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

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Experiments were made on the effects of the toxicity of mineral oils and solvent emulsifier (oil dispersant) on the eggs of common sea bass, japanese parrotfish and japanese flounder, and the values of TLm and 50 % hatching rate (HR 50) were figured. The comparison with reference to 24 hr TLm has clearly shown that the tolerance of the eggs of common sea bass is stronger than those of japanese parrotfish and japanese flounder, and that the toxicity of mineral oils becomes higher when solvent emulsifier is added, since their toxicants diffuse in the water.
Keywords: oil pollution, TLm, hatching rate

The incidence of oil spills and their resulting water pollution, especially their inevitable adverse effects on marine resources, have been taken up as one of the present day's serious social problems. In 1982, therefore, the authors studied and reported the effects of the toxicity of mineral oils and solvent emulsifier upon marine fishes.

The present discussion concerns the experiments made on the toxic effects of mineral oils and solvent emulsifier upon the eggs of marine fish, and in the tolerance of the eggs according to the kind of fish.

Materials and methods

The kinds of oil employed in the experiment were crude oil (Arabian light crude oil), heavy oil A and heavy oil B. As solvent emulsifier used was the article NEOS AB 3000, whose main component is nonionic detergent. The rate of mineral oil to solvent emulsifier was 3 to 1 , figured on the concentration of the solvent emulsifier usually spread over the scene in case of actual oil spilling. The rearing water was regulated by 5 -minutes ultrasonication in order to fully suspend the oil in the sea water.

Eggs used were the fertilized eggs of common sea bass (*Lateolabrax japonicus*), japanese parrotfish (*Oplegnathus fasciatus*) and japanese flounder (*Paralichthys olivaceus*). These eggs had been bred separately, 20 eggs each, in a 2 liter glass beaker. During the experimental period, the rearing water had been fully aerated, with dissolved oxygen 4～6 ppm and pH 8.0～8.2. To regulate the rearing water, the sea water of salinity 33～34‰ was used.

Usually, the recommended index of relative toxicity is the median tolerance limit (TLm), or the concentration at which just 50 percent of the test animals are able to survive, for a specified period of exposure (24, 48 or 72hrs). The period from spawning to hatching varies with fishes. In the present experiment, therefore, the concentration at which just 50% of the eggs came to hatching (HR 50) was used as the index of toxicity. For the calculation of TLm and HR 50 DOUDOROFF's method (1951) was adopted.

Results

Table 1 shows the kinds of mineral oil and eggs used in the present experiment.

Table 1. Toxicity test for eggs of marine fish in sea water containing mineral oils and solvent emulsifier.

Substances / Eggs of fish	C.O.	C.O.+S.E.	H.A.	H.A.+S.E.	H.B.	H.B.+S.E.	S.E.
Common sea bass			○	○	○	○	○
Japanese parrotfish	○	○	○	○	○	○	○
Japnese flounder			○	○	○	○	○

○, tested eggs; C.O., crude oil; H.A., heavy oil A; H.B., heavy oil B; S.E., solvent emulsifier

I. Effects on the eggs of common sea bass

The egg of common sea bass is a floating egg with a single oil globule, and its diameter is 0.34～0.38mm. Usually it comes to hatch, at a water temperature of 14 °C, in 4 or 5 days. The eggs used in the experiment are those which were artificially fertilized from the matured fish caught in the set-net on Jan. 13, 1983.

The eggs in the morula stage, 6 hrs after fertilization, were exposed in water 12.8～14.6 °C. After the exposure started, the eggs came to their hatching peak in 108 hrs, and the experiment was over in 120 hrs. Kinds and concentration of mineral oil used in the experiment, and the mortality and hatching rates are shown in Table 2. TLm and HR 50 figured out of the latter are shown in Table 3.

1. Heavy oil A

Under concentrations below 10 ppm, over 80% of the eggs hatched, the oil effect on the hatching rate being rather slight.

2. Heavy oil B

At the lowest concentration 0.1 ppm, the hatching rate came out 70%, comparatively lower than the control. Heavy oil B, higher in its viscosity than heavy oil A,

Table 2. Effects of mineral oil and solvent emulsifier on morula stage eggs of common sea bass (*Lateolabrax japonicus*), 6 hrs after spawning; changes in mortality and hatching rates.
W.T., 12.8~14.6℃; D.O., 4~6 ppm; pH, 8.0~8.2

Substances	Hour / % / ppm	24		48		72		96		108		120	
		E	H	E	H	E	H	E	H	E	H	E	H
H.A.	10^{-1}	0		5		15		15		20	75	20	80
	10^{0}	0		5		15		15		20	75	20	80
	10^{1}	0		10		20		20		20	60	20	80
	10^{2}	5		5		35		35		40	60	40	60
	10^{3}	10		65		65		70		70	20	75	25
H.A.+S.E.	10^{-1}	5		5		10		15		15	85	15	85
	10^{0}	5		10		20		40		40	30	45	55
	10^{1}	5		5		20		40		40	45	45	55
	10^{2}	10		30		55		60		60	10	90	10
	10^{3}	20		40		90		90		100			
H.B.	10^{-1}	0		10		10		25		30	65	30	70
	10^{0}	10		20		25		40		40	55	40	60
	10^{1}	10		30		45		55		55	30	55	45
	10^{2}	30		40		50		80		90	0	95	5
	10^{3}	25		60		100							
H.B.+S.E.	10^{-1}	5		15		20		20		40	60	40	60
	10^{0}	5		20		30		30		40	50	40	60
	10^{1}	10		35		35		55		70	15	75	25
	10^{2}	30		45		65		80		100			
	10^{3}	40		80		100							
S.E.	10^{-1}	0		10		15		15		15	80	15	85
	10^{0}	0		10		15		15		15	80	15	85
	10^{1}	10		15		30		30		30	60	30	70
	10^{2}	10		15		25		30		35	55	40	60
	10^{3}	35		35		35		35		55	40	55	45
Control		0		0		5		5		10	85	15	85

E, cumulative mortality of eggs (%) ; H, cumulative hatching rate (%)

Table 3. TLm and HR 50 of mineral oil and solvent emulsifier on the eggs of common sea bass (*Lateolabrax japonicus*).

Substances	TLm (ppm) 24 hr.	48 hr.	72 hr.	HR 50 (ppm)
H.A.	>1,000	560	320	190
H.A.+S.E.	>1,000	1,000	74	13
H.B.	>1,000	>320	100	4.6
H.B.+S.E.	>1,000	>140	32	1.9
S.E.	>1,000	>1,000	1,000	480

HR 50, 50% hatching rate

remains in minute viscous particles, suspended in the water, which stick to the egg membrane and exercise an unfavorable effect on the embryonic development.

3. Solvent emulsifier

At the concentration below 1 ppm, the hatching rate was 85 %, which may confirm that the effect of solvent emulsifier is very little; at the highest concentration 1,000 ppm, no less than half of the eggs hatched. This proved the toxicity of solvent emulsifier to be much lower than that of mineral oil.

4. Heavy oil A plus solvent emulsifier

At the lowest concentration 0.1 ppm, the hatching rate showed no difference from the case of heavy oil A only, but at the concentration 1 ppm and above, the toxic effect on the eggs showed a more damaging tendency, caused by adding solvent emulsifier.

5. Heavy oil B plus solvent emulsifier

Compared with the case of heavy oil B only, no significant difference in egg mortality could be seen up to 96 hrs, but after 108 hrs, the added solvent emulsifier effected a lower hatching rate.

With reference to HR 50, showed the following order of toxicity upon eggs of common sea bass from greatest to least : heavy oil B+solvent emulsifier>heavy oil B>heavy oil A+solvent emulsifier>heavy oil A>solvent emulsifier.

II. Effects on the eggs of japanese parrotfish

The egg of japanese parrotfish, a floating egg with a single oil globule and of 0.89～0.95 mm in diameter, usually comes to hatch in about 36 hrs at a water temperature 20 °C. The fertilized eggs used in the experiment are the eggs naturally spawned in the concrete rearing pond and collected on June 1, 1982.

For the experiment, the eggs in their embryonic stage 18 hrs after spawning were expcsed in the water of 19 ± 1 °C, and after the experiment began they attained the hatching peak and entered upon breeding in 24 hrs. In Table 4 are shown the kinds and concentrations of mineral oil used, as well as the mortality and hatching rates; and in Table 5 are TLm and HR 50 figured out of the latter.

1. Crude oil

In concentration below 10 ppm, eggs showed a high hatching rate of 85 %, and in the highest concentration 1,000 ppm, the greater number, 65 % came to hatching. Because crude oil is highly viscous, when ultrasonicated for 5 minutes there is but scarce suspension in water. This indicates that in a short time the toxicants of the oil hardly dissolve into the water. Since the eggs start to hatch only after 18 hrs exposure in the rearing water, the adverse effects, if any, would be minimal.

2. Heavy oil A

At concentrations below 100 ppm, the hatching rate was over 85%, showing

Table 4. Effects of mineral oil and solvent emulsifier on embryonic stage eggs of japanese parrotfish (*Oplegnathus fasciatus*), 18 hrs after spawning; changes in mortality and hating rates.
W.T., 19± 1 ℃; D.O., 4～6 ppm; pH 8.0～8.2

Substances	Hour / % / ppm	6		12		18		24	
		E	H	E	H	E	H	E	H
C.O.	10^0	0		10		15	80	15	85
	10^1	10		10		15	80	15	85
	10^2	10		20		25	60	30	70
	10^3	15		35		35	60	35	65
C.O.+S.E.	10^{-1}	10		10		10	80	10	85
	10^0	10		10		10	80	10	85
	10^1	10		10		10	70	30	70
	10^2	25		25		25	60	35	65
	10^3	30		40		40	40	60	40
H.A.	10^0	0		0		0	90	5	95
	10^1	0		0		10	90	10	90
	10^2	0		0		10	70	15	85
	10^3	0		0		40	50	40	60
H.A.+S.E.	10^{-1}	0		0		10	80	10	90
	10^0	0		10		10	75	20	80
	10^1	5		10		10	80	10	90
	10^2	10		10		20	80	20	80
	10^3	25		40		70	15	85	15
H.B.	10^0	0		0		0	85	5	95
	10^1	0		0		25	65	30	70
	10^2	10		40		75	20	75	25
	10^3	10		60		80	5	80	20
H.B.+S.E.	10^{-1}	5		5		15	60	30	70
	10^0	5		15		30	45	30	50
	10^1	10		20		60	10	75	25
	10^2	0		30		75	15	85	15
	10^3	5		65		100			
S.E.	10^0	0		0		0	85	10	90
	10^1	0		0		10	75	10	90
	10^2	0		0		10	75	10	90
	10^3	0		0		30	60	30	70
Control		0		0		10	90	10	90

Table 5. TLm and HR 50 (equal to the value of 24 hr TLm in this case) of mineral oil and solvent emulsifier on the eggs of japanese parrotfish (*Oplegnathus fasciatus*).

Substances	12 hr. TLm (ppm)	HR 50 (ppm)
C.O.	>1,000	>1,000
C.O.+S.E.	>1,000	400
H.A.	>1,000	>1,000
H.A.+S.E.	>1,000	290
H.B.	320	28
H.B.+S.E.	380	1
S.E.	>1,000	>1,000

hardly any significant effect of the oil. At 1,000 ppm, the highest concentration, the hatching rate was 60%, indicating that the toxicity is a little lower than that of crude oil.

3. Heavy oil B

At concentrations below 10 ppm, the hatching rate was high, while above 100 ppm the rate hastily fell off and the toxicity rose rapidly.

4. Solvent emulsifier

Even at 1,000 ppm, the highest concentration, the hatching rate was 70 %, probably indicating that the toxic effect is even less than that of the crude oil whose toxicants scarcely dissolve in the water.

5. Crude oil plus solvent emulsifier

These showed a similar tendency to that of crude oil only, but at higher concentrations the dissolution of toxicants in the water caused by the addition of solvent emulsifier is mose or less recognizable, accompanied by a slight lowering of the hatching rate.

6. Heavy oil A plus solvent emulsifier

Compared with the case of heavy oil A only, at 1,000 ppm, the highest concentration, the effect on the eggs caused by the addition of solvent emulsifier, is markedly recognizable and the hatching rate reduces to only 15 %.

7. Heavy oil B plus solvent emulsifier

Compared with the case of heavy oil B only, at each experimental concentration, effects caused by the addition of solvent emulsifier are recognizable. For example, at 1,000 ppm all the eggs perished and not a single hatching was found.

With regard to TLm and HR 50, the toxicity effect of mineral oils upon the eggs of japanese parrotfish was in the following order: heavy oil B+solvent emulsifier > heavz oil B > heavz oil A+solvent emulsifier > crude oil + solvent emulsifier > crude oil ≒ heavy oil A ≒ solvent emulsifier.

Ⅲ. Effects on the eggs of japanese flounder

The eggs of japanese flounder are floating eggs, each with a single oil globule and 0.8~0.9 mm in diameter. Usually, at water temperature 18~20 °C they come to hatching in 61 hrs. The fertilized eggs used in the experiment were collected on Feb. 4, 1983, as were the japanese parrotfish, from those which had been spawned in the concrete rearing pond.

The eggs in their embryonic stage, 16 hrs after spawning, were exposed for 60 hrs in water of 19±1 °C, and the hatching peak came 48 hrs after commencement of the experiment. The kinds and concentrations of mineral oil used in the experiment, as

Table 6. Effects of mineral oil and solvent emulsifier on embryonic stage eggs of japanese flounder (*Paralichthys olivaceuu*), 16 hrs after spawning; changes in mortality and hatching rates.
W.T., 19± 1 ℃; D.O., 4 ~ 6 ppm; pH 8.0~8.2

Substances	Hour / % / ppm	3		12		24		48		60	
		E	H	E	H	E	H	E	H	E	H
H.A.	10^{-1}	0		0		10		20	75	25	75
	10^{0}	0		0		20		20	70	30	70
	10^{1}	0		0		25		45	45	50	50
	10^{2}	0		15		40		50	15	75	25
	10^{3}	5		15		100					
H.A.+S.E.	10^{-1}	0		5		20		25	70	25	75
	10^{0}	0		10		20		30	70	30	70
	10^{1}	0		10		20		50	40	50	50
	10^{2}	5		10		50		70	20	80	20
	10^{3}	5		30		100					
H.B.	10^{-1}	0		0		5		15	75	20	80
	10^{0}	0		0		15		25	65	30	70
	10^{1}	0		5		20		30	65	35	65
	10^{2}	0		5		20		35	40	50	50
	10^{3}	5		5		30		40	40	50	50
H.B.+S.E.	10^{-1}	0		0		15		15	75	20	80
	10^{0}	0		0		15		30	70	30	70
	10^{1}	0		0		20		30	50	30	70
	10^{2}	0		10		45		60	30	70	30
	10^{3}	5		30		100					
S.E.	10^{-1}	0		0		0		15	75	25	75
	10^{0}	0		5		5		25	75	25	75
	10^{1}	0		5		45		45	55	45	55
	10^{2}	5		5		60		60	40	60	40
	10^{3}	10		20		65		100			
Control		0		0		0		10	90	10	90

Table 7. TLm and HR 50 of mineral oil and solvent emulsifier on the eggs of japanese flounder (*Paralichthys olivaceus*).

Substances	24 hr. TLm (ppm)	HR 50 (ppm)
H.A.	150	10
H.A.+S.E.	100	10
H.B.	>1,000	100
H.B.+S.E.	125	32
S.E.	21	21

well as the mortality and hatching rates are shown in Table 6 ; TLm and HR 50, figured from the latter, are shown in Table 7.

1. Heavy oil A

At 0.1 ppm, the lowest concentration, the hatching rate was comparably higher (75 %), but at 10 ppm it fell to just 50 %, and at 1,000 ppm, the highest concentration, not a single hatching was found.

2. Heavy oil B

The toxicity is lower than that of heavy oil A, and even at 1,000 ppm, the highest concentration, 50 % of the eggs came to hatching.

3. Solvent emulsifier

At a concentration below 1 ppm the hatching rate was 75 %, a comparatively high value, but at 1,000 ppm no eggs came to hatching.

4. Heavy oil A plus solvent emulsifier

Compared with the case of heavy oil A only, a slightly higher toxicity caused by the addition of solvent emulsifier was recognized.

5. Heavz oil B plus solvent emulsifier

Effects of the added solvent emulsifier were recognized in the rise of mortality and in the lowering of the hatching rates. Especially at 1,000 ppm, the highest concentration, the hatching rate in the case of heavy oil B was only 50 %, but atfer the solvent emulsifier was added no eggs came to hatching.

In regard to TLm and HR 50, the toxicity effect of mineral oils upon the eggs of japanese flounder was in this order: heavy oil A+solvent emulsifier> heavy oil A> solvent emulsifier> heavy oil B+solvent emulsifier> heavy oil B.

Discussion

In the present study, as the tested fish varied in species their time required from spawning to hatching also varied. Moreover, the stage of embryonic development differs with species, so it is rather difficult to draw a definite conclusion in comparing the tolerance of the eggs against the oils, under the same experimental conditions. We have come, however, to the following tentative conclusions.

First of all, the toxicity of any oil increases, when solvent emulsifier is added. It can be reasonably surmised that since oil is by nature an insoluble material, when any solvent emulsifier is added to it, its suspension in the water increases, which gives eggs more chance to contact it, and the toxicity of the oil accordingly becomes more damaging.

Second, comparing the tolerance of the eggs of each species against the mineral oils in the values of 24 hr TLm, the tolerance of common sea bass eggs was higher than that of both japanese parrotfish and japanese flounder. It has lately been ascertained that the eggs in the morula stage (in the case of common sea bass) have a higher tolerance against external stimuli than that of eggs in the embryonic stage (in the case of japanese parrotfish or japanese flounder).

Third, with regard to the tolerance of eggs against the toxicity of mineral oils in reference to HR 50, the eggs of common sea bass and japanese parrotfish were the strongest against the toxicity of heavy oil B plus solvent emulsifier; the values being 1.9 ppm and 1 ppm respectively. While in the case of the eggs of japanese flounder, the toxicity of heavy oil A plus solvent emulsifier and that of heavy oil A were higher. These values of HR 50 are 10 ppm.

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