NUREG/CR-0748
UCRL-52499

COPPER SENSITIVITY OF THE NORTHERN ANCHOVY, *Engraulis mordax*, DURING ITS EARLY LIFE HISTORY

D. W. Rice, Jr.
F. L. Harrison

Prepared for
U. S. Nuclear Regulatory Commission
by
Lawrence Livermore Laboratory

NOTICE

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Manuscript Submitted: June 30, 1978
Date Published:

Prepared for

SAFER
U.S. Nuclear Regulatory Commission
Washington, D. C. 20555
Under Interagency Agreement DOE 40-550-75
NRC FIN No. A 0119

by
Lawrence Livermore Laboratory
Livermore, CA 94550

operated by University of California

for the U.S. Department of Energy

FOREWORD

This study is part of a larger research project that has three purposes: (1) to study the behavior of potentially toxic substances introduced into surface waters from nuclear power plants, (2) to determine the magnitude of the impact of these substances on representative economically important aquatic species, and (3) to develop models to predict the behavior and impact of these discharged substances. The initial thrust of the research has been directed toward investigating the impact of cooling systems' corrosion products, in particular copper. Copper is of special interest because of its documented toxicity to aquatic organisms.

This investigation was funded by the Office of Nuclear Regulatory Research, Division of Safeguards, Fuel Cycle and Environmental Research, under FY 1976 Nuclear Regulatory Research Order No. 60-76-144.

The authors wish to thank Dr. Ambrose Jerald, Jr., Mr. Revelle Davis, Mr. John Dawson, and Ms. Rose Carrillo for their assistance in the collection, handling, and observation of the test organisms.

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ABSTRACT

The sensitivity to copper of embryonic and larval stages of the Northern anchovy, *Engraulis mordax*, was determined using a flow-through bioassay system. Anchovy embryos were exposed continuously from 8 to 10 h after fertilization until hatching, and the larvae were exposed within 24 h after hatching until yolk-sac absorption. During the testing, both total copper concentrations and the percent copper in labile forms were determined. From the cumulative mortality vs measured copper exposure data, a series of median lethal concentrations (LC_{50}) were determined. These LC_{50} values were used to construct comparative toxicity curves.

The anchovy life stage most sensitive to copper was the embryonic stage. For the anchovy embryo, the 12-h LC_{50} was 200 µg Cu/l, and the estimated incipient lethal concentration (ILC_{50}) was 190 µg Cu/l; a sensitive period of embryonic development was noted prior to closure of the blastopore. The 12-h, 24-h, and ILC_{50} for anchovy larvae were 460, 400 and 370 µg Cu/l, respectively.

SUMMARY

The effects of copper on the embryonic and larval stages of the Northern anchovy were investigated. Sensitivity to copper was evaluated because copper is known to be one of the most toxic heavy metals to a variety of marine species, and is discharged into coastal waters by the release of municipal waste waters, power plant effluents, and the use of marine anti-fouling paints. Anchovy early life stages were used because natural mortalities that occur during the early stages of marine fish have been suggested to be a major factor in reducing the size of a given year-class of fish. Pollutants that have an impact on the survival of fish embryos or larvae might further reduce the size of a given year-class of fish.

Anchovy embryos and larvae were exposed continuously to different test concentrations in flow-through bioassay systems. The concentrations of the labile as well as the total copper in the exposure water were determined because the chemical form of the copper to which the fish are exposed may play an important role in the toxic response. Complexation of copper to some organic ligands has been shown to reduce toxicity.

The anchovy life stage most sensitive to copper was the embryonic stage. For anchovy embryos, the 12-h LC_{50} was 200 µg Cu/l, and the estimated incipient concentration (ILC_{50}) was 190 µg Cu/l. The ILC_{50} is defined as that concentration allowing 50% of a population to survive for an indefinite period of time. A sensitive period of embryonic development was noted prior to closure of the blastopore. The 12-h, 24-h, and ILC_{50} for anchovy larvae were 460, 400, and 370 µg Cu/l, respectively. The percent of the total copper in the labile (noncomplexed) form for all concentrations used during testing averaged 96%.

During an earlier study, we examined the sensitivity of the early stages of another marine fish, the Pacific herring. The anchovy and herring have very

different spawning strategies. The anchovy spawns a fragile, pelagic egg and the herring spawns a tough, demersal egg. For both species the embryo was most sensitive, though the demersal embryo was six times more sensitive than the pelagic embryo.

Information currently available on marine fishes indicates that the life stage most sensitive to copper is the embryonic stage, but for fresh-water fishes, the larval stage is the most sensitive stage. This difference in comparative sensitivity between embryos and larvae of marine and fresh-water fishes suggests that caution should be exercised in applying the large volume of results using fresh-water fishes to marine fishes.

RECOMMENDATIONS

1. Relationship of Ovoviparous Spawning Strategy to Copper Sensitivity

Experiments should be conducted in which the effect of sublethal adult exposure is related to the embryonic and larval development of ovoviparous fishes. The anchovy and herring represent the pelagic and demersal spawning strategies, respectively. An additional spawning strategy that should be examined is that employed by the ovoviparous fishes. Embryos of such fishes are retained in the lumen of the ovary until shortly before yolk sac absorption is complete. Fewer embryos and larvae are produced compared with other spawning strategies, and sublethal impacts on adults carrying embryos may have gross effects on fecundity.

2. Effect of Copper-Laden Sediments

Experiments should be conducted to determine the roll that copper-laden interstitial waters and sediments play in the toxic exposure of demersally spawned marine fish embryos. Fishes' embryos spawned onto near bottom environments are exposed not only to copper in the water column, but also to sediments that may contain high levels of copper. This copper may be mobilized from the sediments and be present in the sediment water interface at concentrations many times above ambient water concentrations. Based on copper exposure in water only, the demersal spawning strategy, as represented by the Pacific herring embryo, appears to be more sensitive than the pelagic anchovy embryo (ILC_{50} values of 33 µg Cu/l, respectively). Exposures of demersal embryos using substrates contaminated to known levels should be conducted.

3. Effect of Chronic Sublethal Exposure

Experiments are required to examine the feeding success of fishes' embryos and larvae exposed to sublethal copper concentrations. Although there will probably be no accute effects on anchovy embryos and larvae from power plant

copper discharges, except during start up, more information is needed on sublethal effects. Marine fish larvae must begin appropriate feeding behavior prior to yolk absorption. Subtle toxic effects that alter first feeding behavior may have an important impact on the subsequent size of a year-class of fish.

INTRODUCTION

Copper is one of the wastes commonly discharged into coastal waters that has been shown to be toxic to marine fishes (Becker and Thatcher, 1973; Lewis and Whitfield, 1974). Increased copper concentrations in coastal marine waters have resulted from the release of municipal wastewater (Schafer, 1977), power plant effluents (Young, et al., 1977), and marine anti-fouling paints (Young and Alexander, 1977). In polluted waters, concentrations have been recorded as high as 16,800 µg Cu/l in municipal wastewater (Schafer, 1977) and 1,800 µg Cu/l during start-up of a power plant (Martin, et al., 1977).

One important factor in the toxic effect of copper on marine fishes is the life history stage when exposure occurs. Few studies have examined the comparative sensitivities of the major life stages of marine fishes: embryo, larva, and adult. The Spot, *Leiostomus xanthurus*, was found to be more sensitive to copper in the embryonic stage than in the larval stage (Engel, et al., 1976). The incipient lethal concentration (ILC_{50}) for Pacific herring embryos exposed to copper was found to be approximately 30 times lower than the ILC_{50} for herring larvae (Rice and Harrison, 1978) and seven times lower than the ILC_{50} for herring adults (Harrison and Rice, 1979). Natural mortalities that occur during the early life stages have been suggested to be a major factor in reducing the size of a given year class of fish (May, 1974; Cushing, 1975; Vaughan and Saila, 1976). Pollutants that have an impact on the survival of fish embryos or larvae might further reduce the size of a given year class of fish.

In addition to life stage, the chemical form of copper to which fishes are exposed may play an important role in the toxic response (Lee, 1973; Neff and Anderson, 1976; Chapman and McCrady, 1977). Copper in seawater can exist in many forms. Labile forms include ions, ion pairs, and weakly associated organic and inorganic complexes, whereas bound forms include stable organic and inorganic complexes (Batley and Florence, 1976; Harrison, et al., 1979). Although current copper emission standards are defined in terms of the total

copper concentration in the water (Anonymous, 1972 and 1976; Klapow, 1978), complexation of copper has been shown to reduce its toxicity to marine organisms (Lewis, et al., 1973; Davey et al., 1973; Sunda and Guillard, 1976); Knezovich, et al., 1977). Ionic copper has been suggested as the form most toxic to fresh-water fishes (Pagenkopf, et al., 1974). During our testing of the early stages of the Northern anchovy, we determined both total copper concentrations and the percent copper in the labile forms.

Northern anchovies are pelagic, filter-feeding fishes that spawn in upwelling waters along the Pacific coast of the United States and Mexico (Ahlstrom, 1960). During recent years, the anchovy catch has been the third largest commercial catch on the Pacific coast (McAllister, 1976; Pinkas, 1977). Having conducted earlier tests on the sensitivity to copper of Pacific herring during its early life stages (Rice and Harrison, 1978), we set two objectives for the present study: to conduct similar tests on the early stages of Northern anchovies and to compare the sensitivities of these two species of fish that have different reproductive strategies. We continuously exposed anchovy embryos and larvae to copper over a range of concentrations and then constructed comparative toxicity curves.

EXPERIMENTAL METHODS

COLLECTION AND HANDLING OF TEST ORGANISMS

Northern anchovy embryos were collected in San Francisco Bay, California, between the Tiburon Peninsula and Angel Island. Collections and tests were carried out over a period of two y. Collections were made with a 0.5-m, 505-µm nylon plankton net, towed for 2 min just below the surface of the water. Collections from each tow were placed into a plastic bag, the bag was inflated with air, and the bag was then held in an insulated ice chest containing seawater from the collection site. Water temperature at the collection site was between 17°C and 18.5°C; upon arrival at the Lawrence Livermore Laboratory (LLL), the ice chest was kept below 19°C. All transfers of embryos or larvae were carried out with a large-bore, polished glass pipette. During embryo tests, healthy embryos estimated to be 8 to 10 h old (Ahlstrom's stages IV-V, Ahlstrom, 1943) were either placed directly into exposure chambers containing seawater with known concentrations of copper or allowed to hatch in control seawater. Larvae used for test I hatched within the 24-h period preceding the test. Larvae used for tests II and III hatched within the 12-h period preceding the tests. Collected larvae were placed directly into exposure chambers containing seawater with known concentrations of copper. Approximately 50 embryos or 30 larvae were used in each exposure chamber.

FLOW-THROUGH BIOASSAY SYSTEM

Anchovy embryos and larvae were exposed to copper in 500-ml, clear-glass, flow-through exposure chambers (Fig. 1) which, in turn, were immersed in a constant-temperature water bath (mean temperature: 16.8 ± 1.0°C, except larval test I, in which the mean temperature was 12°C). Seawater containing a known concentration of copper was pumped into each chamber from a 19-l plastic jug at a rate of 5 ml/min. Approximately 5 h were required to replace 95% of the water in the exposure chamber; the mixture of seawater and copper in the 19-l plastic jugs was prepared daily.

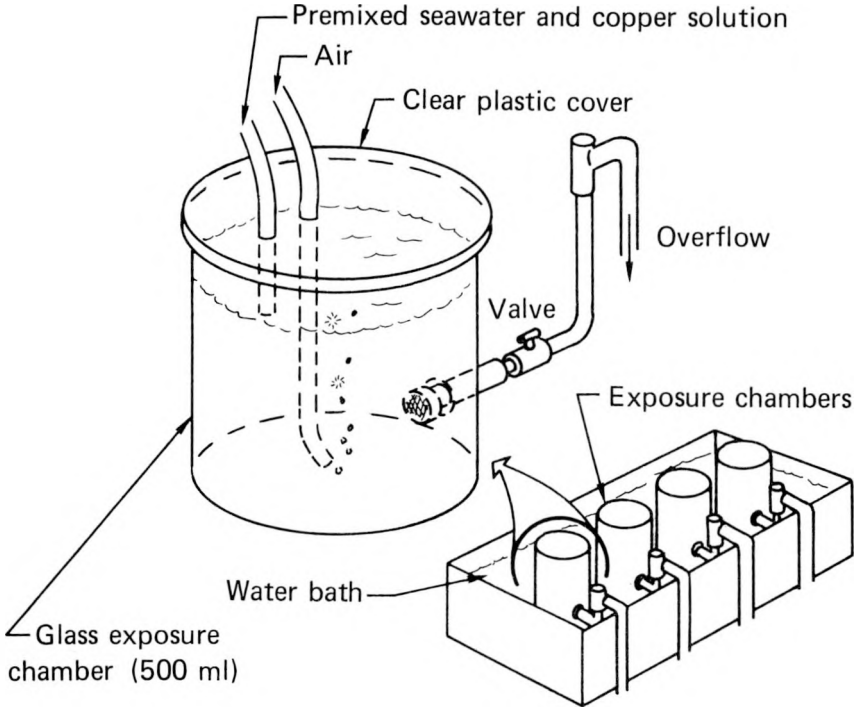

FIG. 1. Diagram of the exposure chamber and flow-through system used to expose anchovy embryos and larvae to copper.

The height of water in the exposure chambers was maintained by a constant-level, outflow siphon. The mouth of the siphon, located at the base of the exposure chamber, was covered with nylon netting (265-µm pore size) to prevent the loss of organisms from the chamber. The bottom outlet from each exposure chamber was fitted with a valve that could be closed to allow removal of the chamber from the water bath. This was important because during each observation the exposure chambers were removed and illuminated from the side. In this manner, both live and dead organisms were examined. Observations were made every 2 to 4 h during the copper exposure. During embryo exposure, a gentle stream of bubbles was delivered to the bottom of the exposure chamber; during larval exposure, no aeration was used. Overhead illumination was provided by the fluorescent lighting in the laboratory and followed the regular ambient photoperiod. The nominal copper concentrations used, the dates on which the tests were conducted, and the total number of embryos or larvae exposed during each test are given in Tables 1 and 2.

Exposures continued until (1) all animals died; (2) in the case of the embryos, until hatching was complete; or (3) in the case of the larvae, until

TABLE 1. Anchovy embryos exposed to copper and results of water analyses.

Nominal concentration, µg Cu/l	Measured total copper ($\bar{X}$ ± S.D.), µg/l	Labile copper, %	Water analysis, no.	Embryos tested
Test I (8/7/76)				
100	116 ± 14	--[a]	4	90
200	171 ± 31	--	4	94
300	285 ± 19	--	4	96
600	589 ± 28	--	4	104
Control	1	--	1	114
Test II (8/23/76)				
150	177 ± 30	--	7	124
200	197 ± 41	--	6	161
250	242 ± 46	--	5	112
600	531 ± 66	--	3	126
Control	1	--	1	112
Test III (7/27/77)				
100	92 ± 8	90.7	2	28
200	190 ± 12	96.1	2	34
250	272 ± 22	93.9	4	66
600	564 ± 39	95.4	2	28
Control	1	--	1	34

[a]Not measured.

TABLE 2. Anchovy larvae exposed to copper and results of water analyses.

Nominal concentration, µg Cu/l	Measured total copper ($\bar{X}$ ± S.D.), µg/l	Labile copper, %	Water analysis, no.	Larvae tested, no.
Test I (8/10/76)				
100	--[a]	--	--	50
200	--	--	--	100
300	--	--	--	100
600	--	--	--	117
Control	--	--	--	50
Test II (7/6/77)				
100	98 ± 1	95	2	56
300	277 ± 6	99	4	71
400	427 ± 7	94	3	61
500	531 ± 42	94	2	60
Control	1	--	1	51
Test III (7/13/77)				
100	143 ± 21	97	2	37
200	198 ± 29	99	2	67
300	289 ± 13	99	2	33
700	724	99	1	26
Control	1	--	1	23

[a]Not measured.

the yolk sac was absorbed. The criterion for embryo mortality was the appearance of opacity of the embryo, and the criterion for larval mortality was failure to respond to a prod with a polished glass rod. Cumulative mortality with time, percent hatching, and the stage of development at mortality were taken to be indices of the toxic effect of copper.

COPPER ANALYSES

Total copper concentrations were measured during each test except the larval test I. Labile copper concentrations were measured during embryo test III and larval tests II and III. Total copper in samples containing $\geq$200 µg Cu/l was determined by direct aspiration of seawater into the flame of a Model 303 Perkin Elmer atomic absorption spectrophotometer (AAS) with deuterium background corrector; total copper in samples containing <200 and $\geq$10 µg Cu/l was determined by direct injection of a sample aliquot into an HGA 2100 model graphite furnace after one-to-one dilution of the sample with ultrapure 2 N HNO_3. Labile copper, defined as that fraction passing through a 0.45-µm filter and retained by Chelex 100 resin, was determined by the method of Riley and Taylor (1968). Eluants from the columns were analyzed directly in the flame or in the graphite furnace of the AAS.

TOXICITY CURVES

The primary measure of toxicity for this study was the copper concentration resulting in 50% mortality over a given time (median lethal concentration, LC_{50}). This toxicity measure was determined by performing a weighted linear regression analysis on the sets of cumulative mortality data, corrected for control mortality, using the logistic function. The straight-line transform of the logistic function was:

$$\text{logit } P = \ln P/Q = \alpha + \beta\chi \quad .$$

Thus, if logit P was plotted against χ, the points fell on a straight line with α as the intercept and β as the slope (Berkson, 1953).

In our calculation of LC_{50}, χ represented the copper concentration. Our method followed that outlined by the American Public Health Association (1976) except that the logit analysis was used in place of a probit analysis. For each observation time, an estimated LC_{50} value was determined. The series of LC_{50} values obtained were used to construct a toxicity curve that was used to estimate the ILC_{50} (Sprague, 1969). Ninety-five percent confidence intervals were calculated for each LC_{50} value.

RESULTS

WATER ANALYSIS

The mean total copper concentration, the mean percent copper in labile forms, and the number of analyses performed are given in Tables 1 and 2.

MORTALITY

Embryo Exposures

Anchovy embryos continuously exposed to copper showed high mortality during the first 8 to 10 h of exposure. After 10 h, the mortality rate was relatively constant until hatching (Figs. 2-4). Two types of developmental abnormalities were noted at mortality. The first type was observed predominantly during the initial 8 to 10 h of exposure and accounted for varying proportions of the total mortality, depending on the copper exposure concentration (Table 3). These embryos appeared to have had their epiboly disrupted; the yolk was naked, and a deformed, opaque mass of protoplasm was found at the animal pole. Embryos with the second type of developmental abnormality appeared similar to normally developing embryos (the embryo encircling the yolk sac), except for an opacity of the embryo. In embryos with either type of developmental abnormality, the chorion was clear at the time the embryos were removed from the exposure chambers. However, during preliminary testing, we noted that the chorion became opaque when dead embryos were allowed to remain in copper concentrations as low as 100 µg Cu/l for a period of time. Embryos with both types of developmental abnormalities were found at the bottom of the exposure chambers, whereas normal-appearing embryos were found at or near the surface of the water except just before hatching, when they tended to sink. The estimated mean hatching time from the start of copper exposure was 32, 33, and 37 h for tests I, II, and III, respectively, indicating that in each

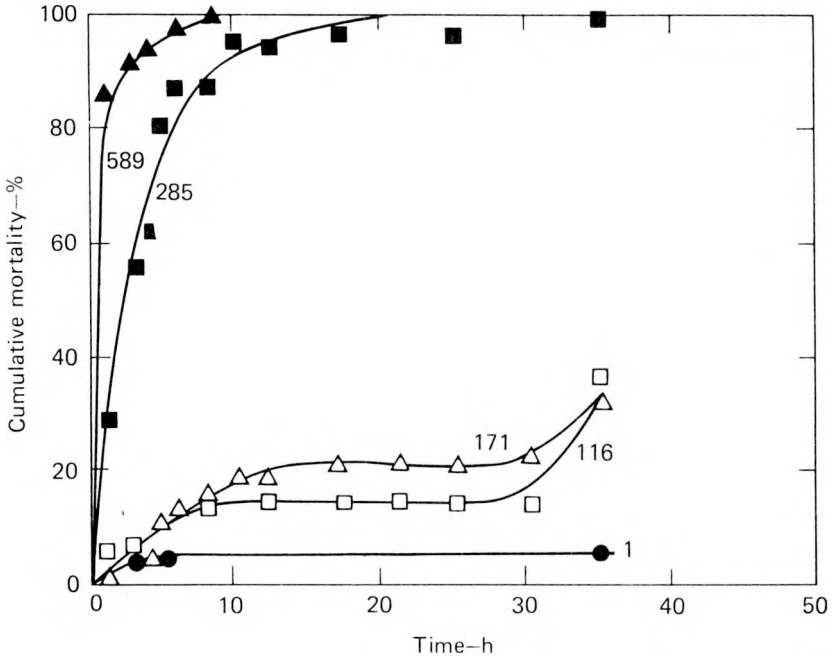

FIG. 2. Percent cumulative mortality of anchovy embryos continuously exposed to copper in Test I. Numbers next to curves give the exposure concentration in µg Cu/l.

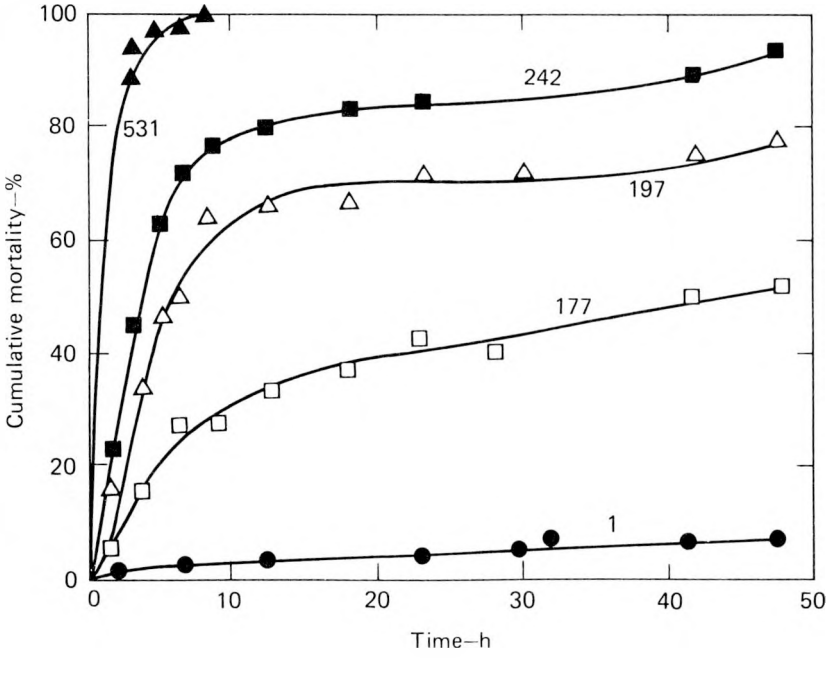

FIG. 3. Percent cumulative mortality of anchovy embryos continuously exposed to copper in Test II. Numbers next to curves give the exposure concentration in µg Cu/l.

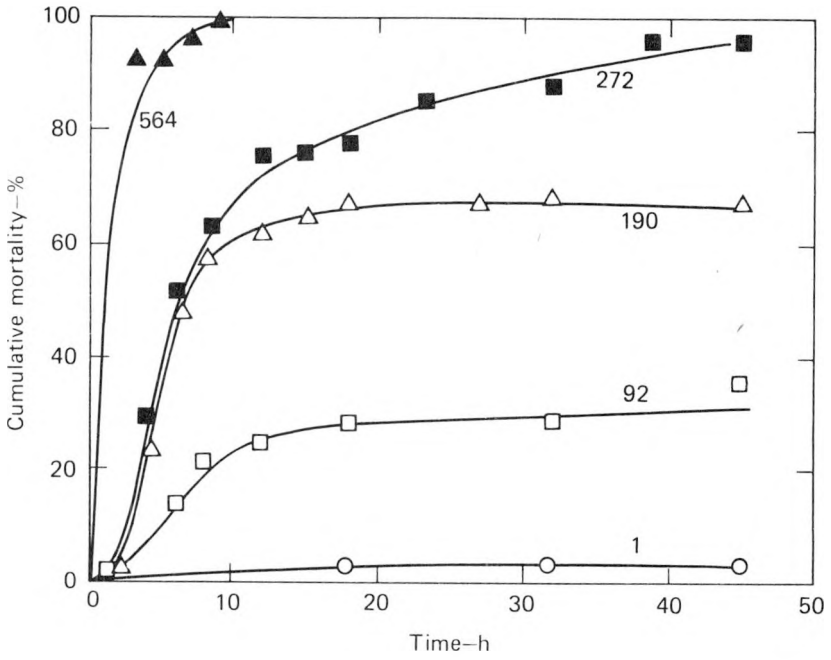

FIG. 4. Percent cumulative mortality of anchovy embryos continuously exposed to copper in Test III. Numbers next to curves give the exposure concentrations in µg Cu/l.

test the embryos were exposed during similar developmental periods. Hatching success was high for controls and decreased with increases in the exposure concentration.

TABLE 3. The results of copper exposure concentration on type of developmental abnormality and the percent anchovy embryos hatching.

Nominal concentration µg Cu/l	Pooled tests	Embryos showing abnormality, type, %		Hatching, %
		type I (disrupted epiboly)	type II (dead after epiboly)	
Control	II, III	3	4	93
100	III	4	32	64
150	II	34	20	46
200	II, III	63	17	20
250	II, III	60	35	5
600	II, III	96	2	2

Larval Exposures

Anchovy larvae continuously exposed to concentrations below 200 µg Cu/l consistently showed better survival than did the controls (Figs. 5-7). Control mortalities were high, but followed the general pattern for larvae not fed during yolk sac absorption (O'Connell and Raymond, 1970; Lasker, et al., 1970). The period of yolk absorption was estimated to be between 24 and 30 h from start of exposure for tests II and III, but later in test I because this test was conducted at a lower temperature. During exposures above 200 µg Cu/l, synergism between copper toxicity and starvation may have played a role in mortality and the shape of the 277 and 289 µg Cu/l mortality curves (Figs. 6 and 7 suggest this effect).

No obvious abnormalities were noted in the dead larvae other than an opacity of the body. Before death, larvae tended to sink to the bottom of the exposure chambers and often exhibited head-shaking movements and whip movements in which head and tail met.

Toxicity Curves

Examples of the cumulative mortality data used to calculate the LC_{50} values and to generate the toxicity curves (Fig. 8) are given in Tables 4 and 5. The 95% confidence interval associated with each calculated LC_{50} is shown, along with a chi-square value.

The comparative embryonic and larval toxicity curves reflected several developmental changes in sensitivity. A slight increase in copper sensitivity can be seen in the embryos during hatching. When we estimated the ILC_{50}, we considered only mortalities before hatching. For embryos, the estimated ILC_{50} was found to be 186 µg Cu/l, and was reached approximately 24 h after the start of copper exposure.

The sudden increase in mortality of the larvae at about 50 h probably was the result of starvation. Only mortalities before this time were considered in the estimated ILC_{50}. The ILC_{50} for anchovy larvae was found to be higher than for embryos, 370 µg Cu/l, and was reached approximately 32 h after the start of copper exposure. The estimated 24-h LC_{50} was 398 µg Cu/l.

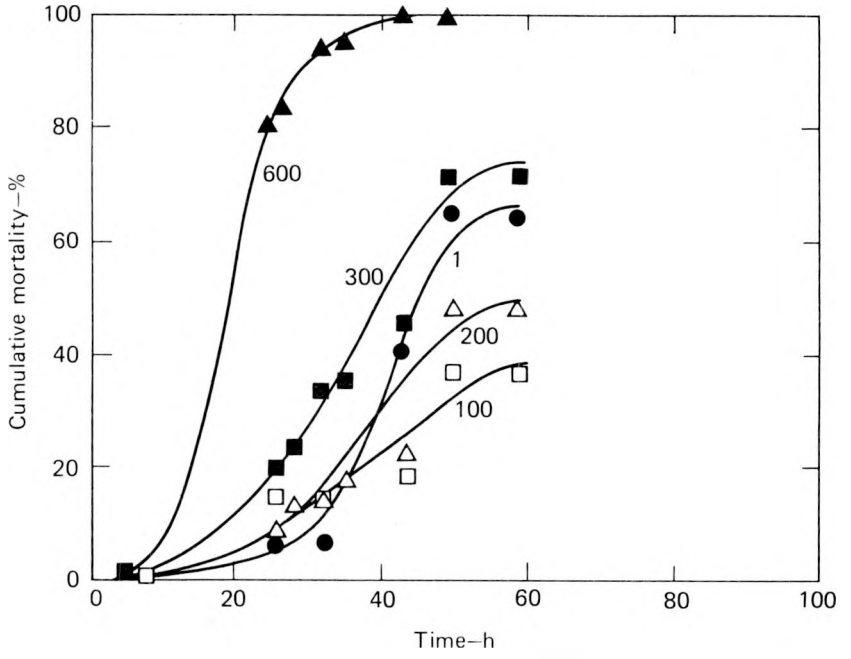

FIG. 5. Percent cumulative mortality of anchovy larvae continuously exposed to copper in Test I. Numbers next to curves give the exposure concentrations in µg Cu/l.

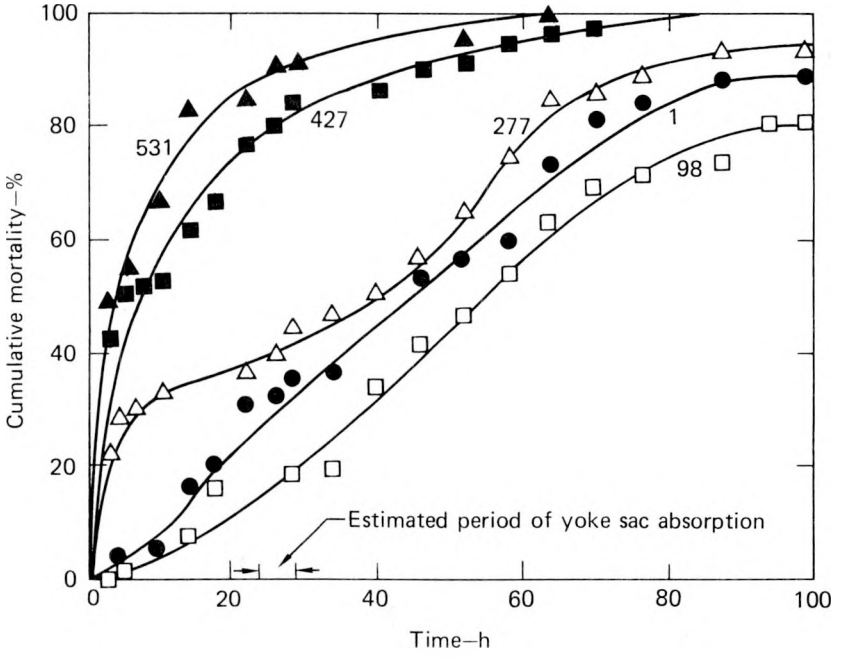

FIG. 6. Percent cumulative mortality of anchovy larvae continuously exposed to copper in Test II. Numbers next to curves give the exposure concentrations in µg Cu/l.

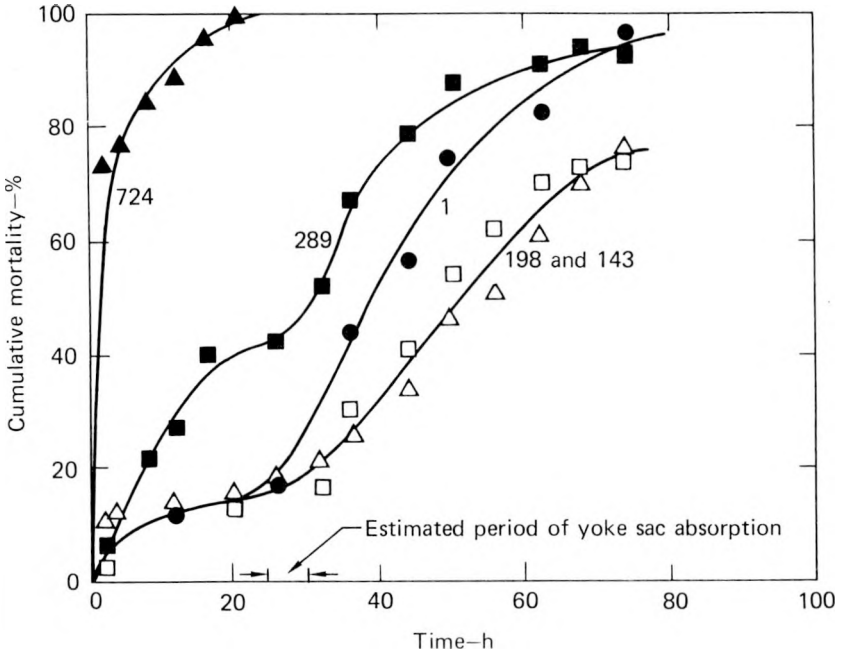

FIG. 7. Percent cumulative mortality of anchovy larvae continuously exposed to copper in Test III. Numbers next to curves give the exposure concentrations in µg Cu/l.

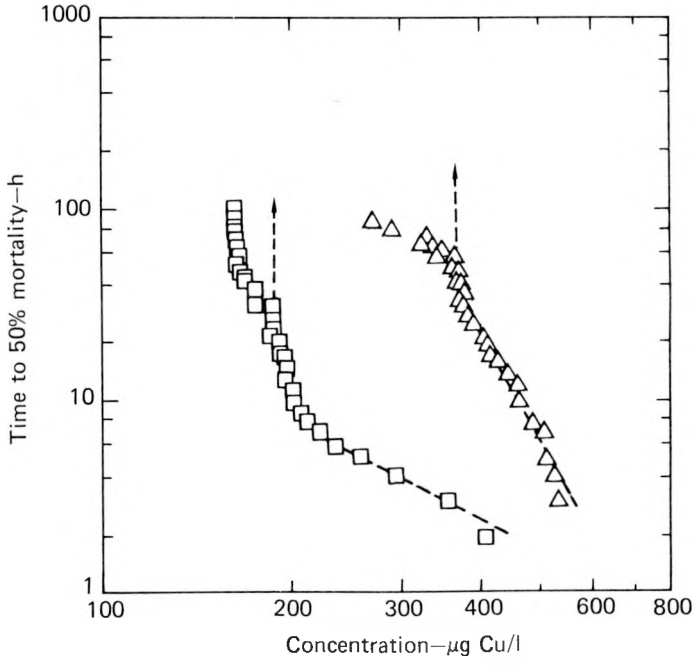

FIG. 8. Toxicity curves for anchovy embryos and larvae continuously exposed to copper. Vertical arrows indicate estimated incipient lethal concentrations.

TABLE 4. Toxicity tables for anchovy embryos showing sample data used to generate LC_{50} values. Data were then used to plot toxicity curve.

Exposure time, h	Proportion dead[a] at measured exposure concentrations, µg Cu/l													Logit-calculated LC_{50},[b] µg Cu/l	Chi-square value	Slope	Degree of freedom
	1	92	113	171	177	190	197	242	272	285	531	564	589				
2	0.00	0.04	0.02	0.00	0.02	0.03	0.16	0.21	0.03	0.27	0.77	0.93	0.85	409 ± 22	35.27	0.01072	10
4	0.00	0.04	0.03	0.01	0.14	0.24	0.32	0.44	0.29	0.61	0.96	0.93	0.94	292 ± 16	64.51	0.01347	11
6	0.00	0.14	0.03	0.08	0.14	0.47	0.44	0.63	0.51	0.86	0.98	0.96	0.98	235 ± 10	127.9	0.01885	11
8	0.00	0.21	0.09	0.12	0.25	0.59	0.49	0.72	0.64	0.88	0.98	1.00	1.00	213 ± 8	72.38	0.02114	11
12	0.00	0.25	0.09	0.15	0.27	0.62	0.62	0.76	0.75	0.95	1.00			199 ± 8	63.97	0.02632	9
18	0.00	0.27	0.09	0.17	0.31	0.67	0.65	0.80	0.78	0.98	1.00			193 ± 7	68.91	0.02737	9
25	0.00	0.27	0.09	0.17	0.38	0.67	0.69	0.84	0.85	0.98	1.00			186 ± 6	63.02	0.02980	9
32	0.00	0.27	0.09	0.17	0.39	0.67	0.70	0.84	0.88	0.98	1.00			185 ± 9	60.96	0.03062	9

[a]Corrected for control mortality.

[b]±95% confidence limit.

TABLE 5. Toxicity tables for anchovy larvae showing sample data used to generate LC_{50} values. Data were then used to plot toxicity curve.

Exposure time, h	Proportion dead[a] at measured exposure concentration, µg Cu/l						Logit-calculated LC_{50},[b] µg Cu/l	Chi-square value	Slope	Degree of freedom
	1	277	289	427	531	724				
4	0.00	0.14	0.02	0.42	0.49	0.76	523 ± 58	9.987	0.008341	4
8	0.00	0.15	0.18	0.49	0.53	0.84	485 ± 54	6.111	0.008296	4
12	0.00	0.16	0.16	0.50	0.65	0.87	457 ± 46	3.853	0.009461	4
20	0.00	0.07	0.30	0.59	0.79	1.00	412 ± 34	9.830	0.01369	4
26	0.00	0.06	0.30	0.71	0.85	1.00	391 ± 31	11.400	0.01591	4
32	0.00	0.08	0.41	0.75	0.87	1.00	375 ± 31	16.080	0.01552	4
40	0.00	0.11	0.41	0.73	0.87	1.00	374 ± 32	12.800	0.01487	4
46	0.00	0.01	0.51	0.79	0.87	1.00	372 ± 30	38.280	0.01652	4

[a]Corrected for control mortality.

[b]±95% confidence limit.

DISCUSSION

EMBRYO VS LARVAL SENSITIVITY

We found the embryonic stage of the Northern anchovy to be more sensitive to copper than the larval stage. This is in keeping with the majority of previous studies of marine fishes' early life stages (Engle, et al., 1976; Blaxter, 1977; Rice and Harrison, 1978). In contrast, studies examining the copper sensitivity of various life stages of freshwater fishes revealed that the larval stages are the most sensitive to copper (Hazel and Meith, 1970; McKim and Benoit, 1971; O'Rear, 1972; Gardner and LaRoche, 1973; Benoit, 1977; McKim, et al., 1978). This difference in comparative sensitivity between embryos and larvae of freshwater and marine fishes suggests that caution should be exercised in applying the extensive results of toxicity tests on freshwater fishes to marine fishes.

ANCHOVY VS HERRING SENSITIVITY

The adult Northern anchovy and Pacific herring are similar in form and in behavior but their reproductive strategies are quite different. The anchovy spawns an eliptical, transparent, pelagic egg, 1.0 × 0.5 mm in size, which hatches in about 48 h at 17 to 18°C into a fragile, unpigmented larva, 2.5 to 3.0 mm long (Ahlstrom, 1956). The Pacific herring spawns demersal, adhesive eggs on shallow intertidal substrates; the 1.3- to 1.6-mm-diam egg is covered by a thick, three-layered chorion (Blaxter, 1963). Herring eggs hatch in 7 to 9 d at 14°C into a pigmented larva, 5.0 to 6.0 mm long (Alderdice and Velsen, 1971). Comparisons of the sensitivities of the early life stages of these two fishes may prove useful for predicting the impact of copper on broad groups of fishes. For comparisons between the copper sensitivity of anchovy and herring embryos and larvae, the data on herring sensitivity are taken from our earlier study (Rice and Harrison, 1978).

It might be expected that the fragile anchovy embryo would be more sensitive to copper than the larger, tougher herring embryo; in fact, however, the opposite appears to be the case. The ILC_{50} for anchovy embryos was approximately six times higher than for herring embryos.

The differences in sensitivity seen between the two embryos may be the result of differences in chorionic structure and developmental period. The chorion of herring (Rosenthal and Sperling, 1974) and another demersal adhesive egg, the Baltic garpike, *Belone belone* (Dethlifsen, et al., 1975) have been shown to concentrate cadmium. The chorion of the herring may be the site of mechanisms to accumulate metals, but these mechanisms may be reduced or lacking in the anchovy. Changes in sensitivity during development were seen in both the anchovy and herring embryos. The high percentage of anchovy mortalities during epiboly suggests that this period of development might be more sensitive to copper than later developmental periods. Increased copper sensitivity during this period was also found for winter flounder, summer flounder, and mummichog (Cardin, 1976). The sensitive period for the herring embryo appeared to be approximately 96 h after fertilization, well beyond epiboly.

Differences in sensitivity were also seen between the two larvae. The fragile anchovy larvae was about three times more sensitive than the herring larvae. Both anchovy and herring larvae displayed spasms before death at the higher copper concentrations to which they were exposed. Such spasms during copper poisoning have been suggested to be similar to those seen in Wilson's Disease (Baker, 1969).

IMPLICATIONS OF SENSITIVITY DIFFERENCES

If the sensitivity differences observed in these two different spawning strategies holds true for other species, then nearshore groups of fishes (e.g., cottids, pholids, stickids, and other groups of fishes that spawn a large demersal adhesive egg; Hart, 1973) might be vulnerable to the pollutants in many coastal outfalls of municipal waste and power plant effluents. Polluted sediments and their interstitial waters associated with such outfalls have been shown to be many times higher in copper than open ocean waters (Duchart, et al., 1973; Greig and McGrath, 1977), and it is onto these surfaces

that demersal eggs are often spawned; such sediment extracts have been shown to be toxic to marine fish larvae (Hoss, et al., 1974). Outfalls sited further offshore, however, might expose different groups of fishes with pelagic spawning strategies; such fishes might be more resistant to the toxic effects of copper.

ACKNOWLEDGMENTS

The authors thank Dr. A. Jearld, Jr.; R. Davis; J. Dawson; and R. Carrillo for their assistance in the collection, handling, and observation of the test organisms.

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