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EFFECTS OF ILLUMINATED EKOFISK CRUDE OIL ON YOLKSAC LARVAE OF COD (Gadus morhua L.)

By

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A thin layer of Ekofisk crude oil was added to the surface of sea water and constantly illuminated with artificial sunlight for several days.

Yolksac larvae of cod were exposed to 100, 50, 20, and 10% of the water-phase stock solution, which was analysed for both polar and unpolar components. Lowest tested concentration of polar components was approx. 0.5 ppm, while the concentration of unpolar components in the stock solution never exeeded 0.7 ppm.

LC_{50} (24h) appeared at 1-2 ppm polar components. The exposure lead to absence of feeding at all tested concentrations.

The results are compared to effects from unilluminated Ekofisk oil, and discussed in relation to possible impacts on fish larvae during spill situations in open seas.

INTRODUCTION

Cod larvae continously exposed to low levels of watersoluble unpolar hydrocarbons (dichlormethan extractable) from Ekofisk oil suffered retarded growth, increased neutral buoyance, impaired feeding ability and reduced oxygen consumption (Tilseth et al. 1981, Solberg et al. 1982ab). During oil spill situations in open seas, however, the oil slick will be subjected to solar radiation, generating formation of polar oxidation products, which readily enters the waterphase and are supposed to be toxic (Zafiriou 1981). Compared to the numerous laboratory experiments concerning the toxicity of unpolar oil components, reports from biotest exposure experiments concerning the toxicity of polar photoproducts are scarce. In the present study we will try to determine the accute toxic level (LC_{50}) of photoproducts from Ekofisk crude oil to cod larvae during shorttime exposure experiments (24 hours).

MATERIAL AND METHODS

The cod larvae used in this toxicity test were taken from the control group of experiments reported in Solberg et al. 1982b).

10 liter of filtered (1 um Millipore filters) and UV-sterilized sea water was filled in each of four similar glass jars with a surface area of $0.03m^2$. Twenty ml of Ekofisk crude oil was added to the water surface in two of the jars. The remaining two were used as controls. All 4 jars were constantly illuminated from a Lightline Osram Powerstar daylight lamp at a distance of 75cm from the water surface.

After 5 days of illumination dillutionseries of 100, 50, 20 and 10% stock solutions from test and control jars were placed in $5^{o}C$ thermostat controlled waterbaths. The watersamples were taken from the same test and control jars

and the remaining two jars illuminated for 3 more days.

Twenty larvae were added to each test beaker and LC_{50} determined after 24 hours exposure. The larvae still alive were tested in feeding experiments, where natural zooplankton was added at 0.5 liter^{-1}. After 1 hour of feeding the larvae were collected and conserved on 4% formaldehyde in 10‰ sea water for later examinations. Three days later (8 days of illumination) the tests were repeated using dillutionseries from the remaining test and control stock solutions.

Prior to each test watersamples were withdrawn for chemical analyses. Unpolar components were extracted with dichlormethan (dcm) and determined the same way as in Solberg et al. (1982a) during experiments with Ekofisk crude oil under normal laboratory illumination. The concentrations of polar components were determined from 1 liter watersamples filtered through 0.45 um glassfiber filters. The analyses were not executed until the day after they had been sampled, but immediately after tapping, they were conserved with sodium azid and kept in a fridge. Total organic material was continously extracted for 5 hours with a solution of ethylacetat, chloroform and hexan. The extract was fractioned on a Sephadex LH-20 column under normal preassure and detected by UV at 254 nm, and the concentration of each fraction determined gravimetricaly.

RESULTS

Chemical analyses

The eluate from the Sefadex column was devided into three fractions which are given in table 1 together with dcm extracted hydrocarbons.

Table 1. Concentration of oil hydrocarbons in watersamples from test and control jars.

	5 days illum.	8 days illum.	
	test	test	control
dcm+ extracted hydrocarbons	0.7 mg/liter	0.5 mg/liter	0.0 mg/liter
fraction 1	2.2 "	6.4 "	0.0 "
fraction 2	1.5 "	2.7 "	0.1 "
fraction 3	0.2 "	0.2 "	0.2 "

+dichlormethan

Fraction 1 originally comprised both untransformed unpolar components and weakly polar components. In the table the dcm extracted hydrocarbons have been subtracted from the original fraction 1 values to give the amont of transferred material only. This consists mainly of hydroperoxides and alifatic alcohols. Fraction 2 comprises carboxylic acids and phenols, while fraction 3 comprices highly polar matter such as sugars. However the concentration of fraction 3 material was not different from the control.

The concentration of dcm extracted compounds was very low, less than 1 ppm, while the total content of transformed polar products were approx. 4 ppm after 5 days illumination and 10 ppm after 8 days. Especially fraction 1 had increased from day 5 to 8 and could from the chromatogram be devided into two subfractions of about same magnitude, one probabely comprising hydroperoxides or similar oxydation products. A closer identification of the products were not executed.

Biological tests

Prior to the second exposure experiments pO_2, pH and salinity were controlled in water samples from both test and

control jars. PH was normal in both, salinity had increased in control from 34.5 to 36 due to evaporation, which did not take place from the oil covered surface. PO_2 was decreased by 30% in both test and control samples, probabely due to temperature elevation during the illumination. However, the availability of oxygen to the cod larvae is not critical until 60-70% reduction (unpublished data) and should not influence the experiments severly. The reduction was equal in both test and control jars, and the dillutions were carried out with $5^{o}C$ fully saturated sea water. After 24 hours exposure the LC_{50} was determined as the concentration where approx. 50% of the larvae had sunk to the bottom and the ones still in the watercolumn showed reduced avoidance reaction when collected with a pipette. Larvae were defined as unaffected when they were randomly spread in the water column, and seemed to have a normal avoidance reaction. Testlarvae definately were effected by the medium and the values are given in table 2.

Table 2. Lethality of cod larvae exposed to different concentrations of stock solutions from test and control jars for 24 hours. N = 30

	5 days illum.		8 days illum.	
% of stock solution	test	control	test	control
100%	all dead	no dead	all dead	no dead
50%	50% "	" "	" "	" "
20%	no "	" "	50% "	" "
10%	" "	" "	no "	" "

In both exposure experiments the LC_{50} (24h) seems to occur at approx. 1-2 ppm polar hydrocarbons.

In both feeding experiments none of the oil exposed larvae showed any feeding activity, while control larvae showed a feeding incidence (% larvae with gut content) of approx. 40 %. Lowest tested concentration of hydrocarbons was approx. 0.5 ppm.

DISCUSSION

The larvae were not added to the test beakers until 1-2 hours after the water had been withdrawn from the stock solutions. Zafiriou (1981) pointed out that if the most toxic agents are shortlived species, such as hydroperoxides this method could underestimate the environmental effects by not allowing the formation process and the biological effects to occur simultanously. This is an objection also applying to the chemical analyses which were executed the day after they had been sampled. On the other hand, at sea most of the cod larvae are found between 10 and 20 meters (Ellertsen et al. 1981), and the most shortlived products would probabely not reach that deep.

The illumination clearly induced formation of toxic products from the oil. The content of dcm-soluble components was far below acute toxic levels (Davenport et al. 1979), and could not have caused the registered effects. The exposure led to abscence of feeding also at lowest concentration, which was approx 0.5 ppm polar products. It therefore seems reasonably to assume that longtime exposure would cause effects at far lower levels than here employd. Although normal laboratory illumination also seem to cause chemical changes in oil (Østgård & Jensen 1982), the numerous reports concerning oil have mainly dealt with unpolar fractions, and will be unsufficient in estimating the total impacts of oil during spill situations in open seas, where the oil slick will be subjected to sunlight. The present study shows the necessity of further investigations in this field.

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