THE TOXICITY OF ZINC AND COPPER TO STRIPED BASS EGGS AND FRY WITH METHODS FOR PROVIDING CONFIDENCE LIMITS

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ABSTRACT

Striped bass eggs, 24 hours after fertilization, and newly-hatched fry were tested to determine their sensitivity to zinc and copper. This study with those of Hughes will provide information on the most sensitive stage of these fish to acute exposure to lethal levels of zinc and copper.

Newly-hatched fry were more sensitive to both zinc and copper than were eggs, but copper, at the lowest concentration used, retarded hatching. Eggs and fry were more sensitive to copper than to zinc.

In order for acute toxicity studies to have a predictive value, confidence limits need to be placed on the TLm values obtained from toxicity studies. Results obtained from three different methods of providing confidence limits are discussed and the relative merits of each method are compared.

INTRODUCTION

Acute toxicity assays provide one means of defining the capacity of an aquatic system to absorb pollutants. These assays provide relative information about different pollutants, species of fish, and differences between life history stages but leave something to be desired in terms of accurate prediction. Some type of confidence limits would improve the use of acute toxicity information. The classic dosage response curves and time response curves (Bliss, 1935, 1937) as well as the median tolerance curves (TLm) provide no measures of variation or confidence limits. Probit analysis (Finney, 1964) and the methods of Litchfield and Wilcoxon (1947) provide confidence limits but these are based on expected not actual measures of variance (Finney, 1964). These methods also require assumptions about the linearity of the data. The present study was designed to provide information on the toxicity of zinc and copper to striped bass (*Morone* saxatilis) eggs and fry and to describe methods for providing confidence limits for TLm information. This study with those of Hughes (1968) and Welborn (1969, 1971) provide continuous acute toxicity information for zinc and copper on striped bass from the egg stage through advanced fingerlings.

METHODS AND MATERIALS

The eggs and fry used in this study were obtained and used at the South Carolina Wildlife Resources Commission hatchery at Moncks Corner, South Carolina, during the 1968 spawning run. This hatchery is used exclusively for striped bass fry production and is equipped with compressed air and a flowing water system.

One-gallon glass jars, containing three liters of water, aerated during the studies, were used as test vessels for both the egg and fry toxicity studies. The characteristics of the hatchery water supply are shown in Table 1. The concentrations used in the individual metal studies are shown in Table 2 with the

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analysis of samples for checking the amount of zinc and copper actually in the test containers.

The general recommendations of Standard Methods for the Examination of Water and Waste Water (1965) were followed. The toxicants used were Fisher Chemical Co. atomic absorption reference standards (zinc and copper oxides in dilute nitric acid) diluted to 100 mg/l zinc and copper respectively with hatchery water. Two drops concentrated hydrochloric acid was added to the standard solution to prevent precipitation. The pH of the test water was lowered no more than 0.2 pH unit by the addition of the toxicants.

The eggs used in toxicity studies were obtained 24 hours after fertilization to eliminate non-fertilization die off. Twenty to sixty eggs were used at each concentration with no more than one gram of eggs per liter of toxicant solution. Two controls were used in each replication.

During the first toxicity test retardation of hatching was noted in the copper test. After this run two categories were established for counting purposes, percent not hatched and percent dead. Percent not hatched is the percentage of eggs dead plus those eggs that have not hatched in the same time period as the controls. Percent dead is the percentage of eggs that have turned opaque or show abnormalities in the embryo.

Fry were obtained when the hatch was approximately 90 percent complete. Forty to 137 fry were used at each concentration with no more than 0.3 gram of fry per liter of toxicant solution. The length of the fry toxicity test was 48 hours.

Table 1. Water quality data for Moncks Corner, South Carolina, striped bass hatchery, 1968 - 1969 spawning seasons.

Test	Mean	Range	N
pH	7.8	7.5- 8.1	5
Alkalinity (Total) mg/l $CaCO_3$	117	140 -200	5
Hardness (Total) mg/l $CaCO_3$	137	110 -150	5
Hardness (Ca) mg/l $CaCO_3$	70	60 - 80	5
CO_2 mg/l	6		2
Conductivity umho/cm^2		600 -750	3
Temperature		58 - 67°F	
Zinc mg/l	0.28 + .03 S.E.		3
Copper mg/l	0.24 + .03 S.E.		3

Table 2. Concentration (mg/l) of toxicants used in individual ion toxicity tests*.

Desired Conc.	N	Zn - Measured Conc. (X + S.E.)	Cu - Measured Conc. (X + S.E.)
5.0	3	5.12 + 0.02	5.21 + 0.06
2.8	3	2.78 + 0.02	2.87 + 0.03
0.9	3	0.87 + 0.02	0.97 + 0.03
0.5	3	0.55 + 0.03	0.47 + 0.03
0.1	3	0.08 + 0.02	0.14 + 0.03
0.01	3	0.05 + 0	0.07 + 0.03

*The mean values for zinc and copper present in the hatchery water have been subtracted from the total amount measured at each concentration.

RESULTS

The raw data for the individual toxicity studies is found in Tables 4 and 5. The 50 percent mortality values obtained by the graphical TLm method, a method by Litchfield and Wilcoxon (1949), and by linear regression are shown in Tables 6 and 7. The tolerance values obtained by graphical TLm and the Litchfield and Wilcoxon methods are within the range of values for zinc and copper toxicities reported by McKee and Wolf (1963). The values obtained by linear regression are only to show the wide variation and a general curve shape. Correlation coefficients for the linear regressions were high: 0.94 and 0.92 for zinc with fry and eggs, respectively; 0.81 and 0.93 for copper with eggs and fry, respectively. A logarithmic transformation of the concentrations of copper had to be used to linearize the results of the toxicity studies. No results were combined in the linear regression studies; each study was treated as a replication for the statistical analysis.

Copper, at every concentration used, retarded hatching in the eggs as much as eight hours over the controls. The comparison of percent mortality and percent not hatched for the second replication of the egg and copper study is shown in Table 8. This effect was not shown in the egg studies with zinc. Grande (1967) reported a similar effect from zinc in salmonid eggs.

Fry show less tolerance to both zinc and copper than do the eggs. Pickering and Vigor (1965) reported that one-day old fathead minnow fry were more sensitive than the eggs to zinc. Hughes (1968) found TLm values for one-week old striped bass fry of 0.1 ppm zinc and 0.05 ppm copper; higher values found in this study are most probably a reflection of the increased calcium in the test water rather than the difference in age.

Table 4. Percentage of striped bass eggs and fry killed at varying concentrations of zinc.

Conc. (mg/l)	Eggs Replication 1	Eggs Replication 2	Fry Replication 1	Fry Replication 2
Control	4	0	16	7
0.01	0	7	26	13
0.1	3	13	13	17
0.5	17	25	24	30
0.9	34	41	26	68
2.8	64	63	88	100
5.0	65	76	67	75

Table 5. Percentage of striped bass eggs and fry killed at varying concentrations of copper.

Conc. (mg/l)	Eggs Replication 1	Eggs Replication 2	Fry Replication 1	Fry Replication 2
Control	4	0	16	7
0.01	11	7	12	8
0.1	6	7	26	7
0.5	24	27	65	64
0.9	77	52	100	71
2.8	84	72	100	100
5.0	95	91	100	100

Table 6. Fifty percent mortality values for striped bass eggs and fry exposed to zinc.

	Method of Calculation				
	TLm	Litchfield and Wilcoxon		Linear Regression	
		ED50	95% C.I.	50% Dead	90% C.I.
Eggs	1.85	1.77	2.51-1.25	3.09	+ 4.07
Fry	1.18	0.67	2.46-0.25	1.34	+ 2.31

Table 7. Fifty percent mortality values for striped bass eggs and fry exposed to copper.

	Method of Calculation			
	TLm	Litchfield and Wilcoxen		Linear Regression*
		ED50	95% C.I.	50% Dead
Eggs	0.74	0.85	1.23-0.605	.35
Fry	0.31	0.42	3.08-0.12	.12
Eggs		-0.4454 + 2.374		90% C.I.
Fry		-0.7696 + 2.138		90% C.I.

*Log transformed 50% mortality and confidence interval.

Table 8. Striped bass egg mortality as a function of varying environmental concentrations of copper ion.

Concentration	Percent Not Hatched*	Percent Dead
Control	0	2.1
0.01 mg/l	54.8	8.6
0.1	78.6	6.4
0.5	54.5	25.4
0.9	68.7	64.5
2.8	100	78
5.0	100	92.8

*Percent not hatched includes live eggs which did not hatch within the same time interval as the controls in addition to the percent dead. This column contains data from the second replication; the percent dead column is the mean of two replications.

DISCUSSION

This study with those of Pickering and Vigor (1965), Grande (1967), and Skidmore (1965) presents convincing evidence that newly hatched fry are least tolerant of zinc and copper. There are apparently two basic mechanisms for heavy metal toxicity (Skidmore, 1964); one operates through effects on the gills (Lloyd, 1965), and the other through longer term effects to liver, kidney, gonads, and spleen (Crandell and Goodnight, 1963). Lloyd (1960) proposes that in acute studies the rate at which the gills are able to prevent the accumulation of the metal ions determines the toxicity threshold. Skidmore (1964) and Baker (1969) reinforce this with reports of pathological damage to the gills in acute studies of zinc toxicity and sub-lethal levels of copper. These studies suggest that the fry, in their very early developmental stages, have not developed regulatory mechanisms for handling the increased zinc and copper. The response could probably be considered as individual cell adjustments. Skidmore (1966) in studying the effects of zin on zebrafish embryos found that unruptured eggs showed lower resistance than ruptured eggs. The unruptured eggs were apparently killed by opaque material forming between the outer egg membrane and the embryo. Skidmore's study used higher concentrations and longer times than were used with the striped bass, but the formation of the opaque material suggests that the material between the outer membrane and the embryo could tie up the metal ions, preventing their entry into the embryo. The shorter time required for hatching in the striped bass and the reduced concentration of zinc and copper could have accounted for not observing the opaque material.

In order to use the results of acute toxicity information for application factors or for relative information, some type of confidence limits need to be provided. The median tolerance limit (TLm) (APHA, 1965), dosage-response curves (Bliss, 1935), and time-response curves (Bliss, 1937) do not provide measures of variation or confidence limits. Probit analysis (Finney, 1964) and the methods of Litchfield and Wilcoxon (1947) provide confidence limits, but the limits are based on expected variances and not actual measures of variance (Finney, 1964). Another problem with probit analysis and the Litchfield-Wilcoxon methods is the assumption about linearizing the data; this assumption is not necessary in either the TLm method or the time-response method. The linear regression method used in this report requires only that the data be linearized through a standard transformation and provides confidence limits based on observed variances, not expected variances. This is the reason for the larger confidence interval reported in the results. A modification of the TLm method could also provide confidence limits using actual variances. The modified TLm method would consist of replications using different batches of fish. A statistical mean with confidence limits could then be calculated; this method requires no assumptions of linearity and the arithmetic is simple. With both the regression method and the modified TLm method, replications are necessary — the more the better — and should not be pooled. One advantage of the linear regression method over the TLm method, however, is the ability to predict values of other than 50 percent mortality.

CONCLUSIONS

1. Newly-hatched striped bass fry are more sensitive to zinc and copper than eggs.
2. Copper, in every concentration used, retarded hatching.
3. Routine replication of TLm studies, without pooling results will provide a simple method for providing confidence limits for acute toxicity studies.

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