COPPER AND ZINC POISONING IN BROOK TROUT (*Salvelinus fontinalis* Mitchell)

By

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INTRODUCTION

The realization that various base metals, including copper and zinc, could readily eliminate fish and other aquatic life has been known for at least a century (Penny and Adams, 1863). In that time, extensive efforts have been made by many investigators to determine maximum tolerated limits. The resulting literature has assumed massive proportions and diversity. Thorough reviews are readily available, however, which catalogue the literature on the toxicity of individual metals to individual fish (Ellis, 1937; Cole, 1941; Doudoroff and Katz, 1953; Jones, 1957, 1959; and Hynes, 1960); consequently, only accounts of investigations pertinent to the current study will be considered.

Two metals, copper and zinc, selected for this study because they are common pollutants. These metals occur not only in waters draining mine wastes but also commonly in the drainage from galvanizing plants, brass works, electroplating industries and certain synthetic plants (Klein, 1957).

The difficulty in determining safe maximum limits of heavy metals soon became apparent because of the great number of variables modifying the toxicity of each metal. It was readily realized, for example, that temperature, dissolved oxygen, pH, and numerous other factors comprising water quality criteria profoundly influenced the toxicity of metal ions. Further investigation revealed that two or more metals would often interact in such a way as to produce a toxicity greater than the sum of the two applied individually (synergism) or could tend to neutralize the toxic effect of each other (antagonism).

Doudoroff (1952) found copper and zinc acted synergistically on minnows (Pimephales spp.). Ellis (1937) noted that calcium and sodium acted antagonistically towards copper on goldfish (Carassius auratus L.). Jones (1938) observed calcium to act antagonistically toward lead and zinc salts on three-spined sticklebacks (Gasterosteus aculeatus L.).

Defferential sensitivity among species of fish was noted by Carpenter (1927) and confirmed by Ellis (1937), but in spite of attendance to these variables, almost no attention was paid to the age or size of the fish tested.

Shelford (1917) pointed out that the injurious effects of a specific fish toxicant should be studied at the weakest stage in the life cycle. Cole (1941) reiterated the same attitude stating:

> "Ecologically it is immaterial how long adults can live in a given concentration of a pollutant is such a concentration prevents the eggs from hatching or stops development of fry or fingerlings."

Certain age or size-based variations in reaction to non-metallic toxicants have been reported, however, only two investigations are known to have made any reference to age variation in response to heavy metals. One of the investigations (Grandell and Goodnight, 1962) makes only passing reference to the problem as an observed, but unexplored, factor. The other report (Jones, 1938) specifically concerns itself with the problem but suffers from inability to maintain a control group and the adoption of uncertain analytical technique.

Aqueous tar extract (phenol, naphthalene, cresol, pyridine, etc.) tolerance in brown trout decreased with age from alevin to yearling, and spermatozoa, ova and zygotes appeared especially resistant (Gardiner, 1927). Carbon dioxide and sulfite waste tolerance had been similarly observed to decrease with age (Wells, 1913; and Nightingale, 1931; respectively, cited by Cole, 1941), however, gas liquors and certain drugs (nicotine and digitaline among them) were tolerated better by adult than juvenile fish (Shelford, 1917; and Sollman, 1905; respectively, cited by Cole, 1941).

Jones' (1938) results indicated that the survival times of young three-spined sticklebacks (18-20 mm) were appreciably longer than for adult sticklebacks when subjected to identical lethal lead solutions. However, as a result of rapid mortality in the adult control fish and his unusual technique for comparing age-based tolerances, his conclusions were to the contrary. In addition, he (Jones, 1939) later confuses matters with an unexplained statement that, "Very small fish proved somewhat less resistant" (than adults), perhaps referring to results from his earlier publication. Crandell and Goodnight (1962) also observed that for two mg. per l. lead sulphate, 5.0 mg. per l. zinc sulphate, or 0.2 mg. per l. sodium pentachlorophenate a higher mortality appeared in immature guppies (Lebistes reticulatus L.) than adults.

However, both the discrepancies and limited range of these investigations indicated clearly that further experimental study was required

to resolve the relation between age and tolerance to heavy metals. Brook trout (Salvelinus fontinalis Mitchell) were used in this study of age-based tolerance to copper and zinc solutions.

In addition, certain discrepancies surrounded the cause of lethal poisoning by heavy metals. The publications of Carpenter (1925, 1927, 1930), Behrens (1928), Dilling, Healey and Smith (1926), Jones (1935, 1938, 1939, 1947), Ellis (1937) and Westfall (1945) all attribute mortality to asphyxia produced by muco-metallic filming of the branchial epithelium. Ellis (1937) tempered his discussion of the cause of mortality by suggesting that in addition to mucous filming, intracellular protein precipitations of the branchial cells might occur. Schweiger (1957) and Parry (1960) found, from histological studies, that heavy metal salts elicited swelling and necrosis of the branchial epithelium, frequently, with desquamation. Neither observed any indication of an increase in the activity of mucous cells. With this disparity in the type of branchial damage, revised histological examinations of the gill platelets were made in this study using yearling brook trout.

Until recently (Saiki et al, 1958; Hibiya and Oguri, 1961; Joyner, 1961) contention surrounded the possibility that heavy metal ions penetrated the body tissues in significant quantities. Carpenter, (1927) could find no trace of lead within the bodies of lead nitrate poisoned minnows after washing them in dilute acetic acid, ashing and then analysing the remains colorimetrically. Behrens (1928), however,

using radio-isotopic lead noted some penetration into internal tissues but considered it insignificant relative to branchial damage. White and Thomas (1912) reported the absorption of copper, and Thomas (1924) the absorption of nickel from sea water by the killifish, Fundulus heteroclites. Dawson (1935) reported tissue damage after extended periods of chronic lead poisoning. His observations included secondary anemia followed by large numbers of immature erythrocytes (erythroblasts) in the general circulation. Associated with the anemia were excess deposits of pigment in the liver, spleen and kidneys (opisthonephros) presumed to be from phagocytosed erythrocytes. Predominant in leukocyte changes were large increases in numbers of monocytes and eosinophiles and the appearance of large numbers of atypical "spindle cells" which Dawson believed may have been of monocytic origin. Other tissue changes reported were a marked proliferation of erythropoietic sites in the cardiac endothelium and formation of large clusters of spindle cells on the surface of the ventricular trabeculae.

Increases in the iron and hemoglobin content of rats subsequent to trace copper ingestion is well known, however, no comparable reports are known for fish immersed in copper or any other metal. Elvehjem and Sherman (1932) found that young rats could be made anemic by feeding only cow's milk, following early weaning (21 days). Iron reserves in the spleen and liver were severely depleted by the rapid animal development and resulted in reduced hemoglobin. Administration of inorganic iron for two weeks quadrupled the levels of hepatic iron

and it doubled the splenic iron but effected less than a five-percent increase in hemoglobin. Administration of 0.05 mg. of copper per day, however, for two weeks increased the hemoglobin concentration over 60 percent and reduced both hepatic and splenic iron concentrations below the anemic level. In addition, in the absence of copper, the hepatic iron accumulation and storage were proportional to iron intake while hemoglobin remained unchanged. When copper was added, however, the hemoglobin formation was roughly proportional to iron intake while hepatic iron concentration remained unchanged until 0.3 mg. was fed daily after which it increased in proportion to the iron intake. Elvehjem and Sherman conclude that copper catalyses the conversion of inorganic iron into hemoglobin. Total iron determinations were performed on the blood of chronic copper and zinc-poisoned yearlings in order to observe whether or not these ions, if absorbed, produced similar effects to the above.

The concentration and accumulation of zinc in tissue has been the subject of several investigations. Feaster et al (1955) determined the major areas of distribution of the zinc radio-isotope (Zn^{65}) in adult rats which indicated the greatest accumulations occurred in the kidney followed in turn by the liver and then the pancreas. Wakely et al (1960) made similar observations with male rats but found the dominant area of deposition to be the prostate, which contained up to 10 times the Zn^{65} concentration of the other tissues. Rapid elimination of peritoneally injected zinc in the fecal

matter led Wakely et al to agree with the conclusions of Sheline et al (1943) and Montgomery et al (1943) that it was excreted into the digestive tract. Very little zinc was found excreted in the urine. Saiki et al (1958) observed active excretion of Zn^{65} in the intestine of the medaka, Oryzias latipes. Similar results were reported from air-bladder injected goldfish, Carassius auratus L., except that high intestinal Zn^{65} concentrations were observed (Hibiya and Oguri, 1961). Appreciable accumulations occurred in the liver, pancreas, air-bladder and pronephros; slightly smaller deposits occurred in the opisthonephros, spleen, gills and vertebrae; still smaller quantities occurred in the muscle and gonads. No apparent variations were observed between male and female gonads. Observations made on the brown bullhead immersed in zinc solutions, containing Zn^{65} as a tracer, (Joyner, 1961) confirmed the results of Hibiya and Oguri that the greatest zinc accumulations are in the gastro-intestinal tract. He (Joyner) found, however, that kidney and gill accumulations contained only slightly less of the isotope after 96 hours immersion. Accumulations in the liver and spleen were extensive but only about half as active as the gut, kidneys or gills. Muscle and bone Zn^{65} activity was less than one-tenth and one-eightieth respectively of the gut. In order to determine whether zinc absorption occurred predominantly in the gills or the gut, Joyner compared tissue Zn^{65} accumulation in normal bullheads and those whose esophagus had been plugged with paraffin. Zinc accumulation in the tissue of fish with plugged esophagus was practically identical to accumulation in corres-

ponding control animals, however, gill Zn^{65} concentration was noticeably lower in animals with the plugged esophagus. Overall, therefore, these results indicate zinc absorption occurs almost entirely in the gills. Another important observation (Joyner, 1961) was the initially rapid gross uptake of zinc for a period of less than twelve hours, followed by a very reduced absorption thereafter. This phenomenon suggests one of two causes: a) absorption continues until reaching a saturation level in the tissues beyond which an equilibrium is established; b) about twelve hours is required to produce a physical change, such as cloudy swelling of the branchial epithelium which reduces permeability to heavy metals.

The absence of other investigations concerned with the actual pathology of chronic zinc and copper poisoning in fish, rather than simply heavy metal accumulations, led to the preparation of certain tissues for histological examination in this investigation. Liver, kidney (opisthonephros), spleen, heart and stomach from yearlings in lethal and sublethal solutions of copper and zinc were examined. Total erythrocyte counts were taken from fingerlings subjected to sublethal solutions of copper and zinc for 11 days and from yearlings in sublethal solutions for 21 days.

1. Survival In Zinc and Copper

(i) Experimental Fish

With the exception of a very small number of yearlings*, all trout were reared from the eyed-egg and maintained, until required for acclimation, in the Whiteshell trout hatchery, operated by the Fisheries Branch, Dept. of Mines and Natural Resources, Province of Manitoba. The eyed-eggs were obtained by the Hatchery from the Ontario government's Dorion hatchery (Port Arthur) or the privately operated hatchery of Mr. K.G. Drew (Spokane, Washington). The thermal history of the eggs until received by the Whiteshell hatchery is not known.

During the holding period, all groups of fish were fed a dry biscuit preparation of an appropiate size every other day and liver sausage once weekly.

The ages of the three groups tested, early fry, young fingerlings, and yearlings are accurately known because of their hatchery confinement:

Early Fry - young fish, two to six weeks past egg-sac absorption, average weight 0.12-0.15 grams.

Young Fingerlings - fingerlings in July and August that were hatched in February and March, held at 5°C. (or less) until late May, thereafter, in water rapidly increasing in temperature to 15°C. about mid-July. Average weight 2.15-2.29 grams.

Yearlings - trout, twelve to eighteen months beyond hatching. Average weight 6.8-8.8 grams.

(*) A small number of yearlings were seined from Stoney Creek, three miles east of Bethany, Manitoba. All specimens caught were either hatchery reared or descendents of hatchery stock.

(ii) Acclimation

Acclimation of the fish to temperature and to the test water were both performed prior to commencement of the tests.

Temperature acclimation was carried out first, with maximum temperature changes of one-half degree Centigrade per day from hatchery temperature (6 - 10°C.) to 10°C., the test temperature. This was followed by a holding period of one week at 10°C. for fry and three weeks or longer for fingerlings and yearlings.

The fish were maintained in ten-gallon aquaria immersed in refrigerated constant-level water baths thermostatically controlled to maintain the temperature at 10°C $\pm$ 0.5°C. Tap water cooled by passage through a long pyrex coil, immersed in the water bath, continuously entered the aquarium. The aquarium overflow syphoned into the water bath.

The fish were acclimated to the soft water (72 p.p.m. T.D.S.) used in test solutions. Trout to be utilized were transferred, 24 hours prior to an experiment, to a mixture of three parts tap water to one part deionized water, then they were transferred to the test water, a mixture of one part tap water to one part deionized water, for a period of at least three days.

(iii) Survivorship

Tests were carried out in static aquaria, the solutions of which were changed once daily. Aquaria of three types were utilized, one for each age group. Fry were tested in 600 ml. pyrex beakers filled to the five hundred milliliter level. Fingerlings were tested in polyethylene

aquaria containing four liters of test solution. Yearlings were tested in eight-liter polyethylene aquaria maintained at six liters.

Constant aeration supplied each aquarium with compressed air that had been bubbled through water. No filtration mechanism was used because of the danger of its constituents absorbing metallic ions from solution.

Temperature was controlled at 10°C. ± 0.5°C. either by immersion of aquaria in the refrigerated water coolers described previously or in a constant-temperature room at 10°C.

Preliminary establishment of upper limits for concentrations of metallic ions was derived from data in Jones (1938) and in Doudoroff and Katz (1953). Maximum concentrations were chosen from those which Jones observed to cause complete mortality in sticklebacks after one day. A series of test concentrations was chosen between this level and the concentration estimated to leave only 70 percent survival at the termination time of 264 hours. Testing was continued until four or more trials were within these limits and at least one more showed a survival greater than 70 percent at the end of testing. Each age group in each metal, therefore, consists of six or more trial groups. A control group for each battery of tests (metal) was also maintained.

(iv) Solutions and Solution Preparation

Stock solutions were prepared by weighing specified amounts of reagent grade copper sulphate ($CuSO_4.5H_2O$) and zinc sulphate ($ZnSO_4.7H_2O$).

Test solutions were prepared by diluting aliquots of stock solution to final test volumes. Water used for dilutions was maintained at 10°C. in

100-gallon reservoirs. Tap water was examined daily for chlorine by the orthotoluidine Flash Test Method (Standard Methods, APHA pp. 91-92) but at no time was it detectable during acclimation or test periods.

Table I

Mean Weight of Trout per liter of Test Solution

Age Group	Metal Series	Number of Fish/Test	Number of Fish/Liter	Mean Weight/Liter
Fry	Copper*	10	20	0.12 grams
Fingerlings		10	2.5	2.3
Yearlings		6	1	6.8
Fry	Zinc*	10	20	0.15
Fingerlings		10	2.5	2.2
Yearling		6	1	8.8

(*) includes the weight of fish in the control solution

Diluent (one part tap to one part deionized water) was accumulated in adequate quantity at one time to supply all aquaria for a complete series of tests. Since diluent was of uniform quality, conductance, alkalinity (methyl-orange test), hardness (EDTA for calcium and magnesium, and also for

calcium alone) determinations were made only once for each series (Table II). Hydrogen ion activity was determined on a Beckman Zeromatic pH meter once for each test concentration immediately after its preparation.

Table II

Experimental diluent quality criteria utilized in copper and zinc survivorship tests. Diluent properties determined were alkalinity (methyl orange test), total dissolved solids (electrical conductivity), EDTA hardness (EDTA and Eriochrome Black T indicator), calcium hardness (EDTA and Murexide indicator); pH determinations were made on final test solutions.

Series	Alkalinity (ppm $CaCO_3$)	Conductivity (mhos per cc)	EDTA Hardness (ppm Ca CO_3)	Calcium Hardness (ppm Ca CO_3)	pH
Zinc	41.1	83.3	56.8	29.8	7.6 ± 0.2
Copper	42.1	83.3	57.5	30.6	7.6 ± 0.2