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Combined effects of cadmium, copper and lead on developing herring eggs and larvae

H. v. WESTERNHAGEN[1], V. DETHLEFSEN[2] & H. ROSENTHAL[1]

[1] *Biologische Anstalt Helgoland (Zentrale); D-2000 Hamburg 50, Federal Republic of Germany, and*
[2] *Bundesforschungsanstalt für Fischerei, Toxikologisches Laboratorium Cuxhaven; Niedersachsenstraße, D-2190 Cuxhaven, Federal Republic of Germany*

ABSTRACT: Eggs of Baltic herring were incubated (10 °C; 16 ‰ S) in sea water containing mixtures of Cd, Cu and Pb at concentrations of 0.56 – 5.0, 0.0167– 0.15, 0.56–5.0 mg metal/l; embryonic survival until hatching, viable hatch and uptake of metals by embryos and early larvae were measured. Negative effects of metals on embryonic survival and viable hatch were additive in the case of Cu and Cd. Pb did not exert detrimental effects. Uptake of metals with exposure time was non-linear in eggs and linear in larvae. Total uptake of Cu and Cd by eggs was subjected to antagonistic or synergistic action of the other two metals present. Accumulation of Pb by eggs was enhanced when Cu was also present.

INTRODUCTION

The effects of heavy metals on aquatic organisms have been the subject of numerous investigations. In most biotests or accumulation studies metals have been used singly rather than in combination with other variables such as salinity and temperature or other pollutants. From the relatively limited number of studies available on the combined effects of metals on aquatic biota it appears that there are three possible reactions when two or more metals are experimentally combined: (1) Combination of toxic metals with other cations may cause mitigated effects and inhibited uptake, as compared to the action of the metal alone. This may be the case when Pb and Cd are used in combination with Ca (Erichsen Jones, 1937; Kinkade & Erdman, 1975; Wright, 1977) or Zn is used in combination with Cd (Rosenthal & Sperling, 1974). (2) The actions of two metals combined may be merely additive, as described by Lloyd (1961) for Zn and Cu and Brown & Dalton (1970) for Zn, Cu and Ni effects on the rainbow trout. (3) The action of metals may be synergistic as described by Barnes & Stanbury (1949) and Corner & Sparrow (1956) for Cu and Hg effects in crustaceans, and by Eisler & Gardner (1973) for the effect of Cd- Cu-Zn mixtures on *Fundulus heteroclitus.*

The combined effects of metals on experimental animals are not always as clear as might appear from the above presentation. In many cases metals show less than additive

effects at low concentrations and above certain concentrations or a certain temperature their action becomes additive (Thorp & Lake, 1974; MacInnes & Calabrese, 1978). Some metals may act additively at low and synergistically at high concentrations (Lloyd, 1961).

Thus it appears that several factors are able to influence effects of toxic metals on aquatic organisms: The actions of otherwise well-known toxicants are more complex and unpredictable when the number of possible interactions in the environment increases. In the present study we try to evaluate the combined effects of Cd, Cu and Pb on developing herring eggs and larvae. The reason for using relatively high concentrations of heavy metal in the experiments is to cause strong and evident effects over a short time scale.

MATERIAL AND METHODS

Eggs of freshly caught Baltic spring spawning herring were artificially inseminated and incubated in the laboratory in brackish water (16 ‰ S) at a temperature of 10 °C. One hour after fertilization, the eggs were transferred into 1 l incubating glass jars containing diluted sea water and test solutions of given Cd, Cu and Pb concentrations (Fig. 1).

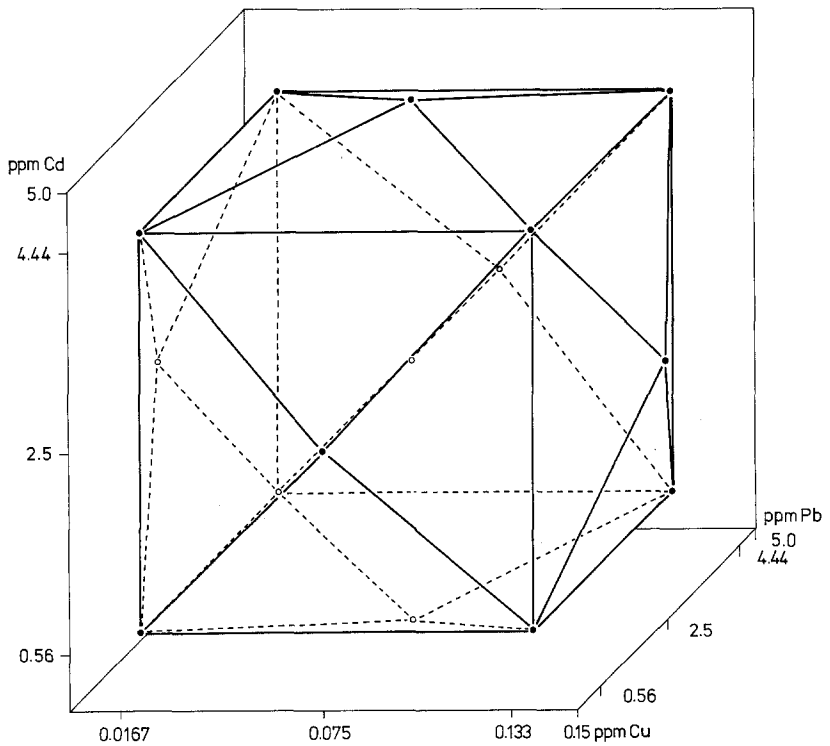

Fig. 1: Three factorial design employed in the incubation of Baltic herring eggs under different metal regimes (10 °C, 16 ‰ S). Open and closed circles indicate combinations used in experiments. Control not included

Each jar contained three glass plates, carrying from between 500 to 1000 eggs in rows. The eggs of the three glass plates were used for (1) the determination of the mortality rate, (2) the determination of the heavy metal accumulation, (3) the measurement of the embryonic activity (to be dealt with in a separate paper).

The incubating medium was contaminated with metals derived from stock solutions of $CdCl_2 \cdot 2\ H_2O$, $CuCl_2 \cdot 2\ H_2O$ and $Pb\ (NO_3)_2 \cdot 2\ H_2O$ added to the experimental sea water in the appropriate amounts to cover all the combinations required (Fig. 1). Test media were renewed every other day, while temperature and salinity (9.15 ±0.08 °C; 16.6 ± 0.16 ‰ S) were determined immediately before and after each renewal. Heavy metal working levels maintained in test solutions throughout the experiments were about ± 10 % of the intended concentrations. At fixed time intervals eggs (5 eggs each sample) and larvae (10 larvae each sample) were removed from the incubating jars, thoroughly washed in double distilled water, placed in polyethylene test tubes for drying (80 °C) and storage. For metal determination eggs, larvae and water samples were digested in a mixture of sulphuric and nitric acid (1:4) before being analyzed by means of flameless atomic absorption spectrophotometry.

Results of incubating experiments (z = % embryonic survival or % viable hatch) and accumulative processes (z = ng heavy metal/mg dry wt) were computed using multiple regressions of the formulae $z = a_0 + a_1x + a_2y$ or $z = a_0x^{a1}\ y^{a2}$ where all parameters were determined at a calculated mean concentration of either Cd, Cu or Pb, so that x and y represented the other two metals employed.

RESULTS

Embryonic survival

In Figure 2 embryonic survival until hatching is depicted be multiple linear regressions at different Cd, Cu and Pb regimes. At Cd and Pb regimes of 0.56, 2.5 and 4.44 ppm (Fig. 2a, c) the strong detrimental effect of Cu on embryonic survival became apparent. With rising Cd concentration Pb tended to increasingly mitigate the negative Cu effects. Within the range of concentrations investigated Pb did not seem to exert any detrimental effects on embryonic survival; in fact, with increasing Pb concentrations survival rates increased (Fig. 2a, b). Table 1 shows the equations for the multiple linear regressions. While Cu and Cd had generally negative effects (Cu between 200 to 800 times stronger than Cd), Pb always acted positively on embryonic survival.

Viable hatch

Figure 3 shows the percentage viable hatch of total hatch of the exposed eggs in a three dimensional presentation as squares located on the Cu planes. For clarity the 2.5 ppm Cd plane is included. Compared to the controls (viable hatch 58 %) in all 15 combinations viable hatch was very low, suggesting strong negative metal effects. The action of specific metal concentrations are illustrated in Figure 4a, which shows the decline in viable hatch

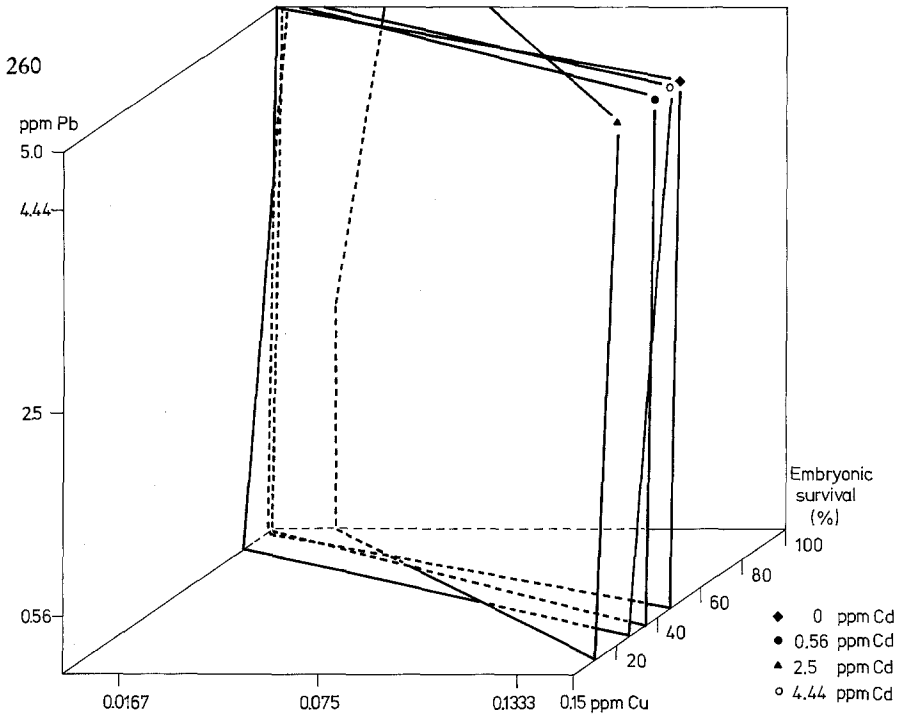

Fig. 2a: Survival of herring eggs incubated in different Cd, Cu and Pb regimes at 10 °C and 16 ‰ S

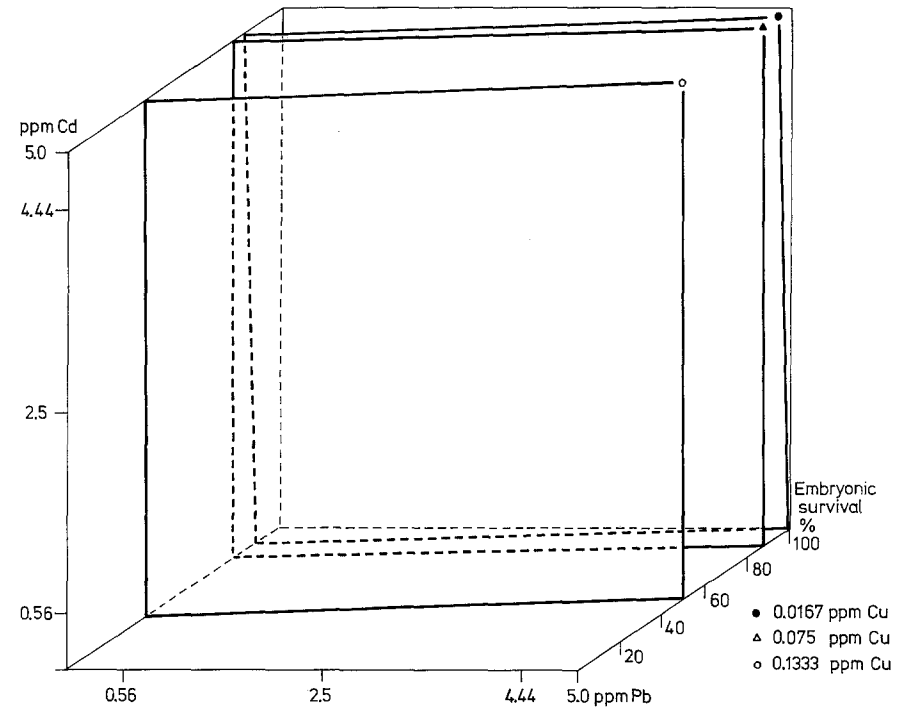

Fig. 2b: Survival of herring eggs incubated in different Cd, Cu and Pb regimes at 10 °C and 16 ‰ S

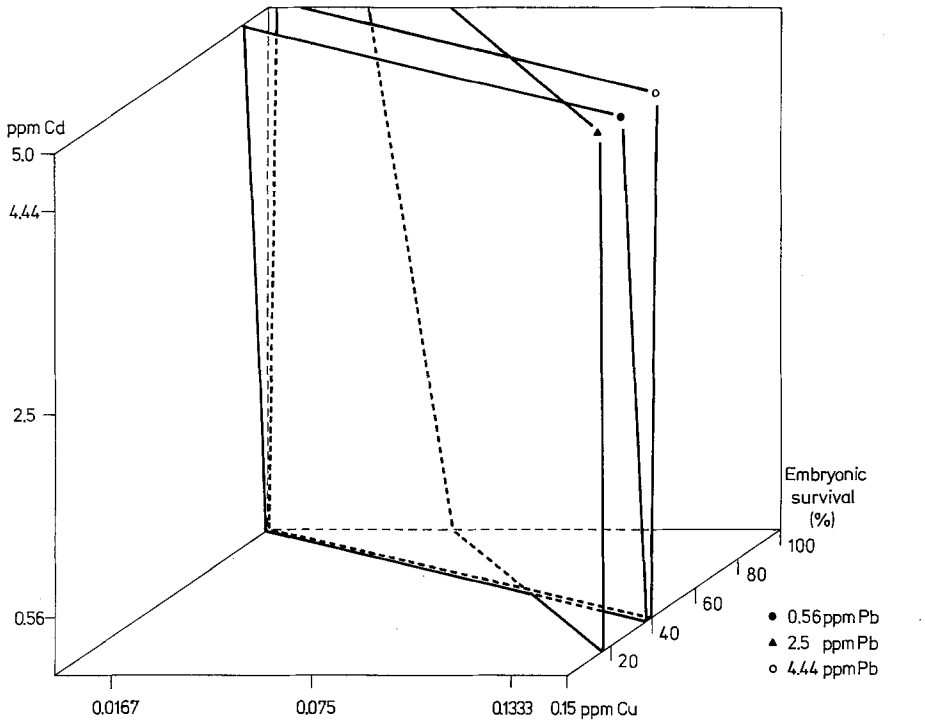

Fig. 2c: Survival of herring eggs incubated in different Cd, Cu and Pb regimes at 10 °C and 16 ‰ S

with increasing Cu and Pb content at various Cd concentrations. At high Cu concentrations viable hatch was drastically reduced. Late embryos became progressively immobilized, so that the hatching enzyme produced by the hatching enzyme glands around the head region would dissolve the chorion in a punctiform manner, causing premature liberation and death of the embryo. In Figure 4b the strong detrimental effects of Cd and Pb on % viable hatch at zero Cu concentration are depicted. The actions of Cd and Pb at a given Cu concentration appeared to be similar, except at 0.0167 ppm Cu where Cd was slightly beneficial to % viable hatch. When no lead was used (Fig. 4c), Cd and Cu were increasingly detrimental to viable hatch with rising concentrations. At 4.44 ppm Pb viable hatch was slightly promoted with rising Cu as well as Cd content of the incubating medium.

Table 2 presents the calculated linear regression equations for the effects of the three metals on viable hatch. It appears that whenever three metals were present, viable hatch was significantly lower than when only two metals were administered. Thus as can be seen from the linear equations, effects of the metals on viable hatch were additive. As already noticed when considering the embryonic survival, Cu was the most toxic of the three metals investigated. From Table 2 it can be seen that negative effects of Cd and Pb on viable hatch are of equal strength.

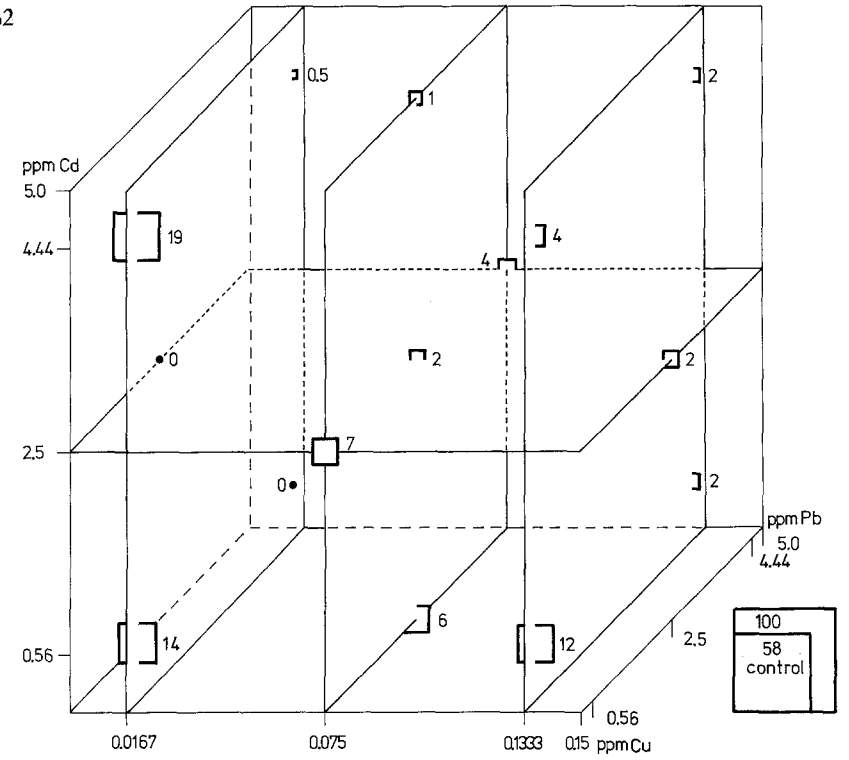

Fig. 3: Squares indicating percent viable hatch of total hatch of herring eggs exposed to Cd, Cu and Pb during incubation at 10 °C and 16 ‰. Figures indicate rounded % viable hatch. 100 % and control in right hand lower corner

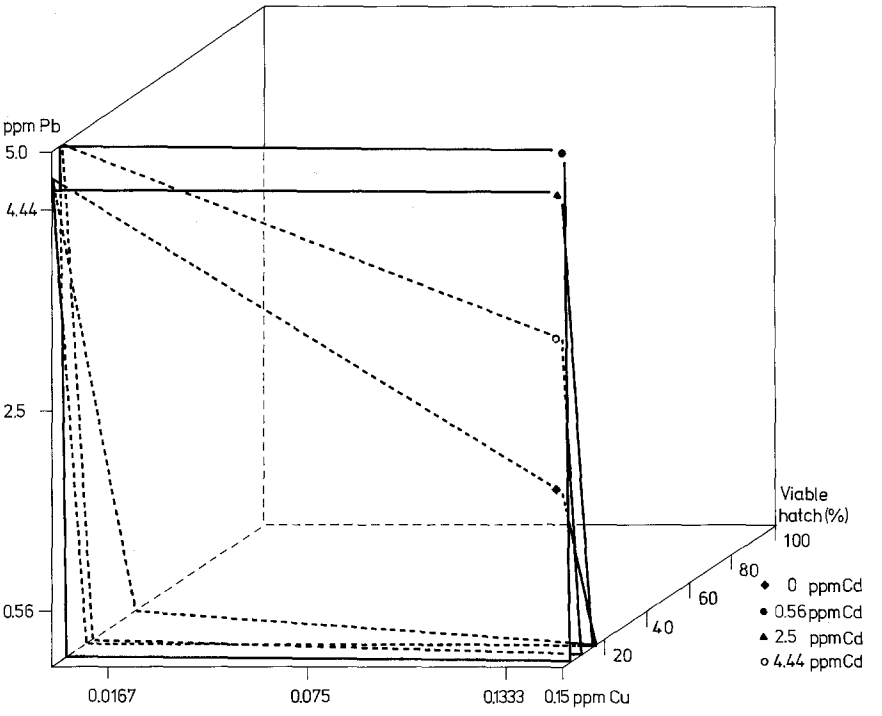

Fig. 4a: Viable hatch of herring eggs incubated in different Cd, Cu and Pb regimes at 10 °C and 16 ‰ S

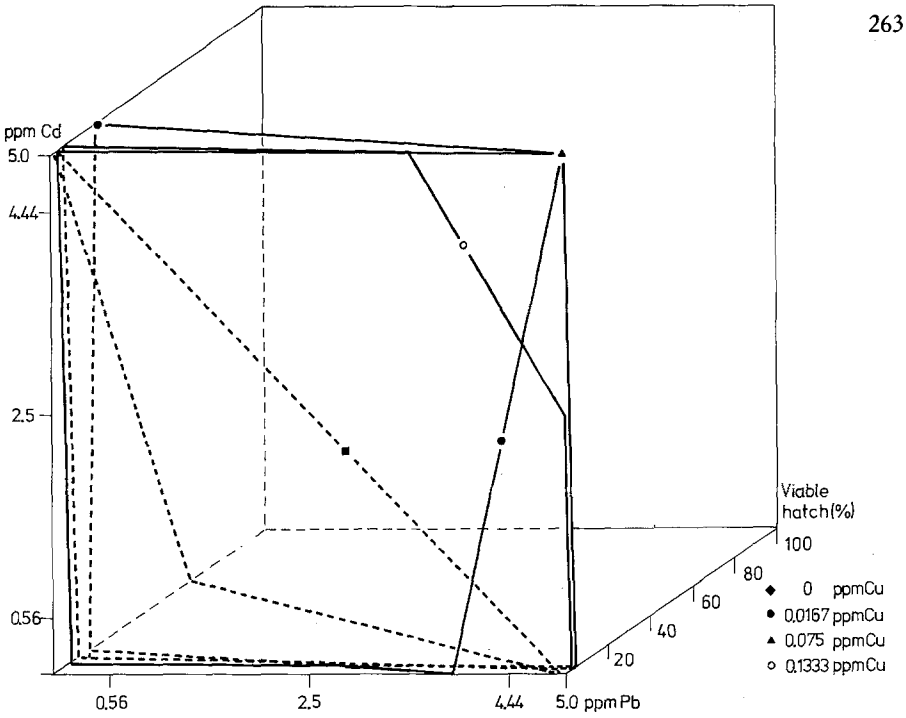

Fig. 4b: Viable hatch of herring eggs incubated in different Cd, Cu and Pb regimes at 10 °C and 16 ‰S

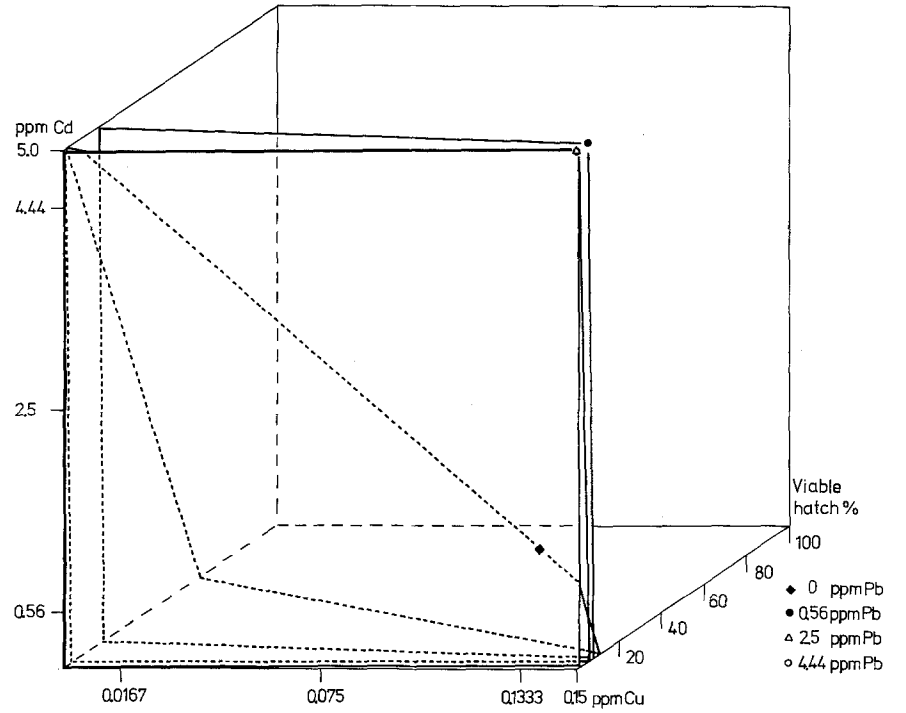

Fig. 4c: Viable hatch of herring eggs incubated in different Cd, Cu and Pb regimes at 10 °C and 16 ‰ S

Table 1

Calculated multiple linear regression equations for % embryonic survival (z) depending on two metal concentrations at a given third metal regime (ppm). * computed equations with the help of the data of von Westernhagen et al. (1974); ** data calculated with the help of values gained from Figure 2c; r = correlation coefficent

ppm	Regression equations	r
Cd		
0	z = 96.7 – 341.2 Cu + 0.8 Pb	0.99**
0.56	z = 98.5 – 431.4 Cu + 1.0 Pb	0.99
2.5	z = 155.2 – 965.3 Cu + 2.0 Pb	0.99
4.44	z = 85.1 – 391.9 Cu + 4.0 Pb	0.99
Cu		
0	z = 98.3 – 12.5 Cd + 11.6 Pb	0.99*
0.0167	z = 88.6 – 1.4 Cd + 2.4 Pb	0.99
0.075	z = 78.1 – 0.2 Cd + 2.0 Pb	0.36
0.1333	z = 37.3 – 0.2 Cd + 2.6 Pb	0.70
Pb		
0.56	z = 99.1 – 414.2 Cu – 2.3 Cd	0.99
2.5	z = 147.9 – 880.0 Cu – 0.2 Cd	0.95
4.44	z = 100.9 – 409.1 Cu + 0.7 Cd	0.99

Table 2

Calculated multiple linear regression equations for % viable hatch (z) depending on two metal concentrations at a given third metal regime (ppm). * computed equations with the help of the data of von Westernhagen et al. (1974); ** data calculated with the help of values gained from Figure 4c

ppm	Regression equations	r
Cd		
0	z = 42.2 – 190.1 Cu – 8.9 Pb	0.84**
0.56	z = 15.1 – 2.3 Cu – 3.1 Pb	0.98
2.5	z = 6.5 – 17.7 Cu – 0.6 Pb	0.63
4.44	z = 17.5 – 59.8 Cu – 2.7 Pb	0.84
Cu		
0	z = 64.7 – 12.6 Cd – 13.3 Pb	0.98*
0.0167	z = 17.3 + 0.7 Cd – 4.2 Pb	0.99
0.075	z = 7.3 – 0.9 Cd – 0.6 Pb	0,78
0.1333	z = 11.6 – 1.1 Cd – 1.7 Pb	0.89
Pb		
0	z = 64.7 – 12.6 Cd – 353.7 Cu	0.98*
0.5	z = 18.9 – 0.4 Cd – 73.7 Cu	0.80
2.5	z = 5.9 – 0.9 Cd – 9.9 Cu	0.88
4.44	z = –0.1 + 0.04Cd + 11.6 Cu	0.98

Accumulation of heavy metals

Cadmium accumulation by eggs and larvae at different ambient metal regimes is depicted in Figures 5 and 6. As found in previous experiments (von Westernhagen et al., 1974, 1975), Cd concentrations in eggs decreased after an initial increase reaching a

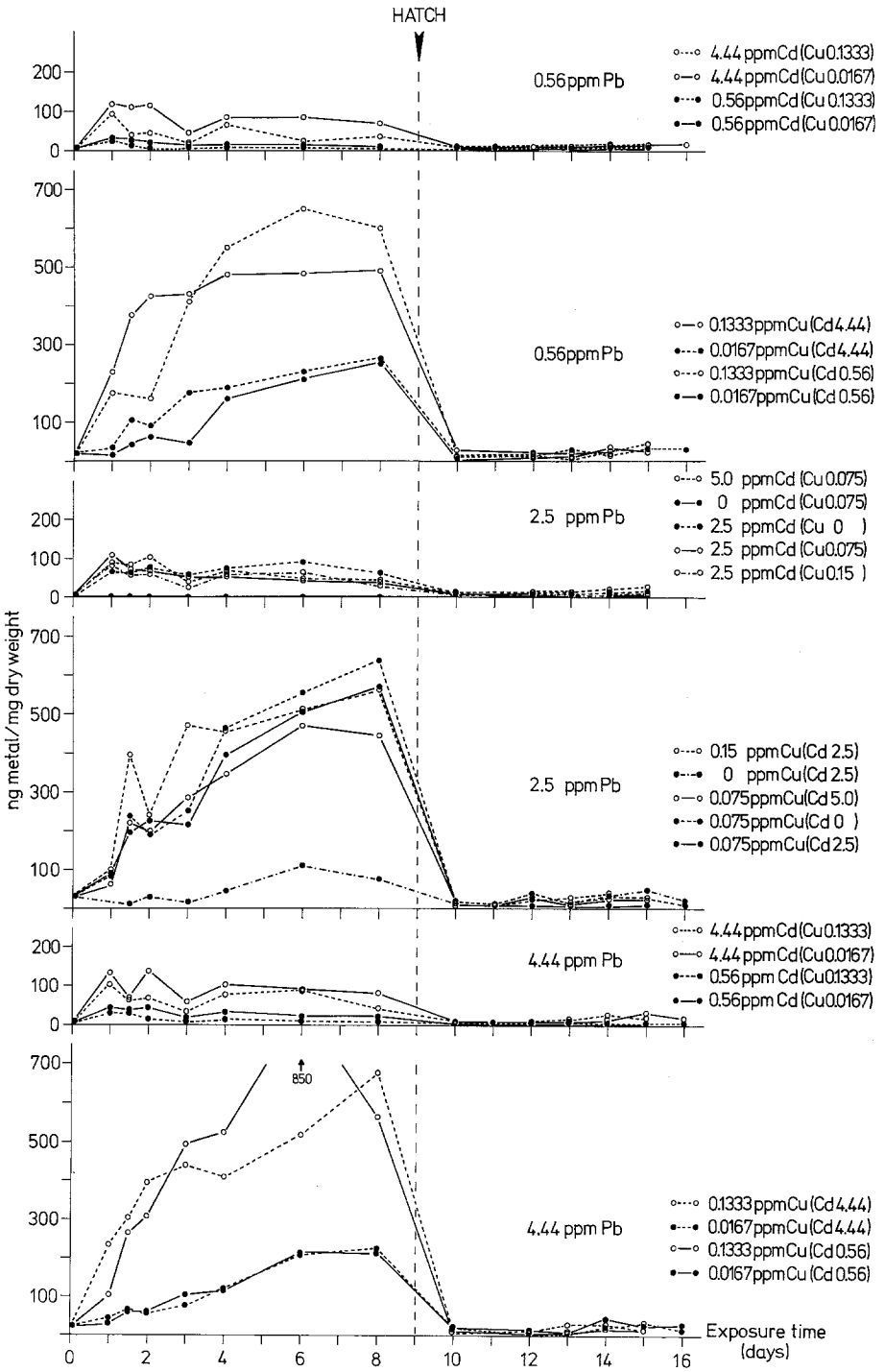

Fig. 5: Accumulation of Cu and Cd by herring eggs and larvae reared in Pb contaminated sea water (10 °C, 16 ‰ S)

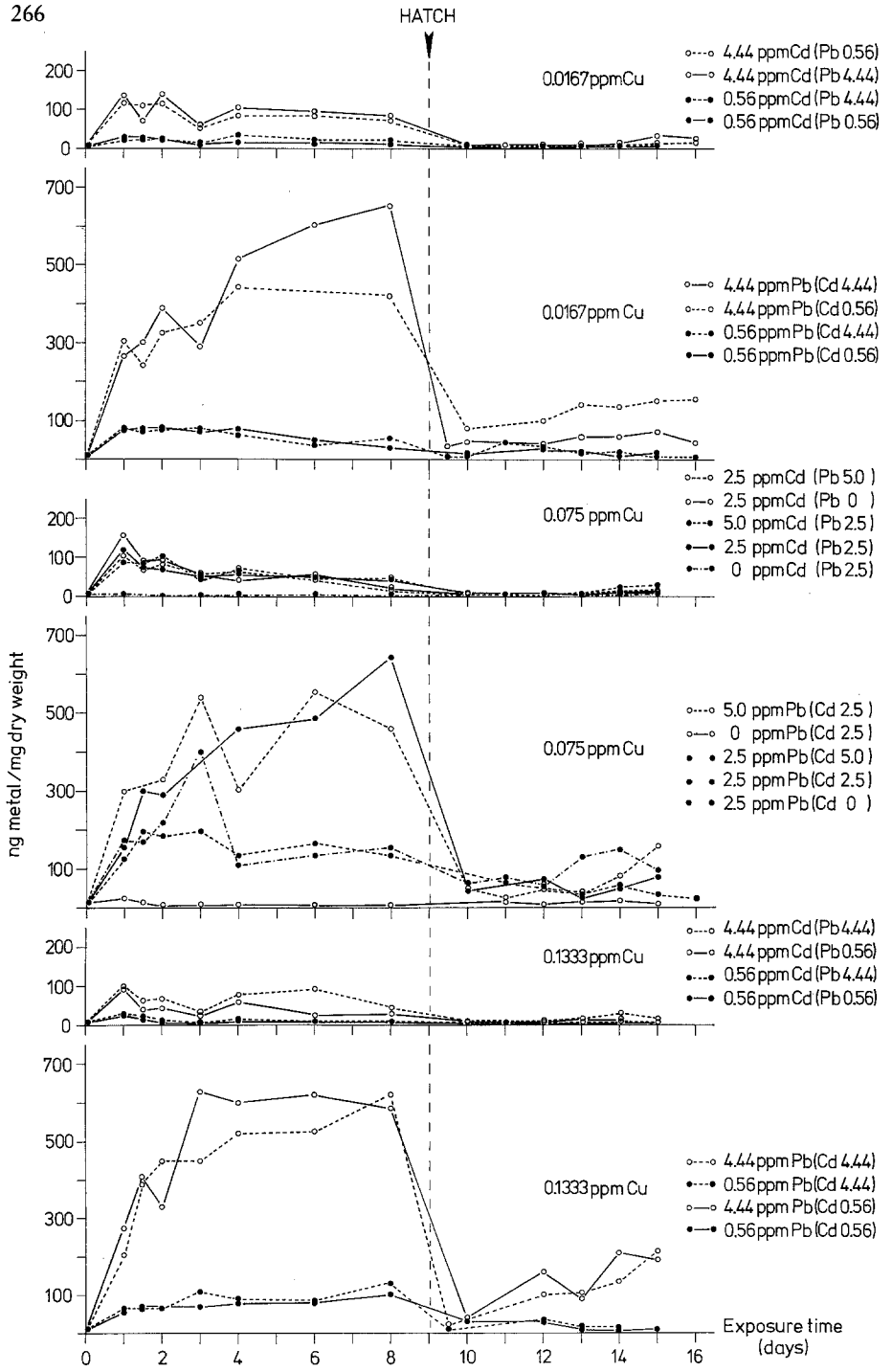

Fig. 6: Accumulation of Pb and Cd by herring eggs and larvae reared in Cu contaminated sea water (10 °C, 16 ‰ S)

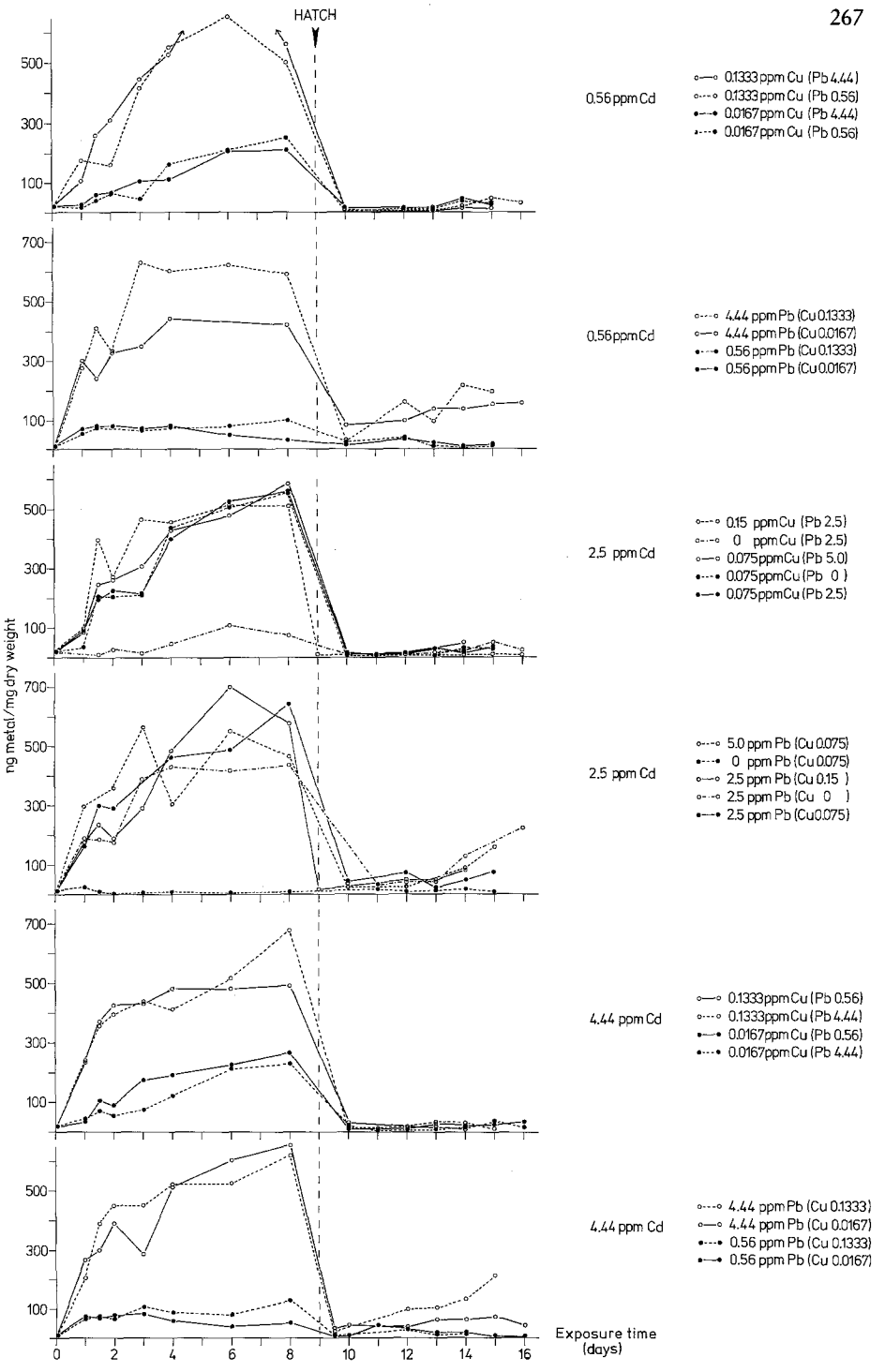

Fig. 7: Accumulation of Pb and Cu by herring eggs and larvae reared in Cd contaminated sea water (10 °C, 16 ‰ S)

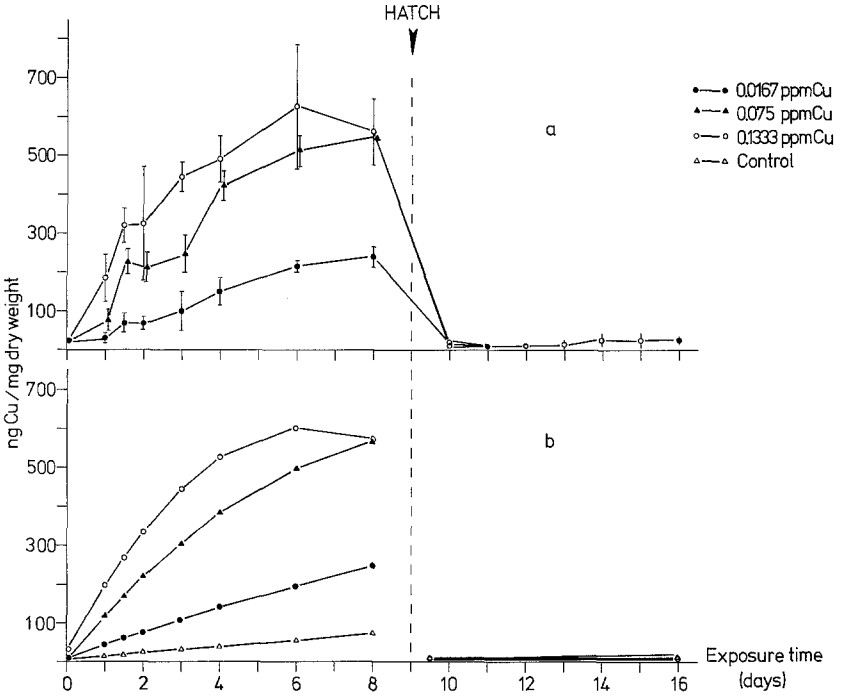

Fig. 8: Pattern of Cu accumulation by herring eggs reared in Pb and Cd contaminated sea water at 10 °C and 16 ‰ S (a) observed, (b) calculated

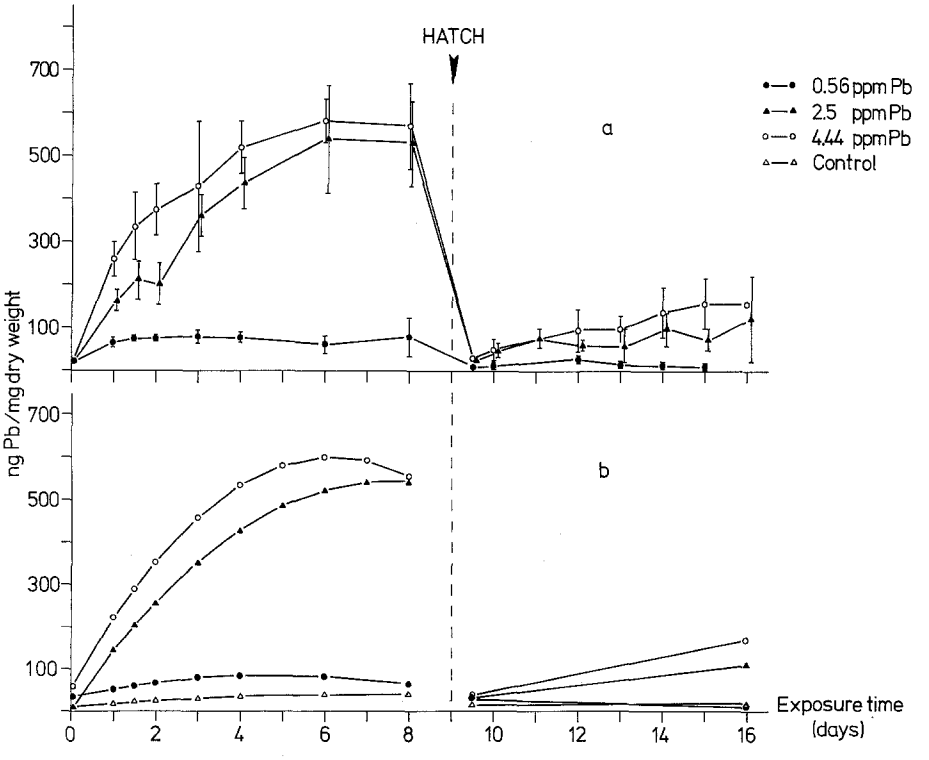

Fig. 9: Pattern of Pb accumulation by herring eggs reared in Cd and Cu contaminated sea water at 10 °C and 16 ‰ S (a) observed, (b) calculated

Table 3

Calculated multiple non-linear regression equations for metal accumulation (z; ng/mg dry wt) by herring eggs, incubated at a given metal regime in the presence of two other metals (ppm)

ppm	Regression equations	r
Cd		
0.56	$z = 6.3\ Cu^{0.73}\ Pb^{1.25}$	0.99
2.5	$z = 56.1\ Cu^{0.96}\ Pb^{0.98}$	0.85
4.44	$z = 33.5\ Cu^{0.79}\ Pb^{1.13}$	0.96
Cu		
0.0167	$z = 122.8\ Pb^{0.93}\ Cd^{1.08}$	0.81
0.075	$z = 323.6\ Pb^{1.00}\ Cd^{0.99}$	0.49
0.1333	$z = 422.3\ Pb^{1.02}\ Cd^{0.99}$	0.92
Pb		
0.56	$z = 84.9\ Cd^{1.07}\ Cu^{1.07}$	0.79
2.5	$z = 349.3\ Cd^{1.16}\ Cu^{1.04}$	0.98
4.44	$z = 578.0\ Cd^{1.05}\ Cu^{1.12}$	0.81

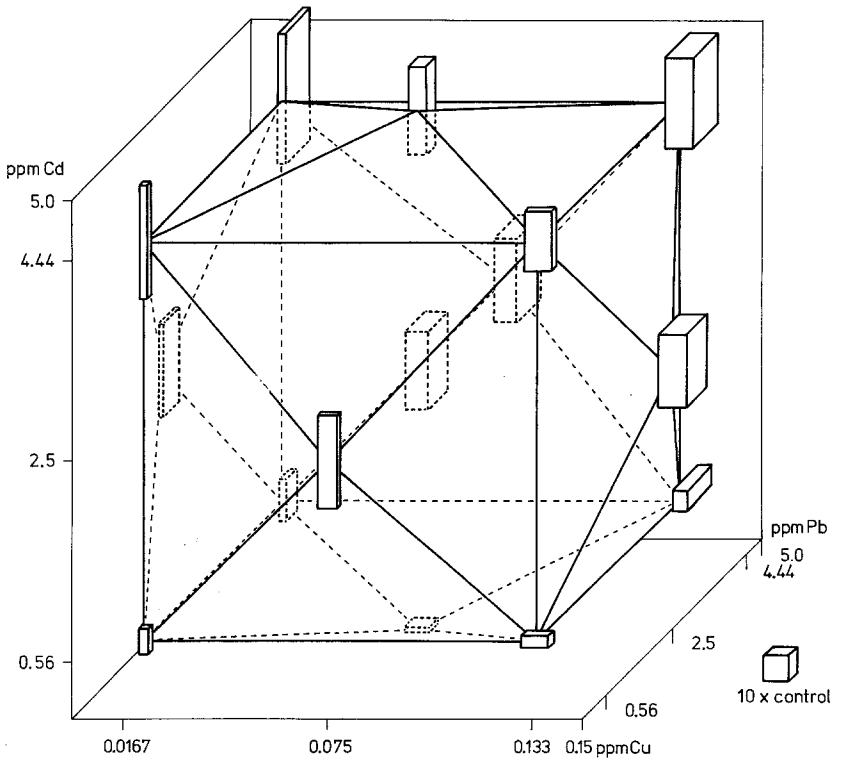

Fig. 10: Mean heavy metal contents of herring eggs exposed for 8 days to different Cd, Cu and Pb concentrations. Sides of rectangular prisms indicate multiples of controls

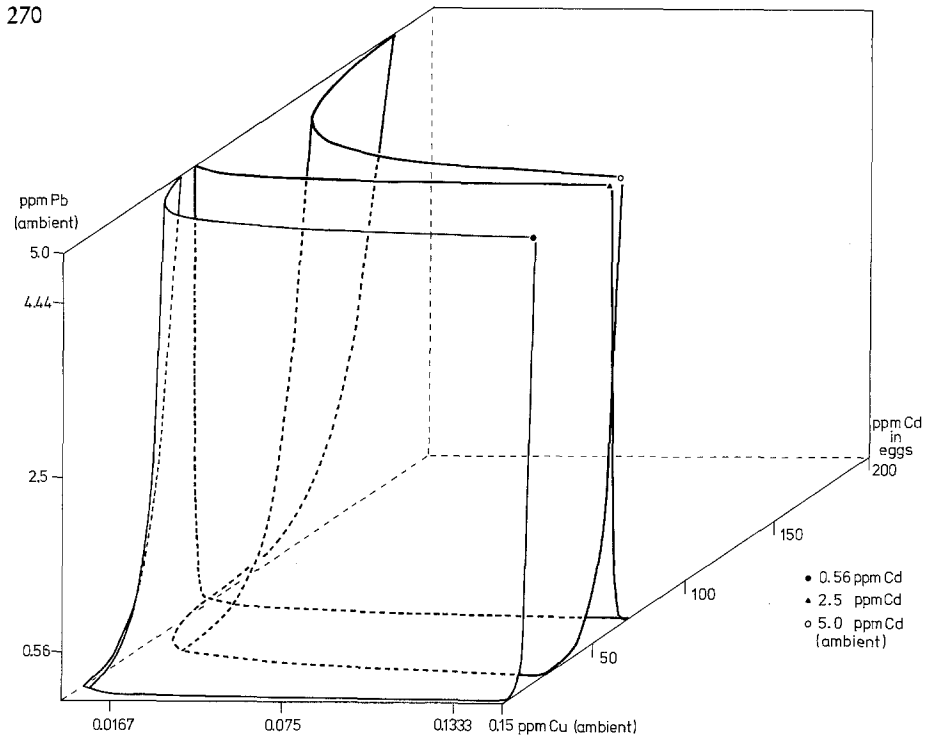

Fig. 11a: Relationship between accumulation of Cd by herring eggs and different Cu and Pb regimes in the incubating medium (10 °C/16 ‰ S)

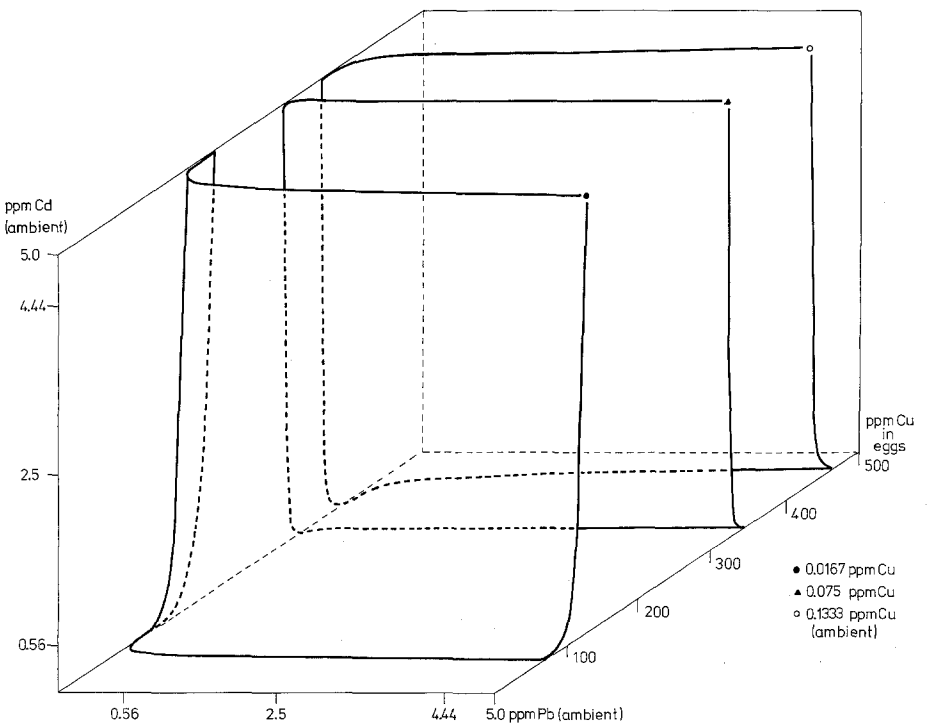

Fig. 11b: Relationship between accumulation of Cu by herring eggs and different Cd and Pb regimes in the incubating medium (10 °C/16 ‰ S)

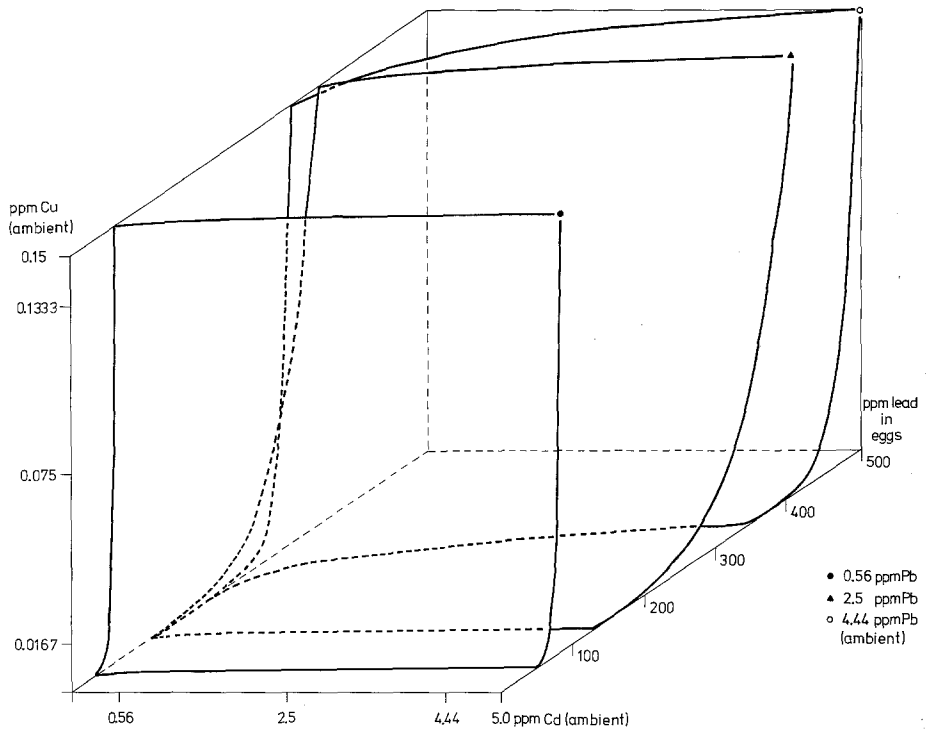

Fig. 11c: Relationship between accumulation of Pb by herring eggs and different Cd and Cu regimes in the incubating medium (10 °C/16 ‰ S)

maximum on the first day of the incubating period. Newly hatched larvae contained very little Cd. Uptake of Cd by larvae was slow but tended to proceed with exposure time. When the two other metals were present in the incubation medium, Cd accumulation by eggs seemed to be somewhat different in eggs exposed to the same Cd concentrations.

In lead we noticed accumulation of the metal in eggs with exposure time. Concentrations in eggs exposed to identical Pb concentrations were similar, but as found with Cd, differences existed. Maximum Pb concentrations at 0.56 ppm were around 75–125 ng Pb/mg dry wt (Figs. 6, 7). At 2.5 ppm ambient Pb concentrations in eggs were between 400 and 700 ng Pb/mg dry wt. Probably due to precipitation of lead in the incubating medium Pb concentrations in two trials (Fig. 6) dropped after the third day. At 4.44 ppm Pb concentrations in eggs did not increase beyond values reached at 2.5 ppm. As found for Cd, Pb concentrations in newly hatched larvae dropped drastically, increasing slowly with exposure time.

Like Pb contents of eggs Cu concentrations in exposed eggs and larvae increased with exposure time, high ambient Cu levels being associated with high Cu content of eggs (Figs. 5 and 7).

Thus generalizing we can say that accumulation of Cd, Cu and Pb by herring eggs and larvae occurs according to the following patterns: (a) Accumulation of Cd is very rapid

shortly after initial exposure. After the building of an early plateau a decrease of Cd contents occurred. Newly hatched larvae contained very little Cd, but larval Cd content increased with exposure time. (b) Cu concentration in eggs reared in Cu contaminated water increased with exposure time (Fig. 8). While accumulation of Cu at the low concentrations (0.0167 ppm) could be approximated by a straight line, Cu uptake at higher concentrations seemed to occur asymptotically within the exposure period of 8 days. Cu uptake by larvae was linear with time. (c) Although Pb concentrations in eggs increased with exposure time, uptake ceased after 7 to 8 days and Pb content of eggs reached maximum values on the 7th day (Fig. 9) or earlier. Larval metal uptake was best described by a straight line.

Figure 10 depicts heavy metal uptake by eggs incubated at the respective metal concentrations employed in the experiments. Sides of rectangular prisms indicate multiples of the controls. With rising ambient metal concentrations accumulation became more pronounced. Relationships between metals or possible interferences or interactions are difficult to detect. In order to be able to trace concentration dependent patterns of accumulation, multiple linear regressions ($z = a_0x^{a1}y^{a2}$) were calculated for uptake of one metal at a given concentration at varying ambient concentrations of two metals. Results are shown in Figure 11. As already mentioned, mean metal accumulation over the total incubation period was employed for calculation. This implies that metal concentrations referred to are slightly below maximum concentrations reached during experiments.

Thus it becomes apparent from Figure 11a that Cd accumulation by eggs was influenced by the levels of the two other metals (Cu and Pb) in the incubating medium. The effect was particularly pronounced, when the other metals were present in only low concentrations; that is Cu from 0 to 0.075 ppm and Pb from 0 to 2.5 ppm. As can be seen (Fig. 11a), the effects are not the same irrespective of the metal species and concentration. In a given Cd regime increasing amounts of Cu in the medium tended to impede Cd accumulation. Addition of Pb had a promoting effect on Cd accumulation at 0.56 and 4.44 ppm. At 2.5 ppm Pb probably exerted a slight inhibitory effect on Cd accumulation. The formulae for the relationships are presented in Table 3.

In a given Cu regime at 0.0167 ppm, the presence of Pb inhibited Cu uptake while additional Cd enhanced uptake of Cu (Fig. 11b). At 0.075 and 0.1333 ppm Cu, Pb contaminated water promoted uptake of Cu while Cd in the water cut down on the Cu uptake (Table 3).

As far as the accumulative behaviour of Pb is concerned, uptake was promoted by the presence of Cd as well as Cu (Fig. 11c, Table 3).

DISCUSSION

Embryonic survival

As shown in Figure 2 embryonic survival was little if at all affected by the Cd concentrations in the incubating medium up to 5.0 ppm, a fact that had previously been found by Rosenthal & Sperling (1974) when incubating herring eggs in Cd contaminated water. Embryonic survival remained high (>80 %). Similarly, the experiments of von

Westernhagen et al. (1974) showed the embryonic survival of herring eggs exposed to Cd was only negatively influenced at 5.0 ppm. Results obtained from incubation experiments with gar-pike *Belone belone* (von Westernhagen et al., 1975) and flounder *Platichthys flesus* (von Westernhagen & Dethlefsen, 1975) confirm that acute effects of Cd on embryonic survival were manifest only at fairly high (1.0–3.0 ppm) concentrations.

Cu effects on embryonic survival were much more pronounced, Cu being between 180 to 5,500 times more toxic than Cd (Table 1). Similar to our experiments in which the toxicity of the Cu could be demonstrated by reduced embryonic survival at 0.0167 ppm (16.7 µg/l, Table 1), Steele et al. (1973) found that artificially inseminated eggs from Clyde herring showed very low survival at concentrations of Cu greater than 10 µg/l. In incubation experiments with herring eggs Blaxter (1977) also found increased embryo mortality at 30 µg/l. The strong deleterious effects of Cu ions on embryonic survival of Pacific herring eggs *(Clupea harengus pallasi)* have been also demonstrated by Rice & Harrison (1978) who could show that at Cu concentrations of more than 35 µg/l embryonic survival was significantly reduced compared to that in controls. At 45 µg/l and above no hatching occurred. They estimated the incipient lethal level for Cu ions for herring eggs to be around 33 µg/l.

Effects of Pb on the developing herring eggs were always beneficial (Table 1); no acute effects on embryonic survival were noticed.

Viable hatch

As mentioned in previous papers (von Westernhagen et al., 1974, 1975) 'viable hatch' is a much more sensitive parameter to be measured for the action of pollutants than embryonic survival. The deleterious effects of water contaminants become more obvious (Fig. 4, Table 2), which is reflected by the low rates of 'viable hatch' at all contaminant combinations used. Using 'viable hatch' as a criterion we see that all three metals eventually exerted detrimental influence on early development of herrings eggs. The two deviations in the 0.0167 ppm Cu and 4.44 ppm Pb regimes (Table 2) are probably artifacts.

Accumulation of metals

In their accumulative behaviour the three metals behaved differently. At 0.56 and 4.44 ppm Cd concentration, contents of Cd in eggs were enhanced by Pb and diminished by Cu. At 2.5 ppm Cd, Cu as well as Pb inhibited Cd accumulation (Fig. 11a, Table 5). In the Cu regime at 0.0167 ppm, Pb inhibited Cu uptake, while in the presence of Cd, Cu uptake increased. At 0.075 and 0.1333 ppm Cu concentrations, uptake of Cu was enhanced in the presence of Pb and inhibited by Cd (Fig. 11b, Table 5). In the Pb regimes, presence of Cd as well as Cu enhanced uptake of Pb at all concentrations investigated (Fig. 11c, Table 5).

The antagonistic and synergistic action of the metals in their accumulation dynamics are not as yet well understood. We know from accumulation experiments that several

Table 4

Mode of action of mixtures of heavy metals in aquatic organisms; ant. = antagonistic; add. = additive; syn. = synergistic

Species	Salinity of medium	Metals	Concentration (mg/l)	Mode of action	Authors
Salmo gairdnerii	fresh	Zn + Cu	3.5–10 Zn 1.1–3.3 Cu	ant., acute tox.	Lloyd (1961)
	fresh	Zn + Cu	35–100 Zn 11–33 Cu	syn., acute tox.	
	fresh	Zn + Cu + Ni	0.14–0.48 Cu 0.68–2.4 Zn 5.44–19.2 Ni	add., acute tox.	Brown & Dalton (1970)
Salmo salar	fresh	Zn + Cu	0.003–4.19 Zn 0.002–0.22 Cu	syn., acute tox.	Sprague & Ramsay (1965)
Oncorhynchus kisutch (eggs)	fresh	Zn + Cu	2.0 Zn 0.002–2.0 Cu	syn., acute tox.	Wedemeyer (1973)
Pimephelas promelas	fresh	Zn + Cu + Cd	0.007–0.36 Zn 0.0019–0.038 Cu 0.0003–0.069 Cd	Cd chronic, ant. Cu chronic, syn. add., acute tox.	Eaton (1973)
Paratya tasmaniensis	fresh	Zn +Cd	1.21–12.1 Zn 0.06–0.6 Cd	add., acute tox	Thorp & Lake (1974)

Clupea harengus (eggs)	16 ‰	Zn + Cd	0.02 Zn, 4.5 Cd	ant., acute tox.	Rosenthal & Sperling (1974)
Fundulus heteroclitus	20 ‰	Zn + Cu + Cd	3.0–60 Zn 1.0–3.0 Cu 1.0–10 Cd	syn., acute tox.	Eisler & Gardner (1973)
Artemia salina *Acartia clausi*	33 ‰	Cu + Hg	0.05–1000 Cu 0.4–50 Hg	syn., acute tox.	Corner & Sparrow (1956)
Tigriopus japonicus	33 ‰	Cu + Cd	0.006–0.64 Cu 0.004–0.44 Cd	syn., growth + reproduction	D'Agostino & Finney (1974)
Chaetogammarus marinus	33 ‰	Cu + Hg	0.2–2.5 Cu 0–40 Hg	syn., acute tox.	Hunter (1949)
			2.5–50 Cu 0–100 Hg	add., acute tox.	
Nitocra spinipes	33 ‰	Cu + Hg	0.026–26 Cu 0.07–4.4 Hg	syn., acute tox.	Barnes & Stanbury (1949)
Crassostrea virginica (embryos)	26 ‰	Zn + Cu	0.05–0.35 Zn 0.005–0.035 Cu	add., acute tox.	Mac Innes & Calabrese (1978)
Cristigera spp.	34.5 ‰	Hg + Pb	0.01 Hg 0.1–9.1 Pb	syn., growth	Gray & Ventilla (1971)
		Cu + Hg	2.0 Cu, 0.01 Hg	syn., growth	

Table 5

Accumulation of Cd, Cu and Pb by herring eggs during incubation. Symbols indicate synergistic (+) or antagonistic (−) effects of the other two metals at a given concentration of the third

ppm	Cd 0.56	Cd 2.5	Cd 4.44	Cu 0.0167	Cu 0.075	Cu 0.1333	Pb 0.56	Pb 2.5	Pb 4.44
Cd				+	−	−	+	+	+
Cu	−	−	−				+	+	+
Pb	+	−	+	−	+	+			

factors such as pH, temperature, salinity (Wedemeyer, 1968) or concentration of the metals under question have some bearing on the nature of the observed effects.

The reactions of the metal ions are not always as clear as mentioned above. Wedemeyer (1968) found that when exposing eggs of the salmon *Oncorhynchus kisutch* to Cu and Zn ions, Zn uptake was inhibited by Cu in the range of 0 to 2.0 ppm Cu, but facilitated above 2.0 ppm. Investigating metal uptake by *Fundulus heteroclitus* Eisler & Gardner (1973) showed that uptake of Zn from the medium was markedly inhibited by 1.0 and 10 mg/l of Cd^{2+} at Zn^{2+} levels of 12, 36 and 60 mg/l. When computing the raw data presented by Eisler & Gardner (1973) we found that addition of Zn (range 0–12.0 ppm) to the medium decreased Cu content in specimens exposed to 1.0 mg Cu/l. In contrast to our findings with herring eggs, Cd in the medium (range 0–10.0 ppm) caused increased tissue Cu content of mummichog at a given (1.0 mg Cu/l) concentration. There seems to be no general uniformity regarding the accumulative behaviour of individual metals when used in mixtures; uptake depends on composition of mixtures, test medium and organisms employed.

Mode of action of the metals tested

As evident from the linear nature of the multiple regressions describing embryonic survival and viable hatch at different concentrations, toxic action of the three metals employed was strictly additive. Whenever non-linear regressions were computed, correlation coefficients were lower than in the linear equations. For accumulation action was either antagonistic or synergistic. When comparing our results with literature data, we are faced with a fairly large number of investigations and the difficulty of weighing the different findings which are far from conforming with one another (Table 4). Yet the majority of the reports referring to action of mixtures of heavy metals describe synergistic effects whenever two or three metals were used in experiments. Again comparison of results becomes difficult, since the combination Cd, Cu, Pb as used by us has not been tested before. Thus we are left with the possibility of comparing data derived from experiments in which two of the metals employed by us were present.

In freshwater exposure of fathead minnow *Pimephales promelas* to mixtures of Zn, Cu and Cd (Eaton, 1973) led to the deduction that at chronic concentrations the effects caused by Cd were mitigated and those caused by Cu were enhanced. At acute levels the action of all metals was strictly additive. The effects of a mixture of Zn, Cu and Cd on

Fundulus heteroclitus in 20 ‰ brackish water at acute toxic levels could be described by the synergistic action of the metals in use (Eisler & Gardner, 1973). Similarly D' Agostino & Finney (1974) described synergistic action of Cu and Cd mixtures on growth and reproduction of the copepod *Tigriopus japonicus* in natural sea water. Strictly additive effects of metals in sea water have been described by McInnes & Calabrese (1978) for Zn and Cu and for Hg and Ag mixtures at 30 °C (*Crassostrea virginica* embryos) and Hunter (1949) for the acute toxicity of high levels of Cu and Hg on *Chaetogammarus marinus*.

We are as yet not able to explain why at certain environmental conditions metals act either antagonistically, additively or synergistically. There remains the quest for further investigation on the mode of action of mixtures before we shall be able to predict possible actions of heavy metal pollutants in the aquatic environment.

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