same ones as those found originally.

In order to see if the infection during the last 5 years has caused any changes in cell characters of the infected strain, I examined the nucleocytoplasmic compatibility between the normal and infected strains. The in-