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Göran Gyllenberg and Gunnar Lundqvist

Some effects of emulsifiers and oil on two copepod species

Helsingin Yliopiston
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SOME EFFECTS OF EMULSIFIERS AND
OIL ON TWO COPEPOD SPECIES

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Abstract

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The effects of emulsifiers and oil on *Acartia bifilosa* and *Cyclops (Mesocyclops) oithonoides* were studied by examining: The formation of large lipid droplets in the body issue after death, the mean lethal dose, and the response in terms of oxygen consumption.

At the least toxic concentrations of emulsifier and emulsifier + oil mixtures in filtered sea water, the animals contained significantly more soluble lipids than animals freshly caught from the sea, the amounts of lipids increasing with the time of survival in the test media.

The concentrations giving the maximal amounts of lipid droplets differed with the test substances (Finasol S.C., Finasol OSR-2 and mixtures with oil), suggesting difference in their toxicity. This was confirmed by toxicity tests, which demonstrated (1) that Finasol S.C. was more toxic to both *Acartia* and *Cyclops* than Finasol OSR-2, (2) that Finasol OSR-2 + oil was less toxic to *Acartia* than Finasol OSR-2 alone and (3) that Finasol S.C. + oil was more toxic than pure Finasol S.C.

When exposed to emulsifiers or oil, the animals first performed escape movements. In *Cyclops*, these movements were random. A well-defined "activity period" was observed when oxygen consumption was measured. After this increase in locomotor activity, the animals sank to the bottom and entered a condition of "narcosis". None of them died during the 10—12 hour "narcosis" period, but some succumbed during the "activity period". In *Acartia*, the escape movements were directed away from the pollutant source. After the initial "activity period", the mortality rate was constant, and the specimens died 10—20 minutes after they had entered the stage of "narcosis".

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I INTRODUCTION

Emulsifying substances (or emulsifiers) increase the speed of emulsification and are therefore often used to remove oil slicks from the surface of the water. The emulsifiers used earlier contain about 60—80 % hydrocarbon solvent, with a high aromatic content (for example Finasol S.C., BP 1002, Essolve), and 8—30 % non-ionic surfactant. Because the aromatic hydrocarbons are the most toxic to animals (see e.g. WILSON 1970), the proportion of these substances has been reduced in recent emulsifiers (such as Finasol OSR-2). The present study treats the effects of one highly toxic emulsifier, Finasol S.C., and one less toxic emulsifier, Finasol OSR-2, on the metabolism of copepods. (A description of the composition of these emulsifiers is available from FINA, 19 Rue de General Foy, Boite postale 752-08, F-75008 Paris 8, France.)

The experimental animals chosen were one typically pelagic marine species, *Acartia bifilosa* Giesbr., and one freshwater species, *Cyclops* (*Mesocyclops*) *oithonoides* G.O.S. *C. oithonoides* also occurs frequently in the brackish water zooplankton in the inshore waters

of the Gulf of Finland (see e.g. HALME 1958).

The aim of the study was to perform some simple toxicity tests with the two emulsifiers, and with mixtures of oil and emulsifier. We also wished to study the amount of lipid droplets formed after the death of the animals from the lipid pools in their bodies, and to determine whether they had metabolized lipids or absorbed hydrocarbons from the surrounding test medium. MORRIS (1974) has observed that in copepods exposed to crude oil or oil products, the proportion of hydrocarbons in their lipids increase.

Some simple tests were made to investigate the escape movements of the animals from the point of discharge of the emulsifier.

In a series of experiments run with polarographic equipment, fitted with a continuous recording device, we also examined the response of the animals to the test substance in terms of oxygen consumption (for details, see GYLLENBERG 1973). During these experiments we observed the behaviour of the animals, and compared our visual observations with the oxygen consumption values.

II METHODS

In the toxicity tests, 20 animals were placed in each of five Petri dishes (diameter 10 cm). The test media consisted of sea water with four different concentrations of emulsifiers or emulsifiers plus oil. A dish of pure sea water was used as the control. After certain periods the number of dead animals was recorded and the animals removed from each Petri dish.

Records were also made of the survival of the animals tested in the respiration chamber of the polarographic system. The dead individuals could be recognized as they bent their pereopods backwards and ceased to perform filter movements, often also bending their urosoma upwards in a sharp angle to the prosoma.

In the tests in which the formation of lipid

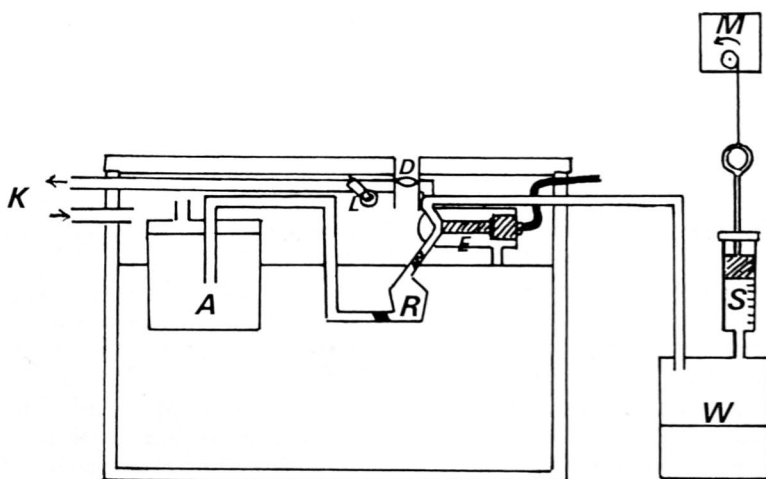


FIG. 1. The modified part of the polarographic equipment (cf. Fig. 1 in GYLLENBERG 1973). A = reservoir bottle for emulsifier solutions, D = magnifying glass for visual observations of the copepods in the respiration chamber (R), facilitated by the use of a lamp (L). E = oxygen electrode E5046 in thermostat cell D616 (Radiometer). K = water flow from and into the crystal Lauda K4R, maintaining the temperature of the water bath and the thermostat cell at $\pm 0.01^\circ\text{C}$. M = synchronous motor, S = glass syringe, W = waste water container.

droplets was studied, the animals were placed in a medium with a known concentration of emulsifier or emulsifier and oil. Parallel tests were run with filtered sea water, in order to observe the formation of lipid droplets in animal in a pure water culture. When the animals had died, or immediately before they died, they were picked up from both types of media and the amount of lipids contained in their bodies was measured.

The lipid droplets formed in the body after death were stained with Fettrot 7B (Ciba) and Oil-Red-O by the techniques presented by Romeis (1968:258—259). The Oil-Red-O and Fettrot 7B methods stain the total amount of lipid material, including lipoids and certain hydrocarbons, providing that these substances occur as fluids forming droplets.

The volume of the lipid droplets was estimated as the sum of several spheres, by measuring the radius of each sphere with the aid of a micrometer. The volume of the animals was calculated as the sum of an ellipsoid (prosoma) and three cylinders (urosoma and the antennulae, see GYLLENBERG 1973). The volume of the lipid droplets is given as a percentage of the volume of the animals.

The test chamber used for studying the escape movements had a volume of 2.1 ml and was constructed from two object glasses, which formed the larger sides, and a piece of silicon tubing bent to form the smaller sides and the bottom. Within the chamber the animals could move in two directions: horizontally and vertically. A droplet of concentrated Finasol S.C. solution (0.025 ml) was

discharged into an upper corner of the water body in the chamber, and the positions of all the animals were recorded at regular intervals.

A detailed description of the polarographic equipment used in the experiments is given in GYLLENBERG (1973). The recorder was a REC51 servograph (Radiometer/Copenhagen) with the high-sensitivity module REA112.

The following modifications were made to the apparatus (cf. Fig. 1):

1. The route leading the reservoir water past the respiration chamber was eliminated. Control values for the reservoir water were obtained by replacing the respiration chamber containing animals with the empty chamber. This arrangement reduced pressure differences in the system.

2. The water flow from the electrode was directed into a waste-water container (W in Fig. 1), which was covered with a lid. The air in the container was sucked out at a constant rate through a tube in the lid, by a glass syringe (S) connected to a synchronous motor (M). This arrangement produced an extremely stable water flow through the polarographic system.

The waste water could not be led directly to the syringe, since this was found to interfere with the movement of the piston. The piston was greased with paraffin oil.

3. The outlet tube from the reservoir bottle (A) with pollutants was placed in the middle of the bottle, so that the concentration of the mixture would be unchanged, since it was observed that the emulsifiers or oil tended to precipitate after

several hours. For the same reason only freshly homogenized mixtures of emulsifiers or oil with sea water could be used.

The surface of the reservoir bottle was kept at the level of the pO₂-electrode throughout the experiment, in order to avoid pressure differences in the system, which might affect the pO₂ values.

4. The tube from the respiration chamber to the pO₂-electrode was shortened, so that the lag between oxygen pressure changes in the respiration chamber and registration by the electrode was minimized. This also reduced possible oxygen diffusion through the walls of the plastic tube. The water flowing out of the respiration chamber reached the electrode within one minute.

5. Only the oxygen electrode was used in 1974, since we found that the pH values measured by the pH electrode could not be transformed into pCO₂, as explained in GYLLENBERG (1973). The pH is probably affected both by excretion products of the animals (ammonium products increasing pH), and by the emulsifiers (progressively depressing pH, see PERKINS 1968).

6. In the experiments with crude oil, the oil was put on the surface of the water in the respiration chamber. A different type of chamber was used, in which the water flow was directed from the middle of the chamber downwards. This left a non-turbulent space at the top of the chamber, which could be filled with oil.

The oil used was sample A 28905 from Nynäs-hamn, Sweden, originating from Venezuela, with a sulphur content of 1.9 %. The emulsifiers tested were Finasol S.C. and Finasol OSR-2.

The experimental animals were caught at Storfjärden immediately outside the Zoological Station of Tvärminne, Finland, and by Drumsö bridge, Helsinki. The *Acartia* specimens could not be cultivated in the laboratory, since they require recirculating water flow systems (cf. ZILLIUX & LACKIE 1970) and fresh flagellate cultures as food (cf. CORKETT 1970, HEINLE 1970), which could not be obtained. The *Acartia* specimens survived for about 48 hours in filtered sea water. Cultures of *Cyclops oithonoides* could be maintained for up to a year, by introducing algae (*Scenedesmus*) into the culture jars.

III RESULTS

A. Preliminary tests with the polarographic equipment

Tests were performed to find out

whether the emulsifiers dissolved in sea water affected the pO₂ values recorded by the oxygen electrode. Table 1 presents the means and standard errors of

TABLE 1. Results of test runs with maximum concentrations of emulsifiers or oil and with filtered sea water: mean (\bar{x}) in mm Hg, standard error (S.E.), number of readings (N), t-value and corresponding probability (P). The oil was introduced on the surface of the water in the respiration chamber.

		\bar{x}	S.E.	N	t-value	P
run 1	sea water	159.3	0.05	4	1.19	0.2 < P < 0.3
	sea water + oil 0.1 ml/l	159.0	0.21	10		
run 2	sea water	131.9	0.46	6	0.33	0.7 < P < 0.8
	sea water + 10 000 ppm Finasol OSR	149.8	0.53	6		
run 3	sea water	132.3	0.96	4	1.38	0.2 < P < 0.3
	sea water + 10 000 ppm Finasol S.C.	148.7	0.62	4		
	sea water + 10 000 ppm Finasol OSR	142.1	0.29	3		

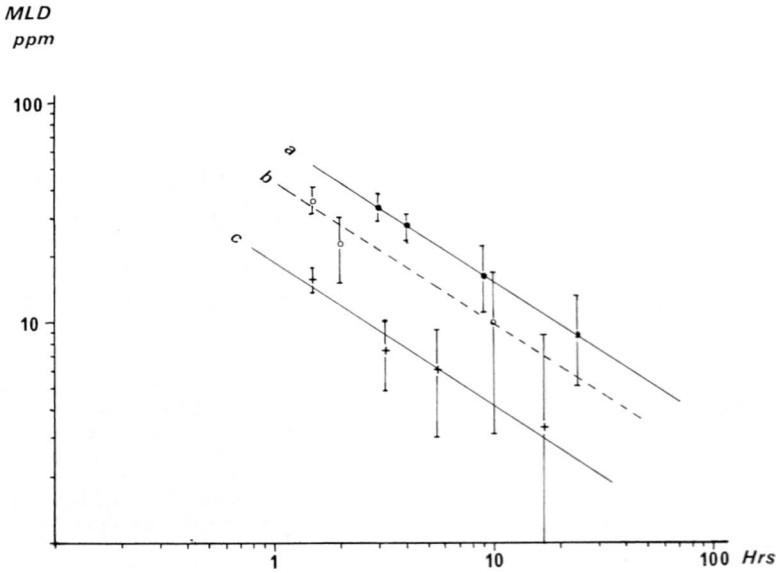


FIG. 2. The relation between mean lethal dose (MLD) values (from probit analyses) and time in hours on a double logarithmic scale for Finasol S.C. solutions. Standard errors (s_y) are indicated. line a (asterisks) = MLD values from the continuous water flow system with *Acartia bifilosa* line b (open circles) = MLD values from Petri dishes for *Cyclops oithonoides* line c (plus signs) = MLD values from Petri dishes for *Acartia bifilosa*

the means of the pO_2 values read every tenth minute with the highest concentrations of oil and emulsifier used and with pure filtered sea water. The t-test gave a significant divergence for the emulsifier Finasol OSR-2 on the third run. It was observed that extensive use of this emulsifier affected the pO_2 values in concentrations above 1000 ppm. For this reason only solutions up to 100 ppm could be used in the experiments. The standard error is within the 0.5% limits of the mean.

The movement of the water current directed into the respiration chamber was observed by staining sea water with a water resistant dye (Evans blue). The dye was spread evenly all through the chamber as it entered from the inlet tube, and did not leave any spaces with unstained water where the animals could escape from exposure to the emulsifier.

B. Escape movements

The measure used for the distance that the animals had reached from the starting point of diffusion of the emulsifier (P) was the length of a vector in a polar coordinate system with P in origo. The vectors were classified into four frequency distribution categories (for the distances 1–14 mm, 15–28 mm, 29–42 mm and 43–56 mm). The empirical frequency distribution was tested against the theoretical distribution (assuming random dispersal) with the χ^2 test (Table 2). With the *Acartia* individuals, the χ^2 values were significantly different after 10 minutes' exposure onwards, indicating that the animals actively avoided the source of pollution. All the readings were done on living animals. The corresponding χ^2 values for *Cyclops* individuals show that this animal moves randomly around until death, regardless of the diffusion of emulsifier into the water.

TABLE 2. The distances reached by the test animals from the outlet point (P) of the emulsifier Finasol S.C., measured as the length of a vector in the polar coordinate system with P in origo (see text). The empirical test values (E) were compared with the theoretical frequency distribution (T) (assuming random movement) for different frequency classes, and for the time periods indicated.

A. <i>Acartia bifilosa</i> (30 animals)																
distance in mm from P	pre-exp. situation		start		2 min.		5 min.		10 min.		20 min.		45 min.			
	E	T	E	T	E	T	E	T	E	T	E	T	E	T		
1—14	6	8	5	8	5	8	2	8	1	8	1	8	0	8		
15—28	11	9	9	9	12	9	7	9	7	9	10	9	10	9		
29—42	8	7	8	7	6	7	11	7	11	7	8	7	12	7		
43—56	5	6	8	6	7	6	10	6	10	6	11	6	8	6		
χ^2	1.254		1.935		2.435		7.397		11.52		10.55		12.35			
prob. P	0.5 < P < 0.7		0.5 < P < 0.7		0.3 < P < 0.5		0.05 < P < 0.1		0.005 < P < 0.01**		0.01 < P < 0.025*		0.005 < P < 0.01**			
B. <i>Cyclops oithonoides</i>																
distance in mm from P	pre-exp. situation		start		5 min.		15 min.		60 min.		2 hrs		4 hrs		10 hrs (all dead)	
	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T
1—14	7	8	6	8	7	8	6	8	5	8	7	8	6	8	5	8
15—28	8	9	10	9	8	9	12	9	11	9	8	9	10	9	11	9
29—42	9	7	8	7	10	7	5	7	5	7	5	7	5	7	7	7
43—56	6	6	4	6	5	6	7	6	9	6	10	6	9	6	7	6
χ^2	0.808		1.421		1.688		2.238		3.641		3.474		2.683		1.736	
prob. P	0.8 < P < 0.9		P = 0.7		0.5 < P < 0.7		0.5 < P < 0.7		0.3 < P < 0.5		0.3 < P < 0.5		0.3 < P < 0.5		0.5 < P < 0.7	

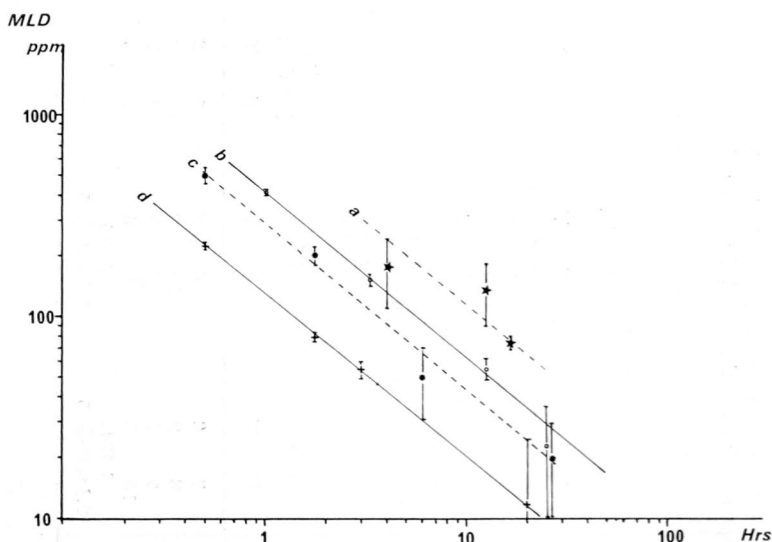


FIG. 3. The relation between mean lethal dose (MLD) values (from probit analyses) and time in hours on a double logarithmic scale for Finasol OSR-2 solutions. Standard errors ($s\dot{y}$) are indicated
 line a (stars) = MLD values from Petri dishes, Finasol OSR-2 + oil, for *Acartia bifilosa*
 line b (open circles) = MLD values from Petri dishes for *Cyclops oithonoides*
 line c (asterisks) = MLD values from the continuous water flow system for *Acartia bifilosa*
 line d (plus signs) = MLD values from Petri dishes for *Acartia bifilosa*

Apparently the emulsifier affects only the intensity of its movements (cf. flowing water polarographic experiments).

C. Toxicity tests

The results of the toxicity tests are shown in Figs. 2—3. For every reading of the number of dead individuals a probit analysis was performed (see SAUNDERS & FLEMING 1971), by plotting the logarithm of the emulsifier concentration against the number of dead animals in a probit scale. This analysis gave mean lethal dose (M.L.D.) values for given periods, and $s\dot{y}$ (standard error of the concentration value measured at the probit value 5.0). These M.L.D. values and standard errors are plotted against time in Figs. 2—3. The following interpretations can be made:

1. The relation between the M.L.D. values and time is linear on a double

logarithmic scale. The slope of all the lines is the same.

2. The *Acartia* individuals survived longer in the continuous water flow system in the polarographic experiments.

3. The mixture of oil and Finasol OSR-2 was less toxic for *Acartia bifilosa* than Finasol OSR-2 alone. The mixture of oil and Finasol S.C. killed all the *Acartia* specimens within one hour at all concentrations (lowest concentration 10 ppm), for which reason no M.L.D. value could be calculated.

4. Finasol S.C. was much more toxic for both *Acartia* and *Cyclops* than Finasol OSR-2.

D. Content of fluid lipids

The experiments in which the content of lipids was determined were performed with copepodites and young adults, which normally store fluid li-

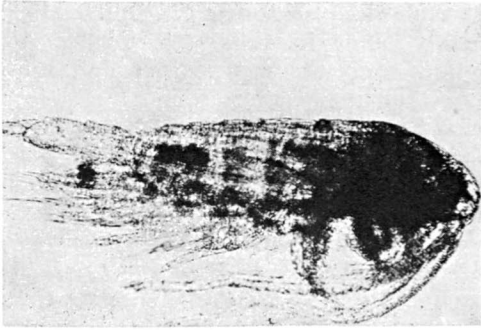


FIG. 4. *Acartia bifilosa*, freshly caught young adult, stained with Oil-Red-O after death. No lipids are visible in this animal.

pids as food reserves (see e.g. WESEBERG-LUND 1939 and BENSON & LEE 1975). Adult females use all their lipid reserves for egg production, and when freshly caught do not contain any lipid droplets at all (BENSON & LEE 1975). However, a control series with only females in different concentrations of emulsifiers revealed that after exposure to emulsifier solutions they always contained fluid lipids in addition to the egg lipids (cf. Figs. 9—11 for *Acartia* females in Finasol OSR-2 with oil solutions), although the proportion of soluble lipids was slightly lower than for copepodites. According to WESEBERG-

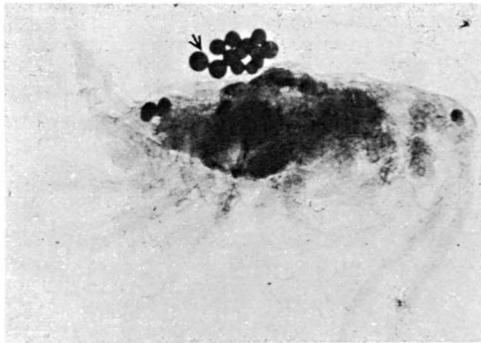


FIG. 5. *Acartia bifilosa* exposed to 500 ppm Finasol OSR-2, stained with Fettrot 7B and Meyers haematoxylin. The dark circles (indicated by arrows) are lipid droplets. The animal shows extensive degradation of body tissue, and the lipids are caught under its exoskeleton.

LUND (1939), copepods constitute an important link in the food chain, since they store lipid oils from diatoms, and these oils are later stored as fats in fish and birds.

The finding that Finasol OSR-2 was less toxic when mixed with oil (see toxicity tests) was confirmed by determinations of the amount of lipid droplets formed after death. Formation of large lipid droplets after death is a

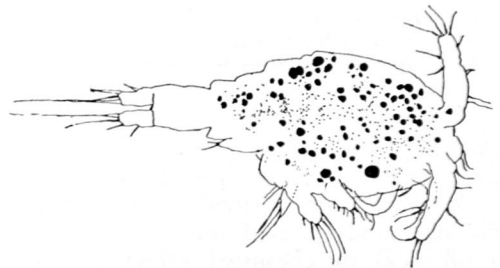
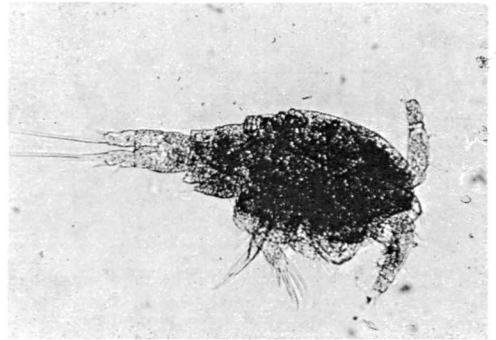


FIG. 6. *Cyclops oithonoides* exposed to 100 ppm Finasol S.C., stained with Oil-Red-O. All the dark spots are lipids. The lipids are distributed throughout the body immediately after death. The distribution of lipid droplets in the animal is elucidated by a drawing.

purely physical process, since small lipid reserves in the cells and tissues join into larger droplets, following the law of surface tension (compare Fig. 6 with Fig. 7). The animals in mixtures with oil had a higher TD_{50} (time to 50 % death) values and subsequently contained larger amounts of lipids. The results are documented with a selection of photographs (Figs. 4—11). The dark

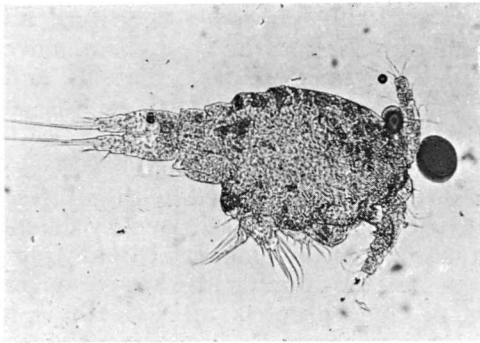


FIG. 7. *Cyclops oithonoides*. The same animal as in Fig. 6, observed one hour later in the test medium (100 ppm Finasol S.C.). The lipids have collected in larger droplets outside the animal, indicating a rupture in the exoskeleton. The total amount of lipids is unchanged. Their distribution in the body is elucidated by a drawing.

stained areas, often demarcated by a dark border, are the lipid droplets.

A test was performed to check that the surrounding medium did not have a physical or chemical effect on the

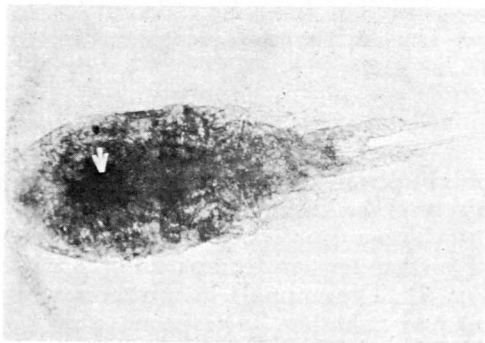


FIG. 8. *Cyclops oithonoides* exposed to 10 000 ppm Finasol S.C. All the dark stained areas (Oil-Red-O) in the middle of cephalothorax represent lipids (indicated by an arrow).

amount of lipids forming droplets after death. Some animals were picked up immediately before they died and analysed for lipids; other animals were left in the medium for about 24 hours after death (cf. Fig. 6 with Fig. 7). The amounts of droplets formed did not differ significantly.

Comparison with freshly caught individuals of both *Acartia* and *Cyclops*, used as controls (Fig. 4), showed that the amounts of lipids forming droplets were significantly higher at the least toxic concentrations of each pollutant mixture (Table 3).

The relation between TD₅₀, the con-

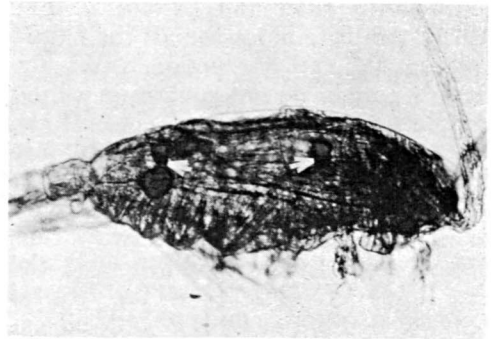
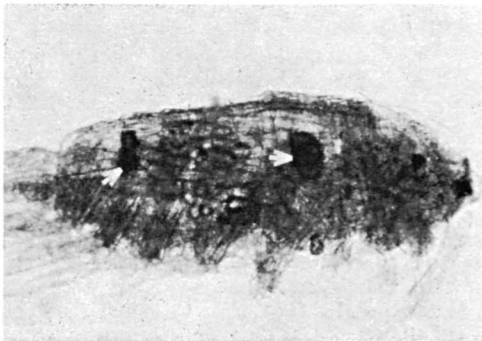


FIG. 9. *Acartia bifilosa*, female, exposed to 10 ppm of Finasol OSR-2 with oil (stained with Oil-Red-O). The dark stained droplets are lipids (indicated by arrows), the light structures are eggs developing in the oviduct.

centration of the emulsifier, and the percentage of lipid droplets formed in the animals is illustrated in Figs. 12—15. As might be expected, the TD₅₀ decreases as the concentration of the emulsifier increases. The relatively amount of lipid droplets increases from the percentage in freshly caught animals (controls in Table 3) to a maximal value at different concentrations for different mixtures (maximum values at 10 ppm Finasol S.C. for *Acartia*, at 1000 ppm S.C. for *Cyclops*, at 10 ppm Finasol OSR-2 for *Acartia*, and at 200 ppm OSR-2 plus oil for *Acartia*).

TABLE 3. Relative lipid volumes (% of body volume) in copepodits II—IV and young adults exposed to different concentrations of emulsifiers: mean value (\bar{x}), standard error (S.E.), and number of observations (N). The t-test was performed between freshly caught animals (controls) and test animals exposed to different emulsifier concentrations, P = corresponding probability.

A. Finasol S.C., <i>Cyclops oithonoides</i>					
concn. ppm	lipid percentage		N	t-value	P
	\bar{x}	S.E.			
10000	0.43	0.16	3	0.07	P > 0.9
1000	5.39	1.26	4	3.75	P < 0.01
100	3.09	1.46	3	1.74	0.1 < P < 0.2
10	0.205	0.205	2	0.59	P ≈ 0.6
B. Finasol S.C., <i>Acartia bifilosa</i>					
concn. ppm	lipid percentage		N	t-value	P
	\bar{x}	S.E.			
20	0.1	0.05	3	1.07	0.3 < P < 0.4
10	1.99	0.4	3	3.79	P < 0.01**
C. Finasol OSR-2, <i>Acartia bifilosa</i>					
concn. ppm	lipid percentage		N	t-value	P
	\bar{x}	S.E.			
100	0.58	0.19	3	1.00	P ≈ 0.4
10	2.03	0.665	3	2.49	0.02 < P < 0.05*
D. Finasol OSR-2 + oil, <i>Acartia bifilosa</i>					
concn. ppm	lipid percentage		N	t-value	P
	\bar{x}	S.E.			
1000	1.315	0.14	4	4.26	P < 0.01**
200	2.36	0.538	5	3.59	P < 0.01**
50	1.57	0.46	3	2.53	P ≈ 0.05*
10	1.17	0.12	2	3.83	P ≈ 0.01**
E. Controls, from sea water					
species	lipid percentage		N	t-value	P
	\bar{x}	S.E.			
<i>Cyclops</i>	0.46	0.38	3		
<i>Acartia</i>	0.31	0.19	5		



In contrast to the freshly caught controls, which presumably contained the same amount of body lipids as animals in the sea, the animals kept in sea water for 20—24 hours never contained lipid droplets (see also BENSON & LEE 1975). The animals kept in the test

FIG. 10. *Acartia bifilosa*, female, exposed to 50 ppm of Finasol OSR-2 + oil (dye Oil-Red-O). The dark stained droplets are lipids (indicated by arrows).

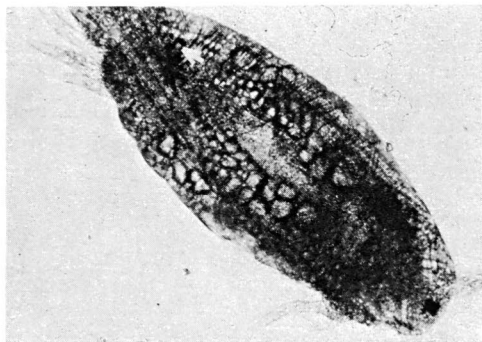


FIG. 11. *Acartia bifilosa*, female, exposed to 1000 ppm of Finasol OSR-2 +oil (dye Oil-Red-O). The light droplets are eggs developing in the oviduct, lipids can be discerned as small dark spots at the front and back of the cephalothorax.

media may therefore be assumed to have used some of the lipids for maintenance metabolism. As the animals survived for longer periods at low emulsifier concentrations these specimens should accordingly have used more lipids for maintenance than the animals in higher concentrations. Table 4 shows the results of a test performed with the values for lipids, in which they

were converted to calories (9.5 cal/mg lipid) and added to the amount used for maintenance during the survival period (TD₅₀). The latter amounts was calculated from the routine metabolic rate (RMR; GYLLENBERG 1973) and converted to calories (oxycaloric coefficient 5 cal/ml O₂ consumed). The t-test showed significant differences between the lipid material in the freshly caught animals (copepodites) and the total amounts obtainable for the animals exposed to the least toxic emulsifier concentrations. However, an analysis of variance showed that the values did not differ significantly between the different concentrations (as for the Finasol S.C. series, *Cyclops*, and Finasol OSR-2 plus oil series, *Acartia*). In these tests it was assumed that the animals used only lipids for maintenance metabolism.

The animals kept in filtered sea water survived for about 48 hours. The total amount of calories used until their death was calculated, and found to be 1.5 to 2 times the energy obtainable as

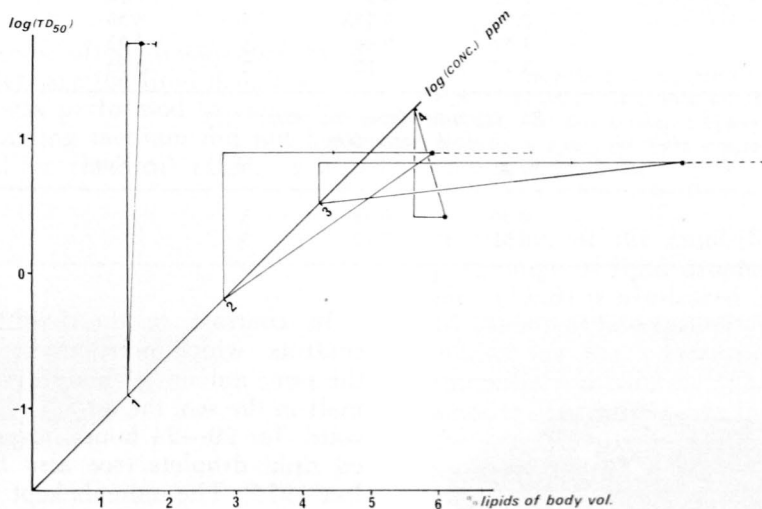


FIG. 12. The relation between TD₅₀ in hours, the concentration of the emulsifier Finasol S.C. in ppm, and lipids as a percentage of body volume. Experimental animal: *Cyclops oithonoides*. The standard errors are indicated for the lipid values (cf. Table 3). Figs. 12—15, 19 and 21 are three-dimensional diagrams. Two of the dimensions are illustrated by the smaller sides of the triangles (x-value and y-value). The position of the triangles on the z-axis gives the third dimension. The asterisks (*) are the actual measurements.

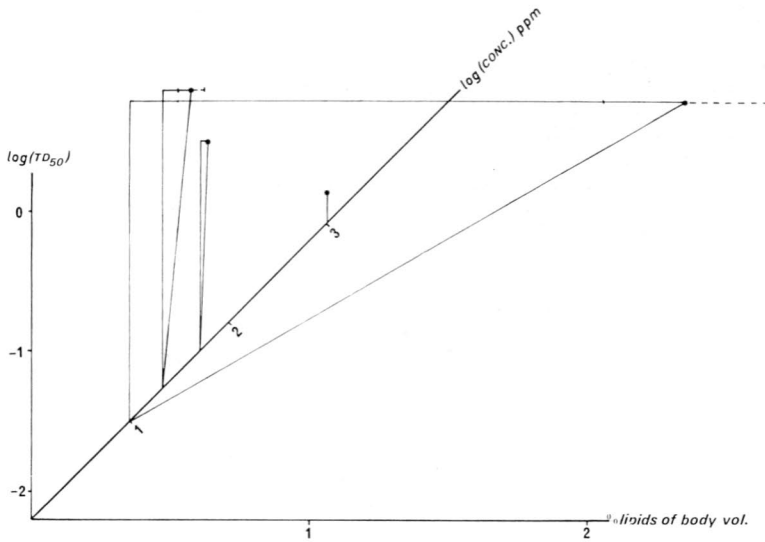


FIG. 13. The relation between TD_{50} in hours, the concentration of the emulsifier Finasol S.C. in ppm, and lipids as a percentage of body volume. Experimental animal: *Acartia bifilosa*. The standard errors are indicated for the lipid values (cf. Table 3).

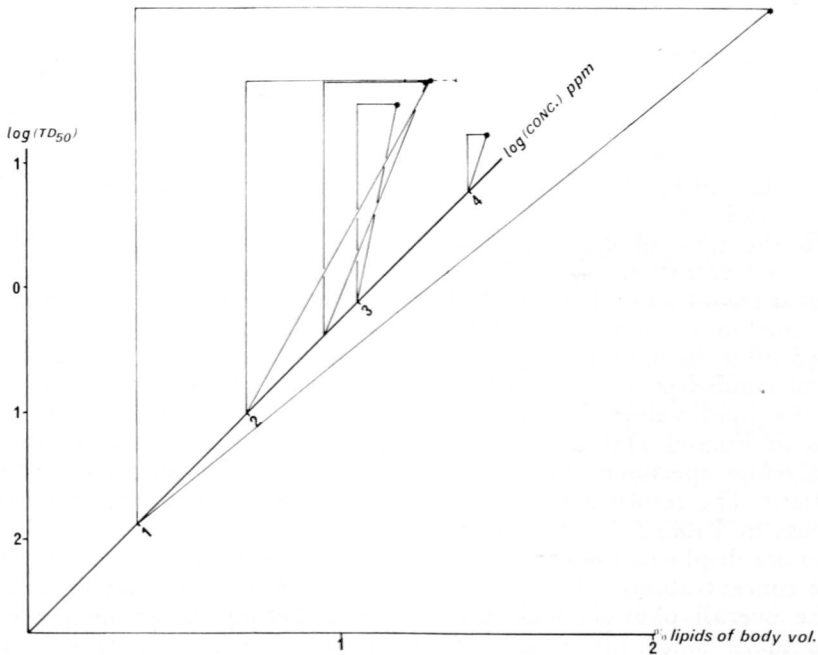


FIG. 14. The relation between TD_{50} in hours, the concentration of the emulsifier Finasol OSR-2 in ppm, and lipids as a percentage of body volume. Experimental animal: *Acartia bifilosa*. The standard errors are indicated for the lipid values (cf. Table 3).

TABLE 4. Maximum amounts of calories obtainable as lipids during the time of survival (TD₅₀), calculated by converting relative lipid volumes to calories/mg body weight and adding the amounts of calories consumed (derived from RMR values of oxygen consumption). \bar{x} = mean value, S.E. = standard error, d.f. = degrees of freedom. t-tests were performed between freshly caught copepodites and copepodites exposed to different emulsifier concentrations, and the analysis of variance was made between different concentrations of the same emulsifier. P = corresponding probability.

species and test medium	total amount lipids obtainable \bar{x}	cal/mg S.E.	d.f.	t-value	P
<i>Cyclops</i>					
Finasol S.C.					
1000 ppm	0.5391	0.126	5	3.747	0.01 < P < 0.02**
100 ppm	0.3278	0.155	4	1.23	0.1 < P < 0.2
10 ppm	0.066	0.066	3	0.263	P ≈ 0.8
freshly caught controls	0.046	0.038	3		
<i>Acartia</i>					
Finasol S.C.					
10 ppm	0.268	0.034	6	6.08	P < 0.001***
Finasol OSR					
10 ppm	0.238	0.078	6	2.58	0.02 < P < 0.05*
Finasol OSR + oil					
200 ppm	0.278	0.053	9	4.36	0.001 < P < 0.01**
50 ppm	0.166	0.049	6	2.58	0.02 < P < 0.05*
10 ppm	0.177	0.018	5	5.58	0.001 < P < 0.01**
freshly caught controls	0.031	0.019	5		
analysis of variance between 1000, 100, 10 ppm Finasol S.C. for <i>Cyclops</i> gives F = 3.41, P > 0.05					
analysis of variance between 200, 50, 10 ppm Finasol OSR with oil for <i>Acartia</i> gives F = 2.2, P > 0.05					

fluid lipids. The animals thus had to burn other substances in order to survive for the 48 hours.

As both the time of exposure and emulsifier concentration affected the survival of copepods, an additional test was performed in which all the animals were killed after 30 minutes' exposure to different emulsifier solutions. Table 5 shows the lipid values for *Acartia* specimens in Finasol OSR-2 plus oil, and for *Cyclops* specimens in Finasol S.C. solutions. The results are comparable to those in Table 3, but the maximal values are displaced towards lower emulsifier concentrations. This may be due to the overall physiological state of the animals, since the results in Table 3 were obtained with animals caught in September, but those in Table 5 with animals taken in January.

E. Flowing water polarographic experiments

1. *Cyclops oithonoides*

Fig. 17 presents a respiration curve typical of the *Cyclops* individuals. The animals were first acclimatized in sea water without emulsifier for 1—2 hours (dotted line in Fig. 17). The respiratory rate of the animals then corresponded to the routine metabolic rate (RMR). After that, the animal chamber was removed for a short period (5—10 min), the sea water being led past the chamber with a connecting tube. The curve level before the emulsifier was introduced thus represents values without animals. When the animal chamber was connected again, the emulsifiers were introduced. The short period

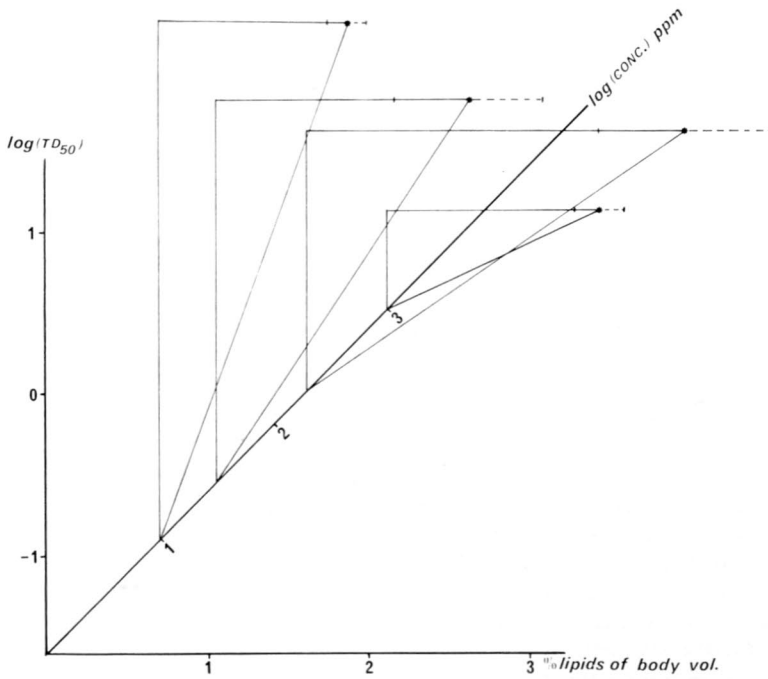


FIG. 15. The relation between TD_{50} in hours, the concentration of the emulsifier Finasol OSR-2 + oil in ppm, and the per cent of lipids of body volume. Experimental animal: *Acartia bifilosa*. The standard errors are indicated for the lipid values (cf. Table 3).

TABLE 5. Relative lipid volumes (% of body volume) in copepodites II—IV and young adults exposed to different concentrations of emulsifiers, mean value (\bar{x}), standard error (S.E.), and number of observations (N). In these experiments all the animals were killed after 30-min exposure to the emulsifier. The t-test was performed between freshly caught animals (controls) and test animals exposed to different emulsifier concentrations. P = corresponding probability.

concn. ppm	A. Finasol OSR-2 + oil, <i>Acartia bifilosa</i>				
	\bar{x}	S.E.	N	t-value	P
200	0.634	0.137	6	3.822	0.001 < P < 0.01**
50	1.663	0.097	8	13.576	P < 0.001***
10	0.529	0.187	6	2.262	0.02 < P < 0.05*
control	0.102	0.025	6		
concn. ppm	B. Finasol S.C., <i>Cyclops oithonoides</i>				
	\bar{x}	S.E.	N	t-value	P
1000	0.750	0.131	6	3.170	P = 0.01**
100	1.128	0.116	8	6.046	P < 0.001***
10	0.774	0.156	8	2.433	0.02 < P < 0.05*
control	0.328	0.028	6		

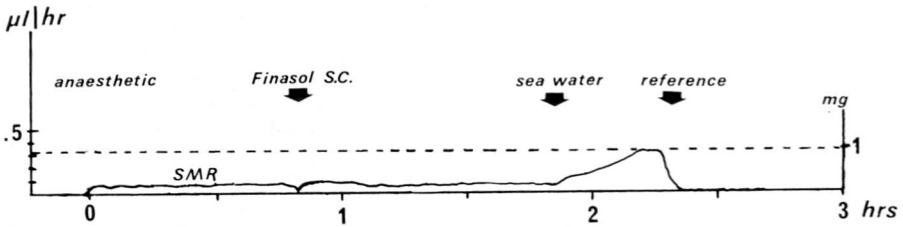


FIG. 16. Recorder outputs of oxygen consumption values measured from the time of introduction of an anaesthetic substance (5×10^{-5} g/ml physostigmium salicylinum), for *Cyclops oithonoides*. The oxygen consumption values in μl per hour are given on the left-hand axis, and the biomass of the animals in mg (broken line) on the right-hand axis. Changes in the composition of the water led into the respiration chamber are indicated by arrows: to sea water with 1000 ppm of Finasol S.C., to pure sea water, and to reference (control). SMR = standard metabolic rate. Temperature 14.6°C , salinity 6 ‰, water flow 3.5 ml/hours. For details, see text.

when the animal chamber was removed caused a slight decrease in the oxygen values measured, but this decrease was insignificant and could not be traced on the recorder output curves.

An increase in oxygen consumption occurred immediately after the emulsifier or oil was added to the respiration water. This increase was not seen when anaesthetized animals were used (Fig. 16), and was therefore not the direct effect of the emulsifier or oil. However, the introduction of these substances caused an increase in locomotor

activity. This increase lasted for different periods in different emulsifier concentrations (cf. Fig. 19). The length of this "activity period" was determined by taking the interval between the point where the respiratory rate rose above the RMR level and the point where it reached this level again (as in both *Acartia* and *Cyclops* at the lower emulsifier concentrations), or fell abruptly to the standard metabolic rate (as in *Cyclops* at the higher concentrations).

We have adopted the definitions of

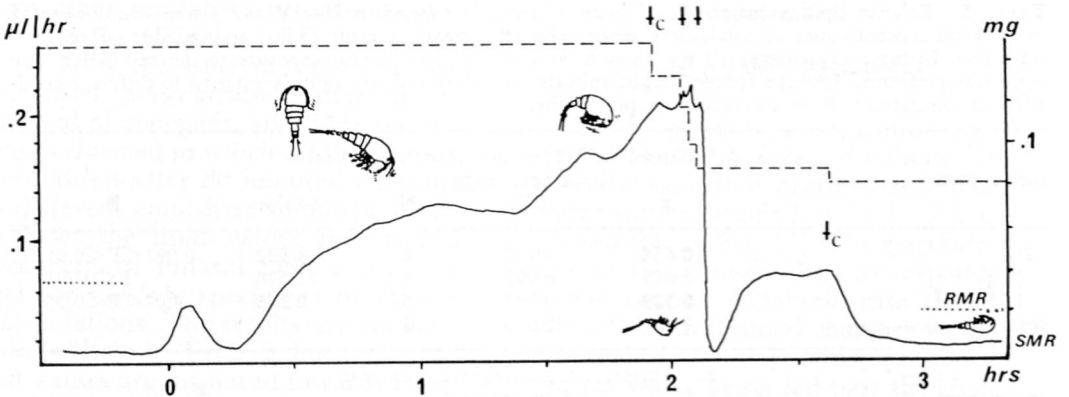


FIG. 17. A typical example outputs of oxygen consumption values measured from the time of introduction of the emulsifier solution, for *Cyclops oithonoides*. The oxygen consumption values in μl per hour are given on the left-hand axis, and the biomass of the surviving animals, in mg (broken line) on the right-hand axis. \dagger = peak in oxygen consumption, indicating death of adults, $\dagger c$ = peak in oxygen consumption, indicating death of copepod. Dotted line = RMR control values calculated for the living biomass at the beginning and end of the experiment. Emulsifier Finasol S.C. 1000 ppm, temperature 11.5°C , salinity 6 ‰, water flow 6 ml/hour. For details, see text.

respiratory rates given by KINNE (1970). Standard metabolic rate (SMR) refers to the minimum energy requirements for the maintenance of all vital functions, routine metabolic rate (RMR) to energy demands for spontaneous normal activity, and active metabolic rate (AMR) to energy requirements during sustained forced activity. It should be pointed out, however, that the standard metabolic rate is equivalent to the "basal metabolic rate" as defined by NEWELL & NORTHGROFT (1967) and BLAZKA (1971) for poikilothermic marine organisms. This fact was established by a simple experiment in which the *Cyclops* individuals were anaesthetized with 5×10^{-5} g/ml physostigium salicylinum in order to remove any possible muscle activity during the oxygen consumption process (GLIWICZ 1968). This experiment established that the standard metabolic rate is equivalent to the metabolic rate of the *Cyclops* individuals during a condition of "narcosis" after AMR.

The anaesthetizing substance (physostigium salicylinum) blocks the synapses of the peripheral neuromuscular system (GLIWICZ 1968). The effect was observed after 20 minutes incubation of the animals in this anaesthetizing medium, and as is seen in Fig. 16, it is exactly the same as the effect of the 1000 ppm solutions of Finasol S.C. When fresh sea water was pumped into the animal chamber, the respiratory rate returned to the RMR level (see Fig. 16).

It appears that the activity response may vary between experiments with the same emulsifier solution, and that the occurrence of peaks in oxygen consumption may merely depend on when the animals are agitating each other (about 20 individuals used in each experiment). The peaks could occur at the beginning or end of the activity period, as is seen in Fig. 18, which shows the rates recorded with different concentrations of Finasol S.C.

Some conclusions can be drawn from the results illustrated in Fig. 19:

1. The length of the activity period decreases as the concentration of emulsifier increases.

2. The total amount of energy used for activity during the activity period

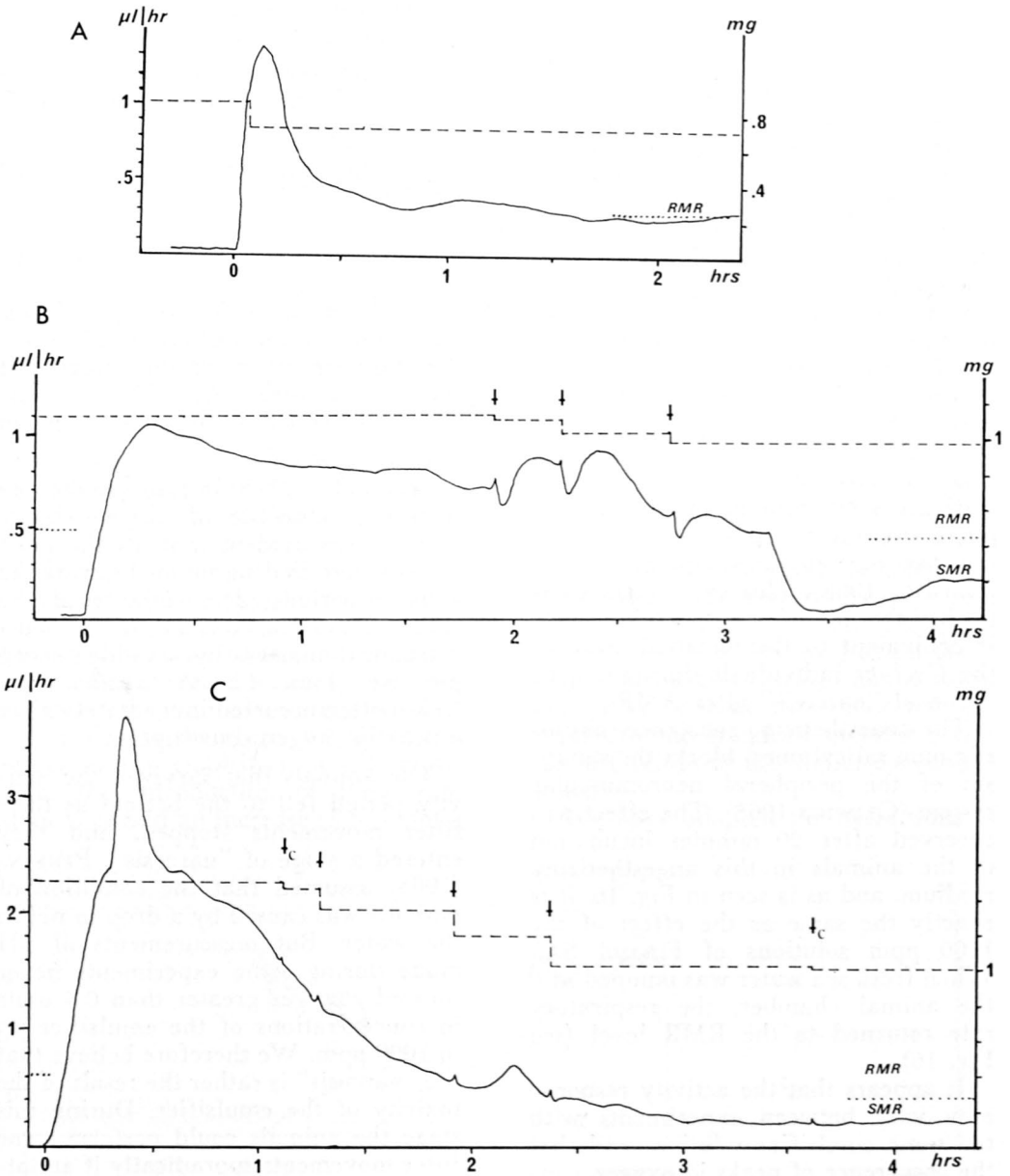
$\left(\int_0^{t_a} R_{t_a} \right)$ reached a maximum in solu-

tions of 500 ppm, decreasing at lower and higher concentrations. Fig. 19 gives the standard errors of the integral at three concentrations, whereas the length of the activity periods is a mean value.

From Fig. 17 it is also possible to trace the behaviour of single individuals. It was evident that all the individuals that died succumbed during the activity periods. The collapse of the energy-regulating mechanisms is indicated in the curves by a sudden sharp increase (Figs. 17—18, symbol +). Death often occurred immediately after a peak in oxygen consumption.

The animals that survived the activity period fell to the bottom as their filter movements stopped, and they entered a stage of "narcosis". PERKINS (1968) assumed that the condition of narcosis was caused by a drop in pH in the water. But measurements of pH made during some experiments never showed changes greater than 0.3 units in concentrations of the emulsifier up to 1000 ppm. We therefore believe that the "narcosis" is rather the result of the toxicity of the emulsifier. During this stage the animals could perform some filter movements sporadically if agitated by knocking on the respiration chamber. The "narcosis" could last for at least 10—12 hours, and the animals recovered when put in pure sea water.

The rate of respiration during "narcosis" is quite close to the standard metabolic rate (cf. Figs. 17—18), but *Cyclops* individuals in lower concen-

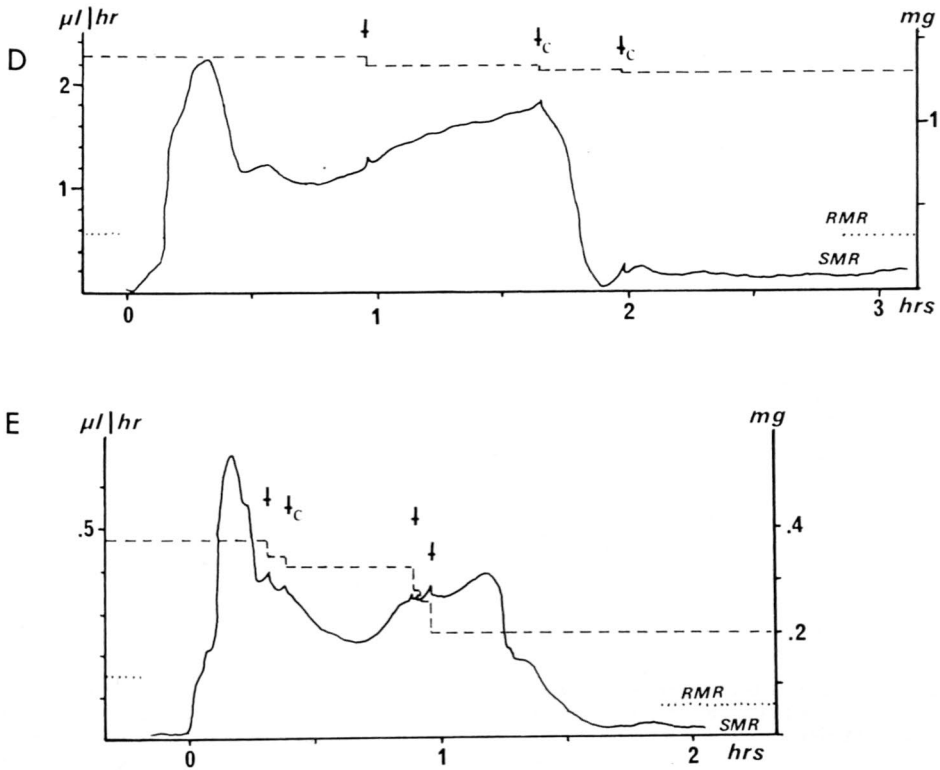


trations (below 50 ppm) of Finasol S.C. always performed filter movements, thus respiring at the RMR. The amount of oxygen consumed during activity was lower in high concentrations, probably owing to the toxicity of the emulsifier, whereas the animals seemed to

acclimatize to lower emulsifier concentrations.

2. *Acartia bifilosa*.

The type of response was quite diffe-



← ↑
 FIG. 18. Recorder outputs of oxygen consumption values measured from the time of introduction of the emulsifier Finasol S.C., for *Cyclops oithonoides*. The oxygen consumption values in μl per hour are given on the left-hand axis, and the biomass of the surviving animals in mg (broken line) on the right-hand axis. + = peak in oxygen consumption, indicating death of adults, +c = peak in oxygen consumption, indicating death of copepodites. Dotted line = RMR values for the living biomass. Concentrations: A. 50 ppm, B. 200 ppm, C. 500 ppm, D. 2000 ppm, E. 10000 ppm. Temperature 11.5°C, salinity 6 ‰, water flow 6 ml/hour. For details, see text.

rent in populations of *Acartia* (about 20 animals used in each experiments), as demonstrated by Fig. 20. The initial increase in oxygen consumption due to activity was followed by a gradual decrease. The animals first sank to the bottom of the respiration chamber, and performed filter movements. After a while the filter movements stopped, and the animals entered "narcosis". As is shown in Fig. 20, animals died throughout the experiment, but the individuals surviving after two hours were in a state of "narcosis" and respired at

a rate of 0.1 $\mu\text{l/hr/mg}$, whereas the individuals surviving after one hour had a rate of 0.26 $\mu\text{l/hr/mg}$ (extrapolated from Fig. 20). The normal respiratory rate (RMR) is about 0.3 $\mu\text{l/hr/mg}$ (GYLLENBERG 1973). This type of response was typical for both emulsifiers and all the concentrations used.

In Fig. 21 the amount of oxygen consumed for activity $\left(\int_0^{t_a} R_{t_s} \right)$ has been plotted against the activity period (in hours) and Finasol S.C. concentration.

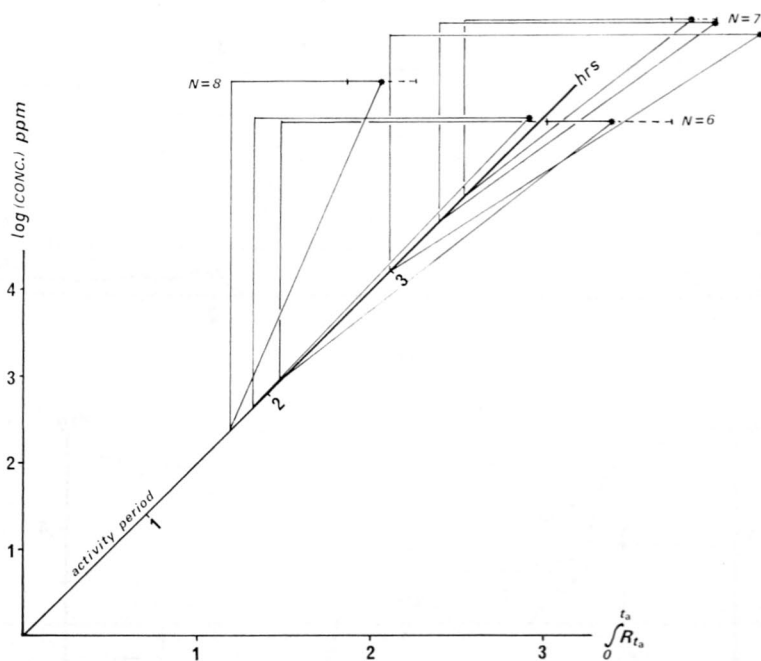


FIG. 19. The relation between the concentration of the emulsifier Finasol S.C. in ppm, the integral of respiration due to activity over the activity period in hours, and the activity period in hours (mean value). Experimental animal: *Cyclops oithonoides*. The standard error for the equivalent number of experiments (N) is given for the integral value (broken line).

Both the length of the activity period and the integral for respiration decreased with increasing emulsifier con-

centrations, indicating a directly toxic effect of the emulsifier Finasol S.C. in higher concentrations.

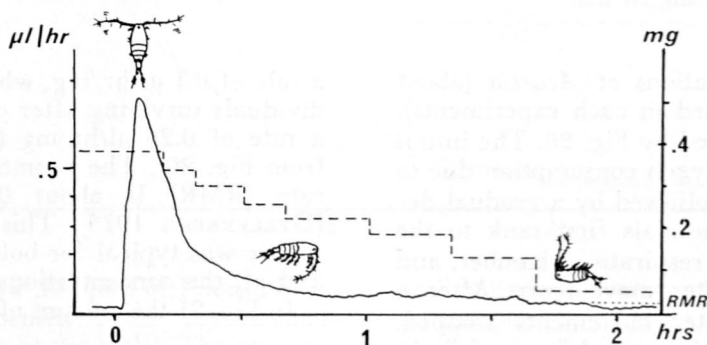


FIG. 20. A typical example of recorded output of oxygen consumption values measured from the time of introduction of the emulsifier solution, for *Acartia bifilosa*. The oxygen consumption values in $\mu\text{l/hr}$ are given on the left-hand axis, and the biomass of the surviving animals in mg (broken line) on the right-hand axis. Dotted line = RMR values. Emulsifier used Essochem OSD 9517 1000 ppm, temperature 11.5°C , salinity 6 ‰, water flow 6 ml/hour. For details, see text.

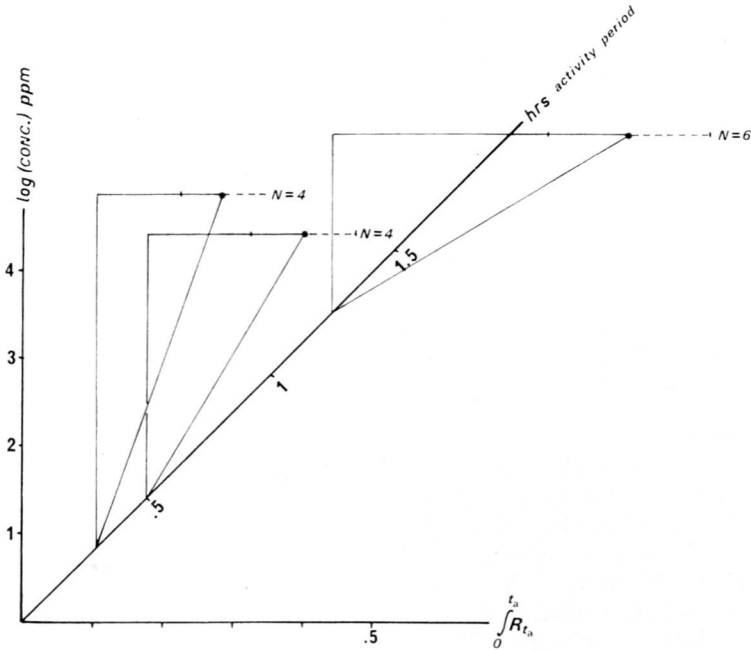


FIG. 21. The relation between the concentration of the emulsifier Finasol S.C. in ppm, the integral of respiration due to activity over the activity period in hours, and the activity period in hours (mean value). Experimental animal: *Acartia bifilosa*. The standard error for the equivalent number of experiments (N) is given for the integral value (broken line).

IV DISCUSSION

In this study the effects of emulsifiers and emulsifiers plus oil on *Acartia* and *Cyclops* were studied by examining the production of liquid lipids in the body tissues, the mean lethal dose, and the response in terms of oxygen consumption.

Oil-Red-O and Fettrot 7B are intensive dyes for material consisting solely of lipids (ROMEIS 1968), but lipids forming compounds with other substances, as lipoproteins, do not stain. This is also true of the lipids becoming part of the eggs formed in the oviduct (cf. Figs. 9, 11). Besides lipids, these dyes stain certain hydrocarbons, and as the hydrocarbons that are ingested by marine organisms become part of their

lipid pool (BLUMER & al. 1970a), they are likely to be stained.

It appears that copepods normally ingest small amounts of non-aromatic hydrocarbons (BLUMER & al. 1970a, 1970), but when they are exposed to crude oil or oil products the proportion of hydrocarbons in their lipids increase (MORRIS 1974). It is possible that the increase in liquid lipids was caused by increased ingestion of non-aromatic hydrocarbons in our experiments. In this case, as BLUMER & al. (1970b) have demonstrated that hydrocarbons are stable compounds in copepods, these animals would be predestined to become carriers, concentrating large amounts of hydrocarbons that are derived from

emulsifiers or oil but resemble natural biochemically important hydrocarbons.

Another explanation can also be found for the increased total amount of lipids: As BLUMER & al. (1970b) pointed out, the non-aromatic hydrocarbons would maintain the lipids in a liquid form for ready metabolism. Thus they could affect the proportion of lipids present in the form of liquid droplets after death.

The other components of emulsifiers and oil may also affect lipids. NELSON-SMITH (1972) observed that the aromatic hydrocarbons and the surfactant fractions both have marked effects on lipoproteins. The aromatic solvent has been found to be most toxic to animals (WILSON 1970) and may interfere with the activity of enzyme systems and other proteins (MANWELL & BAKER 1967). The surfactant probably damages the intercellular membranes (BAKER 1970).

Our results show clearly that emulsifiers and oil have pronounced effects on the lipid metabolism of *Acartia* and *Cyclops*. Whether this is due to a total increase in lipid material, or just the result of the physical effects on the lipids of the portions (hydrocarbons) of the emulsifiers absorbed by the copepods is not clear at this stage of investigation. However, the fact that females exposed to emulsifiers also contain fluid lipids indicates that the animals probably metabolize components of the emulsifiers and store them as lipids.

In the toxicity tests, comparison with the survival of animals kept in pure sea-water (surviving for about 48 hours) showed that both emulsifiers (Finasol S.C. and Finasol OSR-2) are toxic to *Acartia* and *Cyclops*, their toxicity increasing with their concentration. The TD₅₀ increased significantly for *Acartia* in a continuous water flow system, indicating clearly that these animals are favourably affected

by water currents (cf. GYLLENBERG 1973).

Toxic effects of emulsifiers have been demonstrated earlier by, e.g., CAPART (quoted by NELSON-SMITH 1970), who showed that 1000 ppm concentrations of Finasol (probably S.C.) were highly toxic and 100 ppm concentrations still somewhat toxic to *Daphnia pulex*. It appears that the emulsifiers only blocked muscular activity in *Cyclops*, but probably had a directly poisonous effect on the *Acartia* individuals, as they did not survive for more than 10–20 minutes after inactivation.

Different views have been expressed on the question whether mixtures of oil and emulsifiers are more or less toxic to marine animals than the emulsifier alone (cf. PERKINS 1968 and PORTMANN & CONNER 1968). NELSON-SMITH (1972) assumed that the effect of combining emulsifier with oil would depend on whether or not the emulsifier increased the contact of the oil with the organism or facilitated its entry into the organism. Our results show that a mixture of oil and Finasol S.C. (in equal proportions) was more toxic to the *Acartia bifilosa* individuals than the emulsifier alone, whereas Finasol OSR-2 was less toxic to *Acartia* when mixed with oil. The last finding was confirmed by the measurements of the amount of lipid droplets.

The oxygen consumption measurements reflected the behaviour of *Acartia* and *Cyclops* when exposed to emulsifiers. The types of oxygen curve and the activity observed in the respiration chamber were different for the two animals. They first performed escape movements, either at random (*Cyclops*), or away from the oil droplets introduced at the top of the respiration chamber. Similar behaviour was observed in the tests of escape movements: the *Acartia* individuals moved away from the point of diffusion of the emulsifier.

All the *Cyclops* individuals that died succumbed during the activity period. In the surviving individuals the normal filter movements gradually stopped and they sank to the bottom and respired at a lower metabolic rate than the normal routine metabolic rate (RMR).

In this condition of "narcosis", they could survive for at least 10—12 hours. The exoskeleton of *Cyclops oithonoides* probably affords protection against the emulsifier medium, and as the animals do not filter new material into the gut system, toxic compounds of the emulsifier are no longer ingested into the body tissue. It is possible that they could survive as long as material for maintenance metabolism was available.

The interpretation that the exoskeleton affords protection is supported by the observations of WILSON (1970), who reports that the chorion of fish embryos offered a protective shield against emulsifiers, and that a dramatic reduction occurred in LD₅₀ after hatching. SIMPSON (1968) found that young stages of animals are somewhat more susceptible to toxic substances than are adults, which agrees with our results, since the younger nauplius instars with thinner exoskeletons succumbed more rapidly to the emulsifier than the adults.

It is possible that the exoskeleton of *Acartia bifilosa* does not offer good protection against emulsifiers. After they entered "narcosis" and the filter movements stopped, the animals did not normally survive for more than 10—20 minutes. This type of behaviour

was also reported by WILSON (1970) for larvae of fish. He found that the toxic effects of emulsifiers were reversible in the "narcosis" condition, if the animals still responded when touched. Dead larvae often showed extensive tissue damage caused by the emulsifier (cf. also Fig. 5).

In summary, the responses of *Acartia* and *Cyclops* individuals to emulsifiers or oil are completely different. The *Acartia* individuals swim away from the point of discharge, and if they come into contact with the emulsifier, the effect is toxic.

The *Cyclops* individuals exhibit increased activity, but this activity is completely random. After a while the animals sink to the bottom and respire at SMR. This type of response also seems to occur when the animals are exposed to other external stimuli, such as high light intensities and high temperatures. When the animals sink to the bottom of the sea they escape both intolerable light and temperature. Possibly the emulsifier initially acts purely as a stimulus and the animal adopts the same mechanism for escape as with natural stimuli in the sea.

Acknowledgements

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References

- BAKER, J. M. 1970: The effects of oils on plants. — Environ. Poll. 1:27—44.
- BENSON, A. A. & LEE, R. F. 1975: The role of wax in oceanic food chains. — Scient. Amer. 232:77—86.
- BLAZKA, P. 1971: Physiological basis of secondary production. — In: EDMONDSON, W. T. & WINBERG, G. G. (eds.): A manual on methods for the assessment of secondary productivity in fresh waters: 222—228.

- Blackwell Scient. Publ., Oxford and Edinburgh.
- BLUMER, M., SOUZA, G. & SASS, J. 1970a: Hydrocarbon pollution of edible shellfish by an oil spill. — *Marine Biology* 5:195—202.
- BLUMER, M., MULLIN, M. M. & GUILLARD, R. R. L. 1970b: A polyunsaturated hydrocarbon (3, 6, 9, 12, 15, 18 — heneicosahexane) in the marine food web. — *Marine Biology* 6:226—235.
- CORKETT, C. J. 1970: Techniques for breeding and rearing marine calanoid copepods. — *Helgoländer wiss. Meeresunters.* 20:318—324.
- GLIWICZ, Z. M. 1968: The use of anaesthetizing substance in studies on the food habits of zooplankton communities. — *Ekol. Pol.(A)* 16:279—279—295.
- GYLLENBERG, G. 1973: Comparison of the Cartesian diver technique and the polarographic method, an open system, for measuring the respiratory rates in three marine copepods. — *Commentat. Biol.* 60:1—13.
- HALME, E. 1958: Planktologische Untersuchungen in der Pojo-Bucht und angrenzenden Gewässern. — *Ann. Zool. Soc. Zool. Bot. Fenn. 'Vanamo'* 19(3):1—62.
- HEINLE, D. R. 1970: Population dynamics of exploited cultures of calanoid copepods. — *Helgoländer wiss. Meeresunters.* 20:360—372.
- KINNE, O. 1970: Temperature: Invertebrates. — In: O. KINNE, (ed.): *Marine ecology Vol. I. Environmental factors*: 407—514. Wiley Interscience, London, New York, Toronto, Sydney.
- MANWELL, C. & BAKER, C. M. 1967: A study of detergent pollution by molecular methods: starch gel electrophoresis of a variety of enzymes and other proteins. — *J. mar. biol. Ass. U.K.* 47:659—675.
- MORRIS, R. J. 1974: Lipid composition of surface films and zooplankton from the eastern Mediterranean. — *Marine Pollution Bull.* 5:105—109.
- NELSON-SMITH, A. 1970: The problem of oil pollution of the sea. — In: RUSSEL, F. S. & YONGE, M. (ed.): *Advances in marine biology* 8: 215—306. Academic Press, London.
- 1972: *Oil pollution and marine ecology.* — Paul Elek (Scientific Books) Ltd, London, 260 pp.
- NEWELL, R. C. & NORTHCROFT, H. R. 1967: A re-interpretation of the effect of temperature on the metabolism of certain marine invertebrates. — *J. Zool., Lond.* 151:277—298.
- PERKINS, E. J. 1968: The toxicity of oil emulsifiers to some inshore fauna. — *Suppl. to Vol. 2 of Field Studies*: 81—90.
- PORTMANN, J. E. & CONNOR, P. M. 1968: The toxicity of several oil spill removers to some species of fish and shellfish. — *Marine Biology* 1:322—329.
- ROMEIS, B. 1968: *Mikroskopische Technik.* — 16. edition. R. Oldenbourg Verlag, München and Wien. 570 pp.
- SAUNDERS, L. & FLEMING, R. 1971: *Mathematics and statistics.* — 2nd edition. The Pharmaceutical Press, London. 309 pp.
- SIMPSON, A. C. 1968: Oil, emulsifiers and commercial shellfish. — *Suppl. to Vol. 2 of Field Studies*: 91—98.
- WESENBERG-LUND, C. 1939: *Biologie der Süßwassertiere.* — Springer Verlag, Vienna, 814 pp.
- WILSON, K. W. 1970: The toxicity of oil-spill dispersants to the embryos and larvae of some marine fish. — *FAO technical conference on marine pollution and its effect on living resources and fishing.* Rome 9—18 December 1970.
- ZILLIQUOX, E. J. & LACKIE, N. F. 1970: Advances in the continuous culture of planktonic copepods. — *Helgoländer wiss. Meeresunters.* 20:325—332.

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