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Embryotoxicity and Hatchability in *Cichlasoma nigrofasciatum* (Guenther) Eggs and Larvae Briefly Exposed to Low Concentrations of Zinc and Copper Ions

Patrick T. E. Ozoh and Carl-Olof Jacobson
Institute of Zoology, Uppsala University, Box 561, S-751 22 Uppsala, Sweden

In the life processes zinc and copper are essential elements but they are toxic in high concentrations. Aquatic biota accumulate metal ions and as a result of this accumulation metallic body burdens increase in these animals. A mixture of zinc and copper has a more than additive effect (highly synergistic) in fish (DOUDOROFF AND KATZ 1953, SPRAGUE 1970). The synergism may be attributed to zinc-copper couple effect or to some unknown mechanisms. There is a lack of information on whether the synergism of a mixture of zinc and copper which exists with adult fish is also valid for developing eggs of fish. Many reports are available on the effect of copper or zinc on the eggs of fish. These include works of SKIDMORE (1964), of BRUNGS (1969), of MCKIM AND BENOIT (1971), and of BLAXTER (1977).

It seems desirable to investigate the effect of copper and zinc singly and in combinations on the very sensitive embryonic period in fish life. Many effluents from industrial wastes and the mines contain copper and zinc. These discharges find their ways eventually into aquatic environment, where their metal contents interact simultaneously with all forms of biota. It is known that the effect of one factor and combinations of factors can be studied effectively by the use of factorial design (FISHER 1942). In this study, the orthogonal factorial method of DAVIES (1967) was applied in assessing the effect of single additions of copper and zinc ions together with their combinations on the eggs of CICHLASOMA nigrofasciatum Guenther. The eggs were exposed to the metal ions for 48 h, and then transferred back to tap water for incubation.

MATERIAL AND METHODS

Cichlasoma nigrofasciatum (Zebra ciclid) eggs spawned in the laboratory were used in the bioassay. The eggs were exposed to toxicant concentrations 6 h after being ovulated to ensure that the eggs used were fertilized. The eggs that were not fertilized turned whitish with time and were discared before the experiment. Reagent grades of zinc and copper nitrate salts supplied the metallic ions. Nine large glass vessels were provided for housing the toxicants and for renewing the toxic solutions in the dishes which housed the eggs. The concentrations of 0, 16, and 32 ppb of zinc and copper alone were prepared singly and in combinations. A

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total of nine combinations were made and 0 x 0 combination served as the control. The alkalinity of distilled water used in preparing the media was 1.6 ppm by weight of calcium carbonate. The concentrations of zinc and copper increase orthogonally, that is with identical increases (SNEDECOR AND COCHRAN 1967). All the concentrations of the toxicants were prepared one day before use and were analysed in an atomic absorption spectrophotometer on the second day to determine the accuracy of the prepared concentrations. Three days later the same solutions were analysed again, but no difference in the concentrations was noted.

Zebra ciclid eggs were reared in petri dishes at 25 $\pm$ 0.5^{o}C. The number of eggs in each dish was 127. Artificial aeration was provided. The eggs were exposed to the metal ions for 48 h and then transfered to tap water of the same temperature for the rest of the incubation period. Dead eggs were removed daily from the dishes. The incubation media were renewed daily from the main solutions. By these precautions accumulation of metabolites was kept down and the spread of infection was also minimized. The experiment was replicated 5 times for each of the nine combinations. Morphologic malformations seen in the pro-larvae and larvae were recorded. However, zebra ciclids have abundant yolk which make it difficult to observe the malformations when the pro-larvae are in the chorions. The hatchability was analysed, toxicity of zinc and copper ions was compared.

Analysis of variance was carried out on the hatching percentage and significance of each factor determined separately and in combinations with other factors. A regression equation of the type Y in function of X_1 and X_2 was carried out (X_1 was the concentrations in copper and X_2 was the concentrations in zinc).

RESULT

Embryotoxicity of the ions

The presence of copper ions in distilled water affected the eggs and larvae of zebra ciclids. The pro-larvae and larvae exposed briefly to copper ions for 48 h showed many morphological malformations. The malformed larvae have abnormal nervous systems. The area of the nervous system around the rhombencephalon was observed not as a straight and continuous tube as in the normal larva. Rather, the nervous system was invaginated into the yolk. This invagination caused the yolk structure to be bifurcated ventrally. But the zinc ions caused no observable malformations to the eggs or larvae. Only higher embryonic mortalities occurred in solutions of zinc ions than in either the control eggs or the eggs exposed to copper ions.

Effect of the ions on hatching

Exposure of Cichlasoma nigrofasciatum eggs to the concentrations of zinc and copper ions for 48 h affected hatching success. The 0 x 0 combination gave the mean hatching of 73%. However, fungus or bacteria were seen on eggs which died and were not removed until about 6 h later. But the copper concentration of 16 ppb yielded a

TABLE 1

Hatchability of Cichlasoma nigrofasciatum eggs exposed to different concentrations of metal ions (3 x 3 factorial design).

$Zn^{2+}(X_2)$ \ $Cu^{2+}(X_1)$	0 ppb	16 ppb	32 ppb
0 ppb	77 61 47 94 87	74 87 85 71 83	70 79 63 63 77
16 ppb	55 61 50 69 55	69 86 80 90 58	80 72 79 54 83
32 ppb	44 39 81 32 25	19 19 20 14 67	39 35 86 59 50

higher hatching (80%) than the control. Higher concentration of copper gave the mean hatching of 70%. The better hatching result in 16 ppb of copper was due to the bacteriostatic and fungistic properties of copper on the eggs. The reduced hatching percentage obtained in higher copper concentration (32 ppb) was due to the toxicity of copper. A similar higher hatching percentage was obtained also in low copper concentration by BLAXTER (1977) with herrings. Bacteriostatic effect of copper increased hatching since early egg mortality due to fungus and bacteria attack was reduced by copper ions. BLAXTER (1977) noticed bacteriostatic property of copper ions on herring eggs. The concentration of 16 ppb of zinc gave the mean hatch of 58%, and 32 ppb of zinc ions gave the much reduced hatching of 44%. Comparatively, the toxicity of zinc ions on the eggs was felt earlier than that of copper ions. The eggs affected by zinc toxicity died in their egg shells, while the majority of the eggs affected by copper ions reached the hatching stage with morphological malformations.

For the two factor interactions of zinc and copper, concentrations of 32 ppb of zinc x 16 ppb of copper gave the lowest hatching mean of 28%. The highest concentration of copper tested (32 ppb) gave the mean hatch of 70%, and the highest zinc concentration tested gave 44%, which on a purely additive basis gave 70% + 44% =114%. But table 1 shows that the highest combinations of

copper and zinc gave only 54% (bottom left). Higher embryonic mortalities that occurred in copper/ zinc ion mixtures were due probably to the synergistic effects of both zinc and copper on the eggs. A complete analysis of variance of table 1 was given in table 2.

TABLE 2

Complete analysis of variance of table 1 (hatching percent in 3 x 3 factorial with 5 replicates, L = linear, Q = quadratic).

MAIN EFFECTS	Df	Sum of Squares	F ratio
Cuppric (Cu^{2+})			
Cu L	1	399.24	1.67
Cu Q	1	6.60	0.03
Zinc (Zn^{2+})			
Zn L	1	7996.15	33.49^{xx}
Zn Q	1	1226.63	5.14^{x}
Two factor interactions			
Cu L x Zn L	1	188.68	0.79
Cu L x Zn Q	1	1445.60	6.06^{x}
Cu Q x Zn L	1	257.72	1.08
Cu Q x Zn Q	1	636.83	2.67
Remainder (Error)	37	8833.53	238.74

xx = Highly significant at p 0.01.
x = significant at p 0.05. Df = degrees of freedom.

Analysis of variance showed that an increase of copper ions from 0 to 32 ppb in distilled water has no significant linear or quadratic effect on the hatching of zebra ciclid eggs exposed to copper ions for 48 h. But exposure of zebra ciclid eggs for 48 h in zinc concentrations of 0 to 32 ppb have significant linear and quadratic effects at p 0.05 on the hatching of zebra ciclid eggs. Only the linear zinc concentration is highly significant at p 0.01.
The equation representing the effects is $Y = 64 + 3.65X_1 - 16.33X_2 + 0,27X_1^2 - 3.69X_2^2 + 3.07X_1X_2 + 4.91X_1X_2^2 - 2.07X_1^2X_2 + 1.88X_1^2X_2^2$.
It was not intended in this experiment to optimize hatching percent since the eggs were exposed briefly to the toxicants. Instead, it was intended to see how the toxicants affected the eggs and larvae.

DISCUSSION AND SUMMARY

The discharge of metals from industrial and mining effluents into aquatic environments imposes stresses on aquatic biota. SPRAGUE (1965) discovered that the disturbed migration of atlantic salmon was caused by sublethal pollution of the river by zinc and copper. A preliminary study was conducted using factorial design to determine the effect of single and combined copper and

zinc on the eggs of zebra ciclids. Zinc and copper mixtures gave high mortalities of the eggs. The synergistic actions of copper and zinc were found in fish by DOUDOROFF AND KATZ (1953), and by SPRAGUE (1970). The presence of copper ions in distilled water affected the nervous systems of zebra ciclid pro-larvae and larvae. The structures of the yolk were altered as the result of the malformed nervous systems. Similar malformation in the nervous system has been found in zebra fish pro-larvae and larvae exposed to copper ions (OZOH, in press: Bull. Environ. Contamin. Toxicol. 1978). The malformation in zebra fish was much more drastic than that found in the zebra ciclids. It would appear that the degree of malformation induced by copper ions was modifiable by the quantity of yolk possessed by the egg. Zebra fish eggs have small quantities of yolk compared to zebra ciclids. Besides the malformation of the nervous system induced by copper ions, both zinc and copper are known to cause stunted growth in fish (MCKIM AND BENOIT 1970, BRUNGS 1969).

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