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.

A. D. McINTYRE
K. J. WHITTLE

Rapp. P.-v. Réun. Cons. int. Explor. Mer, 157–165. 1977.

EFFECTS OF PETROLEUM HYDROCARBONS ON THE GROWTH OF MARINE ORGANISMS

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Several species of marine organisms, ranging from phytoplankton to fish, have been tested for various growth and reproduction parameters in response to exposure to two crude (Kuwait and South Louisiana) and two refined oils (No. 2 Fuel Oil and Venezuelan Bunker C). The growth of oysters (*Crassostrea virginica*) and brown shrimp (*Penaeus aztecus*) was not affected by oil exposures, while 3·5 mg/litre of total dissolved hydrocarbons (from No. 2 Fuel Oil) reduced embryonic heart beat and hatching success of two fish species and 0·3 to 0·7 mg/litre decreased the growth rates of larval *Palaemonetes pugio*, juvenile *Neanthes arenaceodentata*, and three species of phytoplankton.

INTRODUCTION

As a result of concern over increased inputs of petroleum to the marine environment, effects studies on marine organisms have received considerable emphasis. However, recent reviews (Moore et al, 1973; Evans and Rice, 1974) have noted the need for further work on the sublethal effects of chronic exposures of marine organisms to petroleum hydrocarbons. The effects of oil and detergents on the reproductive success of marine invertebrates has received little attention (Davis, 1972). Sprague (1971) has discussed sublethal effects of pollutants and concluded that growth should be monitored in all chronic exposures. He has also pointed out that reproduction may be a very sensitive physiological parameter and one which is clearly meaningful in the environment.

Some workers have exposed crustacean larvae and marine fish eggs to hydrocarbons from various crude oils. Mironov (1969) has obtained deaths of crab and shrimp larvae at 1 µg/ml concentrations. Mironov (1972) found zooplankton able to tolerate 1 µg/ml but not 100 µg/ml of "oil products". Kühnhold (1972) exposed herring larvae (*Clupea*), to oil at 100 µg/ml by volume of oil added. The exposure caused deformed larvae and the young fish did not seem to avoid oil dispersions. Young larvae were less resistant to the hydrocarbons than embryos (Kühnhold, 1972). More recent work (Kühnhold, 1974) has shown that embryonic development of cod eggs (*Gadus*) was altered by exposure to water soluble fractions (WSFs) of crude oil at total hydrocarbon (TH) levels of 0·015–3·5 µg/ml. Lethal effects were observed at 1–12 µg/ml TH. These concentrations are in agreement with work by Rice et al (1975) with salmon fry. They determined that 6 µg/ml TH from a Prudhoe Bay crude oil was lethal to fry in 96 h tests and growth was decreased after 10 days exposure to 0·73 µg/ml.

Since oysters are an important commercial species and are found in areas where oil is produced they have been studied for effects on growth by crude oils (Mackin and Hopkins, 1962). No adverse effects of a Louisiana crude oil were found from these field studies.

Over the past few years various investigators in our laboratory have been conducting studies on the effects of hydrocarbons on the survival and physiology of marine and estuarine organisms. A portion of these investigations has been concerned with the effects of hydrocarbons from oil on the growth and reproduction of marine organisms. The results of these studies will be summarized below.

MATERIALS AND METHODS

All organisms were collected from Galveston Bay, Texas, or the coastal zone of Galveston Island and transported to the laboratory in College Station. They were held in the laboratory for periods of one week to one month before being utilized in experiments. All sea water was prepared from synthetic sea salts (Instant Ocean) by the addition of deionized water and the salinities were determined by use of an optical refractometer. Unless otherwise noted, laboratory temperatures were 20 ± 1C.

Oil-water mixtures were prepared in the fashion described by Anderson et al (1974a) from four API reference oils. Two of these oils were crudes (Kuwait and South Louisiana), while the other two were refined (No. 2 Fuel Oil) or residual oil (Bunker C). One type

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of exposure mixture was prepared by slowly stirring one part of oil over nine parts sea water for 20 hours and extracting the water phase (water soluble fraction, WSF). The other preparation utilized was an oil-water dispersion (OWD) which was produced by shaking a measured quantity of oil in water vigorously for 5 minutes.

As discussed by Anderson et al (1974a) the mixing of 1 part oil over 9 parts water for 20 hours resulted in water phases which contained from 6—20 µg/ml total hydrocarbons (TH). Even the vigorous shaking of oil with water to prepare OWDs did not introduce much more than 50 µg/ml of TH into the water phase of test solutions, while the calculated concentrations were as high as 10 000—100 000 µg/ml (vol. oil added to water). It should therefore be kept in mind that rather high amounts of oil are required to produce solutions which contain from 1 to 50 µg/ml of total hydrocarbons.

Since numerous toxicity and sublethal effects studies have demonstrated that the high aromatic (38 %) No. 2 Fuel Oil is the most toxic of the four oils it has been more widely utilized in our experiments. It should be noted that both the WSFs and OWDs from the two crude oils (Kuwat and S Louisiana) are considerably less toxic to all the species tested. As the aromatic compounds collectively referred to as naphthalenes (naphthalene, methylnaphthalenes, dimethylnaphthalenes) are particularly high in the Fuel Oil and WSFs prepared from it, analyses of these compounds in the exposure water and animal tissues have been quite useful (Neff and Anderson, 1975).

Each experimental condition varied with the species utilized and the details will be noted next.

RESULTS

PHYTOPLANKTON

A detailed description of the effects of petroleum hydrocarbons on three species of phytoplankton was reported by Mills (1974). The organisms investigated were *Isochrysis galbana* Parke (haptophyte), *Glenodinium halli* Freudenthal and Lee (dinoflagellate) and *Cyclotella nana* Hustedt (diatom). All three species were exposed to two types of oil-water mixtures and the levels which reduced growth during the log phase were reported. Growth was measured as the increase in cell number and increase in quantities of chlorophyll-*a*. Table 49 is from Mills (1974) and summarizes the effect of the various oil-water-dispersions and water-soluble fractions on all three species of phytoplankton. The 72 hour EC_{50} values represent the concentrations of hydrocarbons causing a fifty percent reduction from the control growth rate over the specific period of time. From Table 49, it should be noted that there is relatively good agreement between the values as determined by growth rate and those from chlorophyll-*a* measurements. For both the OWDs and WSFs, the refined oils were significantly more toxic than the crude oils.

Table 49. The 72-hour EC_{50} values determined from interpolation of graphed growth rate data (*K*) and chlorophyll-*a* (Chl *a*) measurements for three species of phytoplankton using the four test oils (from Mills, 1974). Levels of hydrocarbons in the WSF are based on the dilution of a well-characterized water extract, while those of the OWD are calculated from the v/v addition of oil (see Anderson et al, 1974a)

	Isochrysis galbana		*Cyclotella nana*		*Glenodinium halli*	
	K	Chl *a*	*K*	Chl *a*	*K*	Chl *a*
Oil			WSF (µg/ml)			
Kuwait crude	7·8	5·3	12·6	7·8	13·0	6·6
Louisiana crude	4·4	2·8	3·6	2·7	2·4	2·2
No. 2 Fuel Oil	0·7	0·6	0·7	0·3	0·7	0·5
Bunker C	0·7	0·4	1·1	0·7	1·0	0·7
			OWD (µg/ml)			
Kuwait crude	70	*	58	*	20	*
Louisiana crude	13	4·4	18	4·0	13	8·2
No. 2 Fuel Oil	1·3	0·9	2·7	1·8	1·6	1·5
**						

* – No chlorophyll readings taken—interference in determinations from oil droplets in samples.

** – Because of persistant droplets, Bunker C dispersions were not tested.

In additional studies, surviving algal cells exposed to the highest concentration of hydrocarbons were transferred to clean medium and after subsequent transfers the growth rates of these subcultures were equal to those of control cultures transferred through the same series.

POLYCHAETES

The marine polychaetous annelid, *Neanthes arenaceodentata,* has been cultured in our laboratory for approximately three years from a stock supplied by Donald J. Reish. Organisms were separated from the culture containers and juveniles were utilized in growth studies. They were exposed in large culture dishes, placed at different levels such that a water-soluble fraction prepared in a mixing chamber was constantly but slowly moving through the first dish (higher concentration) into the lower culture dish (lower concentration). The two concentrations employed in this particular exposure system were 182 ± 30 µg/litre and 95 ± 20 µg/litre total naphthalenes. The naphthalenes (naphthalene, methylnaphthalenes, and dimethylnaphthalenes) were derived from the mixing of water beneath

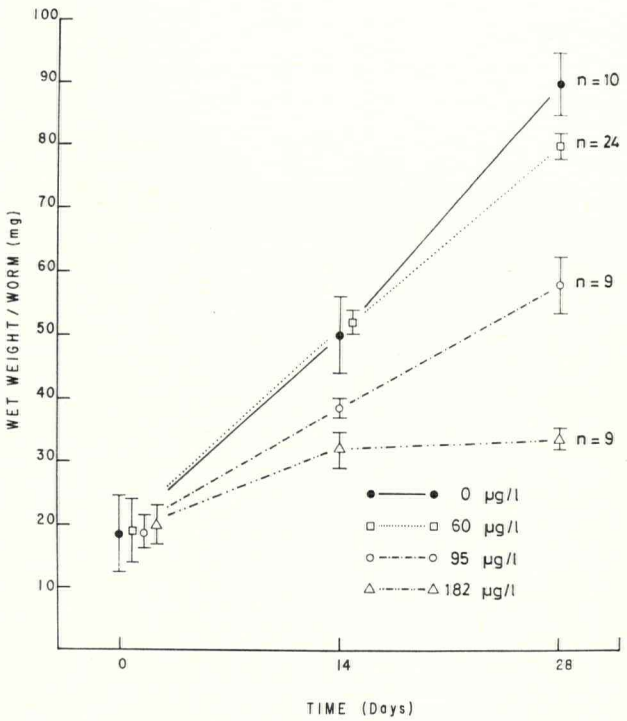

Figure 96. Effect of water-soluble fractions (WSFs) of No. 2 Fuel Oil on the growth of *Neanthes arenaceodentata*. The concentration of total naphthalenes (TN) in the exposure water are shown. Each finger bowl contained 20 worms, and the *n* value represents the number of bowls in each group.

a layer of No. 2 Fuel Oil. A similar system which generated a concentration of 60 µg/litre total naphthalenes (TN) was utilized in an earlier study. In both experiments, cultured organisms were placed in a flow-through control system duplicating the exposure condition with the exception of the presence of hydrocarbons.

The results of wet weight measurements made on worms from each group after 14 and 28 days are shown in Figure 96. It should be noted that those organisms exposed to total naphthalenes at 60 µg/litre and the control animals grew at approximately the same rate and achieved nearly the same size. The group of worms exposed to 182 µg/litre total naphthalenes exhibited significantly reduced growth and achieved a weight of less than half that of control organisms. The response of organisms exposed to the 95 µg/litre concentration was intermediate between the above two groups.

In examining the levels of naphthalenes required to suppress growth (95 µg/litre or greater), it should be noted that the full strength WSF from No. 2 Fuel Oil contains approximately 2 µg/ml TH. This solution is prepared by mixing 1 part oil over 9 parts water for 20 hours and the parent oil is considerably higher in these aromatic compounds than crude oils. When one prepared OWDs from an addition (v/v of 1000 µg/ml oil and shook for 5 min, the resulting water phase contained total naphthalenes at concentrations of 4 µg/ml (No. 2 Fuel Oil), 0·3 µg/ml (South Louisiana crude), and 0·1 µg/ml (Kuwait crude). It is clear that considerable amounts of oil are required to produce a water content of 100 µg/litre total naphthalenes.

CRASSOSTREA VIRGINICA

Since earlier studies by our laboratory and by other researchers have demonstrated that oysters are extremely tolerant to short-term exposures to oil, rather unrealistic concentrations of oil were used in these growth studies. It is obvious that oysters, as other bivalves, may resist exposure to chemicals in the water by shell closure and initiation of anaerobic metabolism. Therefore it is likely that during the four days of exposure to 1 % (10 000 µg/ml) levels of the four test OWDs, the oysters remained closed for more than normal periods of time. However, it has been demonstrated that under these conditions, oysters accumulate high levels of naphthalenes and other petroleum hydrocarbons from the water (R. D. Anderson, 1975). Since there is no doubt that the tissues become contaminated to a high degree during the exposure, the effect of contamination on growth was tested.

As shown in Table 50, the oil-exposed oysters did not exhibit any reduction in growth over the 105 day period examined. While the exposures took place in the laboratory, growth was measured while the animals were maintained in a flowing sea water system on Galveston Island. In similar studies it has been shown that heavily contaminated oysters release the accumu-

Table 50. Effect of a 96 hour exposure to 1 % concentration of four oils on the shell growth of oysters. Daily average and total growth are recorded. (From R. D. Anderson, 1975)

Oil	Growth
No. 2 Fuel Oil	4·9 mm/105 days 0·05 mm/day
Venezuelan Bunker C	5·4 mm/105 days 0·05 mm/day
South Louisiana crude	5·7 mm/105 days 0·05 mm/day
Kuwait crude	5·0 mm/105 days 0·05 mm/day
Control No. 1	4·8 mm/105 days 0·05 mm/day
Control No. 2	3·1 mm/105 days 0·03 mm/day

lated hydrocarbons, which are largely naphthalenes, at some point between 13 and 52 days in clean water (Anderson, 1975).

PENAEUS AZTECUS

As it was shown that No. 2 Fuel Oil was the most toxic of the four oils (Anderson et al, 1974a) and contained a greater proportion of the naphthalenes, water-soluble extracts from this oil were used in growth studies on the commercial brown shrimp, *Penaeus aztecus*. Three groups of postlarval shrimp were separated from a stock which measured 1·36 ± 0·06 cm in length and weighed 2·5 ± 0·4 mg dry weight. While one group provided determination of normal growth, one exposure group was initially exposed to 6·0 µg/ml (70 % WSF) of total hydrocarbons from No. 2 Fuel Oil for 2 hours and returned to clean water for 35 days (acute exposure). It should be noted that 5 µg/ml of this mixture was shown to be the 24 hour TLm for postlarvae. The third group was exposed to lower doses of WSF (chronic exposure) at weekly intervals for 28 days and growth measured for 35 days. As the initial exposure to 2·6 µg/ml (30 % WSF) for 2 hours was judged to be near acute, the subsequent for weekly exposures were reduced to 1·3 µg/ml total hydrocarbons.

The results of molting frequency and growth measurements on the *Penaeus aztecus* postlarvae are summarized in Table 51. It is evident that growth and molting were not significantly affected by either exposure condition. These results are not surprising, considering additional research by Cox (1974) which is summarized in Anderson (1975). Both postlarval and adult *Penaeus aztecus* have been shown to accumulate significant quantities of naphthalenes from No. 2 Fuel Oil WSF's,

Table 51. The effect of chronic and acute exposure to the WSF of No. 2 Fuel Oil on the molting frequency and growth of *Penaeus aztecus*[1] (from Cox, 1974)

	Control	Acute exposure	Chronic exposure
Molting frequency (days per molt)	6·9 ± 1·9	7·4 ± 2·2	6·6 ± 1·2
Final weight (mg dry wt)	29·2 ± 9·4 [2]	31·4 ± 9·5	29·2 ± 10·6
Final length (cm).....	2·86 ± 0·32[2]	2·97 ± 0·30	2·86 ± 0·35

[1] n = 15 for each condition in molting studies and n = 60 for each condition in growth studies. Ten shrimps were sacrified each week under each condition for length-weight determinations.

[2] The overall mean and standard deviation for the initial length and weight of all test animals were 1·36 ± 0·66 cm and 2·5 ± 0·4 mg dry weight.

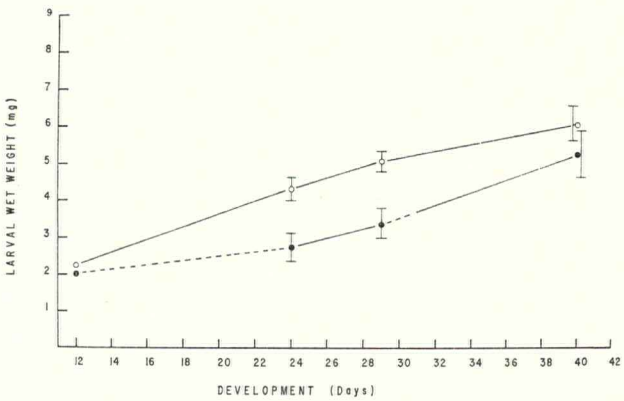

Figure 97. Effect of WSFs of No. 2 Fuel Oil on the growth of *Palaemonetes pugio* larvae. The dashed lines indicate the periods when larvae were exposed to 0·5–0·8 µg/ml TH and 0·3 µg/ml TN. Each value represents the mean weight of a larva derived from the analysis of 10–12 larvae, with standard errors shown as vertical bars (from Tatem, 1975). Open circles refer to control animals, while closed circles indicate exposed shrimp.

but when placed in clean water these petroleum hydrocarbons are released from the tissues in from about 4 days to one month (Cox, 1974). It was apparent that release of naphthalenes by postlarvae was somewhat slower than for adults, and this differential deserves further examination. From the results shown in Table 51, it is likely that the time periods after the initial acute exposure and between the chronic doses were sufficiently long for the postlarvae to rid themselves of the hydrocarbons and thus abnormal growth was not exhibited.

PALAEMONETES PUGIO

Extracts from No. 2 Fuel Oil were also used to test the effects of petroleum hydrocarbons on the growth of larvae from grass shrimp, *Palaemonetes pugio*. The larvae produced from two females were reared in the laboratory for two weeks and then sixty larvae (less than 0·5 cm in length) were divided between four culture dishes. After determining the wet weight of the group from a sample of ten animals, two dishes were established as controls, while the other two were exposed daily to hydrocarbons from a WSF of No. 2 Fuel Oil. Hydrocarbon content in the two dishes of the exposure group varied between 0·5 and 0·8 µg/ml total hydrocarbons and contained approximately 0·3 µg/ml total naphthalenes. Both groups were fed *Artemia* nauplii daily.

As shown in Figure 97, after 12 days of exposure (24 days of development) those larvae exposed to petroleum hydrocarbons exhibited growth which was significantly less than that of control animals. At this time

period some of the exposed larvae had died and all remaining larvae in both groups had developed into postlarvae. All postlarvae were then transferred to clean water for 5 days, and the subsequent growth of both groups was determined. From the slopes of the two curves it can be seen that while in clean water both the control and previously exposed groups grew at the same rate. Next, those postlarvae in the exposure group were again exposed to the same level of hydrocarbons for two days and finally transferred to clean water for the remaining 10 days of the experiment. It would appear that this latter short exposure did not interfer with growth and indeed the slope of the growth curve during the last 12 days was slightly steeper than that of the control animals. The final weights after 40 days were not significantly different, as shown by the overlap of standard errors for the two groups. It appears that the initial reduction in growth exhibited by the exposed larvae was counteracted by the postlarvae during periods in clean water, even though they were subjected to a 2 day re-exposure.

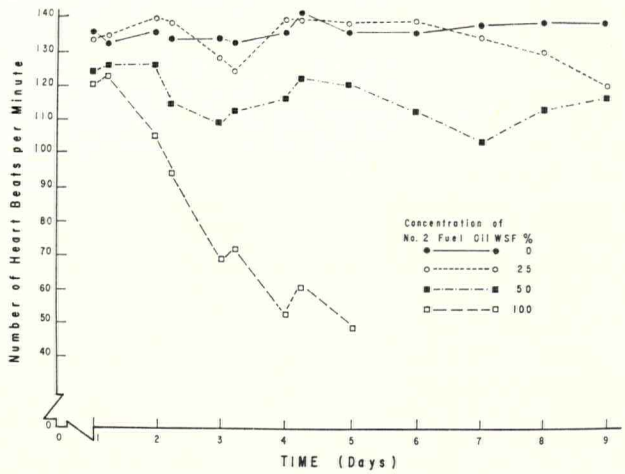

Figure 98. Effect of WSFs of No. 2 Fuel Oil on the heart beat rate of *Fundulus heteroclitus* embryos. The 100% WSF contained approximately 8·0 µg/ml TH and 1·9 µg/ml TN. Each value represents the mean rate of 5 embryos (from Anderson et al, 1976.)

FISH

Live embryos of *Cyprinodon variegatus* and *Fundulus heteroclitus* were obtained from the breeding stock of males and females by stripping and fertilizing the ova in the laboratory (Trinkaus, 1967). The embryos were kept in glass fingerbowls prior to use in experiments. Only healthy looking individuals of apparent similar stages were utilized for the experiments. Required concentrations of WSF from No. 2 Fuel Oil were obtained by diluting the 100 % stock with 20 ‰ S Instant Ocean immediately before use. The 100 % WSF of No. 2 fuel oil contains approximately 7 µg/ml total petroleum hydrocarbons (Anderson et al, 1974a).

Unhatched embryos of *Cyprinodon variegatus* and *Fundulus heteroclitus* approximating to an age of 130 hours, were exposed in groups of five to eight to various concentrations of WSF of No. 2 Fuel Oil. The number of heart beats per minute were counted periodically until the embryos hatched or died. The embryos were maintained at approximately 22°C in glass fingerbowls. No changes of water were made during the course of the experiment nor was the water aerated. Concentrations of the WSF used were 0, 25, 50 and 100 %.

The control group of embryos of *Fundulus heteroclitus* showed a very steady rate of heart beat for a period of nine days (Fig. 98). By the ninth day, all embryos of the control group had hatched. Embryos in 25 % WFS of No. 2 Fuel Oil showed a pattern of heart beat similar to that of control embryos for the first seven days. After that time the observed decrease in rate was due to a single embryo with lower heart rate that did not hatch until the ninth day. Heart beats of embryos in 100 % WSF decreased on the very first day and continued to decrease until the sixth day when all embryos were dead. The embryos in 50 % WSF exhibited a response which was intermediate between the responses of those in 100 and 25 % WSF. A gradual decrease in rate of heart beat was observed in embryos in 50 % WSF. Only 3 of the 5 embryos in this group hatched. No mortality occurred in the surviving fry that were placed in clean sea water after hatching.

The effect of exposure to different concentrations of No. 2 Fuel Oil WSF on rate of heart beat of *Cyprinodon variegatus* was very similar to that observed for *F. heteroclitus* (Fig. 99). The control groups and em-

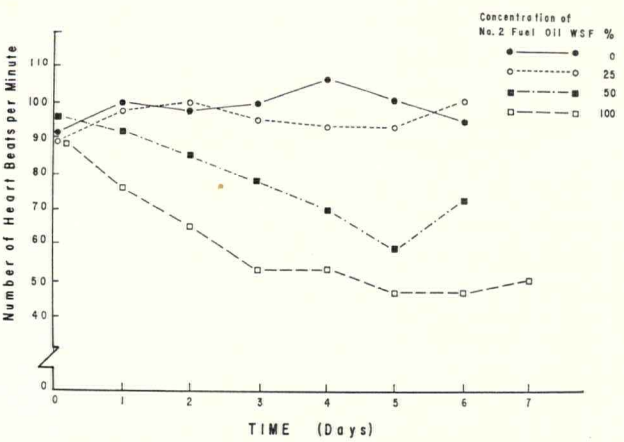

Figure 99. Effect of WSFs on No. 2 Fuel Oil on the heart beat rate of *Cyprinodon variegatus* embryos. Each value represents the mean rate of 8 embryos (from Anderson et al, 1976.)

bryos in 25 % WSF had a steady rate of heart beat for a period of six days. By the end of the sixth day all surviving embryos had hatched. Only one of the eight embryos in 25 % WSF died after the fifth day; the rest hatched normally. Heart rate of embryos in 100 % WSF decreased the very first day and continued to decrease until the fifth day. From the fifth day onwards until the seventh day, the embryos in 100 % WSF maintained slow heart beat, averaging 48 beats/minute. All embryos in this highest concentration were dead by the eighth day of exposure. Embryos in 50 % WSF exhibited an intermediate response to that of embryos in 100 and 25 % WSF. A gradual decrease in heart beat was noted until the fifth day. On the sixth day there was a sharp increase in heart rate, since a single embryo with a slow rate died after day 5. Only 3 out of 8 embryos hatched in the 50 % WSF. A comparison of the two species indicates that both species exhibit similar effects on heart rate and hatching when exposed to WSF of No. 2 Fuel Oil.

DISCUSSION

In order to compare these various growth studies it is perhaps best to examine the effects of the water-soluble fractions of No. 2 Fuel Oil. Earlier investigations have demonstrated that this oil contains a high proportion of the aromatic hydrocarbons collectively referred to as naphthalenes. These compounds have been shown to be (a) toxic in the high µg/litre range (80—1000 µg/litre = 24 hour TLm), (b) accumulated in the tissues rapidly and to the greatest extent of any specific hydrocarbons tested, (c) retained longer than any other petroleum hydrocarbons analyzed. It should be noted that fuel oils contain a significantly higher amount of these aromatics than crude oils and the specific fuel oil tested had a higher than normal (approximately 38 %) aromatic content. The full strength water-soluble extract of this oil (WSF) contained about 7 µg/ml of total hydrocarbons and approximately 1·9 µg/ml of total naphthalenes (Anderson et al, 1974a).

A summary of the effects of No. 2 Fuel Oil on the growth of the organisms tested is presented in Table 52. While there are considerable differences in the exposure periods, it is obvious that oysters are the most resistant organisms as they exhibited normal growth rates after 4 days exposure to a 1 % OWD (approximately 40 µg/ml). Studies by R. D. Anderson (1975) have shown that during this exposure period the oysters accumulate relatively large amounts of naphthalenes, but during a period of 13 to 52 days in clean water contamination decreased to below the level of detection (0·1 µg/ml). Stegeman and Teal (1973) and Lee et al (1972a) have also reported release of petroleum hydrocarbons from oysters and mussels, respectively, but the point of complete release was not observed. With bivalves it is difficult to determine the extent of time that the organism is actually exposed to the contamination in the water. The remaining species have no mechanism to resist exposure and thus the results are more readily comparable.

Fish embryos are somewhat protected from the water by their chorionic membrane, but removal of this membrane did not result in an alteration in larval survival (Anderson et al, 1976). It would appear that the fish embryos tested are relatively resistant to the fuel oil fractions as nearly a 50 % WSF (3·5 µg/ml total hydrocarbons) was required to significantly reduce heart beat and hatching success. While the ability of embryos and larvae to metabolize or detoxify petroleum hydrocarbons has not been studied, Lee et al (1972b) have reported on the capabilities of adult marine fish.

It is interesting to compare the results of studies on the two species of shrimp. The tolerances of the two species are relatively similar (Anderson et al, 1974a), so it is likely that differences in the conditions of exposure produced the contrast in results. Short term exposures (2 hours) of *P. aztecus* to even very high levels of hydrocarbons (6·0 µg/ml) did not produce a reduction in growth or molting. Exposures separated by one week in clean water very likely provided ample time for the shrimp to release previous contamination (Cox, 1974; Anderson, 1975) and grow normally for a period, before re-introduction of the contaminant. When the grass shrimp (*P. pugio*) larvae and post-larvae were subjected to constant hydrocarbon exposure at a lower level (0·8 µg/ml) for 12 days, a significant reduction in growth was observed. The period of 5 days in clean water following the above exposure likely enabled this species to release accumulated hydrocarbons (Tatem, 1975; Anderson, 1975) and resume normal growth. A two day exposure period interposed between a 5 and the final 10 day intervals in clean water had no effect on growth. On return to clean water the grass shrimp grew at a somewhat accelerated rate such that the final weights of animals from the exposure group were not significantly different from those of control animals.

The most adversely affected test organisms were the phytoplankters, since a 3-day exposure to 0·7 µg/ml total hydrocarbons produced a 50 % reduction from normal growth rates. Other evidence of reduced phytoplankton growth in response to extracts of No. 2 Fuel Oil has been reported (Gordon and Prouse, 1973). Of course one cannot study the effects of long-term exposure on single-celled organisms with such short life spans, so most experimentation has been restricted to exposures of a few hours to 2 to 4 days. Mills (1974) attempted to evaluate the long-term effects of oil exposure by transferring aliquots of cells from a culture,

Table 52. Comparison of the effects of No. 2 Fuel Oil WSF on the growth of selected marine organisms

Organism	Exposure period	Concentration µg/ml TH	TN	Growth parameter
Phytoplankton				
Isochrysis galbana	3 days	0·7	0·2	Growth reduced to 50% of normal (EC_{50}). Subcultures of cells exposed to the highest concentrations resumed normal growth rates after transfers through clean media (Mills, 1974).
Cyclotella nana	3 days	0·7	0·2	
Glenodinium halli	3 days	0·7	0·2	
Polychaete				
Neanthes arenaceodentata	28 days	0·4	0·1	Growth reduced by approximately 30%.
Oyster				
Crassostrea virginica	4 days	40[1]		No reduction in growth after 105 days in clean water.
Brown shrimp				
Penaeus aztecus	2 hours	6·0	1·3	Neither "acute" nor "chronic" exposures produced a reduction in growth or molting rate.
	2 hr/week	2·6 + 4 × 1·3	0·6 + 4 × 0·3	
Grass shrimp				
Palaemonetes pugio	12 days	0·8	0·3	Reduced growth after 12 days constant exposure.
	2 days	0·8	0·3	Resumed normal growth over 17 days in clean water even with a 2 day re-exposure.
Fish (Embryos)				
Fundulus heteroclitus	2–3 days[2]	3·5	0·9	Reduced embryonic heart beat and hatching success.
Cyprinodon variegatus	2–3 days[2]	3·5	0·9	

[1] 40 µg/ml is the calculated amount of hydrocarbons in the water produced from a 1% OWD of No. 2 Fuel Oil (Anderson et al, 1974a).

[2] Without replenishing the hydrocarbons, it is likely that levels were below the limit of detection after 2 to 3 days.

which had exhibited marked reduction in growth, to clean media. After several subsequent transfers of both control and previously exposed cells, the growth rates of both populations were equal. These findings would indicate that cells surviving exposure from spills would produce daughter cells capable of dividing at the same rate as normal cells.

Simulating a constant or chronic exposure the polychaete, *Neanthes arenaceodentata,* was grown in water containing 0·4 µg/ml of total hydrocarbons for 28 days. Approximately 25 % of this concentration consisted of the aromatic naphthalene compounds. The worms grew at a slower rate and reached a size which was about 30 % smaller than that of control animals. Observations by Rossi (unpublished data) indicate that during oil exposure the worms are ingesting smaller quantities of food. Additional research has shown that these polychaetes are capable of releasing accumulated naphthalenes and have exhibited increased tolerance to these compounds after long-term pre-exposure. These related findings suggest that if the worms were removed from the contamination, normal growth would resume. Further experimentation is planned which should answer this question.

From the data presented it is clear that the growth of marine organisms is inhibited by long-term exposure to petroleum hydrocarbons in the range of 0·4 to 0·8 µg/ml, particularly when aromatic compounds represent a major portion of the total (0·1 to 0·3 µg/ml total naphthalenes). The suppression of growth exhibited by phytoplankton and animals after constant exposure to low levels of hydrocarbons indicates that these growth parameters are more sensitive than other physiological measurements conducted in earlier studies (Anderson, 1975; Anderson et al, 1974a; 1974b). The effects cannot be directly compared, however, as earlier experiments generally involved shorter exposure periods.

It is particularly important to consider the differences between different oils and the amounts of these products required to produce the hydrocarbon mixtures used in these studies. No. 2 Fuel Oil has been utilized extensively since it is the most toxic of the four oils, and contains a high level of naphthalenes which are quite toxic, accumulated and retained significantly and are readily measured by a simple technique (Neff and Anderson, 1975). To produce a sea water solution containing 0·3 µg/ml of total naphthalene the amount of various oils needed are as follows:

No. 2 Fuel Oil	—	15 % of the WSF
		9 % of a 1000 µg/ml OWD
Bunker C	—	30 % of the WSF
South Louisiana	—	100 % of the WSF
		100 % of a 1000 µg/ml OWD
Kuwait	—	≥ 100 % WSF (0·071 µg/ml TN)
		> 100 % of a 1000 µg/ml OWD

With these analytical values in mind one can understand the differences observed in tests with crude and refined oils. We are presently utilizing South Louisiana crude in similar growth and reproduction studies to provide a better means of comparison. While it appears that naphthalenes are major contributors to the toxicity exhibited by No. 2 Fuel Oil, their content in crude oils may or may not be significant. The toxicities of various crude oils and petroleum products might be compared on a basis of their specific hydrocarbon content in an effort to identify particularly toxic classes of compounds.

The usefulness of laboratory derived data in evaluating the significance of a pollutant in the environment is frequently a subject of debate. The data become more meaningful when experiments are conducted on a longer time scale and when the test concentrations are compared to those reported to occur in the natural environment. Because of analytical difficulties associated with determinations of specific hydrocarbons at very low concentration in sea water samples, nearly all values reported in the literature are in terms of total hydrocarbons or saturated vs. aromatic hydrocarbons. Recently, Gordon et al (1974) have summarized the reported levels of hydrocarbons in sea water and the methods used in the determinations. Total hydrocarbon values generally range from 10 to 50 μg/litre, with the higher levels associated with the areas where tanker lanes approach larger cities. Aromatic hydrocarbons are usually reported to be less than 5 μg/litre, with a few higher values found in particularly polluted regions.

There are a few instances in which one would expect hydrocarbon levels to reach extremely high concentrations. One such case is during or soon after an oil spill from a vessel or drilling operation. McAuliffe et al (1975) reported on the chemical and biological data derived from an extensive study of a 1970 spill (65 000 barrels) from a Chevron offshore production platform. As dispersants were utilized much of the oil was present in oil-in-water emulsion form and occurred at concentrations between 1 and 70 μg/ml (near platform). The highest level of dissolved hydrocarbons measured was 200 μg/litre, while most other samples contained 20 μg/litre or less.

One would also expect that areas containing oil drilling and separator platforms would exhibit high levels of saturate or aromatic hydrocarbons. Templeton et al (1975) reported on an extensive study of Lake Maracaibo, Venezuela, where oil has been produced for approximately 60 years. Ninety per cent of the water samples collected showed less than 1 μg/ml extractable organic material of which less than 15 % could be attributed to saturate or aromatic hydrocarbons. No methylnaphthalenes were detected in the water samples. In field studies conducted over the past year in Galveston Bay, Texas, we have as yet not detected specific aromatic hydrocarbons in the water near an oil-separator platform while significantly high levels have been found in the sediments near the platform (Anderson et al, unpublished data).

Of course measurable quantities of specific aromatic compounds have been found when field experiments have been conducted within a closed system. Cox et al (1975) found water levels of total naphthalenes reached a peak of 0·3 μg/ml when 113 litres of No. 2 Fuel Oil was spread on a 0·2 hectare shrimp pond of about 1·2 m depth. While the sediment contained approximately 3·0 μg/ml of total naphthalenes after 96 days, the water content had decreased to less than 10 μg/litre.

Without discussing the various means by which hydrocarbons can be removed from the water column, it is perhaps sufficient to state that the content of coastal and open ocean water is generally quite low. Considering the quantities of oil required to produce the hydrocarbon levels shown in this paper to reduce normal growth or development, and the relatively short residence time of hydrocarbons in sea water, marine organisms would not seem to be threatened at this time. Another factor to consider is the capability of nearly all organisms studied to recover when petroleum hydrocarbons are no longer present in the sea water. In some cases the recovery is likely the result of available detoxification pathways, as in crustaceans (Corner et al, 1973) and fish (Lee et al, 1972b), while in other instances hydrocarbons may be lost with sloughed cells or slowly flushed from the tissues (bivalves and polychaetes). When considering the effects of petroleum on the marine environment it is clear that a chronic low-level input of hydrocarbons into shallow water is likely to be more deleterious to marine life than a single spill.

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