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THE INFLUENCE OF BYPRODUCTS FROM VINYLCHLORIDE PRODUCTION ON FERTILIZATION, DEVELOPMENT AND LARVAL SURVIVAL ON PLAICE, COD AND HERRING EGGS

by

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Introduction

Of the vast amount of industrial wastes poured out into the sea, the waste products from vinylchloride production, namely chlorinated aliphatic hydrocarbons, have drawn especially attention during the past year. Jensen et al. (1970) showed how widespread these pollutants are in the open seas. In Norwegian waters the industrial wastes are often poured out into fiord systems with only very low rates of exchange causing accumulation of the waste products.

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As it is normally believed that fish eggs and larvae are more susceptible to toxic substances than adults, the aim of this work was to study the effect of chlorinated aliphatic hydrocarbons on the fertilization process, egg development, hatching and larval survival of several species of salt-water fish.

Material and methods

Test material

The industrial wastes tested were byproducts from Norwegian vinylchloride production and were used partly as the crude waste and partly as a distillate of the product, omitting the tar fractions. The main components of both the crude waste and the distillate are: 1.2 dichloroethane, 1.2 dichloropropane and 1.2.3. trichloropropane.

Test concentrations were between 0.01 and 100 ppm, the actual concentrations due to the differences in solubility and volatility of the compounds were lower.

Analysis

The test solutions in concentrations up to 10 ppm were made up by dilution of stock solutions of about 10 or 20 ppm in 2 l amounts. Stock solutions for the continuos flow unit (200 l) were made up to a concentration of 50 ppm. Concentrations of 100 ppm were used only for a few tests and were made up directly by disolving the calculated amount of test material in fresh sea-water.

Stock solutions were regularly analysed for actual concentrations of the different components by use of a Perkin Elmer 900 Gas Chromatograph with ECD detector (Jensen and Palmork, in prep., and Jensen et al., in prep).

Test organisms

Eggs and larvae of plaice (Pleuronectes platessa L.), cod (Gadus morhua L.) and herring (Clupea harengus L.) were used as test organisms. The parent fishes were all from the Bergen area of the Norwegian west coast.

Fertilization

Fertilization was carried out in 1000 ml beakers containing 800 ml of the test solutions. The eggs were taken dry into small beakers or suspended into small amounts of fresh sea-water. They were then equally distributed into the beakers containing the test solutions. Spermatozoa were pressed out into 50 ml of fresh sea-water of 4°C. The emulsions were quickly stirred and about 1 ml added to each beaker of test solution followed by immediate agitation for 10 - 15 seconds. The beakers were then placed in water baths of fairly constant temperature (4 - 5°C). The fertilization percentages were calculated after 24 hours incubation by counting representative egg samples.

Development and survival

Development of eggs and larval survival were followed according to two different procedures.

1. The developing eggs were held in the 1000 ml beakers kept in water baths changing 600 ml of the 800 ml test solution to begin with every, later on every second day.

2. From the beakers where the fertilization had taken place, the eggs were placed in specially constructed aquaria permitting a gentle continuous flow of water kept on constant toxicity levels by means of a precision dosage pump. A rather similar continuous flow system with constant toxicity levels is described by Swedmark et al. (1971).

Under both systems dead eggs were counted and removed and the development stage of the eggs was noted daily. For plaice eggs the development stages were described according to the schema of von Westernhagen (1970) and for cod eggs according to Rollefsen (1929). Survival of the larvae was recorded until absorption of the yolk sac.

Results

Fertilization per cents

Table 1. Fertilization percentages and total counts after 24 hours in the crude waste.
Plaice 3406 x plaice, eggs dry, not washed, 10 drops of spermatozoa.

C_1		C_2		0.01 ppm		0.1 ppm		1 ppm		10 ppm	
F %	T	F %	T	F %	T	F %	T	F %	T	F %	T
94	64	92	61	92	54	91	57	92	52	92	66

Table 2. Fertilization percentages and total counts after 24 hours in the distillate.
Plaice 3349 x plaice 3338, eggs washed, only floating eggs used.
10 - 15 drops of spermatozoa, temperature 5°C. Temperature of test solutions: 100 ppm: 7°C, 50 ppm: 9°C, 10 ppm and controls: 8°C.

C		10 ppm		50 ppm		100 ppm	
F %	T	F %	T	F %	T	F %	T
26	85	21	108	18	84	32	74

Table 3. Fertilization percentages and total counts after 24 hours in the distillate.
Cod x cod, eggs washed, only floating eggs used, great excess of spermatozoa in emulsion to each test concentration. Temperature in test solutions 5 - 6°C.

C		0.1 ppm		0.5 ppm		1 ppm	
F %	T	F %	T	F %	T	F %	T
64	90	67	93	63	101	65	95

Table 4. Fertilization percentages and total counts after 24 hours in the distillate.
Herring x herring, eggs pressed out on to plastic slides of about 5 x 6 cm. As the herring eggs are demersal the previous techniques had to be changed a little. 1 drop of spermatozoa was added to each test solution, stirred and then the slides with eggs were placed in the beakers. After about one min. 1 drop of spermatozoa from another male was added to each beaker followed by stirring. Temperature in test solutions 5 - 6°C. Eggs for estimating fertilization percentages were taken only from parts of the slides where the eggs seemed to be placed in a single layer.

C		0.1 ppm		1 ppm		2.5 ppm		10 ppm	
F %	T	F %	T	F %	T	F %	T	F %	T
74	39	92	87	75	99	91	74	83	40

Development and survival

Test 1.

Plaice eggs and larvae held in 1000 ml beakers in water bath of fairly constant temperature. The fertilization percentages for this assay are the ones given in table 1. Test material the crude waste, test concentrations 0.01, 0.1, 1 and 10 ppm, 2 controls.

Fig. 1a gives the variations in the concentrations of the three main components of the stock solutions used to make up the test concentrations during the assay. The upper graph on fig. 1 gives the variations in the temperatures of the water bath during the same period. The peak of 10.2°C on the 10th day due to a failure in the cooling water system, did not seem to have any influence on the developing eggs.

Table 5 gives the daily count of the dead eggs and larvae, the number of eggs and larvae examined for development stages (in the period of both eggs and larvae, the count of larvae in brackets), and the development stages of the eggs in one of the controls and the test solution of 10 ppm. The other concentrations tested gave quite similar results. Stage 4_3, the last stage before hatching, seemed to be reached by most

eggs after 14 days. Hatching began on the 15th day, but even if stage 4β was reached, hatching was partly delayed. The last eggs hatched in 10 ppm after 27 days. This spreading of hatching of evidently "ripe" eggs was almost the same for all test concentrations.

Table 5. Daily count of dead eggs and larvae, number of eggs and larvae examined for development stages and development stages of the eggs in one of the controls and the test solution of 10 ppm.

	C			10 ppm		
day	dead	exam.	dev. stage	dead	exam.	dev. stage
0						
1	85			145		
2	16	20	1aβ	24	14	1aβ
3	21	9	1bα	43	3	1bα
4	52	3	1bγ - 2α	46	32	1bγ - 2α
5	39	8	2β	18	10	2β
6	8	5	2γ - δ	14	13	2γ - δ
7	3		2γ - γ	27		2α - 2γ
8	9	14	3α - β	32	21	3α - β
9	37	9	3β	1	17	3β
10	69	7		8	13	
11	46	7	3γ	11	35	3γ
12	30	7	3γ	12	18	3γ
13	26	9	4γ	33	17	4γ
14	32	11	4β	18	20	4β
15	23	8		22	14	
16	21	9		24	14	
17	2	4		(1) 24	(1) 11	
18	(1) 10	15		(4) 15	(2) 16	
19	(4) 22	7		(4) 21	(4) 6	
20	(10) 38	(3) 15		(20) 55	(5) 12	
21	(4) 10	(2) 5		(7) 21	(4) 14	
22	(1) 2	(4) 10		(23) 31	(8) 9	
23		(2) 4		(21) 36	10	
24	8			20		
25	5			16		
26	4			15		
27	2			13		
28	1	2		7	6	
29		4		17	2	
30	2	2		7		
31				7		
32				4	1	
33		1		5	4	
34		57			215	

Table 6. Total number of dead eggs, total number of eggs investigated and mortality percentages of eggs during the entire experiment. Eggs removed for examination of the development stages were not put back into the beakers.

	C_1	C_2	0.01 ppm.	0.1 ppm.	1 ppm.	10 ppm.
Dead eggs	698	601	674	839	711	601
Total	865	756	935	1066	893	896
Mortal. %	80.7	79.5	72.1	78.7	79.6	67.1

Table 7. Number of dead larvae, total amount of larvae and mortality percentages of larvae in the different concentrations.

	C_1	C_2	0.01 ppm.	0.1 ppm.	1 ppm.	10 ppm.
Dead larv.	7	42	104	92	53	191
Total	70	119	229	203	261	443
Mortal. %	10.0	35.3	45.4	45.3	20.3	43.1

Test 2.

Plaice eggs held in 1000 ml beakers in water bath of 3.5 - 5°C in high test concentrations, 100, 50 and 10 ppm of the distillate, and in continuous flow aquaria of about the same temperature range in low test concentrations, 1, 0.5 and 0.1 ppm and control. The fertilization percentages of this experiment are given in table 2.

The development of the eggs was followed to the hatching stage (VC) and the results are given in fig. 2. Due to an over-flow most eggs in the 1 ppm solution were lost after 5 days.

Test 3.

Cod eggs and larvae held in continuous flow aquaria in a temperature range of 4.5 - 6.5°C in the distillate in concentrations 1, 0.5 and 0.1 ppm, with 1 control. A rather large amount of eggs were tested as each water bath contained three test aquaria with the same test concentration.

Table 8. Development stages of the eggs in 1 ppm and the control. The two lower concentrations gave exactly the same picture.

Day	C		1 ppm	
1				
2				
3	13	B 3 - 4	11	B 3 - 4
4	9	D 1	10	D 1
5	9	D 2 - 3	23	D 2 - 3
6	21	E 2	16	E 2
7	24	E 4	12	E 4
8	16	E 4 - F 1	23	E 4 - F 1
9	54	F 1 - 2	31	F 1 - 2
10	25	F 3 - 4	28	F 3 - 4

Rollefsen (1929) records the development of cod eggs to stage F 4, in this experiment reached on the 10th day after fertilization. The hatching started on the 15th day and all eggs were hatched the 19th day.

Table 9. The number of dead eggs and larvae, total number of eggs and the mortality per cents in one aquaria of each test concentration in the experiment.

	C	0.1 ppm	0.5 ppm	1 ppm
Dead	652	381	387	631
Total	2127	1706	2093	2550
Mortal. %	30.7	22.3	18.5	24.7

ICES, C.M. 1971/E:11 deals with the acute toxicity of vinylchloride production byproducts on adult Gadidae. As it is normally believed that adults are less susceptible to toxic materials than are eggs and larvae, an acute toxicity test on newly hatched cod larvae was made. Test concentrations were 100, 50 and 10 ppm of the distillate with 1 control. 400 ml of the test solutions (changed every second day) in 1000 ml beakers, containing 30, 38, 36 and 32 larvae respectively, were used. Table 10 shows the mortality percentages of these larvae.

Table 10. Mortality percentages of cod larvae in the distillate.

Time	C	10 ppm	50 ppm	100 ppm
1 hr				0
18 hrs	0		0	10
24 hrs	0	17	5	13
48 hrs	0	17	5	13
96 hrs	0	17	74	93
5 days	0	17	89	93
6 days	0	17	92	93
8 days	25	25	97	100
11 days	100	89	100	

Test 4.

Herring eggs and larvae were held in 1000 ml beakers in water bath of 4 - 5°C. Fertilization percentages are listed in table 4. Test material the distillate, test concentrations 10, 2.5, 1 and 0.1 ppm, 1 control. Variations in the concentrations of the 3 main components of the stock solution are shown in Fig. 1 b.

As the herring eggs are demersal and were aggregated on plastic slides, it was not possible to follow the development stage by stage, as was done with the plaice and cod eggs. Only a few observations were taken: after 7 days control eggs and eggs in 10 ppm seemed to have reached the same developmental stage, the same was observed again after 13 days. Hatching began on the 21st day in all concentrations except in 10 ppm where the first hatching occurred after 23 days.

The hatched larvae were counted daily and transferred to beakers of fresh test solutions. The dead larvae were counted and the mortality percentages calculated.

Table 11. Count of live and dead larvae and the mortality percentages during the observation period.

	C	0.1 ppm	1 ppm	2.5 ppm	10 ppm
Dead larv.	15	43	3	16	27
Total	94	196	53	97	81
Mortal. %	16	21.9	5.7	16.5	33.3

Discussion and conclusion

Fertilization

The fertilization process is a critical stage in the life-cycle of fishes, spermatozoa being the more susceptible of the gamets. Their activity period is affected by the osmolarity of the water (Weisel 1948, Hines and Yashouw 1971), the temperature (Lindroth 1947) and the ionic composition (Elster and Mann, 1952).

In this work the effect of chlorinated aliphatic hydrocarbons on the fertilization of some marine teleost species was tested. The results given in tables 1 - 4 show clearly that the fertilization percentages in controls and different test concentrations within each experiment are almost identical. Between the four experiments, however, the success of fertilization shows marked differences. This is mainly the effect of using fish kept in captivity for long periods. It is always difficult to stipulate the best moment for stripping the eggs and if mature eggs remain in the ovary for some days, many will be unfertilizable. Solemdal (1970) found the fertilization percentages in flounders (Platichtys flessus L.) kept in captivity for long periods to vary between 12.6 and 91.5. In contrast running females stripped immediately after being caught, usually gave fertilization percentages between 90 and 100 (Holliday and Blaxter, 1970, von. Vesternhagen, 1971).

The four experiments must therefore be evaluated separately. On this basis it is concluded that by the techniques used the test concentrations including the high concentration of 100 ppm of the waste products, did not reveal any harm to the fertilization of plaice, cod and herring.

Development and survival

The four tests on egg development and larval survival in vinylchloride byproducts, gave very similar results. The results are in contrast to the general assumption that fish eggs and larvae are especially sensitive and more susceptible to toxic substances than are the adult animals (Marchetti 1965, Pickering 1966, Swedmark et al., 1971).

That development of the fish eggs is apparently not affected by concentrations up to 10 ppm of the vinylchloride byproducts tested (table 5, fig. 2) may be explained by assuming that the different components in the test solutions cannot penetrate the egg membranes (Blaxter, 1969).

The fact that marine fish eggs seem especially delicate until completion of gastrulation (Blaxter, 1969) may be the reason why development in plaice eggs in test concentrations of 100 and 50 ppm, were delayed and stopped at stage IIγ, one of the very late stages of gastrulation.

In test 1 development in controls and all test solutions were identical (table 5), but in 10 ppm hatching was several days delayed in proportion to the controls and lower concentrations. This may be due to the fact that from the beginning of the test, the 10 ppm solution was heavier populated than were the control beakers (1339 eggs against 935 and 875 in the controls). The low mortality percentage of larvae in control 1 of this test may have the same explanation (70 larvae against 443 in 10 ppm).

The pronounced lower mortality in the 1 ppm solutions in tests 1 and 4 might be the result of a certain bactericidal effect of the compounds tested. An investigation on this possibility is in progress.

In the present paper the term "larva" refers to the yolk-sac stage. The larvae were not fed and survived for about 15 days (cod). The long survival of the larvae in the high concentrations of the test solutions compared with the I and II group fish (saithe) (ICES, C.M. 1971/E:11) cannot be explained at the present moment. Also the two experiments are not wholy comparable as the test animals did not belong to the same, but to very nearly related species.

Stock solutions for the tests were calculated by weight per volume. It must be emphasized that later analysis showed actual test concentrations to be lower than the theoretical ones. ICES, C.M. 1971/E:10 gives the variation in calculated (theoretical) and analysed concentrations in experiments with similar solutions.

The conclusion based on the results obtained must be that under experimental conditions the byproducts from vinylchloride production up to concentrations of 10 ppm, are not as dangerous to fertilization, egg development and larval survival as first expected. However, the experiments were carried out over short intervals only, and the question of the consequenses of a long time exposure to low concentrations has yet to be investigated.

Abstract

The effect of byproducts from Norwegian vinylchloride production on fertilization, egg development and larval survival was tested. Eggs and larvae of plaice, cod and herring apparently seemed unaffected of concentrations up to 10 ppm, but concentrations of 100 and 50 ppm delay and stop development of plaice eggs in an early stage. Cod larvae seemed to survive high concentrations of the toxic substances for far longer periods than did I and II year group of saithe.

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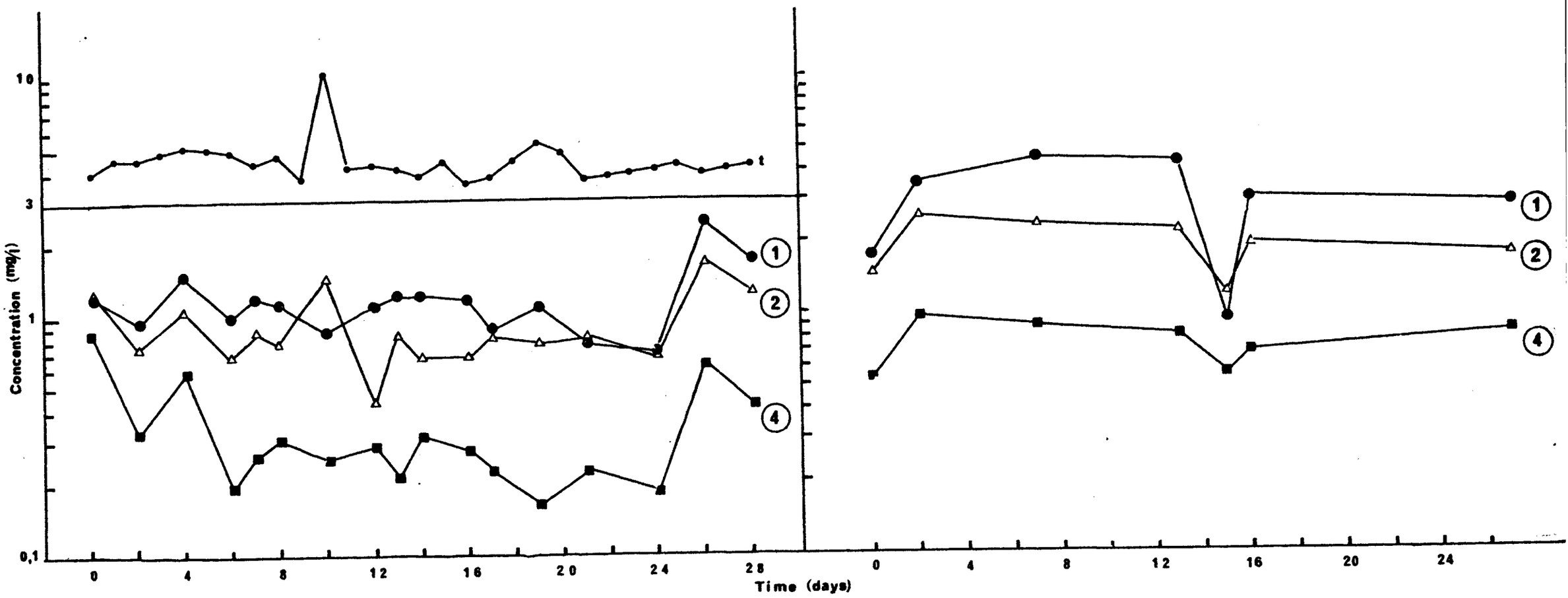

Fig. 1.

●——● 1.2. dichloroethane
△——△ 1.2. dichloropropane
■——■ 1.2.3. trichloropropane

a) The variations in the concentrations of the 3 main components of the stock solutions of the crude waste. The upper graph gives the temperature variations.

b) The variations in the concentrations of the 3 main components of the stock solutions of the distillate.

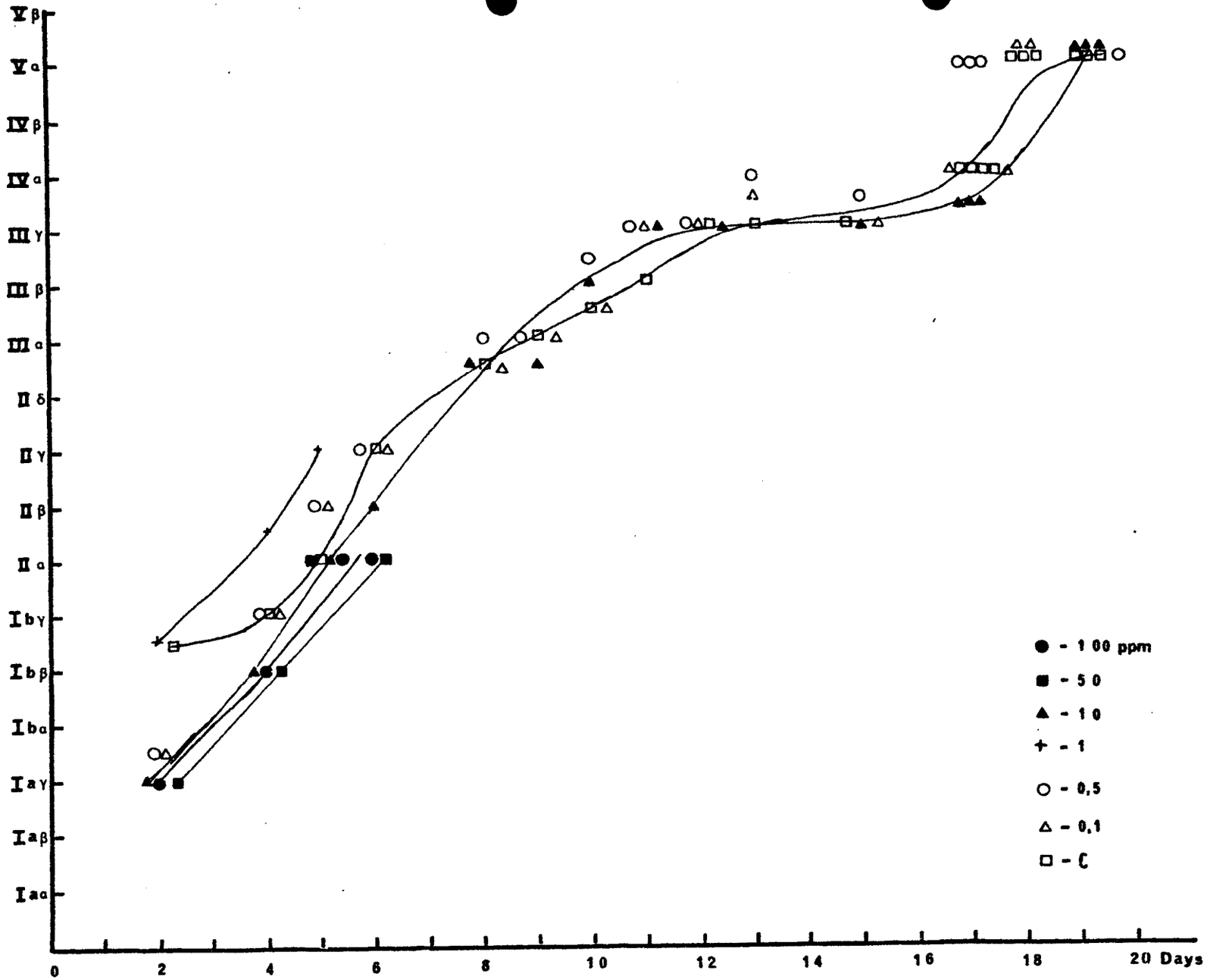

Fig. 2. The development of plaice eggs in different concentrations of the distillate.