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Effects of the Toxicity of Mineral Oil and Solvent Emulsifier upon the Larvae and Young of Marine Fish

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The present experiments were made to learn the toxic effects on the young of black sea-bream (1, 2 and 3 months after hatching), the young of Japanese flounder (2 months after hatching) and larvae of stone flounder (24 hours after hatching) by rearing them in sea waters containing heavy oils A and B, solvent emulsifier and a mixture of the emulsifier with oils A and B, respectively.

The results of the experiments showed that in all these young and larvae the toxic effect of heavy oil A was higher than that of heavy oil B, and in the case of the solvent emulsifier mixed with each of the heavy oils the effect was greater than in the case of oils A and B alone. It is noted that in the black sea-bream the toxic effect of the solvent emulsifier alone worked most strongly on the 1-month-old young but most weakly on the 2-and 3-month-old ones. The strong toxic effect of the same emulsifier was also found in the 2-month-old young of Japanese flounder and the larvae of stone flounder.

Key words : oil pollution, TLm

Oil spill accidents from ships and tankers at sea have been more frequent of late. The resulting water pollution with its inevitable adverse influence upon marine organizms is so serious that the need for countermeasures is now being taken up as one of the pressing social problems. There are a considerable number of papers and reports on these oil-spill accidents and the damage they caused. Moreover, it has often been discussed with alarm that the toxicity of the solvent emulsifier used on the water to clean up the oil-spills could itself cause even greater damage.

The solvent emulsifier is a chemical whose function is not chemical in reaction to decompose or detoxicate mineral oils, but a physical one (OOKUBO, 1976). That is, it emulsifies oils into corpuscles to suspend them in the sea water. This corpusclar oil then needs to be decomposed and eliminated by marine bacteria and by reacting with sea water. Solvent emulsifier is composed of a solvent to dissolve the spilt oils and a detergent to emulsify them. Very few studies, however, have been done on the toxicity of solvent emulsifiers used for such a function. In 1982 the authors reported on the toxicity

of solvent emulsifier and oils on adult marine fish, and in 1983 on the effects of the toxicity of mineral oils and solvent emulsifier upon the eggs of marine fish.

The present paper will outline our experiments on the effects of the toxicity of mineral oills and solvent emulsifier on the larvae and young of marine fishes.

Materials and Methods

The marine fishes used in the experiments were the young of black sea-bream, *Acanthopagrus schlegeli,* and of Japanese flounder, *Paralichthys olivaceus,* as well as the larvae of stone flounder, *Kareius bicoloratus.* The oills used in the experiments were heavy oil A, heavy oil B, and solvent emulsifier, NEOS AB 3000(trade name), the principal ingredient of which is the diethanol amide of nonionic detergent. The fish larvae and young were kept and observed for 48 hours in sea water, suspended with those oils and the solvent emulsifier used separately or mixed.

The ratio of mineral oil to the mixed solvent emulsifier was 3 to 1, and to heighten the suspension of the oils and solvent emulsifier in the water, each experimental solution was treated with ultrasonication for 5 minutes (TOKUDA, 1976). Each solution used during the experimental period had been fully aerated, the dissolved oxygen was kept 4 ~ 6 ppm, pH value 8.0~8.2, and the salinity of the sea water used for the adjustment of the breeding water was 33 ~ 34‰. During the experimental period, no food was given to the fish.

Results

I. Effects on black sea-bream young

Generally speaking, in the early life stage, fish have a only feeble tolerance against toxicity. In the present experiment the tolerance was compared among the young of 3 different sizes; the young of 1 month after hatching, 9.97 mm in mean total length and 0.008 g in mean body weight; the young of 2 months old, 16.90 mm and 0.066 g; the young of 3 months old, 35.75 mm and 0.705 g. Experiments were made with 10 test fish in each glass tank of 2 litres, with water temperature at 20 ± 1°C. Only in case of the young of 2 months old, experiments were made with 20 test fish.

1. Black sea-bream young of 1 month old

The concentration of the mineral oils and solvent emulsifier used in the experiments, the hourly cumulative mortality of the test fish, and the estimated TLm values, in 24 and 48 hours, are shown in Table 1. For the estimation of TLm values, DOUDOROFF's method (1951) was adopted.

The 24 hr and 48 hr TLm values in each experimental section were within 24 ~ 37 ppm and 10 ~ 32 ppm, respectively, which proves that the oils and emulsifier show a very high toxicity, with little differences among the TLm values. Comparisons of mortality, however, showed that heavy oil A has higher toxicity than heavy oil B. It was also made clear that when emulsifier is added to either of the oils, the toxicity of

Table 1. Cumulative mortality (%), 24 and 48 hr TLm (ppm) of 1-month-old black sea-bream, *Acanthopagrus schlegeli*, in mineral oil and solvent emulsifier

substance	ppm \ hour	1	2	3	6	10	12	18	24	36	48	TLm $^{24}/_{48}$
H.A.	1,000	90	100									$^{32}/_{32}$
	100	70	80	100								
	10										0	
	1										0	
H.A. + S.E.	1,000	30	100									$^{24}/_{15}$
	100		90	90	100							
	10				10	20	20	20	20	20	40	
	1					10	10	10	10	10	20	
H.B.	1,000		70	90	100							$^{32}/_{32}$
	100				40	70	100					
	10										0	
	1										0	
H.B. + S.E.	1,000		90	100								$^{37}/_{18}$
	100			30	50	70	70	80	80	80	80	
	10							10	10	10	40	
	1										0	
S.E.	333			40	80	90	90	90	90	100		$^{22}/_{10}$
	33					10	10	60	60	60	80	
	3									10	20	
	0.3										0	
control											0	

H. A., heavy oil A ; H. B., heavy oil B; S. E., solvent emulsifier.

the mixtures rises much higher. The toxicity of the solvent emulsifier used by itself was, by 24 hr and 48 hr TLm values, 22 ppm and 10 ppm, respectively, which proved extremely toxic for the black sea-bream young of 1 month old. Lastly, we conclude that toxicity in the order of strength is: solvent emulsifier > heavy oil A + solvent emulsifier > heavy oil A > heavy oil B + solvent emulsifier > heavy oil B.

2. Black sea-bream young of 2 months old

The concentration, cumulative mortality, and each TLm value are as shown in Table 2.

With reference to TLm values and cumulative mortality, the toxicity of heavy oil A + solvent emulsifier is the highest, almost twice that of the second highest heavy oil A. In other words, with emulsifier added, the toxicity of heavy oil A is doubled. However, in case of heavy oil B, the toxicity is hardly raised at all by adding emulsifier. Heavy oil A also shows a toxicity 5 times that of heavy oil B. The cumulative mortality in 48 hours being 15 % at the highest 333 ppm of the set-up concentration, the toxicity of solvent emulsifier is lower than those of oils and their mixture. Accordingly, the toxicity in the order of its strength is: heavy oil A + solvent emulsifier >

heavy oil A > heavy oil B + solvent emulsifier > heavy oil B > solvent emulsifier.

Table 2. Cumulative mortality (%), 24 and 48 hr TLm (ppm) of 2-month-old black sea-bream, *Acanthopagrus schlegeli*, in mineral oil and solvent emulsifier

substance	hour / ppm	1	2	3	6	10	12	18	24	36	48	TLm $^{24}/_{48}$
H.A.	100							45	55	55	60	$^{78}/_{60}$
	10							10	10	10	15	
	1										5	
H.A.+S.E.	100			5	15	40	85	85	85	85	95	$^{37}/_{32}$
	10							5	5	5	5	
	1										0	
H.B.	1,000			30	70	70	70	80	80	90	100	$^{380}/_{280}$
	100					5	5	10	10	10	10	
	10							10	10	10	10	
	1										0	
H.B.+S.E.	1,000		10	20	80	80	80	90	100			$^{200}/_{200}$
	100							5	10	10	10	
	10								10	10	10	
	1										0	
S.E.	333									5	15	$^{>333}/_{>333}$
	33										5	
	3										5	
	0.3										0	
control											0	

3. Black sea-bream young of 3 months old

The concentration, cumulative mortality and each TLm value are shown in Table 3.

The toxicity, compared with reference to TLm values, showed a similarity to that of the experimental results from the black sea-bream young of 2 months old. That is, heavy oil A + solvent emulsifier was the most highly toxic: about 2 to 6 times that of heavy oil A when used by itself. Though heavy oil B and heavy oil B + solvent emulsifier showed the same valve in TLm, when we refer to the changes in mortality, the latter shows a somewhat higher toxicity on account of the emulsifier added to it. The toxicity of the solvent emulsifier, shown by the TLm values in 24 and 48 hours, was over 3,333 ppm, the lowest toxicity figured in the present experiment. Accordingly, the order of toxic strength is: heavy oil A + solvent emulsifier > heavy oil A > heavy oil B + solvent emulsifier > heavy oil B > solvent emulsifier.

Next we observed the changes in the toxicity of mineral oils and solvent emulsifier which were possibly attendant upon the growth of the black sea-bream. For convenience, each TLm value for the black sea-bream young, 1, 2 and 3 months old is collectively

registered in Table 4.

Table 3. Cumulative mortality (%), 24 and 48 hr TLm (ppm) of 3-month-old black sea-bream, *Acanthopagrus schlegeli*, in mineral oil and solvent emulsifier

substance	ppm \ hour	1	2	3	6	10	12	18	24	36	48	TLm $^{24}/_{48}$
H.A.	5,000	20	80	100								$^{1,800}/_{760}$
	1,000							10	20	60	60	
	100										0	
	10										0	
H.A. + S.E.	5,000	100										$^{320}/_{320}$
	1,000			20	50	100						
	100										0	
	10										0	
H.B.	5,000		20	100								$^{2,200}/_{2,200}$
	1,000										0	
	100										0	
	10										0	
H.B. + S.E.	5,000	100										$^{2,200}/_{2,200}$
	1,000										0	
	100										0	
	10										0	
S.E.	3,333										0	$^{>3,333}/_{>3,333}$
	333										0	
	33										0	
	3										0	
control											0	

Table 4. 24 and 48 hr TLm (ppm) of young black sea-bream *Acanthopagrus schlegeli* (three different sizes)

TLm / stage / substance	24 hr			48 hr		
	①	②	③	①	②	③
H.A.	32	78	1,800	32	60	680
H.A. + S.E.	24	37	320	15	32	320
H.B.	32	380	2,200	32	280	2,200
H.B. + S.B.	37	200	2,200	18	200	2,200
S.E.	22	> 333	> 3,333	10	> 333	> 3,333

①……1 month after hatching; total length 9.97 mm, body weight 0.008 g
②……2 months after hatching; total length 16.90 mm, body weight 0.066 g
③……3 months after hatching; total length 35.75 mm, body weight 0.705 g

As the table clearly shows, the tolerance of the fish against toxicity becomes stronger with growth. For example, the 24 hr TLm value to figure the tolerance against heavy oil B toxicity shows that the tolerance of the young becomes twice as great in 2 months

as that in 1 month, and, further, in 3 months 6 times as high as that in 2 months. The toxicity of solvent emulsifier works very strongly upon the young of 1 month old (10 ppm by 48 hr TLm value), but it falls away remarkably (over 3,333 ppm by 48 hr TLm value) on the young in and after 3 months post hatching.

II. Effects on Japanse flounder young

The Japanese flounder young used in the experiment were 2 months old, 24.75 mm in mean total length, 0.159 g in mean body weight. Experiments were carried out with 20 test fish put into each glass tank of 2 litres, with water temperature at 20 ± 1°C. The concentration, the cumulative mortality and TLm values are shown in Table 5.

Table 5. Cumulative mortality (%), 24 and 48 hr TLm (ppm) of 2-month-old Japanese flounder, *Paralichthys olivaceus*, in mineral oil and solvent emulsifir

substance	hour / ppm	1	2	3	6	10	12	18	24	36	48	TLm $^{24}/_{48}$
H.A.	5,000	90	100									$^{190}/_{190}$
	1,000	70	100									
	100							20	30	30	30	
	10							10	10	10	10	
H.A.+S.E.	5,000	70	100									$^{42}/_{24}$
	1,000	70	100									
	100							80	80	90	100	
	10									20	20	
H.B.	5,000	50	100									$^{320}/_{320}$
	1,000		30	70	80	80	80	100				
	100										0	
	10										0	
H.B.+S.E.	5,000	60	100									$^{320}/_{280}$
	1,000		30	50	50	100						
	100									10	10	
	10										10	
S.E.	3,333					40	60	80	80	80	90	$^{170}/_{120}$
	333							70	70	70	80	
	33										10	
	3										0	
control											10	

With reference to TLm values, the toxicity of heavy oil A is somewhat stronger than that of heavy oil B. Both oils, with solvent emulsifier added, show a rise in their toxicity, the degree of which is especially marked in heavy oil A: the toxicity by the 48 hr TLm value rises 8 times as high by adding solvent emulsifier. The toxicity of solvent emulsifier is also quite high, 120 ppm by 48 hr TLm. Lastly, the order of toxicity strength is: heavy oil A + solvent emulsifier > solvent emulsifier > heavy oil A > heavy

oil B + solvent emulsifier > heavy oil B.

III. Effects on stone flounder larvae

The larvae used were 24 hours after hatching out of eggs which had been artificially fertilized. As the test fish were so small, 3.10 mm in mean total length, to facilitate observation, each 20 of the larvae were put in 100 ml of experimental water in narrow glass tubes of 30 mm in diameter. The water temperature was kept at 12 ± 1°C, to conform it to the condition of the spawning ground of the fish. The concentration, the cumulative mortality and TLm values are shown in Table 6.

Table 6. Cumulative mortality (%), 24 and 48 hr TLm (ppm) of 24-hour-old stone flounder, *Kareius bicoloratus*, in mineral oil and solvent emulsifier

substance	ppm \ hour	1	2	3	6	10	12	18	24	36	48	TLm $^{24}/_{48}$
H.A.	1,000	100										
	100	10	15	30	50	50	50	50	50	50	55	$^{100}/_{72}$
	10			15	15	15	15	15	15	20	20	
	1										0	
H.A. + S.E.	1,000	5	5	20	80	100						
	100			30	70	90	90	100				$^{19}/_{5}$
	10			10	20	30	30	30	30	30	40	
	1										0	
H.B.	1,000	5	5	30	60	65	65	80	80	80	80	
	100		5	5	5	10	15	25	25	25	45	$^{290}/_{140}$
	10			5	10	10	10	25	25	25	25	
	1										0	
H.B. + S.E.	1,000		10	20	90	100						
	100			10	20	40	60	60	60	60	90	$^{10}/_{7}$
	10				10	30	50	50	50	50	60	
	1										0	
S.E.	333	5	5	20	65	95	95	100				
	33		5	10	20	20	30	30	30	30	30	$^{64}/_{64}$
	3			5	5	5	5	5	10	10	10	
	0.3										0	
control											0	

The toxicity, compared with reference to TLm values, proved stronger in heavy oil A than heavy oil B, and it showed a remarkable rise when solvent emulsifier was added to those oils. Especially, in the case of heavy oil B, its 24 hr TLm value of 290 ppm falls to 10 ppm once emulsifier is added to it; the difference amounts to 30 times as high. The toxicity by TLm values, comparing heavy oil A + solvent emulsifier with heavy oil B + solvent emulsifier, showed higher in the latter, but referring to the changes in the cumulative mortality, it proved a little stronger in the former. The solvent emulsifier also showed a strong toxicity. Thus the order of toxicity strength is: heavy oil A +

solvent emulsifier > heavy oil B + solvent emulsifier > solvent emulsifier > heavy oil A > heavy oil B.

Discussion and Summary

The results of the experiments show that the toxicity of heavy oil A affected the fish more strongly in their early life stages than that of heavy oil B, and the toxicity was further raised by adding solvent emulsifier. The solvent emulsifier when used by itself had a much greater toxic effect on the fish in their early life stages than the mineral oils.

An outline of the experimental results:

1. The toxicity of mineral oils and solvent emulsifier, affecting the black sea-bream young of 1 month old, is remarkably high. As the bream young become 2 and 3 months old, their tolerance against the toxicity of oils and solvent emulsifier notably increases.

2. The toxicity of mineral oils and solvent emulsifier affecting the Japanese flounder young of 2 months old shows values approximating the TLm values of the black sea-bream young, with the exception of the experimental cases of heavy oil A and of solvent emulsifier.

3. In case of the stone flounder larvae 24 hours after hatching, the toxicity notably rises when solvent emulsifier is added to the mineral oils. That tendency is especially notable in case of heavy oil B. Solvent emulsifier when used by itself also shows as strong a toxicity as it does on the black sea-bream young of 1 month old and on the Japanese flounder young of 2 months old.

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