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Effects of Ekofisk crude oil on an enclosed planktonic ecosystem

E. Dahl[1], M. Laake[2]*, K. Tjessem[3], K. Eberlein[4] and B. Bøhle[1]

[1] Statens Biologiske Stasjon Flødevigen, N-4800 Arendal, Norway
[2] Institute of Microbiology and Plant Physiology, University of Bergen, Allégatan 70, N-5014 Bergen-Universitetet, Norway
[3] Kjemisk Institutt, Universitetet i Bergen, N-5014 Bergen-Universitetet, Norway
[4] Institut für Organische Chemie und Biochemie, SFB 94, Universität Hamburg, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, Federal Republic of Germany

ABSTRACT: There has been increasing concern about effects of petroleum hydrocarbons and their derivatives on the highly productive North Sea waters. This study reports on possible effects of Ekofisk crude oil on planktonic communities enclosed in medium-scale controlled ecosystems. A 5-mm layer of oil was added to 1 of 2 parallel bags filled before the diatom bloom started in March, and the development followed for 4 wk. Population sizes of bacteria, algae, and zooplankton were estimated; chlorophyll *a*, mineral nutrients and hydrocarbons were analysed, and primary productivity was measured. In the oil-polluted bag, growth of diatoms and copepods was inhibited, while planktonic bacteria, choanoflagellates and tintinnid ciliates increased. Phosphate probably limited the growth of both algae and bacteria, and was rapidly reduced to very low levels in the bags. The measured concentration of petroleum hydrocarbons and alteration products averaged 470 $\mu g\ l^{-1}$. This level may be considered acutely toxic to diatoms. The reasons for the bacterial developments observed, their role in hydrocarbon mineralization, and the predominance of flagellates and tintinnid ciliates are discussed. It is concluded that results from bag experiments may provide important clues to fate and effects of oil pollution at sea.

INTRODUCTION

A major part of the research activities related to oil pollution in recent years has centered on acute and chronic effects of petroleum and specific petroleum hydrocarbons on aquatic organisms and ecosystems. Deleterious effects of oil pollution on benthic ecosystems have been observed in field studies (Mann and Clark, 1978), and laboratory studies have shown that petroleum may exert acute toxic effects on marine phytoplankton (Corner, 1978). Major effects of oil pollution on planktonic ecosystems in the sea have not yet been detected (Smith, 1968; Straughan, 1971; Berge, 1977; Lännergren, 1978). The interplay between marine organisms and oil pollution represents a situation which is influenced by the complexity of nutritional and antagonistic interrelations within a planktonic food web. Seasonal variations may change the sensitivity of the organisms to oil pollutants in as of yet unknown ways. In addition, crude oil and oil products vary widely in their chemical composition and physical properties, and therefore affect marine species in different ways (Corner, 1978). To gain further knowledge and to try to bridge the gap between results obtained in laboratory and field, a few studies have been undertaken in enclosed, marine planktonic ecosystems (Lacaze, 1974; Lee et al., 1977; Davies et al., 1980).

In April 1977 about 20 000 tons of Ekofisk crude oil were released into the North Sea by a blow out and caused a slick of about 4000 km^2 (Mackie et al., 1978). Field studies, however, revealed no dramatic effects on phytoplankton (Lännergren, 1978). Laboratory studies have shown Ekofisk crude oil concentrations of 300 $\mu g\ l^{-1}$ and less to exert acute effects on ^{14}C carbon fixation in marine phytoplankton (Dahl et al., in prep.). The present experiment was initiated to acquire more knowledge about possible effects of Ekofisk crude oil at the community level. Enclosed ecosystems were captured from the North Sea while the planktonic

* Present address: Norges Veterinærhøgskole, Institutt for Farmakologi og Toksikologi, Postboks 8146, Dep. Oslo 1, Norway

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community was in a pre-bloom stage of development. Alteration of the main spring bloom of diatoms may influence phytoplankton species succession and thereby affect herbivorous zooplankton and other trophic levels (Greve and Parsons, 1977). We also measured the succession of general organotrophic bacteria in the sea following an oil spill.

MATERIALS AND METHODS

Experimental design

Two plastic bags, in design similar to that described by Brockmann et al. (1974), 1 m in diameter and 12 m deep with closed bottoms, were filled with sea water by hauling from 12 m to the surface on March 6, 1979. The experiment was carried out in the Rosfjord on the southern coast of Norway. Further information on the experimental site and the physical, chemical and biological conditions are given separately (Brockmann et al., 1981). Water temperature and solar radiation were monitored continuously outside the bags.

The 2 bags were filled within 2 h and contained nearly identical water masses. Sampling was carried out daily from March 7 until April 3 at 0.2, 3, 6 and 10 m depths. On March 14 and 15, sampling had to be omitted due to strong currents stretching the bags horizontally. On March 9, Ekofisk crude oil was layered on top of the sea surface surrounded by the 30 cm high plastic walls, but freely exposed to the air. The thickness of the oil layer was at the start approximately 5 mm. Samples from the oil polluted bag were taken through a 19 cm diameter, 65 cm long PVC tube mounted through the surface, in order to eliminate contamination of the sampling bottles. A sterile gravitation-flow sampler was used for bacteriological samples, while all other samples were taken with air-filled snap-off stoppered bottles (Meyer-bottles).

Determination of salinity, nutrients and hydrocarbons

Samples were analysed directly or treated within a few hours. Oxygen was analyzed once at the end, by sampling with a Ruttner bottle and Winkler titration. Salinity was measured as conductivity. Subsamples for nutrients were filtered through a 0.45 µm Millipore HA membrane filter and analysed within a few hours on Technicon Autoanalyzer. Nitrate + nitrite, ammonium, ortho-phosphate, and reactive silicate were determined (Eberlein et al., 1983). One litre subsamples were filtered on Whatman GF/C filters and extracted with 2 × 50 ml dichloromethane, followed by 50 ml chloroform; the extracts were pooled and frozen until analysis. Following concentration by evaporation (Rotavapor, Büchi) to a few ml and further concentration by a gentle stream of nitrogen, an aliquot of the extract was analysed on a Hewlett-Packard 5880 gas chromatograph equipped with a FID detector. The GC conditions were as follows: temperature programming 80 to 285 °C at 12 C° min^{-1}, glass column 1/4" O. D. and 6' length, packed with 3 % SP-2100, 80/100 Supelcoport; N_2 was used as carrier gas.

The quantitative aspects of the GC procedure used are based on the method published by Grahl-Nielsen et al. (1980) which omits the high molecular weight hydrocarbons, the very low boiling fraction of hydrocarbons and most of the polar degradation products.

Measurement of chlorophyll and primary productivity

Chlorophyll *a* was measured according to Strickland and Parsons (1968) by using a Turner filter fluorometer. Fluorescence was determined before and after addition of hydrochloric acid to allow calculation of pheopigments.

Primary productivity was measured by adding 1µCi ^{14}C-bicarbonate to 50 ml samples in screw-capped bottles (Sovirel) and incubating for 6 h around noon at the sampling depths and adjacent to the bags (Steemann-Nielsen, 1952). After incubation, samples were filtered through Gelman GN membranes filter (pore size 0.45 µm) and the filters stored frozen until fumed with HCl and counted by liquid scintillation.

Total carbon dioxide content was estimated according to Strickland and Parsons (1968, p. 27–34) and, when calculating the primary productivity, the dark fixation was assumed to be 6 % of the light fixation (Dahl, unpubl.).

Bacterioplankton, phytoplankton and zooplankton

Total counts of bacteria were obtained by epifluorescence microscopy (Hobbie et al., 1977). Subsamples of 1 to 5 ml were fixed by adding formaldehyde, filtered on black Uni-Pore membranes of 0.4 µm pore size, stained with acridine orange and counted with a Nikon Labophot EF microscope. Colony-forming bacteria were estimated by spreading 0.1 ml samples from serial dilutions on nutrient-poor seawater agar (Dahle and Laake, 1982) in triplicate plates and incubating at 10 °C for 2 wk. Even very small colonies were counted.

Samples for phytoplankton counting were taken

daily, fixed in neutral formaldehyde (Throndsen, 1978a), and counted in a Palmer-Maloney cell (Palmer and Maloney, 1954) after concentration by centrifugation (Throndsen, 1978b).

At termination of the experiment, a 55 µm mesh size vertical net tow was taken within the bags, the samples fixed with formaldehyde, and the numbers of phytoplankton, microzooplankton and copepods later determined.

RESULTS

Hydrography and solar radiation

The 2 bags were filled with only slightly stratified water masses with a salinity of about 33 ‰ S. During the experiment the salinity in both bags decreased to 31 to 32 ‰ S and the water was more stratified. The temperature was constant at 5 °C until March 13 when it dropped during 2 d to less than 2 °C and remained low until March 23 (see also Brockmann et al., 1982). The temperature then increased about 1 C° and fluctuated between 2 and 3 °C for the rest of the experiment, except for 2 incidents of higher temperature (4 °C) at 10 m depth on March 24 and 25. Only at that time was there a pronounced temperature gradient with depth. On March 29 oxygen concentrations were measured. The values increased with depth from 8.4 to 9.2 ml $O_2\ l^{-1}$ in the control bag, and decreased from 6.7 to 6.3 ml $O_2\ l^{-1}$ in the oil polluted bag, corresponding to 105 to 120 % and 87 to 82 % saturation respectively.

The daily solar radiation from above (spectral range 395 to 720 nm) measured at 5 m depth is shown in Fig. 1.

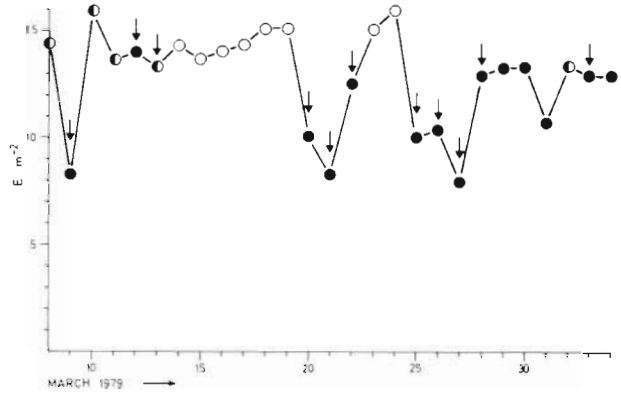

Fig. 1. Daily incident light (395 to 720 nm) at 5 m depth. Filled circles: cloudy days; half filled circles: half cloudy days; open circles: clear days. Arrows indicate days with rain or snow

Hydrocarbons and derivatives from petroleum

The concentration of petroleum constituents accommodated in the water column has been found to vary substantially in the vicinity of the emulsified oil (Table 1). These changes in concentration with time, however, did not appear to show any systematic trend. This can be explained by the interplay of constantly changing physical conditions in the sea, due to prevailing climatic conditions and the changing nature of the oil itself. A general trend of decreasing concentration with increasing depth was usually observed, but the number of samples at 6 and 10 m depths was too small to lend significance to the variation in the distribution of petroleum hydrocarbons and alteration products. Because of the climatic conditions it is presumed that the oil was accommodated into the water column also in the form of small droplets.

Table 1. Concentration of total, accommodated hydrocarbons (µg l^{-1}) determined by gas chromatography. Hydrocarbons with retention times less than 20 min were quantified by comparison with extracts from Ekofisk crude oil, as sum of peak areas

Date	Time of sampling	Sampling depths (m) 0.2	3	6	10
March 9	0900	Ekofisk crude oil added to the surface			
March 9	1500	505	–*	–	–
March 9	1800	250	–	–	–
March 10	0900	550	–	–	–
March 10	1800	1250	–	–	–
March 11	0900	1050	–	–	–
March 11	1800	900	900	–	–
March 12	1800	70	300	–	–
March 13	1730	350	450	200	200
March 20	1830	1400	450**	650	200
March 28	1630	10	150	450	30
April 3	1530	–***	600	300	100

* Depths not sampled for hydrocarbon analysis
** Evaporation to dryness may have caused some loss of material
*** Sample lost during preparation

Mineral nutrients

The water in both bags was rich in phosphate, nitrate and silicate at the beginning of the experiment. In the control, levels of nitrate and silicate remained high until March 25, while phosphate decreased rapidly during the first week (Fig. 2). At termination the levels

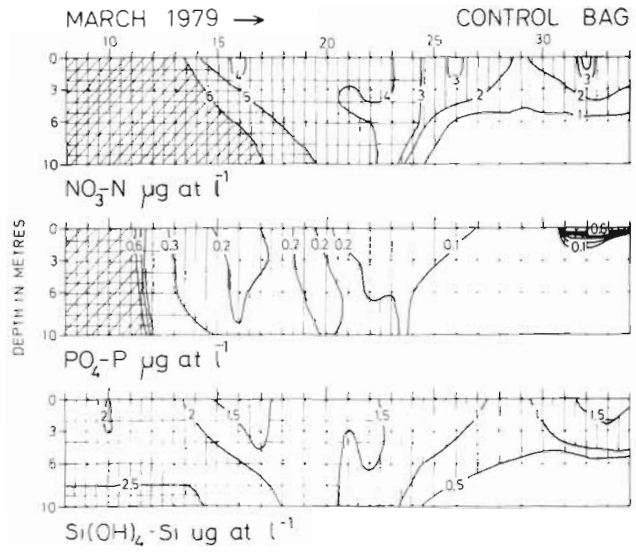

Fig. 2. Nutrients in control bag

of all 3 nutrients were low, most pronouncedly at 6 and 10 m depths. In the oil bag (Fig. 3), levels of both nitrate and phosphate dropped rapidly 3 d after oil addition, from March 12 to 14. Nitrate was reduced to 1 to 2 µgat NO_3-N l^{-1} and phosphate was nearly below the level of detection. Silicate, on the other hand, remained high throughout the experiment, except for a small decrease at the end. Ammonium showed fairly uniform and low values in the range 0.6 to 2.2 µgat NH_4-N l^{-1} for most of the period, except when precipitation or nitrogen depletion caused extremely high or low values, in the range 4.0 to 0.2 µgat NH_4-N l^{-1}, in the last half of the experiment. Evidently, phosphate, nitrate and ammonium were all supplied to some extent with snow or rainfall, particularly during the

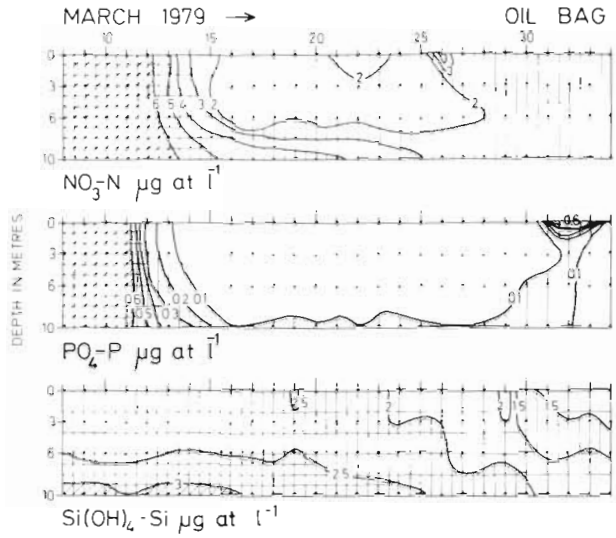

Fig. 3. Nutrients in oil bag

last week (Fig. 2 and 3). The levels of both nitrate and ammonium were very low in the control at 6 and 10 m in this period. Apart from this, both bags were phosphate limited after March 13, when the N : P ratio (nitrate + nitrite versus phosphate) increased from 9 at the beginning to 30 to 40 in the oil bag and 20 to 30 in the control. This ratio fluctuated to very high values after March 21.

Planktonic bacteria

The total numbers of bacteria, as estimated from epifluorescence counts, did not reveal any immediate response to oil exposure (Table 2), till March 14 there was no significant difference in the oil bag compared with the control. After March 15 the number in both bags increased from a mean value of $6.5 \cdot 10^5$ cells ml^{-1} to 2.8 10^6 ml^{-1} in the oil bag, compared to $1.4 \cdot 10^6$ cells ml^{-1} in the control. However, single measurements in that period varied unsystematically one order of magnitude and there was no trend with time or depth in these data. In contrast, colony-forming

Table 2. Acridine orange total counts of bacteria (10^5 cells ml^{-1}) in oil bag, control bag and sea. Data presented as variation and arithmetic means for 0.2, 3 and 10 m depths, separated in 3 periods with distinct succession patterns or interrupted sampling

Date	Period	Oil bag	Control bag	Sea
March 7– 9	Before oil was added	5.4– 9.6 6.7	2.9– 8.9 6.1	6.3– 7.7 6.9
March 9–14	First 5 d after oil addition	2.2–17.5 6.5	2.5–17.5 6.6	2.5–10.2 6.8
March 15–26	Next 6 to 17 d after oil addition	9.5–300 27.8	7.5–230 13.5	5.9–37.9 12.5

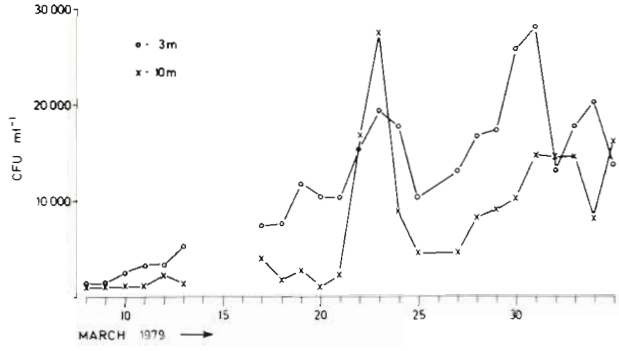

Fig. 4. Concentrations of bacteria as colony forming units (CFU) in oil bag

units (CFU) on seawater agar did show a rapid response to oil amendment and a trend with depth and time in both bags (Fig. 4 and 5). In the control there was a general increase in CFU with time, particularly at 3 m (Fig. 4), which correlated with increased productivity and chlorophyll *a* (Fig. 6), but in part also may have been due to seeding of the water from wall-growing bacteria. However, the 2 major peaks observed on March 23 and 30 to 31, reaching $2.8 \cdot 10^4$ CFU ml^{-1}, occurred after peaks of chlorophyll *a*. The particular groups of planktonic bacteria that are able to form colonies on agar therefore seem to be associated with phytoplankton growth.

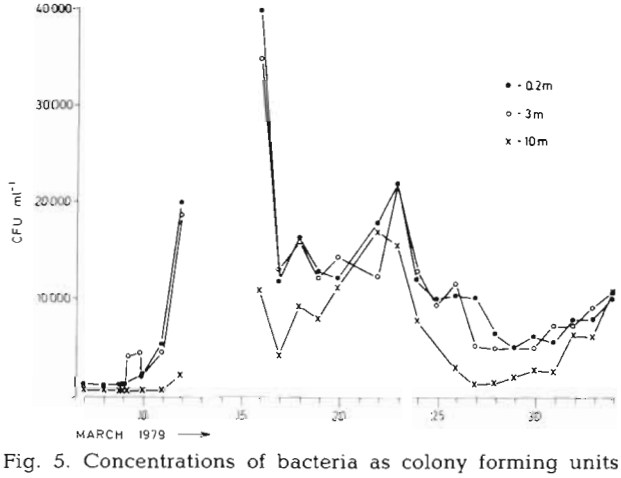

Fig. 5. Concentrations of bacteria as colony forming units (CFU) in oil bag

In the oil bag (Fig. 5) there was a rapid build-up of CFU at 0.2 and 3 m during the first 3 d with oil exposure, March 9 to 11, and then a remarkable parallel development throughout the experiment at these depths. At 10 m a similar build-up started 2 to 3 d later. The major peak was unfortunately lost, but from more than $4 \cdot 10^4$ CFU ml^{-1} it decreased rapidly after March 16. Later some secondary peaks developed. It is not known to what extent these bacteria were crude-oil degraders, but their energy source either primarily or secondarily were certainly hydrocarbons. The increased number of colony-forming bacteria coincides with a rapid reduction of oil constituents from March 11 to 12, and with a second reduction from March 20 to 28.

Chlorophyll *a*, primary productivity and assimilation rates

Measurements of chlorophyll *a* and carbon fixation confirm that the water masses were in a pre-bloom stage when enclosed. In the control bag (Fig. 6) the levels of chlorophyll *a* and productivity remained

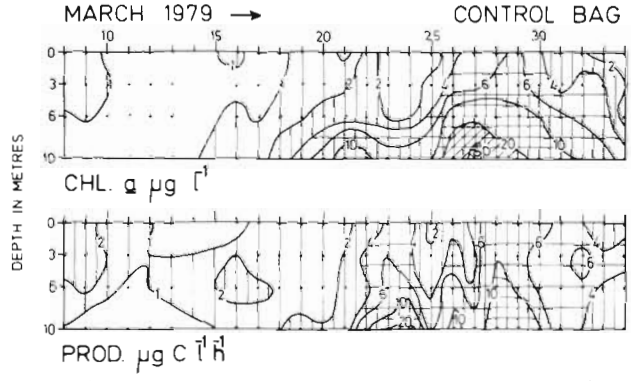

Fig. 6. Chlorophyll *a* and primary productivity in control bag

nearly unchanged until March 20. Then a peak in both parameters developed, most pronounced at 10 m, followed by a second peak from March 26 to 29.

In the oil bag (Fig. 7) no bloom of phytoplankton developed; instead, the levels of chlorophyll *a* and productivity decreased, except for a short period around March 25.

Pheopigments never exceeded 10 % of chlorophyll *a* in either bag and are not reported. The assimilation rates varied little with time in either bag except for a

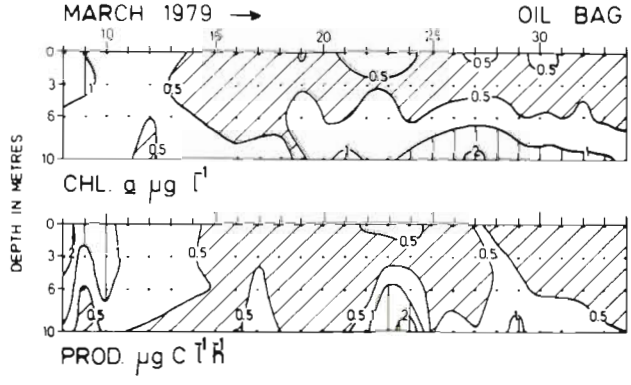

Fig. 7. Chlorophyll *a* and primary productivity in oil bag

peak on March 23 to 24, which coincides with an increase in light and temperature. The mean values generally decreased with depth, and were obviously lower in the oil bag than in the control bag (Table 3).

Table 3. Assimilation rates (mg C mg chl. a^{-1} h^{-1})

Bag	Depth (m)	Mean	Standard deviation	Range
Control	0.2	1.67	0.72	0.46 – 3.47
	3	1.58	0.75	0.29 – 3.55
	6	1.44	1.09	0.20 – 5.05
	10	0.79	0.80	0.13 – 3.38
Oil	0.2	1.03	0.38	0.55 – 2.42
	3	1.15	0.47	0.40 – 2.37
	6	0.95	0.50	0.24 – 1.98
	10	0.76	0.48	0.16 – 1.97

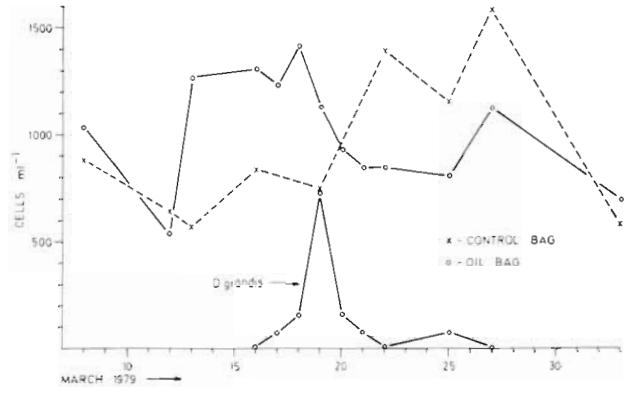

Fig. 8. Naked monads in control bag (cross) and oil bag (circles) at 3 m depth. *Diaphanoeca grandis* is included in total number

Phytoplankton and zooplankton

Tables 4 and 5 list cell numbers for the dominant phytoplankton species as mean values of water samples from 4 depths. At the start species composition was similar in the 2 bags, with *Skeletonema costatum* as the dominant diatom, although naked monads, the majority 2 µm or less in diameter, dominated by numbers (Fig. 8).

In the control bag, *Skeletonema costatum* almost disappeared during the experiment, but other diatoms grew up in high numbers. The most abundant were *Chaetoceros debilis* and *Thalassiosira nordenskioeldii*. Naked monads were the most numerous during the whole period (Fig. 8), but the diatoms certainly dominated in terms of biomass.

Skeletonema costatum also decreased in numbers in the oil bag, but only a few other diatoms succeeded (Table 5). In contrast with the control bag, naked monads seemed to be more numerous in the oil bag from March 13 to 19, with a peak of the choanoflagellate *Diaphaoeca grandis* on March 19 (Fig. 8). Data on chlorophyll *a* and primary productivity in the oil bag (Fig. 7) show that the monads only played a modest role as primary producers. In this report naked monads thus include autotrophic as well as heterotrophic cells,

Table 4. Phytoplankton in control bag (cells ml^{-1}); mean numbers of 0.2, 3, 6 and 10 m depths

Taxon	March 8	March 16	March 21	March 27
Diatoms				
Chaetoceros affinis			2	7
C. borealis		1	2	1
C. debilis	1	40	180	225
C. diadema			1	3
C. similis			+	
Eucampia zodiacus				+
Leptocylindrus danicus		+		+
Navicula sp.	+		1	2
Nitzschia seriata			1	1
Porosira glacialis	+	+		
Rhizosolenia alata	+	+	+	+
R. hebetata f. *semispina*		+		+
Skeletonema costatum	175	6	2	1
Thalassionema nitzschioides	1	6	4	5
Thalassiosira anguste-lineata		1	+	+
T. nordenskioeldii	+	40	153	254
Pennate diatoms	1	+		+
Other groups				
Peridiniales		+		+
Gymnodiniaceae	+	+	1	+
Euglenophyceae	+			

Table 5. Phytoplankton in oil bag (cells ml^{-1}); mean numbers of 0.2, 3, 6 and 10 m depths

Taxon	March 8	March 16	March 21	March 27
Diatoms				
Chaetoceros affinis				1
C. borealis				+
C. debilis	3	2	20	50
C. diadema				+
C. similis				+
Leptocylindrus danicus	+			
Navicula sp.	2	1	1	1
Nitzschia seriata				+
Porosira glacialis	+			
Rhizosolenia alata		+		
R. hebetata f. *semispina*	+			
Skeletonema costatum	151	19	9	6
Thalassionema nitzschioides	2	+	1	+
Thalassiosira nordenskioeldii	+	+	7	2
Pennate diatoms				+
Other groups				
Peridiniales		+		
Gymnodiniaceae				+
Euglenophyceae	+			+

and it should be stressed that quantification of naked monads from formaldehyde fixed samples is uncertain and speculative. The lorica of *D. grandis* is, however, recognizable even after formaldehyde fixation.

On March 27 naked monads were still evenly distributed in the bags, while diatoms were concentrated at the bottom, probably due to reduced vertical mixing in the bags (Fig. 9).

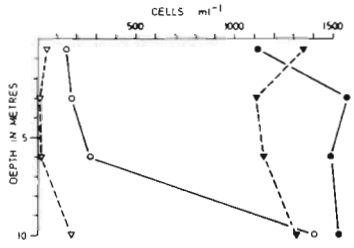

Fig. 9. Vertical distribution of phytoplankton in control bag (circles) and oil bag (triangles) on March 27. Open symbols: diatoms; filled symbols: naked monads

The species composition of the net tows from April 4 confirmed the previous observations (Table 6). The control bag was very rich in diatoms, with *Chaetoceros debilis* and *Thalassiosira nordenskioeldii* as the dominant species. In the oil bag only a small number of diatoms were observed, but the species composition was similar in the 2 bags.

The difference in zooplankton populations observed in the net tows from April 4 was striking. In the control bag, copepods and copepod naupliae were quite common, while tintinnids (planktonic protozoa with lorica) dominated in the oil bag.

Table 6. The most common planktonic organisms observed in the vertical net haul collected by a 55 µm mesh size net from control bag and oil bag on April 4

Taxon	Control bag	Oil bag
Diatoms		
Chaetoceros affinis	+	+ +
C. borealis	+ +	+ +
C. debilis	+ + +	+ + +
C. decipiens	+	
C. diadema	+	
C. laciniosus	+	+ +
Coscinodiscus cf. *concinnus*	+	+
Navicula sp.	+ +	
Porosira glacialis	+	+
Rhizosolenia alata	+	+
R. hebetata f. *semispina*	+	
R. setigera	+	
Thalassiosira nordenskioeldii	+ + +	(+)
Thalassionema nitzschioides	+ +	
Other groups		
Protoperidinium cf. *divergens*	+ +	+
P. pellucidum	+	+
Tintinnids	+	+ + +
Copepods and naupliae	+ +	(+)

Net tow from oil bag also contained much bacterial detritus and small 'tar balls'

Subjective symbolizing indicates relative abundance within each bag; + + + dominating, + + common, + less common, (+) rare

DISCUSSION

All parameters measured confirm that the bags were filled with nearly identical water masses. The decline in the salinity in both bags during the experiment cannot be explained by precipitation only. There may have been some input of freshwater to the bags also through small non-located rifts in the plastic walls. This, however, is assumed to be of minor importance to the general results.

The control bag developed in a way similar to a typical spring bloom in natural waters. *Chaetoceros debilis* and *Thalassiosira nordenskioeldii* were the most abundant diatoms in the bloom population both inside and outside the bags (Brockmann et al., 1982). The oil bag developed very differently, with no bloom of diatoms, an increased number of bacteria and protozoa, and a disappearance of copepods.

Temperature and salinity regimes were nearly identical and could not account for any difference. According to Brockmann et al. (1974) the plastic foil reduces incident light 10 to 20 % depending on the solar angle, however, this will alter light conditions in both bags in a similar way. The 5 mm thick layer of oil obviously reduced the amount of vertical incident light in this bag for a period, but with the small diameter of the bags used, large volume to surface ratio, lower solar angle at this latitude and high transparency of the water masses, this probably had no significance except for the upper meters. During the first week the oil slick formed tar balls or was adsorbed to the walls to a great extent. Thus the light conditions in the oil bag were soon comparable to the control but not identical, since there is always a blue sheen associated with emulsified oil which to some extent blocks incident light. While the light conditions in the oil bag were adjusting back to normal, there was no further growth in the control until March 20 when a bloom started. This lag phase was probably due to a change in temperature from 5 to 2 °C, but inhibition by non-complexed heavy metals may be an other explanation (Brockmann et al., 1982). In conclusion, the bloom in the oil bag was probably not inhibited due to bad light conditions.

Hydrocarbons from oil may have affected phytoplankton growth in the oil bag directly. Laboratory studies have shown that water soluble fractions of Ekofisk crude oil may affect ^{14}C-carbon fixation in phytoplankton at 300 to 500 $\mu g\ l^{-1}$ (Dahl et al., in prep.), and experiments with other oils point to the same concentration range (Corner, 1978). However, only a few, if any, of the diatoms were completely wiped out in the oil bag, as shown by the species composition in net tows and by growth experiments at termination. The oil may have prolonged the lag phase (Winters et al., 1976) and curbed phytoplankton activities such as photosynthesis and growth. The lower assimilation rates in the oil bag may indicate this, but also indicate other stress factors as for example nutrient deficiency. In the control bag the bloom obviously culminated at the end of March due to nutrient deficiency. At the time any growth of diatoms could have started in the oil bag, however, the levels of nitrogen and phosphate were already low due to heavy growth of bacteria, which may then have had a secondary competition effect on phytoplankton. The results point to phosphate as the primary limiting nutrient in both bags. This is supported by the results from algal bioassays performed on water from 0.2, 3 and 6 m depths in the control bag at the start of the experiment (Källqvist, unpubl.). Additions of nitrogen, phosphate or EDTA as complexing agent in cultures of *Skeletonema costatum, Phaeodactylum tricornutum* and *Dunaliella tertiolecta* established phosphate as growth limiting for all species, and nitrogen as a secondary limiting nutrient. It should also be mentioned that the use of a 6 % dark fixation figure in calculations overestimates primary productivity in the oil bag, because when measured (only March 21 and 29) this value ranged from 13 to 75 %. In any case, since the fixation was very low, the values must be considered rough estimates.

The most evident direct effect of oil addition was rapid stimulation of bacterial growth. This was probably due to low molecular-weight fractions being immediately available as carbon and energy sources without requiring any adaptation period (Lee et al., 1977). When oil is spilled at sea the levels of nitrogen and phosphorus in seawater may limit microbial degradation of oil (Colwell and Walker, 1977), and further biodegradation may be stimulated upon supplementation of nutrients (Atlas and Bartha, 1972). Hodson et al. (1977), however, reported inhibition of bacterial glucose assimilation by crude oils. The rates of nutrient uptake were not directly measured during the experiment; however, it seems difficult to explain the very rapid consumption of nitrate and phosphate as being due to bacterial growth only; from the isopleths (Fig. 2 and 3) it seems evident that phosphate limitation may have been the reason for the abrupt reduction in viable counts of bacteria on March 16 to 17. Because total numbers remained high during this period, unselective grazing can be eliminated as the cause. This reduction in viable counts may, in part, have resulted from photochemical actions on the surface sheen emanating from the emulsified oil. It is known that formation of secondary, possibly very toxic and water soluble, material increases very rapidly with decreasing thickness of the oil film (Burwood and Speers, 1974; Aaberg and Tjessem, pers. comm.). However, levels of accommodated petroleum-derived constituents in the water became

very low in this period. Except for a small secondary peak probably due to 'new' supply of petroleum constituents, there is a further slow decrease in viable bacteria until March 31, when phosphate addition at the surface probably caused new stimulation. Thus removal of phosphorus from the water column by sedimentation, and the possibilities of resuspension and regeneration are of utmost importance to the rates of microbial degradation.

Selective determination of oil degraders was, unfortunately, not possible. However, plate counts on nutrient media of low selectivity may be considered representative of bacteria viable and active in hydrocarbon degradation (Hagstrøm, 1977). This technique may be even better for this purpose than either the MPN-techniques for oil degraders used by many investigators (e. g. Gunkel and Trekel, 1967), or the acridine orange total counts, which do not discriminate between living and dead cells. We feel that our work supports this view strongly, and that in combination with measurements of generation times, e. g. by tritiated thymidine incorporation (Tobin and Anthony, 1978), plate counts may give the best estimates of bacterial production. Alternatively, total counts and measurements of division frequency by microscopy might be useful (Hagstrøm et al., 1978). In this study, growth rates may be estimated from the graphs directly, as has been done by general and specific counting methods in other bags (Dahle and Laake, 1982). When the data from Fig. 5 are plotted on a semilogarithmic scale, maximum specific growth rate (μ_{max}) can be estimated to be 1.35 d^{-1} or 0.0564 h^{-1}; this is surprisingly fast at 4 to 5 °C. The sudden increase in CFU at 0.2 m, later also observed at 3 m, may have been due to bacteria associated with settling algae. The lag-phase for hydrocarbon utilizers can be estimated from Fig. 5 to be only 20 h.

The Rosfjord location is virtually unpolluted from local sources and boat traffic; it receives water from the North Sea and Skagerrak. Even though the standing stock of bacteria utilizing hydrocarbons directly or indirectly was low ($3 \cdot 10^2$ CFU ml^{-1} if the experimental increase is extrapolated back to time zero), the population increased to approximately 10^5 CFU ml^{-1} in 4 d and then may have been significant in hydrocarbon mineralization, provided nutrients were available. Conservative estimates, based on observed bacterial population strength and growth rate, range in the order of magnitude of milligrams of oil hydrocarbons consumed per litre per day. Laake and Tjessem (in prep.) calculated an average mineralization rate of 0.06 mg oil d^{-1} in the water column. The observed reductions in dissolved hydrocarbons from March 10 and from March 20 may thus have been due to microbial degradation in combination with sedimentation.

Nanoflagellates may graze heterotrohic bacteria (Hass and Webb, 1979; Sieburth, 1979), and these colorless flagellates constitute a substantial fraction of microplankton biomass in the sea under natural conditions (Throndsen, 1970). Tintinnid ciliates probably graze bacteria and flagellates (Spittler, 1973; Heinbokel and Beers, 1979), and may be very active in phosphate remineralization (Johannes, 1965). Both flagellates and tintinnids were more abundant in the oil bag, which probably was based on bacteria as food source. CEPEX experiments also showed rapid growth of bacteria and predominance of flagellates and tintinnids in oil-polluted model ecosystem (Lee et al., 1978). These workers report a marked increase of tintinnids after addition of fuel oil (1975) and naphthalenes (1976) to the system, and supposed that the ciliates fed on nanoflagellates and bacterial aggregates. Particles of 3 to 5 µm diameter are most efficiently grazed and retained by tintinnids (Spittler, 1973). Lee et al. (1978) reported no effect on naupliar copepods of fuel-oil addition, while naphthalenes addition reduced their numbers. Davies et al. (1980) concluded that the addition of oil in their bag had a direct inhibitory effect on the development of copepod eggs and naupliae.

Thus the different development of protozoans and zooplankton in oil versus control bag may be explained as an effect of oil, acting directly on eggs and naupliae of copepods and indirectly, via bacteria, on tintinnids. There is, however, also the possibility that different phytoplankton species are essential food for different herbivore groups. It seems, for example, reasonable that the large chain-forming diatoms which dominated in terms of biomass in the control bag are more suitable food for copepods than for the rather small tintinnids. Greve and Parson (1977) speculated on possible links between photosynthesis and fish production, i. e. that big species of phytoplankton are converted to fish production through only a few trophic levels, in contrast to the small monads which are converted through many trophic levels, and may end up as jellyfish biomass.

The results of our study on the effects of oil on an enclosed planktonic ecosystem showed much similarity with effects observed by Lee et al. (1977, 1978) and Davies et al. (1980). The effects observed by us, however, seem somewhat more dramatic. This may be due to the higher concentration and different type of oil applied in our experiment.

The use of different pollutants, i. e. whole crude oils, fuel oils, naphthalenes or only water-soluble fractions, and/or design of the experiment may also be important to the effects observed. For example, while Davies et al. (1980) added nutrients every week, we added no nutrients. The fact that the experiments were conducted during different seasons may have been the

main reason for the differing results. Oil pollution may be more harmful to ecosystems in cold than in temperate climates, partially due to the retarded evaporation of volatile components of crude oil at low temperatures.

Accommodation of oil in sea water is highly variable and depends on biological, physical and meteorological conditions. Crude oil is accommodated in the sea both as water-in-oil and oil-in-water emulsions. True solution occurs mainly for low molecular weight fractions. The incorporation of small droplets of oil from the emulsion into the water column is directly related to the degree of turbulence, and the degree of photochemical impact on the emulsified oil is directly related to the climatic conditions (Aaberg and Tjessem, pers. comm.). Photo-oxidation yields more soluble and presumably more toxic products (Larson et al., 1979).

In areas with newly spilled oil on the sea surface during the BRAVO-blowout, Grahl-Nielsen et al. (1977) estimated the concentration of hydrocarbons to be up to 300 $\mu g\ l^{-1}$ in the upper 5 m. Concentrations in our oil bag were generally even higher (Table 1) and may to some extent reflect the relatively large amount of oil initially layered on top of the bag.

Artefacts created by the bags are: increased sedimentation rates, particularly of diatoms (Fig. 9), and reduced vertical mixing (Eppley et al. 1978). Horizontal dispersion and surface spreading were hindered, though wave action could be quite vigorous inside the plastic walls at the surface. Possibly, also evaporation was hindered to some extent. Due to these factors, both vertical fluxes and observed maximum concentrations are high compared to those observed during oil spills in the open sea. However, one cannot exclude that such high concentrations could occur after oil spills in closed areas. In contrast to open-sea studies, where detrimental effects, e. g. on phytoplankton, are difficult to observe (Lännergren, 1978), the history of the water body is known. In the open sea, an oil slick moves primarily along wind trajectories, while subsurface water predominantly follows ocean currents and may move in a different direction. Although one should always have the differences between enclosed ecosystems and natural situations in mind, bag experiments may provide important clues to the understanding of processes, rates and mechanisms involved in the fate of oil pollution at sea and their effects on marine planktonic communities.

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