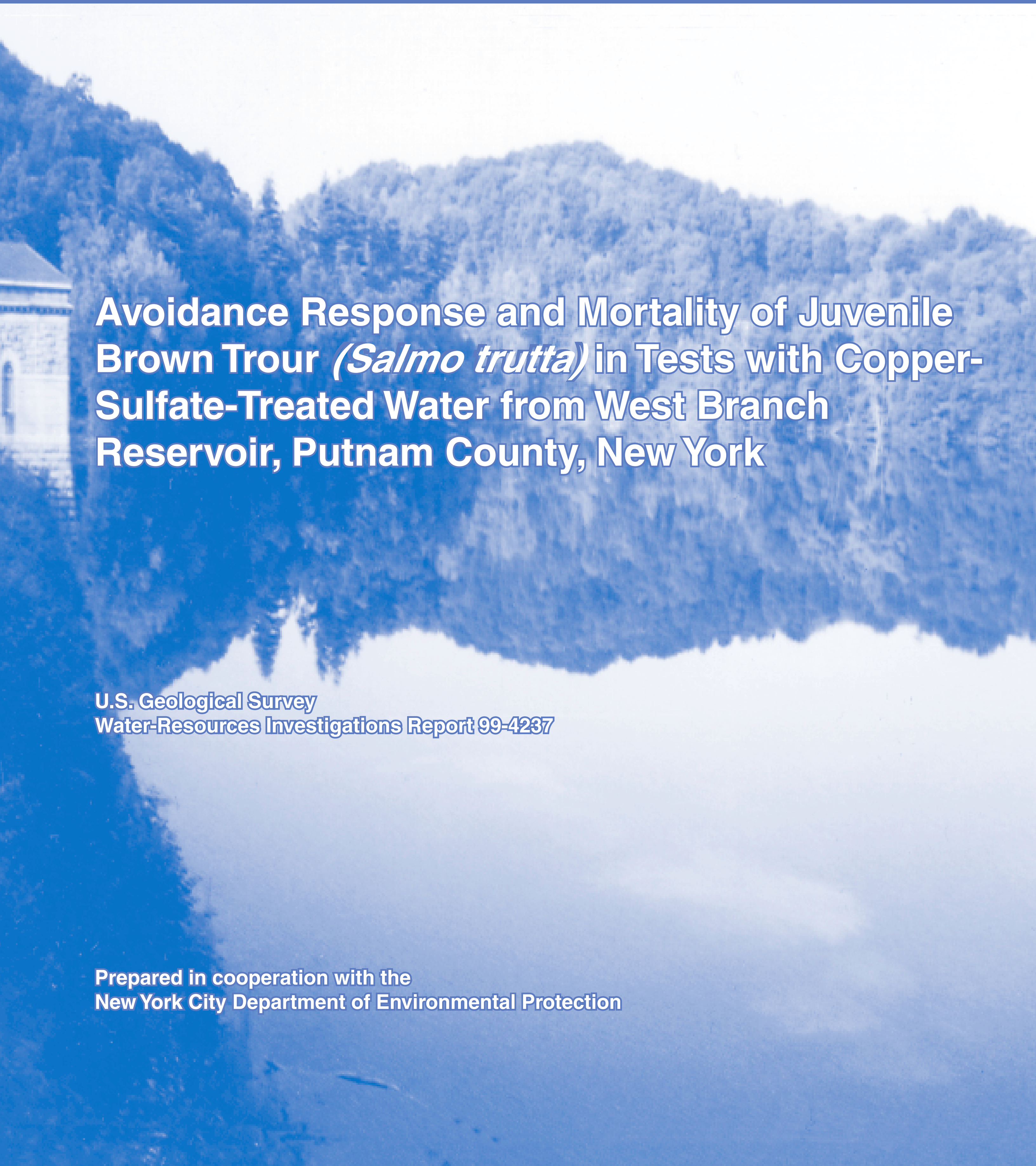
USGS
science for a changing world



Avoidance Response and Mortality of Juvenile Brown Trour *(Salmo trutta)* in Tests with Copper-Sulfate-Treated Water from West Branch Reservoir, Putnam County, New York

U.S. Geological Survey
Water-Resources Investigations Report 99-4237

Prepared in cooperation with the
New York City Department of Environmental Protection

Copper Avoidance and Mortality of Juvenile Brown Trout (*Salmo trutta*) in Tests with Copper-Sulfate-Treated Water from West Branch Reservoir, Putnam County, New York

By Barry P. Baldigo and Thomas P. Baudanza

Water-Resources Investigations Report 99-4237

Prepared in cooperation with the
New York City Department of Environmental Protection

Troy, New York
2001

U.S. Department of the Interior
BRUCE BABBITT, SECRETARY

U.S. Geological Survey
Charles G. Groat, Director

For additional information, write to:
District Chief
U.S. Geological Survey
425 Jordan Road
Troy, New York 12180-8349

U.S. Geological Survey
Branch of Information Services
Box 25286
Federal Center
Denver, CO 80225-0286

CONTENTS

Figures

Figures

Tables

CONVERSION FACTORS, VERTICAL DATUM, AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
gram (g)	0.03527	ounce
kilometer (km)	0.6214	mile
liter (L)	1.057	quart
liter (L)	0.2642	gallon (gal)
meter (m)	3.281	foot
milligrams per liter (mg/L)	1.	parts per million
micrograms per liter (µg/L)	1.	parts per billion
gallon (gal)	3.785	liter (L)

Temperature is given in degrees Celsius (°C) which can be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32$$

Vertical datum: In this report, “sea level” refers to the National Geodetic Vertical Datum of 1929 (NGVD of 1929)—a geodetic datum derived from a general adjustment of the first-order level nets of the United States and Canada, formerly called Sea Level Datum of 1929.

Abbreviated water-quality units used in this report: Chemical concentrations and temperature are given in metric units. Chemical concentration is given in milligrams per liter (mg/L) and micrograms per liter (µg/L). Milligrams per liter is a unit expressing the concentration of chemical constituents in solution as weight (milligrams) of solute per unit volume (liter) of water. One thousand micrograms per liter is equivalent to one milligram per liter. Loadings are reported in kilograms per hectare (kg/ha) and grams per hectare (g/ha).

Specific conductance of water is expressed in microsiemens per centimeter at 25 degrees Celsius (µS/cm). This unit is equivalent to micromhos per centimeter at 25 degrees Celsius (µmhos/cm), formerly used by the U.S. Geological Survey.

Sample volumes are given in liters (L).

Quantities of chemical elements or substances are also reported in this report as micromoles (µmol). A mole is defined as the sum of the atomic weights of all the atoms shown by the chemical formula of a substance, expressed as grams of each element. For example, one mole of copper sulfate ($CuSO_4$) is defined as the sum of the gram-atomic weights of 1 atom of copper (63.546 g), 1 atom of sulfur (32.06 g) and 4 atoms of oxygen (15.9994 g/atom or
63.9976 g); a total of 159.6036 g per mole of copper sulfate. A micromole of an substance is one-one millionth of a mole.

Avoidance Response and Mortality of Juvenile Brown Trout (*Salmo trutta*) in Tests with Copper-Sulfate-Treated Water from West Branch Reservoir, Putnam County, New York

By Barry P. Baldigo[1] *and* Thomas P. Baudanza[2]

ABSTRACT

Copper-avoidance tests and acute-toxicity (mortality) tests on hatchery-reared, young-of-the-year brown trout (*salmo trutta*) were conducted with water from West Branch Reservoir to assess the avoidance response to copper sulfate treatment, which is used occasionally by New York City Department of Environmental Protection to decrease phytoplankton populations in the reservoir. Avoidance-test results indicate that juvenile brown trout tend to avoid dissolved copper concentrations greater than about 55 µg/L (micrograms per liter), which is the approximate avoidance-response threshold. The mean net avoidance response of brown trout to dissolved copper concentrations of 70 and 100 µg/L, and possibly 80 µg/L, was significantly different (at $\alpha = 0.1$) from the mean net avoidance response of fish to control (untreated) water and to treated water at most other tested concentrations. Mortality-test results indicate that the 96-hr median lethal concentration (LC_{50}) of dissolved copper was 61.5 µg/L. All (100 percent) of the brown trout died at a dissolved copper concentration of 85 µg/L, many died at concentrations of 62 µg/L and 70 µg/L, and none died in the control waters (7 µg/L) or at concentrations of 10, 20, or 45 µg/L. The estimated concentration of dissolved copper that caused fish mortality (threshold) was 53.5 µg/L, virtually equivalent to the avoidance-response threshold.

Additional factors that could affect the copper-avoidance and mortality response of individual brown trout and their populations in West Branch Reservoir include seasonal variations in certain water-quality parameters, copper-treatment regimes, natural fish distributions during treatment, and increased tolerance due to acclimation. These warrant addi-tional study before the findings from this study can be used to predict the effects that copper sulfate treatments have on resident fish populations in New York City reservoirs.

[1]U. S. Geological Survey.

[2]New York City Department of Environmental Protection.

INTRODUCTION

The water supply for New York City serves 9 million people (half the population of New York State), including 1 million residents outside the city limits. The water-supply system contains 19 upstate reservoirs and 3 interlinked lakes and has a combined storage capacity of 550 billion gallons. All surface waters that are used as a public water supply must meet water-quality standards as set forth under the Federal Safe Drinking Water Act and the Clean Water Act Amendment of 1990 and the New York State Surface Water Treatment Rule (New York City Department of Environmental

Protection, 1997). Reservoir waters occasionally require chemical treatment to protect against waterborne diseases and to meet water-quality standards as prescribed by city, State, and Federal regulations (New York City Department of Environmental Protection, 1997). Waters from certain reservoirs are treated periodically with copper sulfate by the New York City Department of Environmental Protection to decrease populations of phytoplankton. During treatments, the concentrations of total and dissolved copper can reach levels known to be chronically or acutely toxic to certain species of resident fish (New York City Department of Environmental Protection, 1997). Mortality levels of about 38 percent are estimated for brown trout (*Salmo trutta*) populations in West Branch Reservoir after the inflow waters from the Rondout Reservoir are treated to attain a dissolved copper concentration of 0.36 mg/L (New York City Department of Environmental Protection, 1997). Predictive models generally are based only on laboratory toxicity tests and do not consider possible active or passive avoidance responses of fish that may occur within a treated system and, therefore, can overestimate fish mortality during reservoir treatments.

No major fish kills resulting from copper sulfate treatments in West Branch Reservoir have been reported to date (Ronald Pierce, New York State Department of Environmental Conservation, oral commun., 1998). Though copper treatments produce potentially lethal concentrations in local reservoir waters, no extensive fish surveys of the reservoir have been performed during copper sulfate applications. The absence of reported fish mortality could be from the lack of direct observations of fish before, during, and after copper treatments. Several factors may act separately or together to decrease or even eliminate the potential for brown trout mortality during exposure to high copper concentrations in this reservoir; among these are

(1) an active avoidance of areas with acutely toxic copper concentrations;

(2) decreased sensitivity through acclimation from repeated copper exposures, and

(3) decreased concentrations of biologically available copper through the reaction of free copper ions with inorganic (mineral clays) and organic (humic acids) constituents, or through increased water hardness.

Study Objectives

The ability of brown trout to avoid acutely and chronically toxic concentrations of copper has not been well documented under laboratory or field conditions (New York City Department of Environmental Protection, 1997). In 1997, the U.S. Geological Survey (USGS), in cooperation with the New York City Department of Environmental Protection, conducted a study to test the hypothesis that brown trout will actively avoid such concentrations in reservoir waters. The study was designed primarily to evaluate the avoidance response of juvenile brown trout to a narrow range of copper concentrations in water from West Branch Reservoir. An avoidance response could lessen the mortality rate in reservoir trout populations during copper treatments and could account for the lack of observed brown trout mortality during prior copper applications in West Branch Reservoir.

The study examined the effects of several different concentrations of copper on avoidance behavior and mortality of juvenile brown trout in waters pumped directly from an outflow aqueduct at West Branch Reservoir. The major study objectives were to:

(1) assess the ability of young-of-the-year (YOY) brown trout to actively avoid toxic (lethal and sublethal) concentrations of dissolved copper in water pumped directly from the reservoir,

(2) determine (a) the lowest observed-effect (avoidance and mortality response) concentration (LOEC) and (b) the highest no-observed-effect (avoidance and mortality) concentration (NOEC),

(3) evaluate the short-term avoidance response of YOY brown trout to dissolved copper concentrations that exceed NOEC and the acutely toxic thresholds that are anticipated in New York City reservoir waters during copper sulfate treatments, and

(4) provide preliminary acute-toxicity data to (a) quantify the sensitivity of the test-fish population (brown trout from the New York State Catskill Hatchery) to dissolved copper and (b) assess the mortality response (LC_{50}) of YOY brown trout exposed to copper-treated water from West Branch Reservoir.

Purpose and Scope

This report (1) describes the design of the avoidance and acute-toxicity tests; (2) presents a statistical analysis of results; and (3) discusses major findings of both tests, particularly factors that could have affected the results and that could similarly affect actual avoidance and mortality responses of fish in West Branch Reservoir. It also provides suggestions for (1) improvements in the design and implementation of avoidance tests and (2) further investigations of mortality and avoidance of copper by resident fish in treated reservoir waters.

STUDY METHODS

The study was conducted at a temporary laboratory set up at the New York City Department of Environmental Protection Shaft 10 building at West Branch Reservoir, about 2 km northwest of Carmel, N.Y. (fig. 1). The reservoir is on the east side of the Hudson River and receives most of its water from the West-of-Hudson Catskill reservoir system through the Rondout Reservoir. The avoidance and acute-toxicity tests were conducted from June 26 through July 3, 1997. Control water for all tests was pumped directly from the aqueduct where it leaves the reservoir (fig. 1).

Young-of-year (YOY) brown trout were obtained from the New York State Department of Environmental Conservation Catskill Hatchery near Debruce, N.Y., on June 11, 1997, and transferred to an artificial stream at a Troy, N.Y., laboratory of the New York State Museum and held for about 10 days until test equipment was set up. The fish were transported to the test facilities in aerated coolers 4 days before testing was started and were placed in an acclimation tank containing aqueduct water.

Copper-avoidance tests used a two-channel fluvarium (fig. 2) (Ecological Analysts, 1996) to expose 10 YOY trout for 15-min periods to flowing water that was treated with six different copper dilutions in one channel and untreated (control) water in the other. Each copper-avoidance test (one Cu dilution) consisted of exposing 10 fish to the target copper concentration in one channel of the fluvarium and to control water in the other, then reversing the source water for each channel and re-exposing the same fish to the same copper concentration in opposite channels. The process was repeated two more times, using 10 fresh fish each time, so that three sets of 10 fish were used for each of three test replicates. Because 10 fish were exposed to copper twice during each replicate, fish were provided a choice of channels with control or copper-treated water six times (essentially six replicates) for each copper-avoidance test. Acute-toxicity tests used water-bathed 12-liter tanks to expose 20 YOY trout for 96 hrs to each of six copper concentrations and to untreated water in accordance with standard static-renewal test methods (U.S. Environmental Protection Agency, 1993).

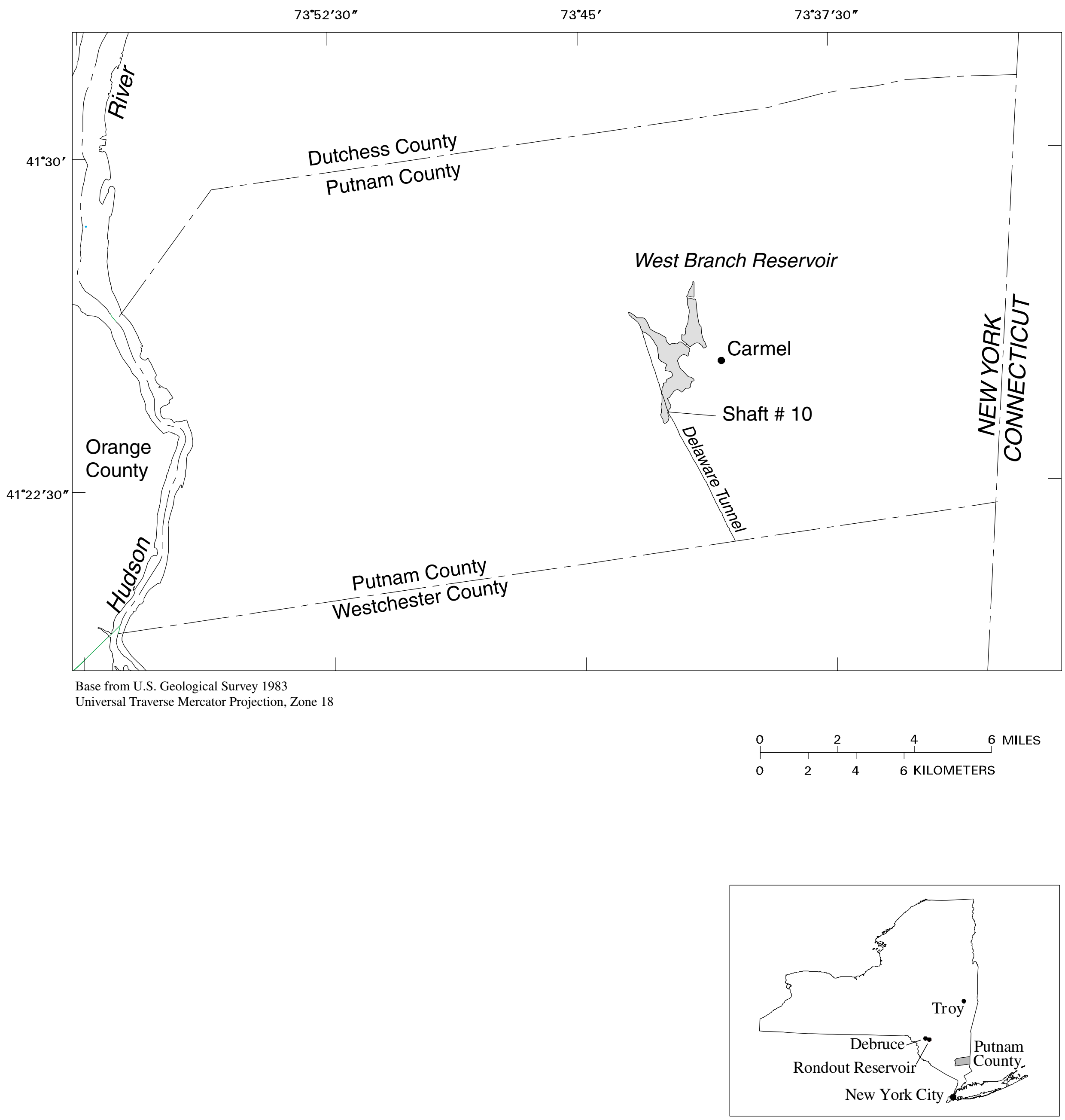


Figure 1. Location of the test facilities at West Branch Reservoir, Putnam County, N.Y.

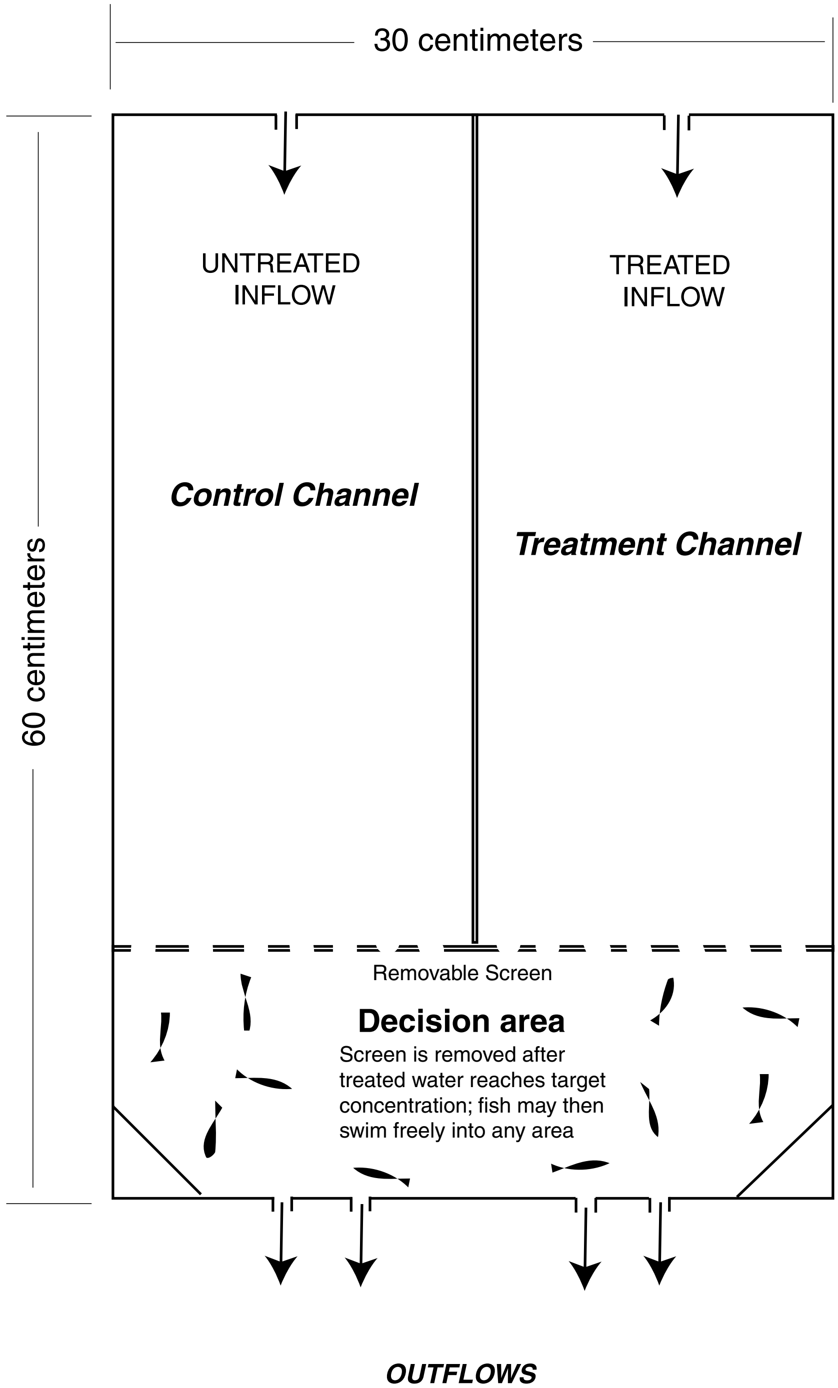


Figure 2. Plan view of trout-exposure fluvarium used in copper-avoidance tests at West Branch Reservoir, Putnam County, N.Y., June 26 through July 3, 1997.

Target concentrations of total recoverable copper (as Cu^{2+}) for the avoidance and acute-toxicity tests were 0, 15, 46, 62, 76, and 92 µg/L (ppb). Each solution was prepared by appropriate dilutions of a stock copper sulfate solution (1 g Cu^{2+}/L) made with anhydrous $CuSO_4$. Additional target dilutions of 3 µg/L and 152 µg/L of copper also were used in the acute-toxicity and avoidance tests, respectively. Wastewater generated by the experiments was held in 55-gal drums prior to appropriate disposal.

Water Quality

The pH, dissolved oxygen (DO) concentration, specific conductance, and temperature for each copper dilution were measured daily in the acute-toxicity test and at the start and end of each avoidance-test replicate in both fluvarium chambers. The quality of field measurements was assessed daily through low-ionic-strength quality-control-check solutions (pH and conductance) or comparison with a DO-saturated reference solution (temperature and dissolved oxygen); instruments were recalibrated when measures exceeded a 5-percent (+/-) error. Dissolved (0.45-µm filter) copper concentrations were determined from water samples taken from each copper dilution in the acute-toxicity test and from the treatment channel before and after each avoidance-test replicate. Occasional measures of copper and DO concentrations, pH, specific conductance, and temperature also were obtained before or after fish exposures in the control channel of several avoidance-test replicates. Additional water samples were collected from the treatment channels and analyzed for concentrations of total recoverable copper, dissolved copper, total organic carbon, calcium (Ca), and magnesium (Mg) through standard methods at the USGS National Water Quality Laboratory (NWQL) in Denver, Colo. (Fishman and Friedman, 1989) and at the USGS Low-Ionic-Strength-Water laboratory in Troy, N.Y., (Lawrence and others, 1995). Water hardness was estimated as the sum of the average concentrations of Ca (2.497 mg/L) and Mg (4.118 mg/L) (American Public Health Association, 1989). Most of the dissolved copper analyses of individual avoidance-test dilutions were run at the New York City Department of Environmental Protection laboratory at Kenisco, N.Y.

Copper-Avoidance Tests

Copper-avoidance-test procedures generally followed those described by Ecological Analysts (1996), Birge and others (1993), and Ramey and Colton (1986). Control (untreated) water was pumped directly from the aqueduct to an intermediate holding cooler, then by a peristaltic pump to the right or left fluvarium channel. Copper solutions for each avoidance test were prepared 15 min before the start of each test in two 20-L cubitainers and kept in a cooler with ice to maintain temperatures equal to that of aqueduct (control) waters. Copper-treated water from the cubitainers was pumped into the right or left fluvarium channel by a peristaltic pump during each test period. Flow rates were maintained at about 250 mL/min and resulted in a turnover of fluvarium water (volume equal to about 10 L) about every 20 min.

Brown trout were exposed to each of six different target copper concentrations (dilutions) in the fluvarium to assess their potential avoidance response to each copper concentration. Each copper-avoidance test (one Cu dilution per test) basically consisted of (1) exposing 10 fish to the copper-treated water in one channel of the fluvarium and to control water in the other, (2) reversing the source water for each fluvarium channel and then reexposing the same fish to the same copper-treated and control waters in opposite channels, (3) repeating this process two more times, using 10 new fish each time. Since 10 fish were exposed to copper twice during each replicate, fish were provided a choice of channels with control or copper-treated water six times for each copper-avoidance test. Thus, each copper-avoidance test consisted of three replicates with parts A and B (essentially six replicates). The order that target copper concentrations were tested was determined randomly.

Before each copper-avoidance test replicate was begun, fish were confined to the fluvarium's decision area (DA) by a removable screen. When the screen was removed, the fish could swim freely between the untreated channel, the copper-treated channel, and the decision area. For each replicate exposure, (1) fish were acclimated to the control water in the DA for 10 min, (2) the screen removed and fish exposed for 10 min to untreated (control) reservoir water in both channels to determine potential treatment-channel "preference," (3) fish were exposed to increasing copper concentrations while confined to the DA for 20 min, and (4) the screen removed and fish exposed for 15 min to copper-treated water in one channel and untreated water in the other to determine copper "treatment" avoidance or attraction. The numbers (positions) of fish in the decision area, treatment channel, or control channel were observed and recorded every 30 sec during the preference and treatment phases.

Computation of Avoidance Response

Gross and net avoidance responses (expressed as percent) for each dilution were calculated from the summed frequency of fish positions within the untreated or control (*C*) and copper-treatment (*T*) channels during the treatment and preference phases for each replicate (Ecological Analysts, 1996; Birge and others, 1993). For both estimates, the frequency of fish in either the *T* or the *C* channel is equal to the number of fish observed in each channel, divided by the total number of fish-position observations during either the treatment or preference phase. A total of 300 fish-position observations were made for each copper-treatment phase (10 fish x 2 observations per min x 15 min of exposure); 200 fish-position observations were made for each channel-preference phase (10 fish x 2 observations per min x 10 min of exposure).

The gross copper-avoidance response (*GR*) for each replicate is

$$GR = ((C - T) / 300) \times 100,$$

where:

T = sum of fish observed in the copper-treatment channel,

C = sum of fish observed in the control channel, and

300 = total number of fish observations for each 15-min-treatment replicate.

The net copper-avoidance response (*NR*) for each replicate reflects the gross response adjusted for the observed treatment-channel preference (*TCP*), which is calculated from the channel-preference phase that precedes each copper-treatment phase. The percent net response for each replicate is

$$NR = GR - TCP,$$

and the treatment-channel preference is

$$TCP = PCD - PTD,$$

where:

PCD = percentage of fish distributed in the control channel, and

PTD = percentage of fish distributed in the treatment channel.

The *TCP* is actually a measure of bias that exposed trout may have had for a specific channel just before its treatment with copper. A positive (+) net response indicates avoidance of, and a negative response (-) indicates attraction to the tested copper solution.

The *GR* for the control avoidance test (exposure of fish to untreated water, a target copper concentration of 0.0 μg/L) was calculated from the right- and left-channel fish distributions during the 36 channel-preference tests, regardless of which channel was to be used in the treatment phase that followed the preference phase. In this manner, the net responses to copper treatment (which already were adjusted for treatment-channel bias) were simply compared with the right- or left-channel preferences, not with data previously used to calculate the *NR*. The *NR* for the control avoidance test was equal to the *GR* for the control avoidance test because the *NR* could not be adjusted for bias.

The *GR* for the control avoidance test is

$$GR = ((R - L) / 200) \times 100,$$

where:

R = sum of fish observed in the right channel,

L = sum of fish observed in the left channel, and

200 = total number of fish observations for each 10 min preference-test replicate.

The Threshold-Effect Concentration (TEC) is the lowest estimated copper concentration that elicits an effect (avoidance or mortality response) in brown trout; it is calculated as the geometric mean of (a) the lowest copper concentration that elicits a significant response (LOEC) and (b) the highest copper concentration that causes no significant response (NOEC) (Birge and others, 1993).

Statistical Analysis of Avoidance Response

The statistical significance of the gross and net avoidance response for brown trout was calculated through a comparison of gross and net avoidance responses of fish among the six different copper-concentration tests (six replicates) by comparing responses to that of fish from the control avoidance test (36 replicates) ($\alpha = 0.05$ to 0.20) through one or more statistical approaches.

Analysis of Variance (ANOVA) and Student *t*-tests were used to compare the mean gross and net avoidance responses among the six copper-treatment dilutions (Ott, 1992; Velleman, 1996). Normality of responses (about their means) was evaluated by (1) calculating the probability-plot correlation coefficients (PPCC) (Helsel and Hirsch, 1992), and (2) determining that they were not significantly different from a slope of one. Hartley's test (Ott, 1992) was used to test the assumption of equal variances for the copper-treatment test responses (s^2 among groups). Results indicated that the responses for the various copper-treatment tests generally were distributed normally, but that the variances of responses for several copper-treatment tests (groups) were unequal. The loss of statistical power (ability to detect differences between means) decreases the confidence in results of the group ANOVA but does not greatly affect results of individual *t*-tests. To account for the lack of equal variances, the non-parametric Kruskal-Wallis (or Wilcoxon) test (Ott, 1992; Velleman, 1996) was used to assess differences in the median net avoidance responses among copper-treatment test groups. This analysis, like the ANOVA, does not identify the copper-treatment dilution with responses that might differ from the others.

The General Linear Model (GLM) was used to test for different mean net avoidance responses among the seven copper-treatment-test groups (Ott, 1992; Velleman, 1996) to meet statistical assumptions and to account for unbalanced data (sample sizes); it also provided additional analyses, such as Fisher’s Least Significant Difference (LSD) test and Duncan’s Multiple Range test, which were able to designate the specific groups with significantly different ($\alpha = 0.05$ and higher) means and medians (Ott, 1992; SAS Institute, 1988).

Acute-Toxicity (Mortality) Tests

One 96-hr static-renewal (daily water change) acute-toxicity test was conducted from June 26 through June 30, 1997, with a subsample of the YOY (mean length of 5.0 cm) brown trout population (unexposed to copper) that were used for the copper-avoidance tests. In this test, 10 trout were placed into each of two 12-L aquariums (two replicates) for each of six copper dilutions (target copper concentrations of 15, 46, 62, 76, 92, and 152 µg/L) and into duplicate control-water (untreated) aquariums. Fish mortality in each aquarium was recorded daily; dead fish were removed. Test aquariums were bathed continuously in reservoir water to maintain ambient reservoir-water conditions and aerated to optimize dissolved oxygen concentrations. The acute-toxicity tests followed standard static-renewal methods (U.S. Environmental Protection Agency, 1993).

The median lethal copper concentration (LC_{50}) and upper and lower 95-percent confidence intervals were calculated from (1) observed mortality at the end of the 96-hr exposure, and (2) the average measured dissolved copper (Cu^{2+}) concentration in each dilution through the Trimmed Spearman-Karber method (Hamilton and others, 1977). Copper concentrations, corresponding to TEC, NOEC, and LOEC for YOY brown trout mortality were calculated as in the avoidance tests.

AVOIDANCE RESPONSE AND MORTALITY OF JUVENILE BROWN TROUT IN TESTS WITH COPPER-SULFATE-TREATED WATER

The results of water-quality analyses, copper-avoidance and mortality tests, and a discussion of factors which may mitigate copper toxicity in the reservoir or affect avoidance-test results and warrant additional study, follow. The standard water-quality data provided below relate primarily to control water in the avoidance tests and acute-toxicity tests. Measurements of copper concentration from copper-avoidance and acute-toxicity tests are presented separately.

Water Quality

Measured temperature, DO concentration, pH, and specific conductance of water used in both channels of the avoidance tests (at six copper concentrations) are summarized in table 1. None of these variables in the treatment channel differed significantly ($\rho < 0.05$) from those in the control channel during any avoidance test, but they differed among the six tests. Mean concentrations and the standard deviations for selected constituents of control (untreated) water are summarized in table 2. Water hardness averaged 15.8 mg/L as $CaCO_3$.

Water Temperature

Water temperatures approximated those in the reservoir and ranged from 15.2 to 18.7°C throughout the day. The standard deviation (*s*) about the mean was less than 1.0°C, except in the 46-µg/L copper-avoidance test, in which it was 1.2°C. Temperatures in this test varied more than in the others because it was conducted over a 2-day period and therefore encompassed extreme late-day and early-morning temperatures. Mean treatment-channel temperatures were 0.1 to 0.5°C greater than those in the control channels because copper-treated water was stored in the 20-L cubitainers for as much as 45 min prior to and during each test.

Dissolved Oxygen Concentration, pH, and Specific Conductance

Dissolved oxygen concentrations ranged from 9.3 to 11.4 mg/L among avoidance tests (table 1). Dissolved oxygen concentrations were generally about 0.1 mg/L lower in the treatment channel than in the control channel as a result of temperature differences. Water pH ranged from 6.80 to 7.14. Although small fluctuations in reservoir-water pH were anticipated, the range of measurements could reflect normal variations in water pH and (or) instrument error. Specific conductance (temperature compensated) measurements ranged from 54 to 57 µS/cm.

Table 1. Statistical summary of selected water properties in control channel and treatment channel of fluvarium during copper-avoidance tests at six target concentrations at West Branch Reservoir, Putnam County, N.Y., June 26 through July 3, 1997

[µg/L, micrograms per liter]

Variable	Target concentration (µg/L)	Channel	Statistics				
			Mean	Standard deviation	Minimum	Maximum	Number of samples
Temperature (degrees Celsius)	15	treatment	17.2	0.6	16.1	18.2	12
		control	16.9	0.5	16.0	18.1	24
	46	treatment	17.0	1.2	15.2	18.7	10
		control	16.5	0.4	16.0	17.3	10
	62	treatment	16.5	0.6	15.9	17.9	12
		control	16.1	0.6	15.2	17.3	16
	76	treatment	17.2	0.7	16.3	18.1	12
		control	16.8	0.6	16.1	17.9	22
	92	treatment	17.0	0.5	16.2	18.0	12
		control	16.8	0.7	15.8	18.0	22
	152	treatment	16.7	0.5	16.2	17.8	12
		control	16.7	0.6	15.8	17.8	22
pH (standard units)	15	treatment	7.01	0.06	6.90	7.10	10
		control	6.99	0.06	6.89	7.06	10
	46	treatment	7.03	0.05	6.97	7.14	9
		control	7.06	0.06	6.99	7.13	4
	62	treatment	7.02	0.05	6.90	7.10	11
		control	6.97	0.09	6.80	7.06	10
	76	treatment	6.98	0.05	6.91	7.06	9
		control	6.98	0.06	6.82	7.02	10
	92	treatment	6.99	0.05	6.93	7.10	12
		control	6.97	0.05	6.90	7.03	8
	152	treatment	7.00	0.02	6.96	7.04	11
		control	6.97	0.08	6.82	7.06	11
Specific conductance (microsiemens per centimeter at 25 degrees Celsius)	15	treatment	54	0.9	54	57	10
		control	54	0.4	54	55	18
	46	treatment	55	0.3	55	56	10
		control	54	0.5	54	55	10
	62	treatment	54	0.5	54	55	12
		control	54	0.5	54	55	16
	76	treatment	54	0.5	54	55	12
		control	54	0.5	54	55	21
	92	treatment	55	0.5	54	55	12
		control	54	0.5	54	55	21
	152	treatment	54	0.5	54	55	12
		control	54	0.3	54	55	16

Table 1. Statistical summary of selected water properties in control channel and treatment channel of fluvarium during copper-avoidance tests at six target concentrations at West Branch Reservoir, Putnam County, N.Y., June 26 through July 3, 1997—Continued

Variable	Target concentration (μg/L)	Channel	Statistics				
			Mean	Standard deviation	Minimum	Maximum	Number of samples
Dissolved oxygen (milligrams per liter)	15	treatment	10.7	0.2	10.2	11.1	12
		control	11.0	.2	10.7	11.4	20
	46	treatment	9.6	.2	9.3	9.8	10
		control	9.8	.2	9.5	10.0	10
	62	treatment	9.7	.2	9.4	10.1	12
		control	9.8	.2	9.4	10.1	16
	76	treatment	10.6	.1	10.3	10.8	12
		control	10.8	.1	10.5	11.1	22
	92	treatment	10.6	.2	10.3	10.8	12
		control	10.7	.1	10.4	11.0	22
	152	treatment	10.0	.3	9.4	10.3	12
		control	10.1	.3	9.6	10.6	16

Table 2. Mean concentrations and standard deviations for selected constituents of control water during copper-avoidance and acute-toxicity tests at West Branch Reservoir, Putnam County, N.Y., June 26 through July 3, 1997.

[Statistics are computed using eight samples. Mean concentrations are in micromoles per liter except as noted; μeq/L, microequivalents per liter; standard deviations are in parentheses]

Sulfate	Nitrate as Nitrogen	TOC[a]	ANC[b] (μeq/L)	Al_{tm}[c]	Chloride	Magnesium	Calcium	Potassium	pH
68.3	18.7	134.6	141.9	0.54	135.0	45.7	112.1	13.9	7.00
(0.71)	(0.46)	(5.92)	(2.13)	(0.06)	(3.60)	(.057)	(0.71)	(0.24)	(0.09)

[a]TOC, total organic carbon.
[b]ANC, acid-neutralizing capacity.
[c]Al_{tm}, total monomeric aluminum.

Dissolved Copper Concentration

Dissolved copper concentrations (Cu^{2+} passing through a 0.45-μm filter) in the avoidance tests differed slightly from the target total-recoverable Cu concentrations, as anticipated. The differences were because of (1) stock aliquot or treatment-solution measurement error, (2) natural binding of the dissolved copper by organic and (or) inorganic constituents, (3) natural background copper concentrations of about 5 μg/L in reservoir waters (New York City Department of Environmental Protection, 1997), (4) possible contamination from laboratory instruments and test chambers, and (5) normal levels of error in laboratory instruments and measurements. In copper-avoidance tests, the mean measured dissolved copper concentrations for total Cu target concentrations of 0, 15, 46, 62, 76, 92, and 152 mg/L were 10, 18, 40, 70, 80, 100, and 183 µg/L, respectively (table 3). Measured dissolved copper concentrations differed significantly among most avoidance tests except the 62- and 76-µg/L dilutions (fig. 3).

The measured dissolved copper concentrations in the acute-toxicity (mortality) tests also were close to target total-recoverable copper concentrations; the differences are attributed to the reasons noted above. In mortality tests, the average measured dissolved copper concentrations for total Cu target concentrations of 0, 3, 15, 46, 62, 76, and 92 µg/L were 7, 10, 20, 45, 62, 70, and 85 µg/L, respectively.

Table 3. Target concentrations, mean measured concentrations, and standard deviations of dissolved copper in control channel and treatment channel of fluvarium during copper-avoidance tests at West Branch Reservoir, June 26 through July 3, 1997

[Concentrations are in micrograms per liter]

Target concentration	Mean measured dissolved copper concentration	Standard deviation	Number of samples
0	9.7	5.4	21
15	18.1	3.8	12
46	40.4	8.4	10
62	70.2	10.2	9
76	79.6	25.2	10
92	99.9	12.5	11
152	183.3	31.5	12

Copper-Avoidance Responses

Cumulative frequency of fish in left and right channels, right- or left-channel preference, and treatment-channel preference during exposure of juvenile brown trout to control water during the six treatment-channel preference tests are summarized in table 4. The cumulative frequency of fish in control and treatment channels and in the decision area, treatment-channel preference, gross avoidance response, and net avoidance response of fish during the six copper-treatment (avoidance) tests are summarized in table 5.

Channel Preference

Results of the channel-preference phase (exposure to untreated reservoir water) that preceded the treatment phase of each avoidance test are summarized in table 4. Right-channel preferences ranged from 32.5 to -99.5 percent, with a mean of -10.8 percent. The results indicate a moderate preference for the left channel, regardless of which channel was to be treated in the subsequent treatment phase. The reason that brown trout preferred the left channel is unknown, but the tendency did not strongly affect channel preferences during the treatment phase of each copper concentration tested. Treatment-channel preferences ranged from 60.5 to -99.5 percent, with a mean of 1.9. The treatment channel preference indicates a minor bias favoring the channel that would receive water treated with copper immediately after each preference phase.

Gross Avoidance Response

The gross distribution (not adjusted for channel preference) of fish in the control channel, treatment channel, and decision area during each test is shown as a histogram in figure 4. These results initially suggest that brown trout are attracted to low (18 and 40 µg/L) and high (183 µg/L) dissolved copper concentrations and that they may avoid intermediate (70 and 80 µg/L) concentrations. Behavioral responses other than avoidance, such as anesthetization, could have affected fish distributions in the fluvarium during the tests and, thus could affect the magnitude and statistical significance of the observed responses.

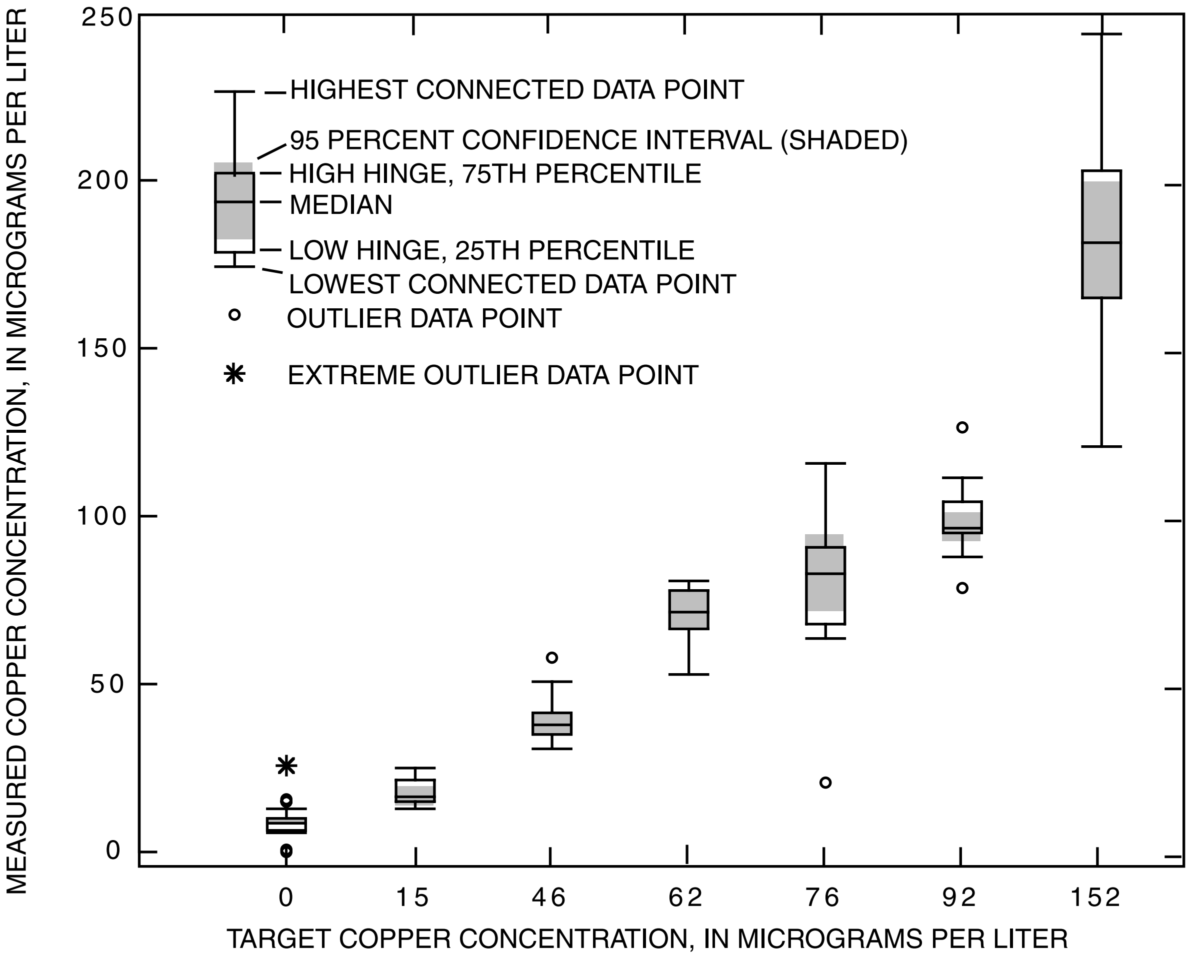


Figure 3. Box and whisker plots showing measured dissolved copper concentrations from treatment and control (0 mg Cu/L target) channels during the six copper-avoidance tests in water from West Branch Reservoir, Putnam County, N.Y., June 26 through July 3, 1997.

In all avoidance tests, the percentage of fish in the decision area increased steadily with increasing copper concentration in the treatment channel (fig. 4). At control (zero) Cu concentrations, fish appeared to spend similar amounts of time in both channels and in the decision area but, as copper concentrations increase, the fish spend more and more time in the decision area and less time in the control and treatment channels. Fish spent almost all of the time in the decision area during the test with the highest copper concentration. This could indicate that the fish were partially narcotized (immobilized) by the high dissolved copper concentrations. If high copper concentrations reduce activity levels of brown trout in a lake system, any avoidance response would be impaired and might explain why fish failed to avoid copper at concentrations higher than 70 µg/L.

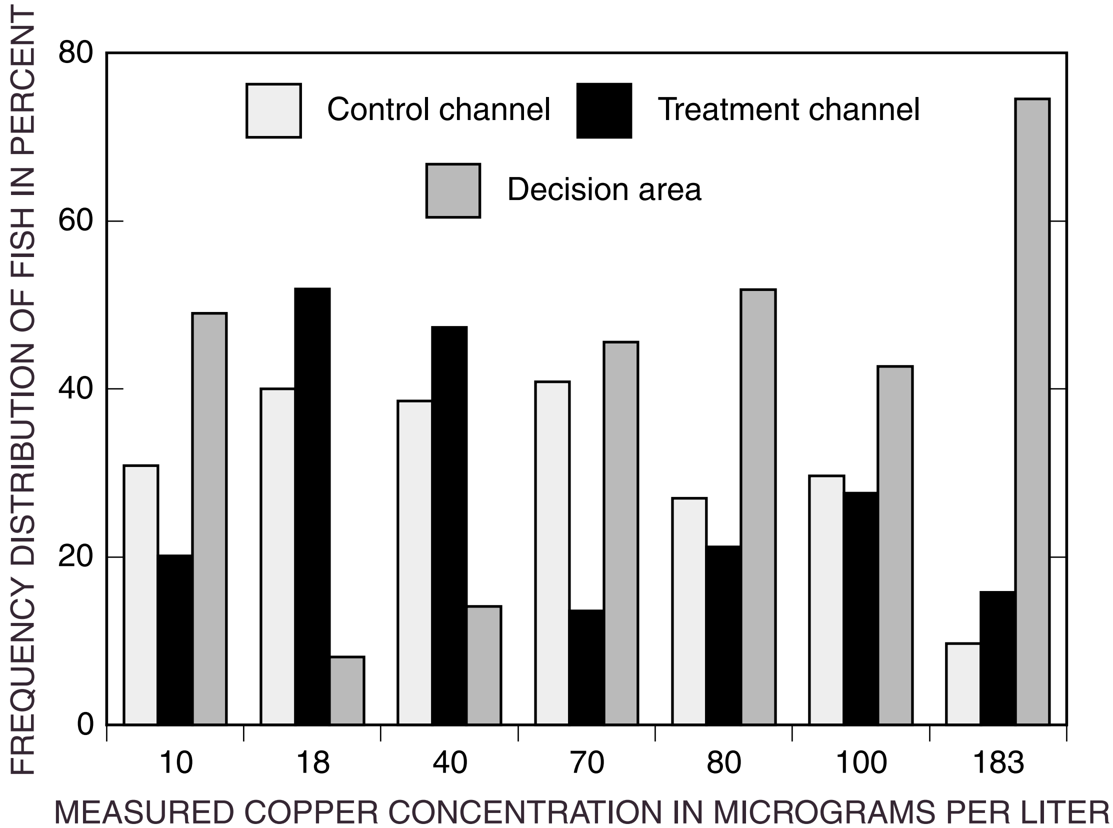


Figure 4. Cumulative frequency of brown trout distributions in treatment and control channels and in the decision area during avoidance tests (replicates are combined) in control exposures and at six copper concentrations in water from West Branch Reservoir, Putnam County, N.Y., June 26 through July 3, 1997.

Net Avoidance Response

The net avoidance responses (table 5) and the box and whisker plots in figure 5 indicate no significant ($\rho \leq 0.05$) median attraction or avoidance response to the six median dissolved copper concentrations. The mean net response and standard error bars in figure 6 depict the central tendency of the responses to copper in the avoidance tests. The ANOVA, GLM, and the nonparametric Kruskal-Wallis test results indicate a significant difference in the net response of fish avoidance tests only at $\rho = 0.126$. Results of Fisher's LSD and Duncan's Multiple Range tests indicate that the mean net avoidance response of brown trout to copper concentrations of 70 and 100 µg/L, and possibly to 80 µg/L, is significantly different (at $\alpha = 0.1$) from the mean net response to the control (untreated) water and to the treated water at most other tested copper concentrations.

Avoidance Threshold Concentration

The highest-no-observed-effect (avoidance response) dissolved copper concentration (NOEC) was about 40 µg/L, and the lowest-observed-effect dissolved copper concentration (LOEC) was about

Table 4. Frequency distribution of brown trout location, right-channel preference, and treatment-channel preference in fluvarium during exposures to untreated (control) water before each copper exposure in avoidance tests at the West Branch Reservoir, June 26 through July 3, 1997

[µg/L, micrograms per liter. All values are in percent. * indicates which channel was subsequently treated with copper]

Target copper concentration	Test replicate	Fish-location frequency distribution			Right-channel preference	Treatment-channel preference
		Left channel	Right channel	Decision area		
15 µg/L	A	10	* 40.5	49.5	30.5	30.5
		* 60	15	25	-45.0	45.0
	B	38	* 58	4	20.0	20.0
		* 32.5	61	6.5	28.5	-28.5
	C	12	* 37.5	50.5	25.5	25.5
		* 28.5	39.5	32	11.0	-11.0
46 µg/L	A	99.5	* 0	0.5	-99.5	-99.5
		* 0	0	100	0	0
	B	* 67.5	11	21.5	-56.5	56.5
		39	* 54.5	6.5	15.5	15.5
	C	* 74	21.5	4.5	-52.5	52.5
		30.5	* 60.5	9	30.0	30.0
62 µg/L	A	47.5	*.5	52	-47.0	-47.0
		* 16.5	49	34.5	32.5	-32.5
	B	* 52	7	41	-45.0	45.0
		19	* 0	81	-19.0	-19.0
	C	* 55	15.5	29.5	-39.5	39.0
		4.5	* 0	95.5	-4.5	-4.5
76 µg/L	A	66	* 2	32	-64.0	-64.0
		* 74.5	14	11.5	-60.5	60.5
	B	58.5	* 10.5	31	-48.0	-48.0
		* 4	4	92	0	0
	C	* 27.5	3.5	69	-24.0	24.0
		11.5	* 23.5	65	12.0	12.0
92 µg/L	A	54	* 4.5	41.5	-49.5	-49.5
		* 15	2.5	82.5	-12.5	12.5
	B	28	* 29	43	1.0	1.0
		* 12	20.5	67.5	8.5	-8.5

Table 4. Frequency distribution of brown trout location, right-channel preference, and treatment-channel preference in fluvarium during exposures to untreated (control) water before each copper exposure in avoidance tests at the West Branch Reservoir, June 26 through July 3, 1997—Continued

Target copper concentration	Test replicate	Fish-location frequency distribution			Right-channel preference	Treatment-channel preference
		Left channel	Right channel	Decision area		
92 µg/L	C	* 35.5	56	8.5	20.5	-20.5
		7.5	* 23.5	69	16.0	16.0
152 µg/L	A	25	* 27.5	47.5	2.5	2.5
		* 0.5	0	99.5	-.5	.5
	B	* 0	2.5	97.5	2.5	-2.5
		1	* 10	89	9.0	9.0
	C	* 0	2.5	97.5	2.5	-2.5
		5.5	* 15.5	79	10.0	10.0
Mean of all replicates		30.9	20.1	49.0	-10.8	1.9
Standard deviation of all replicates		26.2	20.2	32.7	33.5	35.2
Standard error of all replicates		4.4	3.4	5.5	5.6	5.9

70 µg/L for juvenile brown trout. The estimated threshold avoidance (effect) dissolved copper concentration (TEC) is about 55 µg/L. Confidence in these estimates is qualified by the avoidance response at 70 µg/L, which is only significant at $\rho \leq 0.126$.

Mortality Response

Mortality-test results (fig. 7) indicate that 100 percent of the brown trout died at the 85-µg/L dissolved copper concentration during the 96-hr exposures and that many fish succumbed at concentrations of 62 and 70 µg/L. No mortality was observed in the control water (7 µg/L) or at concentrations of 10, 20, or 45 µg/L.

The 96-hr median lethal concentration (LC_{50}) of dissolved copper was 61.5 µg/L, and the lower and upper 95-percent confidence limits were 58.5 and 64.6 µg/L. The highest-no-observed-effect (mortality) dissolved copper concentration (NOEC) was 45 µg/L, and the lowest-observed-effect dissolved copper concentration (LOEC) was 62 µg/L. The estimated threshold mortality (effect) dissolved copper concentration (TEC) is about 53.5 µg/L.

Implications of Copper Treatment for Brown Trout Populations in West Branch Reservoir

Key findings from this study were that juvenile brown trout (1) showed a slight tendency to avoid moderate concentrations of dissolved copper, and (2) decreased their activity or became immobilized in avoidance tests (a possible narcotic response) as copper concentrations increased. The slight avoidance response to dissolved copper concentrations of 70 µg/L, an LC_{50} of 62 µg/L, and 100-percent mortality of fish at 85 µg/L (fig. 7) indicate that (1) treatment of West Branch Reservoir water with dissolved copper concentrations of about 46 µg/L should result in very little mortality of resident trout, (2) treatment with copper concen-

Table 5. Brown trout avoidance responses to exposure to water containing copper sulfate at six concentrations in fluvarium tests at West Branch Reservoir, June 26 through July 3, 1997

[µg/L, micrograms per liter; all values are in percent]

Replicate test	Position of treatment channel		Percentage of fish in: Control channel	Treatment channel	Decision area	Treatment-channel preference	Gross avoidance response	Net avoidance response
15-µg/L copper concentration:								
A	Right		48.7	49.3	2.0	30.5	-0.6	-31.1
	Left		49.0	50.0	1.0	45.0	-1.0	-46.0
		Mean	48.9	49.7	1.5	37.8	-.8	-38.6
B	Right		25.7	73.0	1.3	20.0	-47.3	-67.3
	Left		31.3	65.0	3.7	-28.5	-33.7	-5.2
		Mean	28.5	69.0	2.5	-4.3	-40.5	-36.3
C	Right		46.3	47.3	6.4	25.5	-1.0	-26.5
	Left		39.0	26.7	34.3	-11.0	12.3	-23.3
		Mean	42.7	37.0	20.4	17.3	5.6	-24.9
		Total mean for all means	40.0	51.9	8.1	13.6	-11.9	-33.3
		Standard error of means	6.0	9.3	6.1	12.5	14.4	12.0
		Total mean for all replicates	40.0	51.9	8.1	13.6	-11.9	-33.2
		Standard deviation of replicates	9.8	16.0	13.0	27.7	23.1	31.6
		Standard error of replicates	4.0	6.5	5.3	11.3	9.5	12.9
46-µg/L copper concentration:								
A	Right		0	81.0	19.0	-99.5	-81.0	18.5
	Left		35.3	18.4	46.3	0	16.9	16.9
		Mean	17.7	49.7	32.7	-49.8	-32.1	17.7
B	Left		77.3	11.0	11.7	56.5	66.3	9.8
	Right		54.7	43.3	2.0	15.5	11.4	-4.1
		Mean	66.0	27.2	6.9	36.0	38.9	2.9
C	Left		45.3	52.0	2.7	52.5	-6.7	-59.2
	Right		18.7	78.3	3.0	30.0	-59.6	-89.6
		Mean	32.0	65.2	2.9	41.3	-33.1	-74.4
		Total mean for all means	38.6	47.3	14.1	9.2	-8.8	-17.9
		Standard error of means	14.3	11.0	9.3	29.5	23.8	28.5
		Total mean for all replicates	38.6	47.3	14.1	9.2	-8.8	-17.9
		Standard deviation of replicates	27.2	29.3	17.1	57.4	53.9	45.5
		Standard error of replicates	11.1	12.0	7.0	23.4	22.0	18.6
62-µg/L copper concentration:								
A	Right		74.0	6.3	19.7	-47.0	67.7	114.7
	Left		75.0	6.0	19.0	-32.5	69.0	101.3
		Mean	74.5	6.2	19.4	-39.8	68.4	108.1
B	Left		3.3	0	96.7	45.0	3.3	-41.7
	Right		0.7	0	99.3	-19.0	.7	19.7
		Mean	2.0	0	98.0	13.0	2.0	-11.0

Table 5. Brown trout avoidance responses to exposure to water containing copper sulfate at six concentrations in fluvarium tests at West Branch Reservoir, June 26 through July 3, 1997— Continued

Replicate test	Position of treatment channel		Percentage of fish in: Control channel	Percentage of fish in: Treatment channel	Percentage of fish in: Decision area	Treatment-channel preference	Gross avoidance response	Net avoidance response
62-µg/L copper concentration—Continued:								
C	Left		77.0	2.0	21.0	39.0	75.0	36.0
	Right		15.0	67.3	17.7	-4.5	-52.3	-47.8
		Mean	46.0	34.7	19.4	17.3	11.4	-5.9
		Total mean for all means	40.8	13.6	45.6	-3.2	27.2	30.4
		Standard error of means	21.1	10.7	26.2	18.3	20.7	38.9
		Total mean for all replicates	40.8	13.6	45.6	-3.2	27.2	30.4
		Standard deviation of replicates	38.1	26.5	40.6	37.8	51.5	68.7
		Standard error of replicates	15.6	10.8	16.6	15.4	21.0	28.1
76-µg/L copper concentration:								
A	Right		0.7	74.3	25.0	-64.0	-73.6	-9.6
	Left		65.3	2.7	32.0	60.5	62.6	2.1
		Mean	33.0	38.5	28.5	-1.8	-5.5	-3.8
B	Right		35.0	8.7	56.3	-48.0	26.3	74.3
	Left		47.4	7.3	45.3	0	40.1	40.1
		Mean	41.2	8.0	50.8	-24.0	33.2	57.2
C	Left		6.4	30.3	63.3	24.0	-23.9	-47.9
	Right		7.3	3.7	89.0	12.0	3.6	-8.4
		Mean	6.9	17.0	76.2	18.0	-10.2	-28.1
		Total mean for all means	27.0	21.2	51.8	-2.6	5.8	8.4
		Standard error of means	10.4	9.0	13.8	12.1	13.7	25.4
		Total mean for all replicates	27.0	21.2	51.8	-2.6	5.9	8.4
		Standard deviation of replicates	26.3	27.9	23.2	46.3	49.0	42.8
		Standard error of replicates	10.7	11.4	9.5	18.9	20.0	17.5
92-µg/L copper concentration:								
A	Right		16.0	83.0	1.0	-49.5	-67.0	-17.5
	Left		24.7	1.7	73.6	12.5	23.0	10.5
		Mean	20.4	42.4	37.3	18.5	-22.0	-3.5
B	Right		74.7	17.3	8.0	1.0	57.4	56.4
	Left		23.3	10.0	66.7	-8.5	13.3	21.8
		Mean	49.0	13.7	37.4	3.8	35.4	39.1
C	Left		22.3	46.0	31.7	-20.5	-23.7	-3.2
	Right		17.0	7.7	75.3	16.0	9.3	-6.7
		Mean	19.7	26.9	53.5	-2.3	-7.2	-5.0
		Total mean for all means	29.7	27.6	42.7	-8.2	2.1	10.2
		Standard error of means	9.7	8.3	5.4	5.2	17.2	14.4
		Total mean for all replicates	29.7	27.6	42.7	-8.2	2.1	10.2
		Standard deviation of replicates	22.3	31.3	33.6	24.3	42.7	26.5
		Standard error of replicates	9.1	12.8	13.7	9.9	17.4	10.8

Table 5. Brown trout avoidance responses to exposure to water containing copper sulfate at six concentrations in fluvarium tests at West Branch Reservoir, June 26 through July 3, 1997—Continued

Replicate test	Position of treatment channel		Percentage of fish in: Control channel	Treatment channel	Decision area	Treatment-channel preference	Gross avoidance response	Net avoidance response
152-µg/L copper concentration:								
A	Right		37.0	32.3	30.7	2.5	4.7	2.2
	Left		12.0	1.7	86.3	.5	10.3	9.8
		Mean	24.5	17.0	58.5	-1.5	7.5	6.0
B	Left		.7	23.0	76.3	-2.5	-22.3	-19.8
	Right		.3	25.0	74.7	9.0	-24.7	-33.7
		Mean	.5	24.0	75.5	3.3	-23.5	-26.8
C	Left		7.0	9.0	84.0	-2.5	-2.0	.5
	Right		1.0	3.7	95.3	10.0	-2.7	-12.7
		Mean	4.0	6.4	89.7	3.8	-2.4	-6.1
		Total mean for all means	9.7	15.8	74.5	2.8	-6.1	-9.0
		Standard error of means	7.5	5.1	9.0	.7	9.1	9.6
		Total mean for all replicates	9.7	15.8	74.5	2.8	-6.1	-9.0
		Standard deviation of replicates	14.2	12.7	22.7	5.5	14.3	16.2
		Standard error of replicates	5.8	5.2	9.3	2.2	5.8	6.6

trations of 61 µg/L could produce trout mortality in the range of 40 to 50 percent, and (3) treatment with copper concentrations of at least 91 µg/L could produce nearly 100 percent mortality of resident brown trout (see fig. 7). These estimates are higher than the 11-percent and 38-percent mortality estimates given in the draft environmental impact statement (New York City Department of Environmental Protection, 1997) for brown trout populations in West Branch Reservoir after being treated with copper sulfate at doses (240 and 360 µg/L) that yield total copper concentrations of 61 and 91 µg/L. Differences in mortality predictions between findings from this study and estimates from the New York City Department of Environmental Protection could be because of several factors (listed in the next section) that warrant additional study before either finding could be considered valid.

Toxicity-Mitigating Factors that Warrant Further Consideration

Copper treatment of West Branch Reservoir waters may be less lethal to brown trout than indicated by the results of the acute-toxicity and avoidance tests for several reasons. The adverse effects of toxic concentrations of copper in reservoir populations of brown trout might be mitigated by

(1) greater tolerance of older individuals than juveniles to heavy metals, whereby resident fish populations might respond more conservatively to acutely toxic copper concentrations than the YOY trout used in this study;

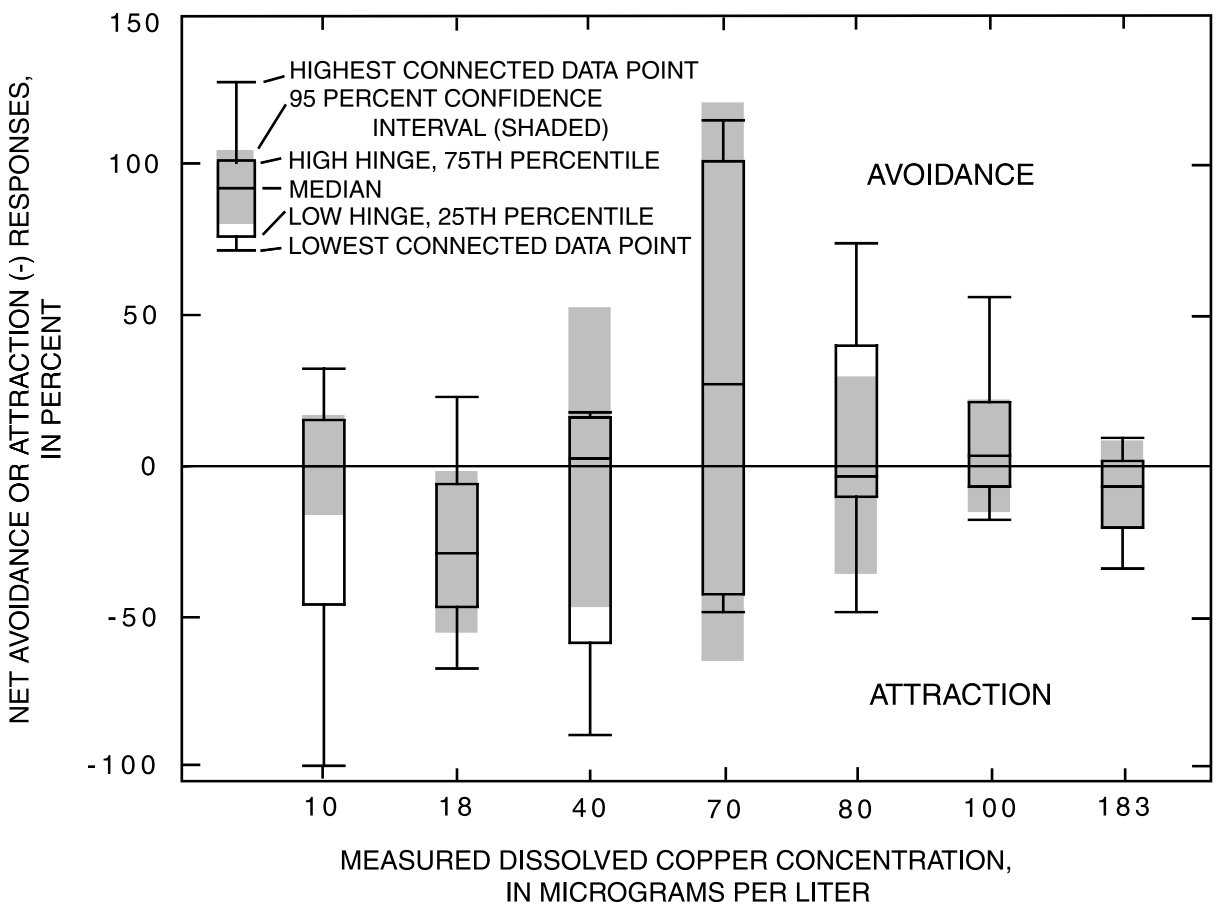


Figure 5. Box and whisker plots showing frequency distribution of median net avoidance or attraction (-) responses of juvenile brown trout exposed to six different dissolved copper concentrations and to control water from West Branch Reservoir, Putnam County, N.Y., June 26 through July 3, 1997.

(2) an effective avoidance response to copper-treated waters;

(3) acclimation of individuals to repeated or slowly increased copper concentrations;

(4) chemical reactions that decrease the toxicity of copper within the reservoir waters; and

(5) a spatial (vertical and horizontal) distribution of individuals that could minimize the population's total exposure to copper during treatments.

The effects of possible mitigating factors on brown trout populations during copper treatment are discussed below.

Age and Size of Fish

Brown trout populations in the reservoir are sustained by a stocking program (New York City Department of Environmental Protection, 1997), and stocked fish are older and larger (1-2 years old and about 23 cm in length) than the YOY trout (about 5 cm in length) used in this study. Older life stages and larger individuals are likely more

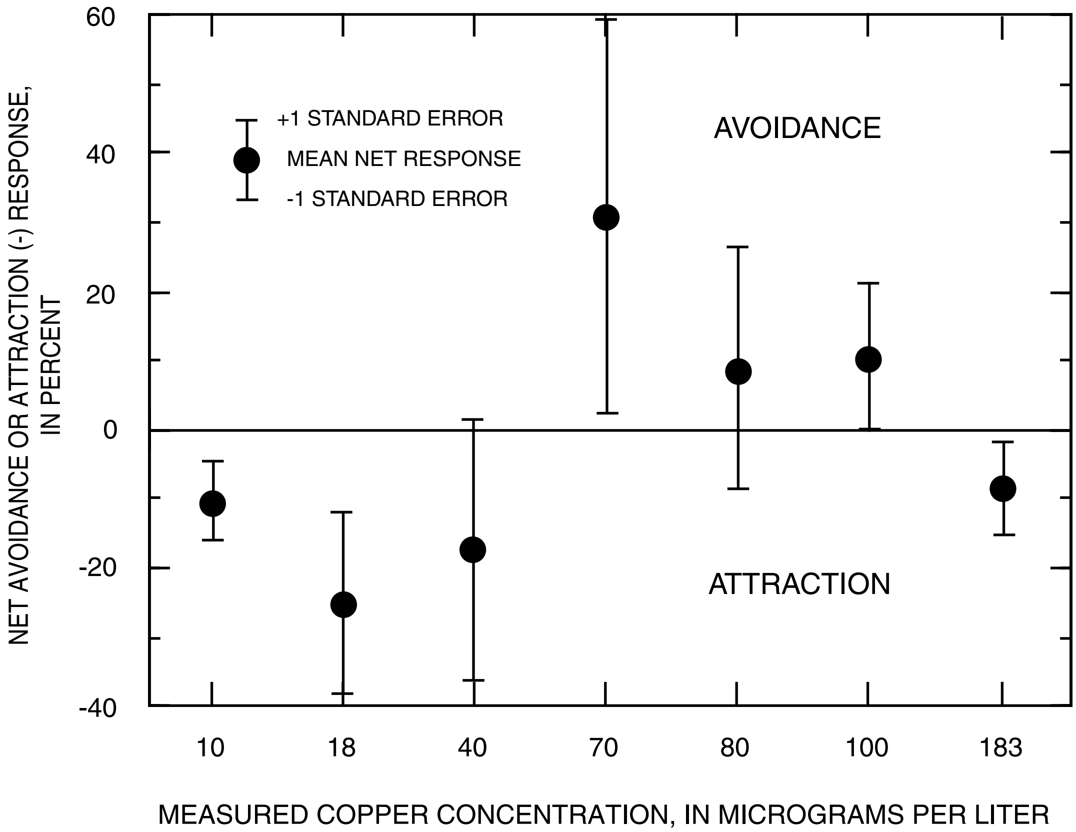


Figure 6. Mean net avoidance or attraction (-) response (and standard error bars) during exposure of brown trout to six different dissolved copper concentrations and to control water from West Branch Reservoir, Putnam County, N.Y., June 26 through July 3, 1997.

tolerant than juveniles to elevated metal concentrations (see review of the effects of pH and aluminum on fish by Baker and others [1990]) and, thus, they also may be able to sense and avoid areas with elevated copper concentrations. Additional copper-avoid-ance and acute-toxicity tests with stockable-sized brown trout could indicate whether large fish are less affected than juveniles are by the proposed treatment concentrations.

Avoidance Capability

The apparent failure of juvenile brown trout to actively avoid the high dissolved copper concentrations is not surprising in that other salmonids have been shown to avoid copper at extremely low concentrations (1-3 µg/L) (Sprauge, 1964) but not at higher concentrations (44-350 µg/L) (Hansen and others, 1995). Brown trout probably are able to actively avoid dissolved copper at certain concentrations; thus, their failure to do so in this study could have been because of the design of the fluvarium, to insufficient exposure periods, or to test conditions that were not representative of those in the reservoir during copper treatments. Although the experiments were conducted onsite with water pumped directly from the reservoir, the 15-min exposures may have been too short for the fish to show an active avoidance response. This possibility is exemplified by the response observed at copper concentrations of 70 and 85 µg/L (and higher) during the 96-hr acute-toxicity tests—fish attempted to jump out of the

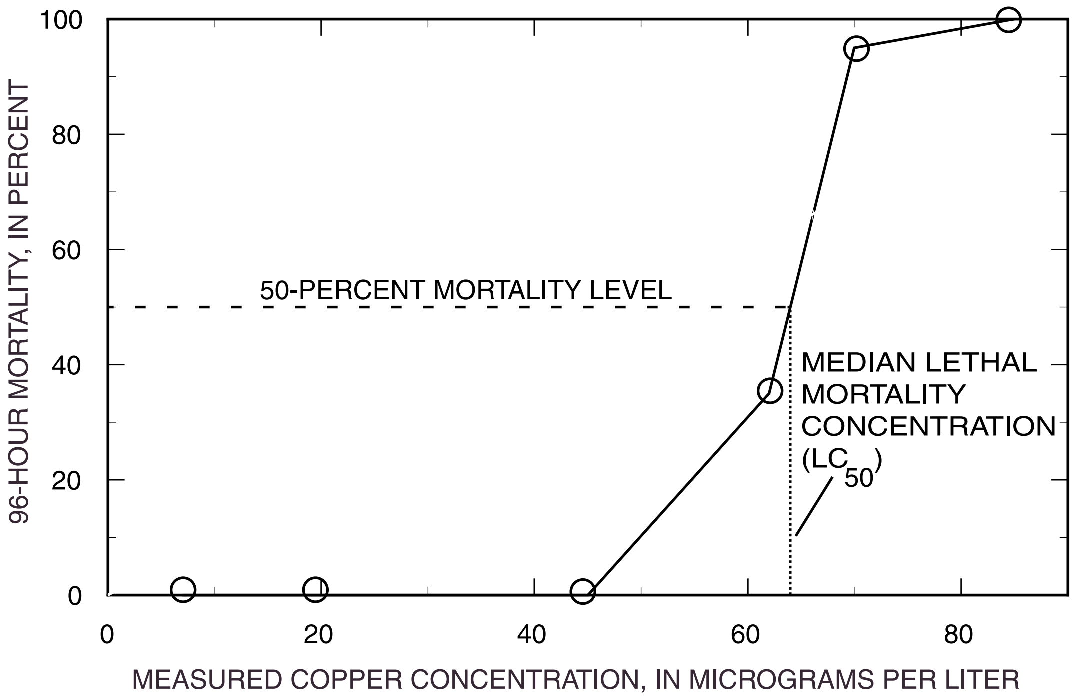


Figure 7. Percent mortality of juvenile brown trout after 96 hours of exposure to six dissolved copper concentrations and to control water water during acute-toxicity (mortality) tests at West Branch Reservoir, Putnam County, N.Y., June 26 through 30, 1997.

aquariums within minutes to hours, after their first exposure to copper-treated water, whereas fish in the 15-min avoidance exposures did not significantly ($\rho \leq 0.05$) avoid dissolved copper concentrations of 70, 80, 100, or 183 µg/L.

These observations indicate that a 15-min test period is probably too short to elicit the avoidance response of brown trout exposed to elevated copper concentrations. Increasing the length of avoidance-test exposures to 30 min, or longer, might allow the fish to react in a manner that would be more comparable to copper exposures in treated reservoirs. The response of fish to toxic waters also might be more rapid and sustained and, thus, more detectable than in these tests if the target exposure concentrations were reached more quickly and if the size of the decision area were smaller. The waters would reach target concentrations more quickly in each channel if the volumes of control (reference) and treatment channels used in the fluvarium were decreased. The fish also would be forced to select either the control channel or the treatment channel during exposures if the size of the decision area was decreased. The relatively large decision area used in this study appears to have hindered an avoidance response—as copper concentrations in successive avoidance tests increased, the fish spent increasing amounts of time in the decision area. Some alternative avoidance-test chambers described in the recent literature (Woodward and others, 1995) do not have a decision area; thus, test fish are forced to select either control water or treated water.

Modification of the fluvarium and (or) use of alternative designs (Woodward and others, 1995), and extending the exposure duration to elicit a clear avoidance response, would be appropriate for additional study because avoidance-detection methods are relatively new and are not standardized. Only after completion of such tests, followed by field verification of findings, could the avoidance of toxic concentrations of copper be confirmed as a factor affecting the resident brown trout populations in New York City reservoirs.

Acclimation to Metals

Even though the acute-toxicity tests indicate that YOY brown trout may die when dissolved copper concentrations exceed 45 µg/L, acclimation to copper by individuals potentially could decrease the effects of copper treatment on trout populations in West Branch Reservoir. Brown trout that had been acclimated to chronically elevated concentrations of metals (mixtures that included copper) had lower mortality rates than nonacclimated fish when exposed to acutely toxic concentrations of these metals; this indicated that their resistance to metal toxicity had increased (Marr and others, 1995b; Marr and others, 1995a). These studies also showed that rainbow trout were more tolerant than brown trout to mixtures of metals. The rainbow trout were less able to acclimate to elevated concentrations of toxic-metals mixtures and ultimately were less resistant to those mixtures than metals-acclimated brown trout (Marr and others, 1995b). Therefore, repeated pulses, or slowly increasing concentrations, of copper during copper treatment of reservoir waters potentially could decrease the mortality rates of brown trout populations if individuals could acclimate to copper before the concentrations became acutely toxic. Acute-toxicity tests to verify the ability of brown trout to acclimate to toxic concentrations of copper would need to be conducted before acclimation could be considered a mitigating factor, however.

Seasonal Water-Quality Changes

Toxicity of copper to resident brown trout populations might be affected by the timing of treatments. For example, seasonal changes in water temperature, hardness, and concentrations of total and dissolved organic carbon (TOC and DOC) and calcium can affect metal availability and the sensitivity of fish to toxic metals (Marr and others, 1995c; Parkhurst, 1987). Water hardness (Ca and Mg) has been shown to alter the toxicity of copper to many aquatic organisms and can be used to modify national water-quality criteria for copper on a site-specific basis (U.S. Environmental Protection Agency, 1986). High DOC concentrations also have been shown to decrease the avoidance response of rainbow trout during copper exposures. DOC concentrations of 8 mg/L and higher have been shown to slightly increase the tolerance of several salmonid species to copper in acute-toxicity tests through the copper-binding affinity of DOC (Marr and others, 1995c). Copper treatments during cooler months, when fish are less active, may result in lower metabolic stresses and lower rates of exposure than in warmer months, when fish are generally most active. Consequently, documentation of seasonal changes in the concentration of chemical constituents that affect fractionation and complexation of copper species in reservoir waters may be warranted.

Horizontal and Vertical Distribution of Individual Fish

The vertical and horizontal distribution of individual fish in the reservoir might affect their exposure and population mortality rates during acutely toxic copper treatments if they are mostly in certain parts of the reservoir or within a specific water-density layer. For example, if copper-treated waters from another reservoir were to enter West Branch Reservoir at temperatures similar to that in the epilimnion, the treated water might not mix with that of the metalimnion or hypolimnion and would not expose the resident fish to the toxic concentrations of copper if they were concentrated at or below the thermocline. Results from surveys of fish distributions and copper concentrations during copper treatments in West Branch and other reservoirs might indicate whether certain fish populations can avoid toxic copper conditions by moving to areas less affected by the treatments. Fish distributions and responses could be best documented through hydroacoustic surveys of fish populations and attendant monitoring of physical and chemical characteristics of the water column before, during, and after copper treatments.

Alternative Exposure—Test Design

One concern in this study was that the design of the avoidance tests (fluvarium and exposure regimes) might have been ineffective in identifying the avoidance response of trout to copper. An alternative approach to studying the effects of copper treatment at the population level would be through field tests with caged brown trout at several locations in the reservoir during copper treatments. Although many factors affect the survival and mortality of fish populations in a lentic system, documented mortality rates of confined fish at selected treatment concentrations would provide an estimate of the worst-case effects of various copper dilutions on resident brown trout populations throughout the reservoir. Use of test cages positioned across the reservoir in a way that would approximate a step increase in copper concentrations during a single treatment would provide the response data needed to estimate mortality under different copper-treatment regimes.

CONCLUSIONS

Results of this study demonstrate that juvenile brown trout may be able to avoid concentrations of dissolved copper greater than about 55 µg/L in reservoirs treated with copper sulfate, but additional (possibly modified) avoidance experiments are needed to verify this finding. The decreased activity (increased time spent in the decision area of the fluvarium) of test fish as copper concentrations increased in avoidance tests was unanticipated and points out the need for alternative fluvarium designs and exposure regimes. High mortality rates at moderate copper concentrations (50-percent mortality at 62 µg of Cu/L) indicate that YOY brown trout die at lower dissolved copper concentrations than conservative models. This suggests that the mortality models, employed to assure the protection of brown trout populations in reservoirs treated with copper sulfate, may not be conservative in regards to YOY fish. Although many factors potentially affect the avoidance and mortality response of individual brown trout and their populations in West Branch and other New York City reservoirs, few are well understood, and most warrant additional study before any of the findings from this study could be used to project the effects of copper-sulfate treatments on resident brown trout populations.

REFERENCES CITED

American Public Health Association, 1989, Standard methods for the examination of water and wastewater, 17th ed.: Washington, D.C., American Public Health Association, 1193 p.

Baker, J.P., Bernard, D.P., Christensen, S.W., Sale, M.J., Freda, J., Heltcher, K., Marmorek, D., Rowe, L., Scanlon, P., Suter, G., Warren-Hicks, W., and Welbourn, P., eds., 1990, Biological effects of changes in surface water acid-base chemistry—NAPAP Report No. 13, Acidic deposition: State of the science and technology: National Acid Precipitation Assessment Program, Oak Ridge, Tenn, 381 p.

Birge, W.J., Hoyt, R.D., Black, J.A., Kercher, M.D., and Robison, W.A., 1993, Effects of chemical stresses on behavior of larval and juvenile fishes and amphibians: American Fisheries Society Symposium, v. 14, p. 55-65.

Ecological Analysts, 1996, Results of avoidance behavior testing with rainbow trout, *Onycorhynchus mykiss*: EA Engineering, Science, and Technology, Inc., 112 p.

Fishman, M.J., and Friedman, L.C., 1989, Methods for the determination of inorganic substances in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations book 5, chap. A1, 545 p.

Hamilton, M.A., Russo, R.C., and Thurston, R.V., 1977, Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays: Environmental Science and Technology, v. 11, no. 7, p. 714-719.

Hansen, J., Bergman, H.L., Meyer, J.S., MacRae, R., Marr, J., Lipton, J., and Cacela, D., 1995, The avoidance of copper by salmonids as affected by metals concentration, organic content, and acclimation, *in* Annual Meeting of Society of Environmental Toxicology and Chemistry: Vancouver, B.C., p. 17.

Helsel, D.R., and Hirsch, R.M., 1992, Statistical methods in water resources—Studies in environmental science: New York, Elsevier Science, v. 49, 522 p.

Lawrence, G.B., Lincoln, T., Horan-Ross, D.A., Olson, M.L., and Waldron, L.A., 1995, Analytical methods of the U.S. Geological Survey's New York District Water-Analysis Laboratory: U.S. Geological Survey Open-File Report 95-416, 78 p.

Marr, J.C.A., Bergman, H.L., Parker, M., Lipton, J., Cacela, D., Erickson, W., and Phillips, G.R., 1995a, Relative sensitivity of brown and rainbow trout to pulsed exposures of an acutely lethal mixture of metals typical of the Clark Fork River, Montana: Canadian Journal of Fisheries and Aquatic Sciences, v. 52, no. 9, p. 2005-2015.

Marr, J.C.A., Bergman, H.L., Lipton, J., and Hogstrand, C., 1995b, Differences in relative sensitivity of native and metals-acclimated brown and rainbow trout exposed to metals representative of the Clark Fork River, Montana: Canadian Journal of Fisheries and Aquatic Sciences, v. 52, p. 2016-2030.

Marr, J.C.A., Lipton, J., Maest, A., Cacela, D., Meyer, J.S., Hansen, J., MacRae, R., and Bergman, H.L., 1995c, Acute lethality and bioavailability of Cu in the presence of DOC, *in* Annual Meeting of Society of Environmental Toxicology and Chemistry: Vancouver, B.C., p. 5.

New York City Department of Environmental Protection, 1997, Draft environmental impact statement—Treatment of the New York City, Delaware, Catskill and Crotan reservoir systems for the control of bacteria, turbidity, algae and zebra mussels, vol. I: CEQR # 92DEP052, 340 p.

Ott, R.L., 1992, An introduction to statistical methods and data analysis, 4th ed.: Belmont, Calif., Duxbury Press, 1170 p.

Parkhurst, B.J., 1987, A comparison of laboratory and in situ bioassays for evaluating the toxicity of acidic waters to brook trout: University of Wyoming, Laramie, Wyo., Ph.D. dissertation, 124 p.

Ramey, B.A., and Colton, L.A., 1986, Effects of acid pH on embryonic and juvenile freshwater fish: U.S. Department of Interior Report, 164 p.

SAS Institute, v. I., 1988, SAS/STAT User's Guide, release 6.03 ed.: Cary, N.C., SAS Institute, Inc., 1028 p.

Sprauge, J.B., 1964, Avoidance of copper-zinc solutions by young salmon in the laboratory: Journal of the Water Pollution Control Federation, v. 36, p. 990-1004.

U.S. Environmental Protection Agency, 1986, Quality criteria for water 1986: U.S. Environmental Protection Agency Report EPA 440/5-86-001, 480 p.

_____1993, Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed.: U.S. Environmental Protection Agency Report EPA/600/4-90/027F, 216 p.

Velleman, P.F., 1996, Data desk handbook, version 5.0: Ithaca, N.Y., Data Description Inc., 319 p.

Woodward, D.F., Hansen, J.A., Bergman, H.L., Little, E.E., and DeLonay, A.J., 1995, Brown trout avoidance of metals in water characteristic of the Clark Fork River, Montana: Canadian Journal of Fisheries and Aquatic Sciences, v. 52, p. 2031-2037.