International Council for the Exploration of the Sea
Montreal, 1975.

C.M. 1975/E:20
Fisheries Improvement Committee
Ref. Pelagic Fish (N) Cttee

SOME EFFECTS OF CADMIUM ON THE METABOLISM OF DEVELOPING EGGS OF PACIFIC HERRING

by

M. Said Mounib, Harald Rosenthal* and Joanne S. Eisan

Environment Canada
Fisheries and Marine Service
Halifax Laboratory
1707 Lower Water Street
Halifax, Nova Scotia, Canada B3J 2R3

*Biologische Anstalt Helgoland
(Zentrale), Hamburg, Germany

SUMMARY

The effect of 10 ppm of cadmium on eggs of Pacific herring, *Clupea pallasii*, incubated at 20‰ salinity and 5°C was tested with particular reference to hatchability, cadmium uptake by eggs, along with the relative activity of four carbon dioxide fixing enzymes (i.e. NAD- and NADP- malic enzymes, propionyl CoA carboxylase and phosphoenol pyruvate (PEP) carboxykinase) at five stages of development. It was found that cadmium-exposed eggs hatched approximately ten days earlier than those of the control; the percentage of viable hatch was 82.00 and 27.3 in control and cadmium-exposed eggs respectively; the average size of viable larvae was 8.46 and 5.49 mm in the same sequence. Like the garpike, cadmium uptake by herring eggs reached its maximum early in the incubation period and was maintained throughout the five developmental stages studied; the total uptake was, however, much greater than that reported for Baltic herring.

The relative activity of all four carbon dioxide fixing enzymes studied was depressed as a result of cadmium exposure; propionyl CoA carboxylase was depressed as early as prior to flattening of blastodisc stage; PEP carboxykinase depression started prior to closure of blastophore stage, whereas NAD- and NADP- malic enzymes were depressed only prior to the hatching stage. It is postulated that the relatively small larvae hatching from cadmium-exposed eggs may be the result of: (1) the depressive effect of cadmium on

the relative activity of the aforesaid enzymes at different stages of development, and/or (2) that in cadmium-exposed eggs, most of the cadmium is bound to the chorion making it vulnerable and thus hatchability is induced earlier than expected and a premature larva is produced.

INTRODUCTION

In view of the recent observation of the effect of cadmium on fertilized Baltic eggs (Rosenthal and Sperling, 1974), and also the recent finding of four carbon dioxide fixing enzymes in fish eggs (Mounib and Eisan, 1973), it was felt appropriate to examine the effect of this heavy metal on Pacific herring eggs at different stages of development with particular reference to these enzymes.

MATERIAL AND METHODS

Fish eggs were obtained, by stripping from Pacific herring, *Clupea pallasii*, that were kept in tanks. The eggs were placed on glass plates in single layers and then submerged into filtered sea water of 20% salinity and 5°C for about 30 min. when sperm were added to make a final density of approximately 1500 - 2000 sperm/ml. Thirty minutes were allowed for fertilization to take place. The eggs were washed twice, with care, and then transferred using filtered sea water, to incubation tanks that contained filtered sea water (20% salinity, 5°C) for the control eggs, whereas eggs exposed to cadmium received in addition 10 ppm of cadmium; this level of cadmium was achieved by adding the appropriate amount of a stock solution of cadmium (4000 ppm) to the water in the incubation tank. Water was renewed every other day and the required amount of the cadmium-stock solution was added to cadmium-exposed eggs. Each incubation tank had 20 litres of water that was continuously aerated. The water temperature was measured 3 times a day, and salinities were checked before and after each water change (Table 1).

Water and egg (2 x 5 eggs) samples were taken at 24 hour intervals to determine the actual cadmium concentration maintained in the incubating medium and the cadmium uptake by eggs. Sampling procedure and cadmium determination were carried out as described by Rosenthal and Sperling (1974).

For biochemical studies, large numbers of eggs were collected at five different developmental stages from both control and cadmium-exposed eggs (Table 2); dead and unfertilized eggs were eliminated and the remaining eggs were suspended in approximately 5 volumes of a cold buffered medium that was composed of: sucrose (0.25 M), tris (0.05 M), reduced glutathione (0.1%, w/v), and EDTA, free acid, (0.1%, w/v), at pH 7.00 (the pH of the medium was adjusted with 1 N HCl) (Mounib and Eisan, 1972a). Eggs in the suspended medium were immediately frozen and kept at -25°C until they were ready for enzyme assays. Concurrently samples were taken from these same five developmental stages to determine the rate of fertilization. Throughout the whole investigation, daily mortality rate, percentage of daily hatch and number of viable larvae were recorded for subsamples that were handled separately.

For the preparation of extracts for enzyme determinations, the frozen eggs were sonicated until they had completely thawed, placed in an ice bath for 15 min., homogenized in the cold, and then sonicated again for a total of 2 min. interrupted for a 3 min. cooling period every 30 sec. (Mounib, 1974). The sonicated homogenate was then centrifuged at 27,000 g in the cold (2°C) for 20 min. and the supernatant fluid was separated for use in enzyme determinations.

Protein concentration in supernates of sonicated homogenates was determined according to Lowry, Rosebrough, Farr and Randall (1951).

Carbon dioxide fixing enzymes were assayed as follows:-

(a) the two malic enzymes requiring NAD- and NADP- respectively (EC 1.1.1. 39 NAD-malic dehydrogenase, decarboxylating; and EC 1.1.1.40 NADP-malic dehydrogenase decarboxylating) were determined as $^{14}CO_2$ produced from the decarboxylation of ^{14}C-4-malate (Mounib, 1974).

(b) propionyl CoA carboxylase ((EC 6.4.1.3, propionyl-CoA: CO_2 ligase (ADP)) was estimated as $^{14}CO_2$ incorporated into methylmalonate-CO_2 following the procedure of Tietz and Ochoa 1962 as modified by Mounib and Eisan 1972.

(c) phosphoenolpyruvate (PEP) carboxykinase (EC 4.1.1.32; GTP: oxaloacetate carboxy-lyase transphosphorylating) was determined as the exchange incorporation of CO_2 into oxaloacetate (Utter & Kurahashi 1955; Mounib and Eisan, 1972).

RESULTS AND DISCUSSION

As in the case of Baltic spring spawners, Pacific herring eggs, when exposed to cadmium started hatching several days earlier than the control (Table 3); the difference between the mean hatching points (50% hatch) was approximately 10 days. Eggs of Pacific herring seemed to be more resistant to cadmium exposure than those of Baltic herring, as in the latter no viable hatch was observed at 20% salinity and in the presence of 10 ppm (Rosenthal and Sperling, 1974), whereas in the present investigation under the same salinity and concentration of cadmium 27.3% viable larvae were obtained (Table 4). In addition the size of larvae from cadmium-exposed eggs was considerably smaller than that from the controls (Table 4). We also noticed that the newly hatched larvae from cadmium exposed eggs were less active than those from the control, as they spent most of their time lying at the bottom of the incubation tank and were typical of a premature hatch.

The uptake of cadmium by Pacific herring eggs reached its maximum early in the incubation period and was maintained throughout the five developmental stages studied (Table 5). These results are in agreement with those of garpike eggs (Westernhagen, Dethlefsen and Rosenthal, 1975). However, it is noted that cadmium uptake by Pacific herring eggs was much higher than that

reported for Baltic herring.

The observation that eggs of Pacific herring were more resistant to the effect of cadmium, and also showed a relatively higher uptake of cadmium than those of Baltic herring, may be due to the fact that the chorion in the former is much thicker than that in the latter. This assumption is supported by the previous finding that most of the cadmium uptake resides in the chorion (Rosenthal and Sperling, 1974). However, it must be borne in mind that in the present investigation a temperature of 5°C was used, whereas in Baltic herring experiments, the incubation temperature was 10°C; thus the possibility that such differences in the incubating temperature played a role in the observed differences between Pacific and Baltic herring eggs should not be precluded.

The relative activity of NAD- and NADP- malic enzymes, in the control eggs, tended to increase from stage A to D and then dropped at stage E (Table 6). Only at stage E of development was the relative activity of NAD- and NADP- malic enzymes higher in the control eggs than that in the cadmium-exposed ones (Table 6).

In the control eggs, the relative activity of propionyl CoA carboxylase was very close at A and B stages of development, but almost doubled at stage C, and was maintained throughout stages D and E (Table 7). It is depicted (Table 7) that eggs exposed to cadmium had lower relative activity of propionyl CoA carboxylase than those of the control at all developmental stages, and this was particularly the case at stages C,D, and E of development.

The relative activity of PEP carboxykinase was very low at stage A but increased sharply at stages B, C and D, and then decreased at E (Table 8). Although exposure of eggs to cadmium had no apparent effect on PEP carboxykinase at stage A, a depressive effect was distinct throughout stages B to E.

From the foregoing results, the evidence is in support of the contention that exposure of Pacific herring eggs to cadmium at 10 ppm in a salinity of 20‰ and 5°C, resulted in a reduction in the relative activity of all four studied enzymes at stage E of development. However, the sensitivity of these enzymes to cadmium treatment was different, e.g. propionyl CoA carboxylase was depressed as early as stage A, whereas the reduction in enzyme activity was observed at stage B for PEP carboxykinase and at stage E for NAD- and NADP- malic enzymes.

Since all of the four studied enzymes are involved in biosynthetic processes, it may be possible that the depressive effect of cadmium on their activity during the different developmental stages of eggs culminated in a small inactive larva. The relatively small larvae hatching from cadmium-exposed eggs could also be attributed to the fact that most of the cadmium is bound to the chorion which makes it vulnerable and thus hatching is induced earlier than its regular time and premature larvae are produced.

ACKNOWLEDGEMENT

Sincere thanks are due to Dr. V. Dethlefsen, Cuxhaven, Germany for cadmium determinations. One of us (H.R.) is supported by the Canadian-German Scientific and Technical Exchange Programme.

REFERENCES

LOWRY, O.H., N.L. ROSEBROUGH, A.L. FARR and R.J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

MOUNIB, M.S. 1974. NAD- and NADP- malic enzymes in spermatozoa of mammals and fish. FEBS, 48: 79-84.

MOUNIB, M.S. and J.S. EISAN. 1972. Fixation of carbon dioxide by the testes of rabbit and fish. Comp. Biochem. Physiol. 43B: 393-401.

MOUNIB, M.S. and J.S. EISAN. 1972a. Mitochondrial and cytosol malic enzymes in the testicular tissue of cod and rabbit. Endocrinology 91: 244-248.

MOUNIB, M.S. and J.S. EISAN. 1973. Fixation of carbon dioxide and some of the enzymes involved in cod eggs. Int. J. Biochem. 4: 207-212.

ROSENTHAL, H. and K.-R. SPERLING. 1974. Effect of cadmium on development and survival of herring eggs. In "The early life history of fish" Edited by J.H.S. Blaxter, Springer, Berlin, pp. 383-396.

TIETZ, A. and S. OCHOA. 1962. Propionyl CoA carboxylase from pig heart. In "Methods in Enzymology", Edited by Colowick S.P. and Kaplan, N.O., Vol. V, pp. 570-575. Academic Press, New York.

UTTER, M.F. and K. KURAHASHI. 1955. Oxalacetic synthesizing enzyme. In "Methods in Enzymology", Edited by Colowick, S.P. and Kaplan, N.O.; Vol. I, pp. 758-763. Academic Press, New York.

WESTERNHAGEN, H. VON, V. DETHLEFSEN, and H. ROSENTHAL. 1975. Combined effects of cadmium and salinity on development and survival of garpike eggs. Helgoländer wiss Meeresunters (in press).

TABLE I. Temperature, salinity and pH values of water during incubation.

Treatment	Temperature		Salinity		pH		Number of Water Changes
	n	Mean ±S.E.	n	Mean ±S.E.	n	Mean ±S.E.	
Control	129	5.06±.055	12	20.87±0.826	18	7.89±0.011	12
With cadmium	131	5.07±0.052	11	20.02±0.013	18	7.87±0.014	11

TABLE 2. Developmental stages of eggs used in biochemical work.

Sample	Developmental Stage	Time after fertilization (days)	Net Wet wt. (g)
CA (control)	Prior to blastodisc flattening (A)	2.5	10.00
Cd A (cadmium-exposed)		2.5	10.00
CB (control)	Prior to closure of blastophore (B)	5.5	10.05
Cd B (cadmium-exposed)		5.5	10.12
CC (control)	Tail bud off yolk, beginning of embryonic activity (C)	7.5	10.10
Cd C (cadmium-exposed)		7.5	10.05
CD (control)	Grey eye pigmentation, open anus, closed mouth, first hatching glands developed (D)	14.5	10.25
Cd D (cadmium-exposed)		14.5	10.05
CE (control)	Prior to hatching (E)	17.0	10.10
Cd E (cadmium-exposed)		17.0	10.48

TABLE 3. Percentages of daily hatch in control and cadmium exposed eggs of Pacific herring.

Days After Fertilization	Hatching Rate (%)	
	Control	Cadmium Exposed
18	0	50.3
19	0	21.1
20	0	14.0
21	0	3.6
22	0	4.6
23	0.3	3.7
24	0	0.7
25	4.7	1.5
26	0	0.7
27	13.9	0
28	42.3	0
29	7.9	0
30	11.7	0
31	5.9	0
32	3.4	0
33	3.6	0
34	2.8	0
35	2.2	0
36	1.2	0
37	0.4	0
38	0.1	0

TABLE 4. Rate of fertilization, number of eggs, mortality, and hatch percentage and length of larvae at 50% hatch in control and cadmium-exposed eggs of Pacific herring.

	Total number of eggs	Fertilization %	Mortality % until hatching	Total hatch %	Viable hatch %	Length of viable larvae (mm) at 50% hatch (Mean ± S.D.)
Control	1553	98.7	13.3	85.3	82.0	8.46±0.30
Cadmium-exposed eggs	1767	99.8	63.9	36.0	27.3	5.49±0.24

TABLE 5. Cadmium uptake by eggs (ng/egg)

Developmental Stage*	Control		Cadmium exposed	
	n	Mean ± S.E.	n	Mean ± S.E.
A	8	0.499±0.189	8	96.941±2.654
B	17	0.564±0.133	16	98.208±2.974
C	18	0.174±0.049	17	109.188±1.875
D	33	0.132±0.056	30	100.117±3.311
E	12	0.344±0.084	14	102.352±1.236

* See Table 2.

TABLE 6. Relative activity of NAD- and NADP- malic enzymes in control and cadmium-exposed Pacific herring eggs at different stages of development*.

Developmental Stage**	NAD- malic enzyme		NADP- malic enzyme	
	Control	Cd-exposed	Control	Cd-exposed
A	15.07±0.22	15.09±0.17	54.13±2.36	51.69±3.10
B	18.23±0.34	18.75±0.19	68.83±1.76	68.49±2.00
C	19.38±0.60	18.60±0.73	72.12±3.09	71.42±3.22
D	20.83±0.27	20.22±1.13	72.12±2.41	73.09±2.66
E	16.16±0.44	12.81±0.37	59.52±1.77	49.19±1.09

*Complete system contained (μ moles in 2 ml): ^{14}C-4-malate, 50 (total activity: 2 μC); $MnCl_2$, 10; NAD (or NADP), 3; Tris, 300; and a suitable aliquot of the tested extract; the final pH was 7.00. Malic enzyme activity is determined as $^{14}CO_2$ produced from the decarboxylation of ^{14}C-4-malate (n-moles of $^{14}CO_2$/mg of protein/5 min.). Mean values of six experiments ± S.E.

**See Table 2.

TABLE 7. Relative activity of propionyl CoA carboxylase in control and cadmium-exposed Pacific herring eggs at different stages of development*

Developmental Stage**	Control Eggs	Cadmium-Exposed Eggs
A	3.86±.06	3.10±0.05
B	3.75±0.11	3.00±0.09
C	6.58±0.13	4.23±0.10
D	6.50±0.16	4.64±0.12
E	6.91±0.20	4.86±0.17

* Complete system contained (μ moles in 3.10 ml): propionyl CoA, 3; ATP, 6; $NaH^{14}CO_3$, 40 (total activity: 2 μC); $MgCl_2$, 25; KCl, 10; Tris 200; and an aliquot of the tested extract; the final pH was 8.00. Propionyl CoA carboxylase is determined as the incorporation of $H^{14}CO_3^-$ into methylmalonyl CoA. All values are expressed as n moles of ^{14}C-methylmalonate CoA/mg protein per 15 min; mean values of six experiments ± S.E.

** See Table 2.

TABLE 8. Relative activity of posphoenolpyruvate (PEP) carboxykinase in control and cadmium-exposed Pacific herring eggs at different stages of development*.

Developmental Stage**	Control Eggs	Cadmium-Exposed Eggs
A	0.82±0.09	0.75±0.10
B	11.52±0.21	8.05±0.17
C	60.47±2.71	40.12±1.86
D	92.34±4.43	64.77±2.86
E	81.11±3.92	55.10±4.00

* Complete system contained (μ moles in 3.00 ml) GTP, 10; $MnCl_2$, 10; oxaloacetate, 10; $NaH^{14}CO_3$, 100 (total activity: 2 μC); KCl, 20, and Tris 300; and an aliquot of the tested extract; the final pH was 7.00. PEP carboxykinase is determined as the exchange incorporation of $H^{14}CO_3^-$ into oxaloacetate. All values are expressed as n-mole ^{14}C-oxaloacetate/mg protein per 5 min; mean values of six experiments ± S.E.

** See Table 2.