FOREWORD

Marine contamination by petroleum, whether by natural seepage or by spills from ships at sea, by accidents in harbour or at offshore installations or by atmospheric or terrigenous input is by no means a new or rare phenomenon. In recent years however, the problems have been highlighted not only by the increased utilisation and marine transport of oil but also by a number of spectacular accidents which have raised questions about possible effects on the ecosystem. A number of detailed studies have been carried out in an attempt to answer these questions. The demands for such knowledge have been further increased by the various questions raised as a result of expansion of offshore exploration and exploitation for oil, particularly in environments hostile to these operations, in regions as far apart as the northern North Sea and the coast of Alaska.

Consequently, diverse aspects of the problem are being studied in several parts of the world by chemists and biologists who are often asking the same questions but using different approaches and sometimes producing conflicting views. Against this background, it seemed timely therefore to bring together a group of scientists from university, industry and government, actively engaged in such work, to examine and discuss common problems relevant to petroleum hydrocarbon contamination of the marine ecosystem and so a Workshop was sponsored by the International Council for the Exploration of the Sea, and held in Scotland at Aberdeen in September 1975.

The Workshop considered methodology, occurrence and fate in the environment, and effects on the ecosystem of petroleum hydrocarbons in the sea. Most of the papers presented and updated where necessary, are brought together in the present volume together with an edited version of the recorded discussion that followed each session. Of necessity, the reportage of the discussion is very brief although the proportion of time available for discussion compared favourably with that set aside for formal presentation of the papers. In preparing the discussion reports, the editors were assisted in particular by Dr R. Hardy, Dr R. Johnston, Mr P. R. Mackie and Dr I. C. White, and by comments from several contributors.

No attempt was made to produce specific recommendations but a study of the papers in this volume does give a clear indication of several lines of research which must be followed up before an adequate understanding can be reached of the effects of petroleum in the sea and it is evident that widespread monitoring operations will be fully effective only when the basis of our knowledge has been thus extended.

A list of participants to the workshop may be found in Appendix I.

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THE FATE AND EFFECT OF PETROLEUM IN CONTROLLED ECOSYSTEM ENCLOSURES

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Water extracts of No. 2 fuel oil were added to CEPEX enclosures during August, 1974 and June, 1975. The approximate initial concentrations of nonvolatile hydrocarbons in 1974 were 10 and 20 µg/litre in two enclosures. At the lower concentration no major effects on phytoplankton or zooplankton were apparent. The 20 µg/litre enclosure had a small bloom of the microflagellate, *Chrysochromulina kappa*, and the growth of a laboratory culture of this flagellate was stimulated by water extracts of fuel oil (Parsons et al, 1976). The 1975 experiments had an initial concentration of 40 µg/litre nonvolatile petroleum hydrocarbons which dropped to 20 µg/litre after 3 days. The losses appeared to be due primarily to microbial degradation and adsorption to sinking particles. The microbial degradation rate of parraffins and low molecular aromatics, but not the degradation rate of high molecular weight aromatics such as fluorene and benzpyrene, increased markedly in the petroleum treated enclosure within one day. Three days after petroleum addition naphthalene degradation rate increased from 0·2 to 2·5 µg/litre/day.

The major changes in populations seen in 1975 after treatment with petroleum were a drastic decline in diatoms followed by a bloom of the microflagellate, *Chrysochromulina kappa*, which replaced diatoms as the dominant phytoplankter. This in turn was followed by an increase in tintinnids and rotifers, presumably feeding on the microflagellates. The control enclosure was dominated by the diatom. *Cerataulina bergonii*, during the course of the experiment (19 days) although for 3 days a small flagellate bloom was also observed. Thus, as a result of the addition of fuel oil there was a major change in the ecosystem of the enclosure in terms of type of primary and secondary producers.

INTRODUCTION

During the past two years, as part of the controlled ecoystem pollution experiments (CEPEX), various pollutants have been added to controlled enclosures in Saanich Inlet, British Columbia, Canada. This facility and the results of earlier replication experiments have been described by Parsons (1974) and Takahashi et al (1975). Several papers describe the general biology, chemistry and hydrology of this coastal fjord (Bary, 1966; Fulton et al, 1969; Herlineveaux, 1962; Parsons and LeBrasseur, 1970; Peiper, 1971).

This report presents the results of adding water extracts of No. 2 fuel oil to quarter scale CEPEX enclosures (ca. 2 m diameter and 15 m deep — 60 000 l) during August, 1974 and June, 1975. Fuel oil, a refined product with a water extract rich in naphthalenes, was selected because of its known toxicity. Crude oils which have been studied to date show effects only at relatively high concentrations (Anderson et al, 1974; Gordon and Prouse, 1973; Hodson et al, 1975; Pulich et al, 1974; Vaughan, 1973).

METHODS

In the experiments of 1974 and 1975 polyethylene enclosures (ca. 2 m diameter and 15 m deep) were filled with 60 000 l of water from Saanich Inlet. The petroleum added to these was a water extract of No. 2 fuel oil (American Petroleum Institute standard) as described by Anderson et al (1974). Carboys containing 17 litres of sea water from the CEPEX site were stirred for 24 hours with 35 ml of No. 2 fuel oil. The phases were allowed to separate for 12 hours; the water extract from each carboy was pumped through a diffusion ring throughout the water column of the enclosure. Biological sampling was carried out at 2 or 3 day intervals both before and after petroleum addition. Chlorophyll, nutrients (nitrate, phosphate and silicate), particle size spectra and photosynthetic productivity were measured on integrated water samples taken with a peristaltic pump from three depth intervals (0—5 m, 5—10 m, 10—13 m). Also studied were the species composition and standing stock of the phytoplankton, microzooplankton and zooplankton. The methods used

for the biological work have been described by Takahashi et al (1975).

MICROBIAL DEGRADATION

Water from various depths in the enclosures was collected with a Niskin sampler. The bacterial degradation of hydrocarbons was measured by adding ^{14}C-labelled hydrocarbons to 100 ml water samples in a 250 ml flask capped with a silicone stopper. After incubation at the *in situ* temperature (12°C) in the dark for 6 to 48 hours, the respired $^{14}CO_2$ was collected and counted in a liquid scintillation counter (Beckman LSC-100). The procedure for collecting $^{14}CO_2$ in sodium hydroxide and transfer to a second flask is described by Hodson et al (1977). Controls were water samples containing 2 ml of 2N H_2SO_4. All samples were run in triplicate for each concentration and time interval. Turnover times were calculated by the equation: turnover time = t/f where t is the incubation time and f is the fraction of the labelled hydrocarbon degraded to $^{14}CO_2$ during the incubation period. The radioactive hydrocarbons used were 2-methylnaphthalene-8-^{14}C (7·98 mci/mmol—California, Bionuclear Corp.), 3, 4-(benz-3,6-^{14}C) pyrene (21 mci/mmol—Amersham-Searle), ^{14}C-1-naphthalene (5·1 mci/mmol—Amersham-Searle), ^{14}C-9-fluorene (2·57 mci/mmol—California Bionuclear Corp.), ^{14}C-(methyl)-benzene (17·5 mci/mmol—Amersham-Searle), ^{14}C(U)-benzene (75 mci/mmol—Amersham-Searle), ^{14}C-1-hexadecane (54·4 mci/mmol—Amersham-Searle), ^{14}C-1-heptadecane (13·5 mci/mmol—ICN), ^{14}C-1-octadecane (25 mci/mmol—Amersham-Searle). All compounds, except benzene and toluene, were purified on silicic acid thin layer chromatograms before use.

CHEMICAL ANALYSIS

For total non-volatile hydrocarbons we used an infra-red procedure similar to those described by Brown et al (1973) and Vaughan (1973). Analysis was at 2930 cm^{-1} using a Perkin-Elmer infra-red spectrophotometer (Model 467). Four litres of sea water were extracted with spectrograde CCl_4. This extract was concentrated to 1 ml and this was treated with activated Florisil to remove nonhydrocarbon material. The infra-red method measured only aliphatic hydrocarbons but was related back to a fuel oil standard to give total hydrocarbons. A second method of measuring total hydrocarbon was used which was based on the ultraviolet method of Levy (1972). Two litres of water were extracted with hexane and the absorption of this extract between 210 and 350 nm was measured after chromatography on Florisil. This method measures aromatic hydrocarbons and in the case of fuel oil most of the absorption is due to naphthalenes (between 210 and 240 nm). The area under the peak is integrated to give total hydrocarbon relative to a fuel oil standard. For gas-liquid chromatography 6 litres of water were extracted with 300 ml of hexane. Hexane extracts were concentrated to a small volume on a rotary evaporator and applied to activated silicic acid thin-layer plates (Merck). A band corresponding to the area of aromatic hydrocarbons, including all the naphthalenes, was removed and eluted with benzene. This fraction was run on a Packard gas chromatograph with an 8′ column of 10 % SP-2100 on 100/120 Supelcoport (Supelco Co.) and temperature programed from 70°C to 220°C. Hydrocarbon standards were used for reference identifications. Naphthalene, l-methylnaphthalene, 2-methylnaphthalene, and dimethylnaphthalenes were quantified by use of an internal standard. Identification of these compounds is tentative since it is based on comparison of retention times with standards. We selected naphthalenes because they are the principal water soluble components of fuel oil (Anderson et al, 1974). The ultraviolet method of quantitating naphthalenes (Neff and Anderson, 1975) gave good agreement with the GLC method. The concentration of benzene, toluene and xylene in water samples was carried out by L. Atkinson using the gas chromatography method of Atkinson et al (1974).

In the 1974 experiments the fluorescence method of Keizer and Gordon (1973) was used to quantify total hydrocarbons in water samples. For the 1975 experiments fluorescence spectroscopy involved extraction of 1·5 litres of sea water with methylene chloride and analysis by a combined high speed liquid chromatograph-fluorescence spectroscopy method (Cretney and Wong, 1974). Excitation was at 308 nm and emission at 383 nm. The readings were related to a chrysene equivalent.

For sediment analysis 100 g of wet sediment collected from the bottom of the enclosure were extracted with chloroform:methanol by the method of Folch et al (1957). The organic phase was concentrated to dryness, redissolved in hexane and the hydrocarbon fraction collected from a silicic acid column.

RESULTS

CHEMISTRY

Hydrocarbons in the water extracts of No. 2 fuel oil have been discussed by Anderson et al (1974) and Boehm and Quinn (1974). Our method of making the stock water extracts was similar to that of Anderson et al (1974) and preliminary analysis of our fuel oil water extracts by gas-liquid chromatography indicated that the concentrations of the major nonvolatile hydrocarbons, namely naphthalene (800 µg/litre), methylnaphthalenes (800 µg/litre) and dimethylnaphthalenes

(200 µg/litre), were similar to those reported by Anderson et al (1974). The major volatile hydrocarbons were benzene (400 µg/litre), toluene (900 µg/litre) and xylene (1000 µg/litre).

After pumping fuel oil extracts into the enclosures water samples were collected at various time intervals for hydrocarbon analysis. During the summer of 1974 two different fuel oil additions were made with one involving the addition of 5 and the other 10 carboys of fuel oil water extract. Analyses of benzene, toluene and xylene were carried out after pumping (Atkinson, 1975) and the fluorometric method of Keizer and Gordon (1973) was used to calculate total hydrocarbon concentration. The concentration of benzene rose to 100 µg/litre immediately after the addition of ten carboys of fuel oil extract but was non-detectable after 2 days. The fluorometric method was found to give extremely erratic results possibly because of interference from naturally fluorescing compounds in the water. Also, satisfactory results with gas-liquid chromatography were not obtained with the 1974 experiments. Using the data of 1975 we calculate that the initial concentration of non-volatile petroleum hydrocarbons in the two experiments of 1974 were 10 µg/litre and 20 µg/litre.

The two enclosures used for 1975 were labelled G (petroleum treated) and F (control). The concentration of non-volatile hydrocarbons in enclosure G after the addition of a water extract of No. 2 fuel oil was 50 µg/litre by the infra-red method. The hydrocarbon concentration by the ultraviolet method was 60 µg/litre. Enclosure F had a hydrocarbon concentration of approximately 10 µg/litre, although this varied from 5 to 20 µg/litre during the course of the experiment. Thus the concentration of petroleum hydrocarbons in enclosure G was approximately 40 µg/litre. After 3 days the hydrocarbon concentration in G had dropped to 30 µg/litre. The concentrations of naphthalene, methylnaphthalenes and dimethylnaphthalenes in G by both gas-liquid chromatography and ultraviolet spectroscopy were 5, 5 and 2 µg/litre, respectively. After 3 days their respective concentrations had dropped to 2, 2 and 1 µg/litre. No naphthalenes were detected in F by gas-liquid chromatography however some uv absorption was seen in the naphthalene range (220–230 nm). Chrysene equivalents rose to 0·13 µg/litre after addition of fuel oil extract compared with a background level of 0·04–0·08 µg/litre (Green, 1975). The chrysene equivalent of the stock fuel oil water extract was 11 µg/litre. Preliminary analysis of sediment collected in the bottom of the enclosure suggests adsorption of hydrocarbons to particles, both living and dead, was an important process in the loss of hydrocarbons from the water.

MICROBIAL DEGRADATION OF HYDROCARBONS

To measure the degradation rate of hydrocarbons various ^{14}C-hydrocarbons were added to water samples collected from the 1975 enclosures. As a result of petroleum extract addition to enclosure G there was a large increase in the degradation rate of octadecane, hexadecane, heptadecane, naphthalene and methylnaphthalene (Table 48). The hydrocarbon degradation rate of different radiolabelled hydrocarbons before the addition of petroleum showed large variations resulting in a high standard error. However, after the addition of petroleum there were only small variations in degradation rates between different water samples of the same depth interval. Both fluorene and benzpyrene were not degraded in the water from enclosure F or in

Table 48. Microbial degradation of ^{14}C-hydrocarbons in water samples from enclosure G

Hydrocarbon and concentration	Collection depth (m)	Incubation time (h)	Time after oil addition (days)	Degradation rate (µg/l/day) $\times 10^2$*	Turnover time (days)
Benzpyrene (16µg/l)	5–10	48	0	0	–
Benzpyrene (16µg/l)	5–10	24	3	1 ± 0·7	1 400
Fluorene (30µg/l)	5–10	48	0	0	–
Fluorene (30µg/l)	5–10	48	3	0	–
Heptadecane (30µg/l)	0– 5	24	0	7 ± 4	400
Heptadecane (30µg/l)	0– 5	16	3	50 ± 3	60
Methylnaphthalene (50µg/l)	0– 5	24	0	10 ± 6	500
Methylnaphthalene (50µg/l)	0– 5	24	3	26 ± 4	200
Naphthalene (50µg/l)	0– 5	24	0	10 ± 3	500
Naphthalene (50µg/l)	0– 5	16	3	250 ± 7	22
Naphthalene (50µg/l)	0– 5	10	4	100 ± 5	57
Naphthalene (50µg/l)	5–10	10	4	500 ± 11	10
Octadecane (30µg/l)	0– 5	24	0	16 ± 7	200

* ± Standard deviation

the water of enclosure G before addition of oil. After additon of oil there was a measurable degradation of benzpyrene but not for fluorene in enclosure G. The degradation rate of naphthalene, a principal component of the water solubles of fuel oil, increased from 0·1 to 2·5 µg/litre/day. However, the degradation of methylnaphthalene, also a major water soluble, increased its degradation rate only from 0·1 to 0·3 µg/litre/day. The turnover time and degradation rates calculated indicate that microbial degradation was responsible for much of the loss of paraffinic and low molecular weight aromatics. However other processes such as adsorption to sinking particles or metabolism by zooplankton are probably more important than microbial degradation for losses of high molecular weight aromatics from the water. Four days after petroleum addition the 5—10 m water had 5 times the naphthalene degradation rate of 0—5 m water. Initial analyses have indicated that the 5—10 m water had a higher concentration of naphthalenes than 0—5 m water.

PHYTOPLANKTON

During August, 1974, the addition of petroleum extract (initial concentration approximately 20 µg/litre of petroleum hydrocarbons) to an enclosure caused an increase in the microflagellate (6—16 µm in diameter) population. The principal species in this bloom was the Haptophyceae, *Chrysochromulina kappa* (Parsons et al, 1976). A second experiment with petroleum at an initial concentration of approximately 10 µg/litre showed no major changes in the species composition or standing stock of the phytoplankton population.

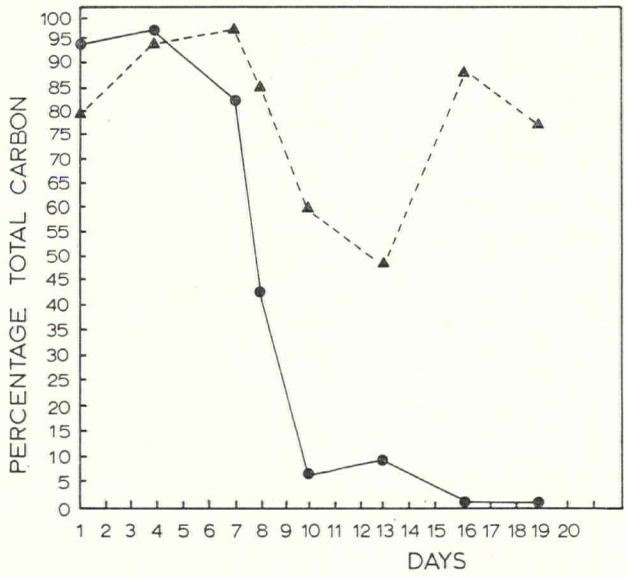

Figure 94. Changes in the population of the diatom, *Cerataulina bergonii*, over 0–10 m, ▲-F—control enclosure, ●-G—contains 40 µg/litre of fuel oil. Petroleum added on day 6.

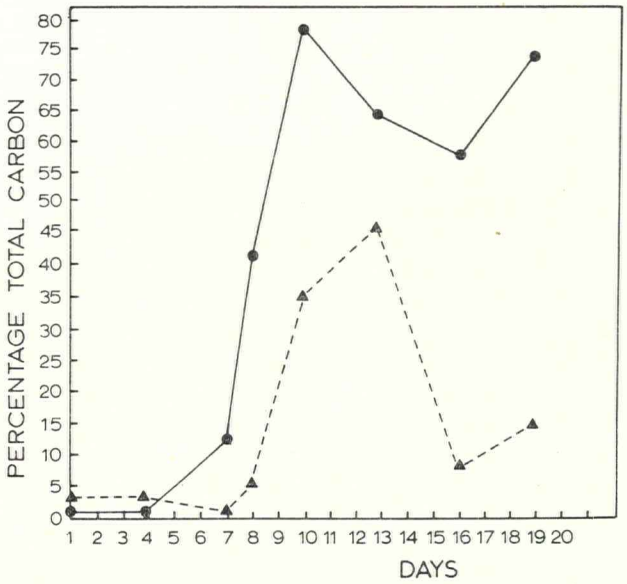

Figure 95. Changes in the population of microflagellates (predominantly *Chrysochromulina kappa*) over 0–10 m, ▲-F—control enclosure, ●-G—contains 40 µg/litre of fuel oil. Petroleum added on day 6.

During June, 1975, the initial petroleum hydrocarbon concentration was 40 µg/litre and more dramatic changes in phytoplankton species were seen. At the start of the experiment the dominant phytoplankton in both G and F was the diatom, *Cerataulina bergonii*. After the addition of fuel oil extract to enclosure G on day 6 there was a rapid decline in the diatom population followed by an increase in microflagellates which reached a peak at day 10 (Figs. 94 and 95). The dominant microflagellate of this bloom was *Chrysochromulina kappa* (kindly identified by R. Waters at the University of British Columbia). In control enclosure F a decrease in diatoms began on day 8 but a recovery began between days 13 and 16. Also microflagellates increased in F between days 8 and 13 but rapidly declined between days 13 and 16. The microflagellates in enclosure F were a mixture of several species, including *Chrysochromulina kappa*.

Productivity relative to chlorophyll (assimilation number) in enclosure G was high between days 10 and 13 averaging 7 µg carbon/µg chlorophyll-*a*/h. Enclosure F was 4 and 1 µg carbon/µg chlorophyll/h on days 10 and 13, respectively. The total phytoplankton biomass in G showed a constant decrease during this period, whereas in F the phytoplankton biomass gradually increased up to day 13 followed by a gradual decrease.

A bioassay on water from enclosure F was used to verify the results from enclosure G. Water from enclo-

sure F was added to two glass containers on day 18 followed by addition of No. 2 fuel oil to one container (final hydrocarbon concentration in the water was 90 µg/litre). The treated container showed a marked decrease in chlorophyll-*a* due to a decline in diatoms, but after 5 days chlorophyll-*a* began an increase due to the growth of microflagellates. As a result of diatom growth the untreated container showed a steady increase in chlorophyll-*a*.

MICROZOOPLANKTON

In the summer of 1974 a sheathed oligotrichous ciliate appeared in the petroleum treated enclosure perhaps grazing on the microflagellate bloom. In 1975 a large increase in the numbers of the tintinnid, *Helicostomella subulata,* and rotifers were seen in enclosure G after the addition of petroleum extract. These organisms feed on small phytoplankters and their increase appeared to correlate with an increase, described above, in the microflagellate, *Chrysochromulina kappa.* The decrease in the standing stock of microflagellates between days 10 and 16 may be due to grazing pressure by the tintinnids and rotifers. The uptake of petroleum droplets by protozoans observed by Andrews and Floodgate (1974) with crude oil extracts may also contribute to their growth. However, our water extracts of fuel oil appeared to lack the droplets of oil often seen in water extracts of crude oil.

ZOOPLANKTON

The species and standing crop of zooplankton in the petroleum treated and control enclosures did not greatly differ in the experiments of 1974. Several groups of zooplankton, including species of amphipods, crab zoeae, copepods and euphausiids collected from outside the enclosures were found to take up hydrocarbons from the water and metabolized various paraffinic and aromatic hydrocarbons to various hydroxylated intermediates (Lee, 1975). Ctenophores and jellyfish did not metabolize hydrocarbons. Up to 22×10^{-4} µg of benzpyrene was ingested by the copepod *Calanus plumchrus,* and a 17 day depuration period resulted in less than 1×10^{-5} µg benzpyrene per copepod.

The analysis of the zooplankton population from the experiments of 1975 have not been completed.

DISCUSSION

The principal events which occurred in enclosure G after the addition of fuel oil extract were a rapid decline in the dominant diatom, *Cerataulina bergonii,* which did not recover during the course of the experiment (13 days). The microflagellate, *Chrysochromulina kappa,* replaced diatoms as the dominant phytoplankton and resulted in high productivity in the enclosure during the course of the experiment. The increase in the tintinnids and rotifers appeared to result from the microflagellate bloom. The large increases in the microbial degradation rate of hydrocarbons suggests changes in the microbial population, probably by an increase in the number of microorganisms able to use hydrocarbons as a carbon source. The small standard error in the hydrocarbon degradation rate after addition of petroleum may be the result of one species dominating hydrocarbon degradation, whereas before addition a mixture of microbial groups are probably responsible for the degradation. Bacteria and fungi were assumed to be the major degraders of lower weight non-volatile hydrocarbons. In unpublished data we have found no evidence of $^{14}CO_2$ production after the addition of the various radiolabelled hydrocarbons to cultures of diatoms (*Skeletonema costatum*), dinoflagellates (*Amphidinium carteri*) or chrysophytes (*Cricospaera carterae* and *Isochrysis galbana*). Soto et al (1975) found no evidence of naphthalene metabolism by the green flagellate, *Chlamydomonas.* However these same studies revealed that phytoplankton were important in the adsorption of hydrocarbons from the water.

Thus the effects of fuel oil on this plankton community, besides changing total productivity, were a selection for certain species of bacteria, phytoplankton and microzooplankton.

Recent papers by Gordon and Prouse (1973), Lacaze (1974), Parsons et al (1976) and Pulich et al (1974) have discussed the effects of petroleum on phytoplankton. Gordon and Prouse (1973) reported that water extracts of No. 2 fuel oil inhibited photosynthesis at hydrocarbon concentrations greater than 50 µg/litre. Photosynthesis was measured by $^{14}CO_2$ fixation of a natural assemblage of phytoplankton from Bedford Basin, Canada, and the dominant phytoplankters were 6 genera of diatoms and dinflagellates. Pulich et al (1974) noted that No. 2 fuel oil above 40 µg/litre stopped photosynthesis in the diatom, *Thalossiosira pseudonana.* However a green and blue-green algae species required higher concentrations of fuel oil to show inhibition of photosynthesis. The report of Parsons et al (1976) summarizes the effects on phytoplankton of fuel oil from the CEPEX experiments of 1974. As noted above the concentration was approximately 20 µg/litre of fuel oil hydrocarbons. The microflagellate, *Chrysochromulina kappa,* which bloomed briefly in the petroleum enclosure was isolated and its response to hydrocarbons studied in the laboratory. Concentrations of No. 2 fuel oil at 50 µg/litre stimulated photosynthesis of this organisms as measured by $^{14}CO_2$ fixation.

A controlled ecosystem, set up in the Rance estuary in coastal France, has been used to study the effects of crude oil (Kuwait) on phytoplankton (Lacaze, 1974).

Several differences between the experiments of Lacaze (1974) and CEPEX are as follows: (1) containers at Rance were 560 l while the CEPEX enclosures had 60 000 l; (2) crude oil, 100 ml, was directly added to the enclosure at Rance while water extracts of No. 2 fuel oil were added to CEPEX enclosures; (3) nutrients were not added at Rance while nutrients were added to CEPEX enclosures as they were removed from the water by plankton. At Rance a sudden drop in primary production was observed in both treated and untreated enclosures and remained low during the course of the experiment (27 days), possibly due to lack of nutrients. The decrease in the primary production was greater in the petroleum treated enclosure.

We can offer no reason for the susceptibility of diatoms and the apparent stimulation of microflagellate production to extracts of fuel oil but the end result is a major change in the structure of the ecosystem as reflected in type of primary and secondary producers and some heterotrophic bacteria. It is of interest that Smith (1968) reports an increase in flagellates, including *Chrysochromulina,* after the Torrey Canyon oil spill. The sequence of events from large diatoms to small flagellates following nutrient exhaustion and eventual return to diatoms is a normal cycle in Saanich Inlet and in control enclosures (Takahashi et al, 1975). Because of the short duration of the petroleum experiments it is difficult to predict if and when the ecosystem would return to a diatom dominated ecosystem. In our experiments a return of diatoms would be expected since hydrocarbon concentrations returned to near baseline levels 9 days after the addition of oil. The selection for a species resistant to or even stimulated by petroleum is similar to the results of copper experiments where an initial decline in phytoplankton was followed by recovery due to growth of copper resistant algae (Thomas and Seibert, 1975).

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