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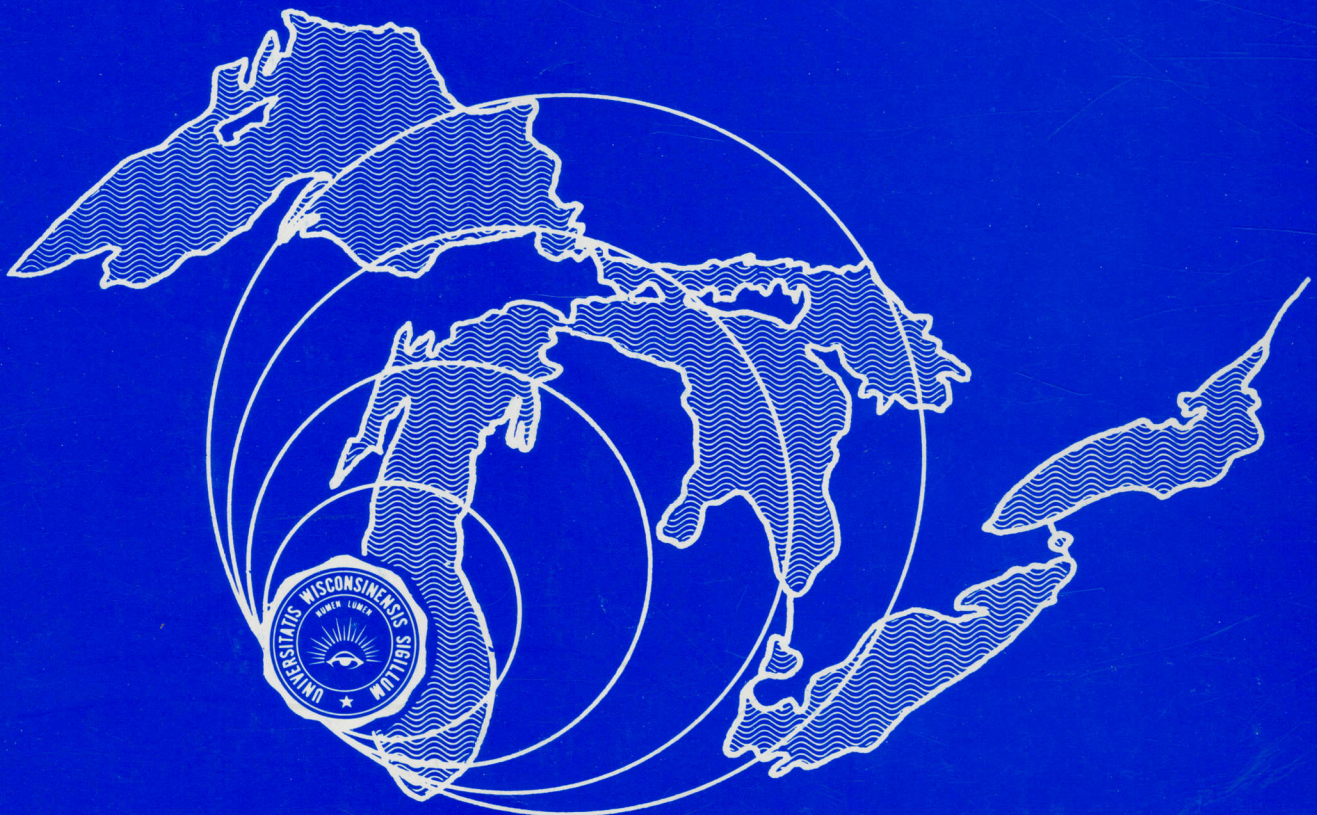

MILWAUKEE, WISCONSIN 53201 U.S.A.

SPECIAL REPORT NO. 31

The Effects of Intermittent Chlorination on the Biota of Lake Michigan

by

A. S. BROOKS and G. L. SEEGERT

Center for Great Lakes Studies
The University of Wisconsin--Milwaukee
Milwaukee, Wisconsin, 53201

January, 1977

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i. ACKNOWLEDGEMENTS

This report represents a final report to the American Electric Power Service Corporation, Consumers Power Company, Northern Indiana Public Service Company, Wisconsin Electric Power Company, Wisconsin Public Service Corporation, and Commonwealth Edison Company who have sponsored the research through a grant to the University of Wisconsin--Milwaukee. We gratefully acknowledge both the financial and technical support provided by the sponsors and their personnel.

We thank the Wisconsin Department of Natural Resources for their contribution of the coho salmon used in these studies. The City of Milwaukee Water Department and the staff of the Linnwood Avenue Filtration Plant were most helpful in supplying untreated Lake Michigan water for several of the experiments.

Completion of the fish experiments was facilitated with the assistance of John VandeCastle, Nancy Liptak and Glen Magyera. Much of the equipment was fabricated by Robert Scott of the CGLS Machine Shop. The invertebrate bioassay work was conducted by David Latimer and Timothy Sharpe as Master's degree research in the Department of Zoology at the University of Wisconsin--Milwaukee. Nancy Liptak and John VandeCastle were of invaluable assistance with the phytoplankton research.

The field surveys were completed through the cooperation

of numerous individuals. We thank Donald Mraz for help in organizing and conducting the cruises, David Baumgartner for assistance with the ship tracking, Scott Burkhardt for help with the chemical analysis and Robert Popp, Captain of the R/V NEESKAY for keeping us afloat and on course. In addition, the cooperation of the plant personnel at each of the survey sites is gratefully acknowledged.

Dr. Kwang Lee of the Department of Mechanics at the University of Wisconsin--Milwaukee was responsible for the plume modeling studies.

We also thank Abe Boman, building manager of the Great Lakes Research Facility where the research was conducted, for his tolerance of wet floors and dead fish and his assistance with numerous aspects of the program. Barbara Kuster and Joan Flores produced the typescript of the report, Kenneth Gradall and Thomas Krischan aided with the final proofing and Ratko Ristic assisted with the figure preparation.

1. Introduction

The intermittent application of chlorine to the cooling water of steam-electric power plants is a common practice employed to control the growth of fouling organisms in the cooling system. Chlorine is a strong oxidizing agent which is toxic to fouling organisms and, thereby, accomplishes effective fouling control. Chlorine is not a specific biocide for fouling organisms, but is toxic to a wide variety of aquatic organisms at all levels of the food chain. Several recent reviews have surveyed the literature and together constitute an excellent bibliography on this subject (Brooks and Seegert, 1976, Brungs, 1973 and 1976 , and Mattice and Zittel, 1976).

At the onset of the present study little was known about the effects of short-term intermittent exposures of chlorine to aquatic organisms and the behavior of chlorine in effluent plumes. This study was designed to investigate the toxicity of intermittent exposures of chlorine to representative species of aquatic organisms commonly found in Lake Michigan. The organisms used in these experiments included natural assemblages of Lake Michigan phytoplankton collected during each season of the year; two species representative of the zooplankton community, the copepods Cyclops bicuspedatus thomasi. and Limnocalanus macrurus an invertebrate representative of the benthic community Pontoporeia affinis; and six species of fish,

Oncorhynchus kisutch (coho salmon) Salmo gairdneri (rainbow trout), Perca flavesens (yellow perch), Alosa pseudoharangus (Alewife), Notropis hudsonius (spottail shiner) and Osmerus mordax (rainbow smelt).

Studies were also undertaken to determine the persistence and dispersion of chlorine in an effluent plume. This phase of the study involved both laboratory and field observations as well as numerical simulation. Each aspect of the overall program, outlined above is presented in more detail in the sections that follow.

2. General Methods

Untreated Lake Michigan water for use in the phytoplankton and invertebrate bioassays as well as the persistence studies was obtained from the City of Milwaukee Linnwood Avenue Filtration Plant (Table 4-1, this report). Fish bioassays were conducted in dechlorinated Lake Michigan water obtained from the Milwaukee municipal system (Table 2-1). Holding and handling procedures for all species tested followed accepted procedures. (Standard Methods 1976, USEPA 1975, National Academy of Sciences, 1973).

Total residual chlorine was determined amperometrically (Standard Methods 1976). A three-electrode cell, consisting of stationary platinum working and counter electrodes and a saturated calomel reference electrode (S. C. E.), was connected to either a Health EUA 19-4-2 or Princeton Applied Research Model 174-A polarograph. The cell potential was maintained at a constant +0.11 volts versus S. C. E. Mixing in the cell was provided by a teflon-coated magnetic stirring bar driven by a 600 r.p.m. synchronous motor. Cell current was graphically recorded by a strip chart recorder. Titrant was delivered by a Metrohm piston buret. The detection limit of the system was 0.001 mg/l residual chlorine. The system could not always effectively discriminate between free and combined chlorine, so measurements were recorded as total residual chlorine. Free chlorine measurements were made using the DPD method (Standard Methods, 1976).

Table 2-1.1974 Average Analysis--Howard Avenue Water Purification Plant, City of Milwaukee. **

Determination	Raw Water	Plant Effluent
pH	8.35	7.60
Turbidity	10.1	.01
Color	3	1
Threshold Odor	3	1
Total Solids*	180	177
Soluable Solids	162	177
Suspended Solids	19	0
Loss on Ignition	64	65
Specific Conductance	229	237
Total Hardness	134	136
Calcium Hardness	90	91
Magnesium Hardness	44	45
Total Alkalinity	112	101
Chlorides	11.3	13.1
Ammonia Nitrogen	0.05	0.16
Organic Nitrogen	0.29	0.15
Nitrate Nitrogen	0.16	0.14
Nitrite Nitrogen	0.009	0.000
Dissolved Oxygen	11.5	12.1
Sulfate	23	33
Phosphate	0.04	0.07
Iron	0.06	0.00
Fluoride	0.05	0.70
Chlorine Residual	.00	0.70
Chemical Oxygen Demand	13.9	10.3
Carbon Dioxide	1.7	5.0
Aluminum	0.01	.04
Sodium	5.8	6.1

*All values below this point are expressed in mg/1.
**Data from City of Milwaukee Water Department.

Mortality data was plotted on log-probability paper. LC-50 values, slopes, and confidence intervals and goodness of fit tests were calculated in accordance with the procedures of Litchfield and Wilcoxon 1949, Finney 1952, and Sprague 1969.

3. FISH

METHODS AND MATERIALS

Fish Collection and Holding Procedures

Young-of-the-year yellow perch were seined from two Wisconsin lakes, Lake Winnebago, Winnebago County, in 1974, and from Pike Lake, Washington County, in 1975. Juvenile rainbow trout were purchased from the Kettle Moraine Springs Trout Hatchery, Adell, Wisconsin. Yearling coho salmon were donated from hatchery stocks by the Wisconsin Department of Natural Resources. Adult smelt were beach seined in Lake Michigan near Milwaukee, Wisconsin, during their April 1976 spawning run. Spottail shiners were seined along Lake Michigan in Door County, Wisconsin. Alewives were lift netted from Lake Michigan near Milwaukee.

All species were acclimated in the laboratory for a minimum of two weeks in large (600-4000 ℓ) circular fiberglass holding tanks. Temperature in these tanks was maintained within 1.5C of the desired test temperature. Acclimation temperatures in the holding tanks were changed at the rate of $1/2^{0}$C per day. Light varied according to the normal seasonal regime. Holding tanks were supplied with dechlorinated Lake Michigan water obtained from the Milwaukee municipal system. Dechlorination was achieved through the use of activated carbon filters followed by the metered addition of a dilute sodium sulfite (Na_2SO_3) solution to remove any remaining chlorine residual. The fish were fed daily except the day before and during a

bioassay. The trout, salmon, shiners, and alewives were fed pelletted "trout chow." The perch were fed ground fish flesh. The smelt received minnows and Gammarus spp.

Experimental Procedures

The bioassays were conducted in 100 ℓ rectangular glass aquaria covered on the sides with opaque plastic. The perch and alewives were tested at temperatures of 10, 15, 20, 25 and 30C; the salmon, shiners, and trout at temperatures of 10, 15 and 20C; and the smelt only at 10C. Temperature control (± 1C) in the bioassay tanks was achieved by connecting the tanks in a flow-through circuit with a 2500 ℓ thermoregulated reservoir tank. Water was continuously pumped from the reservoir tank to the bioassay tanks and returned to the reservoir tank through overflow siphons (Fig. 3-1).

Five experimental and one control group of ten fish each were used in each bioassay (except where noted). The perch, shiners, alewives and smelt were placed in the bioassay (dosing) tanks the day before the test. Initial experiments with the trout and salmon indicated that excessive quantities of nitrogenous waste metabolites were produced during the waiting period which influenced the chlorine species present, so for all subsequent tests these fish were not placed in the dosing tanks until 15-30 minutes before their exposure to chlorine.

To achieve the desired total residual chlorine (TRC) concentration, aqueous sodium hypochlorite (NaOCl) was

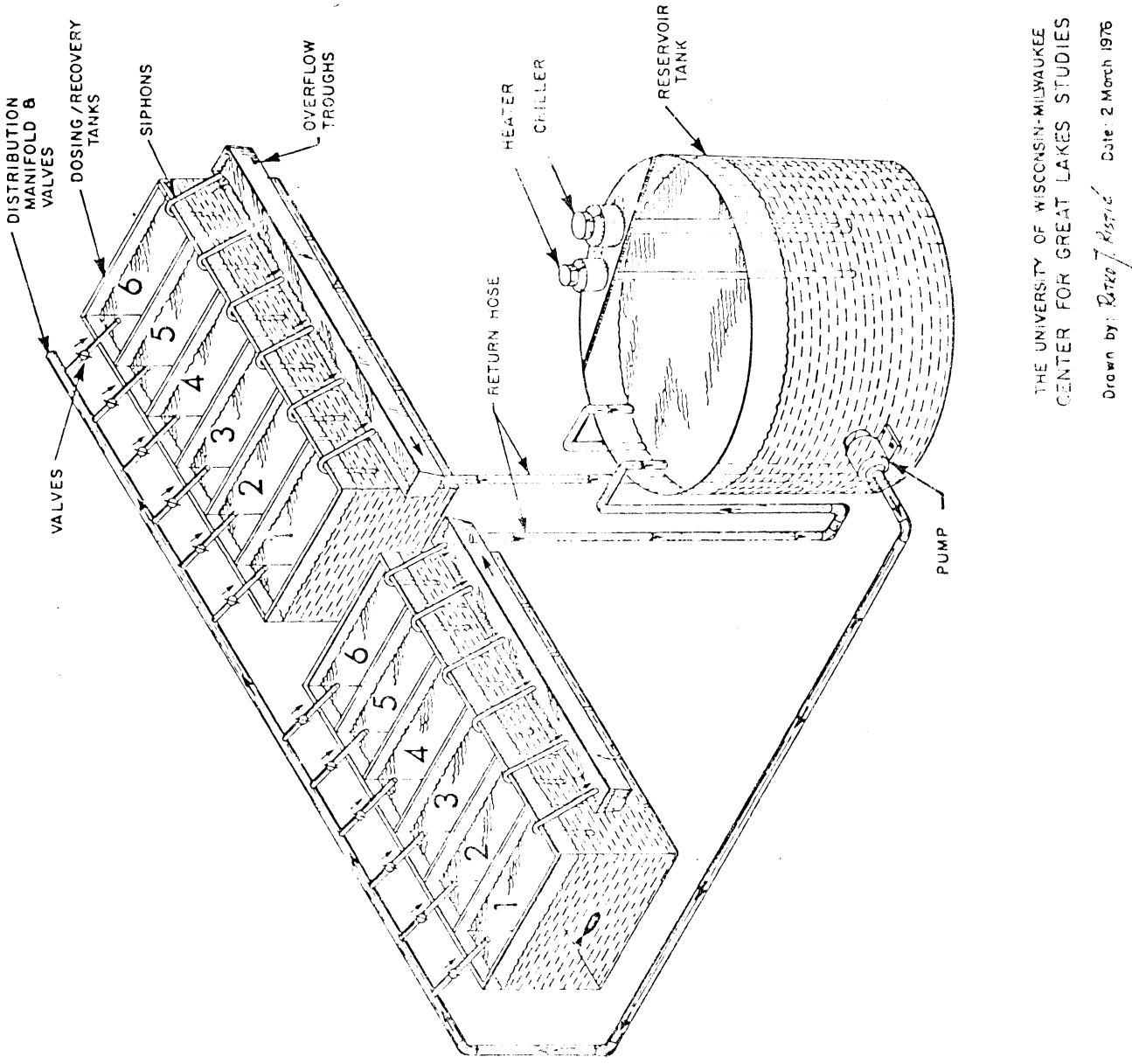

Fig. 3-1 Schematic diagram of the bioassay system.

added to each of the dosing tanks. Prior to the addition of hypochlorite, water flow to the tank being dosed was shut off and the overflow siphon removed to isolate it from the recirculating system. Following the addition of the hypochlorite, the water in the dosing tank was manually stirred for one minute to assure complete mixing. After stirring, samples were collected from each tank for chlorine analysis. Two samples were always analyzed amperiometrically for TRC (Standard Methods, 1976, Seegert, et. al., 1977). Later experiments also used the DPD method to analyze both free chlorine and TRC (Standard Methods, 1976). After the exposure period (5 or 30 minutes) the fish were transferred to 100 ℓ recovery tanks containing chlorine-free water. Additional samples were taken from the dosing tank for chlorine analysis in the manner described above. The initial and final TRC values determined amperiometrically were averaged to give the average TRC concentration to which the fish were exposed. The values derived from the DPD analysis were averaged to determine the percentage of free chlorine in the dosing tanks. During each exposure period the temperature, pH, and dissolved oxygen levels were determined and behavioral observations noted. The multiple 5-minute exposure tests had a 3-hour waiting period between each of three doses. Chlorine values reported for the 5-minute multiple exposure tests represent the average of the three concentrations to which the fish were exposed.

Statistical Analysis

Mortality was assessed in the recovery tanks 24-72 hours after the final chlorine dose. Percent mortality versus TRC concentration was plotted on log-probability paper. LC50 values, slope functions, and confidence intervals were determined according to the graphical methods of Litchfield and Wilcoxon (1949). Their method was also used to determine goodness of fit for the lines drawn and to test for significant differences between LC50 values.

RESULTS

YELLOW PERCH

30-Minute Exposures

Toxic Effects

The resistance of young-of-the-year yellow perch to chlorine varied inversely with temperature (Fig. 3-2). Single-exposure 30-minute LC50 values ranged from 0.70 mg/l at 30C to 8.0 mg/l at 10C (Table 3-1). The biological and chemical parameters monitored during these tests are summarized in Table 3-1. The perch were especially resistant at 10C. No mortalities occurred below 5.1 mg/l while a concentration of 15 mg/l was necessary to affect 100% mortality. At 15C the 30-minute LC50 value was 3.9 mg/l. No mortalities occurred below 1.9 mg/l. At 20C perch exposed to TRC concentrations of 0.5 to 4.6 mg/l had a 30-minute LC50 value of 1.11 mg/l. No mortalities occurred at 0.5 mg/l while complete mortality consistently occurred

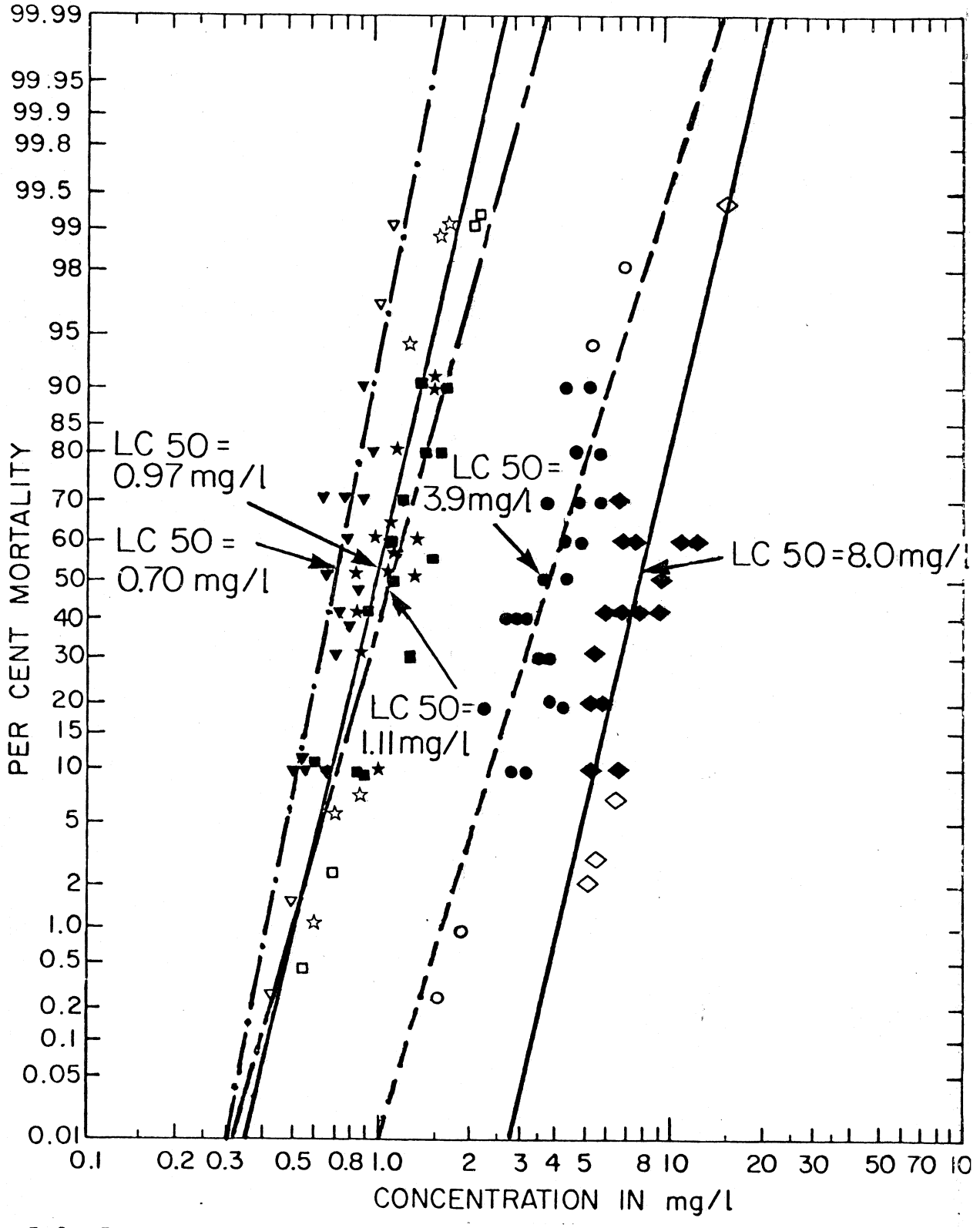

Fig. 3-2 Results of a 30-minute exposure of yellow perch at 10, 15, 20, 25 and 30 C to residual chlorine. Mortality determined 24 hours later after transfer to unchlorinated water for the 10 and 15 C perch and determined 48 hours later for the 20, 25 and 30 C perch. Solid symbols represent observed mortalities; open symbols represent concentrations where 0% or 100% mortality data were transformed: diamonds = 10 C; circles = 15 C; squares = 20 C; stars = 25 C; triangles = 30 C.

Table 3-1. Summary of parameters observed during a 30-minute exposure of yellow perch to residual chlorine.

	Temperature				
	10C	15C	20C	25C	30C
Total length (mm) average (range)	73.5 (60-99)	74.2 (58-90)	79.1 (65-105)	91.2 (69-115)	68.8 (57-81)
Average weight (g)	4.12	4.38	5.48	8.60	3.52
Number fish tested	230	300	190	230	230
pH	8.2-8.7	8.4-8.8	8.2-8.5	8.3-8.5	8.33-8.46
D.O. (mg/liter) average (range)	10.9 (10.2-11.8)	9.8 (8.2-10.2)	9.1 (9.0-9.3)	7.9 (7.0-8.4)	7.7 (7.1-8.6)
Temperature (C) average (range)	10.3 (9.5-11.1)	15.0 (13.5-15.4)	20.0 (19.7-20.3)	25.0 (24.6-25.1)	29.8 (29.1-30.1)
Free chlorine (%) average	NOT MEASURED----------			30 (13-64)	79 (62-90)
No Mortality (mg/liter)	5.1	1.9	0.53	0.68	0.48
100% Mortality (mg/liter)	15	7.1	2.1	1.6	0.95
LC50 (mg/liter) (95% Confidence Interval)	8.0 (6.0-10.8)*	3.9 (2.65-5.7)	1.11 (0.8-1.55)	0.97 (0.72-1.29)	0.70 (0.56-0.88)
Slope function (95% Confidence Interval)	1.34 (1.13-1.59)*	1.47 (1.31-1.65)	1.39 (1.28-1.50)	1.34 (1.25-1.43)	1.26 (1.19-1.31)

*Confidence interval adjusted to compensate for significantly heterogenous data (Litchfield and Wilcoxon, 1949).

above 2 mg/l. The rate of change in sensitivity to chlorine decreased at temperatures above 20C. The 25C 30-minute LC50 value of 0.97 mg/l was only 12% lower than the 20C LC50 value. The drop was still significant, however, at the 5% level. No mortalities were observed at 25C below 0.7 mg/l and complete mortality consistently occurred at concentrations above 1.5 mg/l. Yellow perch at 30C were significantly more sensitive to chlorine than at any of the lower temperatures having a 30-minute LC50 of 0.70 mg/l. No mortalities were observed below 0.5 mg/l while 100% mortality was evident near 1.0 mg/l.

Analysis of the data indicated that the LC50 values were significantly different for each of the test temperatures. Conversely with the exception of the 15C versus 30C groups the slope functions were similar between the groups suggesting that the same toxic mechanism prevailed at all the temperatures. The time to mortality following the 30-minute exposure was temperature related. At 10C and 15C, an average of 88% mortality occurred within two hours of dosing (Table 3-2). Mortality was delayed at higher temperatures. The percentages of mortalities occurring within two hours of dosing decreased progressively to values 23%, 10%, and 5% at 20C, 25C and 30C, respectively (Table 3-2). The majority of mortalities at these higher temperatures occurred 2 to 12 hours after exposure.

Behavioral Observations

Perch exposed to chlorine behaved similarly at all

Table 3-2. CUMULATIVE PERCENT MORTALITY AT SELECTED TIME INTERVALS AFTER EXPOSURE TO CHLORINE

Group	% Mortality time ≤ 2 hrs.	Cum. % Mortality time ≤ 12 hrs.	Cum. % Mortality time ≤ 24 hrs.
Perch 10C	83	94	100
15C	91	93	100
20C	23	73	95
25C	10	72	96
30C	5	62	100
5x3 10C	31	40	62
5x3 20C	50	73	95
Trout 10C(L)	84	100	100
10C(S)	61	83	86
15C	60	87	99
20C(S)	44	100	100
20C(R)	58	85	98
5x3 10C	8	22	74
5x3 20C(R)	83	92	100
5x3 20C(S)	66	99	99
Coho 10C(75)	78	91	97
15C(75)	92	99	100
20C(75)	89	98	100
10C(76)	37	61	87
20C(76)	7	85	100
Alewife 10C	29	76	90
15C	81	90	93
20C	82	90	96
25C	95	99	100
30C	25	73	85
Spottail 10C(75)	79	83	93
10C-M(76)	79	99	100
10C-J(76)	48	57	71
15C	87	97	100
20C	75	94	100
Smelt 10C	29	60	76
5x3 10C	11	21	64

test temperatures. In nonlethal concentrations they were somewhat lethargic but otherwise behaved quite normally. In higher concentrations they typically exhibited exaggerated breathing movements and frequently swam to the surface and gulped for air. While their movements were generally random, they appeared at times to be seeking a way to escape the chlorine. At lethal concentrations the previously described behavior was followed by the fish resting on the bottom and eventually losing equilibrium. Occasional erratic bursts of activity were observed but the fish were generally lethargic during the exposure period. This listlessness usually continued for several hours in the recovery tanks. Only at the highest concentrations did mortalities occur during the 30-minute exposure period. Although specific fish could not be followed individually throughout the bioassay it appeared that any fish losing its equilibrium during the exposure period eventually died. However, fish that did not lose their equilibrium until after transfer to the recovery tanks sometimes did revive. This was particularly evident at 10C where 29% revived following equilibrium loss. Recovery following loss of equilibrium was seen in only 8% of the perch at 15C and rarely (<3%) occurred at any of the warmer temperatures.

5-Minute Triple Exposures

Yellow perch were extremely resistant at 10C to 5-minute doses of chlorine. A single 5-minute exposure killed only one fish out of 100 tested between 9 and 27 mg/l. The

5-minute 3-exposure LC50 value was 22.6 mg/l at 10C (Fig. 3-3 and Table 3-3).

Yellow perch at 20C were also very resistant to chlorine. Their 5-minute 3-exposure LC50 value was 9.0 mg/l (Fig. 3-3). This value is less than one-half of the 10C LC50 multiple-exposure value noted above which is in keeping with the trend established in 30-minute perch experiments where the resistance of perch to chlorine decreases as temperature increases. Yellow perch at 20C were more resistant to a single 5-minute dose of chlorine than to multiple exposures. One hundred fish were tested between 4 and 11.9 mg/l. None died at concentrations between 4 and 10 mg/l while at the highest concentration (11.9 mg/l) about 30% mortality occurred. Observations after the first chlorine dose during the multiple-exposure tests indicated that mortalities began near 10 mg/l and that the LC50 for a single 5-minute exposure was near 12 mg/l. Statistical analysis indicated that the slope functions for the 10C and 20C multiple-exposure groups were similar (Table 3-3), suggesting that the same toxic mechanism prevails at both 10C and 20C.

Although mortalities during the 10C multiple 5-minute tests occurred throughout the observation period, there was a general trend towards delayed mortality (Table 3-2). Sixty percent of the mortalities occurred more than twelve hours after the third dose had been administered.

Mortalities occurred more rapidly during the 20C multiple-exposure perch tests. Forty-five percent occurred

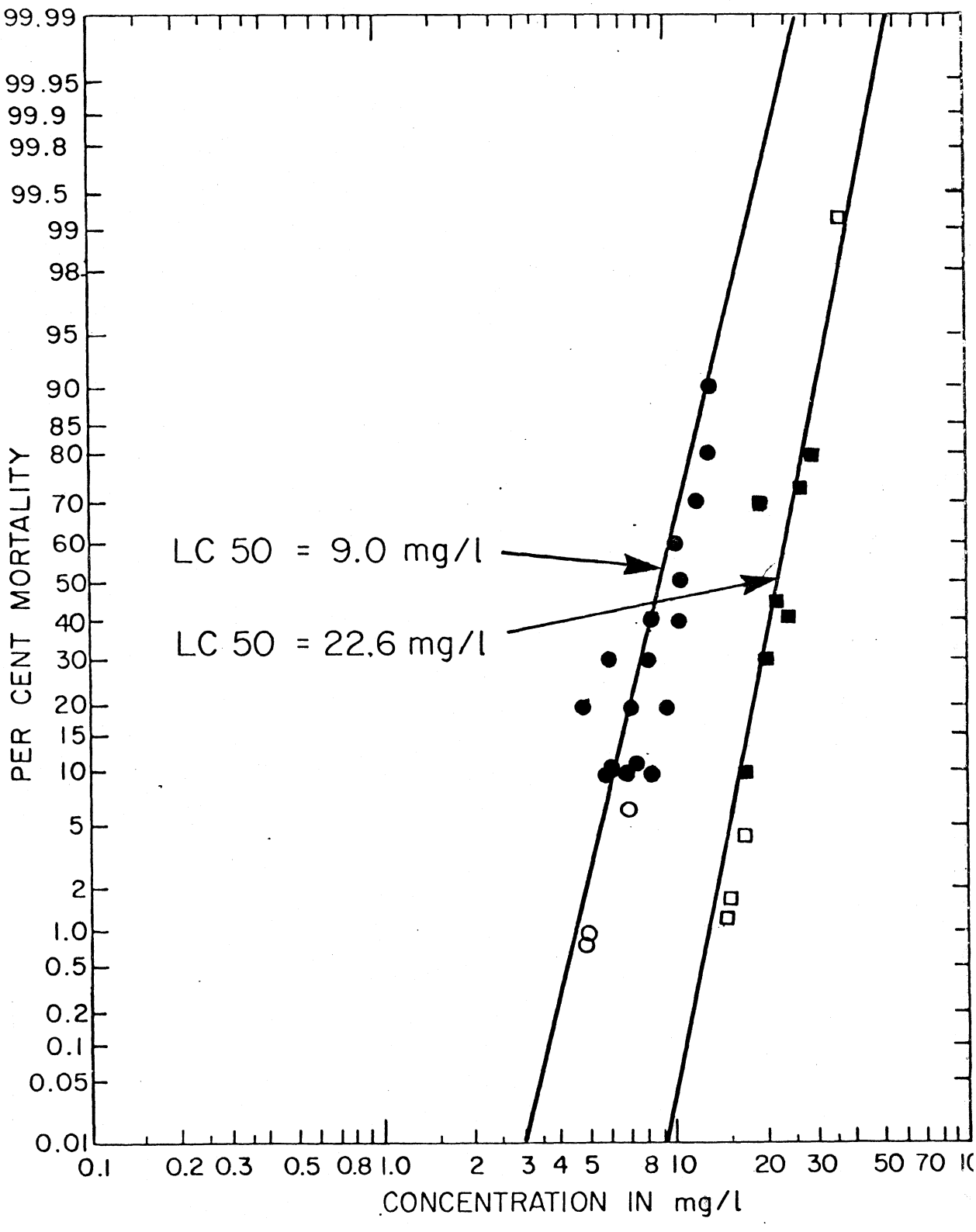

Fig. 3-3 Results of three 5-minute exposures of yellow perch at 10 and 20 C to residual chlorine. Mortality determined 72 hours later after transfer to unchlorinated water for the 10 C perch and determined 48 hours later for the 20 C perch. Solid symbols represent observed mortalities; open symbols represent concentrations where 0% or 100% mortalit data were transformed: squares = 10 C; circles = 20 C.

Table 3-3. Summary of parameters observed during the exposure of yellow perch to three 5-minute doses of chlorine.

	Temperature	
	10C	20C
Length (mm) average (range)	64.8 (57-78)	65.6 (55-80)
Average weight (g)	2.81	2.75
Number fish tested	180	240
pH	8.04-8.41	8.24-8.52
D.O. (mg/liter) average (range)	10.3 (8.8-11.1)	9.0 (7.8-9.8)
Temperature (C) average (range)	10.0 (9.7-10.9)	19.9 (19.2-20.9)
Free chlorine (%) average (range)	95.5 (71-100)	96.5 (79-100)
No Mortality (mg/liter)	1.7	Not Determined
100% Mortality (mg/liter)	37	Not Determined
LC50 (mg/liter) (95% Confidence Interval)	22.6 (18-28.7	9.0 (7.96-10.17)*
Slope function (95% Confidence Interval)	1.26 (1.17-1.36)	1.34 (1.17-1.54)*

*Confidence interval adjusted to compensate for significantly heterogenous data (Litchfield and Wilcoxon, 1949).

before the third dose had been administered and 95% occurred within 24 hours of the third dose (Table 3-2). This contrasts with the delayed mortality seen during the perch 10C multiple-exposure tests described above.

At both 10C and 20C the fish were quite lethargic during both the dosing and observation phases of the test. When fish died they exhibited the same series of reactions that were previously described for the 30-minute perch tests. Only about 7% of those fish losing their equilibrium were able to recover.

RAINBOW TROUT

30-Minute Exposures

Toxic Effects

Two groups of different size rainbow trout were tested at 10C. The data derived from the two groups exhibited similar slope functions, but significantly different 30-minute LC50 values of 2.11 mg/l and 0.99 mg/l for the "large" and "small" fish, respectively (Fig. 3-4 and Table 3-4). Although there was a considerable difference in size between the two groups, the difference in LC50 values was more probably a result of different chlorine species being present in the exposure water (Table 3-4). The 10C "small" group was tested in water containing an average of 75% free chlorine. Although free chlorine measurements were not taken during the 10C "large" trout tests, the accumlation of nitrogenous wastes in the dosing tanks probably produced solutions which were primarily chloramines. The plotted

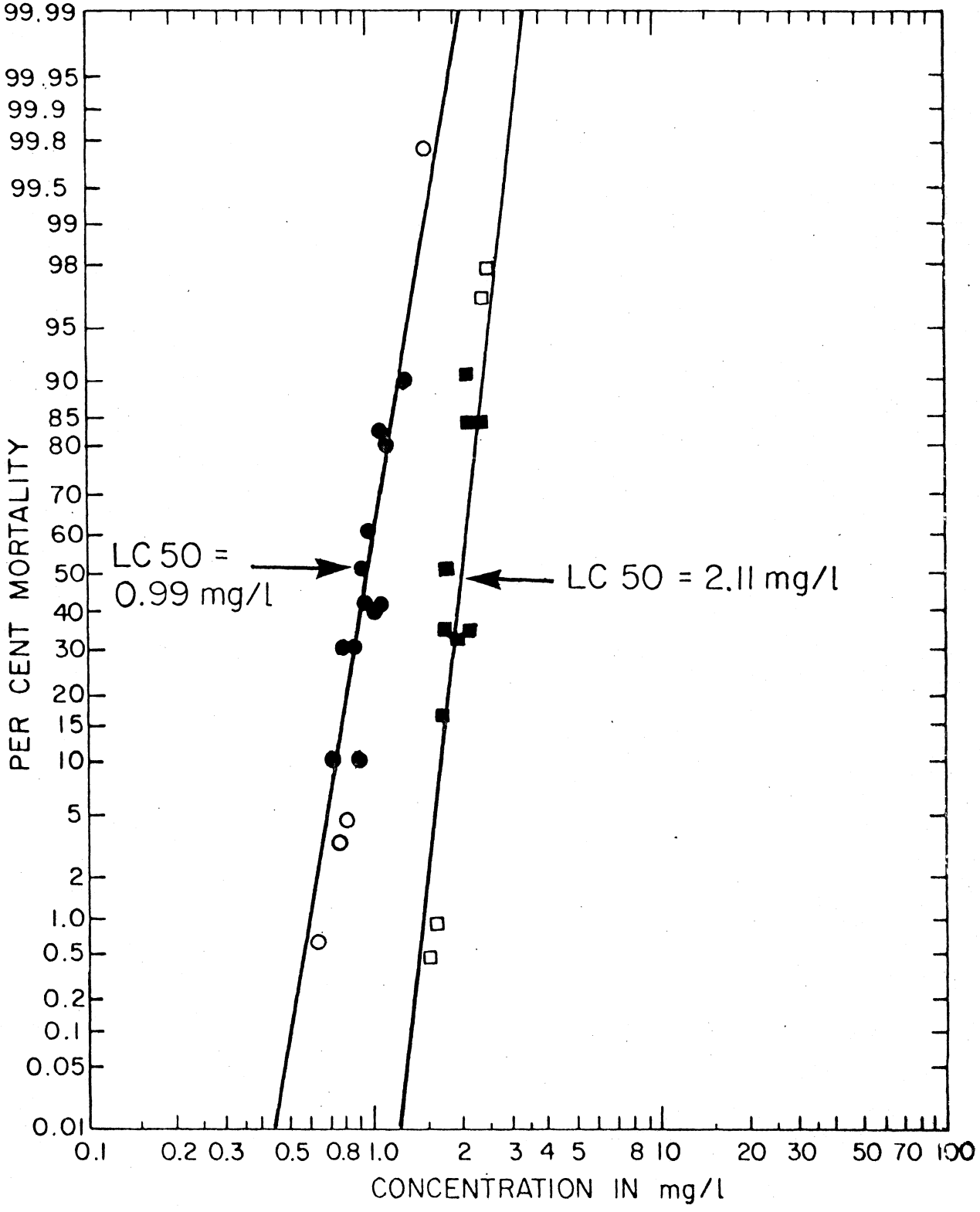

Fig. 3-4 Results of a 30-minute exposure of rainbow trout at 10 C to residual chlorine. Mortality determined 24 hours later after transfer to unchlorinated water for the 10 C "large" trout and determined 48 hours later for the 10 C "small" trout. Solid symbols represent observed mortalities; open symbols represent concentrations where 0% or 100% mortality data were transformed: squares = "large" trout; circles = "small" trout.

Table 3-4. Summary of parameters observed during a 30-minute exposure of rainbow trout to residual chlorine.

	Temperature				
	10C "large"	10C "small"	15	20C "sensitive"	20C "resistant"
Total length (mm) average (range)	281 (190-325)	188 (163-206)	196 (153-228)	172 (143-198)	179 (155-202)
Average weight (g)	268	74.7	83.3	60.7	60.9
Number fish tested	90[a]	190	320	180	130
pH	Not Measured	7.85-8.18	7.81-8.17	7.90-8.33	7.94-8.15
D.O. (mg/liter) average (range)	8.7 (7.8-9.9)	9.1 (8.5-10.0)	8.5 (7.9-9.0)	8.8 (8.4-9.9)	8.3 (7.8-8.8)
Temperature (C) average (range)	10.6 (9.8-11.1)	10.1 (9.5-10.6)	15.1 (14.5-15.6)	20.2 (19.9-20.5)	20.0 (19.6-20.5)
Free chlorine (%) average (range)	Not Measured	75 (63-85)	57 (47-82)	42 (13-82)	56 (40-68)
No Mortality (mg/liter)	1.65	0.65	0.54	0.30	0.45
100% Mortality (mg/liter)	2.5	1.6	1.5	0.56	0.69
LC50 (mg/liter) (95% Confidence Interval)	2.11 (2.00-2.23)	0.99 (0.93-1.07)	0.94 (0.665-1.35)	0.43 (.38-.477)*	0.60 (0.51-0.71)*
Slope function (95% Confidence Interval)	1.15 (1.05-1.23)	1.23 (1.14-1.32)	1.42 (1.29-1.57)	1.22 (1.08-1.38)*	1.18 (1.07-1.30)*

[a] 6 fish tested/tank.

*Confidence interval adjusted to compensate for significantly heterogenous data (Litchfield and Wilcoxon, 1949).

data for both 10C groups had extremely steep slopes (Fig. 3-4). Because the 10C "large" trout were considerably different from all the other groups with respect to both size and the chlorine species to which they were exposed, the results obtained at the other temperatures, presented below, will be compared with the 10C "small" group.

Rainbow trout similar in size to the 10C "small" group had a 30-minute LC50 value of 0.94 mg/l at 15C (Table 3-4 and Fig. 3-5). No mortalities occurred below 0.54 mg/l while 1.5 mg/l caused 100% mortality.

Two groups of similarly sized trout obtained in separate shipments from the same fish hatchery had significantly different 20C LC50 values. The LC50 values were 0.60 mg/l and 0.426 mg/l for what will be termed the "resistant" and "sensitive" groups, respectively (Table 3-4 and Fig. 3-5). Both 20C groups were significantly more sensitive to chlorine than were the trout at 10C. This was also apparently the case when compared to the 15C group, however significantly different slope functions prohibited statistical comparison. The 20C "resistant" group exhibited 100% mortality at 0.69 mg/l and no mortalities at 0.45 mg/l while the "sensitive" group experienced 100% mortality at 0.56 mg/l and no mortality at 0.30 mg/l. The cause for the observed difference in LC50 values is not readily apparent. An examination of the biological and chemical parameters measured during the tests indicated the groups were very similar (Table 3-4).

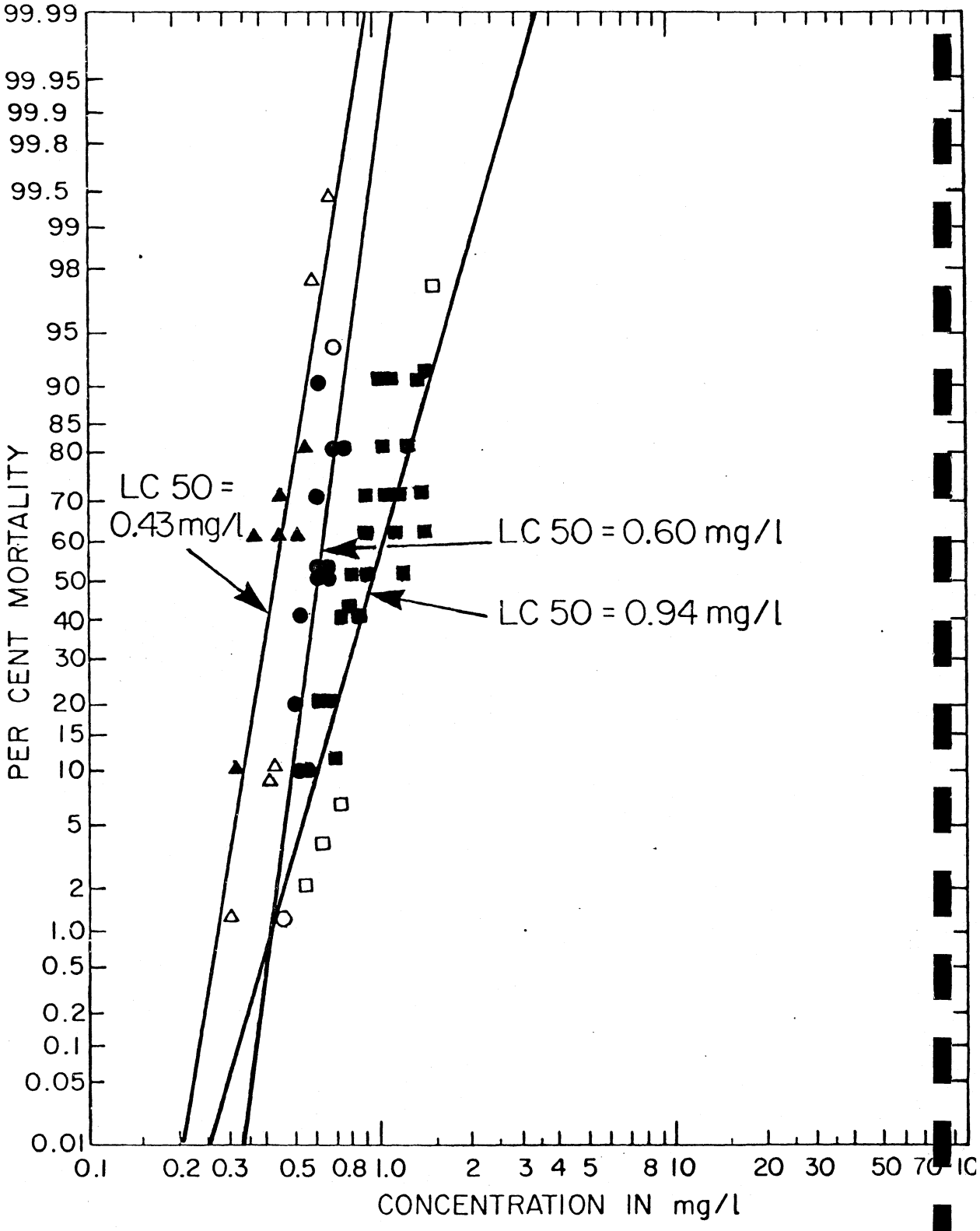

Fig. 3-5 Results of a 30-minute exposure of rainbow trout at 15 and 20 C to residual chlorine. Mortality determined 48 hours later after transfer to unchlorinated water. Solid symbols represent observed mortalities; open symbols represent concentrations where 0% or 100% mortality data were transformed: squares = 15 C; circles = 20 C "resistant"; triangles = 20 C "sensitive."

The temperature-related effects so clearly demonstrated in the perch experiments were less pronounced in the trout tests. There was little difference between the LC50 values for similar size trout tested at 10 and 15C, however the trout were clearly more sensitive to chlorine at 20C than at 10 or 15C (Table 3-4). Delayed mortality observed in the perch tested at higher temperatures was not evident in the rainbow trout experiments. The majority (44 - 84%) of trout mortalities in the five groups tested occurred within 2 hours of the initial dosing and almost all (83 - 100%) the mortalities occurred within 12 hours of dosing (Table 3-2).

Behavioral Observations

The behavior of rainbow trout exposed to chlorine for 30 minutes was similar to that described for yellow perch, however some differences were noted. The trout were generally more active during the exposure period. This was especially noticeable in concentrations which produced rapid mortality. In those cases the trout often swam near the surface exhibiting erratic and sometimes violent bursts of activity. Lower chlorine concentrations produced lethargic behavior. While the perch typically rested on the bottom before dying, the trout often hung in a vertical position (head up) near the surface before finally sinking to the bottom to die. The rainbow trout rarely (<5%) recovered from loss of equilibrium at any of the test temperatures.

5-Minute Triple Exposures

The 10C 5-minute 3-exposure LC50 value for juvenile rainbow trout was 2.87 mg/l (Fig. 3-6 and Table 3-5). No mortality occurred below 1.7 mg/l. One hundred and thirty trout were tested with a single 5-minute dose of chlorine at concentrations from 1.5 to 4.0 mg/l. Since only two fish died in these tests, the LC50 for a single 5-minute dose at 10C was evidently above 4.0 mg/l.

Rainbow trout from the "sensitive" group, as defined for the 30-minute 20C tests, were compared at 20C with a batch of trout from a new shipment. The fish from the "sensitive" group were again significantly more sensitive to chlorine. The 3-exposure LC50 values were 1.65 mg/l and 0.82 mg/l for what will be termed the "resistant 2" and "sensitive" groups, respectively (Fig. 3-6 and Table 3-5). In the "resistant 2" group the chlorine concentrations resulting in 0 and 100% mortality were 1.0 and 2.5 mg/l, respectively, while in the "sensitive" group 0.55 mg/l caused 10% mortality and concentrations greater than 1.3 mg/l consistently caused 100% mortality.

The slope functions for the two 20C groups and the 10C group were similar although the LC50 value for each group differed significantly (Table 3-5). The lower resistance to chlorine shown by the trout at 20C follows the trend of decreased resistance at higher temperatures observed during the 30-minute tests.

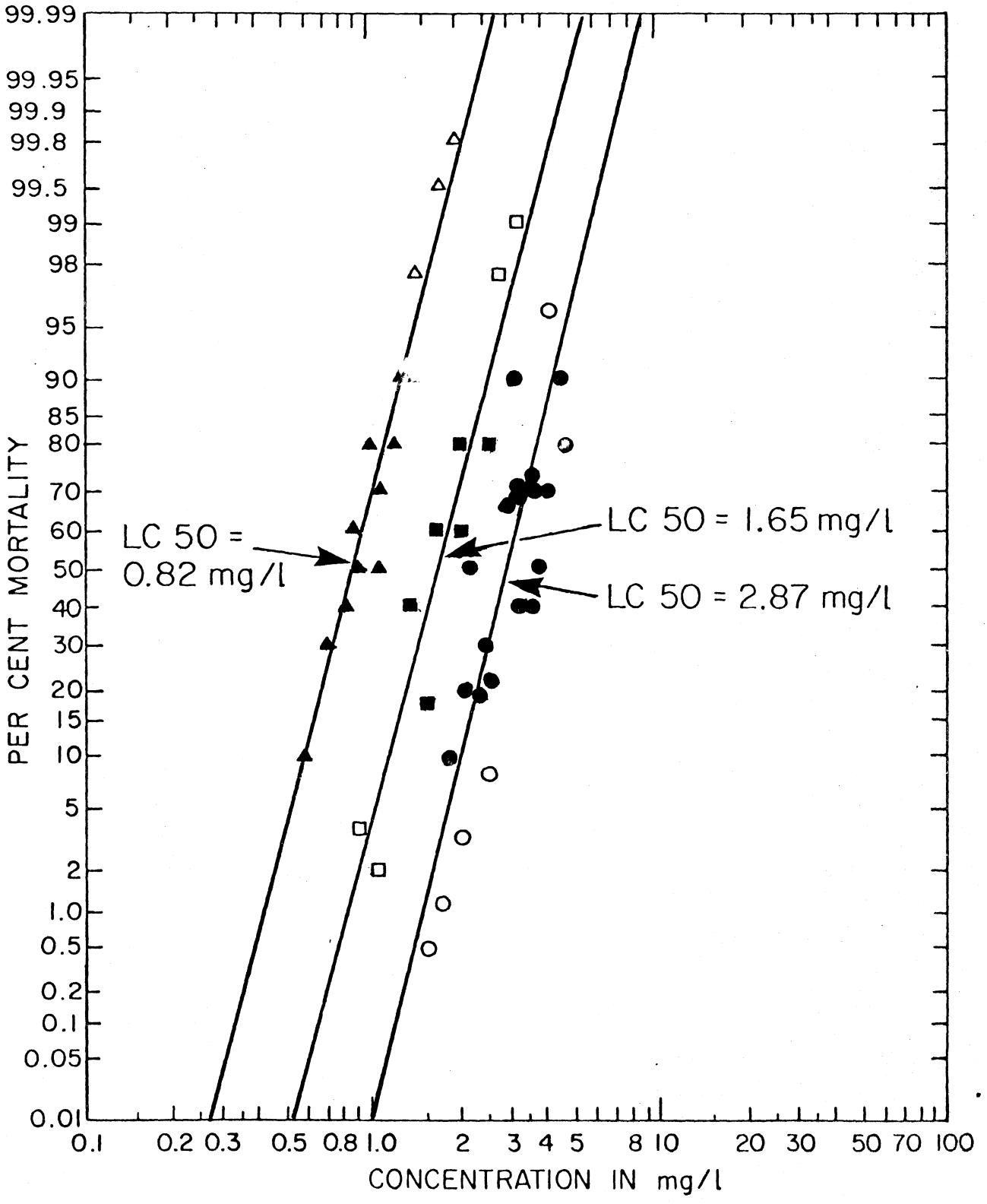

Fig. 3-6 Results of three 5-minute exposures of rainbow trout at 10 and 20 C to residual chlorine. Mortality determined 48 hours later after transfer to unchlorinated water. Solid symbols represent observed mortalities; open symbols represent concentrations where 0% or 100% mortality data were transformed: circles = 10 C; squares = 20 C "resistant 2"; triangles = 20 C "sensitive."

Table 3-5. Summary of parameters observed during the exposure of rainbow trout to three 5-minute doses of chlorine.

	Temperature		
	10C	20C	
		"resistant 2"	"sensitive"
Length (mm) average (range)	138.5 (110-168)	160.9 (135-181)	161.4 (140-197)
Average weight (g)	32.0	46.7	47.9
Number fish tested	280	110	170
pH	7.86-8.30	7.89-8.45	7.87-8.30
D.O. (mg/liter) average (range)	10.1 (9.4-11.1)	7.6 (7.3-8.1)	9.1 (8.2-9.7)
Temperature (C) average (range)	10.5 (9.8-11.3)	19.1 (19.4-20.6)	20.0 (19.6-20.5)
Free chlorine (%) average (range)	95.0 (83-100)	76.3 (47-95)	68.8 (53-88)
No mortality (mg/liter)	1.71	1.01	Not Determined
100% Mortality (mg/liter)	Not Determined	2.5	1.5
LC50 (mg/liter) (95% Confidence Interval)	2.87 (2.56-3.21)*	1.65 (1.46-1.86)	0.82 (.61-1.11)
Slope function (95% Confidence Interval)	1.32 (1.19-1.48)*	1.37 (1.20-1.56)	1.35 (1.23-1.49)

*Confidence interval adjusted to compensate for significantly hetergenous data (Litchfield and Wilcoxon, 1949).

A short series of single 5-minute exposure tests were run at 20C. A difference between the "sensitive" and "resistant" groups was again evident (Fig. 3-7). Although insufficient data prohibited statistical analysis the approximate LC50 values were 1.85 mg/l and 1.3 mg/l for the "resistant 2" and "sensitive" groups, respectively.

In contrast to the rapid mortality which occurred in the 30-minute tests at 10C, mortalities in the 5-minute tests at 10C were delayed. Only 22% of the mortalities occurred within 12 hours after the third dose had been administered. Fifty-two percent of the mortalities occurred during the next 12 hours, while 26% occurred during the final 24 hours of the observation period (Table 3-2). This is similar to the pattern seen in the 10C triple exposure perch tests. At 20C mortalities occurred very rapidly in both the "sensitive" and "resistant 2" groups during the multiple-exposure tests. Fifty-nine percent of the mortalities occurred before the third dose was administered. Most of the remaining mortalities occurred during the first few hours after the third dose (Table 3-2). No fish losing equilibrium recovered at either 10C or 20C. At both 10C and 20C the trout actively swam about during the dosing period but were quite lethargic otherwise. Reactions to lethal concentrations of chlorine were similar to those described for the 30-minute rainbow trout experiments.

COHO SALMON

30-Minute Exposures

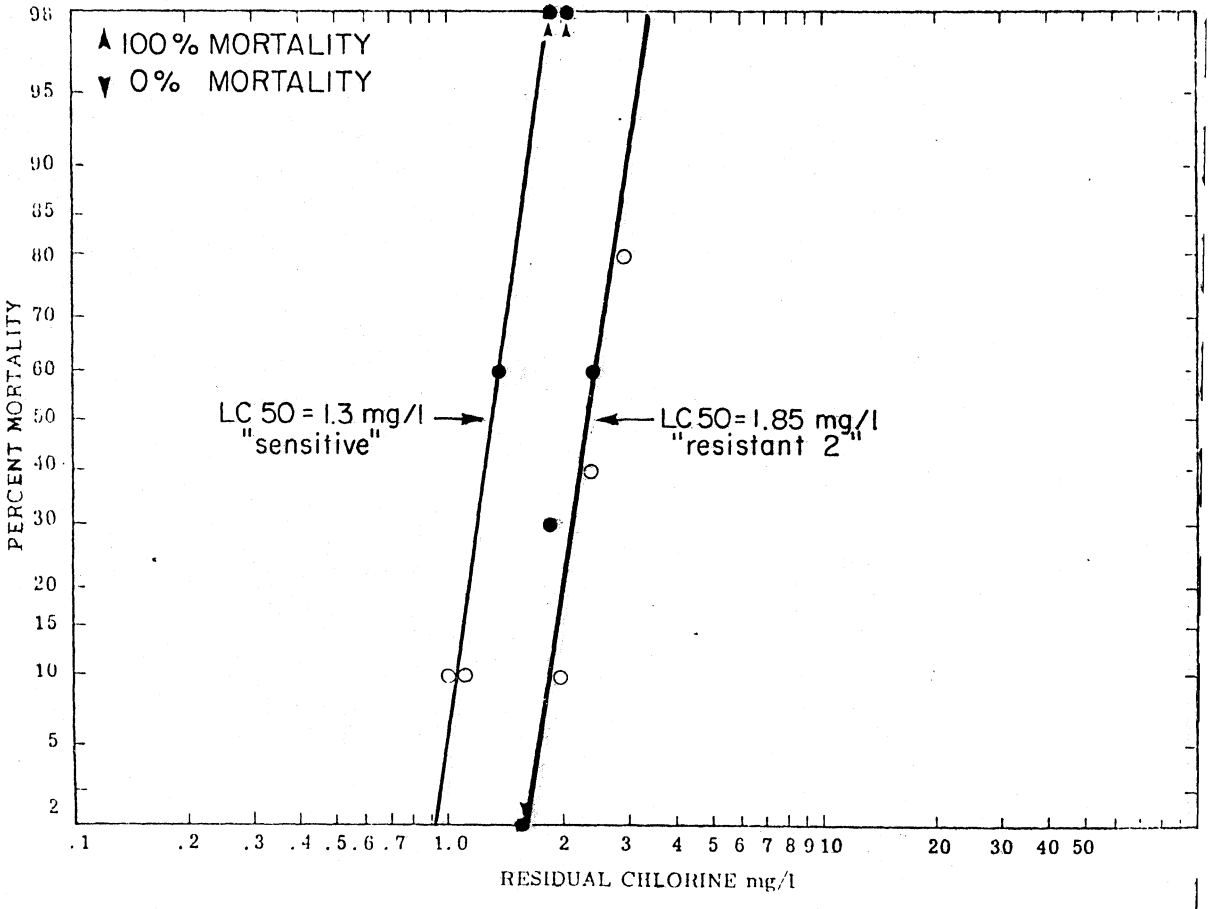

Fig. 3-7 Results of a single 5-minute exposure of rainbow trout at 20 C to residual chlorine. Solid circles represent mortality determined 48 hours later after transfer to unchlorinated water. Open circles represent mortality determined 3 hours later after transfer to unchlorinated water.

Toxic Effects

Coho salmon were the most sensitive of the six species tested. A complete summary of parameters measured for all the coho salmon tests appears in Table 3-6. Tests conducted in 1975 using predominately chloramine solutions yielded LC50 values of 1.26 mg/l, 1.38 mg/l, and 0.9 mg/l at 10C, 15C, and 20C, respectively. Statistical analysis indicated no significant differences between the 10C and 15C LC50 values. At both temperatures little (0 - 10%) mortality occurred at 1.0 mg/l while high (70 - 100%) mortality occurred at concentrations above 1.5 mg/l (Fig. 3-8). Extreme variability in the 20C data precluded statistical analysis. It was quite apparent, however, that at 20C coho salmon were considerably more sensitive to chlorine than they were at the lower temperatures.

Mortalities occurred rapidly at all three temperatures. Percentages of mortalities occurring within two hours of dosing were 78%, 92%, and 89% at 10C, 15C, and 20C, respectively (Table 3-2). Virtually no mortalities occurred after the initial 24 hours of the observation period (Table 3-2).

Because of the high chloramine levels and the extreme variation in the 20C data, 10C and 20C coho experiments were repeated in 1976. The percent free chlorine values in 1976 were much higher (66%) at both temperatures compared to the values (24%) found during the 1975 tests (Table 3-6). As a result the 1976 LC50 values were much lower being 0.56 mg/l; and 0.287 mg/l at 10 and 20C, respectively (Fig. 3-8).

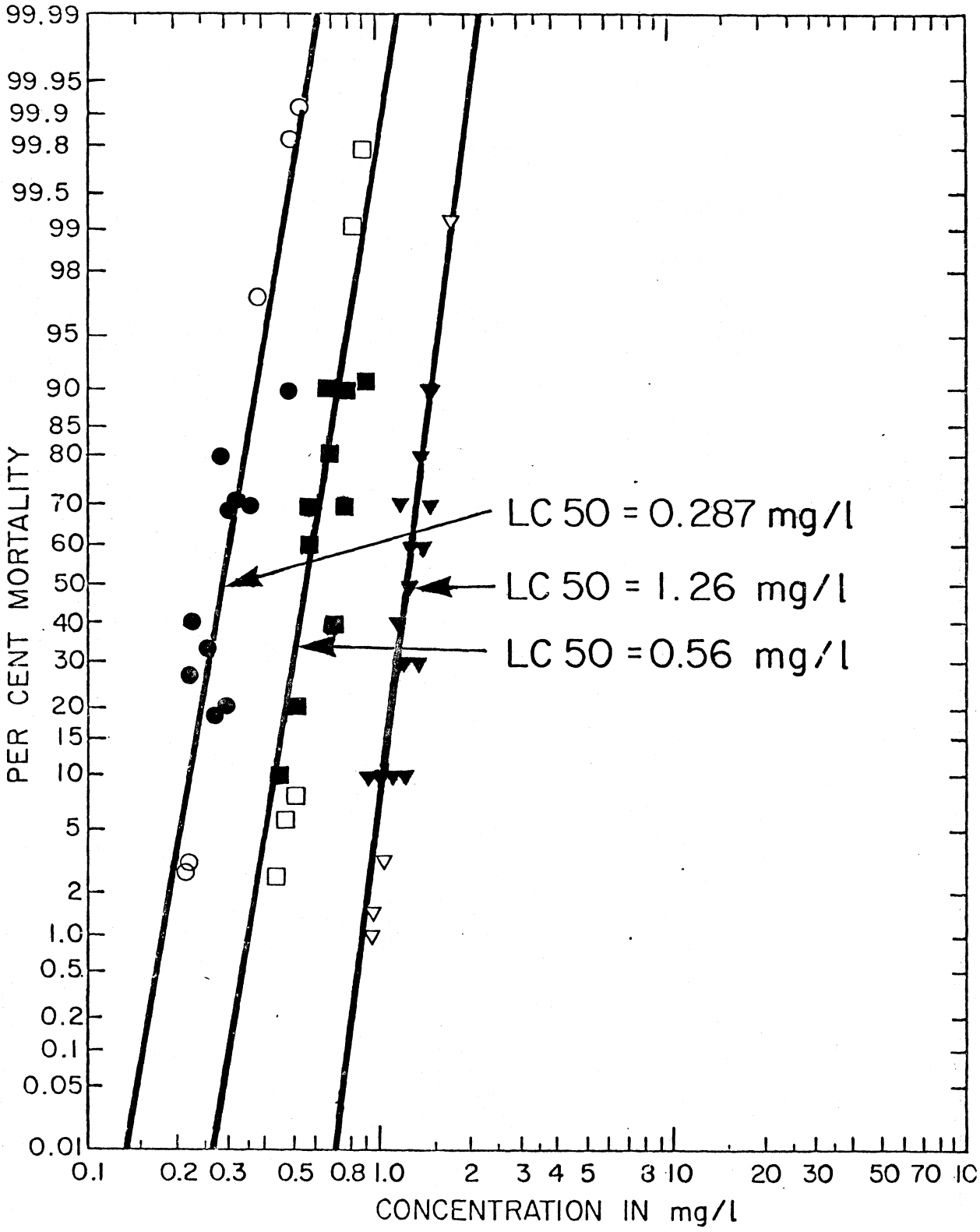

Fig. 3-8 Results of a 30-minute exposure of coho salmon at 10 C and 20 C to residual chlorine. Mortality determined 48 hours later after transfer to unchlorinated water. Solid symbols represent observed mortalities; open symbols concentrations where 0% or 100% mortality data were transformed: triangles = 10 C (1975); squares = 10 C (1976); circles = 20 C (1976).

Table 3-6. Summary of parameters observed during a 30-minute exposure of coho to residual change.

	Temperature				
	10C (1975)	10C (1976)	15C	20C (1975)	20C (1976)
Total length (mm) average (range)	167.3 (141-223)	175.7 (125-224)	172.4 (142-211)	180.0 (140-241)	180.5 (143-226)
Average weight (g)	37.79	46.80	44.10	54.63	46.74
Number of fish tested	220	180	230	240	180
pH average (range)	8.19 (7.6-8.4)	8.26 (8.11-8.42)	8.19 (8.04-8.31)	8.16 (8.02-8.26)	8.33 (8.15-8.42)
Temperature (C) average (range)	10.2 (9.7-10.9)	10.3 (9.9-11.3)	15.0 (14.8-15.5)	20.3 (19.9-20.8)	20.1 (19.8-20.4)
D.O. (mg/liter) average (range)	10.8 (10-11.2)	10.4 (8.7-11.6)	9.0 (7.5-10.2)	8.4 (7.8-9.2)	8.3 (7.2-9.4)
Free chlorine (%) average (range)	24 (6-50)	66 (19-100)	38 (9-49)	23 (5-46)	66 (18-95)
No mortality (mg/liter)	0.91	0.42	0.88	N.C.[a]	0.216
100% Mortality (mg/liter)	1.7	0.81	N.C.[a]	N.C.[a]	0.54
LC50 (mg/liter) (95% Confidence Interval)	1.26 (1.21-1.31)	0.560 (0.505-0.622)*	1.38 (1.26-1.51)	0.9 N.C.[a]	0.287 (0.254-0.324)*
Slope function (95% Confidence Interval)	1.17 (1.14-1.21)	1.22 (1.11-1.34)*	1.31 (1.22-1.40)	N.C.[a] N.C.[a]	1.23 (1.10-1.38)*

[a]Not calculated.

* Confidence interval adjusted to compensate for significantly heterogenous data (Litchfield and Wilcoxon, 1949).

At 10C no mortalities occurred at 0.42 mg/l of chlorine while concentrations above 0.80 mg/l consistently caused 100% mortality (Fig. 3-8). Although there was still significant heterogeneity in the 1976 20C test data the amount of variability was much less compared to 1975. At 20C no mortalities were recorded below 0.21 mg/l of chlorine while concentrations above 0.37 mg/l caused 90 - 100% mortality. The 10C and 20C slopes found during the 1976 tests did not differ significantly from the 10C and 15C slopes found during 1975 tests.

Mortalities were much more delayed during the 1976 tests. Only 37% and 7% of the mortalities occurred within 2 hours of dosing during the 10C and 20C tests, respectively (Table 3-2). At both temperatures most of the mortalities occurred 4 - 24 hours after dosing. Our observations on both trout and salmon have indicated that delayed mortality is often characteristic of high free chlorine concentrations.

Behavioral Observations

Observations both years indicated that coho salmon rarely (<1%) recovered following loss of equilibrium. It was also observed that the fish often reacted quite violently to high chlorine concentrations. Although mortalities were delayed during the 1976 tests, it was found that once the fish did suffer equilibrium loss (E.L.), death followed swiftly. This was especially pronounced during the 1976 20C test where most E.L. individuals died within 15 minutes. This contrasts with our 1975 findings where E.L. fish

sometimes remained alive for hours.

An interesting behavioral reaction was observed during the 1976 10C tests. It was found that when some of the groups were checked during the observation period, the sudden presence of the investigator caused the fish to go into shock. The fish would dart about and often became E.L. This behavior was most noticeable about 12 hours after dosing. These individuals usually died before the observation period was over. This behavior was not observed during the 1976 20C test or during any of the other coho bioassays.

ALEWIFE

30-Minute Exposures

Toxic Effects

The resistance of juvenile alewives to chlorine varied inversely with temperature (Fig. 3-9). Single exposure 30-minute LC50 values ranged from 0.297 mg/l at 30C to 2.27 mg/l at 15C (Table 3-7). Alewives, like the rainbow trout and coho salmon, did not exhibit a significant difference between their 10C and 15C LC50 values. The LC50 values were significantly lower, however, at each of the succeeding warmer test temperatures (20C, 25C, and 30C). At both 10C and 15C concentrations of 1.0 mg/l produced very low mortality while mortality approached 100% at concentrations of 3.5 mg/l (Fig. 3-9). At 20C 10% mortality occurred at 1.2 mg/l while concentrations greater than 2.0 mg/l caused 90 - 100% mortality (Fig. 3-9). At 25C no mortalities occurred below 0.82 mg/l and mortality was normally

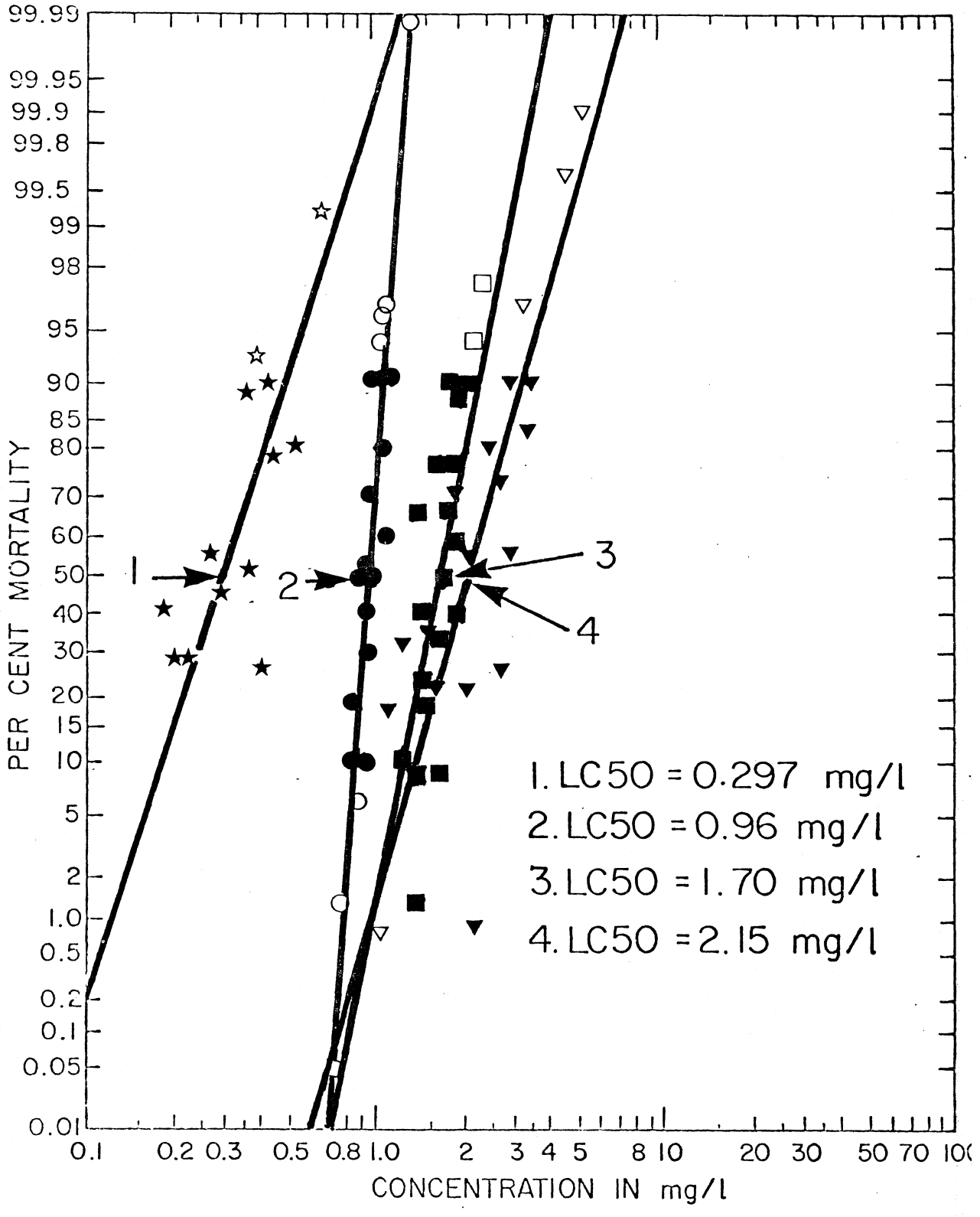

Fig. 3-9 Results of a 30-minute exposure of alewife at 10, 20, 25 and 30 C to residual chlorine. Mortality determined 48 hours later after transfer to unchlorinated water. Solid symbols represent observed mortalities; open symbols represent concentrations where 0% and 100% mortality data were transformed: triangles = 10 C; squares = 20 C; circles = 25 C, stars = 30 C.

Table 3-7. Summary of parameters observed during a 30-minute exposure of alewife to residual chlorine.

	Temperature				
	10C	15C	20C	25C	30C
Total length (mm) average (range)	103.5 (75-132)	108.0 (80-204)	93.0 (75-123)	102.3 (80-121)	100.6 (81-128)
Average weight (g)	9.20	10.3	5.65	7.59	8.48
Number of fish tested	220	290	240	250	155
pH average (range)	8.45 (7.8-8.7)	8.23 (8.00-8.43)	8.36 (8.16-8.45)	8.34 (8.20-8.43)	8.53 (8.43-8.64)
Temperature (C) average (range)	10.5 (9.9-11.4)	15.2 (14.7-15.6)	20.1 (19.8-20.5)	25.1 (24.6-25.5)	29.8 (29.2-30.5)
D.O. (mg/liter) average (range)	10.7 (10.1-11.3)	9.7 (9.0-10.3)	8.0 (7.6-8.4)	7.9 (7.1-8.6)	7.4 (6.8-7.7)
Free chlorine (%) average (range)	Not Measured	89 (78-97)	87 (62-97)	77 (35-95)	61 (16-77)
No mortality (mg/liter)	1.1	0.92	Not Determined	0.82	Not Determined
100% Mortality (mg/liter)	4.6	3.4	2.1	Not Determined	0.63
LC50 (mg/liter) (95% Confidence Interval)	2.15 (1.95-2.42)*	2.27 (2.07-2.49)*	1.70 (1.59-1.82)*	0.96 (0.94-0.98)	0.297 (0.254-0.347)*
Slope function (95% Confidence Interval)	1.39 (1.26-1.53)*	1.41 (1.30-1.39)*	1.27 (1.16-1.39)*	1.10 (1.06-1.14)	1.47 (1.23-1.76)*

*Confidence interval adjusted to compensate for significantly heterogenous data (Litchfield and Wilcoxon, 1949).

complete at 1.20 mg/l (Fig. 3-9). Alewives were much more sensitive at 30C to chlorine than they were at any of the lower temperatures having a LC50 value of 0.297 mg/l (Fig. 3-9). There was a high degree of variability in the 30C data which probably resulted from the effects of high temperature.

Mortalities were delayed at 10C with only 29% occurring within two hours of dosing (Table 3-2). At 15C, 20C, and 25C the mortality percentages for the first two hours progressively increased to 81%, 82%, and 95%, respectively (Table 3-2). Thirty degree results again did not follow the established trend. Only 25% of the mortalities at 30C occurred within two hours of dosing with the remainder being scattered throughout the observation period (Table 3-2).

Behavioral Observations

Alewives at all the test temperatures were normally quite lethargic in their reaction to chlorine. At highly toxic concentrations, however, violent swimming activity was sometimes seen. Dead fish typically (80 - 90% of the time) sank to the bottom of the test aquaria. Alewives rarely (<1%) recovered following loss of equilibrium.

SPOTTAIL SHINERS

30-Minute Exposures

Toxic Effects

Spottail shiners tested during the summer in 1976 showed the same chlorine-temperature interaction previously

described in the other test species. LC50 values were 2.41 mg/l, 1.00 mg/l and 0.535 mg/l at 10C, 15C, and 20C, respectively (Fig. 3-10 and Table 3-8). The biological and chemical parameters measured during these tests are summarized in Table 3-8. At 10C mortality was low (10 - 22%) at 1.6 mg/l but nearly complete (92%) at 3.8 mg/l (Fig. 3-10). At 15C 0.7 mg/l caused slight (5 - 10%) mortality while 1.6 mg/l of chlorine caused nearly complete (91%) mortality (Fig. 3-10). No mortality was seen at 0.38 mg/l during the 20C tests while complete mortality was observed at 0.83 mg/l (Fig. 3-10). Statistical analysis indicated that the LC50 values were significantly different at each of the test temperatures. With the exception of the 10C versus 20C group, the slopes were similar between the groups.

The time to mortality following the exposure period appeared to be temperature related. Mortality was delayed during the 10C tests. Although 48% of the mortalities occurred within two hours of dosing during the 10C tests, 30% of the mortalities did not occur until the second 24 hours after dosing (Table 3-2). At 15C and 20C, however, about 80% of the mortalities occurred within two hours of dosing and no mortalities occurred during the second 24 hours (Table 3-2).

The above results were obtained from spottails collected in Door County, Wisconsin along Lake Michigan. Two groups of spottail shiners from Lake Huron tested at 10C

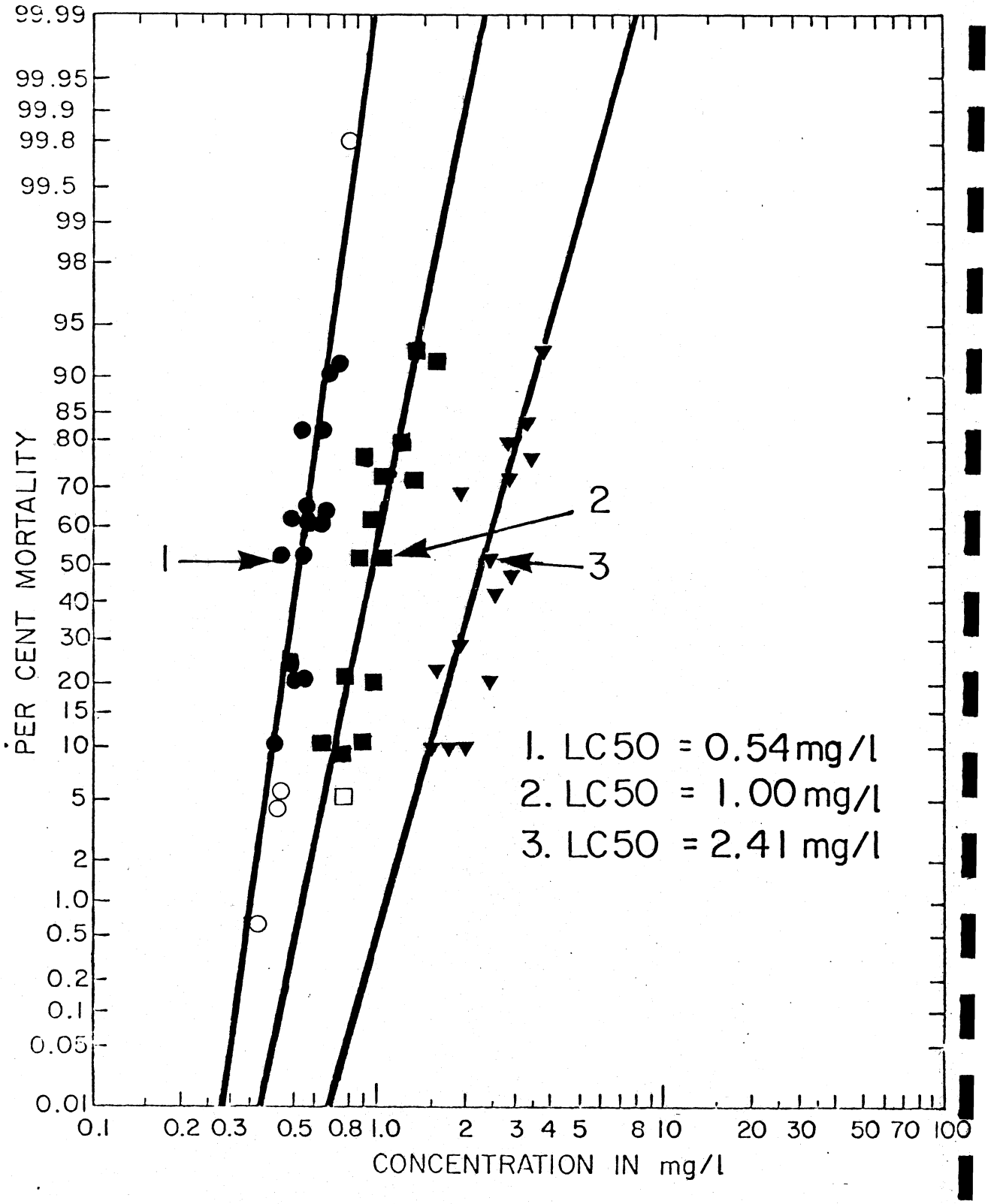

Fig. 3-10 Results of a 30-minute exposure of spottail shiners at 10, 15 and 20 C to residual chlorine. Mortality determine 48 hours later after transfer to unchlorinated water. Solid symbols represent observed mortalities; open symbols represent concentrations where 0% and 100% mortality data were transformed: triangles = 10 C; squares = 15 C; circles = 20 C.

Table 3-8. Summary of parameters observed during a 30-minute exposure of spottail shiners to residual chlorine.

	Temperature				
	10C April 1975	10C March 1976	10C July 1976	15 C 1976	20C 1976
Total length (mm) average (range)	71.7 (56-99)	111.9 (82-130)	62.4 (51-73)	59.6 (46-73)	83.3 (49-110)
Average weight (g)	2.91	12.40	2.07	1.73	5.24
Number of fish tested	160	180	180	180	240
pH average (range)	8.47 (8.3-8.6)	8.22 (8.08-8.42)	8.41 (8.37-8.45)	8.41 (8.34-8.45)	8.28 (8.20-8.36)
Temperature (C) average (range)	10.1 (9.4-10.5)	9.9 (9.0-11.7)	10.1 (10.0-10.4)	15.1 (15.9-15.4)	20.2 (19.8-21.0)
D.O. (mg/liter) average (range)	10.6 (10.1-11.2)	9.8 (8.9-10.8)	10.3 (9.9-10.9)	9.9 (9.4-10.6)	8.7 (7.5-9.4)
Free chlorine (%) average (range)	a	88 (68-98)	94 (83-98)	89 (81-96)	74 (59-94)
No mortality (mg/liter)	1.5	0.54	a	a	0.38
100% Mortality (mg/liter)	5.8	a	a	a	0.83
LC50 (mg/liter) (95% Confidence Interval)	3.21 (2.82-3.66)	1.38 (1.26-1.50)	2.41 (2.21-2.63)	1.00 (0.93-1.07)	0.535 (0.512-0.559)
Slope function (95% Confidence Interval)	1.46 (1.31-1.62)	1.50 (1.38-1.63)	1.40 (1.23-1.58)	1.28 (1.20-1.37)	1.19 (1.16-1.23)

[a]Not determined.

gave divergent results. A group tested in 1975 had an LC50 value of 3.21 mg/l while a group tested one year later had an LC50 value of only 1.38 mg/l (Table 3-8). This disparity, as explained in the discussion section, appears to be related to disease problems and size differences between the groups.

Behavioral Observations

The behavior of spottail shiners exposed to chlorine was similar to that described for the previous species. Like the yellow perch, spottail shiners at 10C showed some ability to recover following loss of equilibrium. But this ability was diminished at warmer temperatures. Percentages of fish recovering after equilibrium loss were 13%, 4%, and 1% at 10C, 15C, and 20C, respectively.

During the 10C July 1976 bioassay experiments a few of the shiners exhibited the shock reaction previously described for the coho salmon. This was not seen during the other tests at 10C or at any of the warmer temperatures.

SMELT

30-Minute Exposures

Toxic Effects

The biological and chemical parameters measured during the smelt tests are summarized in Table 3-9. The 10C 30-minute LC50 value for smelt was 1.27 mg/l (Fig. 3-11 and Table 3-9). Mortality was slight (10%) at 0.72 mg/l but nearly complete (90%) at 2 mg/l (Fig. 3-11). Sixty percent of the mortalities occurred within 12 hours of dosing with the remaining 40% being rather uniformly scattered over the

Table 3-9. Summary of parameters observed during 5- and 30-minute exposures of smelt to residual chlorine.

	Temperature	
	10C 30-Minute	10C 5x3
Total length (mm) average (range)	163.7 (130-245)	157.2 (123-201)
Average weight (g)	25.80	20.29
Number of fish tested	170	180
pH average (range)	8.46 (8.30-8.40)	8.45 (8.28-8.53)
Temperature (C) average (range)	9.9 (9.6-10.3)	10.1 (9.6-10.8)
D.O. (mg/liter) average (range)	10.9 (10.4-11.3)	11.2 (10.0-11.7)
Free chlorine (%) average (range)	87 (69-94)	89 (70-96)
LC50 (mg/liter) (95% Confidence Interval)	1.27 (1.14-1.41)	3.3 (2.92-3.73)
Slope function (95% Confidence Interval)	1.50 (1.30-1.72)	1.68 (1.38-2.05)

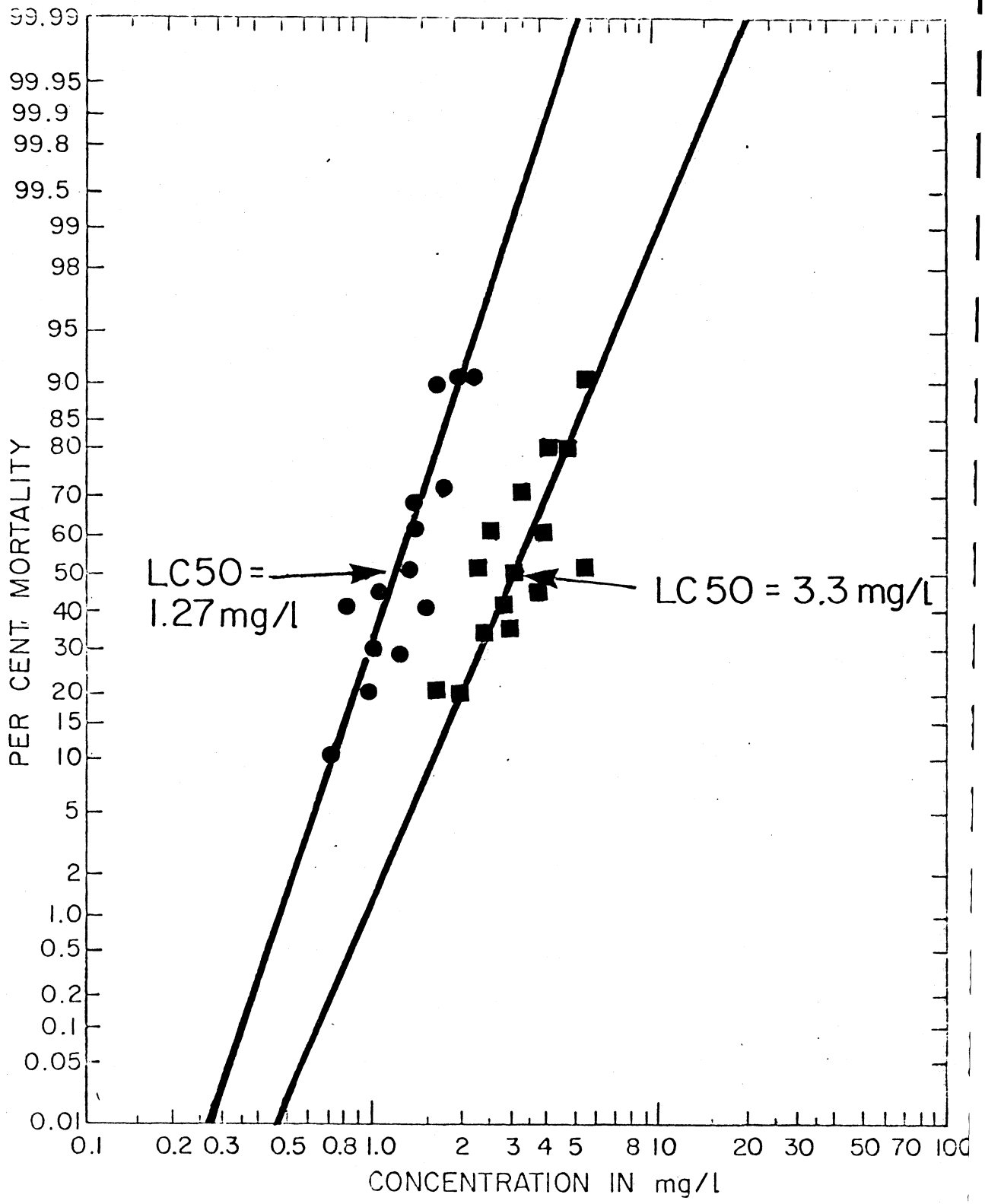

Fig. 3-11 Results of a single 30-minute and triple 5-minute exposures of smelt at 10 C to residual chlorine. Mortality determined 48 hours later after transfer to unchlorinated water for the single exposure group and determined 96 hours later for the triple exposure group. Circles = 30-minute tests; squares = 5-minute tests.

following 36 hours (Table 3-2).

Behavioral Observations

Smelt rarely (<2%) recovered following loss of equilibrium (E.L.). It was found, however, that about 10% of the E.L. fish remained in that state for protracted periods. In an extreme case one smelt remained E.L. for more than seven days.

5-Minute Triple Exposures

Toxic Effects

Table 3-9 summarizes the parameters measured during the 5 x 3 smelt tests. The 10C triple exposure LC50 value was 3.3 mg/l (Fig. 3-11). The dose-response by the 5 x 3 smelt was more varied than in the 30-minute smelt tests. Twenty percent mortality was observed at 1.7 - 2.0 mg/l while 50 - 90% mortality occurred at 5.5 mg/l. Mortalities were delayed compared to the 30-minute smelt tests. Only 21% of the total mortalities occurred during the first 12 hours after the third dose (Table 3-2). The rest of the mortalities were scattered throughout the remainder of the observation period.

Behavioral Observations

Smelt rarely (<3%) recovered following loss of equilibrium. The protracted E.L. period previously noted in the 30-minute smelt bioassays was again evident. The other behavioral responses of the smelt to chlorine were generally similar to those previously described for the other species.

DISCUSSION

The toxicity of chlorine to fish may be influenced by a number of variables such as the duration of exposure, temperature, the chemical characteristics of the water in which the fish are exposed and the individual nature of the fish themselves (Brooks and Seegert, 1976). Considering these variables it is difficult to directly compare our results with previous chlorine toxicity studies. However, several reports are appropriate to consider for general discussion. Arthur, et. al. (1975) found that of seven fish species tested in chlorinated sewage effluent between 12 and 17C, yellow perch had the highest (greater than 0.85 mg/l) one-hour LC50 value. This compares favorably with our findings which indicate that yellow perch are extremely resistant to chlorine at low temperatures.

No results have previously been reported for chlorine toxicity tests on rainbow trout and coho salmon with exposure times as short as those in this study. A seven-day LC50 value of 0.08 mg/l for rainbow trout was reported by Merkens (1958) while four-day LC50 values of 0.14 to 0.29 mg/l were reported by Basch, et. al. (1971). Taylor and James (1928) found that 0.3 mg/l of chlorine was lethal to rainbow trout in two hours. Holland, et. al. (1960) reported that at 14C, 0.15 mg/l of dichloramine caused 100% mortality in coho salmon in 48 minutes but when the salmon were exposed to 0.75 mg/l of a predominantly monochloramine solution it took almost 3 hours to affect 100% mortality.

A considerable disparity exists with regard to the results of short-term chlorine toxicity studies with other salmonids. Pyle (1960) found that 0.5 mg/l concentrations of chlorine produced 50% mortality in brook trout, Salvelinus fontinalis, in 90 minutes. Pike (1971) found that brown trout, Salmo trutta, exposed to 0.04 mg/l suffered 100% mortality in only 2 minutes while Basch and Truchan (1976) found 30-minute LC50 values of 1.19 and 0.56 mg/l for brown trout at 17 and 21C, respectively. Holland, et. al. (1960) found that chinook salmon, O. tshawytscha, in 6C seawater containing 1 mg/l of chlorine began to suffer mortalities after about one-half hour. Stober and Hanson (1974) found that in seawater at 12 - 14C, chinook and pink, O. gorbuscha, salmon had 30-minute LC50 values of 0.25 mg/l.

Field experiments have also been variable in their results. A series of caged fish bioassays at several Lake Michigan power plants indicated that brown trout suffered mortalities when subjected to multiple 30-minute chlorinations at mean TRC values of 0.05 mg/l to 0.40 mg/l (Basch and Truchan, 1976). When these experiments were repeated a year later at comparable chlorine levels using a variety of salmonids no mortality was attributable to chlorine (Basch and Truchan, 1976). No acceptable explanation for this difference in response was found. Basch and Truchan (1976) also found a variety of warm water fish species (mainly centrarchids and bullheads) were able to survive repeated 30-minute exposures at chlorine levels up to 0.5 mg/l.

The authors are not aware of any published papers dealing with the effects of chlorine on alewife, smelt or spottail shiners. Recent studies have indicated that some minnows have 96 hour LC50 values as low as those reported for salmonids (Ward, et. al., 1976).

Temperature Effects

A review of the literature indicates that fish are generally more sensitive to chlorine at higher temperatures (Brooks and Seegert, 1976). The present study has demonstrated this for yellow perch, rainbow trout, coho salmon, alewife and spottail shiner. Other investigators have observed similar results with other species. Eren and Langer (1973) found that Tilapia aurea was more sensitive to chlorine at higher acclimation temperatures. Basch and Truchan (1976) obtained similar results using brown trout. Stober and Hanson (1974) and Wolf, et. al. (1975) have shown that temperature can act synergistically with chlorine.

Brooks and Seegert (1976) and Thatcher, et. al. (1976) have suggested that within a specific temperature range, temperature has little effect on the toxicity of chlorine to fish. This was demonstrated by our results from the rainbow trout, coho salmon and alewife experiments where no change in LC50 value was observed between 10C and 15C but where they were significantly more sensitive at warmer temperatures. Thatcher, et. al. (1976) reported the same phenomenon in brook trout exposed to chlorine for 96 hours. They further reported that 96-hour LC50 values were largely

independent of previous acclimation temperatures but were primarily determined by the temperature at which the fish were being tested.

Individual Differences

Individual fish responded differently to chlorine in all experiments. In 11 of the 31 groups tested the confidence intervals were adjusted to compensate for significant ($P < .05$) heterogeneity in the data (Litchfield and Wilcoxon, 1949)(Tables 3-1 to 3-9). This heterogeneity apparently results from several factors. Since in all the experiments the chemical and biological parameters measured were within accepted limits, (EPA, 1975, Standard Methods, 1976) the main factor appears to be random variability among the fish tested. Since both the alewife and coho exhibited greatly increased variability when tested near their upper lethal temperature it appears that temperature stress was a second factor. The variability seen during the alewives tests may have been compounded by their well known stress reactions when confined. As is discussed later, the variability in the percentages of free chlorine that were present probably also contributed to the heterogeneity. In many of the cases the observed heterogeneity is probably more apparent than real. In most of the tests the resultant slopes were quite steep which allowed small differences in response by the fish to greatly affect how well a line could be fit to the data points. This is illustrated by comparing two different

TRC concentrations tested the same day on yellow perch using water from the same reservoir tank. The two solutions with TRC concentration of 4.924 mg/l and 9.490 mg/l had nearly identical characteristics and both produced 20% mortality. In the case of the 4.924 mg/l concentration the mortality was much higher than expected while the converse was true for the 9.490 mg/l concentration (Fig. 3-3).

The difference between the so-called "sensitive" and "resistant" groups of trout is difficult to explain. In both the 30-minute and multiple exposure tests there were no size differences between the "sensitive" and "resistant" groups (Tables 3-4 and 3-5). Similarly no trends were evident when the chemical parameters were examined nor were the values outside accepted limits (Tables 3-4 and 3-5)(EPA, 1975, Standard Methods, 1976). Holding, handling, and feeding procedures were identical for all groups. Genetic variability does not appear to have influenced the observed test results since both the "sensitive" and "resistant" trout originated from the same hatchery brood stock which has been maintained for several years. Since none of the possibilities discussed above provides a clearcut explanation, the cause of the observed differences in response remains obscure. Since two different lots of trout were significantly more "resistant" when compared with the single lot of "sensitive" trout it appears that the "sensitive" trout exhibited an atypical response to chlorine.

Free Chlorine Effects

The perch, alewife, smelt, and spottail shiners were relatively small and did not produce large quantities of ammonia which would be available to form chloramines. Consequently, free chlorine percentages were relatively constant during the bioassays on these species. The salmonids being considerably larger produced large quantities of nitrogenous wastes thereby affecting free chlorine percentages. Although this problem was partially alleviated by putting the salmonids in the test tanks only 15 - 30 minutes before they were tested, large variations in free chlorine values still sometimes occurred. This was especially noticeable during the 1975 coho bioassays and probably contributed to the extreme variability in the 1975 20C data. The 1975 and 1976 coho groups differed little in most important chemical and biological parameters (Table 3-6). Since the only obvious difference between the two groups was the threefold increase in free chlorine percentages in 1976 it appears that this caused the lower LC50 values observed in 1976. Although the difference in LC50 values between the 1975 and 1976 coho showed that percentages of free chlorine do affect mortality, an attempt to correlate free chlorine percentages at specific concentrations with changes in mortality did not produce any clear cut pattern. Systematic testing using the same TRC value while varying the percentages of free chlorine is probably necessary to fully answer this question.

Miscellaneous Effects

The three groups of spottail shiners we tested at 10C each had significantly different LC50 values (Table 3-8). Several differences between these groups may explain this lack of consistency. The March 1976 group which had the lowest LC50 value (1.38 mg/l) was much larger than any of the other groups we tested (Table 3-8). Additionally these fish had been obtained from Lake Huron and some disease problems were evident during testing. Which (if any) of the above factors caused the low LC50 value is impossible to say. The 10C spottail group tested in 1975 also had disease problems which may have affected the LC50 value. However, the 10C July 1976 group as well as the 1976 15C and 20C groups were all collected from Lake Michigan along Door County, Wisconsin and had no disease problems. Consequently we feel that the July 1976 data is the most reliable of the 10C data.

Alewives at 30C acted erratically in the presence of chlorine. The upper lethal temperature of the alewife ranges from 25 - 32C depending on the age of the fish and their thermal history (Otto, et. al, 1976). It is highly probable that the erratic behavior of the 30C alewives was caused or at least compounded by the high test temperature. This conclusion is supported by the high (up to 33%) control mortality the fish experienced. It should be noted that the fish in the holding tanks were surviving quite well at 30C indicating that the temperature alone was not the sole

toxic factor. Since corrections were made for control mortalities it seems obvious that the chlorine and heat acted synergistically to produce more than additive effects. This view is further supported by the distinctly different slope of the 30C curve compared to the slopes at any of the other test temperatures (Fig. 3-9).

Sublethal Effects

Concern has recently been expressed that various sublethal effects such as disequilibrium and/or increased vulnerability to predation might be overlooked in short-term chlorination studies measuring only LC50 values (Brungs, 1977). In the present study, it was found that, with the exception of the 10C perch, 97% of those fish suffering loss of equilibrium eventually died. This suggests that for fish exposed to short periods of chlorination there is little difference between LC and EC (Effective Concentration) values.

The concern over increased vulnerability to predation does seem warranted. Our studies indicated that fish often swam near the surface when exposed to lethal or near lethal concentrations of chlorine. Basch and Truchan (1976) observed gulls feeding on small fish floundering on the surface during a chlorination period at a Lake Michigan power plant. Similarly, the lethargic behavior previously described would increase the vulnerability of chlorine-exposed fish to piscivorous fish.

Delayed mortalities such as those described here can lead to underestimation of mortality if the observation period following chlorination is not sufficient. In this study 7% of the mortalities occurred at times greater than 24 hours. After the observation period, the fish used in our study were usually returned to holding tanks. Long-term (several weeks) observations indicated that little subsequent mortality occurred, suggesting that a 24- to 48-hour observation period is sufficient to define delayed mortality following short-term chlorination.

Safe Levels

Sprague (1971) reviewed several methods used to establish safe limits for toxic substances. The most commonly used methods involves multiplying an application factor (usually .1 to .4) times a 48-hour LC50 value. Application factors typically have been derived from continuous exposure tests and are not entirely applicable to short-term toxicity studies. Mattice and Zittel (1976) in a literature survey, found for 14 aquatic organisms that for a given concentration of chlorine the maximum exposure time which would not result in any mortality was 37% of that which would cause death to 50% of the exposed organisms. Basch and Truchan (1976) in a field study employed a method using chlorine minutes (CM) to determine the effects of multiple chlorinations on fish. In this method the chlorine concentration (C) is multiplied by the exposure time in minutes (M). They assumed that fish exposed to the same number of CM should

exhibit the same response (i.e., mortality). Assuming this is true the LC50 values determined at the same temperature in our 5- and 30-minute tests should have equivalent CM50 values. Only in the case of the 20C "sensitive" rainbow trout was there good agreement between 5- and 30-minute CM50 values.

Using the results reported in this paper we attempted to determine "safe" levels for the species we tested exposed to chlorine for short periods. "Safe" is defined here as the absence of acute mortality. In 23 of the 32 groups we tested it was possible to determine the highest concentration at/and below which no mortality (N.M.) was observed (Tables 3-1 to 3-9). This concentration when divided by the LC50 value yielded a fraction here called the safe factor (S.F.). The average S.F. values ranged from 0.52 for spottail shiner to 0.72 for coho salmon (Table 3-10). The "safe" levels we determined from these S.F. values were equal to or below the N.M. concentrations actually found for these species indicating that this method of safe level determinations is conservative (Table 3-10). We suggest that a S.F. value of 0.5 be applied to species whose safe factor has not been emperically determined for 30-minute exposures. This has the advantages of simplicity (e.g., 1/2 the LC50 value) and is conservative since it is lower than any of the average S.F. values we determined.

The conservative S.F. value of 0.5 applied to the 30-minute perch and trout LC50 values clearly indicates how

Table 3-10. A comparison of safe and no mortality chlorine levels for 5 species of fish at their lowest LC50 values.

Species	Lowest LC50 Value (mg/l)	Av. S.F.[a] (range)	Safe Level (mg/l)	N.M.[b] (mg/l)
Alewife	0.297	.59(.41-.84)	0.18	c
Coho Salmon	0.287	.72(.64-.76)	0.21	0.21
Rainbow Trout	0.43	.67(.57-.78)	0.29	0.30
Spottail Shiner	0.535	.52(.39-.71)	0.28	0.38
Yellow Perch	0.70	.61(.48-.75)	0.43	0.48

[a]Safe factor.

[b]No mortality level at lowest LC50 value.

[c]Insufficient data.

temperature can affect "safe" limits (Fig. 3-12). When the "safe" concentrations calculated for each of the groups tested were compared with Figures 3-2 to 3-11 it was found that the percent mortality statistically expected for these "safe" concentrations averaged 5.4% (0.01 - 12%). This agrees well with other methods of safe level estimation, which consider 5% mortality to affect an insignificant proportion of the population (Sprague, 1971). The S.F. value of 0.5 we suggest is higher than most application factors (Sprague, 1971) probably because we are estimating a safe level based on mortality caused by a very short exposure to the toxicant. Short-term exposures represent shock concentrations which might be expected to induce an all-or-nothing response. If this is true, this would explain the rapid changes between 0 and 50% mortality levels such as those we observed.

Regulatory Implications

Current effluent limitations for chlorine discharged from steam electric power plants promulgated by the U.S. Environmental Protection Agency limit the free available chlorine concentration to an average of 0.2 mg/l during a maximum of one 2-hour period a day with maximum concentrations of 0.5 mg/l (USEPA, 1974). The 0.5 mg/l maximum discharge concentration currently allowed by the Environmental Protection Agency is above the predicted safe levels for the fishes used in this study. We suggest that chlorine discharge concentrations be limited to an average TRC level

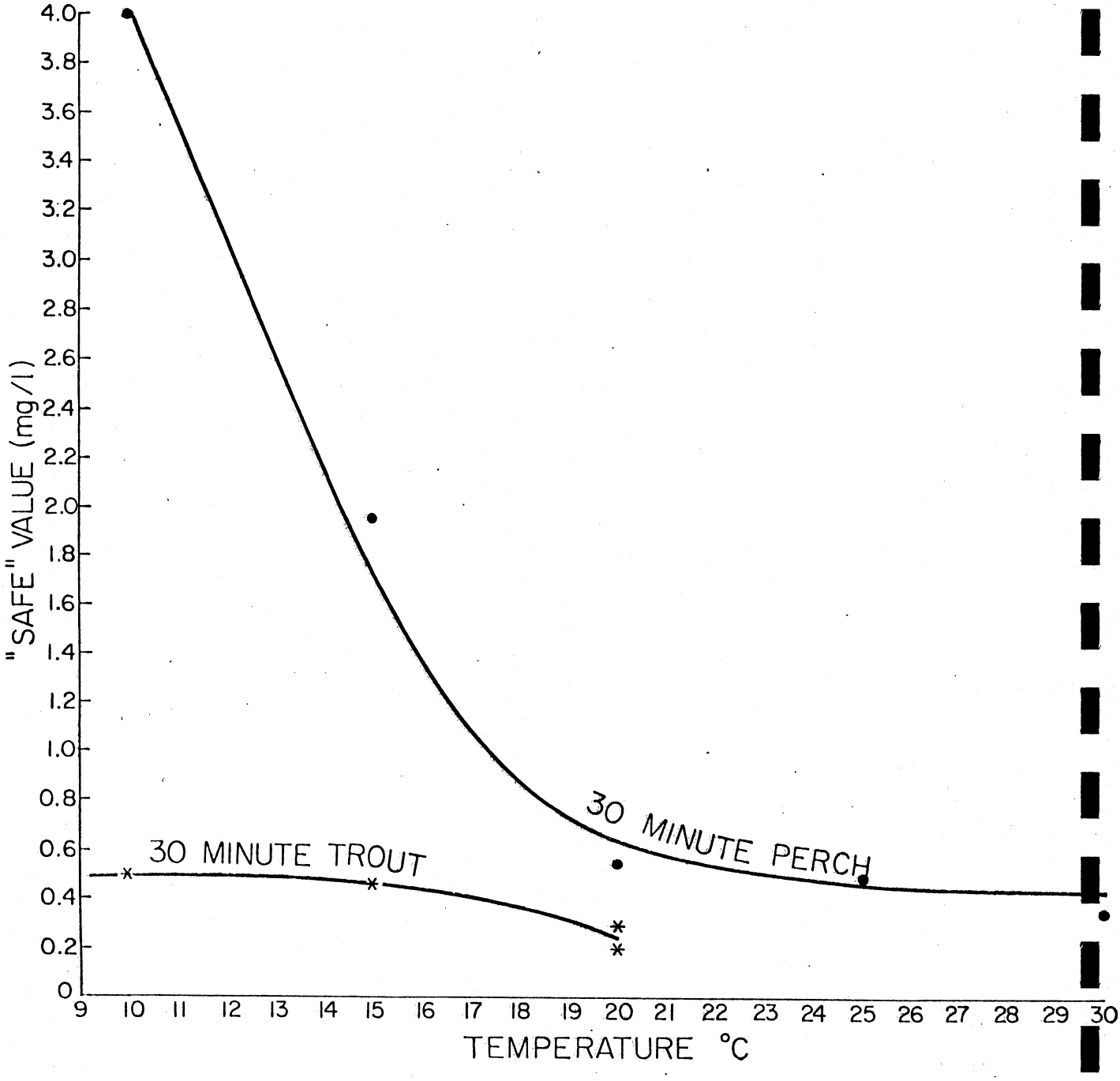

Fig. 3-12 Relationship between "safe" concentrations and exposure temperatures in yellow perch and rainbow trout.

of 0.2 mg/l for periods not to exceed one-half hour. Where longer discharge times are necessary the allowable TRC value should be correspondingly lowered. Where chlorine concentrations of 0.5 mg/l are necessary to accomplish condenser defouling, a series of 5-minute doses could be used and still protect fish.

4. INVERTEBRATES

Materials and Methods

All invertebrate bioassays were conducted in an environmental chamber in which temperature and light were controlled. Cool white fluorescent lighting provided a daily cycle of 16-hours light and 8-hours dark. Light levels were 40 - 60 foot candles at the surface of the test aquaria. Experiments were conducted over a temperature range of 4 - 20C when permitted by the temperature tolerance range of the organisms.

Water used in the bioassays was obtained from the City of Milwaukee Linnwood Avenue Water Purification Plant prior to any treatment. Lake Michigan water was pumped to the treatment plant from an intake crib located 1.6 km offshore at a depth of 17 m. The average chemical composition of this water is listed in Table 4-1. The untreated lake water was collected in non-toxic poly-olefin barrels and transported to the laboratory where it was transferred to glass-lined storage tanks. Prior to use, the water was filtered through 10 micron netting to remove zooplankton.

The bioassay apparatus consisted of two sets of aquaria. A static set containing lake water to which chlorine was added and a set containing unchlorinated lake water which were set up on a flow-through basis. The flow-through system was designed to provide a continuous flow of lake water to the aquaria. The flow rate to the aquaria resulted in a 90% replacement time of 7 hours as recommended by Sprague, 1969. With the exception of the Pontoporeia

Table 4-1 Average chemical characteristics of Lake Michigan water at the Linnwood Avenue Water Purification Plant intake -- 1974 (data from Milwaukee Water Department).

Water Quality Text	Average in mg/l
total alkalinity ($CaCO_3$)	108
chloride	9.7
free ammonia (as N)	0.010
albuminoid ammonia (as N)	0.117
chemical oxygen demand	5
dissolved oxygen	12.6
pH	8.21
dissolved solids	160
suspended solids	7
turbidity (J.T.U.)	3.9
nitrate (as N)	0.287
nitrite (as N)	0.005
phosphate (PO_4)	0.07
coliform bacteria	187 per 100 ml

experiments test organisms were placed in 235 ml glass jars, each of which had a 1 cm hole drilled in the side near the bottom. The hole was covered by nylon mesh which allowed water to flow in and out, but prevented the loss of the test organisms. The jars were placed in the flow-through aquaria in such a way that the lake water flowed directly into the jar (Fig. 4-1). The apparatus used for the Pontoporeia experiments was of similar design but employed 2ℓ polyethylene bottles with screened sides to hold the organisms rather than the 235 ml jars. The polyethylene bottles were placed in 20ℓ aquaria for dosing with chlorine and maintained in 100ℓ aquaria for observation.

Dosing procedures were similar for all experiments. Four to six pairs of toxicant aquaria were set up adjacent to the flow-through aquaria. To all but one pair of toxicant aquaria varying amounts of 5% sodium hypochlorite solution was added which resulted in a range of residual chlorine concentrations. No chlorine was added to the sixth toxicant aquarium, which was maintained as a control. Test zooplankton species were placed in jars within the flow-through aquaria approximately 30 minutes prior to the initiation of the dosing procedure. Pontoporeia were placed in their dosing containers 8-10 hours before exposure. At a predetermined time, two sample jars, serving as duplicates, were removed from their respective flow-through aquaria, and all but about 5 ml of the water contained therein was allowed to drain out through the mesh-covered holes. The jars were then placed in a

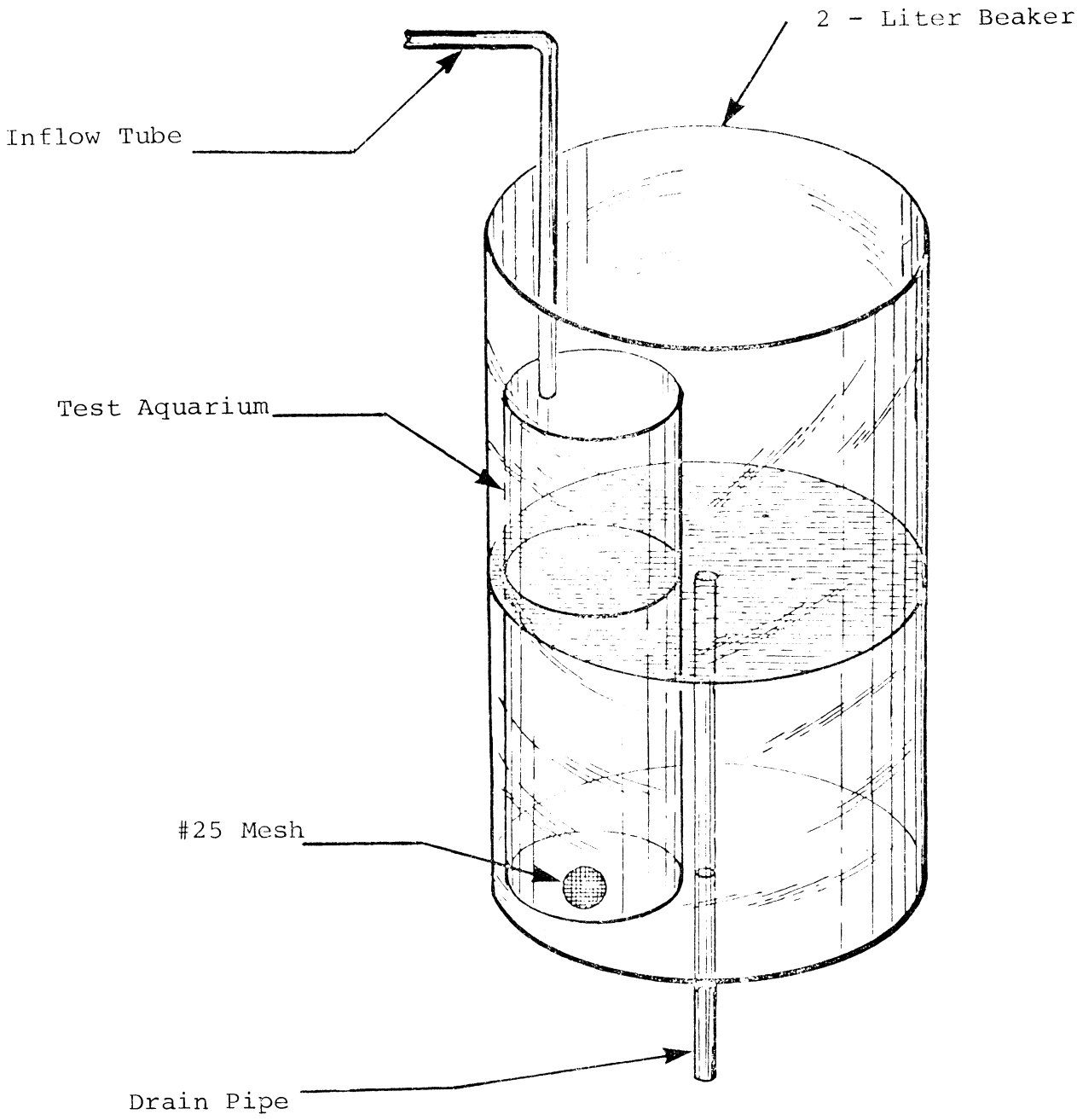

Figure 4-1. Test aquarium used in invertebrate bioassay experiments.

toxicant aquarium. The chlorine solution filled each jar by flowing in through the screened hole. At the end of the 30-minute exposure period, the process was reversed, and the jars were returned to their respective flow-through aquaria. The Pontoporeia holding bottles were gently rinsed by immersing them in a tank of unchlorinated water before being returned to the observation aquaria. The entire dosing and transfer procedure was repeated once per day for four days in the quadruple exposure Pontoporeia experiments.

After a 24-hour observation period, the jars were removed from the flow-through aquaria, and mortality was assessed. Mortality was determined 48 hours after the fourth and final exposure in the multiple exposure Pontoporeia tests. The 9C single and multiple exposure Pontoporeia tests were run simultaneously, hence, the final mortality assessments for the single exposure run was made 5 days after the initial exposure to coincide with the termination of the multiple exposure experiments. The results from duplicate samples were combined and averaged for purposes of analysis.

Chemical tests performed immediately after the organisms were removed from the toxicant aquaria consisted of the determination of total residual chlorine (Section 2 this report) and pH. Dissolved oxygen levels in the dilution water were determined occasionally, and were found to vary between 80 and 100% of saturation levels.

The chlorine species present during the bioassays was

not measured directly during the early experiments. However, the predominant form was most probably the hypochlorite ion, based on a knowledge of chlorine equilibria and reaction kinetics (Morris 1967, Draley 1972, Jolley 1973). The chlorine to ammonia mole ratio in the toxicant aquaria ranged from 2:1 to 100:1, based on the measured chlorine residuals and the average ammonia content, including albuminoid ammonia, of the lake water, as reported by the Linnwood Avenue Water Purification Plant. In all cases the chlorine to ammonia ratio exceeded the 2:1 ratio known to lead to the breakpoint reaction described by Draley (1972). Therefore, the resulting chlorine residual would be expected to be predominantly free chlorine, with traces of monochloramine and dichloramine. At the pH of the lake water (approximately 8.0 - 8.3), about 80% of the free chlorine present would exist in the form of the hypochlorite ion, while the remaining 20% would exist as undissociated hypochlorous acid (Fair et al. 1948). These presumptions are substantiated by the free chlorine measurements which were made during the Pontoporeia experiments using the DPD analytical method.

Biological Methods

The copepods Cyclops bicuspidatus thomasi and Limnocalanus macrurus, were selected as important representatives of the Lake Michigan zooplankton community. Cyclops was obtained by vertical net tows in offshore Lake Michigan. Limnocalanus was obtained by means of horizontal net tows at depths of 30 m or greater. Pontoporeia affinis, an important member

of the Lake Michigan benthic community, was collected from a 100 m deep station northeast of Milwaukee with an epibenthic sled. Pontoporeia was maintained in an aerated 100 l aquaria containing 4 - 6 cm of Lake Michigan sediments. They were fed daily rations of dried leaf and egg powder (Smith, 1972). The organisms were acclimated to the temperature of the bioassay for a minimum of 24 hours prior to testing.

Methods of transferring the test organisms from the holding tanks to the bioassay jars were unique for each species. Cyclops was concentrated on counting slides and examined under a 10X dissecting microscope. Individuals were selected using a disposable dropping pipet and randomly placed in the bioassay jars. A total of twenty organisms were placed in each jar.

The larger size of Limnocalanus and Pontoporeia made microscopic examination unnecessary. A sample was concentrated and placed in a glass petri-dish from which individuals were selected using a wide-orifice dropping pipet. The individuals were placed directly into the jars in the flow-through aquaria.

The conditions upon which an individual was judged alive or dead was somewhat arbitrary. Responses to the toxicant were placed in three categories: actually dead, sluggish, and swimming actively. Individuals designated as actually dead showed no response to probing, and appeared contorted and opaque. Swimming individuals displayed levels of activity similar to the control organisms. The intermediate category

consisted of individuals which appeared to be partially or completely paralyzed, and which displayed responses to probing ranging from active swimming to weak twitches. In all bioassays except those of Cyclops at 20°C, the sluggish individuals were included with the active swimmers to comprise the alive category in the calculation of mortality. For Cyclops at 20°C, mortality was determined in two ways. In the first method, as was done in all the other bioassays, the sluggish individuals were included in the alive category. In the second method, the sluggish individuals were included in the actually dead category.

Results

The 30-minute LC-50 values for Cyclops and Limnocalanus at various temperatures were calculated from the log-probit regression lines (Figures 4-2 to 4-6). These values are shown, along with the values of the regression slope and the 95% confidence limits of both parameters, in Table 4-2. Averaged values for the duplicate tests for each of the bioassays are given in Tables 4-3 to 4-7. The LC-50 value for Cyclops at 20°C (5.76 mg/l) was significantly less than the LC-50 values observed at 10 and 15°C (14.68 and 15.61 mg/l, respectively). For Limnocalanus, no variability in the LC-50 value with temperature was observed. The LC-50 was a constant 1.54 mg/l at both 5° and 10°C. The LC-50 for Cyclops at 20°C was 5.76 mg/l total residual chlorine when mortality was calculated by counting the sluggish individuals as alive.

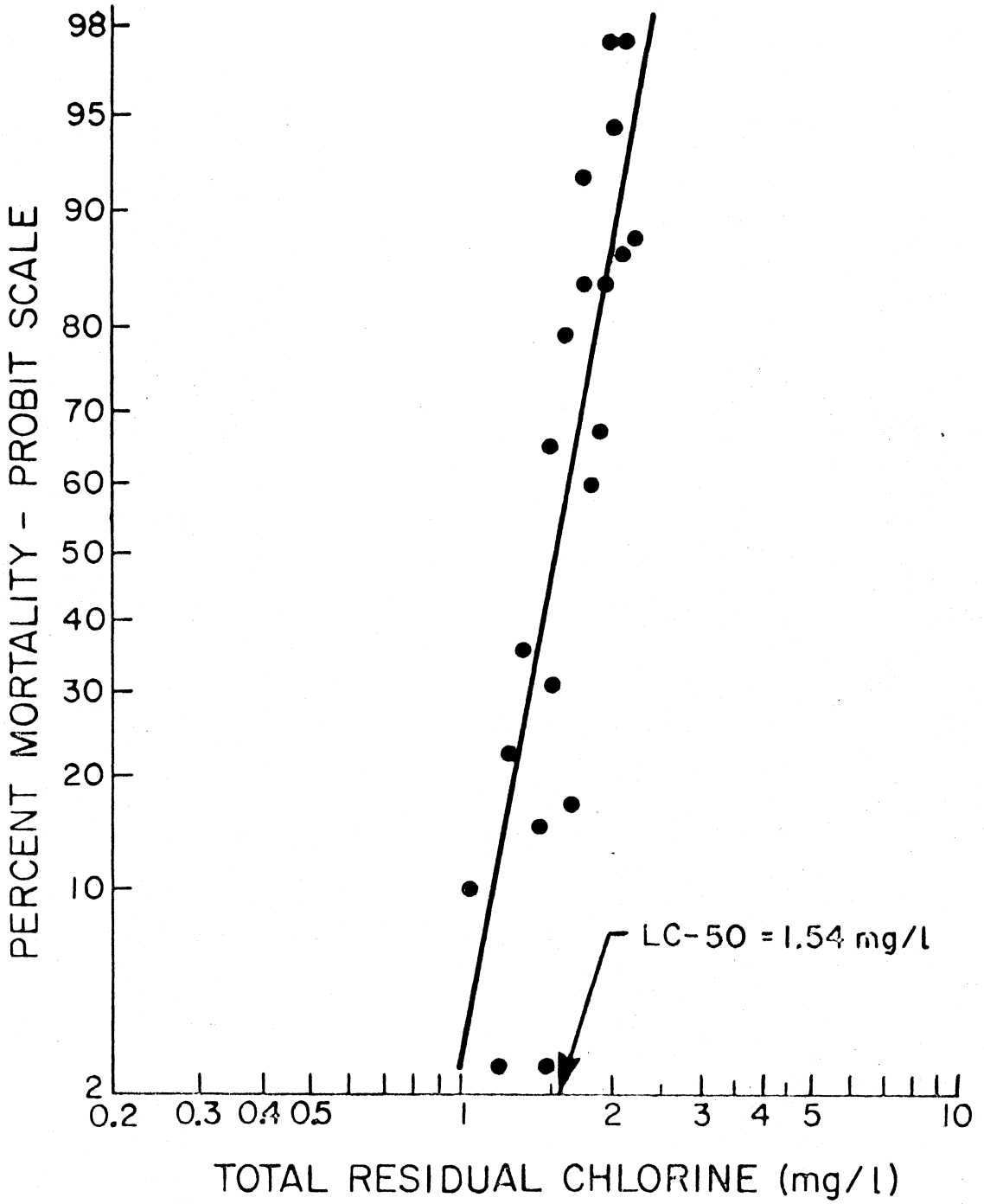

Figure 4-2 Dose-mortality plot for a 30-minute exposure of residual chlorine to Limnocalanus macrurus at 5°C.

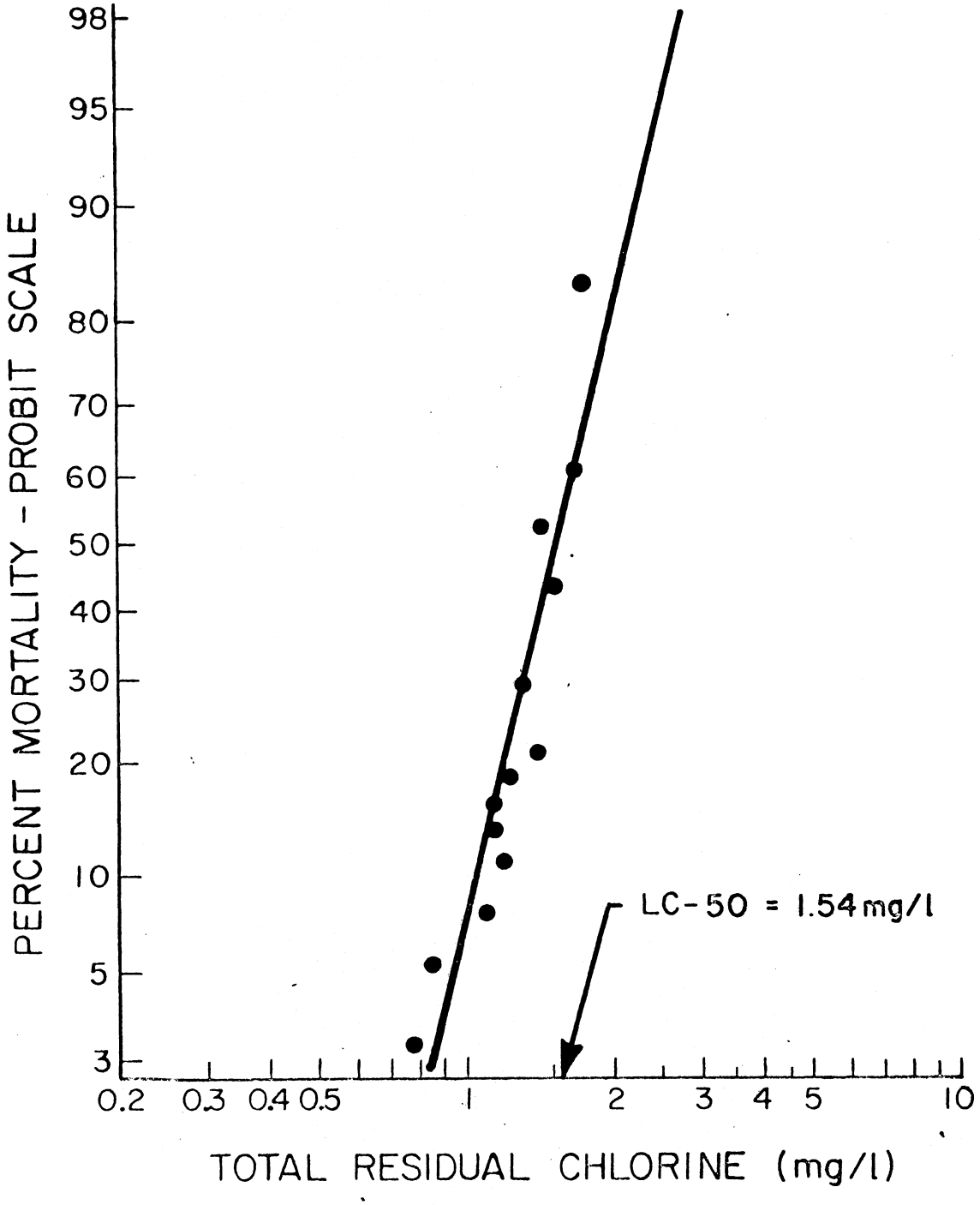

Figure 4-3 Dose-mortality plot for a 30-minute exposure of residual chlorine to Limnocalanus macrurus at 10°C.

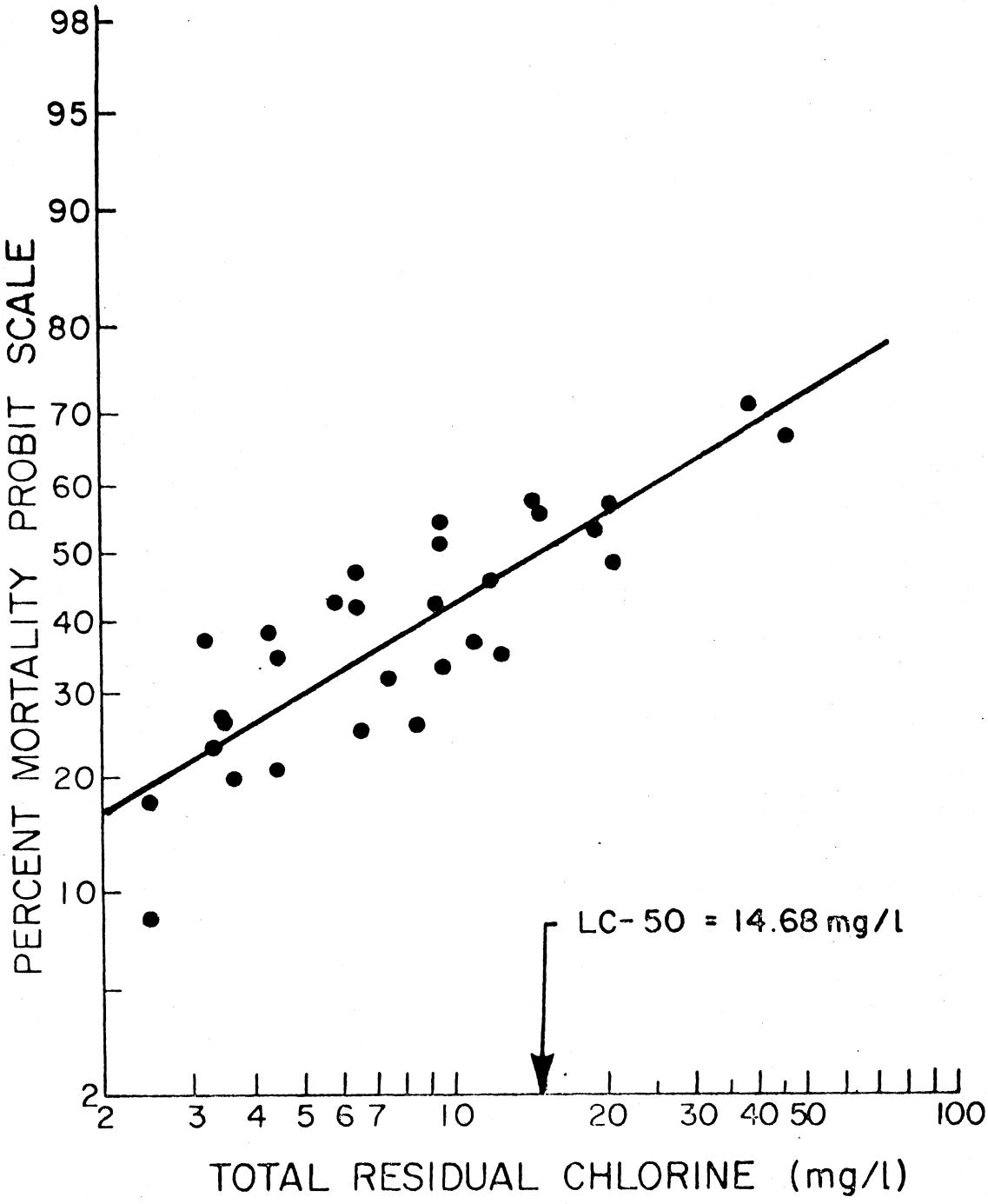

Figure 4-4 Dose-mortality plot for a 30-minute exposure of residual chlorine to Cyclops bicuspidatus thomasi at 10°C.

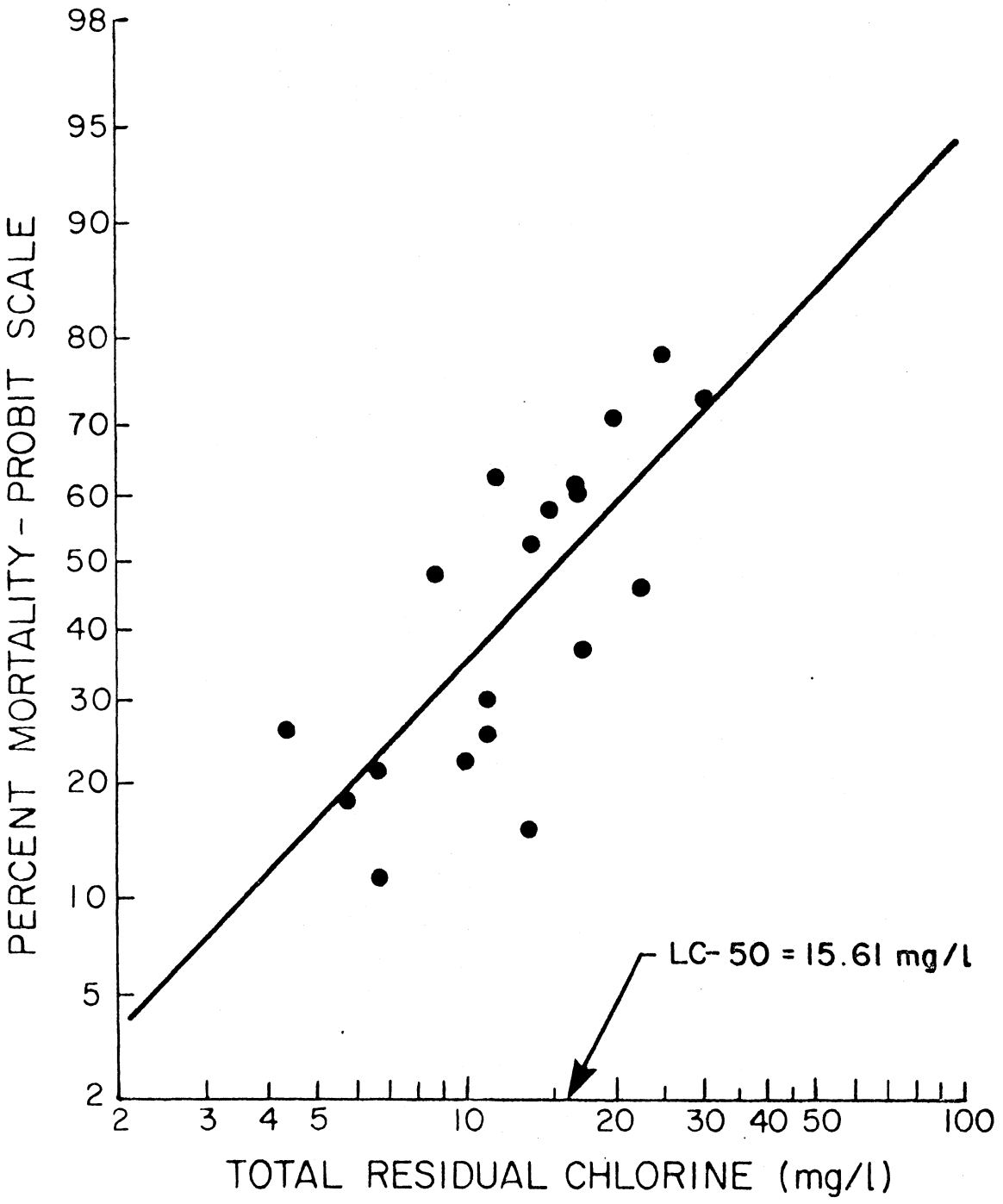

Figure 4-5 Dose-mortality plot for a 30-minute exposure of residual chlorine to Cyclops bicuspidatus thomasi at 15°C.

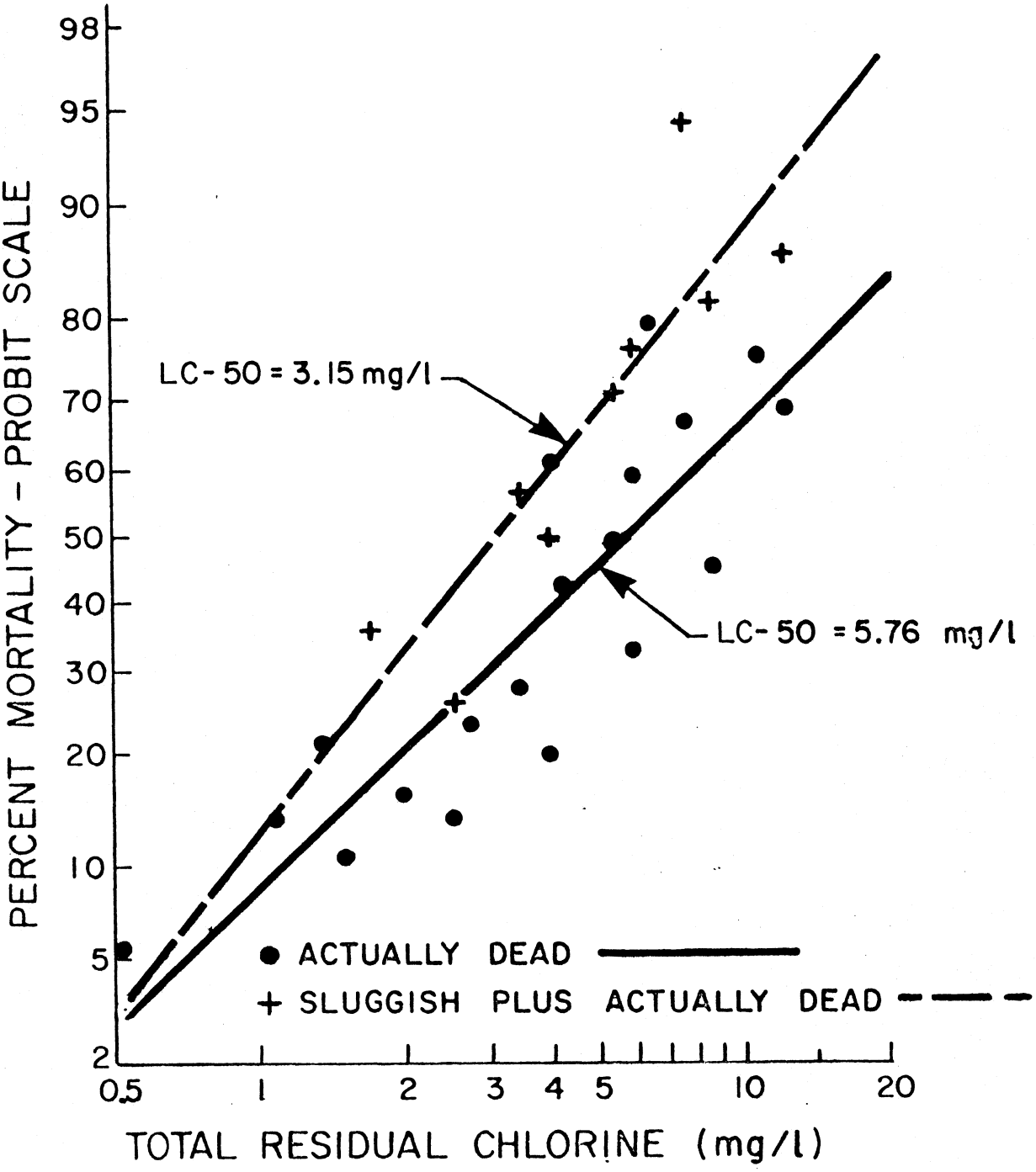

Figure 4-6 Dose-mortality plot for a 30-minute exposure of residual chlorine to Cyclops bicuspidatus thomasi at 20°C.

Table 4-2 Statistical results from bioassays in which Limnocalanus, Cyclops and Pontoporeia were exposed to residual chlorine for 30 minutes at various temperatures.

Species	Temperature (°C)	LC-50 (mg/l)	95% Confidence limits for LC-50 (mg/l)	Regression slope "b"	95% Confidence limits for "b"
Cyclops	10	14.68	12.59 -- 15.29	1.142	0.896 -- 1.388
"	15	15.61	13.96 -- 18.17	2.006	1.386 -- 2.627
"	20	5.76	5.10 -- 6.99	1.814	1.221 -- 2.407
"	20[a]	3.15	2.29 -- 3.67	2.357	1.316 -- 3.397
Limnocalanus	5	1.54	1.51 -- 1.58	7.919	7.097 -- 9.728
"	10	1.54	1.50 -- 1.58	10.41	6.187 -- 14.63
Pontoporeia	4	10.6[b]	7.6 -- 14.8	---	----
"	9	3.2[b]	2.56 -- 4.0	---	----
"	9	20.[c]	9.1 -- 44.0	---	----

[a]Mortality calculated by including sluggish individuals in the dead category

[b]Quadruple exposure

[c]Single exposure

Table 4-3 Bioassay results for exposure of residual chlorine for 30 minutes to Limnocalanus macrurus at 5°C.

Date	Sample Size	Total residual chlorine (mg/l)	Percent mortality	pH
9/30/74	40	0.000	0.00	7.65
	38	1.470	65.78	7.82
	40	1.582	80.00	7.90
	41	1.784	60.72	7.96
	40	1.912	85.00	7.99
	39	2.065	87.37	8.03
9/30/74	39	0.000	0.00	7.91
	40	1.728	85.00	9.04
	39	1.980	94.87	8.07
	42	2.116	97.50	8.09
	39	2.188	100.0	8.12
	39	2.478	100.0	8.15
10/3/74	40	0.000	0.00	7.84
	40	1.017	10.00	8.00
	39	1.297	35.92	8.07
	41	1.402	14.76	8.10
	41	1.869	68.22	8.16
	43	2.185	88.80	8.18
10/12/74	40	0.000	0.00	7.96
	40	0.659	0.00	8.03
	40	0.770	0.00	8.06
	41	0.898	0.00	8.08
	40	1.149	2.50	8.10
	41	1.430	2.50	8.11
10/12/74	41	0.000	0.00	7.72
	41	1.214	22.50	8.08
	42	1.476	30.96	8.15
	41	1.623	17.02	8.17
	40	1.716	92.50	8.11
	41	1.959	97.50	8.15

Table 4-4 Bioassay results for exposure of residual chlorine for 30 minutes to Limnocalanus macrurus at 10°C.

Date	Sample Size	Total residual chlorine (mg/l)	Percent mortality	pH
9/22/74	40	0.000	0.00	7.86
	39	1.134	12.76	7.98
	41	1.436	50.95	8.04
	40	1.740	82.50	8.09
	39	1.888	100.0	8.13
	40	2.108	100.0	8.15
9/22/74	41	0.000	0.00	7.97
	39	0.764	2.63	8.06
	39	0.836	5.00	8.03
	40	1.088	7.50	8.07
	38	1.182	10.52	8.09
	39	1.312	28.29	8.10
9/25/74	40	0.000	0.00	7.82
	40	1.133	15.00	7.95
	40	1.215	17.50	7.99
	40	1.382	20.00	8.02
	41	1.520	41.78	8.03
	42	1.668	59.32	8.07

Table 4-5 Bioassay results for exposure of residual chlorine for 30 minutes to Cyclops bicuspidatus thomasi at 10°C.

Date	Sample Size	Total residual chlorine (mg/l)	Percent mortality	pH
8/20/74	38	0.000	0.00	8.24
	39	2.492	7.90	8.38
	41	3.266	36.43	8.44
	37	4.368	37.58	8.47
	39	6.454	46.32	8.56
	39	9.402	53.82	8.68
8/20/74	40	0.000	5.01	8.24
	36	2.499	16.72	8.28
	38	3.494	26.11	8.33
	41	4.546	34.17	8.38
	39	6.466	41.19	8.50
	40	9.465	50.13	8.53
8/22/74	39	0.000	0.00	8.20
	39	3.548	25.40	8.38
	41	6.552	24.28	8.47
	40	9.556	32.46	8.64
	40	14.76	55.00	8.82
	39	20.42	56.58	8.98
8/22/74	39	0.000	0.00	8.20
	40	3.346	22.50	8.30
	38	5.852	41.94	8.42
	41	9.220	41.55	8.58
	38	11.90	45.00	8.68
	37	14.36	56.91	8.78
8/28/74	40	0.00	2.50	8.27
	40	4.50	20.05	8.49
	38	8.38	25.00	8.72
	43	12.45	44.26	9.00
	38	20.56	47.37	9.26
	41	45.46	65.95	9.52
8/28/74	41	0.00	4.76	8.26
	37	3.69	19.00	8.47
	39	7.41	30.92	8.70
	39	10.91	35.79	8.98
	40	19.02	52.50	9.08
	37	38.64	70.32	9.40

Table 4-6 Bioassay results for exposure of residual chlorine for 30 minutes to Cyclops bicuspidatus thomasi at 15°C.

Date	Sample Size	Total residual chlorine (mg/l)	Percent mortality	pH
10/30/74	39	0.00	2.50	8.10
	39	4.36	25.92	8.30
	38	6.62	20.73	8.41
	38	8.66	48.06	8.48
	39	10.92	29.36	8.55
	40	13.56	52.50	8.63
11/1/74	38	0.00	5.00	8.00
	40	11.51	62.50	8.42
	38	14.74	58.06	8.53
	40	16.76	60.00	8.57
	41	19.92	70.84	8.63
	40	24.91	78.50	8.75
11/3/74	39	0.00	0.00	8.09
	40	5.72	17.50	8.30
	40	11.04	25.00	8.51
	36	16.44	61.30	8.68
	41	30.17	73.34	8.93
11/6/74	39	0.00	2.50	8.18
	37	6.57	10.88	8.40
	37	9.82	21.64	8.55
	40	13.30	15.00	8.65
	41	17.10	36.55	8.76
	41	22.34	46.07	8.85

Table 4-7 Bioassay results for exposure of residual chlorine for 30 minutes to Cyclops bicuspidatus thomasi at 20°C.

Date	Sample Size	Total residual chlorine (mg/l)	Percent actually dead	Percent sluggish and actually dead	pH
9/7/74	39	0.00	2.63	--	7.66
	38	0.52	5.26	--	7.61
	37	1.36	20.88	--	7.71
	39	2.71	23.02	--	7.83
	39	4.04	61.44	--	7.94
	40	6.50	80.00	--	8.07
9/9/74	37	0.00	5.44	--	7.82
	37	1.08	13.30	--	7.92
	39	1.99	15.40	--	8.00
	39	1.50	10.26	--	7.98
	40	4.28	42.48	--	8.22
	37	5.93	59.41	--	8.29
11/15/74	39	0.00	0.00	0.00	8.05
	36	1.70	0.00	36.11	8.12
	37	3.48	24.42	56.72	8.25
	37	5.42	50.59	72.35	8.39
	40	7.66	67.50	95.00	8.47
	38	10.87	76.11	100.00	8.60
11/24/74	40	0.00	2.38	2.38	8.04
	38	2.53	15.56	26.11	8.21
	36	3.96	19.44	50.00	8.29
	40	5.90	32.50	77.50	8.35
	40	8.62	45.00	82.50	8.46
	38	12.38	69.15	86.94	8.57

However, when sluggish individuals were counted as dead, the resulting LC-50 value was 3.15 mg/l, significantly lower.

To determine the fate of the sluggish individuals, one bioassay was performed in which six groups of 15 Cyclops were exposed to 10 mg/l chlorine for 30 minutes, while six groups were maintained as controls. At 24, 48, and 120 hours after the end of the exposure period, 2 control and 2 experimental groups were examined to determine numbers of individuals actively swimming, dead and sluggish (Figure 4-7). While the percent of the individuals of each experiment group actively swimming remained relatively constant at 24, 48 and 120 hours, the percent sluggish decreased significantly and the percent dead increased significantly at 120 hours. No mortality occurred in the control groups at any time.

The pH of the toxicant aquaria was observed to increase as the concentration of residual chlorine increased. The pH of the control chambers and the flow-through aquaria ranged from 7.6 to 8.2, over the course of all the bioassays. An incremental increase in the concentration of chlorine of 10 mg/l generally raised the pH by one-half unit. The pH of the solution with the highest concentration of chlorine tested, 45 mg/l, was about 9.5. The effect of pH alone on Cyclops and Pontoporeia was determined by performing a bioassay in which the toxicant aquaria contained solutions of chlorine-free lake water to which varying quantities of 1N NaOH were added. This resulted in pH solutions as high as 9.6. One

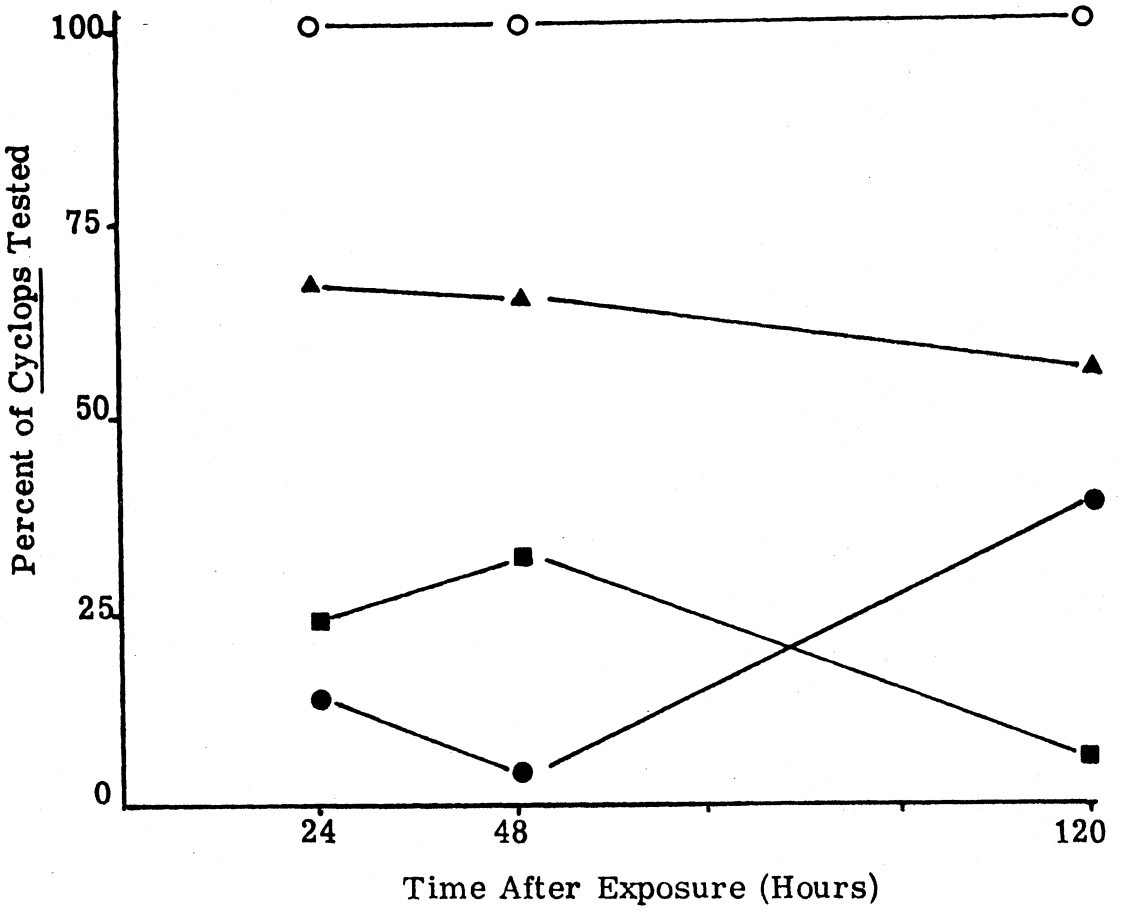

Figure 4-7 Results of a 5-day bioassay conducted to determine the fate of Cyclops rendered "Sluggish" following a 30-minute exposure to 10 mg/l chlorine at 10°C, mortality determined at 24, 48, and 120 hours following exposure.

solution of lake water was maintained as a control (pH 8.2). No mortality was observed at any of the pH values tested. This indicates that the mortality observed in the chlorine bioassays was due to the toxicity of chlorine, and not due to pH.

The results of bioassay experiments with Pontoporeia indicate that this organism is highly resistant to single 30-minute exposures to chlorine. Very low mortality was observed at 4C following exposure to concentrations as high as 25.7 mg/l. (Table 4-8). The 30-minute LC-50 value was estimated to be 20 mg/l at 9°C (Fig. 4-8, Tables 4-2 and 4-8).

Since Pontoporeia is a benthic organism and generally less transient than the planktonic forms tested, the probability of multiple exposure to a chlorinated plume is greater. A series of multiple exposure experiments were conducted following the procedures described above. The LC-50 values determined 48 hours after the fourth and final exposure were 10.6 mg/l at 4°C and 3.2 mg/l at 9°C. (Fig. 4-9, and Table 4-2). The averaged results of the duplicate quadruple exposure experiments for Pontoporeia are contained in Tables 4-9 and 4-10.

Table 4-8 Bioassay results for a single 30-minute exposure of residual chlorine to Pontoporeia affinis

Date	Sample Size	TRC (mg/l)	% Mortality	pH	Temp. (°C)
6/12/75[a]	36	8.579	0	8.7	3.0
	40	4.356	0	8.5	
	38	0.767	0	8.3	
	40	0.000	0	8.2	
6/27/75[a]	41	25.724	2.4	9.1	3.5
	41	18.452	7.3	9.0	
	39	8.829	0	8.6	
	40	0.000	5.0	8.2	
5/27/76-6/2/76[b]	21	27.90	57.1	9.0	9.0
	18	16.20	44.4	8.7	
	20	4.34	5.0	8.3	
	20	0.00	0.0	8.2	
6/22/76-6/27/76[b]	45	20.60	84.4	8.9	9.0
	40	12.60	17.5	8.6	
	40	4.01	10.0	8.2	
	21	0.0	4.8	8.1	

[a]Chlorine measured amperometrically and mortality determined 24 hours after exposure

[b]Chlorine measured by DPD method and mortality determined 5 days after exposure

Table 4-9 Bioassay results for quadruple exposures of residual chlorine for 30 minutes to Pontoporeia affinis at 4C

Date	Sample Size	Avg. TRC[a] (mg/l) (range)	% Mortality	Avg. pH (range)
6/2/75-6/8/75	40	15.148[b] (14.285-15.958)	60.0	8.8 (8.8-8.9)
	40	10.243 (9.756-10.564)	32.5	8.7
	40	5.203 (5.087-5.296)	12.5	8.5
	40	0.000	0.0	8.2 (8.2-8.3)
10/19/75-10/25/75	40	11.70 (8.13-12.94)[c]	60.0	8.8 (8.7-8.8)
	33	7.57 (7.72-7.22)	42.4	8.6 (8.5-8.6)
	40	4.20 (3.91-4.55)	42.5	8.5
	39	0.00	5.1	8.1 (8.1-8.2)
11/1/75-11/7/75	40	13.95 (13.81-14.11)	60.0	8.6 (8.6-8.7)
	42	9.05 (8.90-9.16)	40.5	8.5 (8.4-8.5)
	41	4.41 (4.28-4.53)	34.2	8.2 (8.0-8.3)
	40	0.00	12.5	7.9 (7.7-8.1)
11/15/75-11/21/75	39	14.05 (13.70-14.50)	69.2	8.6 (8.5-8.6)
	39	9.17 (9.02-9.34)	20.5	8.6 (8.6-8.7)
	39	4.48 (4.35-4.63)	30.8	8.4
	40	0.00	10.0	8.0

[a]Average of 4 30-minute doses

[b]Determined by amperometric titration

[c]This and all following values determined with DPD colorimetric method

Table 4-10 Bioassay results for quadruple exposures of residual chlorine for 30 minutes to Pontoporeia affinis at 9C.

Date	Sample Size	Avg. TRC[a] (mg/l) (range)	Avg. % Free Chlorine (range)	% Mortality	Avg. pH (range)
2/19/76-2/25/76	41	14.01 (13.7-14.7)	---	100.	8.7 (8.7-8.8)
	39	6.63 (6.20-6.78)	---	100.	8.4 (8.3-8.5)
	36	0.00	---	5.6	8.1 (8.0-8.1)
3/2/76-3/8/76	40	6.02 (5.96-6.10)	---	85.0	8.5 (8.4-8.5)
	35	2.96 (2.90-3.01)	---	43.9	8.3 (8.2-8.4)
	31	0.00	---	3.2	8.1 (8.0-8.2)
3/29/76-4/4/76	41	5.82 (5.80-5.84)	97.2 (95.2-99.0)	87.8	8.5 (8.4-8.5)
	35	2.80 (2.78-2.83)	93.6 (89.7-95.7)	40.0	8.3 (8.2-8.4)
	40	1.31 (1.25-1.35)	89.9 (81.6-95.6)	7.5	8.3 (8.2-8.3)
	40	0.00	---	10.0	8.2 (8.2)
4/11/76-4/17/76	43	6.16 (6.06-6.20)	97.3 (97.1-97.4)	98.0	8.3 (8.3-8.4)
	36	3.08 (3.00-3.16)	97.1 (96.8-97.4)	38.9	8.2 (8.2-8.3)
	39	1.43 (1.28-1.50)	95.3 (94.6-96.1)	30.8	8.2 (8.1-8.2)
	40	0.00	---	10.0	8.1 (8.0-8.2)
5/3/76-5/9/76	39	5.12 (5.08-5.20)	99.5 (99.2-100)	76.9	8.4 (8.4)
	40	4.18 (4.14-4.25)	98.5 (98.1-99.0)	80.0	8.4 (8.3-8.4)
	39	1.89 (1.75-2.07)	95.2 (90.9-97.8)	15.4	8.3 (8.2-8.3)
	40	0.00	---	10.0	8.2 (8.1-8.2)

[a]Average of 4 30-minute doses

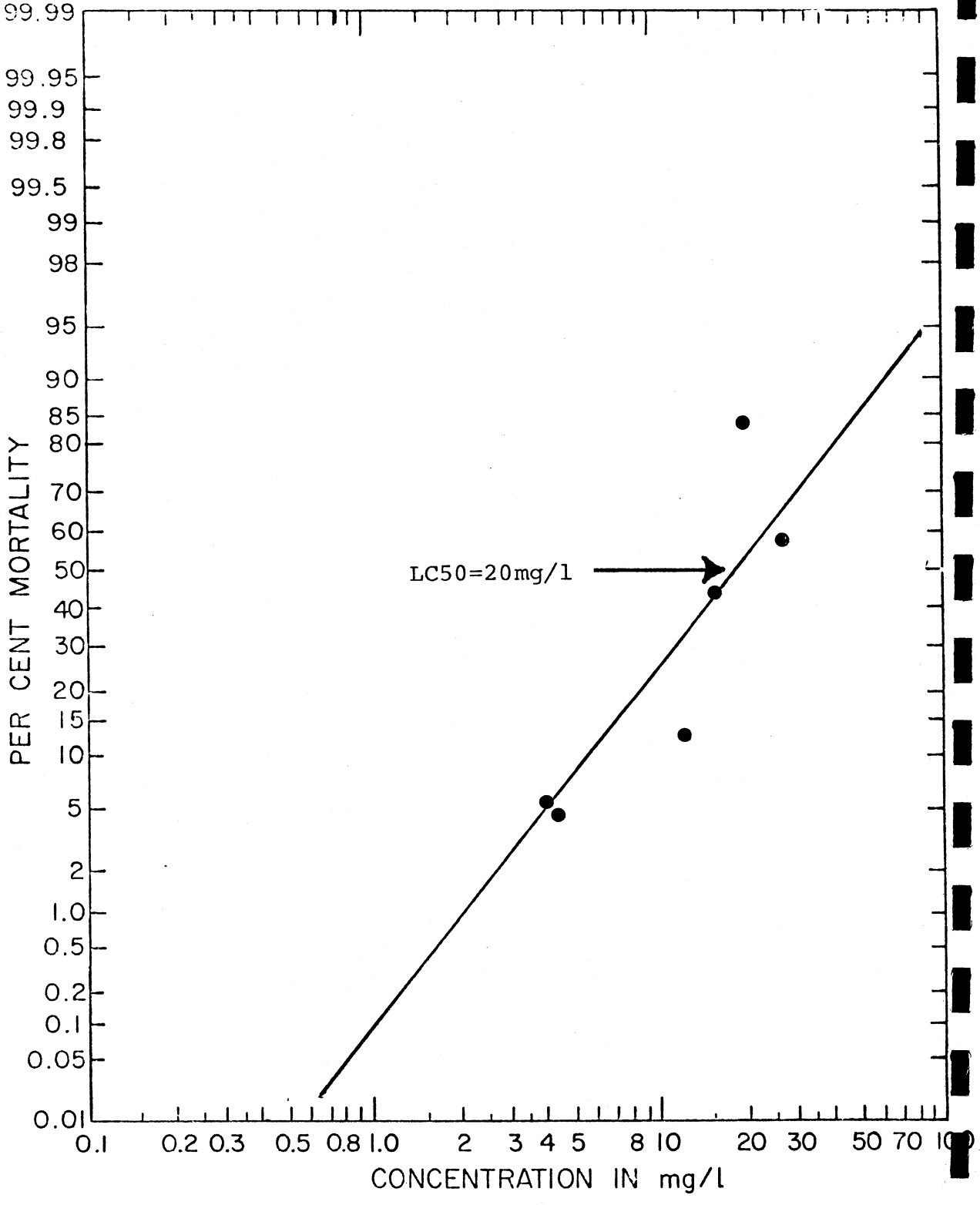

Fig. 4-8 Results of a single 30-minute exposure of Pontoporeia affinis at 9C to residual chlorine

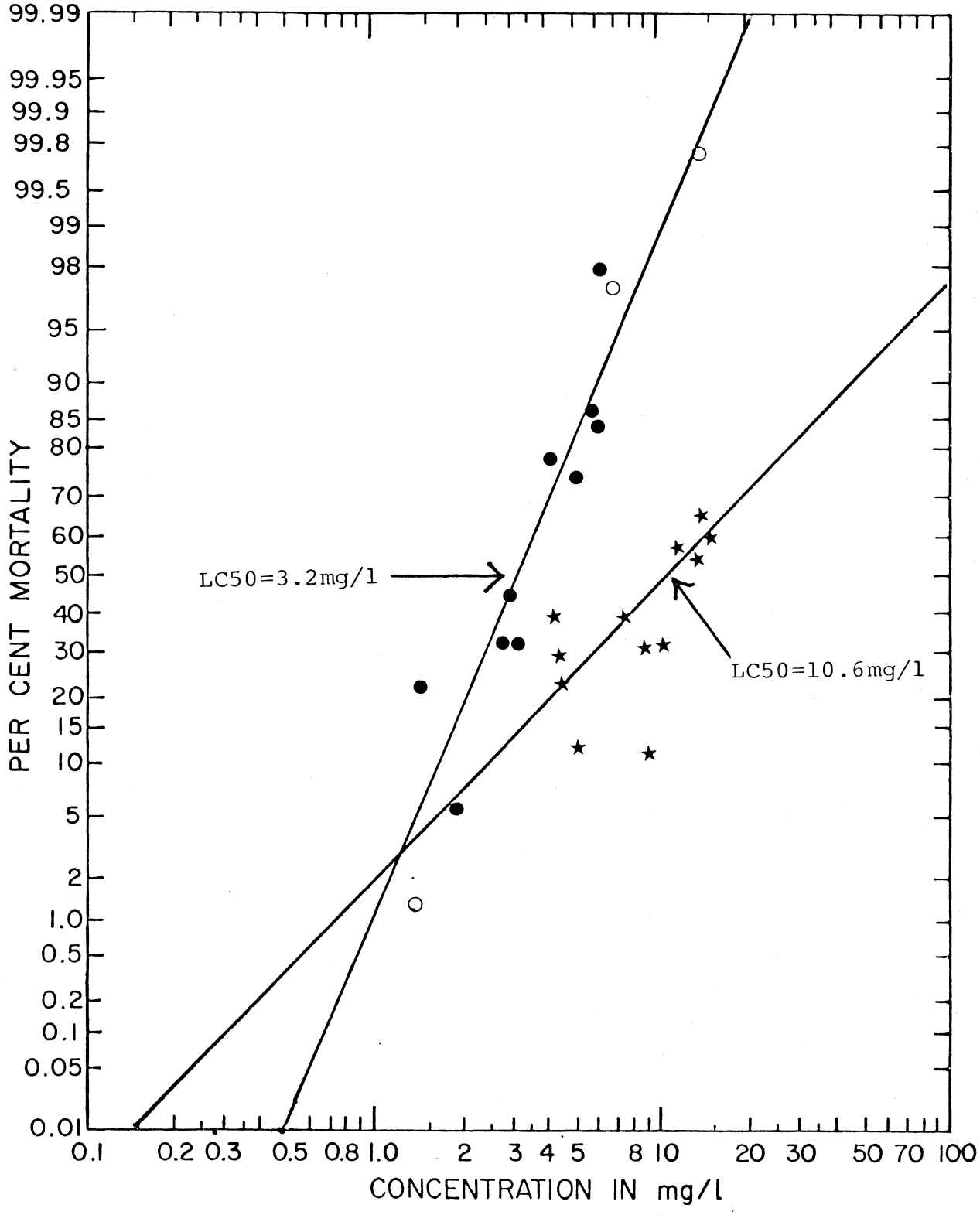

Fig. 4-9 Results of quadruple 30-minute exposures of Pontoporeia affinis at 4C (stars) and 9C (circles). Solid symbols represent observed mortalities; open symbols represent concentrations where 0% and 100% mortality data were transformed.

DISCUSSION

The vast majority of Cyclops bicuspidatus thomasi Limnocalanus macrurus and Pontoporeia affinis were capable of surviving 30-minute exposures to residual chlorine at concentrations exceeding the maximum range of 0.5 - 1.0 mg/l which is typically observed in the effluents of power plants. Considerable differences were observed between the two Copepods species in regards to the LC-50 values and the regression slopes obtained in the bioassays. Cyclops displayed tolerance to concentrations which immediately killed Limnocalanus. Cyclops was able to withstand a much wider range of concentrations than was Limnocalanus. The extreme variability in the sensitivity of Cyclops to chlorine is evidenced by the observation that at 10°C, 25% of those individuals tested were killed by concentrations less than 5 mg/l, while 25% were able to survive concentrations of 45 mg/l. In contrast, about 90% of the Limnocalanus individuals tested at 10°C were killed over the concentration range 1 - 3 mg/l. The high resistance of Cyclops to the 30-minute exposure to chlorine is in contrast to the 96-hour LC-50 of 0.069 mg/l determined by Beeton et al. (1976). This emphasizes the importance of exposure time when considering the toxicity of chlorine to these organisms.

Pontoporeia exhibited very high tolerance to both single and quadruple 30-minute chlorine exposures. There was a significant difference in LC-50 values between 4 and 9 C.

This may have been produced by the longer observation period used at 9 C or it may reflect the fact that 9 C is near the upper temperature tolerance limit for Pontoporeia. A combination of temperature stress and chlorine may, therefore, be responsible for the lower LC-50 value at 9 C.

The literature is relatively devoid of information regarding short exposures of residual chlorine to invertebrates. Several field studies have documented the toxicity of chlorine to invertebrates in power plant effluents and public water supplies. Hale, 1930, reported that Cyclops sp. was killed by a 20-minute exposure to 2 - 3 mg/l residual chlorine in a public water supply. Heinle, 1969, attributed the high mortality rate of the marine copepod Acartia tonsa in a power plant effluent to the chlorination of the cooling water, correlating the apparent periodicity in the mortality rate with the chlorination schedule. Field experiments lack the flexibility and control of laboratory studies, and are generally inadequate for determining exact toxicity levels. Arthur and Eaton, 1971, in a laboratory study, found that the 96-hour TL-50 for the amphipod Gammarus pseudolimnaeus was 0.220 mg/l, and that reproduction was reduced at 0.0034 mg/l. No definitive information could be found regarding the toxicity of chlorine to invertebrates for exposure periods as short as 30 minutes.

No formal attempt was made to determine "safe" levels for 30-minute exposures to residual chlorine for the inverte-

brate species studied. However, Sprague (1971) suggests that safe levels can be predicted by examining the log-probit regression lines and determining the toxicant concentrations which affect a negligibly small percentage of the individuals tested. Concentrations leading to 5% and 1% mortality, the TL-5 and TL-1, respectively, can be used as estimates of safe levels. It was not possible to calculate "no mortality" concentrations as was done for the fish experiments (Section 3 this report) because the range of exposure concentrations employed did not yield sufficient "no mortality data" (Tables 4-3 to 4-10).

The TL-5 and TL-1 statistics were calculated from the equations of the regression lines obtained in this study (Table 4-11). Utilizing the TL-5 values, Cyclops would be protected at 0.5 mg/l, and Limnocalanus at 0.9 mg/l, when exposure periods did not exceed 30 minutes. Based on the TL-1 values, Cyclops would be protected at 0.1 mg/l and Limnocalanus at 0.7 mg/l. The TL-1 and TL-5 values for Pontoporeia were 1.5 and 0.75 mg/l for the four exposure study at 4 C and 1.37 and 1.00 mg/l at 9 C. It is apparent that these safe level estimates generally exceed the chlorine concentration range which is found in most power plant effluents. It is therefore concluded that most power plant effluents would have only minor effect on populations of Cyclops, Limnocalanus, and Pontoporeia provided that exposures were limited to 30 minutes, and that concentrations did not

Table 4-11 TL-5 and TL-1 statistics used in estimating "safe" levels for 30-minute exposures of residual chlorine to Cyclops, Limnocalanus and Pontoporeia.

Species	Temperature (°C)	TL-5 (mg/l)	TL-1 (mg/l)
Cyclops	10	0.533	0.135
"	15	2.364	1.082
"	20	0.713	0.300
"	20*	0.632	0.325
Limnocalanus	5	1.073	0.923
"	10	0.956	0.784
Pontoporeia	4	1.50	0.75
"	9	1.37	1.00

*Mortality calculated by including sluggish individuals in the dead category.

exceed the estimated safe levels. At the present time no information is available regarding the long-term effects of a 30-minute exposure to residual chlorine. Additional research will be necessary to substantiate the proposed safe levels.

5. PHYTOPLANKTON

Introduction

The chlorination of cooling waters has been shown to influence planktonic algae by reducing chlorophyll concentrations and rates of photosynthesis (Morgan & Stross 1969, Hamilton et al. 1970, Brook and Baker, 1972, Carpenter et al. 1972, Brooks, 1974, Fox and Mayer, 1975, Eppley et al. 1976). With one exception (Eppley et al. 1976), these studies have not accurately determined the concentration of chlorine which produced the observed effects. Chlorine was either suspected to be present at the time of observation but not measured, calculated from application rates, or measured with poor analytical methods. Furthermore, the effects of chlorination could not be completely separated from other environmental variables.

Laboratory studies conducted under more controlled conditions have produced conflicting results. Hirayama and Hirano, 1970, showed little or no effect of chlorine on cultures of marine algae while Eppley et al. (1976) reported inhibition of photosynthesis in natural seawater samples similar to results reported in the field studies cited above. The purpose of the present study was to investigate the effects of chlorine on natural populations of Lake Michigan phytoplankton under controlled environmental conditions.

Methods and Materials

Field studies of Lake Michigan have shown that chlorine in chlorinated effluents decays rapidly as dilution and chemical degradation proceed (Beeton, Kovacic and Brooks, 1976. This Report Section 6) Experimental procedures were designed to approximate field conditions whereby the phytoplankton would be exposed to residual chlorine for periods of 30 minutes. Test dates were planned to reflect seasonal changes in physical, chemical, and biological conditions in Lake Michigan. Untreated Lake Michigan water and the phytoplankton contained therein was obtained from the City of Milwaukee Linnwood Avenue Water Filtration Plant. Water is pumped to the treatment plant from an intake crib located approximately 1.6 km offshore at a depth of 17 meters. In the laboratory the water was filtered through #10 Nitex netting to remove larger zooplankton, and transferred to six 4-liter beakers.

All experiments were carried out in incubators at temperatures equal to the lake water at the time of collection. Cool white fluorescent lights provided constant illumination between 60 and 70 microeinsteins $meter^{-2}$ $second^{-1}$. Prechlorination samples from each of the six beakers were taken for chlorophyll analysis, species enumeration, and carbon-14 uptake measurements. The beakers, with the exception of a control, were then dosed with varying amounts of sodium hypochlorite to give total residual chlorine concentrations ranging from 0 in the control beaker to 1.5 mg/l. Thirty

minutes after dosing with hypochlorite, equivalent amounts of sodium sulfite were added to chemically reduce the residual chlorine. Postchlorination data on carbon-14 uptake and pigment concentration were obtained from alequots withdrawn from the beakers at intervals over a 24-hour observation period. The observation period was extended to 47 hours during the summer tests.

Preliminary experiments to assess the effect of sodium sulfite on the phytoplankton followed a procedure similar to that employed for chlorine experiments, except that sulfite alone was added to test beakers. The sulfite alone produced neither positive nor negative effects (α=.05) on C-14 uptake rates or on chlorophyll *a* and phaeophytin *a* concentrations.

Carbon-14 uptake measurements which are indicative of rates of photosynthesis were made following the method of Strickland and Parsons, 1972. Light and dark bottles were incubated for 3 hours under light and temperature conditions identical to those of the 4-liter beakers. To eliminate the effect of diurnal rhythms, carbon-14 uptake data are reported as a fraction of a control value determined at the same time interval. Chlorophyll *a* and phaeophytin *a* were determined fluorometrically after acetone extraction (Strickland and Parsons, 1972). A ratio of phaeophytin *a* to chlorophyll *a* concentration (P/C ratio) was calculated for each sample. Increasing values of this ratio reflect the destruction of photosynthetically active chlorophyll *a* to photosynthetically inactive phaeophytin *a*. Total residual chlorine was

determined amperometrically as described in Section 2 of this report. Sulfite was determined amperiometrically with the same apparatus used for chlorine determinations (Andrew, 1971).

Results

The chlorination of Lake Michigan water for periods of 30 minutes resulted in a reduction of chlorophyll *a*, and an increase in phaeophytin *a* concentrations. These effects, expressed as phaeophytin to chlorophyll ratios, were more pronounced at chlorine concentrations greater than 1.0 mg/l while concentrations less than about 0.1 mg/l produced only slight changes in the P/C ratio (Fig. 5-1). Chlorine levels between 0.1 and 1.0 mg/l generally produced an intermediate response. Virtually no recovery in chlorophyll *a* was observed during the postchlorination observation period.

Carbon-14 uptake rates were reduced immediately following the 30-minute exposure to chlorine. The magnitude of this response was directly related to the chlorine concentration to which the phytoplankton were exposed (Fig. 5-2). Chlorine concentrations greater than approximately 0.5 mg/l produced the most drastic reductions and showed little tendency to recover. Chlorine concentrations less than 0.1 mg/l exhibited an initial reduction down to 20% of the control values, but recovered to within 80 to nearly 100% of the control values over the observation period. Intermediate chlorine levels generally produced greater initial reductions and less

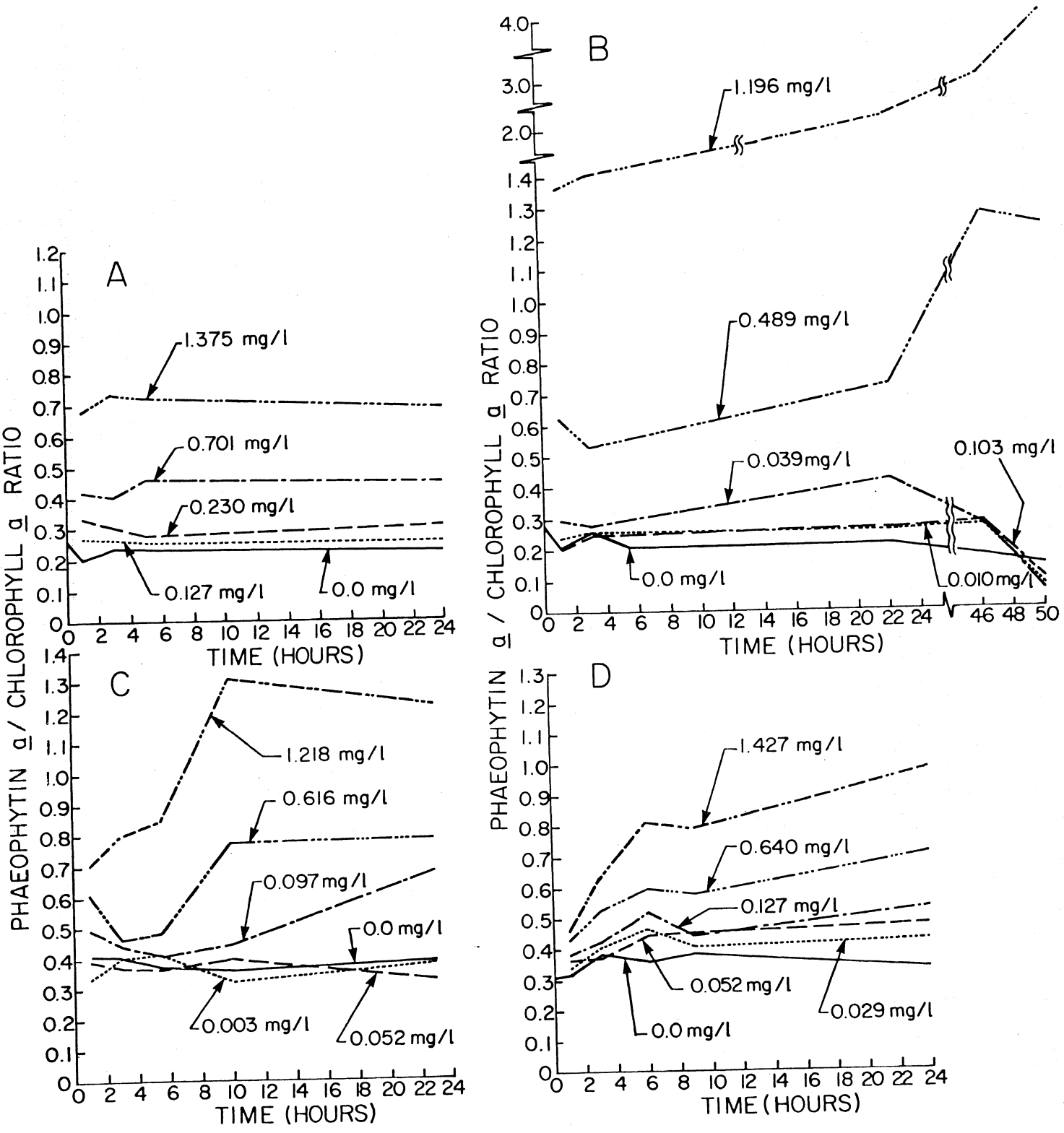

Fig. 5-1 Phaeophytin *a*/Chlorophyll *a* ratio vs. time following a 30-minute exposure to various concentrations of residual chlorine. A. 11-12 June 1974, 10°C. B. 16-18 July 1975, 12°C. C. 23-24 Oct. 1975, 10°C. D. 5-6 Jan. 1976, 2°C.

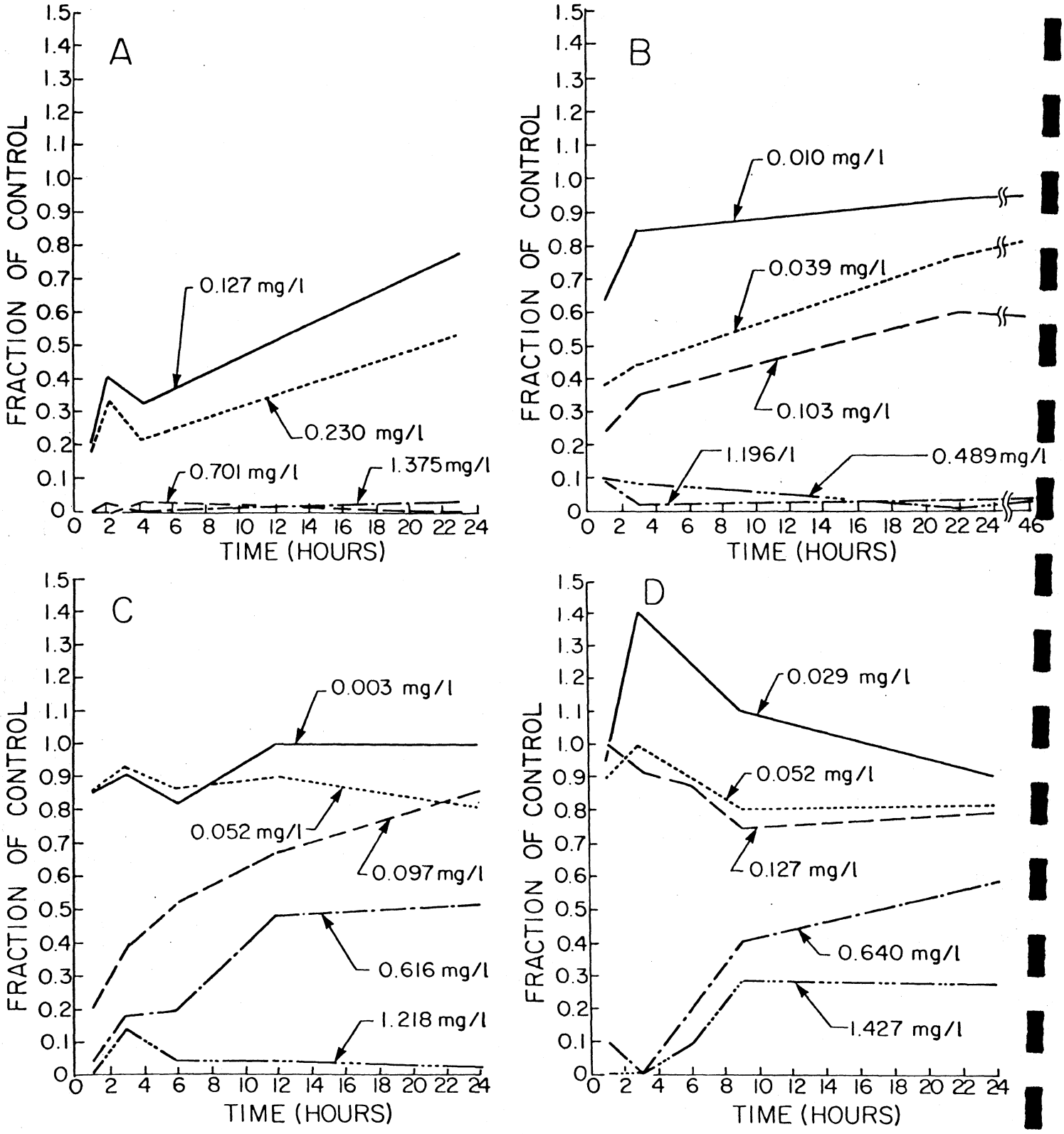

Fig. 5-2 Carbon uptake as a fraction of a control vs. time following a 30-minute exposure to various concentrations of residual chlorine. A. 11-12 June 1975, 10°C. B. 16-18 July 1975, 12°C. C. 23-24 Oct. 1975, 10°C. D. 5-6 Jan. 1976, 2°C.

recovery in carbon uptake rates near the upper end of the concentration range while the converse was true of the lower intermediate levels.

Discussion

Our observations concur with the results of previously cited field studies while providing sound data indicative of the chlorine concentrations which elicit such responses. The specific physiological mechanism which produced the observed responses could not be determined from our studies, however it appears that two possible processes may be involved. At the highest chlorine concentrations active chlorophyll *a* was destroyed by the chlorine resulting in an increase in phaeophytin *a* and a concurrent reduction in rates of carbon uptake. At chlorine concentrations less than approximately 0.1 mg/l no dramatic change in chlorophyll *a* occurred and, following an initial reduction of carbon uptake rates, significant recovery was observed. It appears that at low chlorine levels the photosynthetic reactions of the algae were temporarily interrupted or slowed without irreversibly destroying the entire photosystem.

The results of toxicity tests are frequently expressed in terms of the concentration of a toxicant which produces a measurable effect on 50% of a given population. Since carbon-14 uptake proved to be a sensitive indicator of chlorine activity, the chlorine concentrations which produced a 50% reduction in carbon uptake 24 hours after the initial 30-

minute exposure were calculated. The effective concentrations were 0.275, 0.160, 0.620 and 0.760 mg/l for the spring, summer, fall and winter experiments, respectively.

"Safe" concentrations of a toxicant for higher organisms have been estimated as an arbitrary fraction of concentrations lethal to 50% of the test organisms or by determining the concentration which affects a small percentage of a population, generally 1 - 5% (Sprague, 1971). Such manipulations are not appropriate for phytoplankton since a 50% reduction in carbon uptake does not necessarily indicate that 50% of the phytoplankton have been permanently destroyed and, since we are dealing with organisms having generation times of days or even hours, losses of 1 - 5% of the population will be recovered much more rapidly than with organisms having reproductive cycles on the order of years.

Our results show that nearly full recovery of carbon uptake rates can occur following a 30 minute exposure of phytoplankton to chlorine at levels less than approximately 0.1 mg/l. This finding is significant in that it demonstrates the ability of the phytoplankton to recover from what has heretofore been considered irreversible damage. Studies which do not consider the potential for recovery may overestimate the impact of chlorination on the primary productivity of the aquatic ecosystem. On the other hand, it should be noted that the difference in chlorine concentrations between nearly full recovery and no recovery at all was quite small. This fine line must be carefully evaluated in designing chlorination

procedures and setting regulatory standards to assure effective disinfection and fouling control while protecting the aquatic environment.

6. FIELD SURVEYS

Methods and Materials

Field surveys of chlorinated effluent plumes were conducted at six power plants on Lake Michigan. These plants were the Oak Creek Plant in Wisconsin; the D. H. Mitchell, Bailly and Michigan City stations in Indiana; and the D. C. Cook and J. H. Campbell plants in Michigan. (Fig. 6-0). The surveys were designed to determine the concentration and dispersion rates of chlorine in Lake Michigan waters. Four surveys were conducted at the Oak Creek Plant on a seasonal basis during 1974 while the other plants were surveyed only once during August 1974.

The general procedures followed during each survey called for chlorine to be applied to the cooling water for a period of 30 minutes at a rate sufficient to yield a residual of 0.5 mg/l at the point of discharge to the lake. Sampling commenced 15 minutes after the start of chlorination to allow the chlorine plume to become established in the lake. Sampling continued until chlorine could no longer be detected in the plume area.

With the exception of the surveys at the Bailly and Campbell plants all samples were collected from the circulating water system of the R/V NEESKAY. Water was drawn from a depth of approximately 2 m and pumped to the laboratory where samples were obtained. At the Bailly and Campbell sites, where the water was too shallow for the NEESKAY to

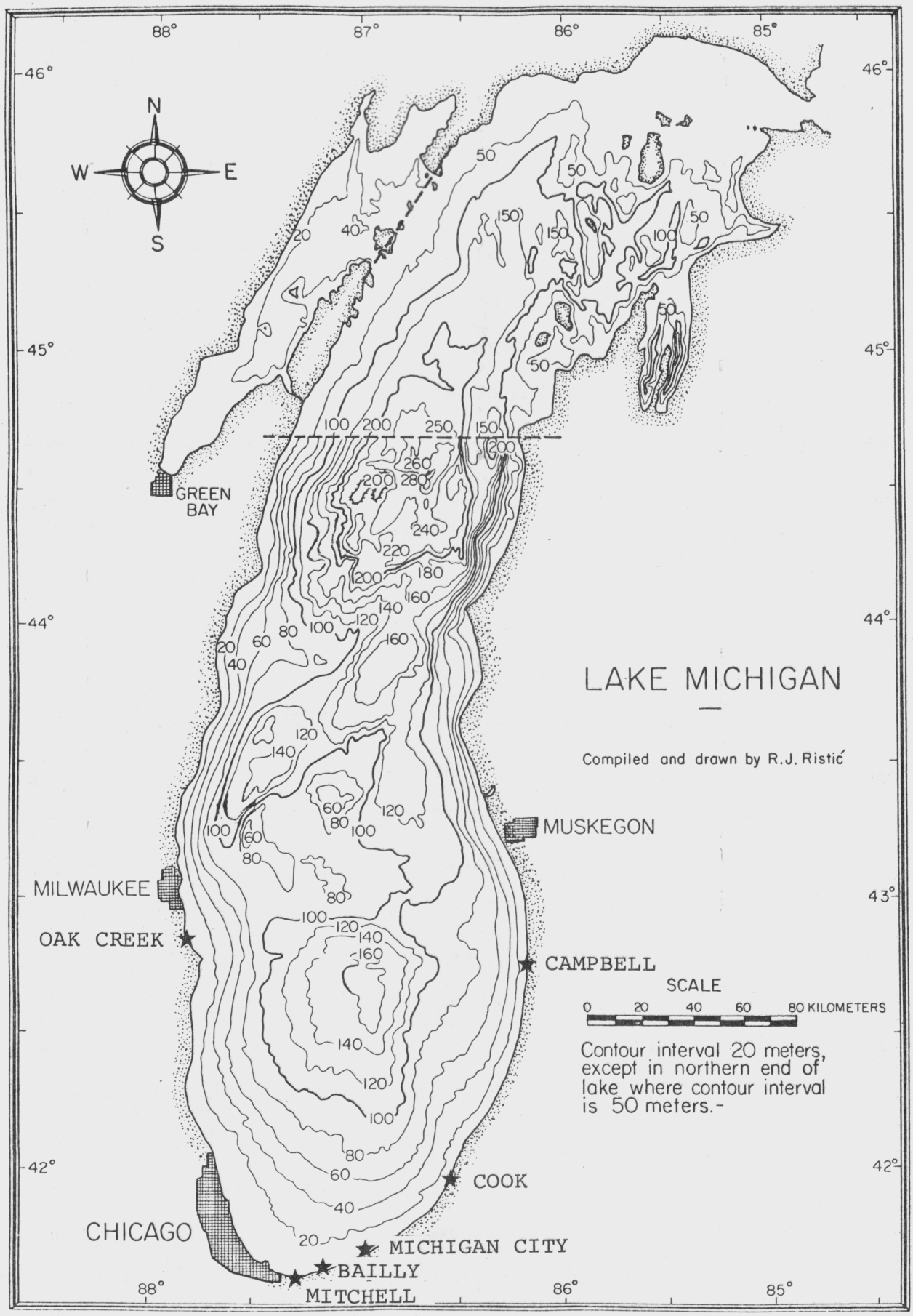

Fig. 6-0 Lake Michigan with locations of power plant sites where chlorine plume surveys were conducted(★).

navigate, samples were collected at the surface of the lake from a small skiff, and ferried to the NEESKAY for analysis. All analyses for total residual chlorine were performed aboard ship by amperometric titration (Section 2 of this report).

Samples collected on the NEESKAY were taken at 2 - 3 minute intervals as the ship cruised through the effluent plume. The location of the sampling points was determined by a Motorola Mini-Ranger radio positioning system which continuously monitored the course of the ship. At the Bailly station sampling points were determined using the Mini-Ranger to measure the distance between the shore and the skiff while a bearing on the skiff was simultaneously obtained with a shore-based transit. The distances and angles were later plotted to fix the position from which samples were obtained. At the Campbell plant the Mini-Ranger monitored the position of the NEESKAY offshore while the skiff ran a series of transects between the point of discharge on shore and the NEESKAY. Four samples were obtained at equidistant points along each transect. Additional samples were also obtained from the NEESKAY as it cruised through the outer zone of the plume.

Temperature data were obtained from a recorder which monitored the temperature of circulating water on the ship. Additional readings were made by thermistor from the skiff at the time samples were collected. With the exception of

the D. C. Cook plant, where the effluent water was not being warmed at the time of sampling, temperature readings were used to keep the ship within the plume area.

Results

The data collected on the plume surveys are presented in Figures 6-1 to 6-9 and Tables 6-1 to 6-9. With the exception of the figure for the D. C. Cook Plant which only illustrates a chlorine plume each figure depicts the estimated outer bounds of the thermal and chlorine plumes. It was not possible to draw isopleths of chlorine concentrations within the plume because of the transient nature of the plume and the fact that samples could not be taken from all areas of the plume simultaneously. The plume boundaries illustrated in the accompanying figures represent the estimated maximum extant of the chlorine plume.

At the Mitchell Station on 6 August 1974 the maximum chlorine concentration observed in the plume was 0.091 mg/l (Table 6-1). Chlorine was observed in the plume area for approximately 98 minutes from the time it was first detected. The area of the chlorine plume was approximately 0.23 km^2 (Fig. 6-1).

The chlorine plume at the Bailly Station surveyed on 6 August 1974 encompassed an area of 0.06 km^2 (Fig. 6-2). The maximum chlorine concentration observed was 0.181 mg/l. (Table 6-2). Chlorine was detectable in the plume area for about 92 minutes.

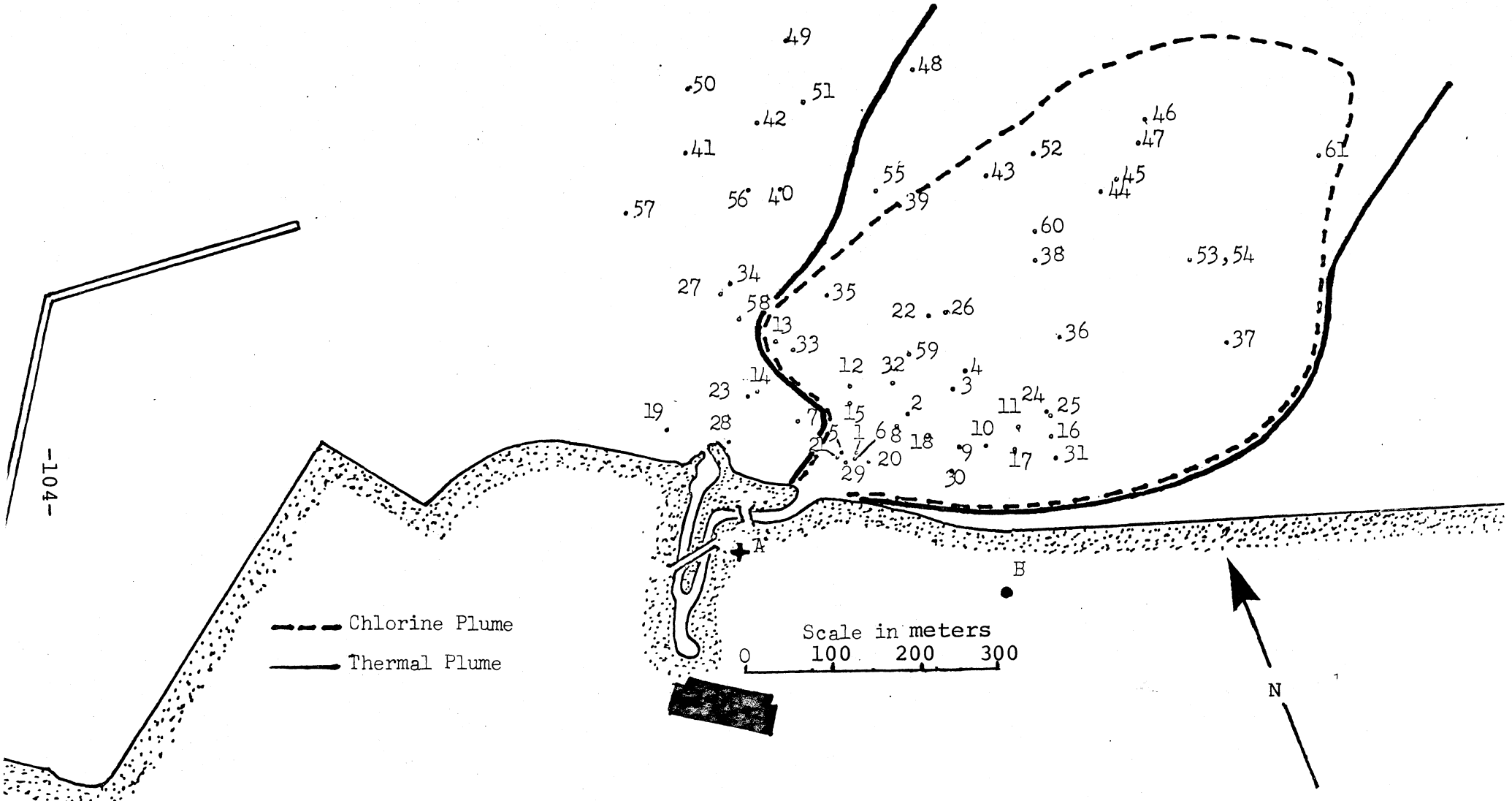

Figure 6-1 Sample station locations in Lake Michigan off the D. H. Mitchell station showing estimated outer bounds of the thermal and chlorine plumes, Aug. 6, 1974. Station numbers correspond to sample numbers in Table 6-1.

Table 6-1. DATA FROM CHLORINATION PLUME SURVEY MITCHELL PLANT - AUGUST 6, 1976

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
1	250	303	0840	23.5	0.015
2	339	309	0845	23.7	0.000
3	412	334	0849	21.6	0.000
4	461	365	0852	22.4	0.000
5	239	304	0900	23.6	0.052
6	232	300	0905	23.6	0.037
7	223	403	0908	21.4	0.000
8	325	305	0910	22.7	0.091
9	391	255	0912	22.7	0.068
10	418	248	0913	22.6	0.020
11	479	274	0915	22.7	0.037
12	302	382	0917	22.7	0.032
13	300	503	0918	22.3	0.008
14	224	467	0920	21.7	0.000
15	290	366	0921	21.4	0.000
16	525	287	0923	22.5	0.064
17	472	246	0925	22.6	0.070
18	347	279	0926	25.0	0.024
19	171	536	0930	21.2	0.000
20	260	277	0932	21.5	0.066
21	458	446	0936	25.6	0.033
22	226	316	0938	25.0	0.053
23	217	474	0940	21.5	0.000
24	523	313	0942	22.2	0.042
25	523	300	0943	22.5	0.054
26	480	448	0946	22.4	0.026
27	366	613	0948	21.8	0.000
28	151	458	0950	21.3	0.000
29	221	316	0951	22.5	0.002
30	369	219	0952	23.5	0.000
31	524	258	0954	25.0	0.057
32	348	368	0956	23.5	0.000
33	298	477	0957	22.3	0.001
34	370	612	0959	21.3	0.000

Table 6-1. Continued

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
35	390	520	1000	21.4	0.000
36	587	425	1002	22.2	0.003
37	812	538	1004	22.9	0.042
38	631	530	1006	23.0	0.038
39	552	612	1008	22.0	0.010
40	530	700	1009	21.8	0.000
41	565	809	1010	21.5	0.000
42	599	780	1012	21.5	0.000
43	674	649	1014	21.7	0.000
44	757	639	1015	22.0	0.000
45	794	667	1016	22.0	0.000
46	878	755	1018	22.2	0.002
47	865	739	1019	22.1	0.000
48	743	805	1021	22.0	0.000
49	729	879	1022	21.7	0.000
50	651	877	1024	21.7	0.000
51	650	797	1025	21.5	0.000
52	730	677	1027	21.7	0.000
53	824	619	1030	22.0	0.000
54	824	619	1030	22.0	0.000
55	552	634	1033	22.0	0.000
56	511	707	1034	21.7	0.000
57	483	778	1035	21.5	0.000
58	314	564	1037	21.3	0.000
59	382	394	1039	22.0	0.000
60	661	571	1041	22.2	0.000
61	1046	831	1043	22.0	0.000

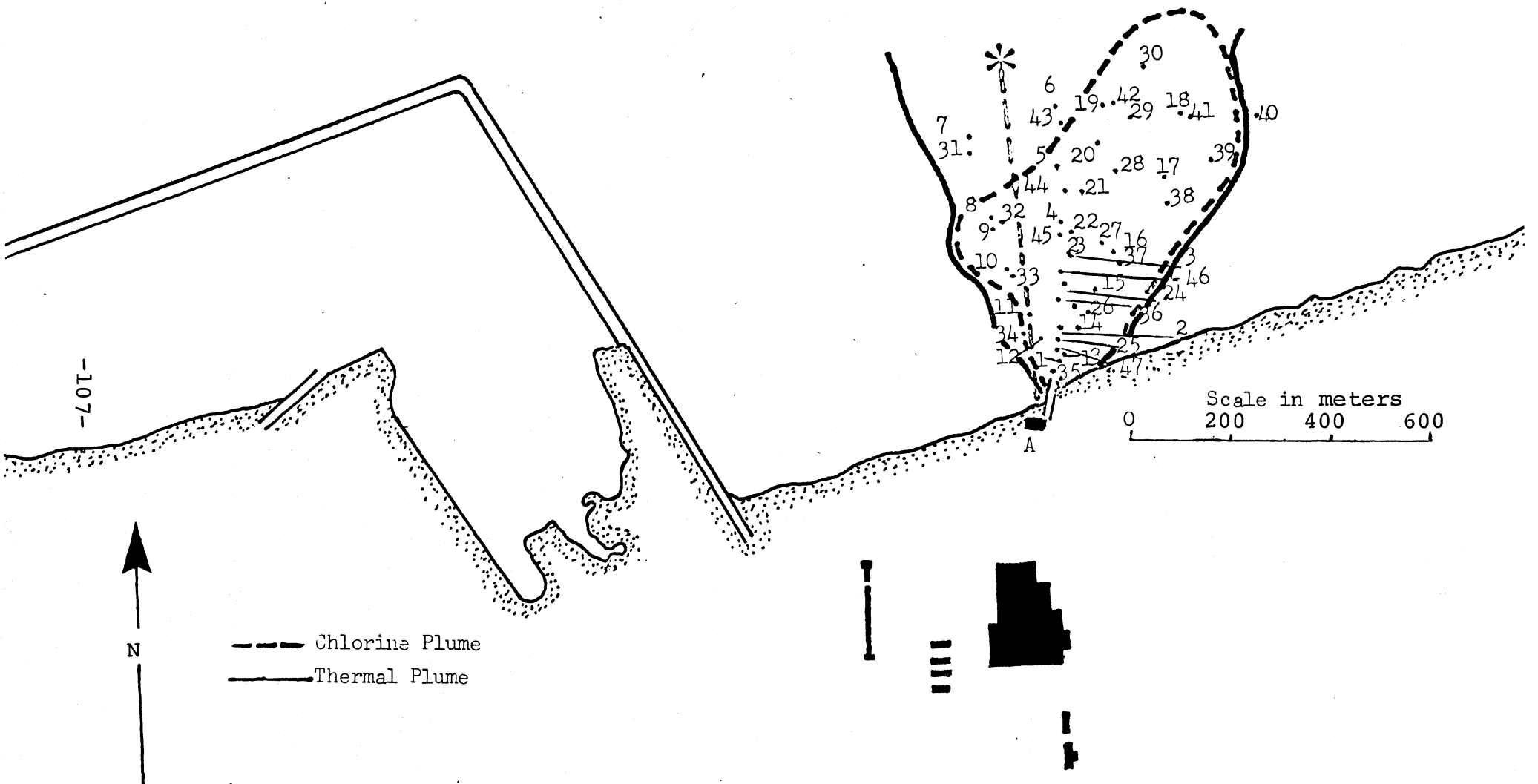

Figure 6-2 Sample station locations in Lake Michigan off the Bailly power station showing estimated outer bounds of the thermal and chlorine plumes, Aug. 6, 1974. Station numbers correspond to sample numbers in Table 6-2.

TABLE 6-2. DATA FROM CHLORINATION PLUME SURVEY BAILLY PLANT - AUGUST 6, 1974

Sample	Range	Bearing	Time	Temperature °C	Chlorine mg/
1	134	4° 55'	1438	28.0	0.000
2	189	6° 45'	1439	28.0	0.000
3	270	6° 30'	1440	28.0	0.000
4	340	5° 12'	1441	28.0	0.000
5	418	4° 20'	1442	27.0	0.000
6	500	4° 00'	1443	27.0	0.000
7	473	348° 47'	1447	25.0	0.000
8	396	349° 15.5'	1449	25.0	0.000
9	348	349° 18'	1450	25.0	0.006
10	278	350° 4.5'	1451	24.0	0.004
11	210	355° 00'	1452	25.0	0.057
12	173	357° 55'	1453	26.5	0.113
13	148	4° 50'	1454	28.5	0.181
14	190	13° 29'	1455	27.5	0.105
15	247	17° 31'	1456	26.5	0.055
16	309	20° 21'	1457	26.5	0.010
17	411	25° 7.5'	1457	25.5	0.000
18	522	24° 36'	1459	25.0	0.000
19	507	11° 23.5'	1507	26.0	0.053
20	446	11° 25.5'	1508	26.0	0.049
21	383	9° 46.5'	1509	27.0	0.090
22	328	7° 53.5'	1510	27.0	0.129
23	295	7° 43.5'	1512	28.0	0.134
24	227	4° 55'	1513	28.5	0.001
25	149	7° 18'	1514	28.5	0.000
26	216	17° 2'	1516	27.0	0.000
27	315	16° 6.5'	1517	27.0	0.024
28	413	16° 7'	1518	26.5	0.045
29	480	16° 19.5'	1520	26.0	0.015
30	571	16° 33'	1522	26.0	0.004
31	450	348° 24.5'	1528	24.5	0.000
32	345	350° 55'	1529	24.5	0.000

TABLE 6-2. Continued

Sample	Range	Bearing	Time	Temperature °C	Chlorine mg/
33	275	351° 12'	1531	24.5	0.000
34	185	350° 00'	1532	22.0	0.000
35	132	4° 37.5'	1533	28.0	0.000
36	220	12° 00'	1535	27.0	0.000
37	300	22° 28.5'	1536	26.0	0.000
38	395	28° 5'	1537	25.5	0.000
39	480	32° 15'	1538	25.0	0.000
40	567	34° 35'	1539	24.5	0.000
41	530	25° 27'	1540	25.0	0.000
42	515	13° 22.5'	1542	26.0	0.000
43	477	5° 10'	1550	27.0	0.000
44	381	5° 46.5'	1551	27.0	0.000
45	324	6° 14'	1552	27.0	0.000
46	255	7° 23'	1554	28.0	0.000
47	179	6° 00'	1555	23.0	0.000

The data from the survey at the Michigan City plant on 7 August 1974 was difficult to interpret. Only 5 out of 48 samples collected had detectable chlorine levels. The maximum concentration observed was 0.147 mg/l (Table 6-3). The time span between the first detection of chlorine and the last was only 13 minutes. The outer bounds of the chlorine plume, which is defined by a small number of points encompassed an area of 0.05 km^2. (Fig. 6-3).

At the Donald C. Cook plant on 7 August 1974 chlorine was present in the effluent area for approximately 62 minutes. The maximum chlorine concentration observed during this period was 0.129 mg/l. (Table 6-4). The estimated area of the chlorine plume was 0.20 km^2 (Fig. 6-4).

The maximum chlorine concentration observed at the D. H. Campbell plant on 8 August 1974 was 0.214 mg/l. (Table 6-5). The chlorine injection rate at the plant was increased above normal operating levels for this survey in order to obtain measurable chlorine concentrations at the point of discharge to the lake. Chlorine was observed in the effluent area for approximately 141 minutes from the time of first detection until all traces had dissipated. The area encompassed by the chlorine plume was approximately 0.17 km^2 (Fig. 6-5).

The surveys conducted at the Oak Creek Plant on 28 May, 11 September, 12 November and 10 December 1974 did not show any significant seasonal variability. The average length of time that chlorine was detectable in the plume area was 54

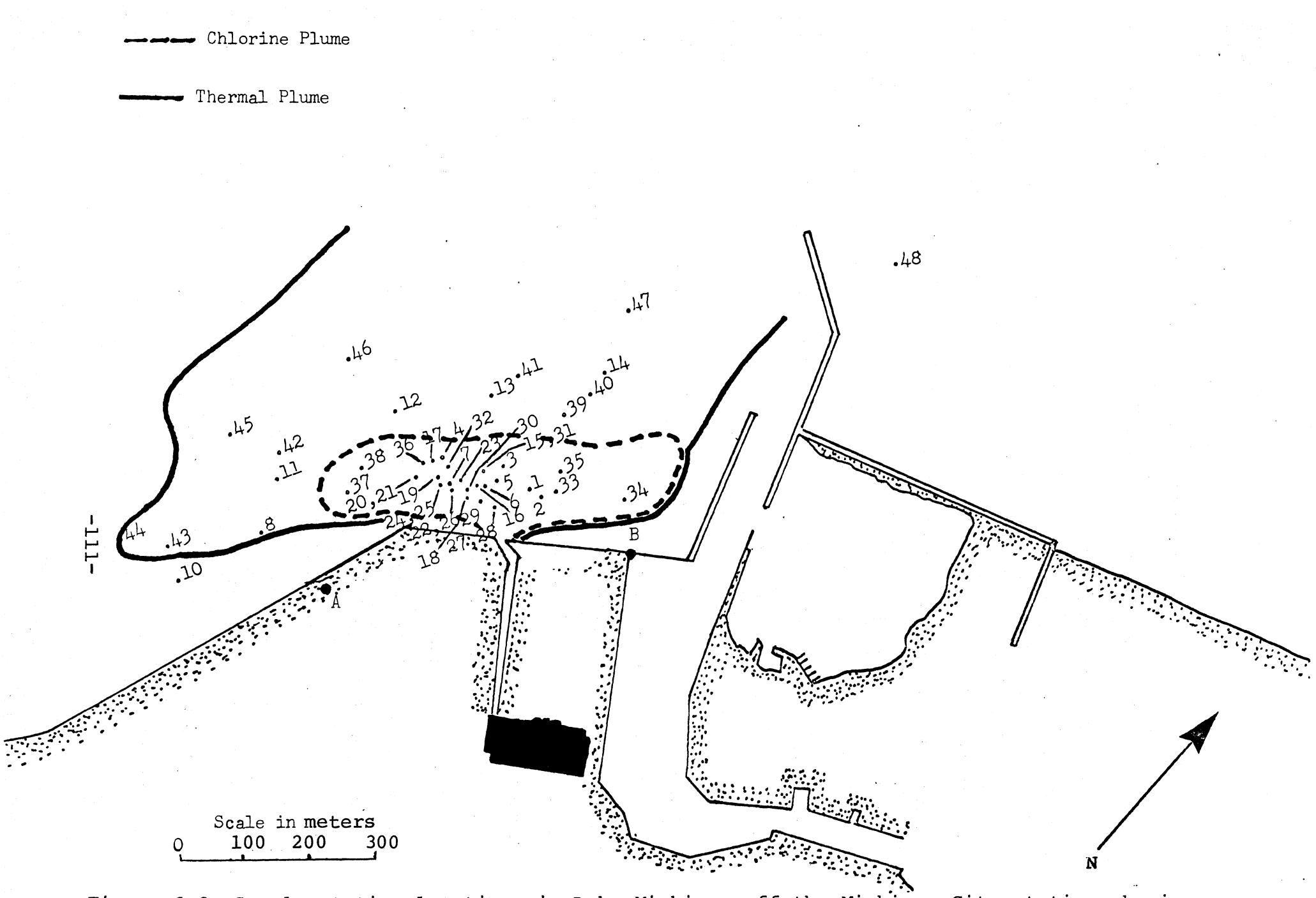

Figure 6-3 Sample station locations in Lake Michigan off the Michigan City station showing estimated outer bounds of the thermal and chlorine plumes, Aug. 7, 1974. Station numbers correspond to sample numbers in Table 6-3.

TABLE 6-3. DATA FROM CHLORINATION PLUME SURVEY MICHIGAN CITY PLANT - AUGUST 7, 1974

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
1	580	253	0857	21.6	0.000
2	593	255	0900	21.6	0.000
3	583	312	0903	22.0	0.000
4	545	358	0909	21.2	0.000
5	537	294	0911	24.0	0.000
6	543	284	0912	24.3	0.000
7	535	319	0914	22.1	0.000
8	381	559	0918	22.8	0.000
9	299	769	0920	22.4	0.000
10	375	687	0922	21.2	0.000
11	472	572	0923	21.3	0.000
12	589	497	0924	23.0	0.000
13	680	442	0925	22.7	0.000
14	789	434	0927	22.7	0.000
15	501	335	0947	23.4	0.000
16	506	325	0949	21.9	0.000
17	506	334	0950	21.7	0.000
18	504	339	0951	21.5	0.000
19	503	357	0953	21.8	0.000
20	507	369	0955	21.3	0.000
21	506	372	0956	21.4	0.000
22	505	319	1001	23.7	0.000
23	512	298	1003	23.9	0.000
24	513	294	1004	21.9	0.000
25	510	295	1005	23.3	0.000
26	508	319	1007	22.7	0.000
27	516	275	1012	23.6	0.000
28	517	275	1013	24.6	0.010
29	511	308	1016	22.2	0.000
30	514	320	1017	22.1	0.000
31	509	336	1018	21.7	0.000
32	522	337	1020	21.9	0.001
33	618	264	1021	21.8	0.000
34	687	231	1022	23.7	0.147

TABLE 6-3. Continued

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
35	643	288	1024	22.2	0.000
36	525	375	1025	22.1	0.007
37	445	475	1026	22.9	0.028
38	495	471	1027	22.3	0.000
39	721	378	1029	22.6	0.000
40	759	408	1030	23.0	0.000
41	724	453	1031	22.9	0.000
42	505	600	1033	23.0	0.000
43	425	723	1034	22.8	0.000
44	479	793	1036	21.6	0.000
45	553	694	1037	21.7	0.000
46	658	610	1038	23.0	0.000
47	926	562	1040	22.9	0.000
48	1156	602	1042	23.1	0.000

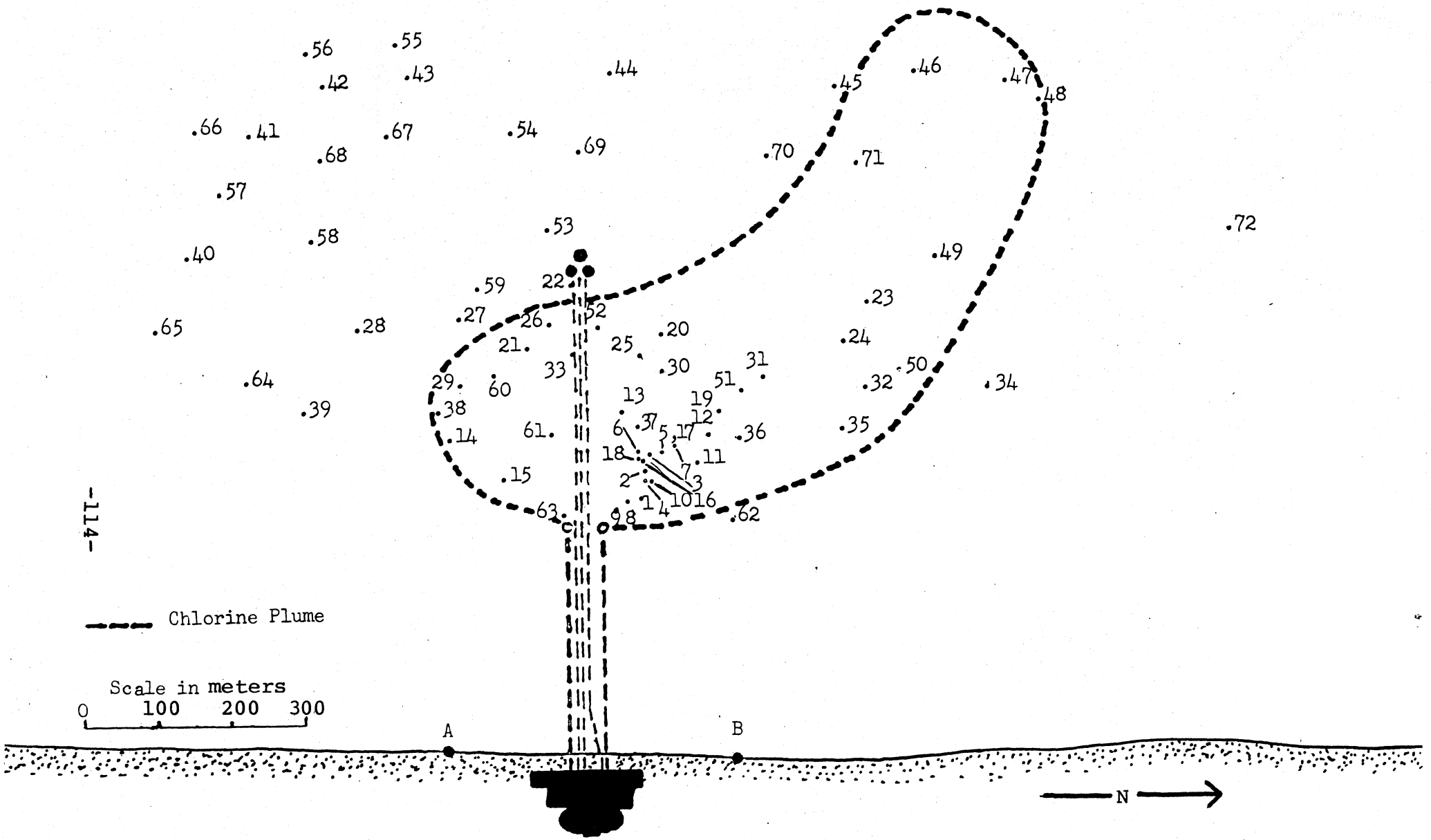

Figure 6-4 Sample station locations in Lake Michigan off the Donald C. Cook plant showing estimated outer bounds of the chorine plume, Aug. 7, 1974. Station numbers correspond to sample numbers in Table 6-4.

TABLE 6-4. DATA FROM CHLORINATION PLUME SURVEY DONALD C. COOK PLANT - AUGUST 7, 1974

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
1	479	458	1321	22.0	0.000
2	501	450	1323	23.5	0.000
3	541	478	1325	23.8	0.000
4	467	447	1330	22.4	0.000
5	524	463	1334	22.2	0.017
6	546	489	1336	22.2	0.024
7	511	453	1338	21.7	0.027
8	455	443	1341	21.6	0.117
9	421	422	1342	21.6	0.129
10	487	438	1343	21.6	0.000
11	580	458	1347	21.6	0.063
12	613	508	1348	21.4	0.000
13	581	566	1349	21.4	0.080
14	484	670	1351	21.9	0.000
15	437	583	1352	22.0	0.000
16	517	468	1354	21.9	0.000
17	525	467	1355	21.5	0.126
18	521	475	1356	21.3	0.104
19	666	537	1358	21.3	0.085
20	663	642	1400	21.4	0.027
21	627	714	1401	21.6	0.000
22	741	776	1404	21.9	0.000
23	924	722	1407	21.7	0.029
24	868	664	1408	21.5	0.000
25	715	669	1410	21.5	0.000
26	676	738	1411	21.4	0.062
27	665	807	1412	21.8	0.000
28	658	880	1413	21.9	0.000
29	570	734	1415	21.9	0.000
30	662	612	1417	22.0	0.000
31	748	599	1418	21.9	0.000
32	853	613	1419	21.5	0.011
33	641	682	1420	21.5	0.000
34	998	690	1421	21.5	0.000

TABLE 6-4. Continued

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
35	776	535	1423	21.6	0.000
36	649	504	1424	21.6	0.000
37	575	548	1425	21.6	0.004
38	527	713	1427	21.6	0.001
39	572	868	1428	22.0	0.000
40	872	1165	1430	22.0	0.000
41	1008	1238	1431	21.9	0.000
42	1045	1225	1432	21.7	0.000
43	1033	1170	1433	21.5	0.000
44	1065	1072	1435	21.5	0.000
45	1198	1061	1437	21.5	0.000
46	1255	1090	1438	21.5	0.005
47	1347	1131	1439	21.6	0.000
48	1336	1103	1440	21.6	0.000
49	1042	810	1442	21.6	0.000
50	897	647	1443	21.7	0.000
51	699	563	1444	21.7	0.000
52	689	706	1447	21.5	0.000
53	809	868	1448	21.9	0.000
54	944	1021	1449	22.0	0.000
55	1097	1230	1451	21.6	0.000
56	1105	1289	1452	21.6	0.000
57	940	1200	1454	21.6	0.000
58	822	1042	1455	21.8	0.000
59	710	833	1456	21.9	0.000
60	593	723	1457	22.0	0.000
61	509	586	1458	22.0	0.000
62	563	367	1501	21.5	0.000
63	408	454	1503	21.7	0.000
64	656	967	1507	21.9	0.000
65	800	1127	1508	22.0	0.000
66	1035	1283	1510	21.9	0.000
67	953	1112	1512	21.8	0.000
68	936	1033	1513	21.9	0.000

TABLE 6-4. Continued

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
69	942	969	1514	21.7	0.000
70	1037	931	1516	21.8	0.000
71	1100	939	1517	21.6	0.000
72	1421	1091	1520	21.7	0.000

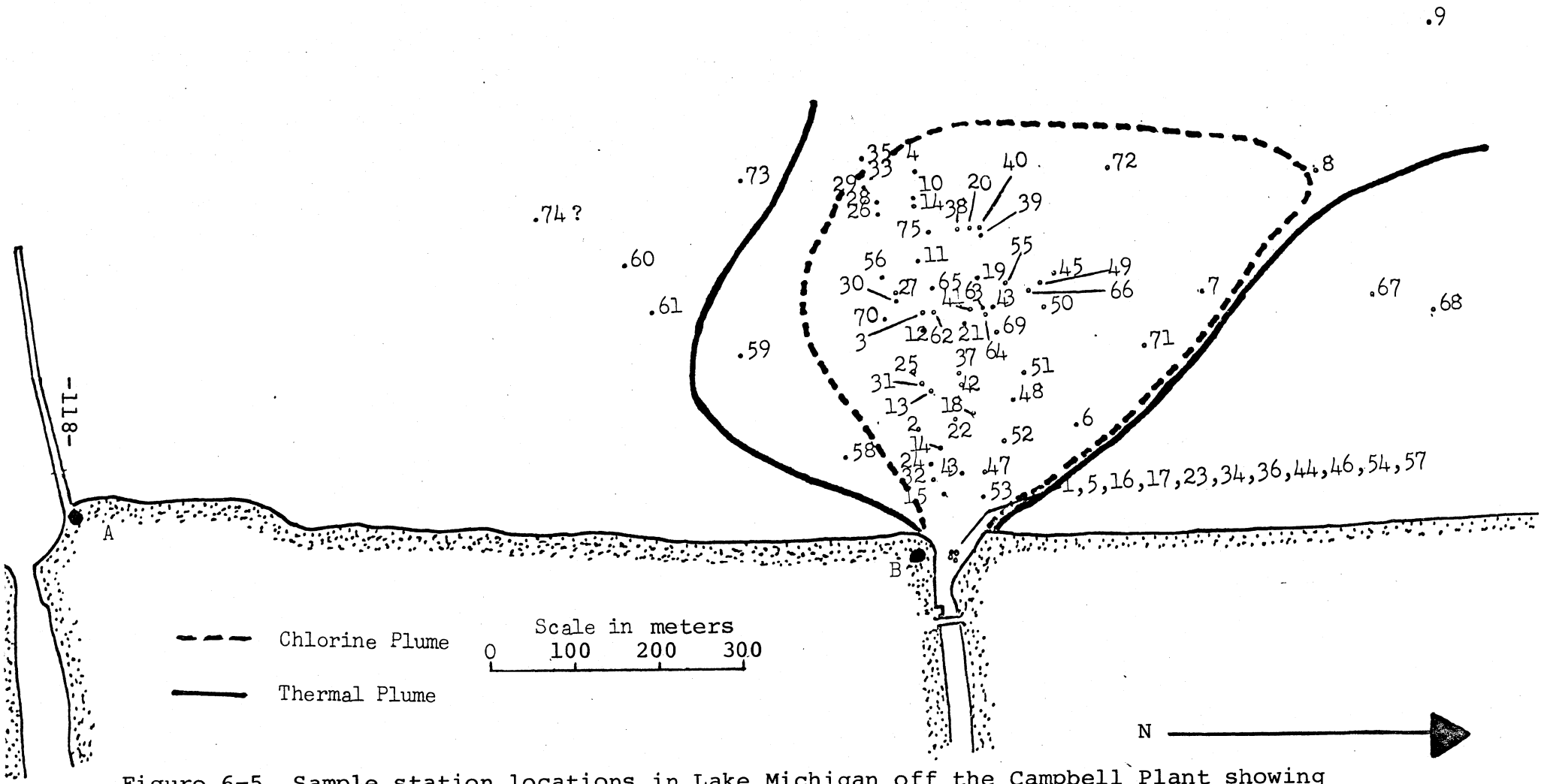

Figure 6-5 Sample station locations in Lake Michigan off the Campbell Plant showing estimated outer bounds of the thermal and chlorine plumes, Aug. 8, 1974. Station numbers correspond to sample numbers in Table 6-5.

TABLE 6-5. DATA FROM CHLORINATION PLUME SURVEY CAMPBELL PLANT, AUGUST 8, 1974

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
1	1244	498	1148	26.0	0.000
2			1150	26.0	0.000
3			1151	25.5	0.000
4			1152	24.0	0.000
5			1222	26.0	0.055
6			1223	26.0	0.047
7			1224	26.0	0.013
8			1225	25.0	0.000
9	2025	1011	1226	25.0	0.000
10	1239	486	1235	24.5	0.007
11			1237	25.0	0.017
12			1238	25.0	0.042
13			1239	26.0	0.061
14			1240	25.5	0.100
15			1241	26.0	0.117
16			1242	26.5	0.214
17			1243	25.5	0.080
18			1244	23.5	0.012
19	1312	379	1245	24.5	0.000
20	1306	450	1247	24.0	0.000
21			1249	25.0	0.007
22			1250	24.5	0.007
23			1252	26.5	0.061
24			1259	25.5	0.055
25			1300	24.5	0.000
26	1204	473	1300	22.7	0.000
27			1301	23.0	0.000
28	1196	488	1302	23.0	0.000
29	1193	516	1307	22.5	0.000
30			1308	22.5	0.000
31			1309	23.5	0.000
32			1310	24.0	0.000
33	1196	533	1310	23.1	0.000
34			1313	26.5	0.001

TABLE 6-5. Continued

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
35	1187	536	1313	23.0	0.000
36			1315	26.0	0.004
37			1316	26.0	0.000
38	1305	448	1317	25.5	0.017
39	1324	448	1318	24.9	0.000
40	1315	451	1323	25.0	0.000
41			1324	26.0	0.000
42			1325	25.0	0.000
43			1326	26.0	0.000
44			1327	26.5	0.000
45	1407	414	1335	24.1	0.000
46			1337	25.5	0.000
47			1338	24.5	0.000
48			1339	24.0	0.000
49	1396	393	1340	23.5	0.000
50	1394	367	1344	24.0	0.000
51			1345	24.5	0.000
52			1346	24.5	0.000
53			1347	26.0	0.000
54			1348	26.5	0.000
55	1347	369	1349	23.5	0.000
56	1185	371	1350	24.5	0.003
57			1352	26.5	0.000
58			1353	26.0	0.000
59			1354	26.0	0.000
60	842	588	1355	25.0	0.000
61	846	522	1358	23.0	0.000
62	1238	326	1410	24.0	0.000
63	1310	337	1411	25.1	0.047
64	1302	340	1414	24.8	0.040
65	1253	357	1421	25.4	0.046
66	1387	382	1422	25.4	0.005
67	1853	705	1425	24.5	0.000
68	1931	770	1426	24.8	0.000

TABLE 6-5. Continued

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
69	1320	312	1443	24.4	0.006
70	1178	317	1444	25.1	0.000
71	1517	399	1457	24.9	0.000
72	1524	575	1503	23.9	0.000
73	1029	593	1506	24.2	0.000
74	751	724	1508	24.7	0.014?
75	1264	438	1516	24.3	0.000

minutes ranging from a maximum of 66 minutes in September to a minimum of 37 minutes in November. The maximum chlorine concentration observed on the Oak Creek surveys was 0.376 mg/l (Tables 6-6 to 6-9). The shape and size of the chlorine plume was dependent on wind conditions at the time of sampling. In May and September both the chlorine and thermal plumes moved offshore, in response to winds with a southerly and westerly component (Figs. 6-6 and 6-7). On the November and December surveys the plume extended southward close to the shore (Figs. 6-8 and 6-9). The area encompassed by the plume was greater at times when it was blown offshore. The estimated plume areas observed on the November and December surveys were 0.09 and 0.12 km^2 respectively as compared to areas of 0.19 and 0.27 km^2 measured in May and September.

Discussion

The sampling scheme employed on the nine plume surveys conducted on Lake Michigan was such that measurements were made in the effluent area until chlorine could no longer be detected. It is reasonable to assume, therefore, that chlorine degredation was complete within the time span and plume areas defined for each survey. It is apparent that the mass of chlorinated water discharged from the plant did not drift off into the lake as a cloud, but was dissipated well within the bounds of the observed thermal plume.

The rate of chlorine dissipation in Lake Michigan waters

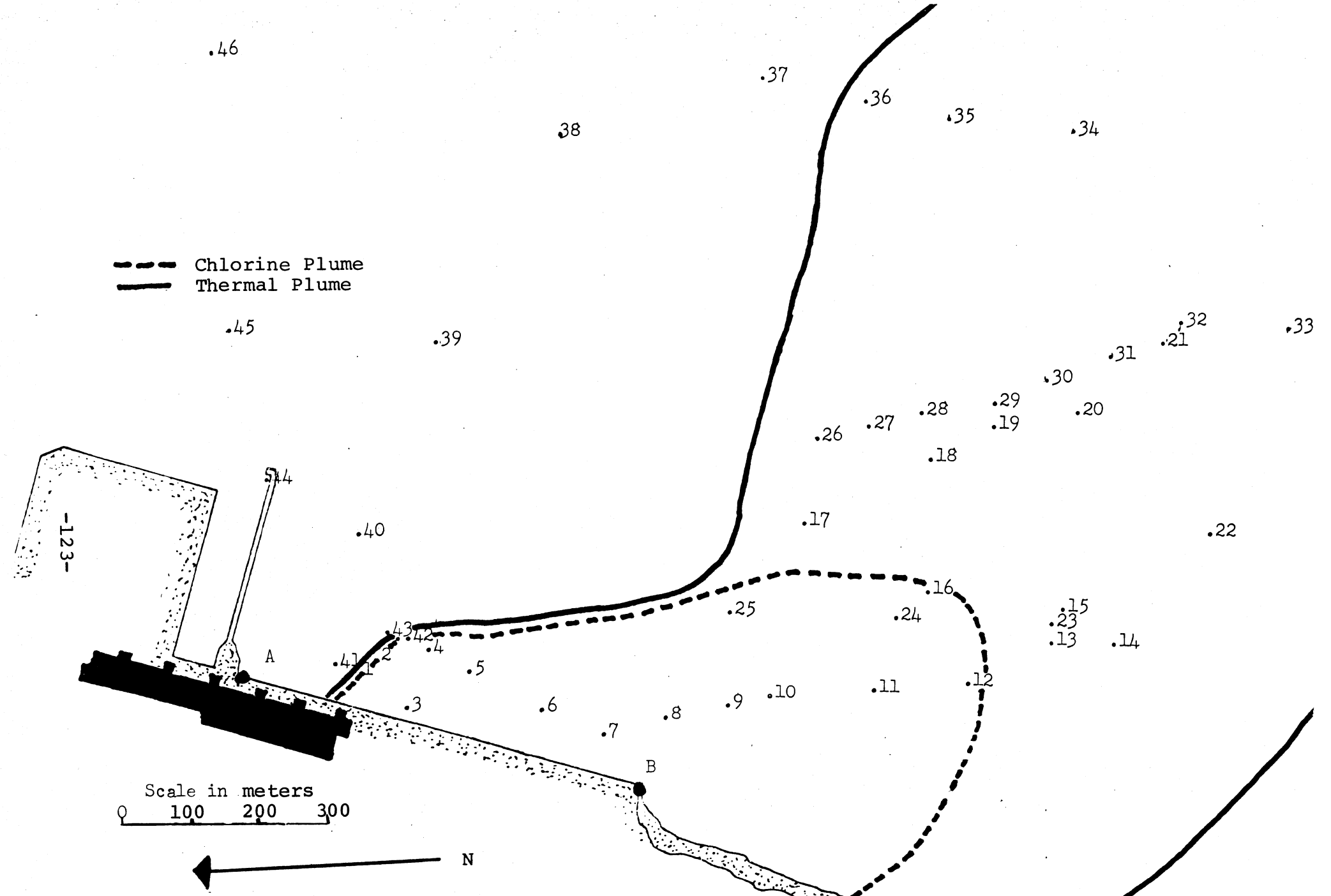

Figure 6-6 Sample station locations in Lake Michigan off the Oak Creek Plant showing estimated outer bounds of the thermal and chlorine plumes, May 28, 1974. Station numbers correspond to sample numbers in Table 6-6.

TABLE 6-6. DATA FROM CHLORINATION PLUME SURVEY OAK CREEK PLANT - MAY 28, 1974

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
1	184	510	1100	14.0	0.000
2	216	443	1102	14.0	0.016
3	248	417	1104	10.3	0.089
4	268	393	1106	10.7	0.075
5	356	315	1108	11.5	0.031
6	494	185	1110	12.0	0.080
7	605	115	1113	12.8	0.085
8	719	120	1116	12.9	0.065
9	824	197	1118	13.0	0.050
10	904	267	1120	11.3	0.000
11	1064	415	1123	12.0	0.000
12	1224	571	1126	11.7	0.000
13	1383	745	1129	11.3	0.000
14	1477	834	1132	11.3	0.000
15	1394	767	1135	9.5	0.000
16	1180	589	1138	10.0	0.000
17	976	512	1141	11.5	0.000
18	1219	729	1144	9.8	0.000
19	1329	835	1147	9.6	0.000
20	1481	961	1150	9.5	0.000
21	1656	1150	1153	9.7	0.000
22	1652	1044	1157	10.5	0.000
23	1366	736	1200	10.8	0.000
24	1126	524	1203	10.9	0.110
25	840	329	1206	10.5	0.002
26	1038	641	1209	10.5	0.000
27	1128	702	1211	10.5	0.000
28	1229	778	1213	10.8	0.000
29	1341	865	1215	10.5	0.000
30	1438	953	1217	10.0	0.000
31	1564	1068	1220	10.3	0.000
32	1680	1178	1222	10.3	0.000
33	1859	1329	1224	10.5	0.000
34	1665	1306	1226	10.6	0.000

TABLE 6-6. Continued

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
35	1509	1225	1228	10.8	0.000
36	1420	1199	1230	10.8	0.000
37	1318	1192	1232	10.9	0.000
38	1041	1092	1235	11.0	0.000
39	636	815	1237	11.1	0.000
40	299	642	1239	11.0	0.000
41	156	557	1241	10.3	0.000
42	215	441	1243	10.3	0.000
43	258	498	1245	11.0	0.000
44	321	819	1248	10.5	0.000
45	569	1041	1250	10.3	0.000
46	1028	1422	1253	10.5	0.000
47	1523	1839	1255	10.0	0.000
48	1838	2112	1258	9.6	0.000
49	2134	2372	100	9.6	0.000
50	2300	2511	102	8.9	0.000
51	2552	2744	104	9.5	0.000
52	2851	3029	106	10.0	0.000
53	3027	3190	108	10.0	0.000

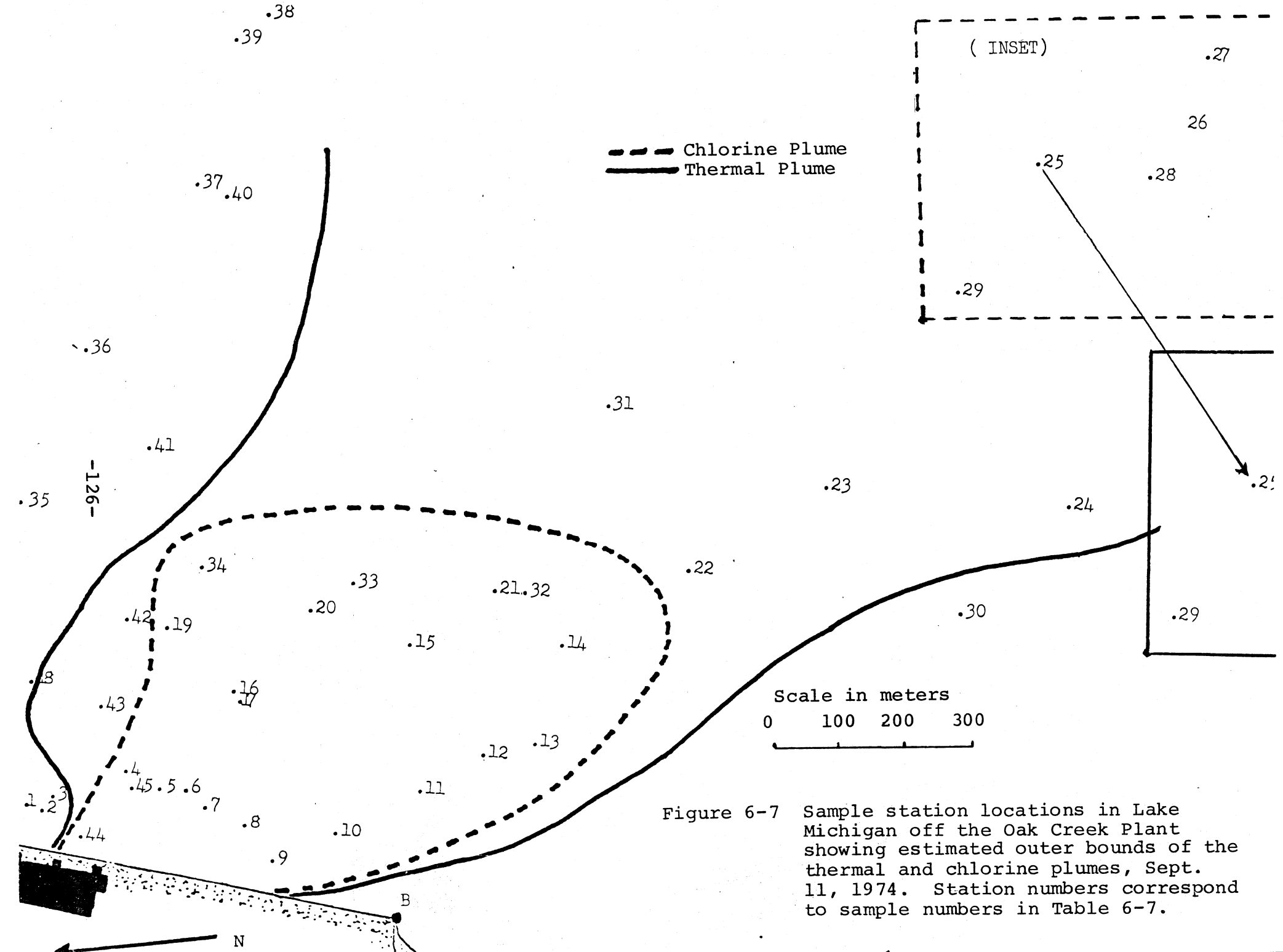

Figure 6-7 Sample station locations in Lake Michigan off the Oak Creek Plant showing estimated outer bounds of the thermal and chlorine plumes, Sept. 11, 1974. Station numbers correspond to sample numbers in Table 6-7.

TABLE 6-7. DATA FROM CHLORINATION PLUME SURVEY OAK CREEK PLANT - SEPTEMBER 11, 1974

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
1	115	584	1051	15.0	0.000
2	117	585	1053	15.0	0.000
3	120	567	1056	16.0	0.000
4	283	476	1109	17.0	0.011
5	277	390	1111	20.0	0.000
6	330	335	1114	20.0	0.078
7	367	310	1116	20.0	0.354
8	429	256	1118	19.5	0.292
9	496	210	1120	18.0	0.195
10	586	170	1122	17.0	0.160
11	723	194	1125	17.0	0.084
12	830	283	1127	18.0	0.044
13	913	346	1129	17.0	0.021
14	980	504	1133	17.0	0.010
15	776	444	1135	17.0	0.002
16	493	447	1137	17.0	0.000
17	254	116	1139	16.5	0.000
18	278	686	1141	17.0	0.000
19	466	596	1143	15.5	0.000
20	659	528	1145	17.0	0.000
21	935	558	1147	17.0	0.000
22	1219	729	1149	17.3	0.000
23	1469	969	1151	16.5	0.000
24	1812	1242	1153	16.5	0.000
25	2077	1486	1156	15.5	0.000
26	2423	1819	1159	15.0	0.000
27	2403	1822	1200	15.0	0.000
28	2241	1632	1201	15.0	0.000
29	1918	1289	1203	16.0	0.000
30	1591	991	1205	15.0	0.000
31	1240	910	1207	16.0	0.000
32	959	566	1210	17.0	0.000
33	738	557	1213	17.5	0.018
34	581	658	1215	17.0	0.005

TABLE 6-7. Continued

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
35	558	913	1216	16.0	0.000
36	844	1083	1218	17.0	0.000
37	1168	1284	1220	15.0	0.000
38	1466	1540	1223	16.0	0.000
39	1413	1503	1225	15.0	0.000
40	1158	1249	1227	16.0	0.000
41	713	882	1230	16.0	0.000
42	434	643	1232	15.0	0.000
43	310	571	1236	16.0	0.000
44	194	515	1238	16.5	0.000
45	233	438	1240	17.0	0.000

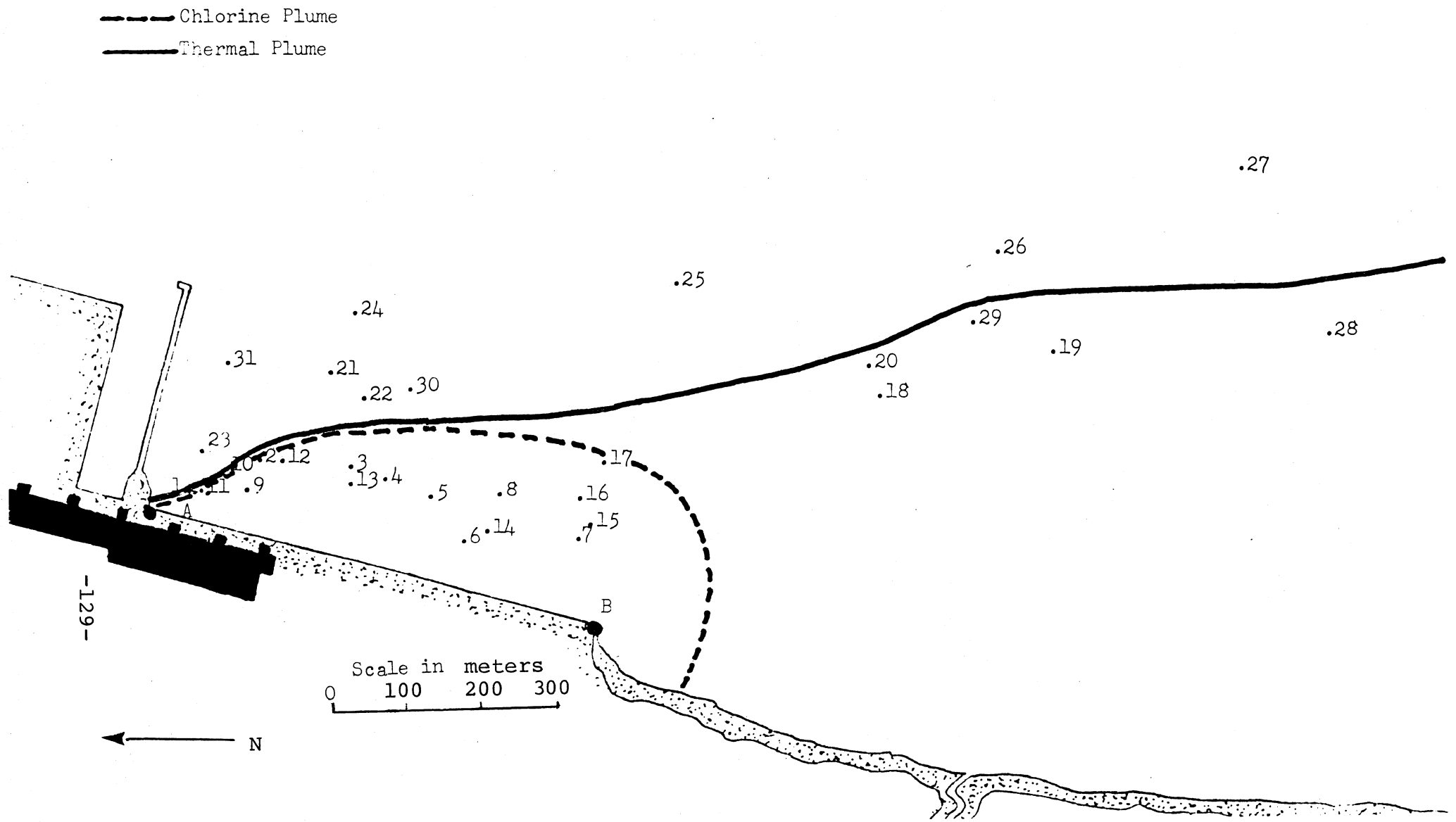

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Figure 6-8 Sample station locations in Lake Michigan off the Oak Creek Plant showing estimated outer bounds of the thermal and chlorine plumes, November 12, 1974. Station numbers correspond to sample numbers in Table 6-8.

TABLE 6-8. DATA FROM CHLORINATION PLUME SURVEY OAK CREEK PLANT - NOVEMBER 12, 1974

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
1	114	580	1051	10.0	0.000
2	160	496	1053	12.0	0.050
3	292	365	1057	13.5	0.020
4	360	299	1059	13.0	0.025
5	435	225	1101	12.5	0.016
6	528	157	1104	13.0	0.014
7	708	115	1106	14.0	0.077
8	566	213	1108	12.0	0.000
9	167	521	1112	11.0	0.000
10	114	566	1114	10.0	0.000
11	114	582	1116	12.0	0.000
12	179	476	1119	13.0	0.156
13	311	358	1122	12.5	0.151
14	546	121	1125	14.0	0.190
15	719	144	1127	13.5	0.069
16	709	176	1130	11.5	0.004
17	735	232	1134	11.5	0.000
18	1167	580	1138	11.0	0.000
19	1450	851	1140	11.0	0.000
20	1158	596	1142	10.5	0.000
21	706	357	1145	10.5	0.000
22	397	450	1147	10.0	0.000
23	263	589	1150	10.0	0.000
24	452	550	1152	10.0	0.000
25	904	523	1155	10.0	0.000
26	1388	855	1158	10.0	0.000
27	1793	1242	1201	10.0	0.000
28	1860	1240	1205	11.0	0.000
29	1330	764	1209	10.5	0.000
30	459	412	1215	10.0	0.000
31	256	638	1218	10.0	0.000

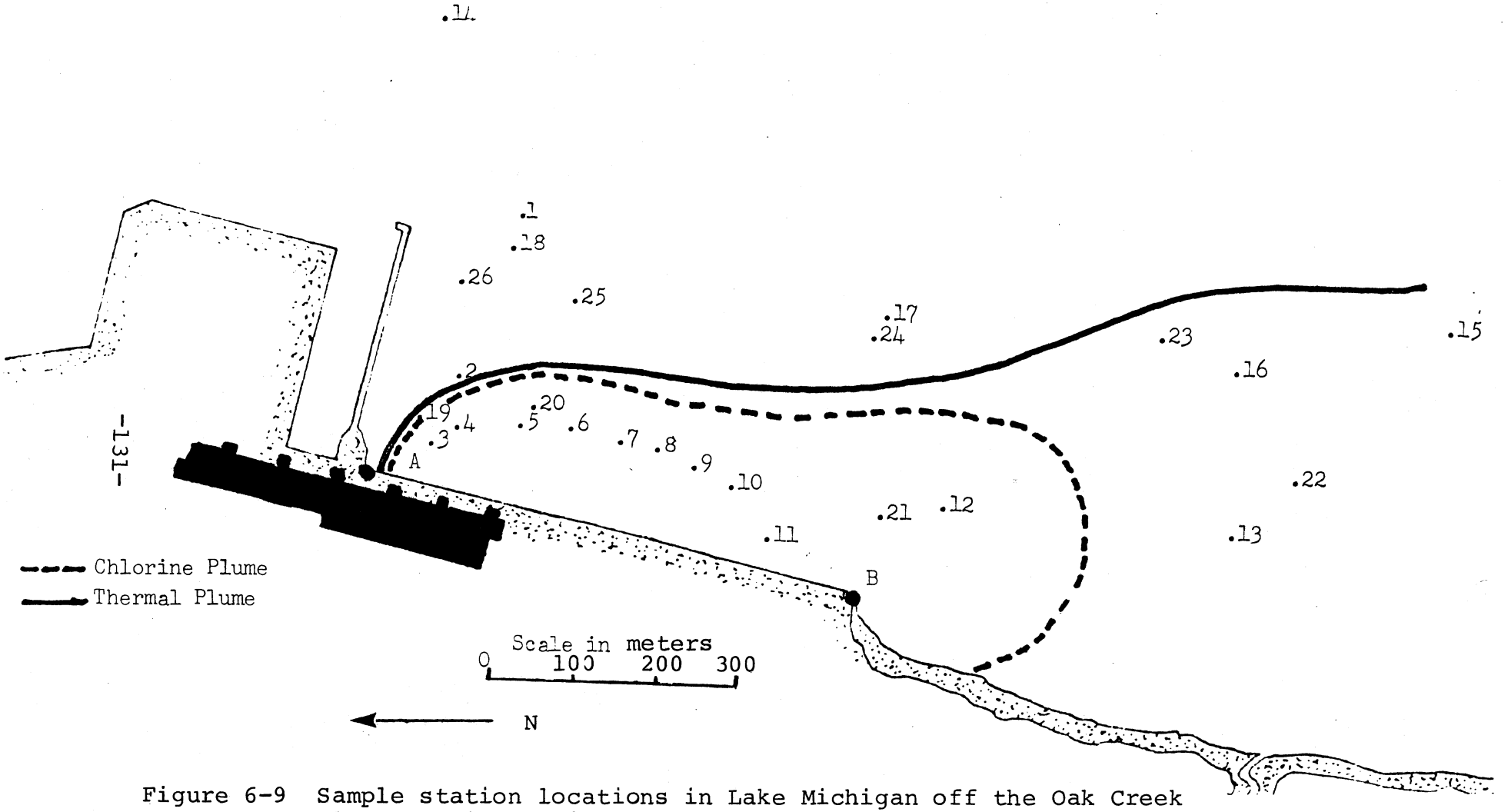

Figure 6-9 Sample station locations in Lake Michigan off the Oak Creek Plant showing estimated outer bounds of the thermal and chlorine plumes, December 10, 1974. Station numbers correspond to sample numbers in Table 6-9.

TABLE 6-9. DATA FROM CHLORINATION PLUME SURVEY OAK CREEK PLANT - DECEMBER 10, 1974

Sample	Mini Ranger Yards A	Mini Ranger Yards B	Time	Temperature °C	Chlorine mg/l
1	397	682	1121	5.0	0.000
2	175	603	1123	5.0	0.000
3	114	596	1126	7.0	0.001
4	114	570	1128	7.0	0.012
5	185	482	1129	8.0	0.112
6	264	397	1131	8.5	0.240
7	328	338	1133	8.0	0.157
8	397	264	1136	8.0	0.345
9	450	213	1138	8.0	0.187
10	502	167	1140	8.5	0.376
11	595	115	1143	9.5	0.343
12	821	183	1145	9.5	0.069
13	1231	556	1148	9.0	0.000
14	1602	964	1150	9.0	0.000
15	1549	938	1154	7.0	0.000
16	1245	641	1157	5.0	0.000
17	765	378	1200	4.5	0.000
18	341	662	1203	4.5	0.000
19	122	625	1208	5.0	0.000
20	192	467	1213	6.5	0.000
21	738	113	1216	8.2	0.100
22	1312	655	1220	8.5	0.000
23	1130	566	1223	5.5	0.000
24	739	354	1226	4.7	0.000
25	366	537	1229	4.0	0.000
26	268	682	1232	4.5	0.000

observed in the field is similar to the duration of the "rapid phase" of degradation determined under laboratory conditions (Section 7 this report). The extended "slow phase" seen in the laboratory experiments (Section 7 this report) was not observed under actual plume conditions. The latter observation demonstrates the importance of dilution in determining rates of chlorine dissipation under field conditions.

7. PERSISTENCE STUDY

Materials and Methods

Untreated Lake Michigan water was obtained from the City of Milwaukee Linnwood Avenue Filtration Plant. Testing was done over four seasons; winter (January, 1975), spring (March, 1976), summer (July, 1975) and fall (October, 1976). The tests were performed in 4 1 beakers under 4 different light and 3 different temperature regimes. To achieve four different light intensities the beakers were either left uncovered (light = L), covered with one layer of cheesecloth (cheesecloth 1 = C1), covered with several layers of cheesecloth (cheesecloth 2 = C2), or covered completely with an opaque plastic (dark = D). Temperature control was achieved by placing the beakers in water baths at 10, 15 and 20C. Each bath contained four beakers, one for each light regime.

The baths and beakers were placed on the roof of the Great Lakes Research Facility in Milwaukee, Wisconsin, under natural light conditions and allowed to equilibrate overnight. Chlorine demand measurements were determined according to Standard Methods, 1976. The following morning sodium hypochlorite (NaOCl) was added to the beakers to produce a calculated TRC of 0.5 mg/l. The first sample from each beaker was taken 5 minutes after the introduction of the chlorine. This sample was considered as time zero. Subsequent samples were taken at about 1, 2, 4, 8, and 24 hours from time zero. After the first day, samples were taken once or twice per day until the chlorine concentration reached zero. Total residual chlorine

concentrations were measured amperometrically (Section 2 this report) and free chlorine concentrations were determined using the DPD method (Standard Methods, 1976). Beaker temperatures and weather conditions were recorded at each sampling period.

Results and Discussion

Both light intensity and water temperature affected chlorine decay rates. Chlorine exposed to ambient seasonal light levels (L) disappeared more rapidly than under dark (D) conditions (Figs. 7-1 to 7-4). Chlorine decay rates under conditions allowing partial light penetration (C1 and C2) were usually intermediate to the rates under the light and dark conditions (Figs. 7-1 to 7-4).

The decay curves typically exhibited an initial "rapid" phase followed by a "slow" phase. The "rapid" decline phase typically lasted about 1-2 hours, while detectable levels of chlorine were observed for several days during the "slow" decay phase. Similar curves for the decline of chlorine have been described both in seawater (Eppley et al. 1976) and in freshwater (Baker, 1970; Bender et al. 1975).

Chlorine demand values for the water used in these experiments averaged 0.1 mg/l. Tests indicated that 70% of the demand was satisfied within 5 minutes. Since the initial sample for TRC analysis at time zero was taken 5 minutes after the chlorine had been introduced about 30% of the demand remained unsatisfied. It seems reasonable to assume that the "rapid" decay phase of the curve reflects both the chemical

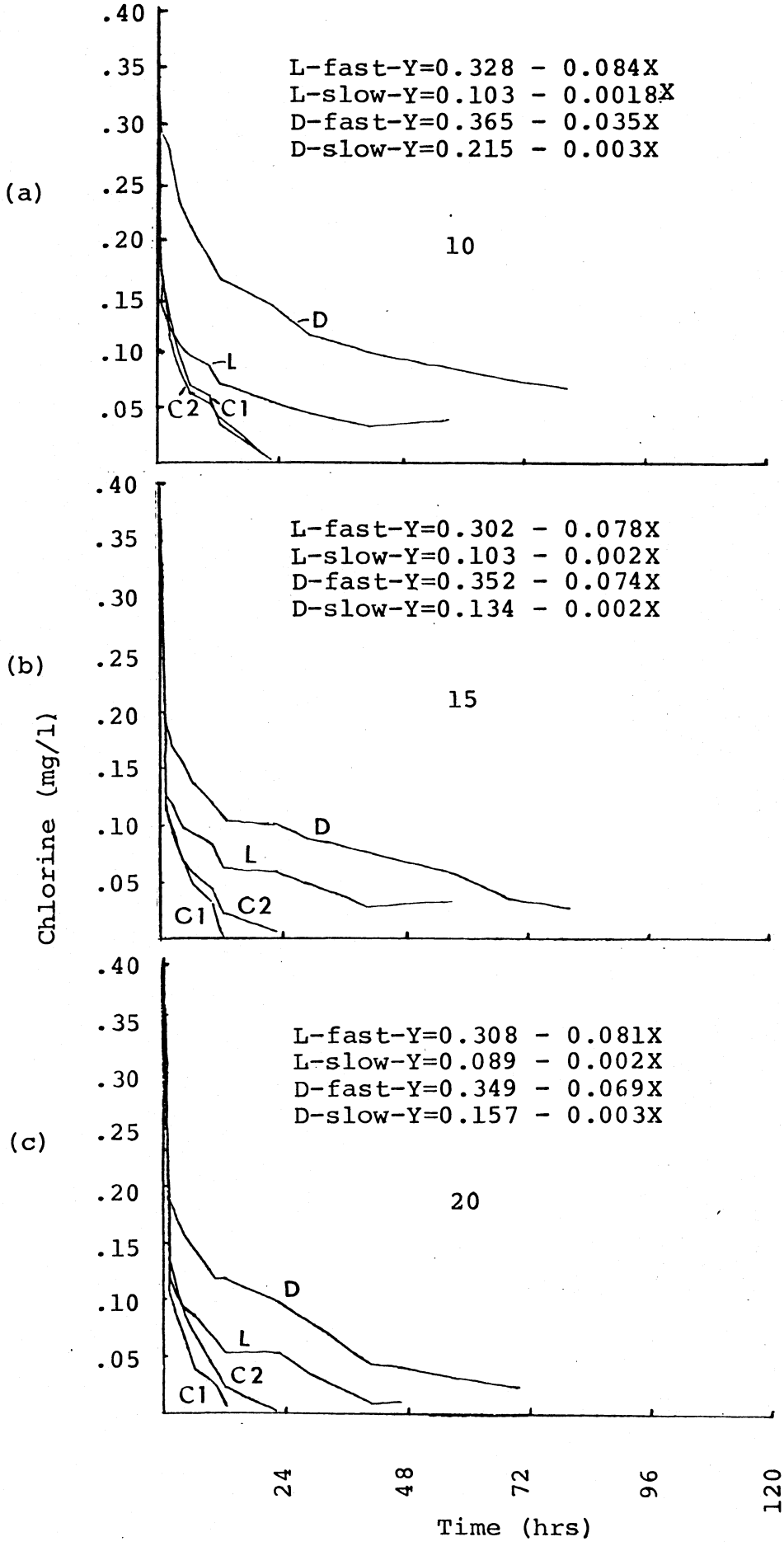

Fig. 7-1 Chlorine decay rates measured during the winter under four light conditions (L, C1, C2, D) at three temperatures. Regression equations are shown for the "fast" and "slow" portions of the decay curves.

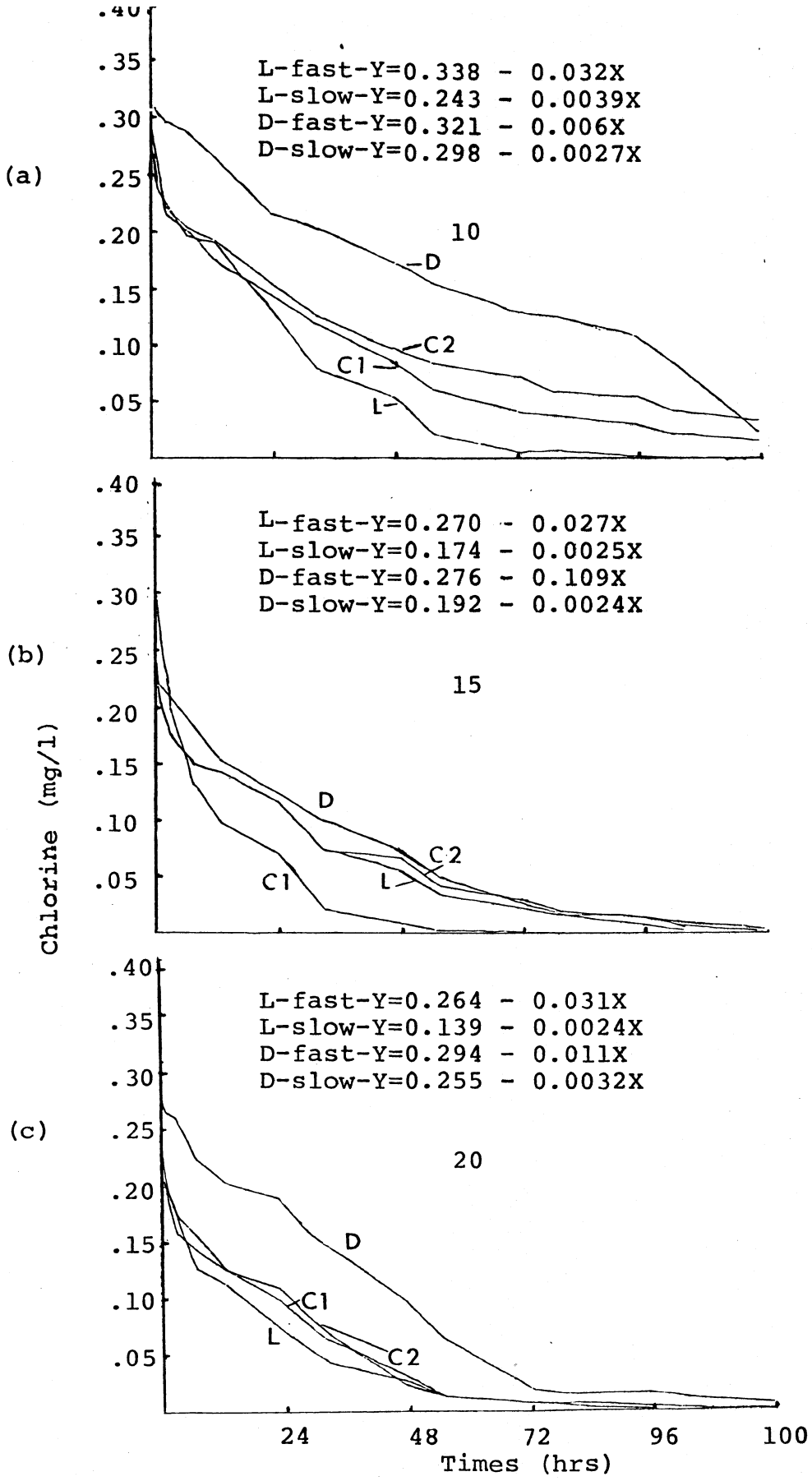

Figure 7-2. Chlorine decay rates measured during the spring under four light conditions (L, C1, C2, D) at three temperatures. Regression equations are shown for the "fast" and "slow" portions of the decay curves.

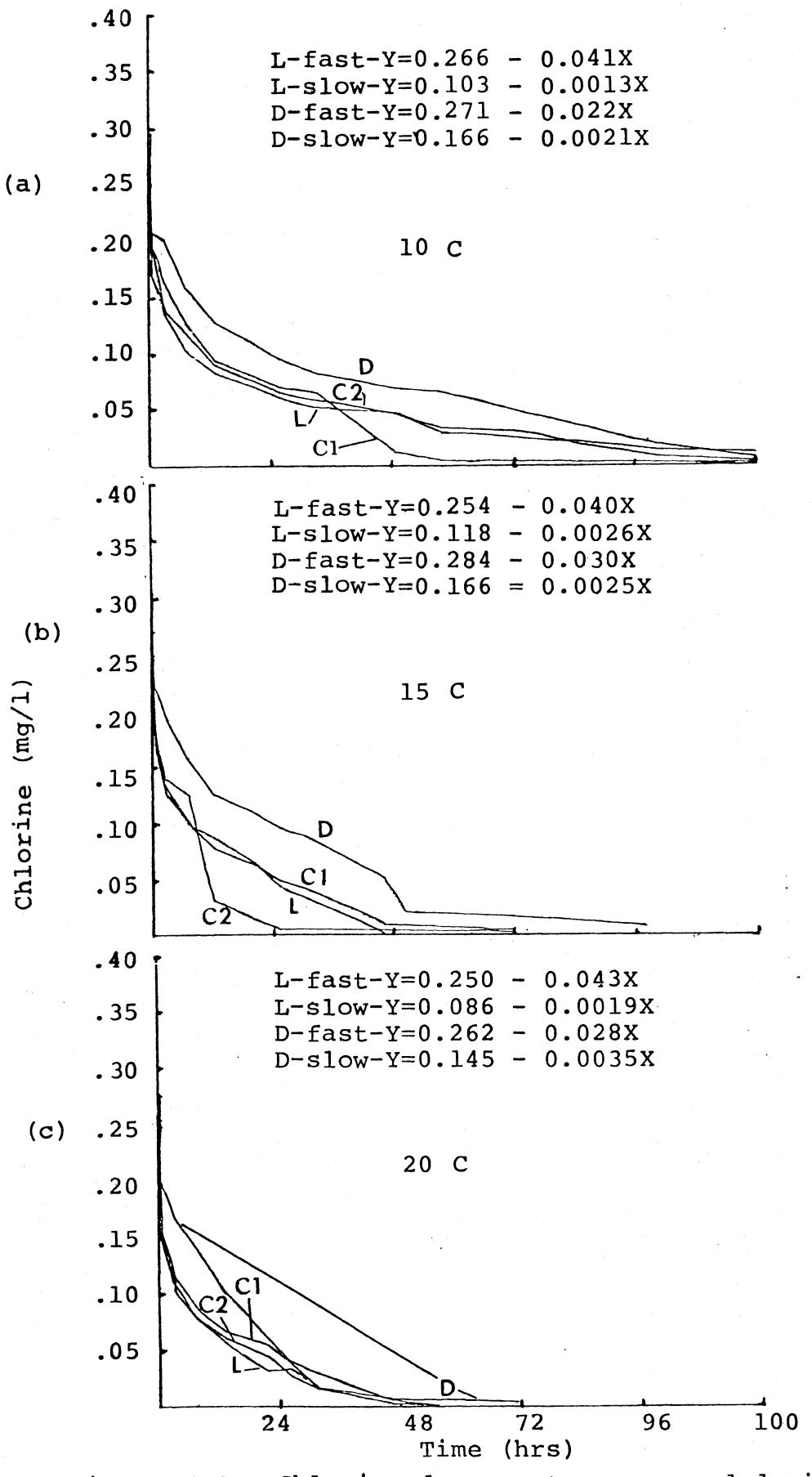

Figure 7-3. Chlorine decay rates measured during the summer under four light conditions (L, C1, C2, D) at three temperatures. Regression equations are shown for the "fast" and "slow" portions of the decay curves.

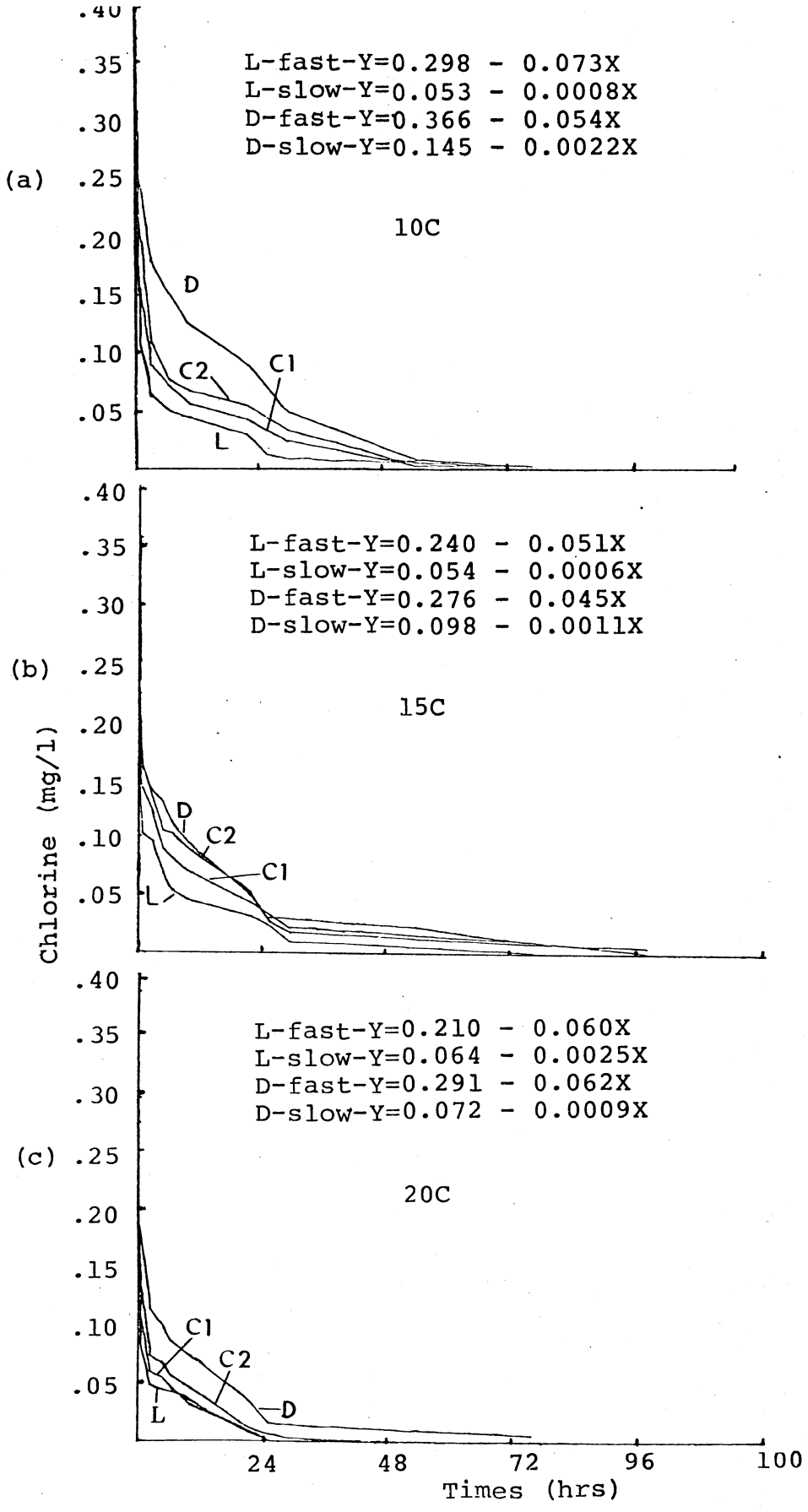

Figure 7-4. Chlorine decay rates measured during the fall under four light conditions (L, C1, C2, D) at three tempe tures. Regression equations are shown for the "fas and "slow" portions of the decay curves.

decay of the chlorine and the quenching of the demand. Johnson (1976) states that "the initial rapid reduction of chlorine on demand is due to the easily ozidized reducing agents present." The "slow" decay phase observed in these tests probably represents the physical-chemical decay of the chlorine after the demand has been essentially satisfied. Regression equations were calculated by season for the "fast" and "slow" phases of the curves for the light and dark samples (Figs. 7-1 to 7-4).

Although calculated chlorine half life times varied greatly under both light (14-1516 min) and dark (109-3245 min) conditions several trends did emerge (Table 7-1). It was found, that at any given temperature or season the samples exposed to light had a shorter chlorine half life than did dark samples. The effect of temperature on decay rates was less clear. Under dark conditions the seasonal eighth life times consistently showed an inverse relationship with temperature while half life times usually showed a similar trend (Table 7-1 and Figs. 7-5 and 7-8). In the light samples however no relationship between temperature and chlorine half or eighth life times was apparent (Table 7-1 and Figs. 7-5 to 7-8).

If, for a given temperature the only factor influencing the chlorine decay rate was the amount of light to which the sample was exposed then the chlorine decay rates in the dark samples should be the same regardless of season. Table 7-1 clearly indicates that this is not the case and

Table 7-1 Half, quarter and eighth life times for chlorine in Lake Michigan water under various seasonal and temperature conditions

		SUMMER		SPRING		FALL		WINTER	
		light	dark	light	dark	light	dark	light	dark
10 C	half life (min)	160	588	1516	3245	58	206	67	578
	quarter life (min)	1321	3090	1945	6310	149	837	597	3778
	eighth life (min)	3293	5081	2974	7020	484	1853	1635	6636
15 C	half life (min)	173	543	928	1162	59	168	14	124
	quarter life (min)	1165	2088	2322	3033	346	913	743	1455
	eighth life (min)	1195	2899	3312	3872	1025	1481	913	3751
20 C	half life (min)	117	798	309	2077	36	109	20	117
	quarter life (min)	643	1043	1532	3143	121	543	572	1517
	eighth life (min)	1702	1840	2263	3228	841	1262	1476	1810

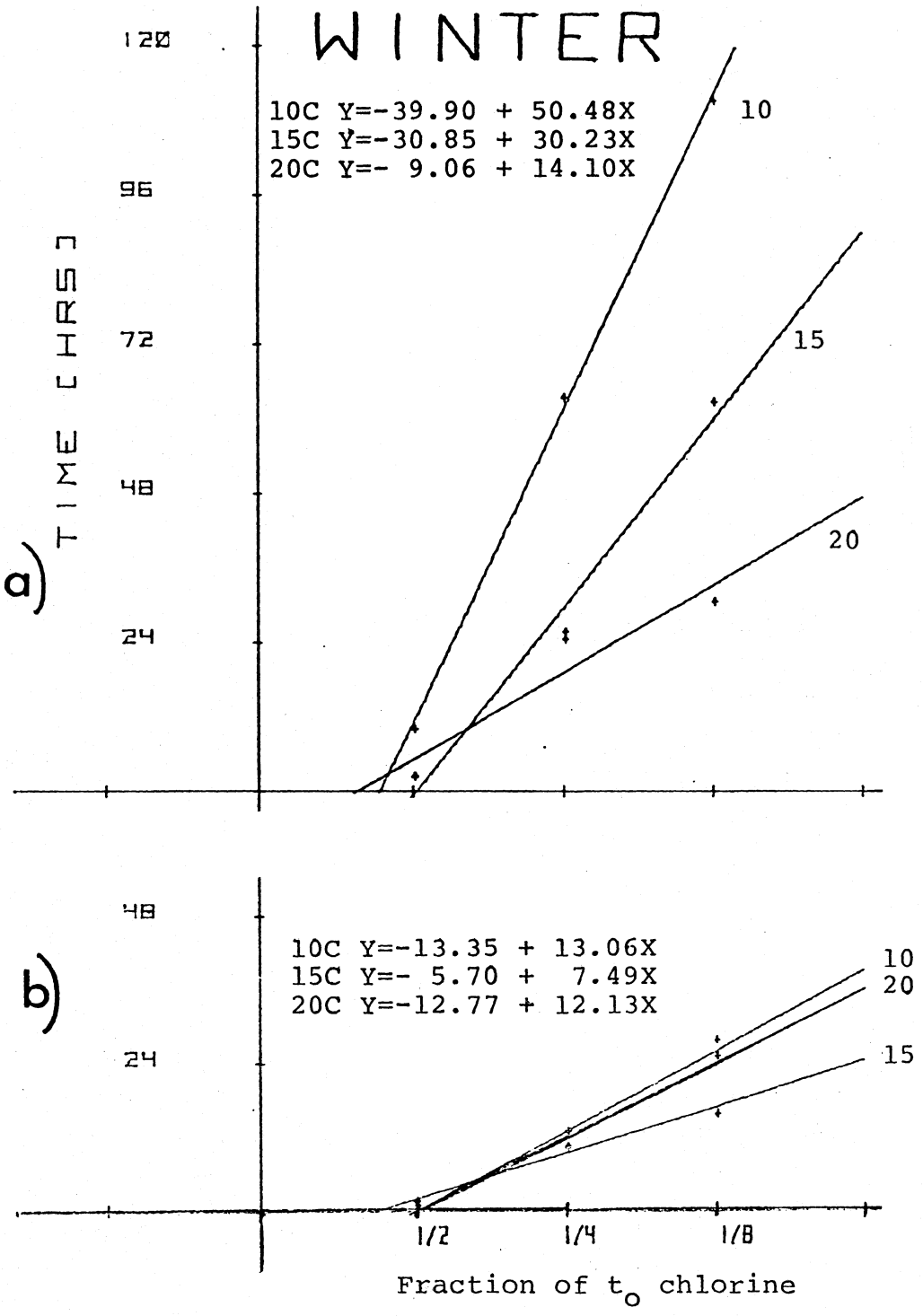

Figure 7-5. Half, quarter and eighth life times of chlorine under dark (a) and light (b) conditions during winter. Regression equations are shown for each of the curves.

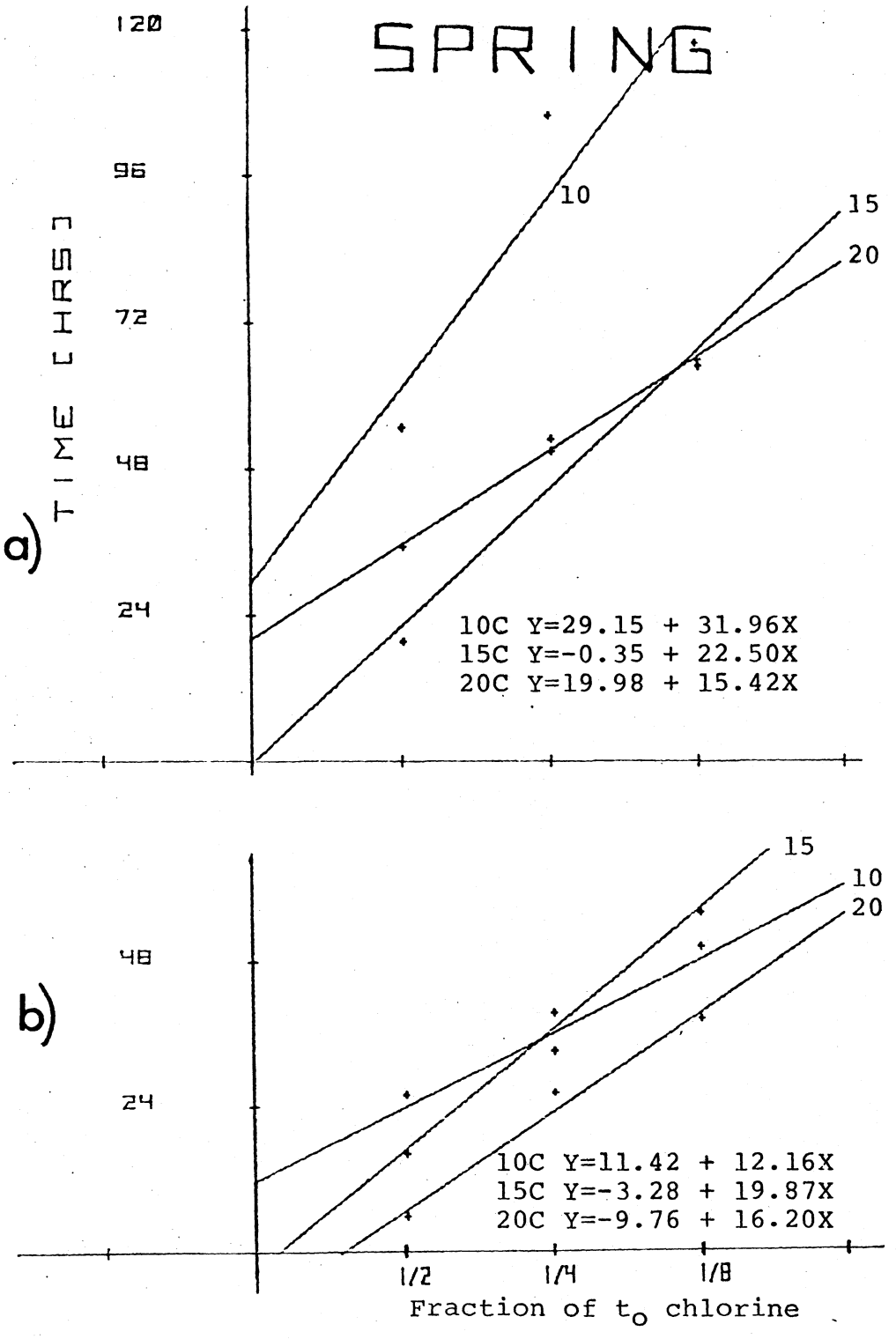

Figure 7-6. Half, quarter and eighth life times of chlorine under dark (a) and light (b) conditions during spring. Regression equations are shown for each of the curves.

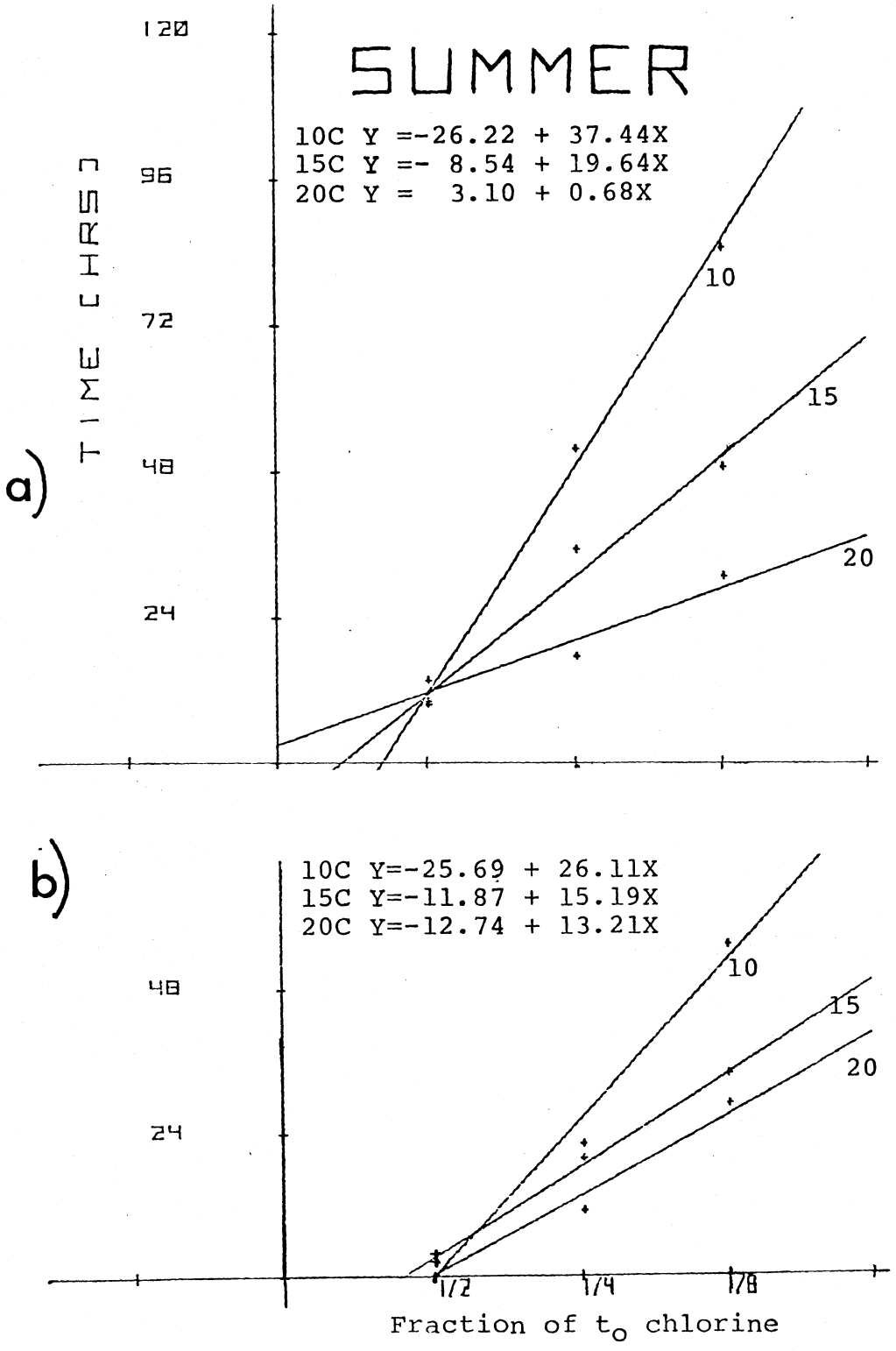

Figure 7-7. Half, quarter, and eighth life times of chlorine under dark (a) and light (b) conditions during summer. Regression equations are shown for each of the curves.

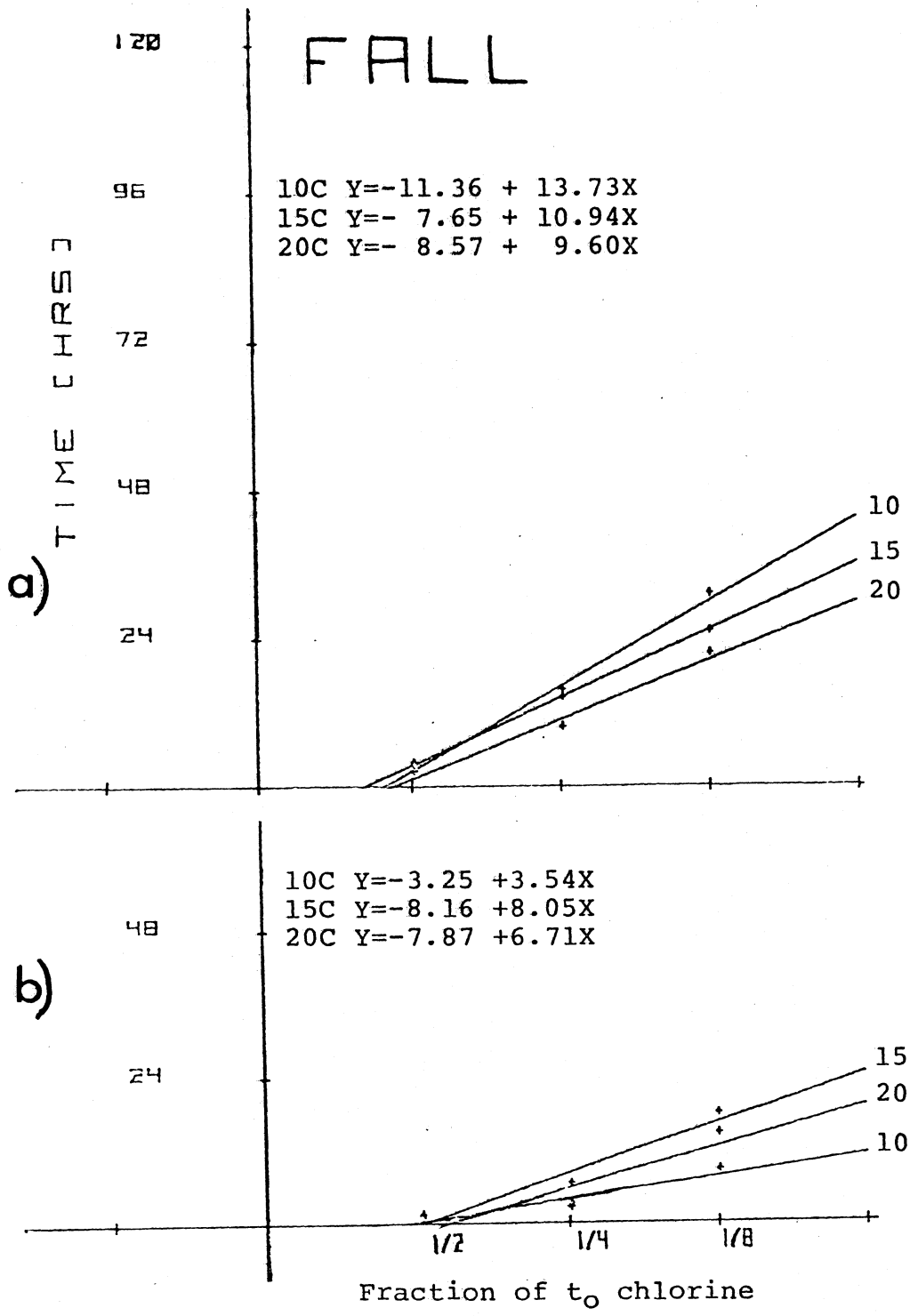

Figure 7-8. Half, quarter and eighth life times of chlorine under dark (a) and light (b) conditions during fall. Regression equations are shown for each of the curves.

that additional factors must be affecting the chlorine decay rates. Reducing chemicals and ozidable organics affect both chlorine demand and decay rates (Johnson 1976).

A general similiarity was found between the summer and fall curves (Fig. 7-3 and 7-4) and between the winter and spring curves (Fig. 7-1 and 7-2). Lake Michigan is vertically mixed from December to June and chemical conditions in the lake are generally similar during January and March, the later encompassing our winter and spring experiments. Similarities in chemical constituents are also found during the summer and fall when the lake is stratified which could account for similar decay curves observed in July and October. Thus, the seasonal similarities and dissimilarities in chlorine decay curves are most probably the result of seasonal changes in the water chemistry of Lake Michigan. This view is consistent with that of Johnson, 1976, who indicates that variations in reducing chemicals and oxidizable organics can affect both chlorine demand and decay rates.

The decay rate of chlorine is also affected by the chlorine species present. Chloramines are more persistent, while free chlorine solutions are more labile (Baker and Cole, 1974; Johnson 1976). The species composition was only determined during the 1976 fall studies. At the start of the experiment free chlorine averaged 72% of the TRC. This value decreased to only 35% after 84 minutes. At 440 minutes no free chlorine remained in any of the samples. Much of

the initial rapid decline observed in the fall experiments was a direct result in the loss of free chlorine.

Several workers (Baker and Cole, 1974; Snoeyink and Markus 1974) have indicated that free chlorine is strongly affected by UV catalized reactions. This was confirmed in our fall studies. At time zero the 10C samples had about 80% free chlorine under each of the 4 light conditions. After 85 minutes the percentages were 8, 29, 54, and 67% for the L, C1, C2, and D samples respectively. Similar results were found at 15C and 20C. Thus a clear trend emerged with the percent of free chlorine progressively increasing as more light was excluded from the sample.

Shipboard Persistance Study

To determine how well our chlorine decay studies under controlled conditions compared with chlorine decay rates under field conditions samples of chlorinated discharge water were collected at several of the power plants during the field surveys (see Section 6). These samples were placed on the deck of the ship in open 60 l polyethylene barrels. Initial TRC values were determined for each sample after which time the TRC levels were periodically measured until the chlorine had disappeared.

The chlorine measurements and half life times are shown in Table 7-2. Because of the small amount of data collected statistical comparisons were not attempted. However, except for the December sample at Oak Creek, the half life times were surprisingly similar (159 - 250 min) Table 7-2). The longer

Table 7-2. Shipboard Chlorine Persistence Study

Station	Date	Sample time	Elapsed time (min.)	Residual Cl_2 mg/l
Mitchell	8-6-74	0908	0	0.041
		0924	16	0.028
Chlorine		0943	35	0.027
1/2 life = 238 min.		1005	63	0.032
1/4 life = 313 min.		1029	87	0.030
1/8 life = 350 min.		1131	149	0.034
		1213	191	0.027
		1417	315	0.010
		1613	431	0.010
		1646	464	0.000
D.C. Cook	8-7-74	1359	0	0.071
		1459	60	0.053
Chlorine		1606	127	0.040
1/2 life = 159 min.		1925	199	0.032
1/4 life = 548 min.		2146	340	0.027
1/8 life = 756 min.		0815	971	0.000*
Oak Creek	5-28-74	1112	0	0.043
		1130	18	0.045
Chlorine		1155	43	0.046
1/2 life = 217 min.		1218	66	0.046
1/4 life = 237 min.		1231	80	0.045
1/8 life = 430 min.		1247	96	0.041
		1305	114	0.031
		1442	211	0.025
		1505	234	0.011
		1555	284	0.006
		1632	321	0.007
		repeat		0.006
		1645	334	0.007
	5-29-74	1700	30 hrs.	0.000
Oak Creek	9-11-74	1123	0	0.184
		1203	40	0.150
Chlorine		1302	99	0.104
1/2 life = 250 min.		1338	135	0.116
1/4 life = 488 min.		1455	212	0.096
1/8 life = 1091 min.		1658	335	0.083
		2136	278	0.054
	9-12-74	1025	23 hrs.	0.012
		1605	29 hrs.	0.004
		1950	33 hrs.	0.002

*rained in barrel overnight

Table 7-2 . (continued) Shipboard Chlorine Persistence Study

Station	Date	Sample time	Elapsed time (min.)	Residual Cl_2 mg/l
Oak Creek	12-10-74	1141	0	0.202
		1235	54	0.205
Chlorine		2000	499	0.166
1/2 life = 1432 min.	12-11-74	1045	23 hrs.	0.145*
		1505	27 hrs.	0.118
1/4 life = 3162 min.		2015	32 hrs.	0.122
	12-12-74	1225	50 hrs.	0.078
1/8 life = ?		1630	54 hrs.	0.062
		1725	55 hrs.	0.050
	12-13-74	1037	71 hrs.	0.019
		1335	76 hrs.	0.008
		1535	78 hrs.	<0.001

*Barrel with layer of ice on top.

half life for the December Oak Creek sample may have been caused by the cold water and ice formation which slowed down the degradative processes.

Chlorine dissipation in Lake Michigan observed under field conditions (Section 6 this report) was similar to the "rapid" phase of decay described in the laboratory. The extended "slow" phase was not observed in the lake reflecting the importance of mixing and dilution on rates of chlorine dissipation. It is important to note that none of the laboratory or shipboard persistence studies involved dilution as a factor in determining decay rates. Dilution would have a two-fold effect on the decay of chlorine in a plume; first by physical dilution of the chlorine with the water surrounding the plume and secondly through the action of the chlorine demand in the dilution water.

8. MATHEMATICAL PLUME MODELING

by Dr. Kwang K. Lee

Introduction

A mathematical model has been developed to predict the distribution of residual chlorine in an effluent plume so that it may be possible to minimize objectional environmental effects and still achieve fouling control. Unlike a thermal effluent which is continuously discharged, chlorine is injected into the cooling water in discrete, interrupted, intervals to provide shock fouling treatment. Since the chlorination is applied intermittently rather than continuously, the residual chlorine plume behaves like a train of transient clouds meandering through the thermal plume.

In addition to advection, dilution, and diffusion within the thermal plume, chlorine also undergoes chemical degradation. The rates of reaction or decay depend on temperature, light intensity, and the chlorine demand of the receiving water (Section 7 this report). Consequently, the effect of thermal plume on the characteristics and distribution of residual chlorine must be coupled with rates of chlorine decay. Without adequate knowledge of thermal plume behavior, and the kinetics of chlorine decay proper assessment of the impact of chlorine residual on receiving waters cannot be determined.

Methods

In view of the interlocking action between a thermal plume and chlorine kinetics, and adequate model of the phenomenon may consist of three steps: namely, the design of a thermal plume model which is capable of including both the dynamic characteristics of thermal plume and the transient behavior of residual chlorine; secondly, a chlorine kinetic model and finally the incorporation of the two interrelated models.

The analysis of chlorine residuals in a thermal effluent discharged by an once-through cooling system must be based on valid mathematical, physical and chemical assumptions in modeling. Most of the available models of thermal discharges are based on the applications of classical theory on steady state turbulent jets. Policastro and Paddock, (1972) provided valid comparison between the available jet models and experimental data. The jet models are generally acceptable for a steady state, initial-momentum dominated jets into an infinitely large receiving water. However, the jet model cannot account for its own influence on the surroundings, and the physical boundary conditions of the receiving water cannot affect the entrainment conditions of the jet. Despite the mathematical simplicity of the jet models, they are not adequate nor compatible with the requirement of valid chlorine residual distribution characteristics. Therefore, a far-field thermal plume model would be the only acceptable alternative

which could incorporate factors such as the individual geographic location, the geometric boundary of the discharge plume, and the unsteady intermittent sequence of chlorination. When chlorine is added to condenser cooling water, it quickly reacts with oxidizable substances leaving a lesser residual concentration in the effluent. The discharge plume then carries the residual chlorine clouds into the receiving water body where further chemical degradation occurs. The rates of these chemical reactions are functions of pH, temperature, and light condition which must be incorporated in the model. The basic chlorine kinetics in the receiving water can be expressed in terms of a system of simultaneous differential equations.

In addition to chlorine kinetics, the concentration of chlorine residuals also dispersed due to advection and turbulent diffusion by the plume and receiving water must be considered. Consequently, the equations that govern chlorine kinetics and the transport and diffusion of chlorine residuals would have to be solved in conjunction with the far-field thermal plume model. The solution of thermal plume model will be derived first at each step and then be substituted into the chlorine reaction and transport models. The results of these models at each stage can later be stored on tape as well as displayed in graphic form by the computer.

Results

As was described previously, a far-field thermal plume model is of primary importance in the modeling framework. The mathematical formulation of the model is based on the principles of mass and energy conservation within each cell in the field which are subdivided into an appropriate number of unequal cells. Numerical routines are developed to tag and track the conservative properties of these cells at each time step. Other physical parameters such as velocities and temperature can then be derived. The model can provide an unsteady two dimensional integrated temperature and velocity field for a thermal plume at any given geometrical or field boundary condition. The primary application of the model is aimed at the Great Lakes environment, otherwise mathematical formulations are similar to that of the model of Eraslan, (1975). The model has been applied and tested in the case of the Oak Creek plant near Milwaukee. Appropriate boundary and physical conditions of that multi-unit plant are submitted into the program. Figure 8-1shows the initial start-up of thermal plume at the end of 1/2 hour.

In addition to the thermal plume model, numerical capability was also tested in the case of dispersion of clouds of residual chlorine in receiving waters. Figure 8-2 illustrates a pocket of an interrupted thermal cloud meandering along with the plume. The numerical method demonstrated herein will surely be applicable to the calculation of the chlorine

residual distribution when a valid chlorine kenetic model is designed using the data from Section 7 of this report and incorporated with the present development. The next step will be the merging of these models followed by field verification. A verified model can be both useful and illuminating in providing necessary information for both the biological and the physical system.

FIGURE 8-1 THERMAL PLUME (1/2 Hr) AT THE OAK CREEK PLANT

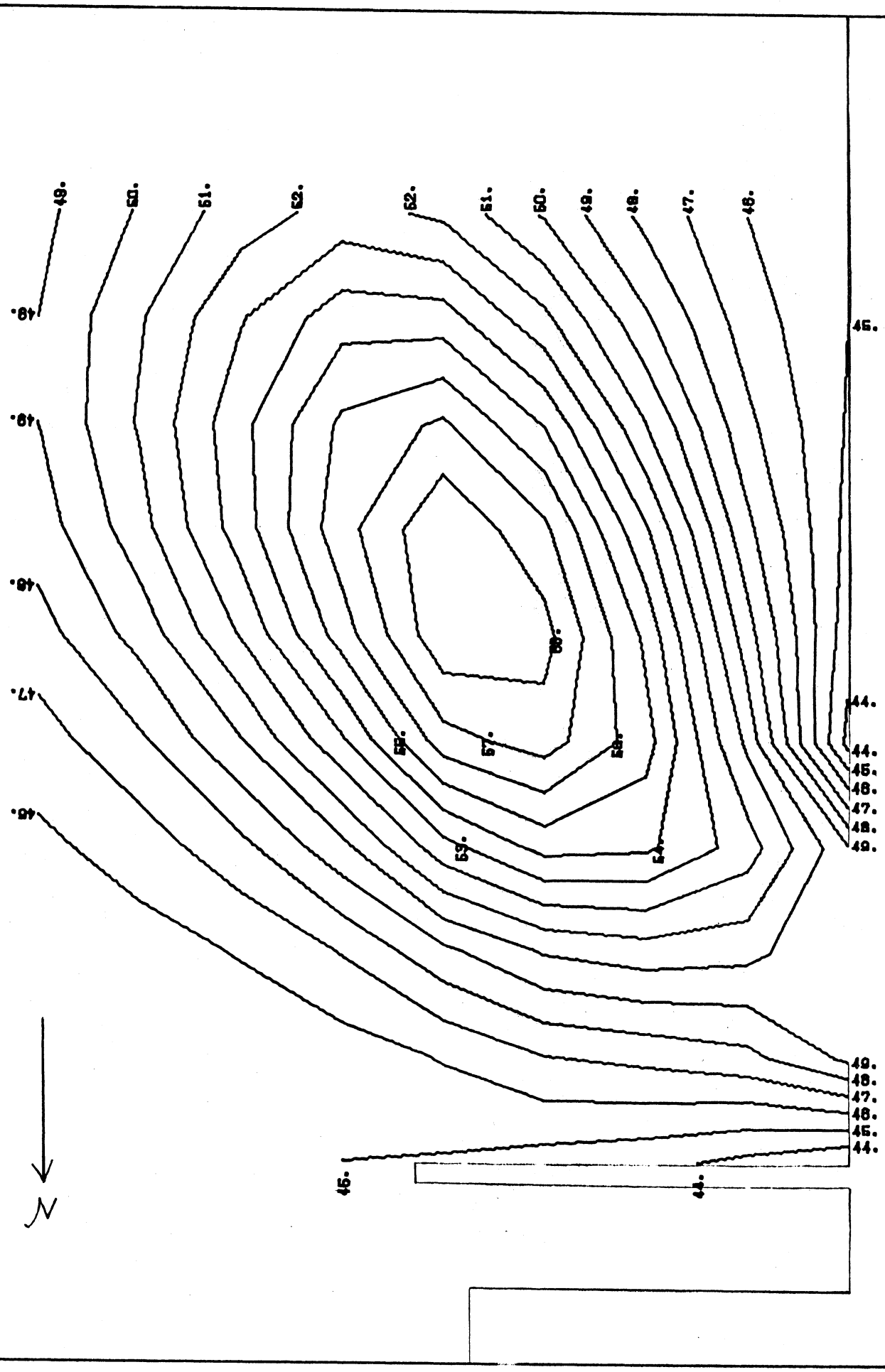

FIGURE 8-2 DISTRIBUTION OF TEMPERATURE DUE TO A IMPULSE INPUT AT 2 HOURS

9. SUMMARY

The results of this research indicate that many Lake Michigan organisms can tolerate intermittent chlorination at concentrations comparable to those observed in the lake.

Toxicity tests with six species of important Lake Michigan fish indicate that the fish are more sensitive to chlorine at elevated temperatures . This is exemplified by the results of tests with alewife and yellow perch where both species exhibited a ten-fold increase in sensitivity as test temperatures were increased from 10 to 30 C. The range of 30-minute LC-50 values for all fish tested was 8 mg/l at 10 C for yellow perch to 0.287 mg/l for coho salmon at 20 C. Estimated no mortality levels were approximately one half the LC-50 values ranging from 0.18 mg/l for the alewife to 0.43 mg/l for the yellow perch.

Behavioral observations indicated that most fish are lethargic while exposed to chlorine with the salmonid species somewhat more active than others. Most species came to the surface of the test aquaria to gulp for air during the exposure period. Mortality was delayed following exposure to chlorine in several of the species tested, however, considerable inter- and intra-specific differences were noted. Except for the perch at 10 C fish losing their equilibrium rarely recovered following exposure to chlorine.

The invertebrate species tested were generally less sensitive to intermittent chlorination than were the fish.

The LC-50 values generally decreased with increasing temperature. The range of LC-50 values observed was approximately 15 mg/l at 10 C for Cyclops bicuspidatus thomasi to 1.54 mg/l at 10 C for Limnocalanus macrurus. Estimated "safe" values (TL-5) ranged from 2.4 to 0.53 mg/l.

Lake Michigan phytoplankton showed a significant loss of active chlorophyll a and a permanent reduction of carbon uptake rates following 30 minute exposures to chlorine at levels above 0.5 mg/l. Chlorine concentrations less than 0.1 mg/l produced only slight losses in chlorophyll a and, following an initial reduction in carbon uptake rates, nearly complete recovery was observed. Concentrations between 0.5 and 0.1 mg/l generally produced intermediate responses.

Laboratory studies to determine the persistence of chlorine in Lake Michigan water indicated that following chlorine addition there is an initial rapid decrease in chlorine concentrations within 1-2 hours to levels approximately one half of the initial concentrations. Low levels of chlorine were detectable for much longer periods. Degradation rates were more rapid under warm lighted conditions as opposed to cold, dark conditions.

Field surveys at six Lake Michigan power plants indicate that chlorine degrades rapidly within the bounds of the thermal effluent plume. The maximum chlorine concentration observed was 0.376 mg/l. Chlorine was generally not detectable longer than 1-2 hours while the maximum area of the chlorine plume was only a fraction of the observable thermal plume.

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