

Combined effects of cadmium and salinity on development and survival of garpike eggs*

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KURZFASSUNG: Kombinierte Wirkungen von Cadmium und Salzgehalt auf Entwicklung und Überleben von Hornhechteiern. Laichreife Hornhechte (*Belone belone* L.) wurden im Frühjahr bei Travemünde (Ostsee) gefangen. Die Eier wurden in unkontaminiertem Seewasser von 15, 25 und 35 ‰ Salzgehalt und etwa 15° C befruchtet und nach 3 Std. in Cadmium-kontaminiertes Erbrütungswasser überführt. Neben den Kontrollversuchen wurden Erbrütungsexperimente mit folgenden Cadmiumkonzentrationen in den 3 Salzgehaltsstufen durchgeführt: 0,05; 0,1; 0,5; 1,0; 2,0 und 5,0 ppm Cd⁺⁺. Die Versuchsmedien wurden alle 2 Tage mit den 800 ml fassenden Inkubationsgefäßen gewechselt. Die Toxizität von Cadmium wurde im Hinblick auf folgende Kriterien bewertet: Embryoaktivität, Herzfrequenz, Überlebensrate bis zum Schlupf, lebensfähiger Schlupf. Die Herzschlagfrequenz und die Ventilationsbewegungen der embryonalen Pectoralflosse waren bei Cadmiumkonzentrationen über 0,5 ppm deutlich beeinflusst. Die Embryonalsterblichkeit war in den Versuchen bis zu einer Konzentration von 1,0 ppm Cd⁺⁺ nicht verschieden von den Kontrollen. In den höheren Cadmiumkonzentrationen (2,0 und 5,0 ppm) nahm die Überlebensrate bis zum Schlupf sowie der prozentuale Anteil des lebensfähigen Schlupfes mit steigendem Salzgehalt zu. Die Cadmiumaufnahme war deutlich konzentrationsabhängig, lag jedoch in der niedrigsten Salzgehaltsstufe am höchsten. Die unterschiedliche Aufnahme von Cadmium durch Hornhecht-, Herings- und Flundereier wird verglichen und ihre Ursache diskutiert.

INTRODUCTION

During the past years concentrations of heavy metals in certain coastal waters have risen to levels several times higher than those found in unpolluted waters, as determined by KRAUSKOPF (1956), GOLDBERG (1965), PRESTON (1973) and others. Concentrations of heavy metals as described by ABDULLAH et al. (1972), ABDULLAH and ROYLE (1974) and BUTTERWORTH et al. (1972) for the Bristol Channel and EUSTACE (1974) for the Derwent estuary in some instances were of one or two orders

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of magnitude higher than in unpolluted waters. Exceptionally high values for cadmium (0.05 ppm), copper (0.07 ppm) and lead (0.66 ppm) (up to 180 times that of natural waters) have been recorded in Hongkong waters (CHAN et al., 1974).

Elevated concentrations of the above mentioned magnitude are usually restricted to confined localities, largely associated with industrial development (PRESTON, 1973), indicating that today heavy metal pollution is essentially a problem of inshore waters. Among the heavy metals cadmium as a pollutant represents a real hazard to human health as is documented by the occurrence of the "itai-itai" disease in Japan caused by the ingestion of cadmium contaminated food and water (KOBAYASHI, 1971).

Numerous experiments have proved the acute and chronic toxicity of cadmium to marine animals such as annelids (BROWN & AHSANULLA, 1971), molluscs (CALABRESE et al., 1973), crustaceans (O'HARA, 1973a) and to fish eggs (ROSENTHAL & SPERLING, 1974; WESTERNHAGEN et al., 1974; WESTERNHAGEN & DETHLEFSEN, 1975). Toxicity of cadmium and its accumulation by test organisms was found to depend greatly on the salinity of the sea water in which the experiments were conducted. Detrimental effects of the metal on test organisms were usually less grave in water of high salinity than in a brackish medium.

Investigations on the susceptibility of eggs of inshore spawning teleosts (*Clupea harengus*, *Pleuronectes flesus*) showed that these eggs proved to be quite resistant to the toxic action of cadmium (ROSENTHAL & SPERLING, 1974; WESTERNHAGEN et al., 1974; WESTERNHAGEN & DETHLEFSEN, 1975), and that there existed species specific differences in the accumulation of cadmium from the sea water.

MATERIAL AND METHODS

Eggs of freshly caught garpike (*Belone belone* L.) from Travemünde (Baltic Sea) were artificially inseminated in unpolluted (15 ‰, 25 ‰ and 35 ‰ S) and cadmium polluted water (25 ‰ S) at a temperature of 14.7 °C. After three hours eggs fertilized in uncontaminated water were transferred into 800 ml incubating jars containing natural sea water of the above mentioned salinities (controls) and test solutions of 0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 ppm Cd⁺⁺ using CdCl₂ as toxicant (Table 1). Incubation temperature was 15 °C. The incubating jars were aereated through PVC air tubes with compressed CO₂-free air, assuring continuous circulation of the test solution.

Embryonic activity and heart beat, hatching rates and percentage viable hatch were determined. At defined ontogenetic stages eggs were removed and preserved for later cadmium determination. The numbers of eggs in each trial varied from 50 to 150. Because of expected high mortalities at 2.0 and 5.0 ppm Cd, these trials were stocked with 100 to 150 eggs each compared to 50 to 100 eggs at lower contamination levels. Water was renewed every two days. Dead eggs were counted daily.

Determination of cadmium in water samples and eggs was accomplished by means of flameless atomic absorption spectrophotometry using a Perkin Elmer Type 300 equipped with an electrodeless discharge lamp. For determination the samples were treated as described by WESTERNHAGEN & DETHLEFSEN (1975).

Table 1

Working levels of cadmium contamination maintained throughout the experiment. * additional trial in 25 ‰ S, eggs fertilized and incubated in cadmium contaminated water. n = number of determinations; x = mean; s = standard deviation. Temperature range: 14.99 – 15.11°C; pH range: 7.93 – 8.21

Experimental design		Working levels					
Salinity (‰)	Cd concentrations intended (ppm)	Salinity (‰)			Cd concentrations determined (ppm)		
		n	x	s	n	x	s
15	Control	26	15.11	0.44	14	0.027	0.027
	0.05	28	15.12	0.39	15	0.062	0.021
	0.1	26	15.15	0.44	14	0.124	0.040
	0.5	28	15.15	0.42	15	0.573	0.143
	1.0	28	15.19	0.41	15	1.034	0.167
	2.0	26	15.21	0.41	13	1.991	0.358
	5.0	12	15.53	0.19	7	4.624	0.202
25	Control	30	25.10	0.13	16	0.023	0.027
	0.05	30	25.13	0.11	16	0.076	0.038
	0.1	30	25.16	0.17	16	0.130	0.045
	0.5	30	25.21	0.31	16	0.613	0.134
	1.0	28	25.19	0.16	15	1.055	0.171
	2.0	28	25.19	0.17	15	2.004	0.253
	5.0	26	25.15	0.26	14	4.308	0.794
25*	0.05	28	25.14	0.15	15	0.066	0.029
	0.1	30	25.16	0.13	15	0.130	0.045
	0.5	24	25.18	0.16	13	0.548	0.119
	1.0	26	25.17	0.24	13	1.074	0.374
	2.0	26	25.12	0.14	13	1.888	0.319
	5.0	26	25.14	0.13	14	4.270	0.719
35	Control	29	34.73	0.34	15	0.035	0.030
	0.05	29	34.78	0.18	16	0.088	0.029
	0.1	28	34.81	0.21	15	0.129	0.038
	0.5	28	34.81	0.21	15	0.581	0.167
	1.0	29	34.80	0.18	16	0.952	0.184
	2.0	29	34.77	0.17	16	1.860	0.290
	5.0	26	34.74	0.17	14	3.988	0.789

RESULTS

Embryonic activity

Contractions of the heart were first detectable in embryos 8 days old and at that time, except for the 5.0 ppm trials, heart beat did not appear to be affected by the toxicant (Fig. 1). At 5.0 ppm contraction frequency tended to be lower than in the other trials containing less cadmium. With advancing development, differences in heart beat rate at different cadmium contamination levels became more pronounced. This was especially evident at 15 ‰ S where 13 day old embryos failed to show any heart

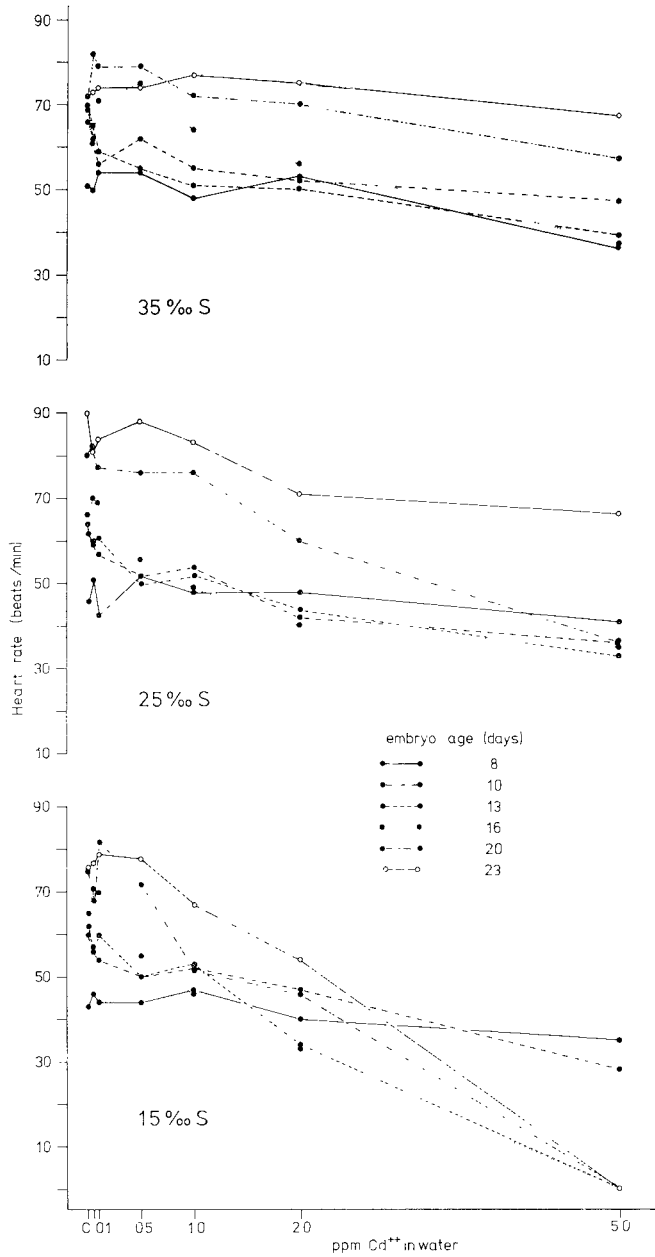


Fig. 1: *Belone belone*. Heart rate of embryos incubated in cadmium contaminated water of different salinities at 15°C. Each point represents the mean of 10 measurements

contractions at 5.0 ppm and contraction frequency at 1.0 and 2.0 ppm was much depressed compared to trials with less cadmium. Although not as pronounced as in the 15 ‰ S trial, this phenomenon was also detectible at 25 ‰ and 35 ‰ S. 20 day old embryos exhibiting more than 80 heart contractions/min in the controls displayed a heart beat frequency of only 60 and 36 at 2.0 and 5.0 ppm. At 35 ‰ S inhibiting

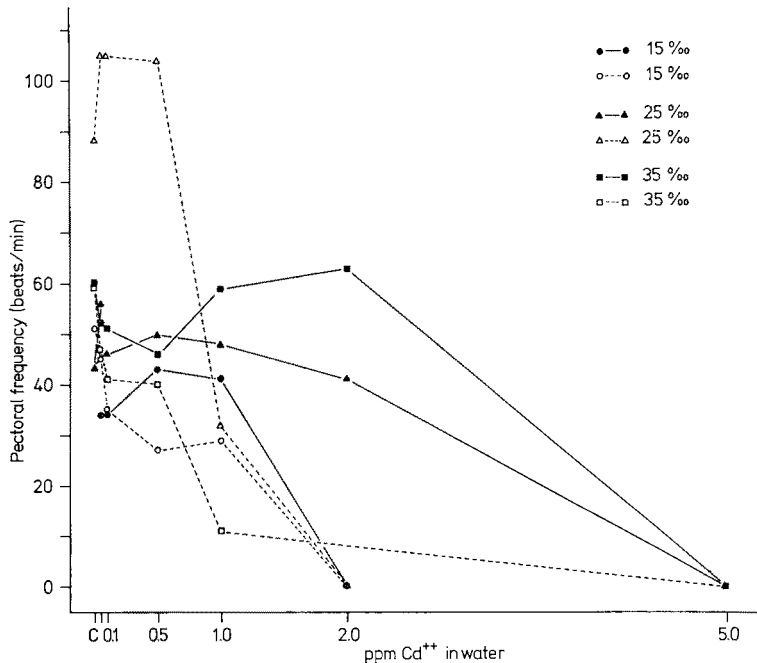


Fig. 2: *Belone belone*. Frequency of pectoral fin movements of advanced embryos (solid lines: 21 days old; broken lines: 24 days old) incubated in different salinity-cadmium concentrations. Incubation temperature 15° C. Each point represents the mean of 10 measurements

effects of cadmium on the heart beat frequency of embryos were least severe and although there was still distinct depression of heart activity at 2.0 and 5.0 ppm, effects were less pronounced than in trials at 15 ‰ and 25 ‰ S.

At a certain stage advanced garpike embryos start to circulate the perivitelline fluid in their egg casings (ROSENTHAL & FONDS, 1973; WESTERNHAGEN, 1974) by means of their pectoral fins. Figure 2 shows effects of cadmium on the pectoral fin activity of 21 and 24 day old prelarvae. As was true for heart activity, pectoral fin activity was completely depressed (at 5.0 ppm) or greatly reduced in cadmium contaminated water. This was particularly evident in the 25 ‰ S trials 24 days after fertilization, in which only at low contamination levels (up to 0.5 ppm) could high activities be recorded. At 1.0 ppm pectoral fin activity was much reduced and was wanting at 2.0 and 5.0 ppm.

Embryonic survival until hatching

Survival of eggs at cadmium concentrations up to 1.0 ppm was comparable to that in the controls (Fig. 3a). It was only in that 25 ‰ S trial in which eggs had been fertilized in cadmium contaminated water that survival was poor (maximum at 0.05 ppm; 38 ‰) even at lower contamination levels.

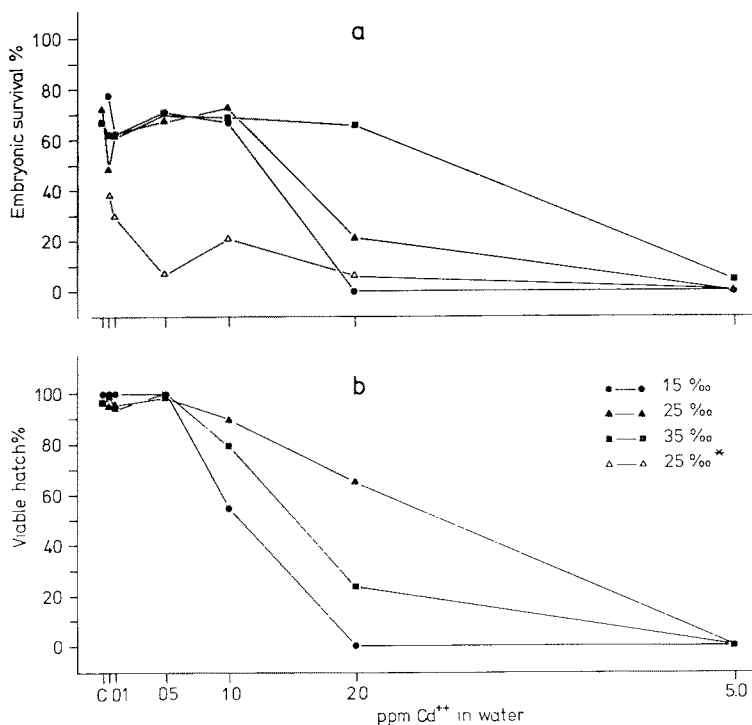


Fig. 3: *Belone belone* eggs incubated at different salinity-cadmium concentrations. (a) Rates of survival (% fertilized eggs) until hatching, (b) viable hatch (% total hatch). Incubation temperature 15°C. * Eggs fertilized and incubated in Cd contaminated water

At 2.0 ppm embryonic survival was zero at 15 ‰ S, 20 ‰ at 25 ‰ S and above 60 ‰ at 35 ‰ S. The 5.0 ppm trials did not yield any larvae at 15 ‰ and 25 ‰ S, at 35 ‰ S a small percentage (4.5 ‰) of larvae hatched, showing the deleterious influence of cadmium on the embryos being stronger in diluted than in normal sea water.

Viable hatch

Viable hatch proved to be a finer indication for the toxicity of cadmium than embryonic survival (Fig. 3b). It was only in cadmium concentrations of up to 0.5 ppm that viable hatch was comparable to that of the controls (94 ‰ to 100 ‰). At 1.0 ppm

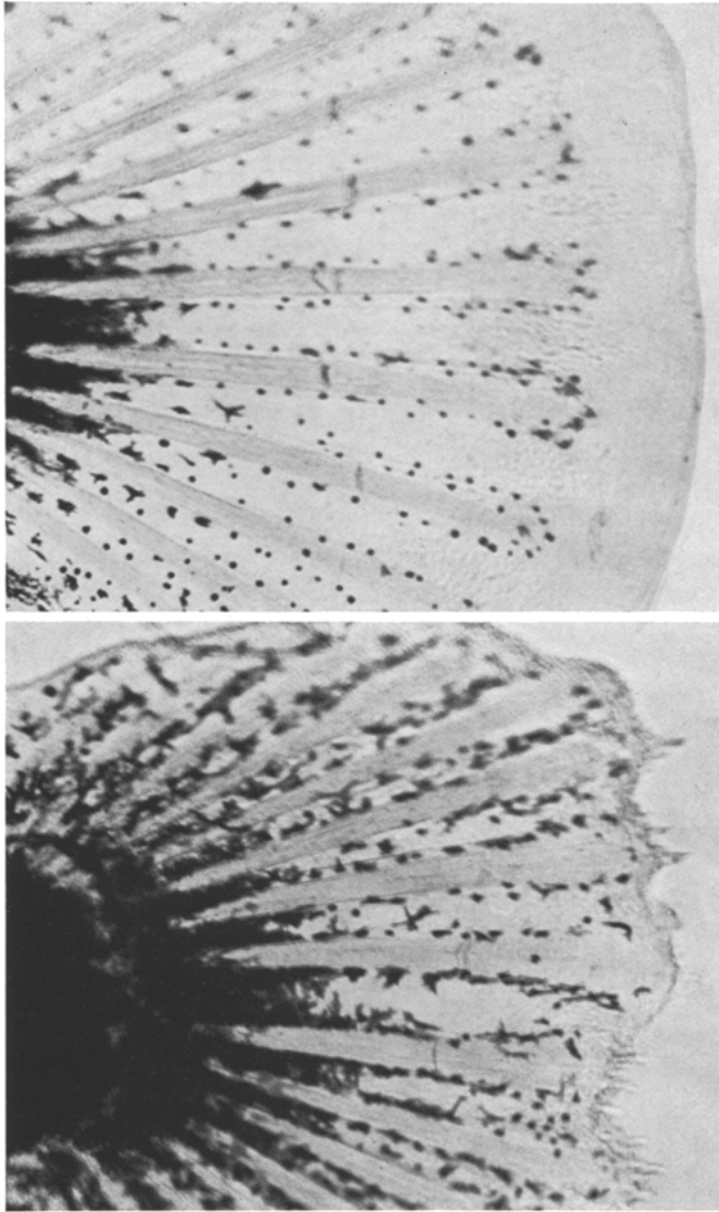


Fig. 4: *Belone belone*. Serrated caudal fin of a newly hatched larva incubated in cadmium contaminated sea water (bottom) as compared to the undamaged primordial fin of a control specimen (top)

percentage viable hatch dropped considerably. Newly hatched larvae exhibited serrated or frayed primordial and caudal fins (Fig. 4) not commonly found in control individuals. At 2.0 ppm no viable larvae were obtained at 15 ‰ S. In none of the salinities tested did at 5.0 ppm cadmium contamination hatch any viable larvae. The few larvae derived from the 35 ‰ S trial were all crippled.

Cadmium uptake during embryonic development

Amount of cadmium accumulated by garpike eggs during embryogenesis under exposure to cadmium ions is shown in Figure 5. Initial cadmium uptake after exposure had to be fairly rapid, because highest metal concentrations in eggs were, except for a few trials, recorded at the first day after fertilization. Prolonged exposure did not lead to increasing cadmium content in eggs. In fact cadmium content in eggs decreased steadily throughout the incubation period, reaching lower values after 24 days than those recorded on the first day.

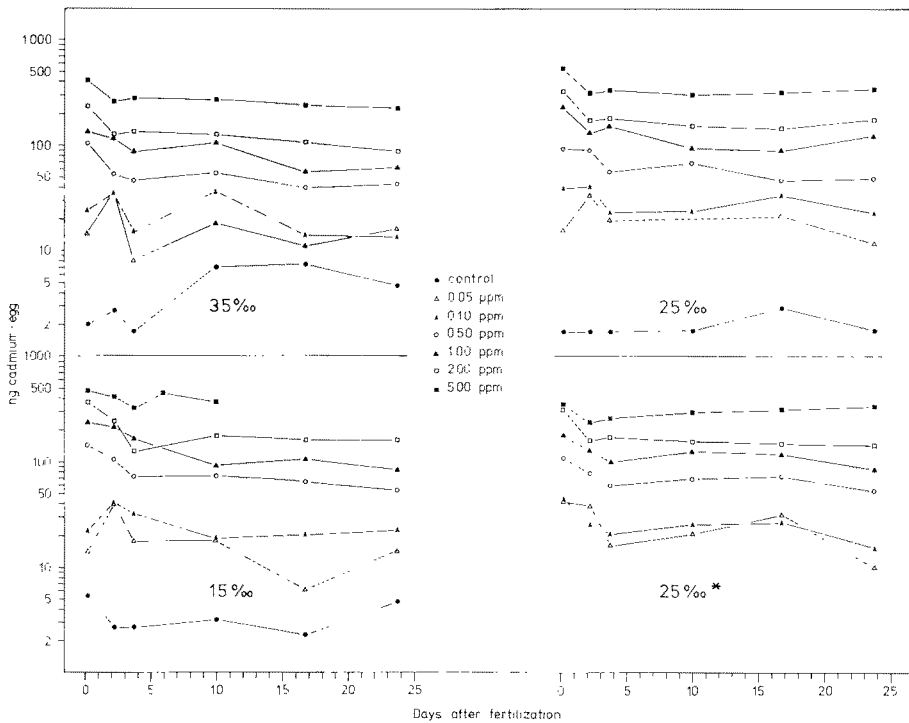


Fig. 5: *Belone belone*. Mean cadmium content of eggs during the course of incubation period in relation to cadmium concentration and salinity of the incubation medium. Incubation temperature 15°C. * Eggs fertilized and incubated in cadmium contaminated water. Each point is based on 5 determinations. Broken line: one value missing

Maximum levels attained depended on the initial concentrations employed. Accumulation was higher at 15 ‰ S (Fig. 5) than at 25 ‰ and 35 ‰ S. No differences in contamination levels could be detected between the two 25 ‰ S series, the eggs in one

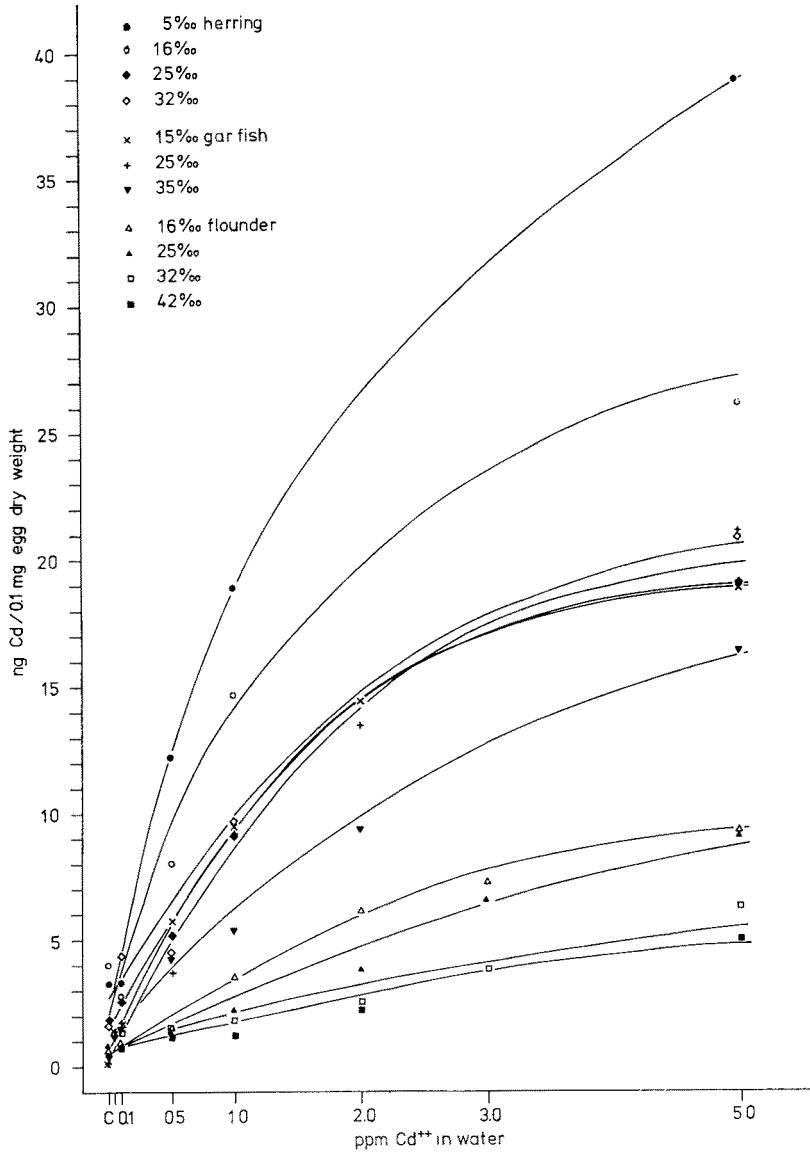


Fig. 6: Accumulation of cadmium in demersal (*Clupea harengus*, *Belone belone*) and pelagic (*Pleuronectes flesus*) marine fish eggs in relation to cadmium concentration and salinity of the incubation medium

of which had been fertilized in contaminated water, while the eggs of the other series were fertilized in an uncontaminated medium.

Newly hatched larvae at all cadmium-salinity combinations contained only small amounts of cadmium.

In Figure 6 maximum amounts of cadmium found in garpike eggs are compared on a 0.1 mg dry weight basis with values recorded from herring (WESTERNHAGEN et al., 1974) and flounder eggs (WESTERNHAGEN & DETHLEFSEN, 1975). Herring eggs appear to accumulate slightly more cadmium than do garpike eggs incubated at respective salinities. Both egg types show much higher accumulation values than are found in flounder eggs.

DISCUSSION

The experiments indicate that toxic action of cadmium on the developing eggs of garpike (*Belone belone* L.) is dependent on the salinity of the incubating medium. Embryonic activity as well as embryonic survival and viable hatch were most adversely affected by cadmium ions during exposure of eggs in diluted sea water. There was a high-low relationship between detrimental effects of the metal and salinity of the incubating medium. The same results have been found by WESTERNHAGEN et al. (1974) when testing the reaction of developing herring eggs to cadmium ions.

They assumed an interaction between Cd^{++} and Ca^{++} ions, which in diluted sea water with less Ca^{++} ions would lead to relatively higher amounts of the toxic Cd^{++} to be incorporated into the egg shell (chorion). The apparent interaction between Cd^{++} and Ca^{++} ions had been pointed out previously by KOBAYASHI (1971) and MALJKOVIC & BRANICA (1971). On the same basis the extremely high cadmium susceptibility of fresh water teleosts described by PICKERING & HENDERSON (1966), BALL (1967) and CEARLEY & COLEMAN (1974) can be explained: freshwater having a much lower content of Ca^{++} ions than sea water.

Several other authors working with heavy metals in sea water, for example OLSON & HARREL (1973) could show that the acute toxicity of mercury, copper and chromium on the bivalve *Rangia cuneata* increased with decreasing salinity and the same was stated by ROESIJADI et al. (1974) working on the toxic action of mercury on the porcelain crab *Petrolisthes armatus*. In contrast to these findings WESTERNHAGEN & DETHLEFSEN (1975) were not able to show a relationship between toxicity of cadmium and salinity of the incubating medium when working with the eggs of flounder, *Pleuronectes flesus*, which at all salinities were distinctly less susceptible to the toxic action of cadmium than were herring or garpike eggs under the same conditions. At all salinities tested (16‰–42‰ S) there was considerable viable hatch (18‰ at 42‰ S) of flounder larvae.

A possible explanation for the different reactions of demersal (herring, garpike) and pelagic (flounder) eggs is given when looking at Figure 6. Eggs incubated at low salinities have accumulated more cadmium than when kept at comparable contamination levels at high salinities. This phenomenon is known for crustaceans exposed to cadmium under various salinity conditions as could be shown by O'HARA (1973b)

working with *Uca pugilator* and HUTCHESON (1974) investigating cadmium uptake by *Callinectes sapidus*.

Cadmium contamination of herring and garpike eggs as shown in Figure 6 is several times higher than that of flounder eggs incubated under the same toxicant conditions. Maximum values for flounder eggs depending on the salinity of the incubating medium (16 ‰–32 ‰), range from 6 to 9 ng Cd/0.1 mg egg dry weight. It should be considered though that incubation temperatures were not the same for all three species.

Table 2

Surface to volume ratio of fish eggs: *Belone belone*, *Pleuronectes flesus*, *Clupea harengus*

Species	Salinity (‰)	Surface to volume ratio of fish eggs (mm ⁻¹)
Garpike	15	1.81
	25	1.91
	35	1.85
Flounder	16	5.47
Herring	5	4.40
	16	4.58
	25	4.71
	32	5.24

Maximum contamination levels for herring eggs for the same salinity range vary from 19 to 26 ng Cd/0.1 mg egg dry weight, and garpike eggs show similar values between 16.5 and 21 ng Cd/0.1 mg egg dry weight. Slightly higher values for herring eggs (at 15 ‰ and 35 ‰ S) are probably due to the higher surface/volume ratio of these eggs (Table 2), herring eggs having a diameter of about 1 mm, while garpike eggs are much bigger (diameter approx. 3 mm).

Although the surface/volume ratio of flounder eggs is similar to that of herring eggs (Table 2) cadmium content of the pelagic flounder eggs is much lower than would be expected from these data. Thus uptake and accumulation of cadmium can only to a certain extent depend on the surface/volume ratio, but appears to be directly related to physical and chemical properties of the egg membrane which actually is the site of cadmium uptake (ROSENTHAL & SPERLING, 1974; WESTERNHAGEN et al., 1974). As evident from Figure 5 uptake was completed shortly after exposure, which suggests binding of cadmium to a complexing agent, the capacity of which at a certain cadmium concentration is set, otherwise further accumulation throughout the experiment would be expected.

The thick chorion of herring (~ 16 µ; BRAUM, personal communication) and garpike eggs (20–25 µ) provide more material and complexing agent to accumulate cadmium than flounder eggs with their thin egg membrane (~ 1.8 µ; LÖNNING & SOLEM-DAHL, 1971). As mentioned by WESTERNHAGEN & DETHLEFSEN (1975) an additional capacity for cadmium accumulation by herring eggs appears to be the adhesive covering the surface of these eggs. Table 3 presents accumulation values of garpike, her-

Table 3

Cd-accumulation by fish eggs incubated at different cadmium-salinity concentrations

Species	Salinity (‰)	Intended Cd-concentration (ppm)	Egg volume mm ³	ng Cd in water body with egg volume	ng Cd/egg (max. values)	Accumulation factors
Garpike	15	C	19.16	—	4.8	—
		0.05		0.958	39.0	40.7
		0.1		1.916	40.8	21.29
		0.5		9.580	145.9	15.23
		1.0		19.160	238.0	12.42
		2.0		38.320	363.0	9.47
		5.0		95.800	473.0	4.94
	25	C	16.21	—	2.9	—
		0.05		0.810	34.3	42.35
		0.1		1.621	41.5	25.60
		0.5		8.105	95.0	11.72
		1.0		16.210	231.0	14.25
		2.0		32.420	338.0	10.46
		5.0		81.050	529.0	6.53
	35	C	17.81	—	7.5	—
		0.05		0.890	36.1	40.56
		0.1		1.781	36.0	20.21
		0.5		8.905	106.0	11.90
		1.0		17.810	136.0	7.64
		2.0		35.620	237.0	6.65
		5.0		89.050	412.0	4.63
Herring	5	C	1.323	—	4.8	—
		0.1		0.132	6.0	45.45
		0.5		0.661	18.7	28.29
		1.0		1.323	27.9	21.09
		5.0		6.615	56.4	8.53
	16	C	1.177	—	5.5	—
		0.1		0.117	4.4	37.61
		0.5		0.588	11.6	19.72
		1.0		1.177	21.2	18.01
		5.0		5.885	38.6	6.56
	25	C	1.078	—	1.7	—
		0.1		0.108	3.3	30.57
		0.5		0.539	8.4	15.58
		1.0		1.078	13.7	12.71
		5.0		5.390	21.5	5.10
32	C	0.780	—	1.7	—	
	0.1		0.078	4.6*	58.9*	
	0.5		0.390	5.2	13.33	
	1.0		0.780	10.7	13.72	
	5.0		3.900	23.3	5.97	
Flounder	16	C	0.690	—	0.079	—
		0.1		0.069	0.252	3.65
		0.5		0.345	0.504	1.46
		1.0		0.690	1.270	1.84
		2.0		1.380	2.100	1.52
		3.0		2.070	2.536	1.23
		5.0		3.450	3.300	0.96

* Extremely high value; probably due to contamination.

ring and flounder eggs incubated under different salinity-cadmium concentrations. The accumulation factor is calculated by dividing the amount of cadmium found in one egg by the theoretical amount of cadmium in a water body having the volume of one egg at the respective salinity. Thus garpike eggs incubated at 15 ‰ S and 0.05 ppm cadmium contained 40.7 times more cadmium than is present in a water body with the volume of one egg (19.16 mm³).

The high accumulation factor of garpike eggs (> 20) incubated at 0.1 ppm Cd⁺⁺ is even surpassed by herring eggs (30.57–45.45). As suggested by WESTERNHAGEN & DETHLEFSEN (1975), this is probably due to complex forming mucopolysaccharides on the sticky egg surface. From Table 3 it becomes apparent that there is only considerable accumulation of cadmium in the demersal egg of garpike and herring. Flounder eggs take up only very minute amounts of cadmium.

In all three species there exists the same general pattern for the uptake of cadmium from surrounding water. The lower the ambient metal concentration, the higher the accumulation factors (Table 3). At 5.0 ppm cadmium concentration in flounder eggs was not higher than in the surrounding medium.

From the above it becomes conceivable to understand the mechanism of cadmium accumulation in fish eggs as binding of the metal to one or several agents the capacity of which is limited. Thus high ambient cadmium concentrations, although leading to high absolute contamination, cause overloading of binding capacities on the egg surface, giving rise to relatively low accumulation factors.

The combined results of the present and the foregoing studies of ROSENTHAL & SPERLING (1974), WESTERNHAGEN et al. (1974) and WESTERNHAGEN & DETHLEFSEN (1975) conducted on marine fish eggs exposed to cadmium enable us to understand the mechanism of cadmium accumulation by fish eggs as a binding of the metal to one or several agents on or inside the chorion. Highest cadmium concentrations were always reached shortly after exposure. Further exposure did not lead to higher accumulation values, suggesting that the binding capacity of the chorion is limited.

The chorion of fish eggs with its binding agents acts as a protecting barrier that prevents cadmium from entering the egg proper. Thick egg membranes as found in herring and garpike eggs accumulate up to 10 times more cadmium than the thin chorion of flounder eggs. Thus at high ambient cadmium concentrations seepage of metal through the metal overloaded egg membrane, a mechanism suggested by DANIELLI & DAVIES (1951) for the reaction of metals on cell surfaces, and consequent intoxication of the embryo is more likely in fish eggs with thick egg membranes. This would explain the relatively low susceptibility of flounder eggs to the toxic action of cadmium as described by WESTERNHAGEN & DETHLEFSEN (1975) compared to reactions of herring and garpike eggs under the same conditions.

SUMMARY

1. Eggs of Baltic Sea garpike (*Belone belone* L.) were incubated in normal and cadmium contaminated sea water (0.05, 0.1, 0.5, 1.0, 2.0, 5.0 ppm) at 15 ‰, 25 ‰ and 35 ‰ S and 15 ° C.

2. Embryonic heart beat and frequency of pectoral fin movements in prelarvae was found to be greatly depressed by cadmium concentrations of more than 0.5 ppm.
3. Embryonic survival and viable hatch were unaffected in cadmium concentrations of 1.0 and 0.5 ppm, respectively. At high cadmium concentrations (2.0 and 5.0 ppm) there were greater embryonic survival and viable hatch at high than at low salinities.
4. Cadmium content of eggs was found to be generally higher in lower salinities than in more saline water at comparable cadmium concentrations.
5. Accumulation factors of cadmium in eggs were inversely proportional to the ambient cadmium concentration. Highest accumulation factors of more than 40 were recorded at 0.05 ppm Cd. At high cadmium concentrations accumulation factors were only around 5.

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