Bull. Environm. Contam. Toxicol. 28, 674–681 (1982)

Sublethal Copper Stress and Susceptibility of Channel Catfish to Experimental Infections with *Ichthyophthirius multifiliis*

M. S. Ewing,[1] S. A. Ewing,[2] and M. A. Zimmer[3]

Departments of [1]Zoology; [2]Veterinary Parasitology, Microbiology & Public Health; and [3]Veterinary Pathology, Oklahoma State University, Stillwater, OK 74078

A variety of approaches to evaluating the effects of sublethal concentrations of toxicants upon fish have been developed. Demonstrated effects of heavy metals include changes in blood chemistry (MCKIM & BENOIT 1971; LEWIS & LEWIS 1971), tissue morphology (BAKER 1969; GARDNER & LA ROCHE 1973), and growth and reproduction (MOUNT 1968; MOUNT & STEPHAN 1969). Because environmental stress is well known to predispose fish to infection, the interaction of sublethal concentrations of toxicants and disease agents promises to be an important focus for study of water quality.

The relationship between the stresses of parasitic infection and concomitant exposure to toxicants has only recently come under study. For example, BOYCE & YAMADA (1977) examined the effect of cestode infection on resistance of salmon to zinc stress, and GUTH et al. (1977) found that the tolerance of snails to acutely lethal concentrations of zinc was reduced by schistosome infection. The influence of copper stress upon disease processes has been studied to a limited degree. Elevation of blood corticosteroid concentrations following experimental exposure to dissolved copper was demonstrated in salmon (DONALDSON & DYE 1975; SCHRECK & LORZ 1978) Effects of copper exposure upon immune response were shown in salmon (STEVENS 1977) and in blue gourami (ROALES & PERLMUTTER 1977). In laboratory studies of steelhead trout KNITTEL (1980, 1981) showed an increase in susceptibility to redmouth infection following exposure to copper. Susceptibility to this bacterial disease was measured by mortality rate.

The present study describes a technique for evaluating the change in susceptibility of channel catfish (Ictalurus punctatus) to infection by the protozoan parasite Ichthyophthirius multifiliis following exposure to sublethal concentrations of dissolved copper. This ubiquitous parasite infects a wide variety of freshwater fishes and causes severe disease problems in catfish. Exposure to sublethal concentrations of contaminants may amplify stress significantly for fish exposed to such disease agents. Therefore, techniques are needed to judge the effect of contaminant stress upon the course of disease, rather than simply death from disease, in fishes.

0007-4861/82/0028-0674 $1.60

MATERIALS AND METHODS

The general design of the experiments is as follows: fish were (1) exposed to sublethal concentrations of dissolved copper for 96 hrs. (4 days), (2) placed in clean water for exposure to a standardized dose of I. multifiliis for 5 days, and (3) examined to assess the extent of resultant infection and histopathologic change. Details of experimental design follow.

Fish

The catfish used in these experiments were obtained as 50-mm fingerlings, brought into the laboratory, held at 20 C for one month, and found to be free of disease. The fish were fed a pelleted ration containing 30% protein until 24 hours before experimental use. Fish, when used in these experiments, were 3.2 gm mean weight and 71 mm mean total length.

Exposure of Fish to Copper

Dilution water was tap water that had been filtered through activated carbon and allowed to stand for two days. Total EDTA hardness was 188 mg $CaCO_3$/liter; pH was 7.6; and dissolved copper concentration was less than 25 ug Cu/liter as measured by flame atomic absorption spectroscopy. Copper was added to dilution water as reagent grade copper sulfate. Preliminary static bioassays had shown the lower limit for 96-hr lethality (LC_0) in this system to be approximately 3200 ug Cu/liter, nominal concentration. Nominal concentrations from 0 to 3200 ug Cu/liter were therefore used in the following experiments.

During each experiment six fish were maintained in each glass aquarium containing 15 liters of solution. Fish were exposed in accord with EPA standard methods for static bioassay (PELTIER 1978), specifically, for 96 hours with continuous aeration and no feeding.

In Experiment A four groups of six fish were exposed to each of the following nominal concentrations: 0, 32, 1000, and 3200 ug Cu/liter. Water temperature was 21.0 C ($\pm$ 1.2). In Experiment B five groups of six fish were exposed to each of two nominal concentrations, 0 and 3200 ug Cu/liter. Water temperature was 20.1 C ($\pm$ 1.2). In both experiments dissolved copper concentrations of duplicate samples from each aquarium were determined one hour after test solutions were added to the aquaria (just before fish were placed in them), at the end of the 96-hr exposure period, and after fish were transferred to clean water for exposure to parasites (see Exposure of Fish to Protozoan). Dissolved oxygen and temperature measurements were made daily throughout the course of the experiments.

Protozoans

A strain of Ichthyophthirius multifiliis collected from hatchery-reared channel catfish was maintained in the laboratory by serial infection of susceptible fish. Fish with 8-day-old infections of the protozoan, i.e., with mature trophozoites, were pithed and placed in dishes of dilution water in order to collect the trophozoites that dropped off the hosts. Trophozoites were maintained for 24 hours at 21 C and underwent repeated mitotic divisions resulting in the formation of tomites, the infective stage. Aliquots of this water containing tomites were placed in counting chambers and the concentration of tomites determined.

Exposure of Fish to Protozoan

Following 96-hr exposures to copper, each group of fish was transferred to a clean aquarium containing 15 liters of dilution water. In preliminary experiments it was verified that trophozoites of the strain with which we were working produce highly variable numbers of tomites, mean 78 (S. E. $\pm$ 26) tomites/trophozoite, an observation consistent with published descriptions of variability in tomite production (MEYER 1974). Therefore, the infective dose was standardized on the basis of number of tomites, the invasive stage, rather than number of trophozoites, the reproductive stage.

In preliminary experiments, groups of fish were exposed to tomites in concentrations of 15, 60, and 115 per liter. Infective doses greater than the last were found to kill experimental fish in five to seven days. Infections were estimated by counting trophozoites embedded in three 0.5-cm^2 areas of skin on each fish after five-day incubation. The variability of response at the lowest level was judged unacceptable. In infections at the highest level parasites were massed in the tissue to the extent that individual parasites overlapped and it was not possible to count them accurately. It was concluded that infection resulting from a moderate infective dose, approximately 60 tomites/liter, could be estimated readily and likely would not be too variable.

In the present experiments, therefore, tomites were added to each aquarium so that the final concentration was 50 tomites/liter, a concentration found to produce infection in normal fish in the range 10-50 trophozoites/cm^2 skin. In addition, one tank of six fish that had not been exposed to copper and one tank of six fish exposed to 3200 ug Cu/liter were maintained as controls, i.e., not exposed to the protozoan.

Fish were maintained for 5 days with constant aeration. At the end of the experimental period, fish were anaesthetized in a solution of 70 mg/liter tricaine methanesulfonate (Finquel®). This treatment quieted the fish sufficiently to minimize dislodgement of trophozoites in handling. Each fish was examined with a dissecting microscope and the number of trophozoites counted in two 0.5-cm^2 sample areas, one on the dorsal surface immediately anter-

ior to the base of the dorsal fin and the second, on the upper portion of the caudal fin.

Histopathology

Following enumeration of parasites at the conclusion of Experiment B, six fish that had been exposed to 0 ug dissolved Cu/liter and six fish that had been exposed to 3200 ug dissolved Cu/liter (nominal concentrations) were fixed in 10% buffered formalin. Because copper is known to cause respiratory and/or osmotic stress (e.g., LORZ & MCPHERSON 1976) and the development of trophozoites of I. multifiliis causes considerable distortion of host gill epithelium, gill was selected as the tissue in which the interaction of toxicant and parasite might most easily be observed histopathologically. Therefore, gill from these fish was embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Gill from control fish, those exposed to no copper and those exposed to 3200 ug Cu/liter, none of which were exposed to the protozoan, was treated similarly. Sections of four gill arches from each fish were read blind for histopathologic change.

Condition Factor

Total length and weight of fish were recorded and used to calculate condition factor (K) as described by Carlander (1969).

RESULTS AND DISCUSSION

Within the sublethal range of nominal concentrations 0-3200 ug and the corresponding measured concentrations <25-1030 ug dissolved copper/liter, a weak (r=.56) though statistically significant positive correlation (P <0.01) existed between exposure concentration and susceptibility to the parasite (Table I). Fish exposed to measured concentrations of less than 25 ug/liter had mean infections of 30 (12-42) trophozoites/cm^2 and fish exposed to 280-1030 ug/liter had mean infections of 39 (22-64) trophozoites/cm^2.

TABLE I

Mean Disssolved Copper Measured at 96 Hours ug/liter	Susceptibility to I. multifiliis No. Trophozoites/cm^2 (± S.E.)
Experiment A:	
<25	37 (±4.6)
280	40 (±4.7)
670	42 (±4.8)
1030	48 (±4.7)
Experiment B:	
<25	23 (±4.2)
620	28 (±1.9)

The variability in susceptibility among fish which are the same age, nearly same length, and from the same hatchery source is considerable. Condition factor (K) was examined in order to see whether part of the variability among apparently homogeneous fish within experimental groups might be attributable to variation in general robustness. It has been suggested that low condition factor might be associated with high susceptibility to disease. For example, a negative correlation between body condition and susceptibility to red-sore disease has been shown for some populations of largemouth bass (ESCH et al. 1976; ESCH & HAZEN 1980).

Condition factors for the fish used in this study ranged from 0.43 to 0.89, values within the normal observed limits for channel catfish of this length (CARLANDER 1969). Although ESCH & HAZEN (1980) reported a significant threshold in terms of K for condition and therefore susceptibility of largemouth bass to red-sore disease, no comparable threshold was observed in this study. In fact, condition factors were not even strongly negatively correlated with susceptibility of channel catfish to I. multifiliis.

Tissue response to experimental conditions is summarized in Table II. Rarely, small cystic spaces and mild epithelial cell hyperplasia were observed in control fish, but these conditions were much more common in fish exposed to I. multifiliis and/or sublethal concentrations of copper. Lethal concentrations of copper commonly have been reported to cause hyperplasia of gill epithelium (e.g., GARDNER & LA ROCHE 1973), but chronic or sublethal exposures have occasionally been reported to cause this condition as well (BAKER 1969).

TABLE II

EXPERIMENT B

SUMMARY OF HISTOPATHOLOGIC FINDINGS

EXPT'L EXPOSURE	Control Control	Copper No Parasite	No Copper Parasite	Copper Parasite
Normal	84%	33%	33%	16%
Cystic Spaces	16%	50%	50%	66%
Epithelial Cell Hyperplasia	16%	50%	33%	66%
Lamellar Fusion	0%	16%	16%	84%

In fish that were exposed for 5 days to I. multifiliis but not copper, the occurrence of epithelial cell hyperplasia, cystic spaces, and fusion of lamellae, was more common than in control fish. These findings are in agreement with observations of older infections (13-day) made by HINES & SPIRA (1974). Among fish exposed to both copper and I. multifiliis, at least two lesions (cyst formation, epithelial hyperplasia, lamellar fusion) were observed in 84% of the fish examined. It appears that the pathologic changes attendant upon exposure to the parasite and those upon exposure to copper are complementary in effect. Exposure to the combination of toxicant and parasite clearly presents greater stress to the fish; fish exposed to the combination were least likely to have normal gills.

The increase in susceptibility of copper-stressed fish to I. multifiliis in this study was expected to be considerably greater than the increase observed. Gill is the major site of epithelial damage from copper exposure (BAKER 1969; MARTIN & STEPHENSON 1977). Swelling and hyperplasia immediately following 96-hr exposure to copper likely cause respiratory and/or osmotic stress (LORZ & MCPHERSON 1976) of importance in lowering host resistance to invasion by the parasite. In the gill itself, the swollen epithelium may provide, in addition, an immediately accessible site of parasite invasion. It is hypothesized that the unexpectedly small difference in susceptibility that was observed is a consequence of recovery of gill from copper stress after the fish were transferred to clean water for exposure to the parasite.

Susceptibility to disease in fish usually has been described in terms of mortality rate. For example, KNITTEL (1981) estimated the effect of copper stress on mortality rate due to bacterial infection (Yersinia ruckeri) in steelhead trout and found that only increases of 20% or more in mortality rate were statistically significant. In the present study, in contrast, the question is asked: what is the effect of sublethal contaminant stress upon infection rate and morbidity? Susceptibility is here described in terms of infection rate and is expressed as numbers of individual parasites per unit area of skin. Differences of 20% in susceptibility to I. multifiliis were observed in comparing infected controls with infected copper-stressed catfish. However, given the variability of response among individual fish, this increase was not statistically significant. Changes in rate of infection and/or morbidity are more subtle than changes in mortality rate, and individual variation such as that described in tissue response is an important consideration in analyzing experimental compar--sons. Such subtle changes are likely of considerable importance in judging sublethal effects of toxicants.

In conclusion, within the sublethal range of measured concentrations <25 to 1030 ug dissolved copper/liter, a weak though statistically significant positive correlation existed between expo-

sure concentration and susceptibility to the parasite. Fish exposed to measured concentrations of less than 25 ug/liter had mean infections of 30 trophozoites/cm^2 skin and fish exposed to 280 to 1030 ug/liter had mean infections of 39 trophozoites/cm^2. It appears that the pathologic changes attendant upon exposure to the parasite and those upon exposure to copper are complementary in effect.

ACKNOWLEDGMENTS

Journal article 4131 of the Agricultural Experiment Station, Oklahoma State University. The research was supported by Grant 901-15-152 from the USDA/CSRS to the Department of Zoology, College of Arts and Sciences, and the Department of Parasitology, Microbiology, and Public Health, College of Veterinary Medicine, Stillwater, Oklahoma, OK 74078. The assistance of Norman Boyd, S.L. Burks and Elaine Stebler of the Water Quality Research Laboratory, and O. Eugene Maughan of Oklahoma Cooperative Fisheries Research Unit is appreciated.

REFERENCES

BAKER, J.T.P.: J. Fish. Res. Bd. Canada 26, 2785 (1969).

BOYCE, N.P., and S.B. YAMADA: J. Fish. Res. Bd. Canada 34, 706 (1977).

CARLANDER, K.D.: Handbook on freshwater fishery biology. 2nd ed. Ames, Iowa: Iowa State University Press (1969).

DONALDSON, E.M., and H.M. DYE: J. Fish. Res. Bd. Canada 32, 533 (1975).

ESCH, G.W., T.C. HAZEN, R. V. DIMOCK, JR., and J.W. GIBBONS: Trans. Amer. Micros. Soc. 95, 687 (1976).

_____, and T.C. HAZEN: Trans. Amer. Fish. Soc. 109, 532 (1980).

GARDNER, G.R., and G. LA ROCHE: J. Fish. Res. Bd. Canada 30, 363 (1973).

GUTH, D.J., H.D. BLANKESPOOR, and J. CAIRNS, JR.: Hydrobiologia 55, 225 (1977).

HINES, R. S., and D. T. SPIRA: J. Fish Biol. 6, 189 (1974).

KNITTEL, M.D.: In Aquatic Toxicology, ASTM STP 707 American Society for Testing and Materials, 321 (1980).

_____, J. Fish Diseases 4, 33 (1981).

LEWIS, S.D., and W.M. LEWIS: Trans. Amer. Fish, Soc. 100, 639 (1971).

LORZ, H.W., and B.P. MCPHERSON: J. Fish. Res. Bd. Canada 33, 2023 (1976).

MARTIN, M., and M.D. STEPHENSON: Calif. Fish and Game 63, 95 (1977).

MCKIM, J. M., and D. A. BENOIT: J. Fish Res. Bd. Canada 28, 655 (1971).

MEYER, F.P.: U.S. Fish and Wildlife Service Fish Disease Leaflet No. 2 (1974).

MOUNT, D.I.: Water Research 2, 215 (1968).

_____, and C.E. STEPHAN: J. Fish. Res. Bd. Canada 26, 2449 (1969)

PELTIER, W.: Methods for measuring the acute toxicity of effluents to aquatic organisms. U.S. Environmental Protection Agency. EPA-600/4-78-012 (1978).

ROALES, R.R., amd A. PERLMUTTER: Arch. Environ. Contam. and Toxicol. 4, 325 (1977).

SCHRECK, C. G., and H.W. LORZ: J. Fish. Res. Bd. Canada 35, 1124 (1978).

STEVENS, D.G.: Survival and immune response of coho salmon exposed to copper. U.S. Environmental Protection Agency. EPA-600/3-77-031 (1977).