## RESEARCH PROJECT TERMINATION REPORT OWRRI PROJECT NO. A-033-OKLA

## THE CHRONIC EFFECTS OF COPPER AND ZINC ON FISHES

Submitted to

The Oklahoma Water Resources Research Institute Oklahoma State University Stillwater, OK

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The work upon which this report is based was supported in part by funds provided by the U.S. Department of the Interior, Office of Water Resources Research, as authorized under the Water Resources Research Act of 1962.

The initial objectives of the research project were

- (1) To determine the effects of sublethal concentrations of copper on the growth rates of fishes raised from egg to juvenile stages in aquaria.
- (2) To determine the effects of sublethal concentrations of zinc on the growth rates of young fishes.
- (3) To determine the synergistic effects of mixtures of sublethal concentrations of copper and zinc on the growth rates of young fishes.
- (4) To evaluate the histological differences between fishes raised for the first three objectives. Any histopathology that can be attributed to the treatments will be described. Since copper is known to retard growth, some specimens can be expected to remain in larval stages for extended periods. The histology of these specimens will be examined to determine if cellular differentiation and organ development is in fact retarded or if differentiation has continued and the structures are just smaller.

It was proposed to use two species of native fishes, the logperch <u>Percina caprodes</u> and the longear sunfish <u>Lepomis megalotis</u>. Because project initiation followed by two months the spawning season of these two native fishes in Oklahoma, the initial ten months of research were devoted to physical preparations of aquaria and analytical apparatus and a comprehensive literature search.

Proposed experimental levels of zinc and copper were tested at four levels: 0.1, 0.2, 0.4, and 0.6 ppm. However, extraneous contamination of both zinc and copper in the laboratory water supply killed all specimens within 25 days with the result that the selected fish species were not available for continued research.

Several attempts to rear artificially spawned logperch in sublethal concentrations of copper were largely unsuccessful because the concentrations that are nonlethal with continuous exposure are so low that they are virtually indistinguishable from the control water available. Larval logperch survived up to 10 days in various copper concentrations that ranged up to 0.2 ppm, while survival to juvenile stages were obtained only when the copper concentration was 0.02 ppm or less. Since continued growth to juvenile stages was hoped for in these experiments, relatively few specimens were fixed while living or fresh enough to be useful for histological examination.

Preliminary experiments using the longear sunfish indicated that the problems would be greater and the results no better than those obtained with logperch. Consequently, attention was turned to the channel catfish, <u>Ictalurus punctatus</u>.

Approximately 200 young catfish were maintained in a static system containing 0.5 ppm copper for a period of 30 days. A few specimens were preserved periodically for subsequent comparison with control specimens collected concurrently.

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Because of their relatively high tolerance to copper, channel catfish were used instead of logperch or sunfish for the remainder of the research. Approximately 1,000 channel catfish fry, about two weeks old, were obtained from a private hatchery and used to evaluate their suitability for laboratory rearing and their tolerance to copper. It was found that they can be reared in aquaria using a commercial fish food, but their growth rate is considerably less than that obtainable in a pond.

Static bioassays were performed following standard methods to determine the tolerance limits of the young catfish. A 48 hour test gave a TLm of 1.4 ppm copper and a 96 hour test gave a value of 0.3 ppm copper. The fish were not fed during the tests and starvation became an obvious factor in the longer test.

Approximately 200 young catfish were kept in a static system containing 0.5 ppm copper for a period of 30 days with food being added three times per day. A constant level of ionic copper could not be maintained because it combines readily with several chemicals that are necessarily present in such a system. Some of the water was replaced daily with fresh copper solution in an effort to maintain some copper in solution. Frequent analysis showed variation between 0.1 and 0.5 ppm copper. A few fish died each day, but this was also true in the control aquarium. A few specimens were preserved periodically for comparison with control specimens collected concurrently.

Since the literature contains no information on the median tolerance limits of channel catfish to copper, and forty yearling catfish were available from another project, a preliminary test of their tolerance was made. A continuous flow system was used to maintain a more constant concentration of copper, and a 48 hour TLm value of 5.5 ppm was obtained. Before 96 hours had elapsed the copper began to precipitate, but an estimated value would be above 4 ppm.

A final effort involving yearling and fry channel catfish were attempted in which the channel catfish were raised from the age of three weeks to seven weeks in 0.1 mg/1 of copper in a continuous flow system. However, the controls became diseased and showed poorer survival and growth than the test specimens, so the results are inconclusive. Static bioassays of channel catfish fry in copper gave 48 hour TLm values of 0.1 and 1.4 mg/1 for specimens one week and two weeks old respectively, but the condition of the fish may have contributed more to the variation than the age difference. Yearling channel catfish were subjected to static and continuous flow bioassays and were found to be much more tolerant to copper than are the fry. The copper precipitated in concentrations above 3.5 mg/1 which is below the TLm value. The deaths that occurred were not the result of asphyxiation from gill obstruction that is typical of metal poisoning in other species of fishes. The presence of copper tends to inhibit feeding, which makes it difficult to interpret the results of long-term studies, especially with young fish. ł

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Because of the repeated failure to successfully test chronic effects of three separate species of fishes to what had been anticipated to be sublethal concentrations of copper, the decision was made to terminate the project rather than continue for another year in hopes that positive results could be obtained. It is the opinion of the writer that unusually rigorous water quality standards must be followed by anyone seeking to determine the long-term chronic effects of sublethal concentrations of metals in aquatic environments. This problem was anticipated neither in the literature or by the principal investigator.