Bull. Environm. Contam. Toxicol. 26, 606-612 (1981)

Survival and Fecundity of *Daphnia pulex* on Exposure to Particulate Oil

C. Kim Wong[1], F. Rainer Engelhardt[1], and J. R. Strickler[2]

[1]*Department of Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5;* [2]*Australian Institute of Marine Sciences, P.M.B. No. 3, Townsville M.S.O., Q.4810, Australia*

In spite of the importance of zooplankton in freshwater ecosystems (FEDERLE et al. 1979) most of our limited knowledge of the effects of oil on zooplankton comes from marine studies (BARNETT & KONTOGIANNIS 1975, BERDUGO et al. 1977, KONTOGIANNIS & BARNETT 1973, LEE & NICOL 1977, MIRONOV 1969). However, because of the differences in species composition, marine and freshwater zooplankters may have different degrees of sensitivity to oil. Further, exposure to petroleum hydrocarbons in a particulate, or emulsion, form has been but little investigated. Part of the oil spilled into an ocean or a lake is broken up by wave action into particles of a size range of 10 to 100 µm, which is similar to the size of phytoplankton (FORRESTER 1971). These particles are of a size to be potentially ingested by zooplankters, and indeed are, as shown by CONOVER (1969). The present study investigates the effects of small particles of oil on Daphnia pulex, a common and important inhabitant of most freshwater lakes. Survival and reproduction parameters are examined.

MATERIALS AND METHODS

D. pulex were obtained from laboratory cultures raised from animals collected from Heney Lake, Quebec. We employed a method of exposure to oil rather similar to that used by O'BRIEN (1978). Animals were enclosed in submerged chambers which were made from a transparent plastic cylinder with nylon mesh screens affixed to both ends to give an enclosed volume of 500 mL. The screens were of a mesh size of 125 µm, allowing free passage of oil particles and algal cells. For each exposure experiment, 10 egg-bearing D. pulex were added to the chamber which was then put into 3 L of filtered lakewater in a beaker. Air was bubbled gently through the system using glass tubing.

To prepare the particulate oil medium, 1 mL of fresh Norman Wells crude oil was placed on 200 mL of filtered lakewater in a beaker and emulsified with a Polytron homogenizer without sonification. For experiments that used weathered oil, the oil/water mixture was stirred slowly with a magnetic stirrer for 24 h before being emulsified. The emulsion was allowed to stand for 1 h, then the portion below the surface layer was removed. This consisted of oil particles in an approximate size range of 1 to 100 µm, with the bulk of the particles of 5 to 10 µm

0007-4861/81/0026-0606 $01.40

range. Aliquots of the emulsion were added to the water in the exposure apparatus to achieve the required experimental concentrations. Since much of the oil appeared to float to the surface in the course of the experiment, the calculated concentration must be regarded as a nominal value. A separate test study by WONG (M.P.), DUEY & ENGELHARDT (in press) of Norman Wells crude oil emulsions has shown that marked losses in concentration occurred by 24, 72, and 96 h after the initial preparation using the above methods. A loss to 30% by 72 h and 20% by 96 h of the nominal concentration was usual. The rate of loss decreased with aging of the oil in water emulsion. In all experiments, the control and experimental emulsion media were renewed every 72 h. To test whether the observed mortality was due to the direct toxic effects or the physical contact of the oil, similar experiments were carried out using inert paraffin oil.

All experiments were carried out at 20 ± 2°C. Food consisted of Chlamydomonas sp. which has a high tolerance to crude oil (HSIAO 1978). Food concentrations were not determined, but equal volumes of algal suspension were added to all beakers.

The first series of experiments studied the effects of exposure for 192 h to emulsions of crude oil and 24-h weathered oil on the survival and reproduction of D. pulex. Nominal oil concentrations of 1, 5, 10, 50, and 100 ppm (µl/l) were used and each test was carried out in duplicate. A second series of experiments determined the longest time interval D. pulex could survive without exposure and also assessed fecundity. Animals were subjected to 50 to 100 ppm concentrations for various durations up to 30 h and then returned to clean oil-free filtered lakewater. All of these cleaning periods also lasted for 192 h and the clean media were renewed after 72 and 144 h. All the animals were removed and examined daily for mortality. Individuals without heartbeats were considered dead. Young produced between the daily examinations were counted and removed. All live animals were returned to the chamber.

RESULTS

The effects of crude oil on the survival of D. pulex are presented in Fig. 1a. Toxic effect increases with concentration. Neither paraffin oil at any concentration, nor crude oil up to 5 ppm, has any significant effect (at 5% level) on survival. Concentrations of 50 and 100 ppm resulted in total mortality within 168 and 72 h, respectively.

Fig. 1b shows the effect of 24-h weathered crude oil on the survival of D. pulex. Survival at concentrations up to 10 ppm was not significantly different from the controls at the 5% level. Comparison with Fig. 1a shows clearly that the weathered oil is less toxic that the fresh oil at nominal concentrations approximately one-half that of the toxicity of fresh oil emulsions.

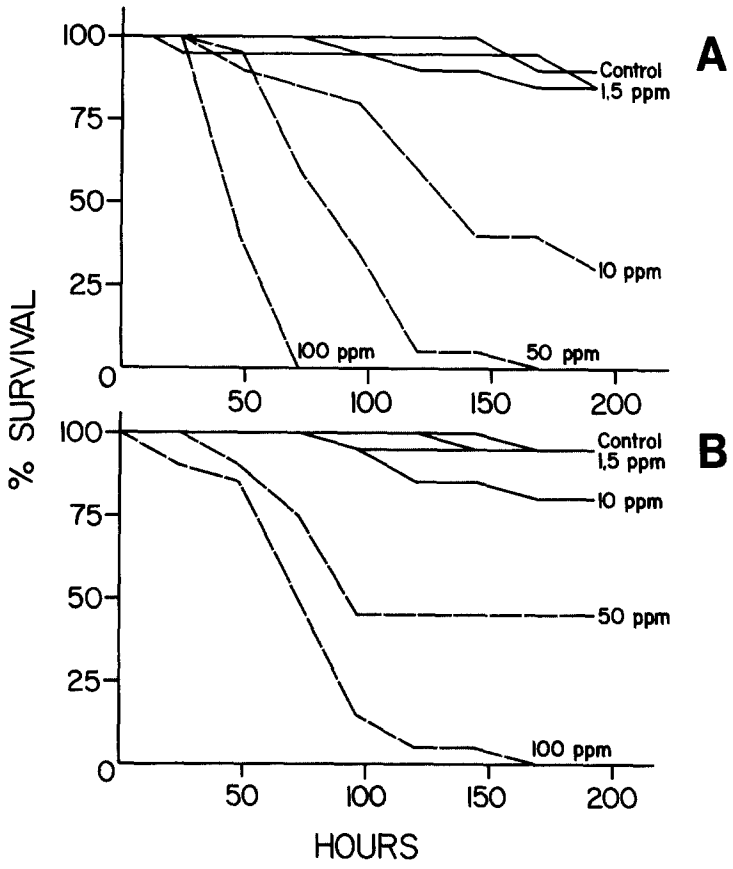

Fig. 1. Survival percentage of D. pulex exposed to (A) fresh crude oil, and (B) 24-h weathered crude oil; broken line plots represent treatments different from control at 5% level of significance (Chi-square test). Paraffin treatments from 1 to 100 ppm were not different from control.

The effect of crude oil, 24-h weathered crude oil, and inert paraffin oil on the reproduction of D. pulex are presented in Figs 2a, 2b, and 2c, respectively. The results are expressed as the cumulative number of young produced in the populations. An analysis of covariance was used to determine the regression coefficient of each curve and to test each slope for difference at the 5% level of significance. Crude oil and 24-h weathered crude oil reduced the number of young produced even at a concentration of 1 ppm. Inert paraffin oil, which had no lethal effect on D. pulex, affected reproduction when presented at concentrations of 50 and 100 ppm. Since this method of expressing reproduction does not account for the effect of mortality, at the end of each experiment the brood size of all surviving D. pulex was determined. The results presented in Table 1 are consistent with those from

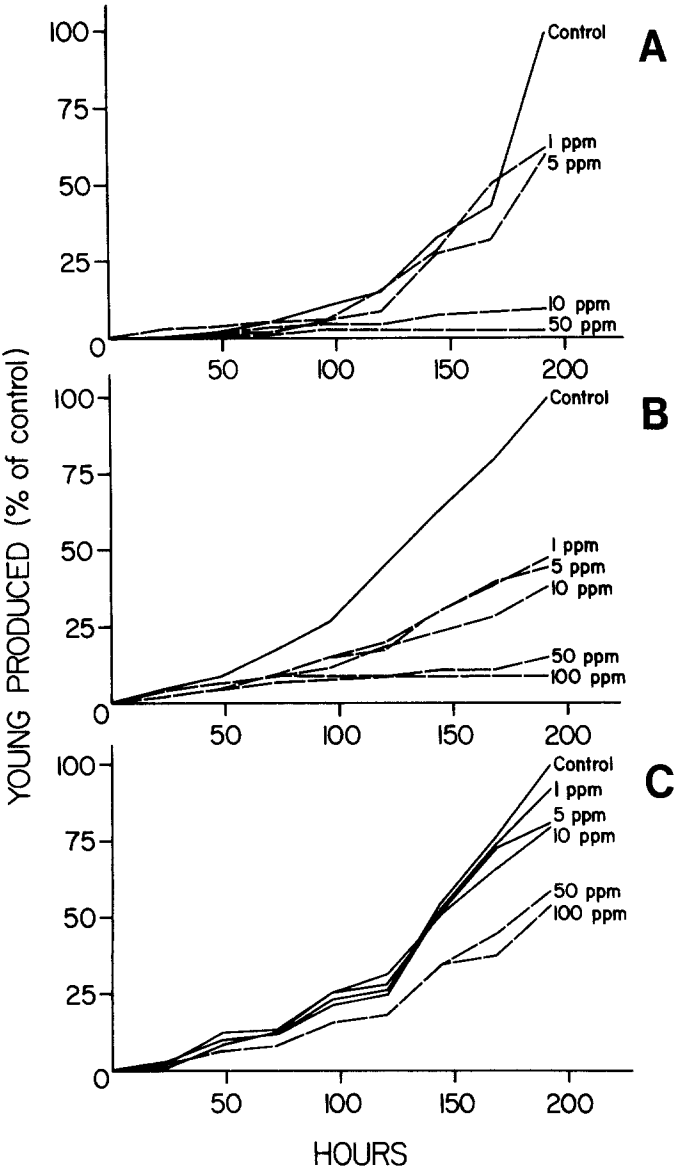

Figure 2. Cumulative number of young produced expressed as a percentage of the control. (A) fresh crude oil, (B) 24-h weathered crude oil, and (C) paraffin oil; broken line plots represent treatments different from control at 5% level of significance.

Table 1. Average brood sizes of D. pulex at the end of the experiment. Underlining indicates differences from control at 5% level of significance (U-test).

	Crude oil		Weathered oil		Paraffin	
ppm	x	SD	x	SD	x	SD
0	13	7	17	7	15	3
1	6	3	14	7	13	4
10	7	4	10	8	12	5
50	0		13	6	9	3
100			8	5	6	5
					8	

the former method. D. pulex were exposed to fresh crude oil for 3, 6, 12, 24, and 30 h and then returned to unpolluted filtered lakewater. To determine the longest exposure interval without deleterious effects, concentrations of 50 and 100 ppm which had caused total mortality within 192 h were used. The results are summarized in Table 2. In general, D. pulex which were alive and swam in response to stimulation at 48 h survived to the end of the experiment. A 30 h exposure to both 50 and 100 ppm concentrations resulted in total mortality. Crude oil at concentrations of 50 to 100 ppm caused 50% mortality after 24 and 12 h and exposure. At both 50 and 100 ppm the percentages of D. pulex showing swimming behaviour after 24 h of exposure were lower than those after 48 h. This suggests that some individuals had regained their swimming ability within 24 h after returning to an unpolluted environment. Populations that were exposed to crude oil produced fewer young during the exposure period. By the end of the experiment at 192 h, however, their average brood sizes were not lower than those of the controls.

DISCUSSION

Results of this study show that crude oil can be lethal for D. pulex. Oil slicks trap the animals and interfere with their filter-feeding behaviour. However, since the survival rate of D. pulex subjected to inert paraffin oil remained high, the lethal effect is due to toxic substances in the crude oil rather than to direct coating effects.

O'BRIEN (1978) found that Prudhoe Bay crude oil at concentration of 12 ppm had no effect on the survival of Daphnia middendorffiana, but concentration of 60 ppm killed all the animals within 120 h. Comparison between results is difficult because O'Brien's animals were exposed to the water soluble fractions of the crude oil, whereas the animals in this study were exposed to the water soluble fractions as well as the oil particles. However, both studies indicate that Daphnia is extremely sensitive to oil.

Table 2. Effects of exposing D. pulex to crude emulsion for various time intervals. Underlining indicates differences from control at 5% level of significance (U-test).

	50 ppm						100 ppm					
Duration of exposure (hours)	0	3	6	12	24	30	0	3	6	12	24	30
End of exposure- % survival	100	100	100	100	100	100	100	100	100	90	100	100
% swimming	100	100	100	100	0	0	100	100	100	90	10	0
48 hours post-exposure- % survival	100	90	100	100	100	30	100	100	90	50	50	80
% swimming	100	90	100	100	50	0	100	100	90	50	30	0
192 hours post-exposure- % survival	100	90	100	100	50	0	100	80	90	50	30	0
% swimming	100	90	100	100	50	0	100	80	90	50	30	0
Young produced (% of control)	100	67	100	78	39	0	100	99	66	20	16	0
Brood size of 192 hr	14	9	16	7	10	0	6	13	13	9	10	0
SD	3	5	4	4	5		4	6	1	6	9	

Toxicity of crude oil decreases with weathering. As the oil weathers, the lower boiling fractions which are more toxic are lost during the evaporative process (MOORE & DWYER 1974). However, the reduction in toxicity could also be due to the loss of total hydrocarbon, as did occur.

The fact that crude oil at concentrations as low as 1 ppm affects reproduction suggests that reproduction is a good indicator of oil stress in D. pulex. In Daphnia, the feeding rate increases with food cell concentration to a critical concentration (MCMAHON & RIGLER 1963). CONOVER (1971) found that zooplankton ingest oil particles. Therefore, mixing algae with oil particles will decrease the algal intake by Daphnia. In addition, oil particles trapped between the filtering legs likely interfere with the filter-feeding process. Therefore, even if the particles have no lethal effects, such as the inert paraffin oil, the animals will still suffer a loss of nutritional intake, which in turn results in lower fecundity. This is supported by our findings that while paraffin particles caused no mortality, the fecundity was decreased. A more drastic dose-dependent decrease in fecundity was evidenced by exposure to particulate crude oil, where both physical effects and chemical toxicities are combined factors.

Acknowledgments. Work was supported by a Natural Sciences and Engineering Research Council Canada Strategic Grant. We thank F. Khochbaf, M.P. Wong, and B. Stacey for technical assistance.

REFERENCES

BARNETT, C.J. & J.E. KONTOGIANNIS: Environ. Pollut. 8, 45 (1975).
BERGUDO, V., R.P. HARRIS, & S.C. O'HARA: Mar. Pollut. Bull. 8, 138 (1977).
CONOVER, R.J.: J. Fish. Res. Bd. Can. 28, 1327 (1969).
FEDERLE, T.W., J.R. VESTAL, G.R. HATER, & M.C. MILLER: Mar. Environ. Res. 2, 3 (1979).
FORRESTER, W.D.: J. Mar. Res. 29, 151 (1971).
HSIAO, S.I.C.: Environ. Pollut. 17, 93 (1978).
KONTAGIANNIS, J.E. & C.J. BARNETT: Environ. Pollut. 4, 69 (1923).
LEE, W.Y. & J.A.C. NICOL: Environ. Pollut. 12, 279 (1977).
MCMAHON, J.W. & F.H. RIGLER: Can. J. Zool. 41, 321 (1963).
MIRONOV, O.G.: Zoo. Zh. 48, 980 (1969).
MOORE, S.F. & R.L. DWYER: Water Res. 8, 819 (1964).
O'BRIEN, W.J.: Arctic 31, 219 (1978).

Accepted March 26, 1981