## 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, 171: 175-183. 1977.

## THE EFFECT OF MINERAL OILS ON THE DEVELOPMENT OF EGGS AND LARVAE OF MARINE SPECIES. A REVIEW AND COMPARISON OF EXPERIMENTAL DATA IN REGARD TO POSSIBLE DAMAGE AT SEA

WALTHER W. KÜHNHOLD

Institut für Meereskunde an der Universität Kiel, D–2300 Kiel, Düsternbrooker Weg 20, Bundesrepublik Deutschland

The paper gives a review of relevant publications. There are astonishingly few papers on the toxicological or metabolic effects on the generally most susceptible stages of the life cycle. Most experiments were carried out with different experimental parameters (temperature, salinity, species). The most important data are lacking: contents and spectrum of hydrocarbons in the test medium. The data given in the papers are tabulated and compared. It is, however, impossible to draw a general conclusion or see a connecting pattern of influence of hydrocarbons. Corresponding field observations on eggs and larvae do not exist.

#### INTRODUCTION

Since people are becoming aware of the extent of dumping, and of the frequency of accidental spillages of oil into the seas, an increasing number of attempts has been made to get an idea of what might happen to animals and plants as they come into contact with the almost unmeasurable spectrum of fossil hydrocarbons, i.e. mineral oils. Of the many experiments only a few were undertaken in a systematic manner. Moore and Dwyer (1974) were the first to make a critical assessment of published data on the effect of oil on marine organisms. Their paper summarises published studies on the effects of mineral oils on living marine organisms.

The authors view their studies (Moore et al, 1973) as a starting point for discussion and iteration towards a model for understanding the impact of petroleum substances on higher levels of biological organisation, i.e. on marine communities, on the ecological web and on food chains.

As our knowledge is still a filigreed picture of the effects on individuals there are only spotlights of understanding. This means that we are still at the starting point for conceptualizing the interference of oil in the marine environment. Moore and Dwyer resume the impact on all "classes" of organisms found in the marine environment. The early stages of animal life cycles, eggs and larvae, usually represent the most susceptible sector of the whole life cycles. So it seems adequate to focus again on this weakest link of the biological chain i.e. on the subsistence of a stock.

The published data of numerous experiments aiming to determine the toxicity of "oils to larvae and eggs" of a variety of species are reviewed in detail and evaluated below. They are supplemented by a discussion of where and how further investigations should start, to come to a better assessment on the impact on life cycles of individuals and the survival of a stock.

#### OIL AS PHYSIOLOGICAL FACTOR

Planktonic eggs and larvae are exposed, on the whole, to all changes of their environment including introduction of pollutants. At these stages usually the whole body surface is unprotected, with the integument acting as osmotic membrane.

Hydrocarbons like other chemical compounds from the surrounding medium can enter the organism to physicochemical equilibrium depending on the lipophilic properties of the membrane and of the tissue compounds. The possible responses to interference with hydrocarbons may be divided into 5 categories, two of which (4 and 5) are applicable to planktonic stages only with certain restrictions:

- (1) Direct lethal toxicity
- (2) Sublethal toxicity
- (3) Accumulation of hydrocarbons
- (4) Avoidance of polluted habitat
- (5) Effects of direct contact with oil films or droplets.

Toxicity means interference with cellular or subcellular processes, which leads to disruption of the normal metabolism.

Hydrocarbons may come into competition with some metabolites or block some pathways. The organism tries to adjust the energy flow system through additional cellular activity, and if adequate mechanisms are available to the organism the toxicant is metabolized (Corner et al, 1973). This means physiological stress at the expense of normal chemical process, i.e. shortage of energy. In the case of developing eggs, this means a deficit of energy for the differentiation of the predetermined ontogenetic pattern. Consequently lethal deformations may occur, or even immediate death if the substances block enzymes and cause an interruption of energy flow. A lower stress may be balanced, with little differentiation damage, and as long as the energy supply is sufficient the toxicity remains sublethal. If the toxicant interferes with some nervous function, this may cause behavioral change which might not be lethal primarily, but may perhaps secondarily lead to death.

Accumulation of hydrocarbons can occur in eggs or larvae, with deposition in any tissue of higher lipophilic properties than the average body tissue. This is usually a fat or oil deposit. Many fish eggs for example have visible oil droplets in the yolk. Hydrocarbons accumulated in such oil or fat deposits may be "inactivated" at first. When the fat reserves are mobilized, however, the hydrocarbons are likely to be liberated and reintroduced into metabolic cycles. The ability and range of active movement decreases with the size of organism. Some large crustacean or fish larvae have a high swimming speed and show an extensive diurnal vertical migration, so that they are not confined to a particular polluted water.

Direct contact with an oil film may occur in tidal zone communities, coating sessile forms, benthic eggs (e.g. some herring races spawn in very shallow waters), settling larvae (e.g. barnacles, polychaetes etc.). Coating effects usually mean mechanical or physiochemical injury besides the metabolic stress. Littoral larvae not rising to the surface can come into direct contact with oil droplets formed by the dispersing action of waves, even without anthropogenic help from "cleaning the sea surface" with chemicals.

## PRESENT STATE OF EXPERIMENTAL EVALUATION

Since hardly any field observations on the effects of oil spillages on eggs and larvae have been made, all publications dealing with the changes in ecosystems in regard to this subject, are based on some of the experiments listed in Table 61. The only field observations of effects on early stages are published by Straughan (1969) and George (1970). Straughan observed that after the Santa Barbara oil spillage, at all localities except the badly polluted Santa Barbara harbour, the barnacle *Balanus* was releasing (planktonic) nauplii, and that settlement of the larval stages occured as well.

George reported that some days after a fuel oil spill-

age off the south coast of England, autumn spawning was normal in a polychaete population. The mortality of the brood was the same as previously observed and eggs collected from that location and incubated in clean water showed normal development. Laboratory experiments up to now have been confined to measuring direct lethal toxicity and survival time. As 34 species were used in these tests a good description of the effects should be expected. As the table however reveals, researchers never used identical experimental methods such as oil type (crude or refined), way of application (sea water extracts, time of extraction, dispersions, size of experimental vessels, time of exposure etc.). In some cases the same oils have been tested, but as the physiochemical behavior on water is a function of many experimental factors, which were never identical, it is clear that the test media differ widely in their properties. The effects are even more difficult to judge as almost no analytical data have been collected. From the experiments and observations of the 16 publications cited only 3 reported any analytical data at all.

The only experiments accompanied by extensive analyses were made by Anderson et al (1974) who gave a comparison of the compositions of the oils tested and those of the water soluble fraction. The concentration of total hydrocarbons dissolved varies for the different oils. A profound finding, however, is that the relative content of aromatics from C10-C17 of the water soluble fraction, is enriched by magnitude of 14 to 125 (under the condition used by Anderson et al) in comparison to their percentage in the parent oil. This is especially important for light crude oils with high percentage of low boiling paraffins (e.g. Kuwait crude). Refined oils such as No. 2 fuel and Bunker C, initially have a higher aromatic than paraffin content, consequently the enrichment factor is lower. Analyses made by Boylan and Tripp (1970), however, without parallel biological experiments gave similar results. (In this connexion it should be mentioned that, besides the investigation of Anderson et al, the only series of biological tests with extensive parallel analyses of hydrocarbon content in the test medium was carried out by Bean et al (1974). The test, however, did not use larval or egg stages.) The results of Anderson et al suggest that, at least in reference to 4 oils tested by them, the toxicity of an oil is a function of its di- and triaromatic hydrocarbon content. Their concentration in water however is generally very low, because of their low water solubility. On the other hand the statement above by Anderson et al is supported by analyses of Kühnhold (unpublished). The concentration of mono- to triaromatics in a seawater extract, prepared from a mixture of these substances, was decreasing with time reciprocal to their water solubility and boiling point when the seawater extract was left standing. The ratio of aromatic to

Species Stage Common Type of Type of Way of Volume Analyses of Authors name tested test oil appliof test test medium (age/days) cation medium or oil Echinodermata Pisaster ochraceous..... E (2) L (17) Luida foliata..... starfish survival No. 2 fuel SWE of 40 ml none Chia, 1973 time 0.50/0 oil Crossaster papposus ..... L (17) L (4) Dendraster executriucus . . . . . . Psammechinus miliaris..... E sea fertilization OWD with ? Kuwait crude none Lönning & urchin development Ekofisk crude film Hagström short time 1975 effect Mollusca Acmaea scutum..... limpet L (3) Haminoea virescens . . . . . . . . . seaslug L (11) survival No. 2 fuel SWE of 40 ml none Chia, 1973 time 0.5%/0 oil L ( 9) L ( 4) L ( 9) Melibe leonina..... Katharina tunicata . . . . . . . . . Crassostrea gigas..... oyster Crassostrea gigas..... Spermato- fertilization oyster Arabian crude "concen-Renzoni, "large none zoa first develop-Venez. crude trated sobeakers" 1973 Crassostrea angulata ..... oyster L ment Russian crude lution" = E swimming acti- Gas oil SWE? vity No. 2 fuel Mytilus galloprovincialis ..... blue mussel Annelida..... Polycha-Л Л S C C A A

Table 61. Selected marine species, of which eggs or larvae have been used in oil resistance tests. L = larvae, E = eggs, SWE = seawater extracts, OWD = oil water dispersion

Nereis vexillosa ragwo Nereis branti	orm L (-) L (4)	survival					
		time	No. 2 fuel	SWE of $0.5^{\circ}/_{0}$ oil	40 ml	none	Chia, 197 <mark>3</mark>
Serpula vermicularis bristle	- (-)			0.5.70 011			
Cirratulus cirratus	(adults, eggs)	field obser- vation, spaw- ning, after- effect	"fuel oil"	spillage		none	George, 1970
Crustacea		011000					
Artemia salina brine shrim		hatching success	Marine diesel	oil film	10 ml	none	Aubert et al, 1969
	L	survival, toxicity limit					
Acartia clausi copep	od L	survival time	fuel oil (heavy)	"mixture" (= OWD)	-	none	Mironov, 1969 a)
Oithona nana copep	od L		,	(			2000 a)
Balanus sp barna	cle L	field ob- servation: releasing, settlement	Sta Barbara crude	spillage	(Sta Bar- bara chan- nel)	(none?)	Straughan, 1969
Balanus cariosus barna	cle L (?)	survival time	No. 2 fuel	SWE of $0.5^{\circ}/_{0}$ oil	40 ml	none	Chia, 1973
Elminius modestus barna	cle L	general observation	Kuwait crude	OWĎ	-	none	Smith, 1968
Neopanope texana	several lar- val stages	- survival time, moulting	Venezuelan crude (light)	SWE of 1º/ <sub>0</sub> (24 h)	"compart- ed plastic boxes"		Katz, 1973
Pachygrapsus marmaratus crab	L	,	Russian crude light fuel oil	"mixture"			
					(	Continued	overleaf)

12

Species	Common name	Stage tested (age/days)	Type of test	Type of oil	appli-	Volume of test medium	Analyses of Authors test medium or oil						
Crustacea													
Pilumnus hirtellus	crab	L	survival time	heavy fuel oil	OWD	-	none	Mironov, 1969b					
Leander adspersus	shrimp	L											
Homarus americanus	lobster	L	LC 50 develop- ment	Venezuelan crude	OWD	-	none	Wells, 1972					
Penaeus aztecus	shrimp	postlarvae		Lousiana crude	OWD	0·5, 0·7 1	IR, GC (detailed!)	Anderson et al, 1974					
				No. 2 fuel Bunker C	SWE of $10^{\circ}/_{\circ}$		,						
Batrachia					10								
Boltenia celosa		L (4) (tadpole)	survival time	No. 2 fuel	SWE of 0.5%/0	40 ml	none	Chia, 1973					
Fish													
Rhombus maeoticus	Blacksea turbot	E L	survival hatching	Russian crude light	OWD	-	none	Mironov, 1967					
Engraulis enrasicholus Scorpaena porcus	anchovy	E E	behaviour mortality	heavy fuel heavy fuel	OWD		none	Mironov, 1969c					
Crenilabrus tinca		Е											
Gadus morhua	atlantic cod	Е	mortality	heavy Bunker C light	SWE of $0.1^{0}/_{0}$ (static &	1,61	none	James, 1925					
				Bunker C	flow through)								
ti		survival time embryoge- neses	Venezuelan crude Iranian crude	SWE of 0.1, 0.01%/0	20 1 1 1, 0·1 1	total hc (by wt), refinery data of oil	Kühnhold 1972, (1973)						
			hatching				relative aromatic content						
		yolksac larvae	behaviour recovery, LC 50	Libyan crude									
Clupea harengus	herring	E L	survival time hatching success morphoge- nesis behavio	Venez. crude Iranian crude Libyan crude fractions of Iranian crude ur	SWE of 1 (2) <sup>0</sup> / <sub>0</sub> , dispersions	11	none refinery data for of	Kühnhold, 1969 ils					

Table 61 (continued)

paraffin in water soluble fractions was calculated by Anderson et al. For the crude oils it is 0.15 to 0.24, clearly different from refined oil (fuel No. 2 and Bunker C) where it is 1.2 to 3.4. These values might be carefully extrapolated to other similar oils, taking into consideration that the way of applying the oil in other experiments varied from those of Anderson et al. For example, the 96LC<sub>50</sub> of 5 µg/ml total hydrocarbons for the larvae of *Penaeus aztecus* (dissolved from No. 2 fuel oil) need not at all express the same resistance as 96LC<sub>50</sub> of 5.2 µg/ml (of Venezuela crude) for cod eggs.

If oil-water dispersions are used there is even less possibility of a comparison, because the dispersions are subject to still more changes with time in their composition than water soluble fractions. There is little evidence on what is affecting the organisms in dispersions, whether it is the oil droplets themselves or the dissolved hydrocarbon leached from the droplets. A short test with herring larvae gave some evidence for the effects of dispersion changing their toxic properties (Kühnhold, 1972). Dispersions prepared from Iran crude were standing 1 and 50 hours before tests begin. Figure 105 shows that after two days the toxicity of the dispersion has decreased (curve 4 versus 1). However, when the dispersions were stabilized by an emulsifier (the emulsifier alone was nontoxic at the concentra-

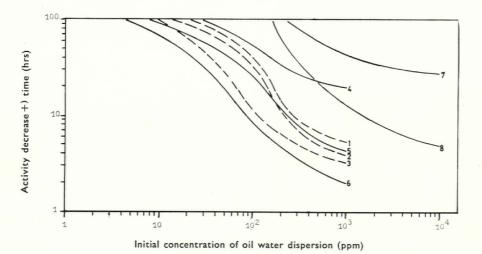


Figure 105. Decrease of swimming activity of 3-day-old Norwegian herring larvae exposed to dispersion of Iranian crude oil. +) = time until decrease of activity to degree 2 = lowest activity level for recovery (Kühnhold, 1972).

Curve 1: dispersion, standing time, without dispersant Curve 2: dispersion, before test ,  $1^{0}/_{0}$  dispersant Curve 3: dispersion, 1 hour ,  $10^{0}/_{0}$  dispersant Curve 4: dispersion, standing time, without dispersant Curve 5: dispersion, before test ,  $1^{0}/_{0}$  dispersant

Curve 6: dispersion, 50 hours, 10% dispersant

Curve 7: seawater extracts of crude oil, extracted for 1 day same initial amounts.

Curve 8: solution of dispersant (Corexit 7664).

tion used (1 and 10 % of oil)) the toxicity increased slightly in the 1 h-dispersion and strongly in the 50 hdispersions. Another effect is that the relative toxicity increases with lower concentration of the dispersions (curve 4 versus 1). However when the dispersions were stabilized by an emulsifier (the emulsifier alone was non-toxic at the concentration used (1 and 10 % of oil)) the toxicity increased slightly in the 1 h-dispersion and strongly in the 50 h-dispersions. Another effect is that the relative toxicity increases with lower concentration of the dispersions (curve 4, untreated dispersion). Therefore, an attempt to compare directly the toxicity data, listed in Table 62, in regard to species resistance and to the different oils, must fail. In spite of this an approach for estimating the toxicity level for larvae has already been made by Moore et al (1973), (Table 63).

It shows the sensitivity of the "class" of larvae to hydrocarbons in comparison to other ecological "classes". The sensitivity grouping also uses estimates of soluble aromatic derivatives (SAD) as a basis for comparison.

Moore and Dwyer (1974) suggest a consideration of these data for only two categories of marine organisms – adults and larvae: for most adults a lethal response can be expected in the range of  $1-100 \,\mu\text{g/ml}$  soluble aromatic derivatives for periods of several hours and for the larval stages at concentrations of  $0.1 \,\mu\text{g/ml}$ soluble aromatic derivatives.

It must be stressed however that this is an estimation for a short exposure time. Over a longer period toxicity levels decrease to a tenth or less as is seen with the cod eggs (Table 62).

Taking the ratio of 0.2 for aromatics/*n*-paraffines (Anderson et al, 1974) as a very rough estimation of the soluble aromatic derivatives involved in the LC<sub>50</sub> for the cod eggs, this would result in a LC<sub>50</sub> range of 5 to 25  $\mu$ g/l soluble aromatic derivatives for early embryonic stages until hatching. Similar extrapolations may be done for other LC<sub>50</sub> data in Table 62, for an exposure over the whole incubation period or longer period of larval development. This indicates a much lower toxic concentration of soluble aromatic derivatives than 0.1  $\mu$ g/ml.

# RELEVANCE OF EXPERIMENTAL DATA TO NATURAL CONDITIONS

For the estimation of any injury of the brood of any species in the sea, some considerations are made in the following. Figure 106 shows the interdependence and interaction of factors which affect the consequences of oil spillages positively or negatively. As previously mentioned the ultimate interest is the resulting concentration of dissolved hydrocarbons in the sea. Although some experimental data on the dissolved total amount of hydrocarbons and the aromatic fraction obtained in seawater extracts are now known, the web of hydrographical and chemical factors affecting dissolution reveals the impossibility of predicting the resulting concentration at sea. In spite of this, only one attempt Table 62. Toxicity data of different oils to various marine species (for authors see Table 61) OWD = oil water dispersion, i. c. = initial concentration, i. a. a. = initial amount applicated (for extraction by seawater).  $^{1}$  = extrapolated from diagrams,  $^{2}$  = interpolated from published tables

Species	Common name	Stage tested	Type of oil	Appli- cation	Degree of toxicity				
Penaeus aztecus	shrimp	postlar-	Lousiana	OWD	96 $LC_{50} > 1000 \ \mu g/ml$				
	T	vae	crude	SWE	$96 LC_{50} > 19.8 \mu g/ml$				
		postlar-	No. 2 fuel	OWD	$96 LC_{50} = 9.4 \ \mu g/ml$				
		vae		SWE	96 $LC_{50} = 5.0 \ \mu d/ml$				
		postlar-	Bunker C	OWD	$24 \text{ LC}_{50} = 3.8  \mu \text{g/ml}$				
		vae			$48 \text{ LC}_{50} = 3.5 \ \mu\text{g/ml}$				
					$96 \text{ LC}_{50} = 1.9 \ \mu\text{g/ml}$				
Acartia clausi	copepod	L	heavy fuel	OWD	$48 \text{ LC}_{50} = 100 - 10 \ \mu\text{g/ml} (i.c.)$				
Oithona nana	copepod	L	heavy fuel	OWD	$48 \text{ LC}_{50} < 1 \mu \text{g/ml} (i.c.)$				
Pilumnus hirtellus	shrimp	Ĩ.	Russ. crude	OWD	$48 \text{ LC}_{50} = 100 - 10 \ \mu\text{g/ml} (i.c.)$				
Leander adspersus	shrimp	ĩ	Russ. crude	OWD	48 LC <sub>50</sub> $\approx$ 100 µg/ml (i.c.)				
Pachygrapsus marmaratus	crab	L	Russ. crude	OWD	$48 \text{ LC}_{50} = 50 \ \mu\text{g/ml} (i.c.)$				
a unigrapsus marmaratus	ciub	-			96 $LC_{50}$ < 1 µg/ml (i.c.)				
Pachygrapsus marmaratus		L	fuel	OWD	$48 \text{ LC}_{50} = 50  \mu\text{g/ml} \text{ (i.c.)}$				
I unygrapsus marmaratus		1	1401	_	$96 \text{ LC}_{50} < 1 \mu \text{g/ml} (i.c.)$				
Pachygrapsus marmaratus		L	heavy fuel	OWD	$48 \text{ LC}_{50} = 1 \ \mu\text{g/ml} \ (\text{i.c.})$				
r unygrapsus marmaratus		1	neuvy ruer	0112	$96 \text{ LC}_{50} = 1 \mu \text{g/ml} (i.c.)$				
Elminius modestus	barnacle	L	Kuwait crud	eOWD	$> 100 \mu g/ml$ : "no effect for several hours"				
Artemia salina	brine	Ē	Marine	SWE	toxicity limits $TL = 300 \ \mu g/ml$ (i.a.a.)				
Artema sauna	shrimp	L	diesel	SHL	hatching				
	smmp		uicsei		survival after TL = $57 \ \mu g/ml$ (i.a.a.)				
					hatch				
		L			survival $TL = 2 \mu g/ml$ (i.a.a.)				
DI I I I I I I I I I I I I I I I I I I	Blacksea	E	Russ. crude	OWD	$48 \text{ LC}_{50} \approx 60 \mu\text{g/ml} (i.c.)^2$				
Rhombus maeoticus		(4-8  cells)	Russ. crude	OWD	$\sim 00 \ \mu g/m$ (i.e.)				
C. I	turbot	E E	Venezuela	SWE	48 LC <sub>50</sub> 12 $\pm$ 5.8 <sup>1</sup>				
Gadus morhua	cod	0.5  days	Venezueia	SVIL	$96 \text{ LC}_{50}$ 2.6 $\pm$ 1.0				
		0.5 days			hatch $0.09 \pm 0.06$				
		2 1	Venezuela		$\begin{array}{cccccccccccccccccccccccccccccccccccc$				
		3 days	VEIIEZUEIA		96 LC <sub>50</sub> $10.0 > \times > 1.0$				
					hatch $0.05 \pm 0.03$				
		10 1	Venezuela		$\begin{array}{cccccccccccccccccccccccccccccccccccc$				
		10 days	venezuela						
					$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$				
		1 days	Tron	SWE	$48 \text{ LC}_{50}$ 70 $\pm$ 30 <sup>1</sup>				
		1 day	Iran	SWE	$96 \text{ LC}_{50}$ 19 $\pm$ 7 <sup>1</sup>				
					hatch $0.3 \pm 0.14$				
		2 down			$\begin{array}{cccc} \text{Hatch} & 0.5 & \pm & 0.14 \\ 48 \text{ LC}_{00} & 100 & > \times > 10^1 \end{array}$				
		3 days			96 $LC_{50}$ 5.2 $\pm$ 2.41				
					50				
		6 hours	Libus	SWE					
		6 hours	Libya	SWE					
		3 days			hatch $2\cdot 2 \pm 0\cdot 5^{1}$				

(known to the author) was made to determine the dissolution of hydrocarbons in a large scale experiment, with an oil slick of 100 t of Kuwait crude (Dodds, 1970). The concentration in the upper 5 m under the slick ranged from 0.01 to 0.45 µg/ml, with 0.1 µg/ml as the mean value. Estimating the soluble aromatic derivatives on the basis given by Anderson et al for Kuwait crude would yield a concentration of 0.18–0.004 µg/ml total soluble aromatic derivatives (0.04 µg/ml being the mean value). 0.03–ca. 0.001 µg/ml soluble aromatic derivatives of C10–C17 may represent the sea water extract some time after the low boiling compounds evaporated. These values almost reach the level

which has been obtained in some oil-seawater extracts on a laboratory scale (Kühnhold, 1974, Tables 62 and 64), and which lie in the range of LC<sub>50</sub> for early stages of cod eggs and exposure time until hatching (Table 62). However, there are no indications of how long these concentrations will stay in the surface layer. Levy and Walton (1973) found levels of 1 to 10 µg/l of total hydrocarbons in coastal waters of the West Atlantic. In Swedish coastal waters and from the open Baltic sea, Carlberg (1973) found concentrations between 0·2 µg/ml and less than 50 µg/l of total (nonpolar) hydrocarbons.

Similar calculations for the soluble aromatic deriv-

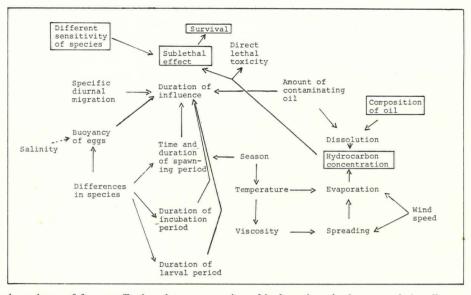


Figure 106. Interdependence of factors affecting the concentration of hydrocarbons in the sea, and the effects on eggs and larvae.

Table 63. Summary of toxicity data (after Moore et al, 1973). The toxicity range for larvae is discussed in the text.

Class of organisms	Estimated concen- tration (µg/ml) of soluble aromatics causing toxicity					
Flora	. 10 –100					
Finfish	. 5 - 50					
Larvae (all species)						
Pelagic crustaceans	. 1 - 10					
Gastropods (snails, etc.)	. 1 -100					
Bivalves (oysters, clams, etc.)	. 5 - 50					
Benthic crustaceans (lobster, crabs, etc.)	. 1 – 10					
Other benthic invertebrates (worms, etc.)						

atives, however, can hardly be made. The possible expected toxic effect is, however, decided largely by the probability of planktonic eggs and larvae appearing and staying in the contaminated water layer. This has for example been studied for planktonic fish eggs. At low wind speeds (0-2 Bft) 10-100 % of the eggs distributed over the whole water column were collected in the upper 10 cm, whereas from 3 Bft the ratio was only 1 %. A limitation of contamination of the water body by little wind and turbulence parellels the accumulation of eggs in the surface layer. On the other hand, depending on the species and their specific buoyancy, planktonic fish eggs concentrate in deeper water at halocline discontinuity layers at depths of 30 to 60 m (Müller pers. comm.; Dannevig, 1945). These organisms cer-

Table 64. Concentrations of total dissolved hydrocarbons (HC) and dissolved aromatic derivatives (SAD) (total and C10-C17-fraction) measured in seawater extracts from  $0.001 \ 0/0-10 \ 0/0$  of oil and beneath an oil slick at sea. <sup>1</sup> = data estimated, on a basis given by Anderson et al, 1974.

$10^{0}/_{0}$ oil		Hydrocarbon co $1^0/_0$ oil			content in seawater ex from $0.1^{0}/_{0}$ oil		xtracts (µg/ml) 0·01º/0 oil			100 t oil slick Kuwait crude				
total HC			total HC			total HC			total HC			total	total	SAD C10-C17
23.76 21.65 1.36 6.28	13·9 10·0 1·28 5·74	0·31 0·08 0·94 2·0	← 20	0 h/20	°C							0.01-0. x=0.1	45 0-04	0.01
			4·80 4·42 0·37	1.71	$0.8^{1}$ $0.7^{1}$	0·83 0·88 0·21	0.31	$\begin{array}{c} 0 \cdot 14^{1} \\ 0 \cdot 1 \end{array}$	0·05 0·05 0·03	0.021	$0.01^{1}$ $0.01^{1}$		1	
	total HC 23.76 21.65 1.36 6.28 2	total total HC SAD 23.76 13.9 21.65 10.0 1.36 1.28 6.28 5.74 24 h/6 °	total total SAD HC SAD C10-C17 23.76 13.9 0.31 21.65 10.0 0.08 1.36 1.28 0.94 6.28 5.74 2.0 24 h/6 °C $\rightarrow$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	total total SAD total total HC SAD C10-C17 HC SAD 23.76 13.9 0.31 21.65 10.0 0.08 1.36 1.28 0.94 $\leftarrow$ 20 h/20 6.28 5.74 2.0 24 h/6 °C $\rightarrow$ 4.80 0.37	total total SAD total total SAD HC SAD C10-C17 HC SAD C10-C17 23.76 13.9 0.31 21.65 10.0 0.08 1.36 1.28 0.94 $\leftarrow$ 20 h/20 °C 6.28 5.74 2.0 24 h/6 °C $\rightarrow$ 4.42 1.71 0.71 0.37	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

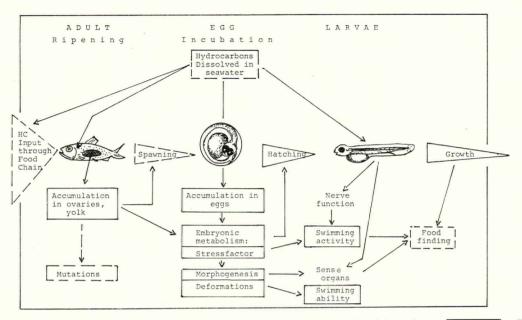


Figure 107. Possible pathway and effects of hydrocarbons at sublethal concentration in adults to larvae \_\_\_\_\_: effects or concentration exactly measurable \_\_\_\_\_: effects not exactly measurable.

tainly are beyond the influence of acute oil pollution. They will only be affected if the whole water column is contaminated at concentrations mentioned above.

Dispersions reaching the toxicity level  $(LC_{50})$  (Table 62) will probably only be obtained when dispersing oil films by means of chemicals. There are no concentration data of dispersions at sea resulting from wave actions.

In bights and firths with low water depth and low water exchange, these concentrations can easily be reached. In this case even benthic eggs (herring, polychaetes) would be influenced.

Estimating the possible decimation of a larval population must also take into account the duration of the spawning period, which can vary with abnormal water temperatures. Consequently the phase of lower or higher sensitivity of the brood to hydrocarbons is shifted in time.

### PROPOSALS FOR EVALUATION OF SUBLETHAL EF-FECTS OF HYDROCARBONS ON EARLY STAGES

As the discussion revealed there is hardly any evidence of direct lethal toxicity of hydrocarbons to larvae in the sea. Therefore it appears to be of less value to go on establishing tables of toxicity levels for more and more species, than to ask for the possible sublethal interference. Further investigations should focus on the effects on eggs and larvae by hydrocarbons, induced by the mother animals through food chain accumulation or uptake of dissolved hydrocarbons. 5 points of major interest may be stressed:

- 1) Accumulation of hydrocarbons in ripening ovaries and incubated developing eggs
- 2) Embryonic development: metabolism rate of eggs with accumulated hydrocarbons, morphogenesis
- 3) Hatching success: percentage of lethal deformities
- 4) Behavior of normally hatched larvae: swimming behavior
- 5) Survival of primary vital larvae: swimming activity, feeding activity, growth.

Figure 107 shows the interdependence of these factors taking as an example the life cycle of fish. Such factors as hydrocarbon accumulation in the food chain and spawning will not be measurable directly. As for the first, the input of hydorcarbons into animals caught at sea is not known and for the second, spawning behavior and efficiency is usually changed in the laboratory milieu. Mutations, which are often quoted in literature are difficult to detect. Accumulation of a wide spectrum of hydrocarbons in herring ovaries was observed, and there are also indications that the early stages of eggs accumulate hydrocarbons from oil-seawater extract of very low hydrocarbon concentration (Kühnhold, in prep.). Both ways of hydrocarbon accumulation, through the mother animal and directly from the water, suggest a physiological stress, as previously mentioned. The embryonic metabolism rate may be expressed in terms of O<sub>2</sub>-consumption or also the amount of hydrating enzymes, corresponding to the cellular activity. Stress during incubation without any obvious sign of morphogenetic deformations may even appear after hatching.

In spite of "normal" embryonic development and successful hatching after short incubation in sublethal oil-seawater extracts observations on reduced survival time were made. The swimming activity of cod larvae decreased to 50 % within one day (Kühnhold, 1972).

Investigations on the abnormal morphogenesis of larval organs and proof of malfunction of sensitive organs, like chemoreceptors and eyes, will require most subtle work. The normal function of nerves, necessary to predation (and escape) can otherwise be measured secondarily through swimming behaviour and food finding, which is then expressed in normal or abnormal growth. This, however, is a main concern for the survival of a stock of any species.

#### REFERENCES

- Anderson, J. W., Neff, J. M., Cox, B. A., Tatem, H. E. & Hightower, G. M. 1974. Characteristics of dispersions and watersoluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Marine Biology 27: 75 -88.
- Aubert, M., Aubert, J., Gambarotta, J. P., Donnier, B., Barelli, M. & Daniel, S. 1969. Etude générale des pollutions chimiques rejetées en mer. Inventaire et études de toxicité, I. Centre d'études et de recherches de biologie océanographique médicale, Nice.
- Bean, R. M., Vanderhorst, J. R. & Wilkinson, P. 1974. Interdisciplinary study of the toxicity of petroleum to marine organisms. Battelle, Pacific Northwest Laboratories Richland, Washington 99352.
- Boylan, D. B. & Tripp, B. W. 1971. Determinations of hydrocarbons in seawater extracts of crude oils and crude oil fractions. Nature, London, 230: 44-7.
- Carlberg, S. R. 1973. On the Baltic oil pollution problem. A presentation of Swedish investigations. Second Swedish-Soviet Symposium on the Control of the Baltic Sea Pollution. Chia, F. S. 1973. Killing of marine larvae by diesel oil. Mar.
- Pollut. Bull. 4 (2): 29–30. Corner, E. D. S., Kilvington, C. C. & O'Hara, S. C. M. 1973. Qualitative studies on the metabolism of naphthalene in *Maia*
- squinado (Herbst). J. mar. biol. Ass. U. K. 53: 819-32. Dannevig, A. 1945. Undersøkelser i Oslofjorden 1936-1940; Egg og lyngel av vårgytende fiskearter. FiskDir. Skr., Serie Havundersøkelser, 8, No. 4, 91pp. (Engl. summary).

- Dodds, E. N. 1970. Report of oil spillage trail. In Coastal Water Pollution—Pollution of the Sea by Oil Spills, Committee on the Challenges of Modern Society No. 1.
- George, J. D. 1970. Sub-lethal effects on living organisms. Mar. Pollut. Bull., 1 (NS), No. 7.James, M. C. 1925. Preliminary investigation on effect of oil
- James, M. C. 1925. Preliminary investigation on effect of oil pollution on marine pelagic eggs. Report of the United States Bureau of Fisheries.
- Katz, L. M. 1973. The effects of water soluble fraction of crude oil on larvae of the decapod crustacean *Neopanope texana* (Sayi). Environ. Pollut. 5: 199–204.
- Kühnhold, W. W. 1969. Der Einfluss wasserlöslicher Bestandteile von Rohölen und Rohölfraktionen auf die Entwicklung der Heringsbrut. Ber. Dt. Wiss. Komm. Meeresforsch. 20: H.2, 165–71.
- Kühnhold, W. W. 1972. Untersuchungen über die Toxizität von Rohölextrakten und -Emulsionen auf Eier und Larven von Dorsch und Hering. Diss. Kiel 1972.
- Kühnhold, W. W. 1974. Investigation on the toxicity of seawater extracts of three crude oils on egg of cod (*Gadus morhua* L.) Ber. Dt. Wiss. Kommn. Meeresforsch. 23: 165–80.
- Lönning, S. & Hagström, B. E. 1975. The effects of crude oils and the dispersant Corexit 8666 on sea urchin gametes and embryos. Norw. J. Zool. 23: 121–9.
- Mironov, O. G. 1967. Wirkung niedriger Konzentration von Öl und Rohölprodukten auf die Entwicklung der Eier des Schwarzmeer Steinbutts. Vop. Ichtiol. 7: 577–80 (Russ. summ.).
- Mironov, O. G. 1969a. The effect of oil pollution upon some representatives of the Black Sea zooplankton. Zool. Zh. 48 (7): 980-84 (Russ., Engl. summ.).
- Mironov, O. G. 1969b. Viability of larvae of some crustacea in seawater polluted with oil products. Zool. Zh. 48: (11) 1734-37 (Russ., Engl. Summ.).
- Mironov, O. G. 1969c. Development of some Black Sea fishes in oil polluted seawater. Vop. Ichtiol. 9 (6): 1136–39 (Russ. summ.).
- Moore, S. F. & Dwyer, R. L. 1974. Effects of oil on marine organisms: A critical assessment of published data. Water Research, 8: 819-27.
- Moore, S. F., Dwyer, R. L. & Katz, A. M. 1973. A preliminary assessment of the environmental vulnerability of Machias Bay, Maine, to oil supertankers, TR No. 162 Ralph M. Parsons Laboratory for Water Resources and Hydrodynamics, Massachusetts Institute of Technology, Cambridge Massachusetts.
- Renzoni, A. 1973. Influence of crude oil, derivates and dispersants on larvae. Mar. Pollut. Bull. 4(1): 9-13.
- Smith, J. E. 1968. Torrey Canyon. Pollution and marine life. Cambridge U. P., 196.
- Straughan, D. 1969. Breeding activity of intertidal species. Mar. Pollut. Bull., No. 13.
- Wells, P. G. 1972. Influence of Venezuelan crude oil on lobster larvae. Mar. Pollut. Bull. 3 (7): 105-6.