

AVOIDANCE OF PETROLEUM HYDROCARBONS BY THE COD (*GADUS MORHUA*) *

By

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ABSTRACT

BØHLE, B. 1986. Avoidance of petroleum hydrocarbons by the cod (*Gadus morhua*). *FiskDir. Skr. Ser. HavUnders.*, 18: 97–112.

The experimental fish were held in two different aquaria, one with three and one with two compartments. Water containing «water soluble fraction» of Fuel Oil No. 2 was introduced in one of the compartments. By means of light- and sound-based detectors, the amount of time spent by the fish in the various compartments was recorded. Although the results were somewhat contradictory, it seems that the fish avoided water containing more than 100 µg/l of the petroleum hydrocarbons.

INTRODUCTION

Concern over marine pollution by crude oil and refinery products has increased steadily over the last few decades. The risk for and occurrence of oil spills in the open sea and chronic oil pollution in inshore waters has increased considerably as the utilization of petroleum products has increased. The increase of petroleum hydrocarbons in the oceans has been estimated to be 4.5 mill tons per year, with the largest increase resulting from transportation and river run-off (ANON. 1975).

In spite of the relatively high chronic increase and occasionally heavy spill of crude oil and oil products, the concentrations of petroleum hydrocarbons in offshore waters are reportedly very low (ANON. 1975). In the open ocean, the concentration of petroleum hydrocarbons at the surface is generally less than 10 µg/l. In subsurface waters, the concentration is even lower. However, in inshore waters close to large harbours, up to 1000 µg/l of total hydrocarbons may occur.

* This paper was first presented at the national symposium «Behaviour of marine animals» held at Solstrand, Os, Norway, 9–10 February 1983.

Though extremely variable, the solubility of petroleum hydrocarbons in the sea water is low. Components with low boiling points evaporate quickly, however, depending on temperature, wind speed, and like influences. Petroleum products spilled on the sea surface may also be emulsified in the water column.

After an oil spill near Stavanger (Norway), concentrations of hydrocarbons were found up to 200 µg/l although concentrations of 20 000 µ/l were found close to areas where oil had gathered in bays (GRAHL-NIELSEN *et al.* 1976). The high values occurred as oil-in-water emulsions.

Several experiments have been performed to study possible effects of petroleum hydrocarbons on marine life. Many of these involved high oil concentrations that only very seldom might be found in the sea, for example, immediately after oil spills (KÜHNHOLD 1969).

RICE (1973) found that fry of Pink Salmon (*Onchorhynchus gorbuscha*) avoided Prudhoe Bay oil at a concentration of 1.6 µg/l. SUAZUKI (1964) found that some fish species (goby, perch, striped mullet) avoided «crude petroleum» at 0.7 µg/l. Other types of oil required much higher concentrations to give the same effects.

Earlier experiments on the behaviour of adult cod (*Gadus morhua* L.) in relation to hydrocarbons are lacking. HELLSTRØM and DØVING (1983) have estimated that cod can detect very low concentrations of petroleum hydrocarbons, of the order of 0.1 µg/l.

It is not known whether realistic concentrations, i.e., those less than 500 µg/l, of petroleum hydrocarbons in the sea may alter the natural pattern of fish behaviour, i.e., migration and catchability. The aim of this study was to investigate any avoidance effects of hydrocarbons due to chronic pollution or oil spills. The experiments were performed at the Institute of Marine Research, Flødevigen Biological Station, Norway, in the period 1979–1981.

METHODS

The experiments were performed with two aquaria. One aquarium is cog-shaped, with three compartments and a hexagonal core. The other is rectangular, with two compartments.

THE COG-SHAPED AQUARIUM

A sketch of a cog-shaped aquarium is given in Fig. 1. Except for the bottom in the central section, which was of acryl, the walls and bottom were made of glass, sealed to a steel frame with silicone glue. The water depth in the aquarium was 30 cm, giving a total volume of 55 l in each compartment.

Sea water was introduced separately in each compartment, and the flow was adjusted to maintain equal volumes. An overflow outlet was placed in the

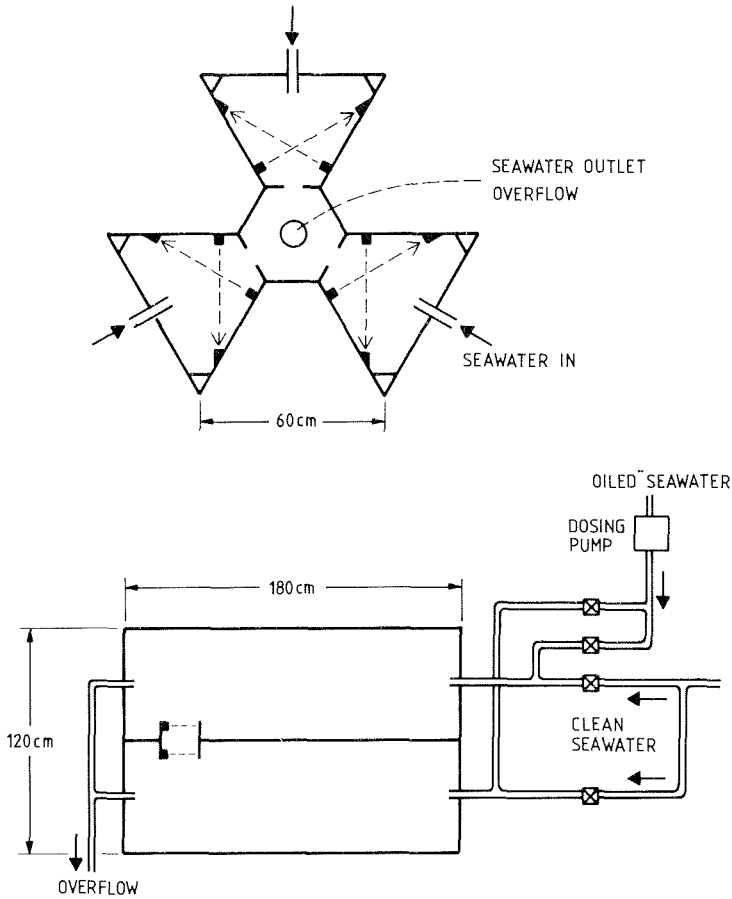


Fig. 1. The experimental aquaria. Top: the cog-shaped aquarium, Bottom: the two-compartment aquarium.

middle compartment. This was separated from the outer compartments by glass, except for openings, 11 cm x 8 cm, through which the experimental fish could move. To minimize the risk of water entering other compartments, the inflows to the several compartments were equalized. Water outlets were arranged as overflow in the middle compartment.

The aquarium was shield from daylight and the side walls covered with black cardboard. Six 25 W lamps above the aquarium and controlled by a dimmer provided equal illumination to each compartment.

In each compartment infrared light sources and photo detectors were mounted for recording where the fish stayed and moved. When the fish interrupted the light beam, an impulse was given to a counter. There were two detectors and two counters for each compartment. The detectors were mounted

identically in each compartment. The numbers on the counters, reflecting the swimming activity in each compartment, were most often logged every two hours.

The size of the aquarium was chosen so that the fish would experience the need for more space or water than was in a single compartment, i.e., the fish would be constantly motivated to move to another compartment. This was necessary to facilitate data-gathering.

The fish were considered to be acclimated to the experimental conditions and unstressed when their swimming mode was relaxed and their pigmentation was natural. This ensured that the fish had relieved any stress due to the capture, transportation, or transfer from the storage tank.

The oily sea water was prepared by letting clean sea water, at a rate of 1–1.5 l/min, flow through a diffusor and sink by gravity as small drops through a column of the lighter fuel oil (Fig. 2). In this way, a relatively large portion of the sea water came in direct contact with the oil. This resulted in a relatively high concentration of hydrocarbons; namely, 2000–3000 $\mu\text{g/l}$ in the outflowing water.

Initially, it was desired to use Ekofisk crude as a source in preparing a «water soluble fraction» (WSF). Because of technical problems in mixing the crude with sea water, this could not be performed. Therefore, the Fuel Oil No. 2 was selected as the pollutant. This is a refinery product which, compared to Ekofisk

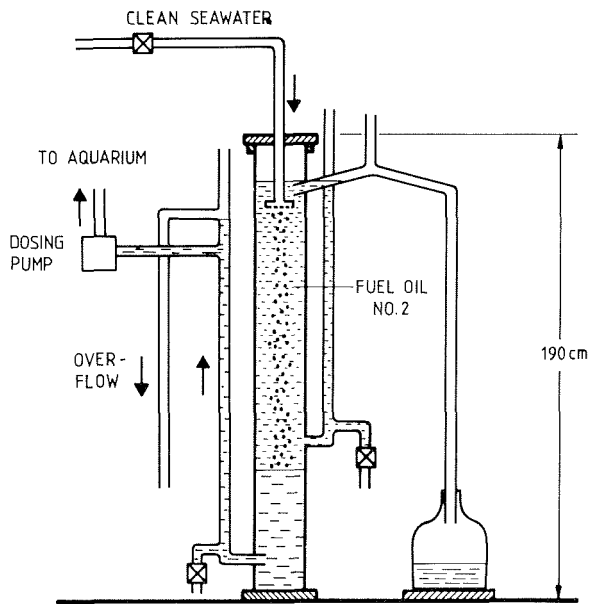


Fig. 2. The petroleum-water mixing arrangement.

crude, lacks only the lowest- and highest-boiling-point components (Fig. 3).

One of the experiences from the Ekofisk blowout in the North Sea in April 1977 was that the hydrocarbons introduced to the sea were both in true solution and in emulsified form (GRAHL-NIELSEN, WESTRHEIM and WILHELMSEN 1977). The oily water in the present study, as indicated on the chromatograph (Fig. 4), contained hydrocarbons both in solution and in emulsion. In this respect, at least, there is a similarity in chemical exposure compared to the Ekofisk blowout (GRAHL-NIELSEN *et al.* 1976).

The experiments lasted from a few days to four weeks. Usually one batch of oil was used throughout the same experiment, sometimes from one experiment to the next one.

It has been argued that the most soluble components of the oil might be «washed out» with time, and that the WSF in the aquarium could shift toward the less soluble components. Tests of the outflowing sea water from the column showed that the content of the most soluble components at the times of measurement was as high as at the beginning of the experiments.

The supply of oily sea water to the aquarium was taken from the outflow of the oil column and delivered at a constant rate by a dosing pump. The concentration of hydrocarbons in the aquarium was altered by adjustments to the dosing pump. The clean and the oily sea water were mixed in a glass funnel to ensure dilution before entering the aquarium.

THE TWO-COMPARTMENT AQUARIUM

The two-compartment aquarium was rectangular, with dimensions 180 cm × 120 cm × 80 cm (Fig. 1). The aquarium was divided into two equal compartments by means of a partition wall of shaded glass. The aquarium was constructed from a steel frame, with bottom and side walls of glass. The end-walls were made from acryl. The aquarium was covered by black cardboard, shaded from daylight, and illuminated from above by a single 60 W lamp.

The inlets of the sea water were placed at the end walls, one in each compartment. The flow was adjusted by valves and controlled separately by flow meters. Two overflow outlets were placed in the opposite end-walls, one in each compartment. There was an opening in the partition wall near the outlets. This opening, 20 cm × 20 cm, was made as small as possible to minimize penetration of water from one compartment to the other, while being large enough to let the fish swim through easily.

Sea water with hydrocarbons was prepared in the oil column (Fig. 2) and delivered by a dosing pump. The pipeline from the oil column was connected directly to the pipeline with clean sea water. The oily sea water was directed to

only one of the two compartments at any one time, but could be shifted from one to the other.

It was not possible to prevent sea water from entering the other compartment. Thus, the fish had the «choice» between oily and less oily sea water.

For monitoring and recording the compartment in which the fish stayed, two ultrasound transmitters were placed in the opening in the partition wall. These were connected to an electronic unit. When the fish moved through the opening, the two sound beams were interrupted. The sequence of interruptions indicated the direction the fish moved, and consequently where the fish had been. A strip chart recorder indicated where the fish stayed. To obtain a quantitative measure of the durations of residence of the fish in the different compartments, the recordings on the chart paper were measured by means of a line meter and computed to give fish distributions in percent of time between the two compartments.

The sea water used in all experiments was taken from 75 m depth, through the Biological Station's sea water system. The salinity range was 30–32 ‰, the temperature varied between 6 and 12° C, though very little within each experiment. In the last two experiments with the two-compartment aquarium, the temperature was held constant at 10° C. To some extent, different temperature levels might explain different levels of total swimming activity and may have influenced how fast a response to changes in water quality could be recorded.

Water samples of 0.8–0.9 l were taken from the aquaria by siphoning into a 1 l separator funnel. Hydrocarbons were extracted by shaking twice for 3 min with 20 ml of distilled dichloromethane. Of the extract, 10–30 ml was evaporated in a Rotavapor to 0.5–1.0 ml. The hydrocarbons with $C_n < 10$ were to a large extent lost during this procedure. This volume was transferred to a 1 ml Microvessel where it was concentrated further by blowing nitrogen. In the first experimental period, the volume was adjusted to 15 μ l. Later on, 40 μ l was used as a standard volume. When the injection in the gas chromatograph was to be performed by an autosampler, the concentrate was transferred to a 0.1 ml V-shaped vial and the volume adjusted to 40 μ l.

The quantification of hydrocarbons in the sea water was performed by gas chromatography (GC). Water samples from the first five experiments with the cog-shaped aquarium were analyzed on a Perkin Elmer 900. The GC was equipped with a 1/4 inch \times 10 feet glass column, packed with 3% SP 2100 on 80/100 Supelcoport. The temperature profile was 80–295° C, at 8° C/min. Carrier gas was nitrogen at 50 ml/min. The GC was equipped with flame ionization detector (FID).

For the quantitative estimates, the area below the chromatographic curve, measured by means of a planimeter, was subtracted from the area below the

chromatographic curve resulting from injection of the same volume of solvent (dichloromethane). As a standard, a solution of Fuel Oil No. 2 in dichloromethane was used. The injection volume was standardized to 2 μ l.

Water samples from the last four experiments with the cog-shaped aquarium and from all the experiments with the two-compartment aquarium were analyzed on a Hewlett-Packard 5880A gas chromatograph. Most of these samples were injected by an automatic sampler. This GC was equipped with FID and 1/4 inch \times 6 feet glass column, packed with 10% SP2100 on 80/100 Supelcoport. The temperature profile was 40–250° C, at 8° C/min. The carrier gas was nitrogen, at 40 ml/min.

From the last experiment with the cog-shaped aquarium and all the experiments with the two-compartment aquarium, the quantitative estimations were performed by the electronic integrator in the HP 5880 system. The calculations were based on the standard of Fuel Oil No. 2 and dichloromethane as solvent as before.

Chromatograms of the Fuel Oil No. 2 standard and a sea water extract are shown in Figs. 3 and 4, respectively. The precision of the analysis is judged to

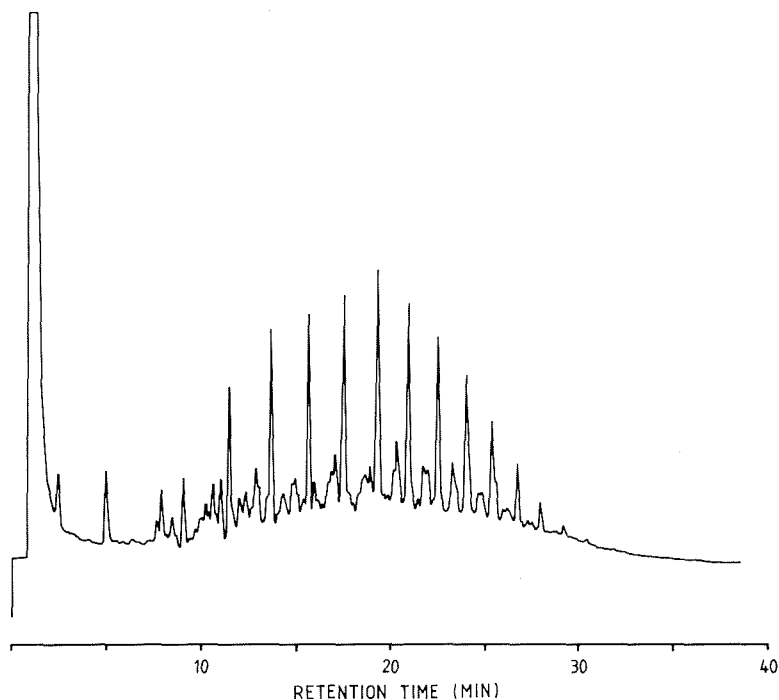


Fig. 3. Chromatogram of the Fuel Oil No. 2, diluted in methylene-chloride. Hewlett-Packard 5880 A.

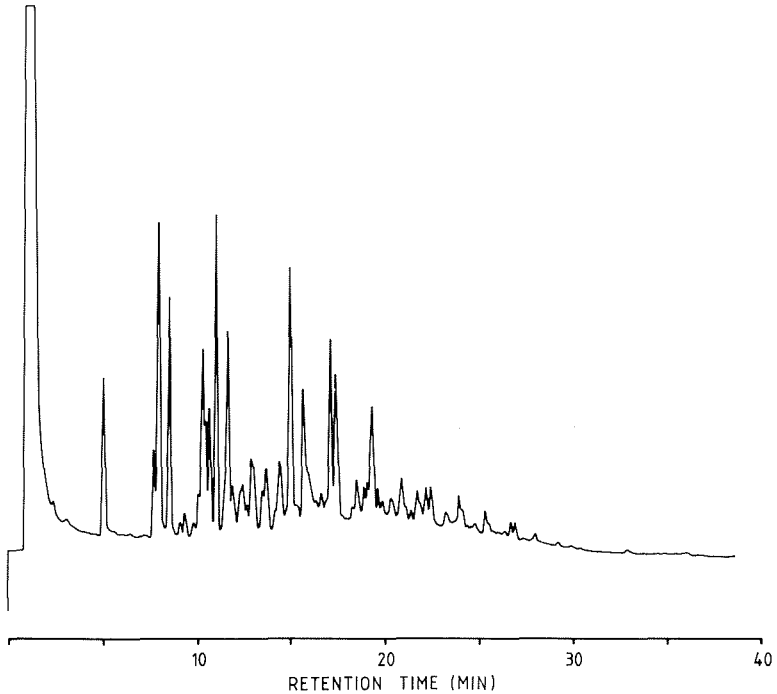


Fig. 4. Chromatogram of the «water soluble fraction» of the Fuel Oil No. 2, introduced to the experimental aquaria. Hewlett-Packard 5880 A.

be of the order of $\pm 20\%$. For several reasons, the sensitivity of the analytical method is judged to be about $10 \mu\text{g/l}$.

By increasing or decreasing the flow of oily water and by recording the fish movements, the fish reaction to different concentrations of hydrocarbons could be studied. With a water flow of 1.5 l/min and volume of each compartment of 675 l , 20 hr elapsed before the hydrocarbon concentration in the aquarium was in balance with the last adjustment.

Most of the fish used in this study were caught in traps along the coast between Arendal and Grimstad (southeastern Norway). In a few experiments one-year-old fish, which had been hatched and bred at the Biological Station in Flødevigen, were used.

The fish held in clean sea water in tanks before being transferred to the experimental aquaria. This should have constituted adequate acclimation to laboratory conditions. The fish needed different time periods to be accustomed to the conditions in the experimental aquaria. This included finding the small opening between the compartments. Some of the fish found these openings rapidly. Later, these same fish could find and move through the openings very easily. Other fish «learned» very slowly to move through the openings. Some

fish apparently were stressed or frightened and never succeeded in moving between the compartments. These were taken out and discarded.

Before introducing the oily sea water, it was desirable that the fish should have an active, but relaxed swimming behaviour, be able to find the opening easily, and spend approximately equal periods of time in the two compartments. The last item was difficult to achieve. Therefore, in most cases, hydrocarbons were introduced to that compartment where the fish apparently preferred to stay.

The time period needed to adaptation and acclimatization varied from 2 days to 3 weeks. There were large differences among the individual fish. Only one fish was used at a time.

Data on swimming activity is not included in this paper. However, in some periods, the activity could be extremely low, and the fish hardly moved from one compartment to the other. In such periods, food items were sometimes introduced to the aquarium divided equally among the several compartments. This usually resulted in increased activity, also between the compartments.

During the study, 30 experiments were performed. However, some of these were pilot experiments and had to be terminated due to stressed fish or to other reasons. Altogether, 16 experiments were executed, 10 with the cog-shaped aquarium and 6 with the two-compartment aquarium.

RESULTS

Recordings of the percentage distribution of the fish among the three compartments are summarized in Table 1 and Fig. 5. Fig. 5 shows that at very low concentrations, below 50 $\mu\text{g/l}$, the fish showed distribution percentages from 5 to close to 100. This reinforces the impression that the fish only to a very small extent noticed the petroleum at concentrations below 50 $\mu\text{g/l}$.

When the fish were offered concentrations 50–100 $\mu\text{g/l}$, they most often stayed for less than 35% of the time. When the fish were offered 100–200 $\mu\text{g/l}$, they stayed there always less than 30% of the time.

Table 1. Number of recordings at each concentration range giving different rates of activity in the cog-shaped aquarium.

Concentration of petroleum hydrocarbons ($\mu\text{g/l}$)	Activity (%)			
	0–15	16–35	36–75	76–100
100–	24	13	4	0
50–99	19	8	4	0
0–49	9	13	15	7

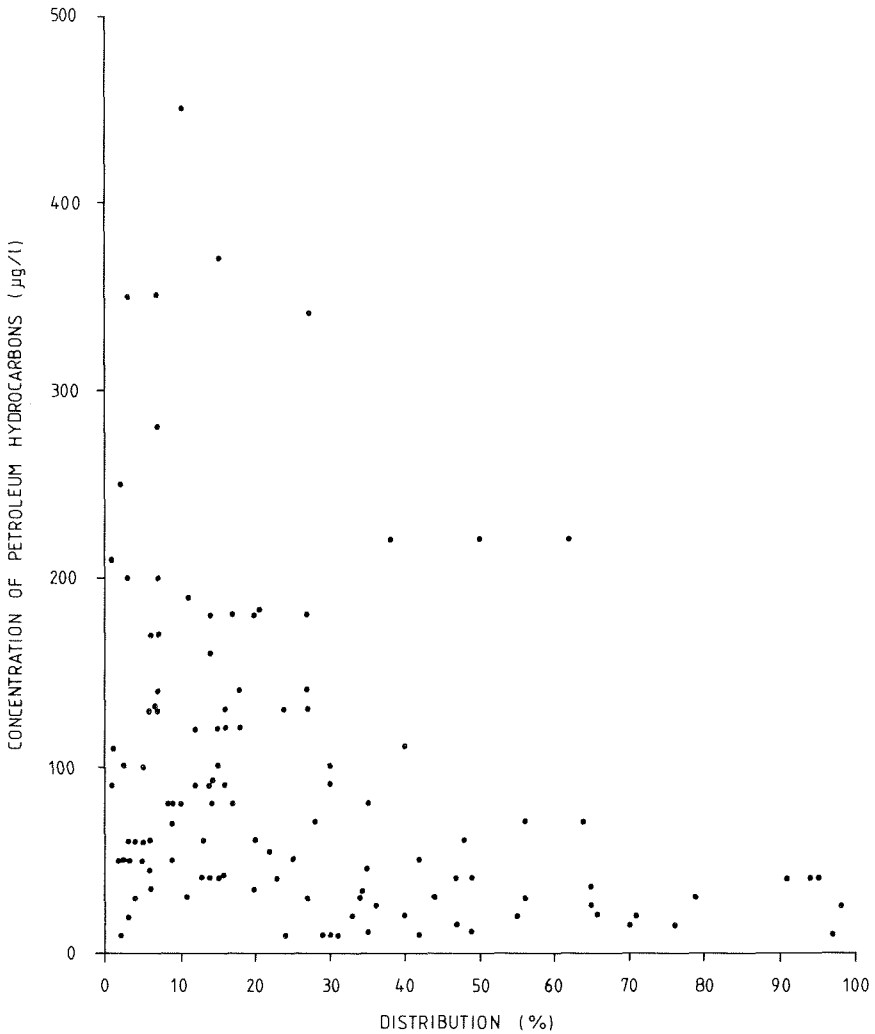


Fig. 5. Recordings of the time spent in water with different concentrations of petroleum hydrocarbons in the cog-shaped aquarium.

A few times the concentrations were increased to 200–400 µg/l. With some exceptions, the fish stayed in such concentrations not more than 10–15% of the experimental periods.

The results from the 10 experiments in the cog-shaped aquarium show that avoidance of petroleum hydrocarbons was observed in all of the experiments (Table 2). Also, responses named «indifference» and «attraction» were observed, though far more scarce. In one case, the avoidance response was recorded at concentrations as low as 30 µg/l. Petroleum concentrations were, to

Table 2. Effects of exposure to petroleum hydrocarbons in the cog-shaped aquarium.

Experiment no.	Concentration of hydrocarbons ($\mu\text{g/l}$)	Behavioural response
8	190-600	Avoidance
11	160-550	Avoidance
	220	Attraction
12	30- 50	Avoidance
	100	(Avoidance)
	200	Avvoidance
13	140-370	Avoidance
	70- 90	Avoidance
	180	(Indifference)
	50- 60	Avoidance
14	80-100	Avoidance
16	50- 70	Avoidance
	90	Avoidance
18	40- 60	Avoidance
	170	Avoidance
	30	Indifference
19	60	Avoidance
	90-120	Avoidance
20	60-100	Avoidance
	30	Avoidance
	10	Attraction
	10	Indifference
21	100-130	Avoidance
	20- 30	(Avoidance)
	20- 40	Indifference

a large extent, maintained in the range 50-100 $\mu\text{g/l}$ during the experiments. A majority of recordings within that range showed the avoidance response.

With one exception, the indifference response was recorded only at concentrations below 50 $\mu\text{g/l}$. From the 10 experiments with the cog-shaped aquarium, it seems that the concentration range of 50-100 $\mu\text{g/l}$ of petroleum hydrocarbons is a border zone between avoidance and indifference.

Recordings of the percentage distribution of the fish between the three compartments are summarized in Table 3 and Fig. 6. Not even at high levels of hydrocarbons did the fish show any distinct response. As seen from Table 4, both avoidance, attraction and indifference were recorded, also at hydrocarbons levels above 100 $\mu\text{g/l}$.

DISCUSSION

The aquaria were constructed in order to separate different types of water without to much intermixing. Hydrocarbons to some extent did penetrate the

Table 3. Number of recordings at each concentration range of petroleum hydrocarbons giving different rates of activity in the two-compartment aquarium.

Concentration of petroleum hydrocarbons ($\mu\text{g/l}$)	Activity (%)			
	0-15	16-35	36-75	76-100
100-	3	6	7	5
50-99	2	5	13	3
0-49	1	8	16	6

other compartments. The situation for the fish was thus most often a «choice» between not quite clean water and water containing a higher concentration of hydrocarbons. Because of the small opening between the compartments, an artificially elevated threshold of stimulus may have had to be exceeded before the fish moved to another compartment. However, in most experiments the fish moved freely through the openings, and it seemed that the openings presented no major hindrance.

The results are variable. Within one estimated level of concentration between different experiments or within one experiment with the same fish, the behavioural responses were observed to vary. This could be explained from natural variation between specimens or from variation or instability in the experimental conditions. How rapidly the fish «learned» or «accepted» the experimental situation in the aquaria seemed to vary from fish to fish. Although obviously stressed fish had been sorted out, some slightly stressed fish may have been included in the experiments.

To some extent, the classification of the behavioural responses was based on subjective judgement. Sometimes, it seemed reasonable to classify the behaviour from a change in fish distribution. In other cases, the classification was based on the actual fish distribution.

Very often a response to a change in the hydrocarbon supply was apparently delayed. This could be due to the need for a period of 20-24 hr to stabilize a new concentration, combined with a possible period for the fish to combine different stimuli («think over») before taking action, i.e., swimming away from the oily water. Such a period would be parallel to what ATEMA and STEIN (1974) called «alert phase» in experiments with lobsters.

A delayed reaction could also be explained by «habitual behaviour», in which the fish continued to stay or maintained a swimming pattern they were used to, despite the environmental conditions becoming unfavourable. Despite such uncertainties, it is concluded that the results support the conclusions below.

A response has been characterized as «avoidance» if the fish stayed away from the concentration to be studied 60-100% of the time. This does not imply that the fish stayed away continuously; the fish could inspect the water or could

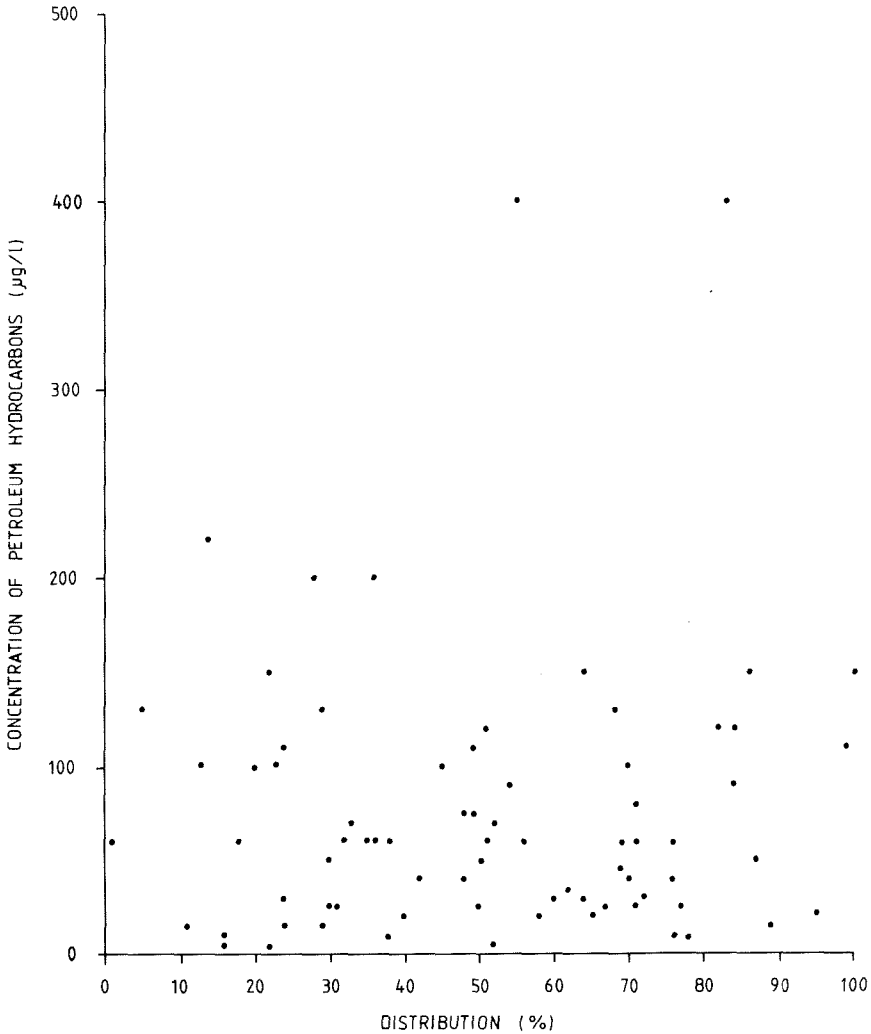


Fig. 6. Recordings of the time spent in water with different concentrations of petroleum hydrocarbons in the two-compartment aquarium.

accept to stay in the water of adverse quality for some time before searching activity for better water quality increased and the fish succeeded or happened to reach water of an acceptable quality. To facilitate data collection, the fish had to have a requirement for movement (and probably food-searching) which resulted in the fish moving into a compartment of adverse water quality.

The fish were not observed to react at concentrations of 10–15 µg/l. Indifference was also noted at 100–130 µg/l. In one of the experiments with the two-compartment aquarium, the fish did not respond to concentrations up to

Table 4. Effects of exposure to petroleum hydrocarbons in the two-compartment aquarium.

Experiment no.	Concentration of hydrocarbons ($\mu\text{g/l}$)	Behavioural response
103	130	Avoidance
	90	Attraction
	90-110	Indifference
	100	Avoidance
104	30- 40	Indifference
	110-150	Attraction
	220	Avoidance
105	110-200	Avoidance
106	25- 60	Attraction
	70- 75	Indifference
	120	Attraction
107	60- 70	Avoidance
	50	Indifference
108	40- 60	Indifference
	130	Avoidance
	100-120	Indifference
	120-130	Attraction
	400	Indifference
	400	Attraction

400 $\mu\text{g/l}$. However, at the end of that experiment, the fish was stressed and exhibited very low activity, hence this recording is doubtful.

The behavioural response characterized as «attraction» was noticed mostly at high concentrations. This characterization is judged to be doubtful when it appeared suddenly during the experiment. However, it has been reported that fish schools have been attracted to oil spilled on the sea surface; it is possible that certain components in the fuel oil may give rise to food-searching activity. Thus, under special conditions, fish may be attracted to petroleum, but this is probably not a «normal» response.

In the two-compartment aquarium, the experimental fish displayed the indifferent response more often than in the cog-shaped aquarium. The reason for this cannot be fully explained. In the two-compartment experiments, larger fish were used. It may be that these fish were less sensitive to petroleum hydrocarbons. It may also be that the size proportions of the two-compartment aquarium were unsuitable for the larger fish.

Despite variable results, the main conclusion is that the cod in these experiments avoided concentrations of total petroleum hydrocarbons down to 50-100 $\mu\text{g/l}$. The question is how these results can be applied for conditions at sea.

It is obvious that the fish's life in an experimental aquarium is different from that in the sea where the fish may be influenced by varying stimuli, for

example, salinity, temperature, current, and light. The total fish behaviour is also a result of feeding activity or food searching, sexual activity, migration, social activity (schooling), and avoidance of predators.

In the sea, behavioural responses due to a pollutant may be overwhelmed by responses due to other stimuli or environmental conditions. In the laboratory experiments, such changing conditions were eliminated, hence the responses which were observed in the aquaria should derive from the concentration of petroleum hydrocarbons alone.

It is impossible to simulate completely in a laboratory what will happen in the sea after an oil spill or during chronic pollution by petroleum hydrocarbons. The hydrographic and atmospheric state to a large extent influences the quality and composition of hydrocarbons entering the water column. In addition, there are almost an infinite number of crudes and petroleum products that may be spilled into the sea.

Within these limitations, then, it seems reasonable to conclude that cod avoid petroleum hydrocarbons at concentrations down to 50–100 µg/l, whether these are in true solution or emulsified as droplets. This may also happen in the sea unless other stimuli should be stronger.

The observed threshold concentrations are well below the concentrations recorded after oil spills in the sea, for example, the Ekofisk blowout in 1977 (GRAHL-NIELSEN *et al.* 1977), where recordings in excess of 300 µg/l were made. As judged from the present results, it seems that the cod would leave or avoid such an area of pollution.

If such concentrations are to have any noticeable effect on fisheries, the concentrations would have to last for weeks or months. Mortality among large fish with high swimming performance is very improbable because these will move away. Mortality on free-swimming fish due to oil spills has hardly ever been recorded. It is also likely that the fish density would have to be very high before any severe effects in practical fisheries could occur.

ACKNOWLEDGEMENT

The author expresses his thanks to the staff at the Department of Biological Oceanography at the Institute of Marine Research, Bergen, for advices and guidance. Thanks are also due to the staff at the Biological Station Flødevigen. Leiv Nilsen developed and constructed most of the sensoric and electronic equipment.

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