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EFFECTS OF CHLORINE AND SULFITE REDUCTION ON LAKE MICHIGAN INVERTEBRATES

Environmental Research Laboratory
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EFFECTS OF CHLORINE AND SULFITE REDUCTION ON LAKE MICHIGAN INVERTEBRATES

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ABSTRACT

The acute toxicity of residual chlorine was determined for the copepod Cyclops bicuspidatus thomasi and the rotifer Keratella cochlearis. The 96-hour TL_{50} value for Cyclops was 0.084 mg/l total residual chlorine added as monochloramine. When Cyclops was exposed to sodium hypochlorite the 96-hour TL_{50} was 0.069 mg/l total residual chlorine. The 4-hour TL_{50} value for Keratella was 0.019 mg/l total residual chlorine added as monochloramine.

Chemical studies determined that sodium sulfite was an efficient, inexpensive chemical agent for reducing chlorine residuals which did not produce undesirable by-products. Complete reduction was accomplished in less than 20 seconds with a calculated k_{min} of 43 sec^{-1}. Bioassay studies indicated that sodium sulfite added to chlorinated water completely eliminated the acute toxicity of residual chlorine to both Cyclops bicuspidatus thomasi and Keratella cochlearis.

Field studies in Milwaukee Harbor and adjacent Lake Michigan indicated that measurable chlorine residuals were confined to a very small area surrounding the effluent from the Jones Island Sewage Treatment Plant. Significant reductions in the populations of benthic organisms were observed in the effluent plume area after the start of chlorination.

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ACKNOWLEDGMENTS

The field study section of this report is based primarily on the Master's thesis of P. Scott Hausmann ("The benthic macrofauna of Milwaukee Harbor and adjacent Lake Michigan," Department of Zoology, The University of Wisconsin-Milwaukee, August 1974).

Studies on the chemical reduction of chlorine were conducted by John W. Strand as Master's thesis research (Part III--"Reduction of chloramines in chlorinated sewage," Department of Chemistry, The University of Wisconsin-Milwaukee, September 1972).

Additional chemical studies were undertaken by Drs. Calvin O. Huber, Roy A. Crochet, F. Ryan Sullivan and Christopher Daniels of the Department of Chemistry.

Bioassay data on the rotifers is from the Master's thesis of Nevin E. Grossnickle ("The acute toxicity of residual chloramine to the rotifer Keratella cochlearis (Gosse) and the effects of dechlorination with sodium sulfite," Department of Zoology, The University of Wisconsin-Milwaukee, August 1974).

Crustacean bioassays were completed with the assistance of R. Scott Burkhardt, Lynn L. Frederick, and David Latimer.

We also wish to acknowledge the assistance provided by the staff of the City of Milwaukee Linnwood Avenue Water Purification Plant in obtaining lake water, and the staff of the Milwaukee Sewage Commission Jones Island Sewage Treatment Plant for providing information on plant operations.

Figures were prepared by Ratko J. Ristić and the manuscript was typed and assembled by Irene Berg.

Contribution number 137, Center for Great Lakes Studies, The University of Wisconsin-Milwaukee, Milwaukee, Wisconsin.

SECTION I

CONCLUSIONS

1. The chlorinated effluent from the Jones Island Sewage Treatment Plant appears to have reduced the populations of benthic organisms in areas of Milwaukee Harbor influenced by the effluent plume.

2. The rotifer Keratella cochlearis is one of the most sensitive species to chlorine residuals having a 4-hour TL_{50} value of 0.019 mg/l total residual chlorine added as monochloramine.

3. The copepod Cyclops bicuspidatus thomasi is among the more sensitive aquatic species to residual chlorine with a 96-hour TL_{50} value of 0.084 mg/l total residual chlorine added as monochloramine and 0.069 mg/l total residual chlorine in a solution of hypochlorite and monochloramine.

4. Sodium sulfite is an effective chemical agent for reducing chlorine residuals. It is not toxic to Cyclops bicuspidatus thomasi or to Keratella cochlearis at levels sufficient to reduce chlorine residuals observed in chlorinated sewage. And, it effectively reduces the toxicity of residual chlorine to these organisms without producing undesirable by-products.

SECTION II

RECOMMENDATIONS

1. A careful evaluation of the use of chlorine as a disinfectant for sewage treatment should be undertaken in terms of its effectiveness as a biocide and its adverse effects on the aquatic environment.

2. The rate of chlorine application to sewage should be carefully regulated in terms of the chlorine demand of the sewage and receiving waters to minimize chlorine residuals in the effluent plume.

3. Total residual chlorine levels should be kept below 0.01 mg/l when applied continuously in order to protect all but the most sensitive species. To protect even the most sensitive species, total residual chlorine levels should not exceed 0.002 mg/l when applied on a continuous basis.

4. Sodium sulfite should be used to reduce chlorine residuals in situations where residuals cannot be maintained within acceptable limits by sound operating procedures and it can be demonstrated that a continuously chlorinated effluent would have adverse effects on important organisms in the receiving water body.

5. Studies should be undertaken to determine if other compounds are formed in the process of chlorinating sewage, such as chlorinated organics, which may not be detectable as residual chlorine yet are toxic to aquatic life and humans.

6. Pilot studies should be undertaken to determine the effects of chlorine reduction by sodium sulfite, and the reaction products formed, on receiving water bodies of varying water qualities.

SECTION III

INTRODUCTION

Chlorine, a strong oxidizing agent, has been, and continues to be used in the treatment of sewage to control pathogenic organisms, reduce odor, and to reduce the biochemical oxygen demand. The Chlorine Institute (1974) estimates that 187,000 tons of chlorine were used for the treatment of sewage in the U. S. in 1973. Chlorination is considered desirable from an operational standpoint because it improves the physiochemical characteristics of the effluent and reduces the bacterial load of sewage although its effectiveness in controlling pathogenic organisms has been questioned (Durham and Wolf 1973). Chlorinated sewage represents a hazard to the receiving waters, however, since the residual chlorine is toxic and chloramine and unknown chlorinated organic compounds are produced which are toxic to aquatic life and generally more persistent than free chlorine.

Numerous studies have been conducted which demonstrate the toxicity of chlorine to various types of organisms. Brungs (1973) has compiled an excellent review on the effects of residual chlorine on aquatic life which summarizes this literature.

This project was undertaken to determine the effect on invertebrates of Milwaukee Harbor and adjacent Lake Michigan of the chlorinated effluent from the Jones Island Sewage Treatment Plant and to determine what additional chemical treatment could be employed to eliminate or reduce the toxicity of the residual chlorine in the effluent.

To accomplish these objectives, three basic approaches were taken. First, field studies were conducted to sample the benthic community of the harbor prior to and subsequent to the start of chlorination. The original research proposal included sampling of fish and plankton, but at the request of the Environmental Protection Agency studies of fish were not included. Data on plankton populations in the harbor

are not included because the frequent exchange of various water masses into and through the harbor make such data meaningless in assessing the effects of a single effluent. The measurement of residual chlorine in Milwaukee Harbor and adjacent Lake Michigan was also included in the field program. Secondly, laboratory bioassay experiments were conducted to determine the toxicity of residual chlorine to copepods and rotifers, which are normally the most abundant organisms within the study area. Bioassays on organisms such as amphipods were not included since the results of the field studies indicated that they were not an important portion of the biota in the study area. The third approach involved laboratory chemical studies which were undertaken to identify compounds which would reduce or eliminate the toxicity of residual chlorine in chlorinated sewage. Bioassay experiments were conducted using the most promising compound to determine its toxicity and effectiveness in reducing chlorine toxicity to the test organisms.

SECTION IV

FIELD STUDIES

INTRODUCTION

Many workers have suggested that changes in environmental quality are inevitably reflected in the composition of the benthos and that the best way to determine the extent and kind of change taking place in the environment is by the study of the benthos (Hynes 1960; Mackenthun 1969). Henson (1966) has stated that maximum sensitivity in detecting environmental changes requires investigation at the species level. Changes in the community structure and abundance of organisms have provided useful indices of pollution (Hooper 1969; Hynes 1960). In several instances changes in the benthic biota have been valuable in documenting eutrophication in the Great Lakes (Beeton 1965, 1966; Howmiller and Beeton 1970, 1971). While warning that no universal indicator species exists, Brinkhurst (1965) suggests that analysis of benthos and substrate will give an indication of the source, nature and extent of pollution.

Fortunately with increasing awareness of the continued decrease in water quality there has been increased research on the macrobenthos of the Great Lakes from which an adequate inventory of the fauna is being built (Brinkhurst 1966a, 1966b, 1967, 1969, 1970; Brinkhurst, Hiltunen and Herrington 1968; Carr and Hiltunen 1965; Henson 1966; Hiltunen 1967, 1969a, 1969b, 1969c; Howmiller and Beeton 1970, 1971; Johnson and Matheson 1968; Robertson and Alley 1966; Schuytema and Powers 1966; Veal and Osmond 1968). From these studies it has been shown that of all the benthic organisms the Oligochaeta are assuming the greatest importance in terms of abundance, especially in areas of organic pollution, e.g., harbors. Oligochaetes have been used to assess increases in pollution (Carr and Hiltunen 1965).

This study was undertaken to document the composition of the benthic fauna, especially that of the aquatic oligochaetes, of Milwaukee Harbor and adjacent Lake Michigan and to determine whether chlorination of sewage from the Jones Island Waste Water Treatment Plant, Sewage Commission of the City of Milwaukee, has affected the benthic community.

SITE DESCRIPTION

Milwaukee Harbor is an artificial harbor formed by man-made breakwaters enclosing approximately 5.84 sq Km of a natural embayment of Lake Michigan (Figure 1). An inland harbor area encompasses about 0.93 sq Km which includes the mouths of the Milwaukee, Kinnickinnic, and Menomonee Rivers. The major tributary of the harbor, the Milwaukee River, contributes an average of 14.6 m^3/sec of water to the system while the Kinnickinnic and Menomonee Rivers discharge about 0.48 m^3/sec each. The dissolved oxygen content of all three rivers may be less than 1.0 mg/l in summer months (Department of Natural Resources 1969).

The Jones Island Sewage Treatment Plant, operated by the Sewage Commission of the City of Milwaukee, is an activated sludge plant which began discharging into Milwaukee Harbor in 1925. The plant began continuous chlorination of sewage on 21 June 1971. At present the average flow of sewage treated by the plant is 8.5 m^3/sec. The chlorine, which is in the liquid state, is vaporized and injected into the sewage through a perforated mixer. From 1300 to 1500 Kg of chlorine are used per day at a rate of 25 l/sec. After chlorination, the effluent flows approximately 225 m before entering Milwaukee Harbor at station 2 (Figure 1). Flow time between the points of chlorination and discharge is 10-20 minutes which results in an average chlorination contact time of 15 minutes. The chlorine residual near the point of chlorination ranges from 0.5 to 1.0 mg/l. Ammonia concentrations range from zero in the summer to 10 mg/l in the winter.

METHODS AND MATERIALS

Benthos samples for this study were taken from 16 sampling stations in Milwaukee Harbor and adjacent Lake Michigan (Figure 1) from the R/V NEESKAY on June 7, 1971, two weeks before chlorination of the Jones Island effluent commenced and July 6 and 7, December 1, 1971, and April 17 and June 20, 1972, after chlorination was started.

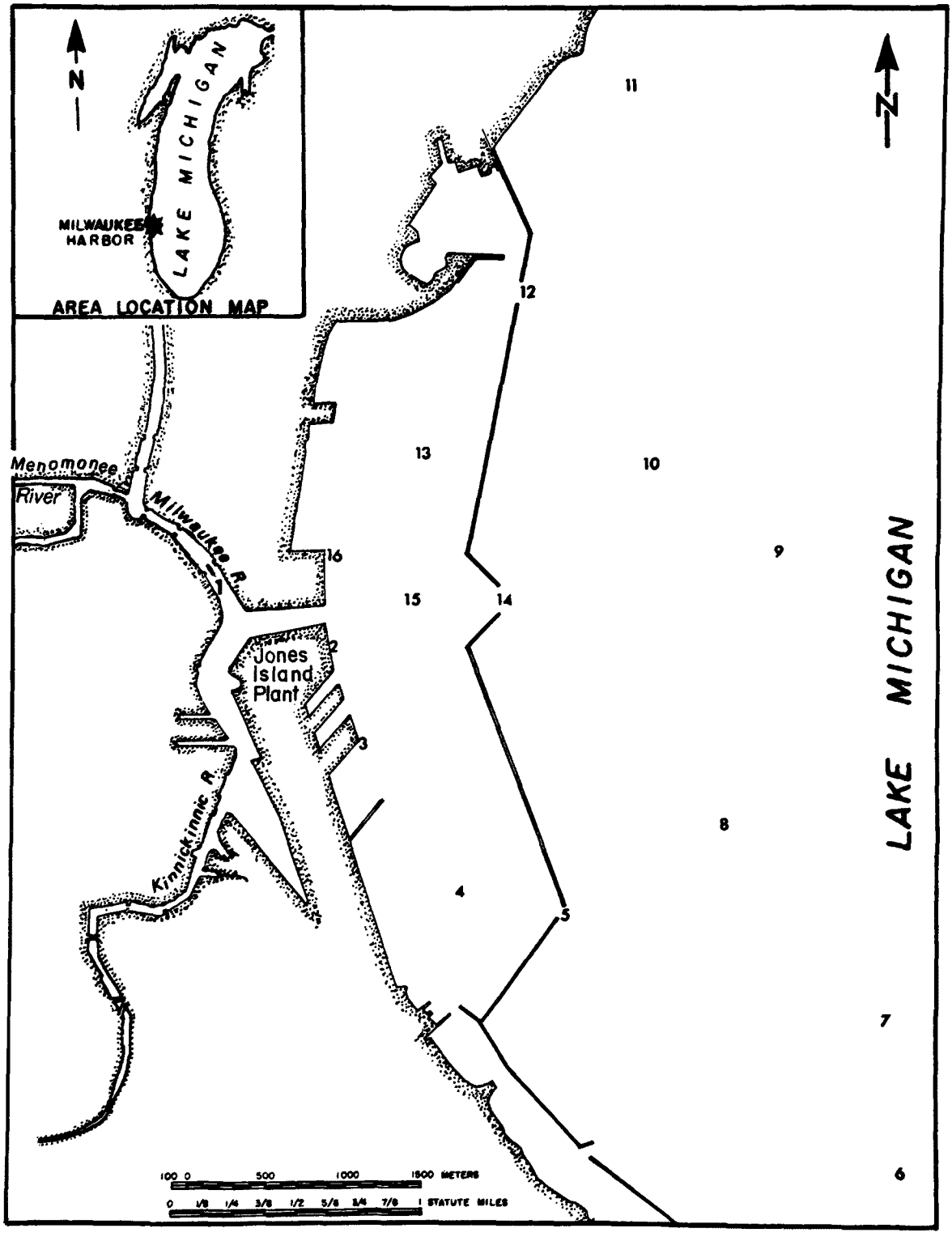

Figure 1. Sampling stations in Milwaukee Harbor and adjacent Lake Michigan.

On each sampling date three samples were taken at each station with a 23 cm x 23 cm Ponar grab. The Ponar grab was selected for this study because of its greater efficiency in a wider variety of sediments than the commonly used Petersen or Ekman grabs (Glannagan 1970; Howmiller 1971; Poers and Robertson 1967). Each grab sample was placed in a large plastic tub and then screened immediately through a U. S. Std. No. 30 mesh (mesh opening 0.62 mm) screening box. Residue remaining after screening was transferred into glass bottles, and preserved in 10% formalin.

At the time samples were collected, sediments were described according to "categories for field evaluation of soil characteristics" (Roelofs 1944) (Table 1). Depth at each station was determined using the recording fathometer on the R/V NEESKAY (Table 1).

In the laboratory the preserved samples were washed in a U. S. Std. No. 60 mesh screen box to remove the formalin solution. Samples were then placed in white enamel pans and all organisms were hand picked and counted using forceps and the aid of a Dazor magnifying lamp.

The abundance of organisms, based on the numbers in the sample, were calculated by multiplying the numbers in the sample by 19 to convert from the area of the sampler to a square meter. Numbers of organisms in this report are, for each station, based on the arithmetic mean of the three samples taken at that station.

A volume displacement method (Anderson and Hooper 1956) was used to determine the abundance of oligochaetes at stations 1, 2, 4 and 16. The volumes displaced by a known number of worms and by the worms in the sample were determined. A specifically modified centrifuge tube with a screen basket insert (Howmiller 1972) was used to rid the worms of externally adhering water.

Oligochaete worms picked from each sample at the 16 stations were examined for species identification. Usually not more than 30 worms per sample (90 per station), were mounted on microscope slides in a mixture of Turtox CMC and Turtox CMCS (this mixture gives the best viscosity for handling and mounting) and were examined at a magnification of at least 100X.

In cases where only a portion of a sample was examined, the worms to be examined were selected at random. The collection was spread out evenly in a pan (in about 1 cm of water), which had a numbered grid layed out on its bottom.

Table 1. PHYSICAL CHARACTERISTICS OF BENTHIC SAMPLING STATIONS, 1971 AND 1972.

Station	Water depth (m)	Bottom type
1	10.4	Oily organic silt, much debris
2	9.7	Organic silt, debris, fly ash
3	10.4	Organic silt, sand, crushed Mollusca shells
4	10.7	Organic silt, clay
5	12.8	Gravel, sand over hard-packed clay
6	9.4	Sand
7	15.5	Sand
8	14.6	Sand
9	16.1	Sand
10	14.6	1971, Sand, clay; 1972, Organic silt over sand, clay
11	4.3	Gravel, sand over hard-packed clay
12	10.7	Gravel over hard-packed clay
13	7.9	Organic silt, Mollusca shells
14	10.7	Gravel, sand over hard-packed clay
15	11.6	Organic silt, debris, ash
16	7.3	Organic silt, sand, crushed Mollusca shells

The grid squares were numbered from 01 to 77. Pairs of digits were read from a table of random numbers and worms from the indicated squares taken for examination until the desired number of specimens were obtained.

The taxonomic works of Brinkhurst (1964, 1965); Brinkhurst and Cook (1966); and Brinkhurst, Hamilton and Herrington (1968) were used for identification of the Oligochaeta. The works of Hiltunen (1967) and Kennedy (1969) were particularly helpful with the genus Limnodrilus. Nomenclature used follows that in the recent monograph of Brinkhurst and Jamieson (1971), except that Limnodrilus cervix-claparedeanus is used here to describe worms that seem almost exactly intermediate between L. claparedeanus and L. cervix. The form of the penis sheath of these worms was similar to that described by Brinkhurst and Cook (1966) and Kennedy (1969) as intermediate between L. claparedeanus and L. cervix and treated by Hiltunen (1967, 1969b, 1970) and Howmiller (1972) as a variant of L. cervix.

All the midge larvae from a sample were examined. The identification of chironomid larvae requires microscopic examination. This is necessary because the structures associated with the larval head are characteristic for each genus. The head was dissected off each larva and mounted ventral side up in CMCS. The body was mounted under a separate coverslip. The taxonomic keys of Hilsenhoff and Narf (1968), Johannsen (1937) and Mason (1968) were used in identification.

No special methods were employed for the identification of Mollusca, Hirudinea, or Crustacea. Sphaeriidae were sorted and counted as a major taxonomic group with no attempt to speciate them. Nematoda were not sampled quantitatively, hence not counted, but their presence or absence was noted for each station. Taxonomic keys in Edmondson (1959) and Pennak (1953) were used for organisms other than oligochaetes and midges.

Water samples to determine chlorine content of the harbor and adjacent lake waters were collected at stations 1, 2, 4, 9, 13, 15, and at several other points in close proximity to station 2 at the Jones Island sewage effluent (Figure 1). Complete surveys of these stations were conducted on April 5, 26, May 31, and August 22, 1973. In addition, station 2 was sampled June 7, 1973, and February 12 and 28, 1974. Samples were obtained from the circulating water system of the ship which draws water from a depth of 2 m. Additional samples were collected at the surface with a plastic bucket and at depths greater than 2 m using a

4 ℓ van Dorn bottle. All samples were analyzed immediately after collection on board the ship using an amperometric titration procedure described in the chemical section of this report.

RESULTS

Organic sediments were found at all stations in the harbor (Table 1). The dominant component of the bottom in this area was an organic silt ranging in thickness, from approximately 2.5 cm to greater than the penetration depth of the grab (10.2 cm). Stations within the inner harbor and near its entrance (stations 1, 2, 15 and 16) contained abundant hair, debris and partly burned materials and ash; these last items probably originated from the City of Milwaukee incinerator, which had stood on the north side of the inner harbor entrance.

Hard bottom was present in each of the passages through the breakwaters. In most cases these hard bottoms were of clay with varying overlays of course gravel.

Gravel and sand were the dominant sediments of the embayment (Lake Michigan). In 1972 a fine layer (.5 to 1.0 cm) of silt appeared at station 10. This layer had not been present during sampling in 1971. Its distribution in just this one area and absence of such sediment in the rest of the embayment suggests that this silt fraction was a current-carried contribution from the harbor.

The dominant organisms within the harbor were the oligochaete worms, accounting for over 90% of the total benthos at all stations (Table 1 to 5, Appendix A). Every station inside the breakwater had a density of worms of 10,000 to >50,000/m^2. Maximum numbers occurred in the mouth of the Milwaukee River and in the immediate area of the Jones Island Sewage Treatment Plant outfall. Minimum numbers of worms were present at the breakwater entrances.

In the embayment proper, worms were present in concentrations usually reported for Lake Michigan. Only in the extreme north end of the embayment (station 11) did the number of oligochaetes exceed the usual range of 0 to about 500/m^2.

Oligochaetes generally were significantly lower in abundance at harbor stations in 1972 (Figure 2). A standard two-tailed test (Elliot 1971) was used to test the significance of difference between means of paired sets of data

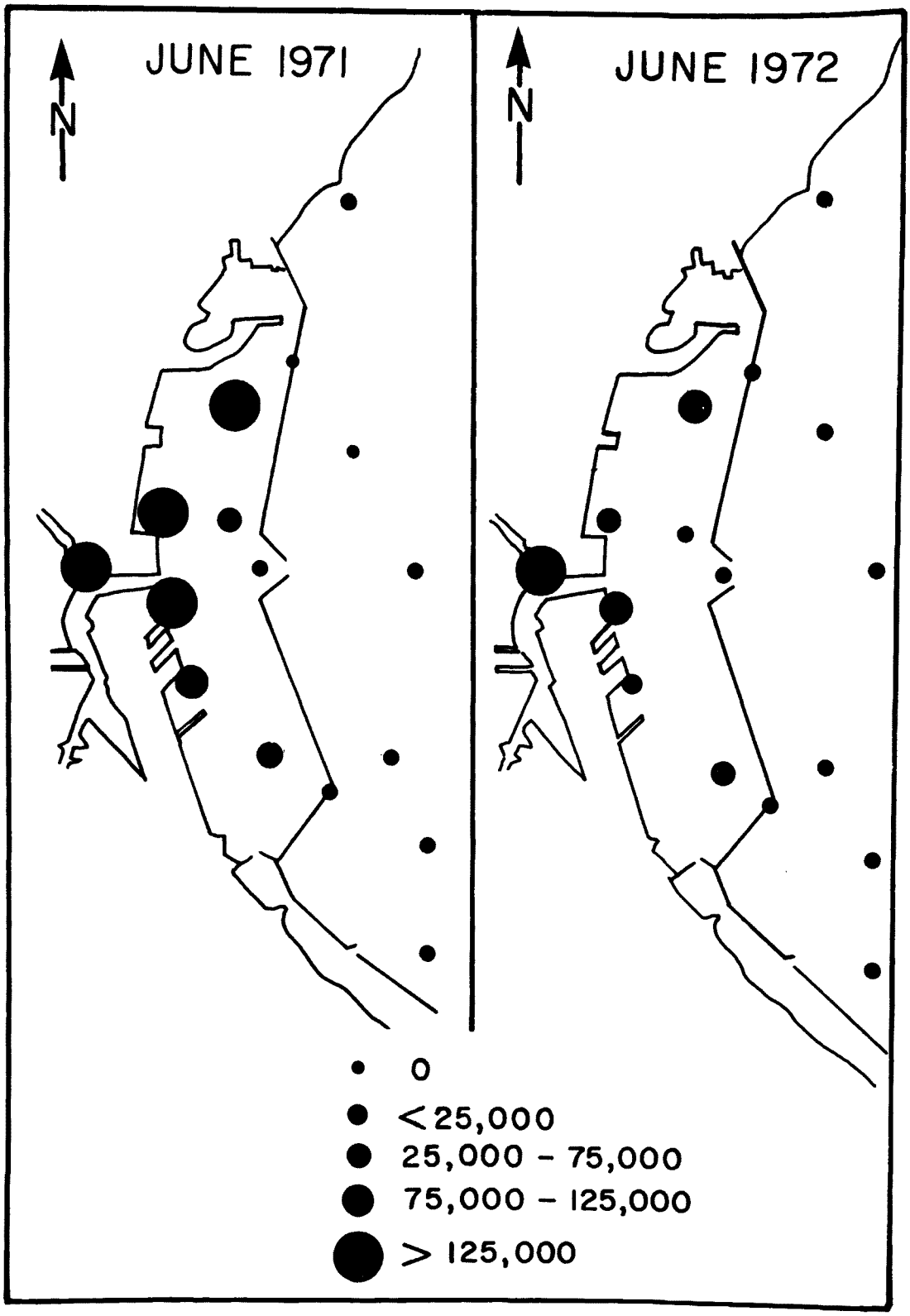

Figure 2. Abundance (#/m^2) of Oligochaeta in Milwaukee Harbor and adjacent Lake Michigan, June 1971 and June 1972.

from June 7, 1971, and June 20, 1972 (Table 2, Figure 3). Stations in mid-harbor and near the Jones Island outfall (stations 2, 14, 15 and 16) all had differences in populations which proved to be significant at the 99% level. Station 13 in the northern part of the harbor also showed a significant decrease (99% level) in worm populations in 1972. Populations of worms at the inner harbor station (#1) and in the extreme northern and southern parts of the outer harbor (stations 4, 5, 12) as well as the embayment stations (6-11) generally decreased, but the differences were not significant. Station 3, located near the municipal docks, also followed this pattern of a non-significant decrease.

The most abundant species throughout the study were Tubifex tubifex, Limnodrilus hoffmeisteri, and Peloscolex multisetosus (Figures 4-6) and (Tables 6-10 Appendix A). Four other species were found: Stylodrilus heringianus, Dero digitata, Limnodrilus cervix-claparedeanus, and Limnodrilus cervix. Stylodrilus heringianus (Figure 7) was restricted to the embayment and breakwater entrance stations, while Dero digitata was restricted to the south and north ends of the main harbor. Limnodrilus cervix-claparedeanus (Figure 7) was generally distributed at main harbor stations, with as many as 17,327/m^2 found at one station. Limnodrilus cervix was found at two stations twice during the study accounting for about 6% of the total worms found at those stations (Tables 7-9 Appendix A).

Because many common tubificids can be positively identified only when they are sexually mature and have genital chaetae and/or penis sheaths, it was impossible to identify many of the worms in the samples, especially those taken early or late in the year. These are listed as undetermined immatures and placed into two groups: those with hair chaetae and those without hair chaetae. One can make a reasonable guess, though, at the identity of immature specimens based upon this knowledge of which species are present in the sample from the positive identification of mature specimens. Thus, the tables in Appendix A list the probable abundance of Limnodrilus hoffmeisteri and Tubifex tubifex. These numbers include mature specimens and a portion of or all of the immature worms which could fit into the category. In cases where a portion of the immature worms could have belonged to another species the immature worms were assigned to the two species on the basis of relative abundance of positively identified mature specimens.

Table 2. COMPARISON OF MEANS OF PAIRED SETS OF DATA OF OLIGOCHAETA FROM 7 JUNE 1971 AND 20 JUNE 1972. THE LEVEL OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEANS ACCORDING TO A TWO-TAILED t-TEST.

Station	Oligochaeta (number/m^2) 7 June 1971	20 June 1972	t-value	Level of significance
1	146357 ± 4660	151145 ± 8873	0.86	n.s.
2	164242 ± 7037	112480 ± 3923	6.42	99
3	84949 ± 7766	72783 ± 7378	1.14	n.s.
4	68590 ± 4200	55436 ± 5648	1.44	n.s.
5	203 ± 44	228 ± 94	0.24	n.s.
6	70 ± 17	19 + 11	1.09	n.s.
7	38 ± 25	38 ± 29	0.00	n.s.
8	101 ± 28	89 ± 50	0.21	n.s.
9	57 ± 22	158 ± 61	1.58	n.s.
10	0	2261 ± 378	----	---
11	1545 ± 290	1995 ± 295	1.09	n.s.
12	0	25 ± 17	----	---
13	138244 ± 5089	99649 ± 5090	7.58	99
14	70 ± 6	19 ± 11	4.07	98
15	33662 ± 1662	13072 ± 1889	8.18	99
16	133285 ± 12191	31863 ± 7422	7.11	99

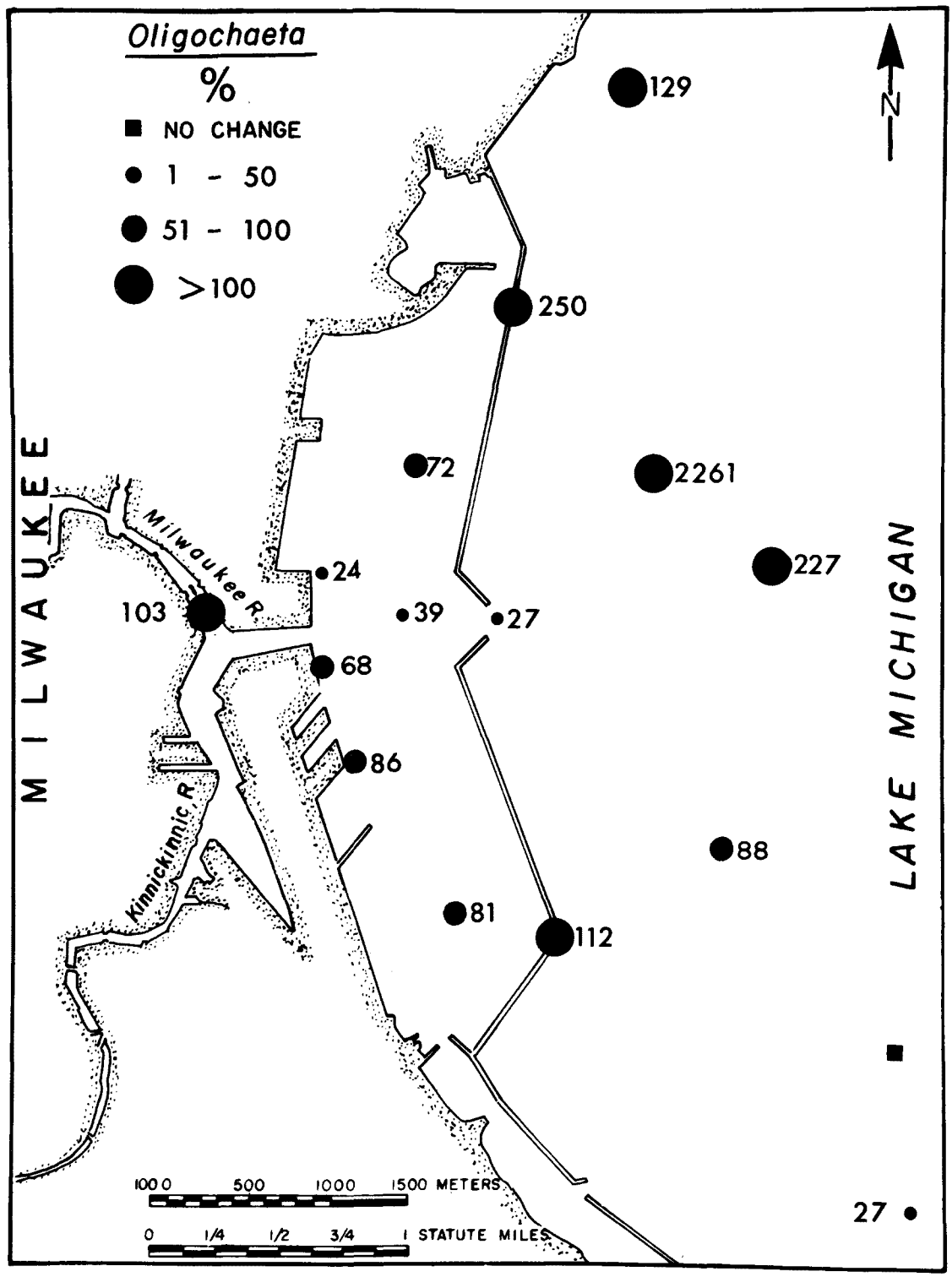

Figure 3. Abundance of Oligochaeta in June 1972 as a percentage of the June 1971 abundance.

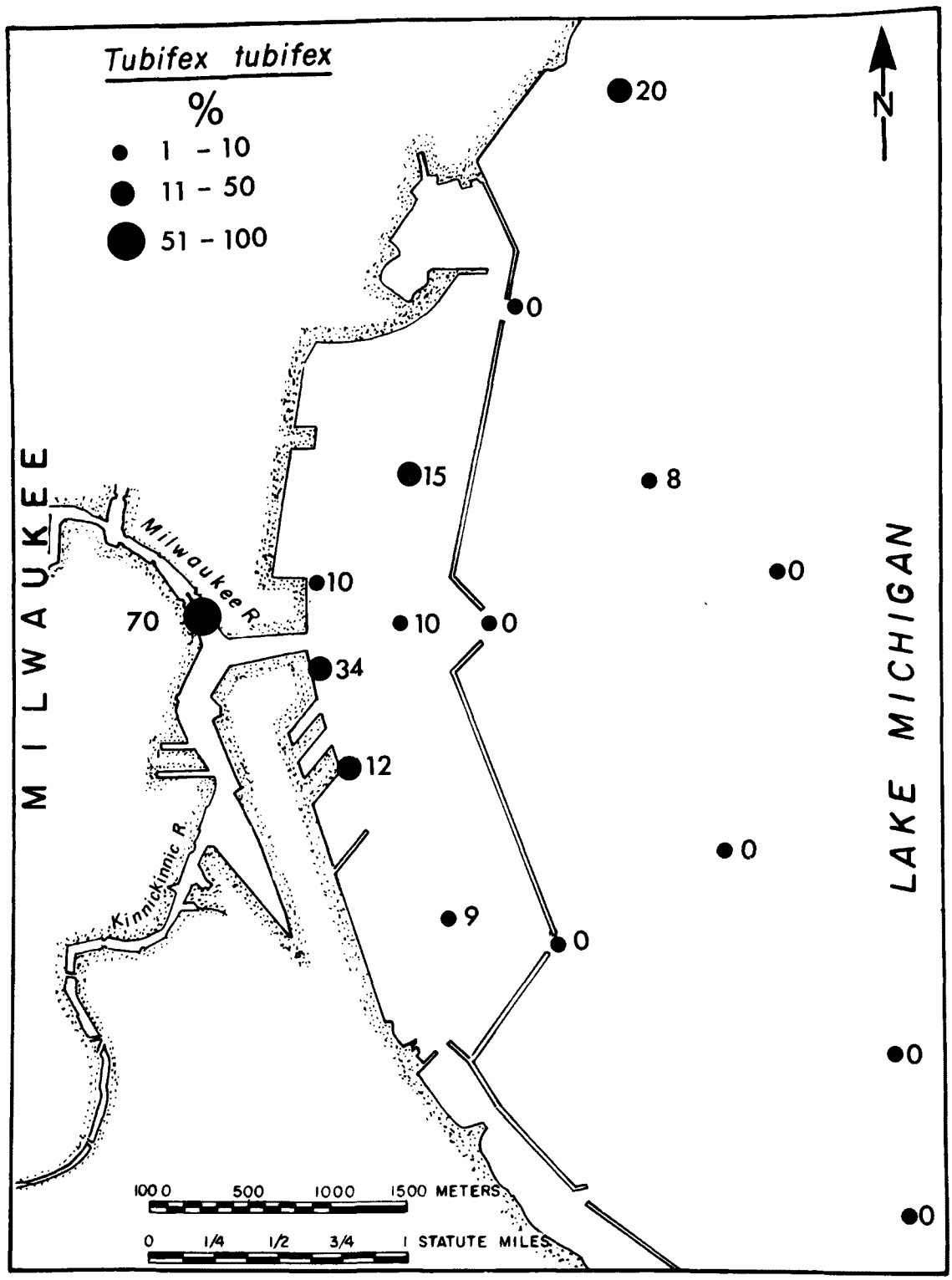

Figure 4. Percentage of Tubifex tubifex in the oligochaete population of Milwaukee Harbor and adjacent Lake Michigan.

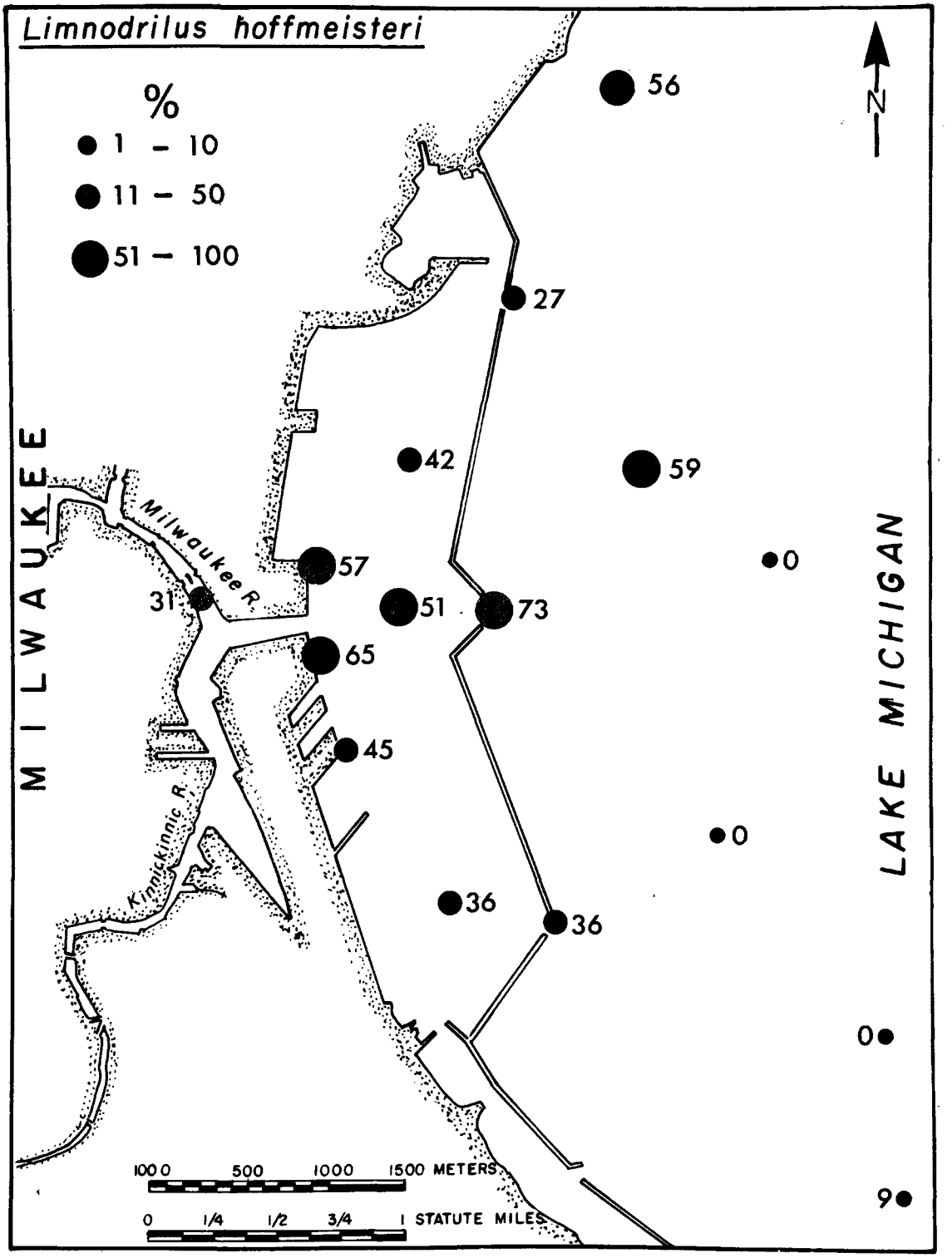

Figure 5. Percentage of Limnodrilus hoffmeisteri in the oligochaete population of Milwaukee Harbor and adjacent Lake Michigan.

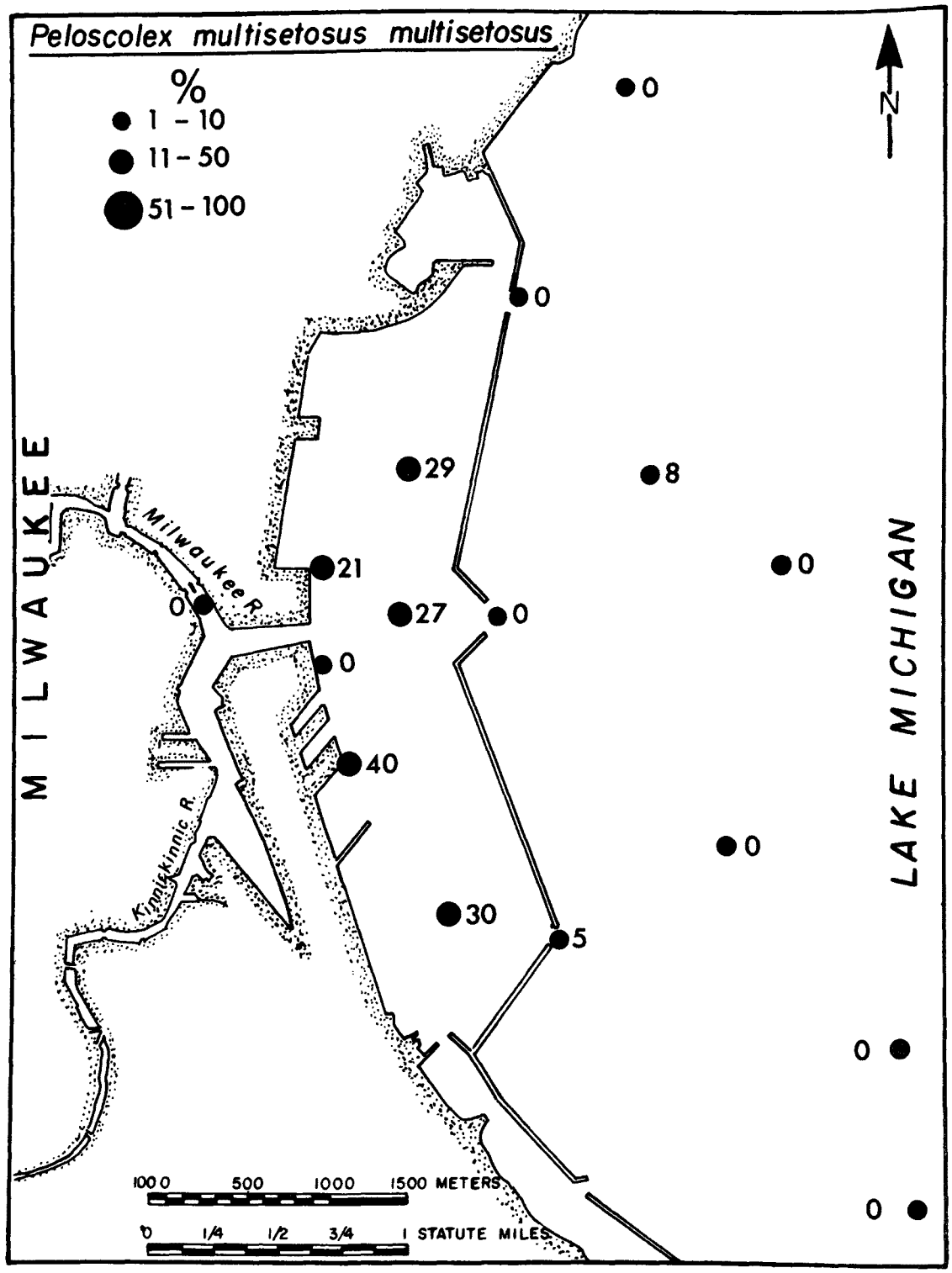

Figure 6. Percentage of Peloscolex multisetosus multisetosus in the oligochaete population of Milwaukee Harbor and adjacent Lake Michigan.

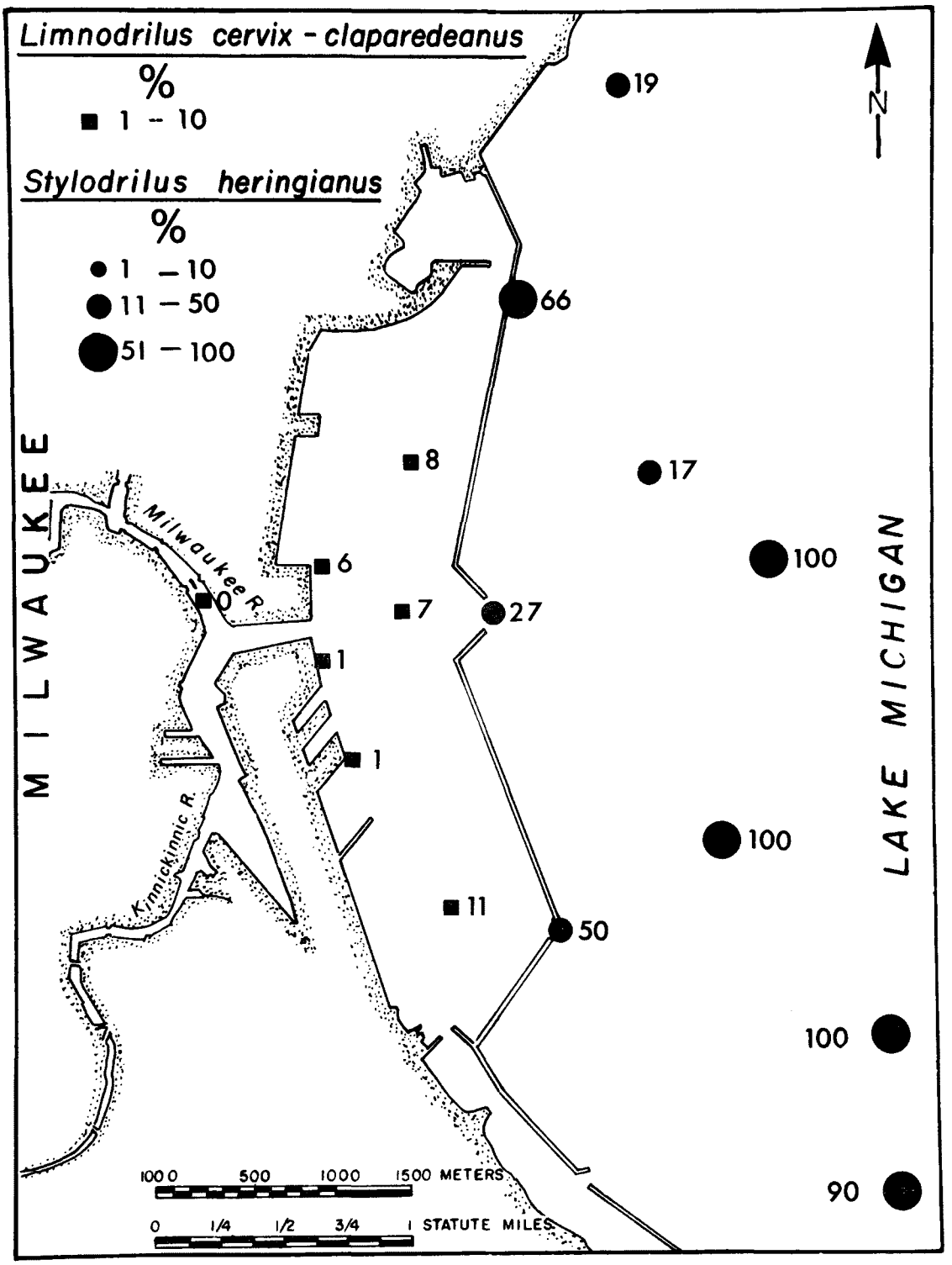

Figure 7. Percentages of Stylodrilus heringianus and Limnodrilus cervix-claparedeanus in the oligochaete population of Milwaukee Harbor and adjacent Lake Michigan.

The distribution of the tendipedids (chironomids, midge larvae) was markedly different from that of the oligochaetes. Near the north and south ends of the area inside the breakwaters and the entrances in the breakwaters, there were small populations (20-100/m^2) of midges (Tables 11-15 Appendix A). Elsewhere few midges were collected. Outside the breakwaters midge abundance was usually less than 50/m^2. Only in the extreme north end of the embayment (station 11) did the numbers of tendipedids approach usual Lake Michigan concentrations, 0 to 500/m^2. Three taxa were identified. The dominant taxon at all stations was Hetrotrissocladius sp. Procladius and Chironomus species occurred in small numbers at stations 11 and 13.

No marked changes in the relative abundance of tendipedids was noticed between 1971 and 1972, primarily because so few were collected within the harbor.

Leeches and snails were minor components of the benthos (Tables 11-15 Appendix A). They were found only in the area inside the breakwater and never in numbers exceeding 63/m^2 or about three individuals per grab. Only one species of leech was found, Helobdella stagnalis. The snails were represented by Vivaparus and Valvata sp. Many empty shells of these taxa occurred at many of the stations within the harbor.

Fingernail clams (Pelecypoda, Sphaeriidae), like snails and leeches, were not very abundant, and found generally in the area north of the main breakwater entrance (Tables 11-15 Appendix A). Maximum numbers (772/m^2) occurred at station 16, just north of the main entrance and near the west harbor wall. Collections included numbers of Sphaerium corneum, S. transversum, and S. lacustre form jayense. Shells of these taxa were at many stations in the harbor, but only about one in six were found alive, the rest being empty shells. No clams were found in the area outside the breakwater, except at station 11, where one to two individuals were found per grab. These clams are believed to be the naiad clam (Unionidae) Lampsilis siliquoidea.

Isopods (Asellus intermedius) and amphipods occurred only in the embayment outside the breakwaters and in the breakwater entrances themselves (Tables 11-15 Appendix A). Usually less than 50/m^2 were collected, if there were any amphipods or isopods at all. Localized areas of higher populations of both lay north and northeast of the north harbor entrance (stations 10 and 11). The amphipods, Gammarus fasciatus, Hyalella azteca and Pontoporeia affinis, were identified from specimens taken at these stations.

The common and abundant benthic nematodes in this study were about 3-4.5 mm long and 0.15 mm in diameter. An animal this size cannot be collected quantitatively using a No. 30 (0.516 mm aperture) screen and thus, nematodes were not counted in the samples. Nematodes were found at all harbor stations and at one embayment station (Tables 2-6 Appendix A).

The results of the chlorine determinations in the harbor and adjacent lake are presented in Table 3. The only area of the harbor where measurable residual chlorine was detected was in the immediate vicinity of the Jones Island Treatment Plant effluent (station 2, Figure 1). The highest concentration of total residual chlorine observed at this station was 0.220 mg/l. Samples obtained 200 m from the effluent at both the surface and the bottom did not contain measurable residual chlorine.

DISCUSSION

Aquatic oligochaete worms, especially the Tubificidae, are probably the most common member of the benthos in eutrophic or organically polluted areas. Pollution biologists have long associated an abundance of oligochaetes, along with a scarcity of other benthic invertebrates, with severe organic pollution.

The abundance and distribution of worms in relation to pollution has been discussed in numerous reports (Brinkhurst 1965, 1966a, 1966b, 1968, 1970; Brinkhurst, et al. 1970; Carr and Hiltunen 1965; Goodnight and Whitley 1960; Howmiller and Beeton 1971). The view held is that worms can be used as indicators of organic pollution and that it is possible to define species regularly associated with organically polluted and eutrophic water and those associated with cleaner oligotrophic systems.

Stylodrilus heringianus (Lumbriculidae) is apparently an oligotrophic species, "typical of wide reaches of the Great Lakes where there is little evidence of eutrophication" (Brinkhurst 1969). It is abundant in Georgian Bay, Lake Huron (Brinkhurst, et al. 1968) and oligotrophic Lake Superior (Beeton and Hausmann, unpublished; Hiltunen 1969). It is common throughout Lake Ontario, except where the environment is considered unfavorable (Hiltunen 1969b) and it is the dominant oligochaete throughout the central basin of Lake Michigan (Hiltunen 1967; Howmiller 1972). Stylodrilus heringianus was the dominant oligochaete at all

Table 3. TOTAL RESIDUAL CHLORINE VALUES OBSERVED IN MILWAUKEE HARBOR AND ADJACENT LAKE MICHIGAN. ALL SAMPLES PUMPED FROM A DEPTH OF 2 m UNLESS DESIGNATED AS SURFACE SAMPLE.

Date	Station no.		Residual chlorine (mg/l)	Temp. (°C)
Apr. 5, 1973	1		0.000	10
	2a	60 m east of outfall	0.000	8
	2b	60 m east of outfall	0.000	-
	2c	30 m east of outfall	0.000	-
Apr. 26, 1973	9	3 mi offshore	0.000	6
	4		0.000	14
	15	south of channel	0.000	14
	2a	30 m east of outfall	0.020	15
	2b	surface 30 m east of outfall	0.020	15
	2c	surface 15 m east of outfall	0.220	15
	13		0.000	15
	15	north of channel	0.000	14
	1		0.000	15
May 31, 1973	9	3 mi offshore	0.000	10.5
	4		0.000	15
	15	south of channel	0.000	14
	15	north of channel	0.000	14
	1		0.000	18
	2	surface 5 m east of effluent	0.136	17
	2	surface 5 m east of effluent	0.131	17
	2	surface 60 m east of effluent	0.030	15
	2	6 m deep 60 m east of effluent	0.000	13
	2	surface 200 m east of effluent	0.000	16
	2	6 m deep 200 m east of effluent	0.000	14.5
	2	surface 200 m S.E. of effluent	0.000	15
	2	6 m deep 200 m S.E. of effluent	0.000	-
	2	surface 200 m N.E. of effluent	0.000	17.5
	2	6 m deep 200 m N.E. of effluent	0.000	14.5

Table 3. (continued) TOTAL RESIDUAL CHLORINE VALUES OBSERVED IN MILWAUKEE HARBOR AND ADJACENT LAKE MICHIGAN. ALL SAMPLES PUMPED FROM A DEPTH OF 2 m UNLESS DESIGNATED AS SURFACE SAMPLE.

Date	Station no.	Residual chlorine (mg/l)	Temp. (°C)
Jun. 7, 1973	2 30 m east of effluent	0.000	21.2
Aug. 22, 1973	1	0.000	23.5
	2 at effluent	0.001	20.5
	2 30 m east of effluent	0.006	20.5
	4	0.000	20
	15 south of channel	0.000	19.5
	15 north of channel	0.000	19.5
	13	0.000	20
Feb. 12, 1974	2 30 m east of effluent	0.000	3.7
Feb. 28, 1974	2 30 m east of effluent	0.000	2.5

stations outside the breakwaters, except station 11, in the present study. Stylodrilus heringianus was also present in about equal abundance with other worm species at the breakwater entrances.

It has been shown that there are two species of Tubificidae that have the least demanding ecological requirements (Brinkhurst and Kennedy 1962) and consistently occur in greatest numbers in highly organic sediments and water highly "polluted" or eutrophic (Brinkhurst 1965, 1966a, 1966b, 1970; Hiltunen 1969; Howmiller and Beeton 1970). These two species are Tubifex tubifex and Limnodrilus hoffmeisteri. in great abundance and in the absence of other benthic organisms, including other tubificid species, indicate gross organic pollution (Brinkhurst 1970). Where conditions are less extreme L. hoffmeisteri is often the most abundant species, generally found together with other Limnodrilus species and Peloscolex multisetosus. Peloscolex multisetosus is the only member of the genus closely associated with organic pollution and highly organic sediments. Howmiller (1972) found that the distribution of P. multisetosus extended far into the southern polluted area of Green Bay. Brinkhurst (1969) has found that in the Great Lakes in general, P. multisetosus appears to be restricted to polluted area.

In Milwaukee Harbor, T. tubifex, L. hoffmeisteri and P. multisetosus dominated the benthos and were present in great abundance. T. tubifex dominated the worm population of the inner harbor (Milwaukee River) and along with L. hoffmeisteri made up about 97% of the benthos near the Jones Island outfall. Brinkhurst (1970) has found a similar distribution pattern in the polluted Toronto Harbor near the mouth of the Don River, which is the major source of organic pollution to the harbor. T. tubifex was absent from all the stations outside the breakwater, except station 11 and in 1972 station 10, both areas possibly affected by dredging spoils.

In the northern and southern areas of the harbor Limnodrilus cervix-claprédeanus was common. Howmiller (1972) reported this form common in lower Green Bay and Hiltunen (1967, 1969) has reported it from enriched areas of Lake Michigan and from western Lake Erie.

Dero digitata, a naidid, was found by Brinkhurst (1967) to be the commonest naidid in Saginaw Bay, Lake Huron, which is influenced by the inflow of the polluted Saginaw River. Howmiller (1972) found D. digitata to be restricted to the lower bay and western shore of Green Bay. D. digitata was found in low numbers at only two stations in the northern and southern ends of Milwaukee Harbor.

The midges, especially the Chironomidae, include many species with larvae and pupae tolerant of the very low oxygen concentrations associated with organic pollution. The aquatic larvae of midges were the second most widespread members of the benthic fauna during this study but nowhere did they reach the abundance reported by Ayers and Huang (1966) in their 1964 study of Milwaukee Harbor. While Ayers and Huang found populations both in the harbor and the embayment, midges were observed only in the extreme north and south ends of the harbor and in the embayment in this study.

Fingernail clams, like the midges, were less abundant in 1971-72 than in 1964. Distribution patterns did, though, follow those found by Ayers and Huang, with clams found everywhere in the harbor except off Jones Island and at the mouth of the Milwaukee River. Rofritz (1973) reported high populations of sphaeriids near the west wall of the north section of the harbor but did not differentiate between living and relict shells. Emmling (1975) observed a distribution pattern dependent on sediment grain size with a maximum abundance of sphaeriids occurring in the extreme north and south portions of the harbor. The lower abundance of

sphaeriids observed in this study is probably a function of an accurate separation of living from relict shells which was not done in previous studies.

While no naiad clams (Unionidae) were reported by Ayers and Huang, Lampsilis siliquoidea was found at several Lake Michigan embayment stations. The presence of naiads generally indicates clean water (Carr and Hiltunen 1965). Howmiller (1972) found these naiads in the sandy substrates of middle and upper Green Bay, and noted that "soft muds . . . are not a suitable substrate for these large clams."

Leeches and snails were minor components of the benthos in the harbor and showed little variation in numbers. Neither leeches nor snails appear able to tolerate decomposition gases at low oxygen concentrations (Pennak 1953). Carr and Hiltunen (1965) concluded that, in western Lake Erie, they were adversely affected by polluted conditions near river mouths.

Amphipods and isopods occurred only at the stations outside the breakwater. Amphipods and isopods did occur occasionally at the breakwater entrances, but their presence at these stations was probably a function of water movements.

Ayers and Huang (1968) believed, based on their measurements of transparency, sulphide levels, and distribution of benthic organisms, that the long-term mean water movement was to the northeast in the embayment. Bellaire (1964) found northward currents along Milwaukee during four days of October 1963. The FWPCA (1966) reported primary northward currents through the embayment during September through March and weaker southward currents during the five months of April through August. The low numbers of benthic organisms in the southern and central part of the embayment, and the tendencies toward higher concentrations of benthic invertebrates to the north and east of the northern harbor opening observed in this survey is taken to indicate a long-term tendency of harbor water to move northeast from the harbor.

The relative abundance of worms, the abundance of certain worm species themselves and the lack of other benthic organisms in the inner harbor and central area of the harbor probably indicate a zone of gross pollution. The appearance of other worm species and invertebrates in the north and south areas of the harbor would appear to indicate dilution of the pollution from the river and sewage effluent with lake water. The northern and southern ends of the harbor

could best be described as mesotrophic areas based on the benthic invertebrate fauna. While the benthos found in this area could only be described as pollution-tolerant, the assemblages and greater species diversity indicate less extreme conditions, as more and more dilution and mixing with lake water entering through the breakwater entrances takes place.

The lower abundance of oligochaetes in the southern end would also seem to indicate that circulation of lake water is better in this area and that the currents carrying the detritus and bacteria-rich harbor water within the harbor were primarily northward.

Emmling (1975) in his study of sphaeriid-sediment interaction in the Milwaukee Harbor found sediment with the highest organic matter primarily in the central portion of the main harbor off Jones Island and extending into the north and south-central regions of the harbor. Both the extreme north and south ends of the harbor had greater percentages of sand and lower organic matter than the sediments of the central region and a greater abundance and diversity of non-oligochaete macroinvertebrates.

Observations of water color within the harbor would seem to confirm these conclusions. Dark brown water enters the harbor from the Milwaukee River and from the Jones Island Sewage Treatment Plant. In the central area of the harbor the water progressively becomes a lighter brown moving away from these sources, as the harbor water is diluted by lake water entering through the central harbor entrance. In the south end of the harbor the water takes on a blue-green-brown color indicating extensive mixing of harbor and lake water. The north end of the harbor remains a light brown color probably because of impaired circulation due in part to the curved internal bulkhead located in the north end and the general northward movement of harbor water.

The significant decrease of oligochaetes in 1972 and the subsequent disappearance of midges from Milwaukee Harbor suggests a major environmental degradation. Investigation of possible spills of toxicants into the harbor or the rivers which might have contributed to the dramatic changes in population levels revealed no reported spills other than a few minor (less than 15 gallons) oil spills in 1971-72 (Department of Natural Resources 1973a).

Monthly Performance Reports for the Jones Island Sewage Plant (Sewage Commission of the City of Milwaukee 1971-72), indicate no periods of time when the 15-minute chlorine

residual measured less than 0.31 mg/l or greater than 1.62 mg/l for the total plant effluent (includes plant bypass). Yearly averages for the plant were 1.01 mg/l in 1971 and 0.65 mg/l in 1972. Total residual chlorine in the harbor, however, was not measurable except directly over the effluent pipe of the plant.

Records of climatological data for the Milwaukee area (U. S. Department of Commerce, 1971, 1972, 1973) in 1970-72 did not show any variations in average wind speed and direction or air temperature great enough to influence any major differences in the harbor environment for the winter of 1971-72 compared to previous years. Water temperature data (Department of Natural Resources 1973b) for Milwaukee Harbor in 1969-72 (Figure 8) shows lower water temperatures in the winter months of 1971-72 than in previous years. It has been shown that in many northern European lakes tubificids are only able to breed during a restricted period in late summer when the water temperature rises above 15°C. This influence of temperature has been reported for Limnodrilus hoffmeisteri (Kennedy 1966a) and L. udekemianus (Kennedy 1966b). Brinkhurst and Jamieson (1971) have stated that where the habitat is very productive the greatest number of breeding specimens were found in the winter months, but a spell of very low temperatures (below 4°C) can cause a temporary cessation in activity.

The fact that the worm populations decreased only in the central and north-central area of the harbor near the Jones Island effluent and not in the rest of the harbor, the rivers, or in adjacent Lake Michigan, suggests adverse conditions associated with the sewage outfall. Although no residual chlorine was measured in these areas of the harbor it is possible that toxic levels of chlorine could have existed there for short periods of time. Learner and Edwards (1968) demonstrated that 0.5 mg/l chlorine was lethal to Nais (Oligochaeta) within a period of 35 minutes. Hart (1957) found that 0.5 mg/l chlorine killed Nais within one hour.

It is also possible that chlorinated compounds formed in the sewage that were undetectable by amperometric analysis may have been responsible for the changes observed.

Natural variations in the benthic community could also have influenced the results. Sampling over a number of years would be required to establish natural fluctuations in the abundance and composition of the benthic community in Milwaukee Harbor.

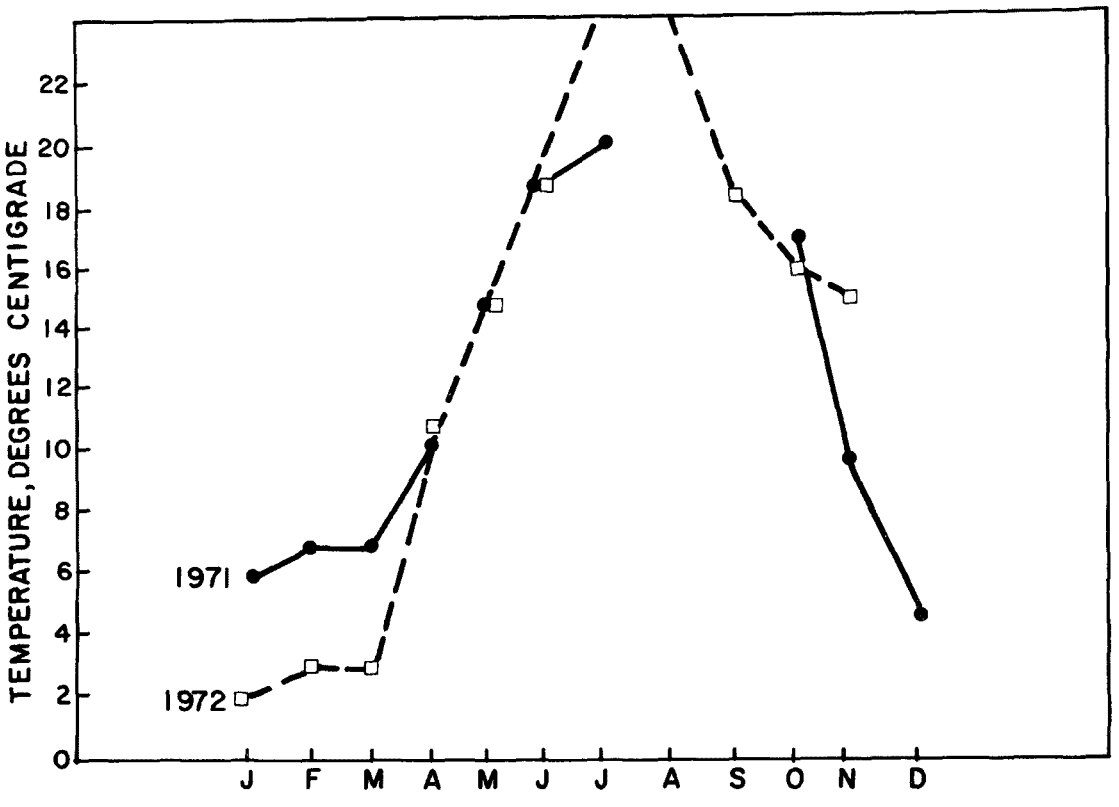

Figure 8. Mean monthly temperatures in Milwaukee Harbor, 1971 and 1972 (adapted from Wisconsin Surface Water Monitoring Data 1969-72).

SUMMARY

Macroscopic benthic invertebrates of Milwaukee Harbor and surrounding Lake Michigan were studied from grab samples taken at 16 stations in 1971 and 1972. Comparison of the present findings with data from studies made in 1964 indicate that many types of invertebrates are less abundant now. These include Hirudinea, Sphaeriidae, Gastropoda and probably Amphipoda and Isopoda. Oligochaeta and Chironmidae were present in similar numbers in 1971, but declined significantly in 1972 in the central and north-central area of the harbor. This change might be indicative of chlorine toxicity and further studies are needed to determine if this is really so.

Oligochaetes were the most abundant macroinvertebrate in the harbor, generally composing over 90% of the total benthos. Seven species were recorded. Species distribution patterns were similar to those found in other studies of polluted areas of the Great Lakes.

Other benthic invertebrates composed only about 10% of the total specimens collected with clams probably being the most abundant, although being concentrated in only certain areas. Chironomids were found at about half of the stations and in subnormal lake concentrations (0-50/m^2). Taxa identified included the genera Chironomus, Procladius and Heterotrissocladius. Subsequent studies of the harbor area (Emmling 1975) have reported the almost complete absence of midge larvae.

This study has been adequate to determine distribution patterns of the dominant elements of the benthic fauna. It thus may serve as a reference for further bioassessment of water quality in the harbor area. Further studies are needed to determine accurately the abundance and distribution patterns of the less numerous benthic species.

SECTION V

CHEMICAL STUDIES

INTRODUCTION

The goal of this section of the study was to find a fast, economical, and safe method for the removal of residual chlorine from chlorinated sewage. To achieve this goal, the following objectives were pursued:

1. To screen selected agents, e.g., sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), sulfur dioxide (SO_2), which are chemically synonymous, and sodium thiosulfate ($Na_2S_2O_3$) as reductants of residual chlorine as monochloramine.

2. To ascertain the kinetics of the reduction of monochloramine by the preferred reducing agent.

Several chemical and physical methods for removal of chlorine and chloramines have been used and reported in the literature. Watzl (1929) exposed chlorinated water to granular charcoal which safely removed the excess chlorine. To remove chloramines from tap water for use in fish tanks, Conventry, et al. (1935) added thiosulfate.

$$2NA_2S_2O_3 + NH_2Cl + 2H_20 \dashrightarrow$$

$$Na_2S_4O_6 + NH_4OH + NaOH + NaCl \qquad [1]$$

Coventry, et al. (1935) also used several other compounds, with some degree of success. These included $NaHSO_3$, Fe^{++} salts and $CH_2 = CH_2$.

Boiling chloramine solutions for 30 minutes had little effect until acid was added while aeration alone removed some chloramine (Adams and Buswell 1933). The use of activated carbon to remove chlorine has recently been reviewed by Magee (1956). Complete utilization of the sorption properties of activated carbon depends on the concentration of ammonia (Itlina 1958). Elimination of free chlorine by heating was most effective with an iron kettle; 0.4-0.8 mg/l were completely removed by heating to 30-50° (Kikuchi, et al. 1958). Elimination by stirring was not effective; stirring for 30 minutes at 75-300 rpm could reduce the level by about one-half, regardless of the initial concentration (Kikuchi, et al. 1958). The addition of $Na_2S_2O_3$ or $NaHSO_3$ could instantly destroy free chlorine (Kikuchi, et al. 1958). The amount of $Na_2S_2O_3$ required to neutralize 1 mg of chlorine ranges from 1.6 to 3 mg over a pH range of 6.2-9 (Al'terman 1958). The removal of up to 0.5 ppm of free chlorine from tap water over long periods of time was affected by the addition of $Na_2S_2O_3$ (McCauley and Scott 1960). This compound had previously been used only on a short-term basis. The compound was added continuously at a constant flow rate as an aqueous solution. The amount needed was calculated by titrating a known volume of tap water with a standard solution of the reagent. The strength of the reducing solution was calculated on the basis of a maximum concentration of free chlorine of 0.5 mg/l. At this concentration, 1.5 g per day of thiosulfate was required for a water flow of 1.1 liters per minute. No ill effects were observed on sea lampreys, goldfish, brown bullheads, and white suckers held in the treated water for several months. The addition of sodium thiosulfate to effluent removed the residual chlorine, which was tested in laboratory and field conditions (Zillich 1972).

The nature of Cl_2-NH_3 reaction depends on temperature, pH, and concentration of ammonia. At a pH above 8, monochloramine is formed, between pH 3-5, dichloramine is generated, and below pH 3, trichloramine results (Corbett, et al. 1953). At any one pH only two N-chloramine species can be present and the points of intersection are isosbestic (Corbett, et al. 1953).

When chlorine is added to water, it hydrolyzes very quickly to produce hypochlorous acid and hydrochloric acid equation [2] (Mark 1963).

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-, \; K_1 = 3.94 \times 10^{-4} \text{ at } 20\text{-}25° \qquad [2]$$

This reaction is nearly instantaneous (Draley 1972). If the $[H^+]$ concentration is taken as 1×10^{-7}M and the $[Cl^-]$ concentration as 3.81×10^{-4}M, the mean values for sewage effluent, the [HOCl]/[Cl] ratio is constant at 1.03×10^{7}. The hypochlorous acid partially ionizes according to equation [3] (Mark 1963).

$$HOCl \rightleftarrows H^+ + OCl^-, \; K_2 = 3.2 \times 10^{-8} \text{ at } 20\text{-}25° \qquad [3]$$

This gives a $[OCl^-]/[HOCl]$ ratio of 3.2×10^{-1}, also considered a constant because the rate is instantaneous. Thus chlorine water at pH 7 is actually approximately 1 part hypochlorite anion and 3 parts hypochlorous acid with essentially no free chlorine. Ammonia or ammonium ion forms chloramine rapidly with a rate constant of 5.1×10^{6} liter $mole^{-1}$ sec^{-1}, (Morris 1967) equation [4] (Mark 1963).

$$NH_3 + HOCl \rightleftarrows NH_2Cl + H_2O, \; K_3 = 3.6 \times 10^{9} \qquad [4]$$

The reaction between ammonia and hypochlorous acid is not inhibited by the ammonia-ammonium equilibrium which is rapidly reversible. If the NH_3 concentration is 5.9×10^{-4}M, the maximum ammonia concentration in the City of Milwaukee effluent, the $[NH_2Cl]/[HOCl]$ ratio is 2.1×10^{4}. The reaction of monochloramine and hypochlorous acid occurs much slower, with a rate constant of 3.4×10^{2} liter $mole^{-1}$ sec^{-1} (Morris 1967). If the [HOCl] concentration is 2×10^{-5}M, the $[NH_2Cl]/[NHCl_2]$ ratio would be 2.7×10^{1} equation [5] (Draley 1972).

$$NH_2Cl + HOCl \rightleftarrows NHCl_2 + H_2O, \; K = 1.33 \times 10^{6} \text{ at } 20\text{-}25° \qquad [5]$$

Although dichloramine is favored in the Milwaukee effluent from equilibrium considerations, little is formed due to the slow rate, and hence monochloramine is the predominant species.

MATERIALS AND METHODS

All chlorine determinations for the field studies and those made in conjunction with the laboratory bioassay studies were determined using a modification of the amperometric titration procedure described in American Public Health Assn. (1971), with the following exceptions:

(1) a more dilute solution of phenylarsine oxide titrant with a normality of 0.0028 was used;

(2) a mechanical Metrohm piston-type burette to introduce the titrant to the titrating vessel was employed;

(3) a 3-electrode cell (platinum test, platinum counter, saturated calomel reference) connected to a Heath model EUA-19-2-4 polarograph for measuring the current in the titration solution was used; and

(4) a strip-chart recorder was used to record current changes in the solution being titrated.

Using the Metrohm burette and keeping the tip of the delivery tube below the surface of the solution being titrated, it was possible to accurately introduce as little as 0.005 ml of titrant at one time. With the normality of the titrant at 0.0028, 0.005 ml of the titrant was equivalent to 0.005 mg/l residual chlorine. Through the use of the strip-chart recorder, and interpolation, it was possible to determine the endpoint of the titration, to within 0.001 mg/l residual chlorine.

In studies undertaken to examine the effects of chlorine on biological species, it is desirable to distinguish between "free" chlorine and that combined as chloramines. The amperometric titration procedure developed for this study was unable to clearly distinguish between "free" and combined chlorine. The o-tolidine test similarly measures a combination of both "free" and combined chlorine. A method which measures "free" chlorine, in combination with other analytical procedures would provide information on the amount of each chlorine compound present in a solution.

Bauer and Rupe (1971) have developed a method for the analysis of "free" chlorine in the presence of bound forms. Syringaldazine was found to be a chomophoric agent which is sensitive to hypochlorite, but insensitive to combined chlorine such as chloramines. The reagent reacts on a mole-for-mole basis with chlorine. Unfortunately, this photometric method did not work well at low concentrations such as those encountered in the lake and in the laboratory bioassay experiments. Hence, all values expressed here are as total residual chlorine.

The concentration of sulfite used in the bioassay experiments was measured spectrophotometrically using the 5,5' dithiobis-(2-nitrobenzoic acid) method of Humphrey, et al. (1970). A Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer with a tungsten lamp was used at a wavelength of 412 nm with no filter. Five-centimeter pathlength cells were employed. Absorbance readings were converted to sulfite concentrations by the Beer's Law data furnished in Table 1 of Humphrey, et al. (1970). Sulfite concentrations were expressed by convention as sulfur dioxide (SO_2).

Standard Preparation of Chloramine for Chemical Studies

To prepare a 1.5 M solution of sodium hypochlorite, 80 g of sodium hydroxide is dissolved in 200 ml of distilled water, and the mixture is cooled by Dry Ice-acetone bath. After the addition of 200 g of cracked ice, chlorine gas is passed into the solution through an 8 mm glass tube. The chlorine addition is continued until the desired increase in weight (142 g) has been attained or until there is no longer a yellow precipitate of mercuric oxide when a sample of the solution is added to mercuric chloride. When the reaction is completed, the pH should be 7. The solution is diluted to 600 ml and titrated with standard 0.1 N sodium thiosulfate. Caution should be exercised to avoid overchlorination.

In a 1-liter beaker is placed 200 ml of 1.5 M ammonia which is cooled to 0°. After addition of 100 g of cracked ice, 200 ml of 1.5 M sodium hypochlorite is slowly introduced with stirring. This yields chloramine of 0.2 molarity.

Decomposition of Chloramine

A fresh solution of chloramine was equilibriated in a constant temperature bath at 25±1°C. At 6-minute intervals during an 80-minute period, 1 ml aliquots were removed and added to a solution of 40 ml of acetic acid, 10 ml of water, and 2 g of sodium iodide. The concentration was plotted

versus time indicating a decomposition rate of -0.00019 mole per minute at pH 9. This experiment indicated that chloramine is stable enough to permit a study of the rate of reduction with various chemical reducing agents.

Reduction of Chloramine

A fresh solution of chloramine (0.208M) was equilibriated at 25° ± 0.1°. Aqueous solutions (0.2M) of ferrous chloride sodium bisulfite, sodium thiosulfate, sodium sulfite, sodium, metabisulfite, and sulfur dioxide were also maintained at the same temperature. Individual experiments were conducted during which the chloramine solution was added to each of the reducing agents with magnetic stirring. Aliquots (1 ml) were withdrawn as rapidly as possible, timed, and quenched in an acidic KI solution. Titration with thiosulfate was used to determine the concentration of chloramine versus time. In this way, the time required for total reduction of chloramine by each reducing agent was determined.

Kinetic Studies

To study the kinetics of chloramine reduction in the mg/l range, it was necessary to use a much more sensitive analytical procedure. Voltammetry with a platinum electrode was used to obtain current-voltage curves for sodium hypochlorite and monochloramine in the mg/l range in order to determine reduction potentials. With hypochlorous acid at 20 mg/l, buffered at a pH of 7, 0.1 M Na_2SO_4 supporting electrolyte, and with deaeration, two waves resulted with maxima at -0.11 v and -0.51 v vs. S.C.E. (Figure 9). When 0.02% Triton X-100 (a surface active agent) was added, a limiting current was obtained with an $E_{1/2}$=+0.42v vs. S.C.E. (Figure 10). The double wave could not be eliminated with gradual drop-wise addition of Triton X-100. No limiting curve or maxima were obtained with oxygen free 200 ppm monochloramine, buffered at pH 7, 0.1 M K_2SO_4. Again when Triton X-100 was added, a limiting current was obtained with a half wave value of -0.15v (Figure 11). The difference in half wave potentials was great enough to distinguish between the two species (HOCl and NH_2Cl).

The current-voltage curves of hypochlorous acid and monochloramine have been obtained using a rotating platinum electrode (Marks and Bannister 1947), and by the dropping mercury electrode method (Heller and Jenkins 1946). A set of double polarographic waves was obtained with the dropping mercury electrode, which were shown to result from simple chemical reaction at the mercury surface (Jenkins 1951).

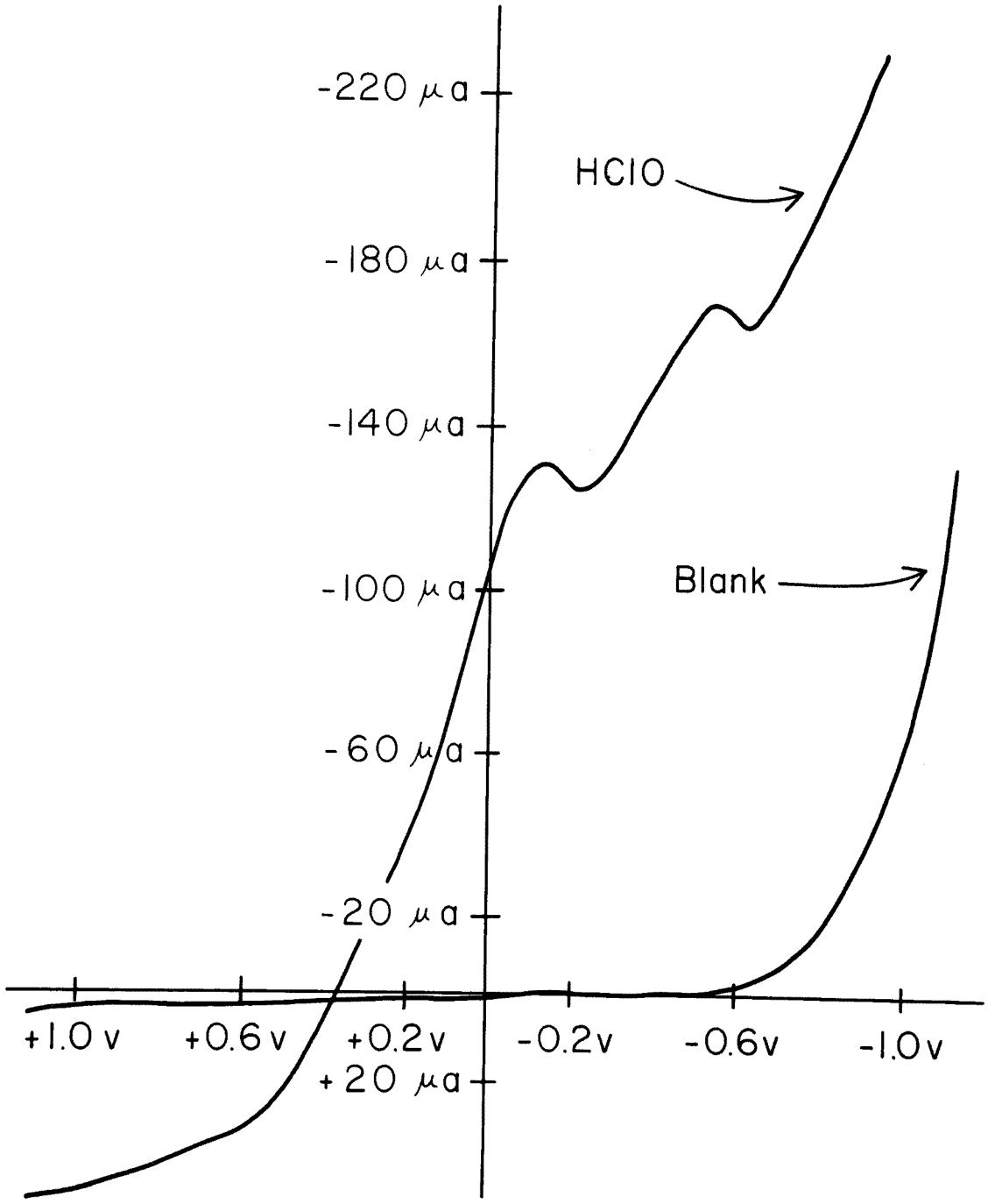

Figure 9. Current-voltage curve of HOCl and blank.

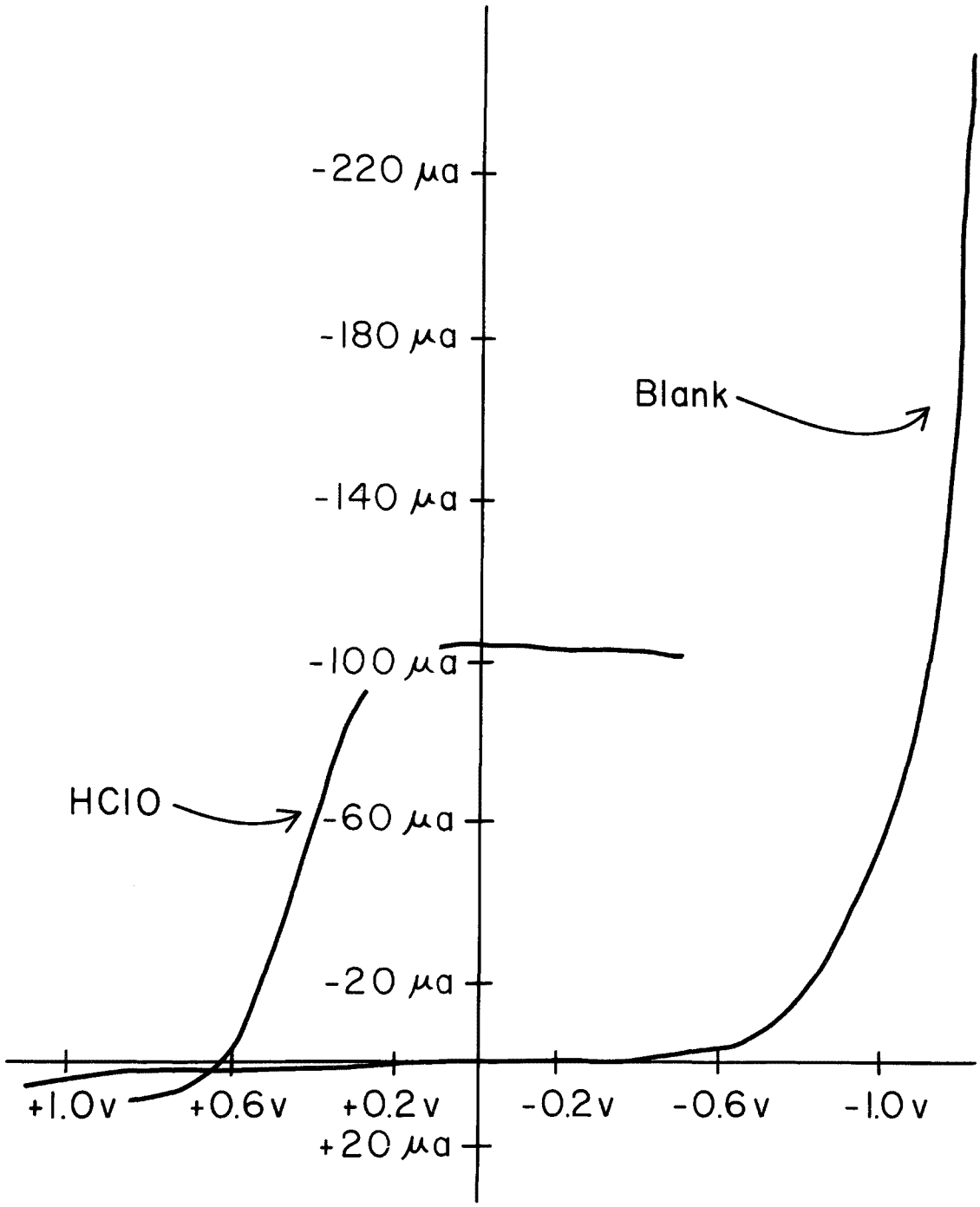

Figure 10. Current-voltage curve of HOCl and blank with Triton X-100 added.

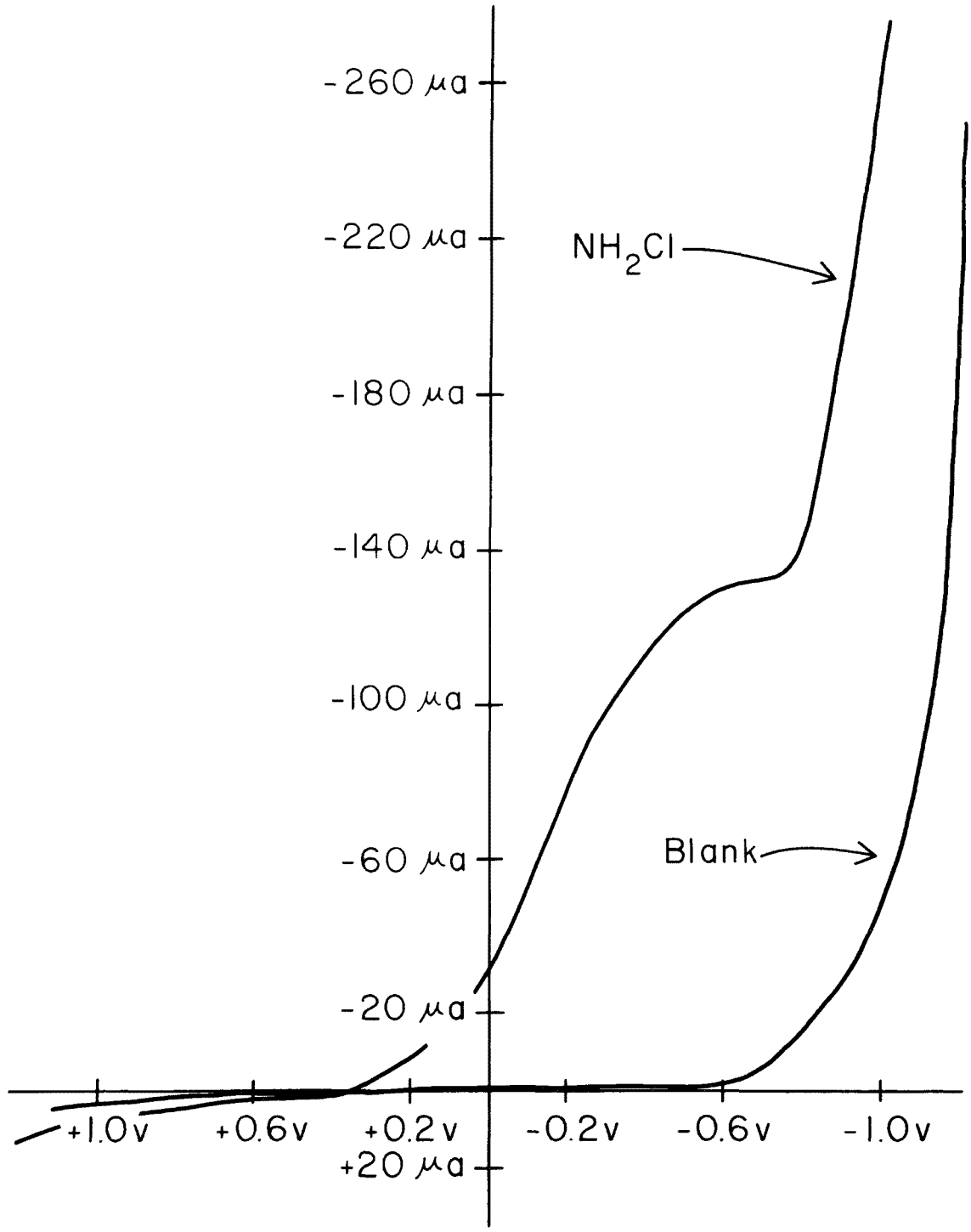

Figure 11. Current-voltage curve of NH_2Cl and blank with Triton X-100 added.

Recently in two papers (Harrison and Khan 1971; Schwarzer and Landsberg 1968) on the reduction of hypochlorous acid at a platinum and a carbon electrode, an oxide film was reported to form in both cases. The oxide on platinum inhibits reduction strongly in the early stages, followed by a relaxation in the oxide structure, after which the inhibition disappears. With the carbon electrode, one part of the oxide layer is reduced with the anion and the rest at a more negative potential. It is believed that the double wave in the current-voltage curve of hypochlorous acid is due to an oxide film on the platinum surface and that, with both hypochlorous acid and chloramine, Triton X-100 prevents formation of the oxide. Since the double wave was not completely eliminated with drop-wise addition of Triton X-100, this suggests there are sites on the surface of the platinum for which oxygen and Triton X-100 are competing.

When a current-voltage curve of Milwaukee sewage effluent was determined, added chloramine could be detected in the ppm range. It appears that the metal cations present are not reduced at this potential. When the current-voltage curve was taken of sodium sulfite, the reducing agent of principal interest, no wave was detected that might be mistaken for the chloramine wave (Figure 12). Also the oxidized product of $SO_3^=$, i.e., $SO_4^=$, does not interfere and is used as a supporting electrolyte.

Voltammetric Limits of Detection

Current-voltage curves were determined for solutions of 20, 10, 5, 1 and 0.5 ppm monochloramine. The plots were used to determine the lower limit of chloramine detectable. To accomplish the lower limit of detection, an electrode pretreatment process was employed. The three-electrode system was placed in the solution, deaerated for 20 minutes with N_2, set at a potential of 0.0v for 30 seconds, 2 drops Triton X-100 (0.02%) added, and deaerated again for 3 minutes with N_2. The current-voltage curves were then determined.

Kinetic studies were done after electrode pretreatment, using 9.6×10^{-5} M (5 mg/l) chloramine solution (0.02% Triton X-100, 0.1 M sodium sulfate, 0.01 M phosphates, pH 7 and under nitrogen). The potential of the polarograph was set on -0.50v on the limiting plateau. A strip-chart recorder monitored current versus time as solid sodium sulfite was added. The recorder followed the chloramine concentration, and resultantly the rate of the reduction reaction. Because the reaction is so fast the instrumentation response time is the limiting factor in precisely determining the rate of reduction.

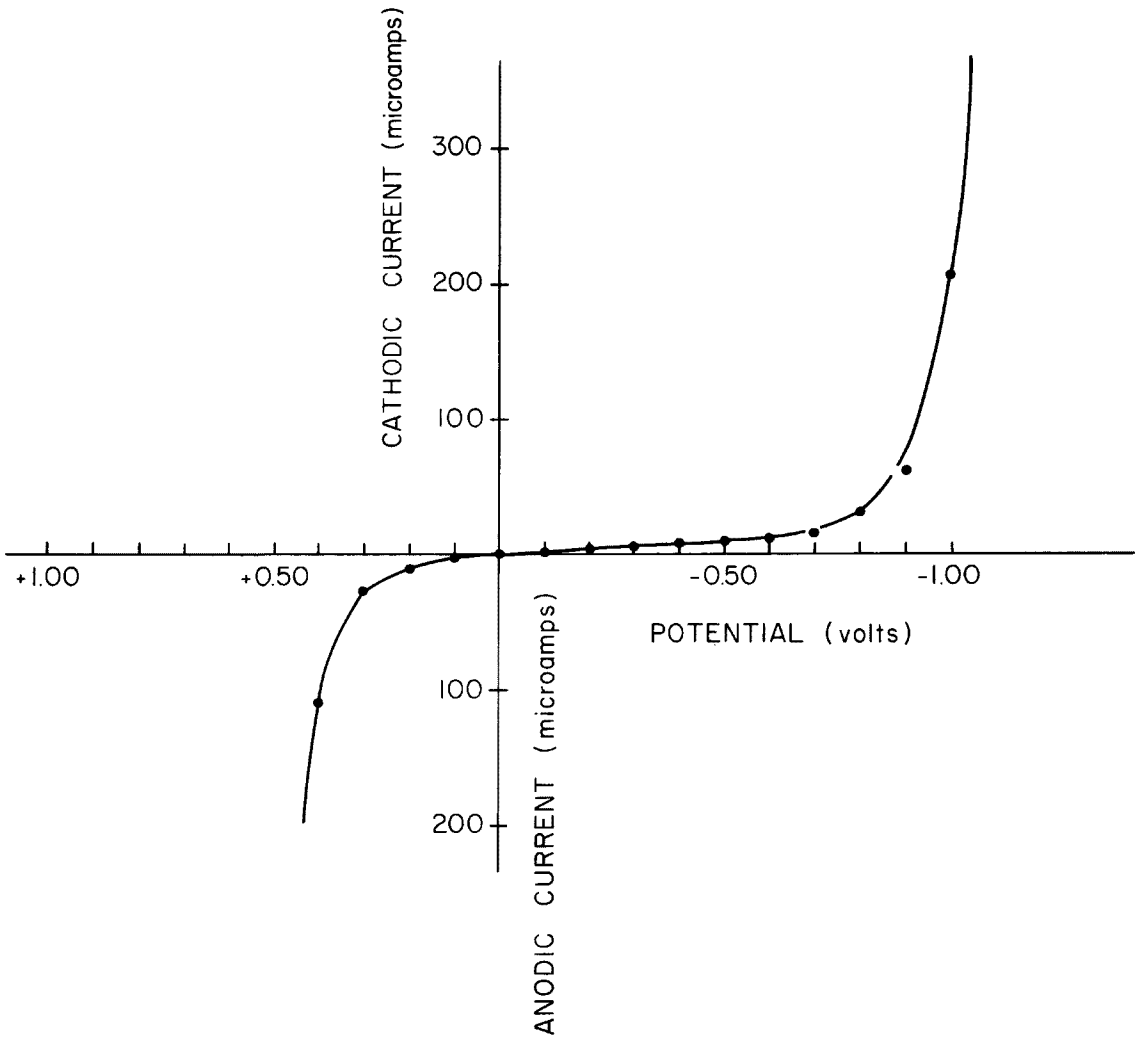

Figure 12. Current-voltage curve for sodium sulfite.

A new approach was tried to determine fast reaction rates. Steady-state systems have often been used to measure rapid rates of reaction. Such a system was constructed (Figure 13). In this system, chloramine was added at various distances from the platinum indicator electrode by an infusion pump. An increase in current showed that chloramine was reaching the indicator electrode before the reduction reaction was complete. Knowledge of rate of addition of chloramine, the stirring rate and distance of the Pt indicator electrode from the point of chloramine entry should allow calculation of the rate constant (k). Once an increase in current was found for a certain rate of addition and distance x, the rate of addition could be decreased to an equilibrium point where the current remained constant. In the final case the rate of addition was proportional to the rate of reaction.

Ninety-five ml of 0.1 M sodium sulfite was placed in the reaction vessel. A solution of 0.05 M chloramine was then forced into the system by the infusion pump through a Teflon tube at various points of entry. The fastest rate of addition was 46.3 ml/min. A synchronous motor stirred the solution at 600 rpm.

RESULTS AND DISCUSSION

Reduction of Chloramine

Ferrous chloride, sodium bisulfite, sodium thiosulfate, sodium sulfite, sodium metabisulfite, and sulfur dioxide all reduced chloramine in less than 60 seconds. With sulfur dioxide, sodium bisulfite, and sodium sulfite, reduction was complete in less than 20 seconds. Sodium nitrate was eliminated as a potential reducing agent since the oxidation product, nitrate, is a nutrient. Ferrous chloride was eliminated because it formed a precipitate. Sodium thiosulfate and sodium metabisulfite were also excluded because of a potentially deleterious effect due to the tetrathionate product. Due to its low cost and efficiency of chloramine reduction, sodium sulfite was the preferred reducing agent.

Voltammetric Limits of Detection

A detectable current-voltage wave was found down to 1 mg/l (1.9×10^{-5} M) (Figure 14). The electrode pretreatment procedure enabled the lower limit to be decreased to 0.5 mg/l (9.6×10^{-6} M) monochloramine (Figure 15). This increase in sensitivity is presumably due to renewal of the electrode surface. Removal of absorbed organics and oxide

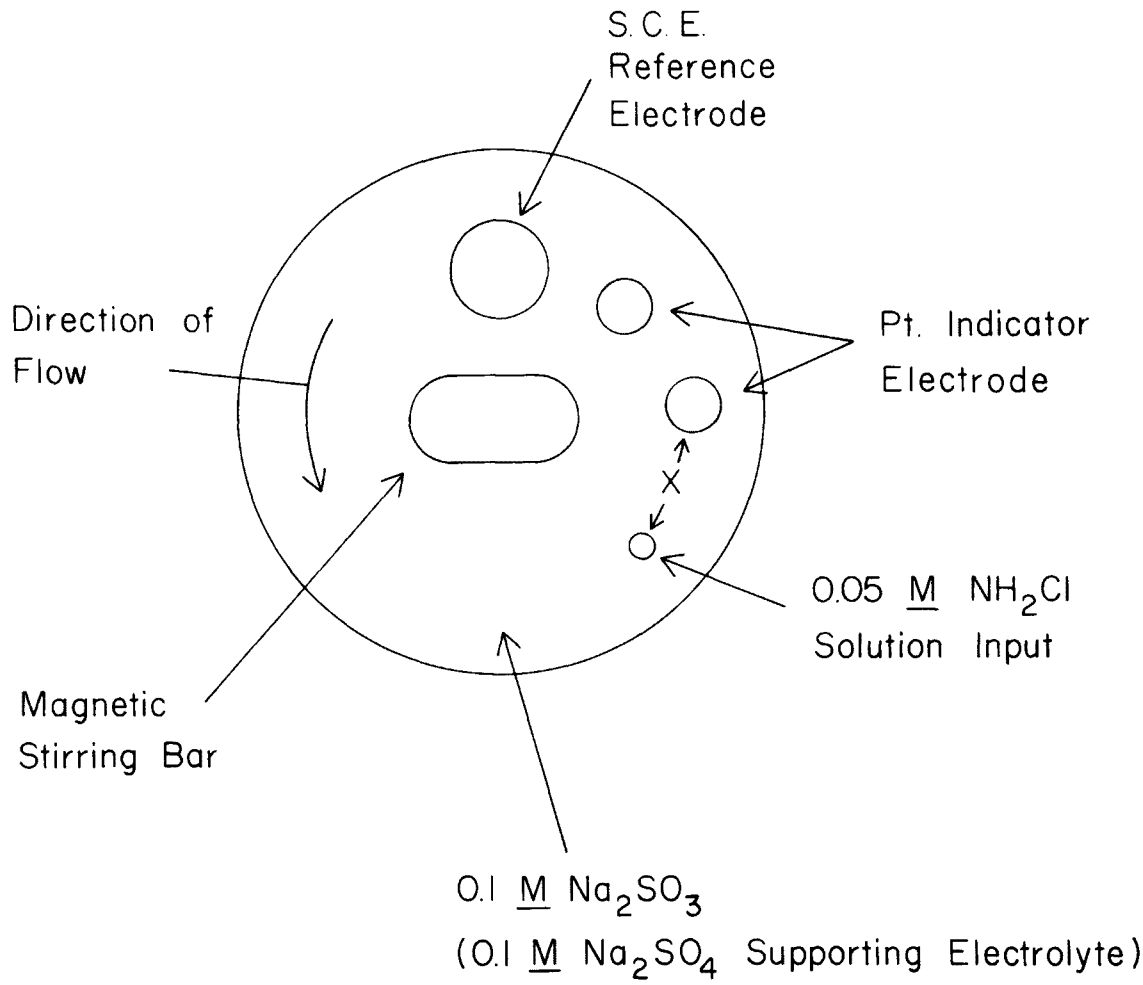

Figure 13. Schematic representation of the three-electrode cell used in obtaining current-voltage curves for chlorine and chloramine.

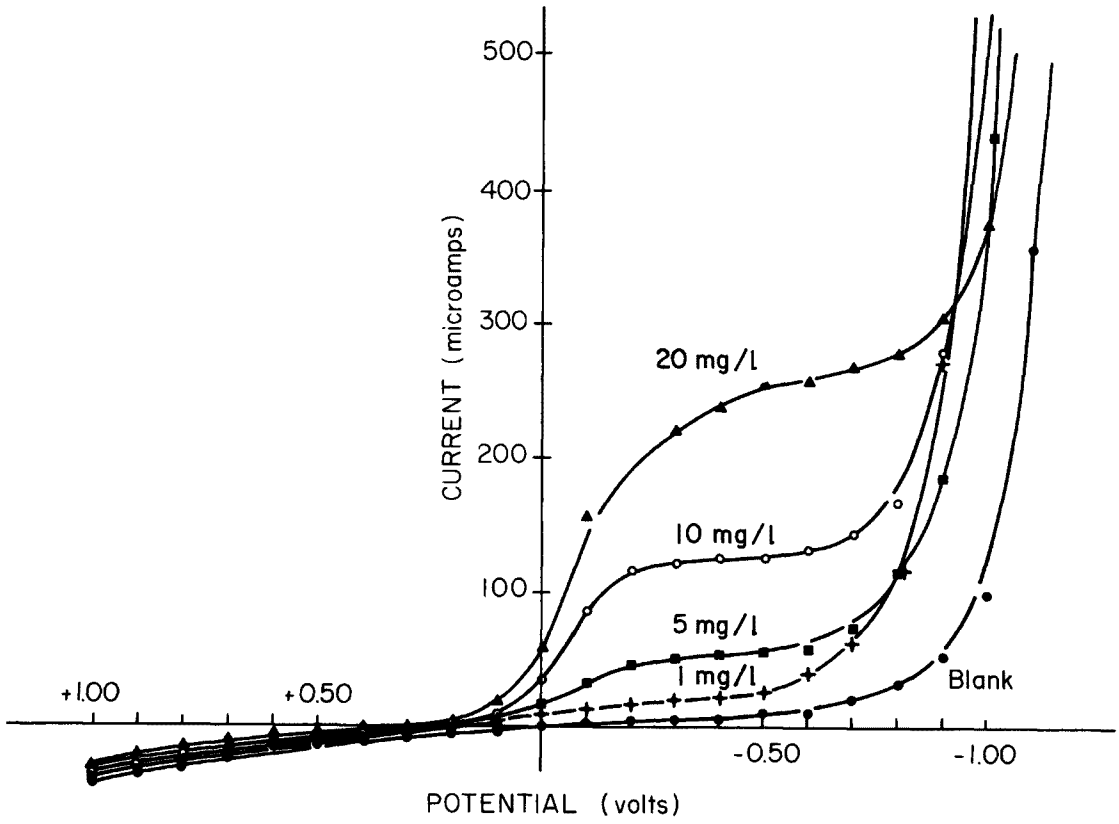

Figure 14. Current-voltage curves for various concentrations of NH_2Cl. Detection limit was 1 mg/l.

formations exposes the active sites on the surface. Figure 16 indicates that at -0.05 volts, on the limiting current plateau, the current is proportional to the chloramine concentration present in solution. On this basis, the monitoring of chloramine concentrations can be done efficiently using voltammetric techniques.

Kinetics of Reduction of Chloramine

Voltammetry was used to monitor the reduction of chloramine by sodium sulfite. Upon the addition of solid sodium sulfite to a 5 ppm chloramine solution the current dropped to zero almost instantaneously. This rapid decrease in current, i.e., extremely rapid reaction, occurred too fast to permit measurement of an absolute rate. An attempt to slow the

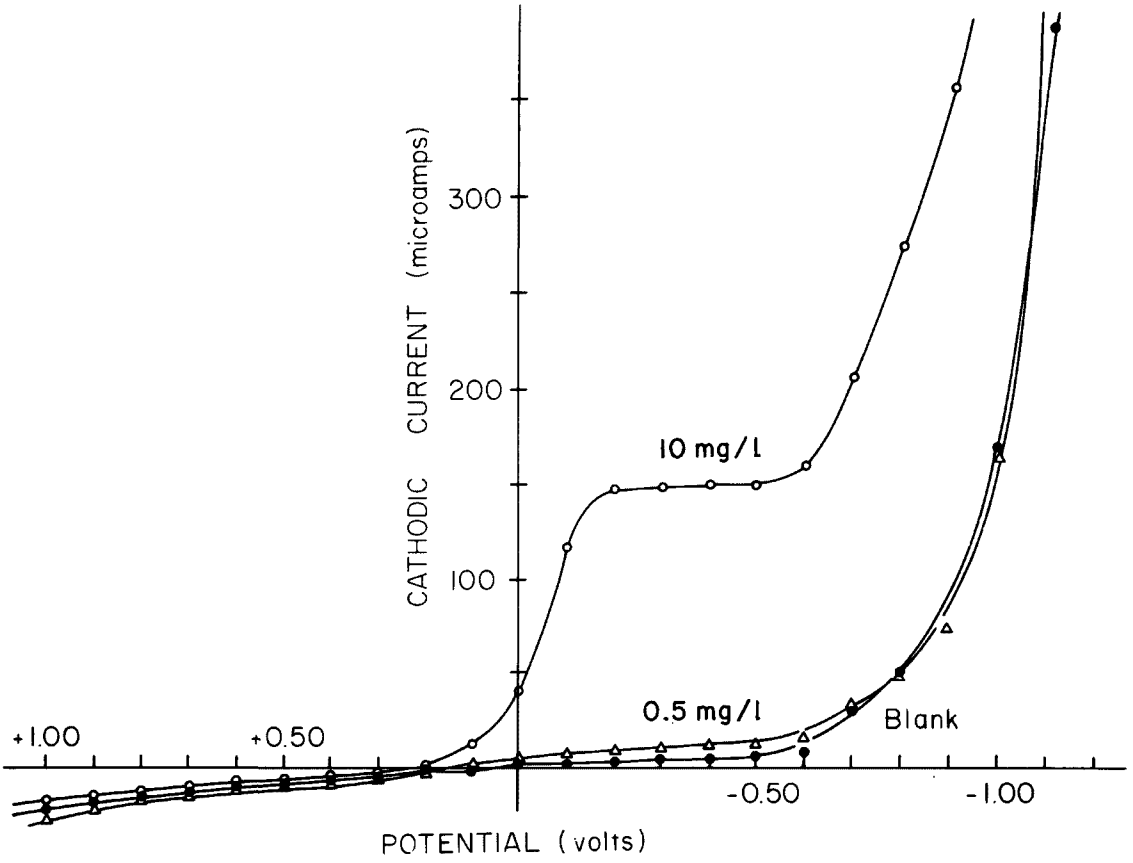

Figure 15. Current-voltage curves for NH_2Cl following electrode pretreatment. Detection limit was 0.5 mg/l.

reaction enough to measure the rate was made by decreasing the temperature. A kinetic run at 0°C displayed the same rapid decrease in current. Because of the fast reaction rate, instrumentation response became the limiting factor. The response specifications for the recorder at 10 in. per sec., allowed a minimum rate constant, k_{min}, to be calculated, namely $k_{min} = 0.40\ sec^{-1}$. The final concentration of sodium sulfite, 9.0×10^{-3} M, was in excess of the chloramine concentration, thus the reaction can be assumed to be zero-order with respect to reducing agent. Assuming the reaction is first-order with respect to chloramine the reaction should follow equation [6].

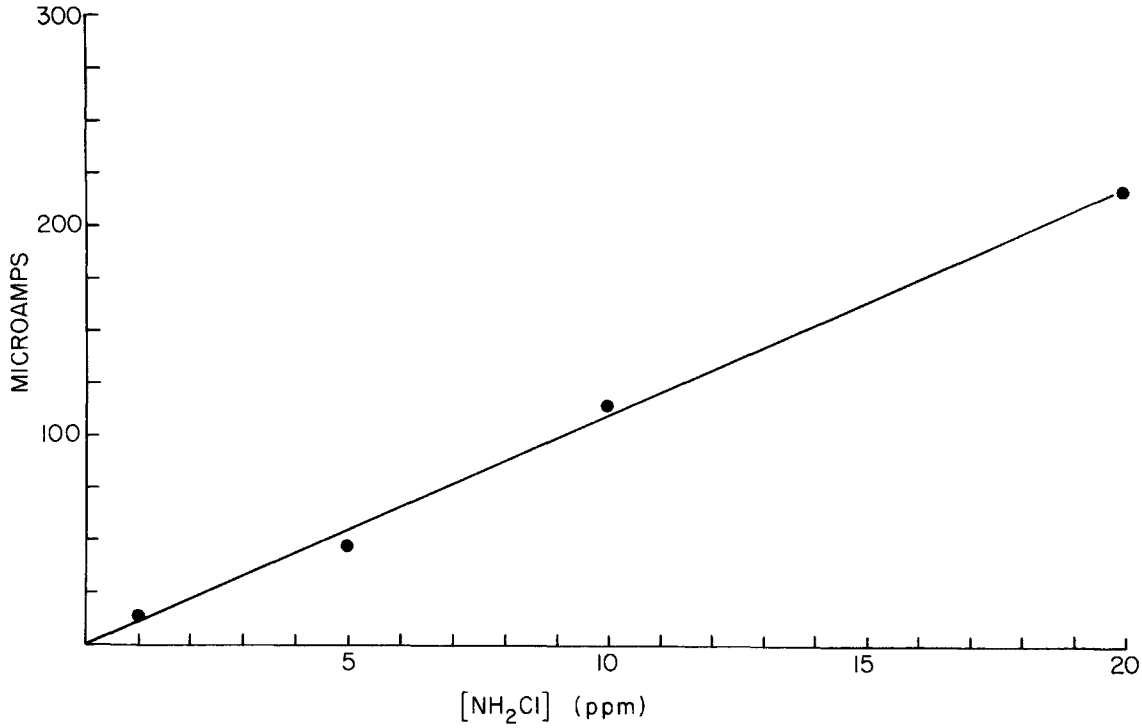

Figure 16. Relationship between current and concentration for NH_2Cl at -0.50 volts.

$$\frac{d[NH_2Cl]}{dt} = -K[NH_2Cl] \qquad [6]$$

The steady-state system (Figure 13) was used to determine the first-order reaction rate constant (k).

When 0.05 M chloramine was added to a solution of supporting electrolyte without reducing agent, the current increased linearly with time. The next experiment involved the addition of chloramine to the reducing agent. A 0.05 M chloramine solution was introduced into a 0.10 M sodium sulfite solution. With the inlet port as close as 10 mm to the indicator electrode and an addition rate at 46.3 ml per second, the maximum rate of the pump, no response was observed. From this data the minimum rate constant was calculated to be $k_{min} = 43\ sec^{-1}$. This value of k_{min} represents a 100-fold increase over the value determined by the earlier method.

The significance of this value is presumably associated with the fast nature of the reaction. It is apparently mass transport limited. Hence mixing is crucial in destruction of chloramine in sewage effluent.

Using this value of the rate constant, k_{min}, and assuming a chloramine concentration of 9.6×10^{-5} M (5.0 mg/l), a half-life value can be calculated using the integrated form of equation [6], i.e., equation [7].

$$\ln[NH_2Cl]_{t=0} - \ln[NH_2Cl]_{t=t_{1/2}} = -k\, t_{1/2} \qquad [7]$$

The half-life calculates to be $t_{1/2} = 0.016$ sec.

Of concern in destruction of chloramine by sodium sulfite is the stability of the reducing agent. Studies on the oxidation of sodium sulfite by dissolved oxygen have been done (Fuller and Crist 1941). The first-order rate constant of oxidation for $[NA_2SO_3] \leq 0.015$ M, saturated with 0_2 at 1 atmosphere was found to be $k_{avg} = 0.013$ sec^{-1} at 25°C (Fuller and Crist 1941). The $k_{min} = 43$ sec^{-1} for reduction of chloramine is ~ 3×10^3 greater, thus added sodium sulfite should react with residual chloramine before any appreciable oxidation by dissolved 0_2 can take place. Trace catalysis by Cu^{2+} was also observed. With $[Cu^{2+}] > 10^{-9}$ M the rate constant was found to be $k_{avg} = 2.5 \times 10^6$ ℓ/mole/sec (Fuller and Crist 1941). However, second-order kinetics are implied by the units and a direct comparison of the values should not be made.

SUMMARY

Sodium sulfite was selected as the preferred reducing agent of chloramine because of its lower relative cost, efficiency and speed of reduction, and lack of reaction products potentially deleterious to the aquatic environment. The minimum first-order reaction rate constant (k_{min}) for the reduction of chloramine by sulfite was determined voltametrically to be 43 sec^{-1}. The half-life of a chloramine solution of 5 mg/l in the presence of sulfite was 0.016 sec. Therefore, complete reduction of chloramine by sulfite in sewage effluents would occur almost instantly.

SECTION VI

BIOASSAY STUDIES

INTRODUCTION

Numerous toxicity tests have estimated the tolerance of certain forms of aquatic life to chlorine residuals (Brungs 1973). Chlorine toxicity tests have been common using fish but aquatic invertebrates have been largely neglected. This study involved two common aquatic invertebrate groups, the rotifers and the cyclopoid copepods, which heretofore have not been studied in chlorine toxicity tests. The purpose of this investigation was to determine the acute toxicity of residual chlorine to these organisms, and to investigate whether the addition of sodium sulfite, a reducing agent, could eliminate or reduce the toxicity of chlorine residuals to the test organisms.

Two test organisms were selected because of their abundance within the study area and their wide distribution throughout the Great Lakes. Previous field work conducted at the Center for Great Lakes Studies indicated that Cyclops bicuspidatus thomasi is one of the more common zooplankton species found in the inshore areas of Lake Michigan. The rotifer, Keratella cochlearis, was shown by Stemberger (1973) to be the most abundant rotifer in the Milwaukee area of Lake Michigan. Another consideration in choosing these test organisms was the fact that both could be maintained in the laboratory without significant mortalities.

METHODS AND MATERIALS

Bioassay experiments were conducted in a continuous flow proportional diluter, modified from the design of Mount and Brungs (1967) (Figure 17). Modifications of the original design were incorporated to overcome the problems of excessive consumption of dilution water, of starting several

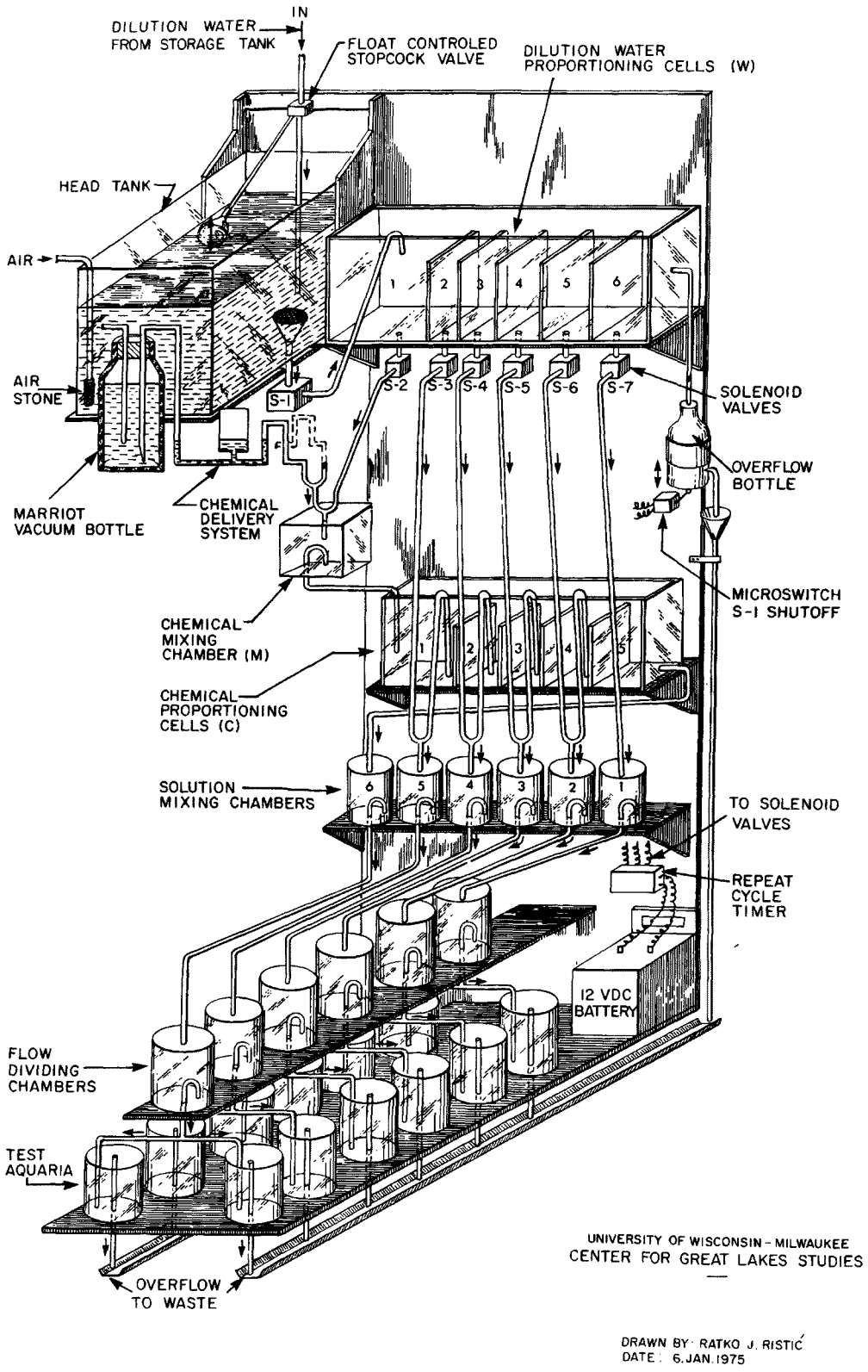

Figure 17. Proportional diluter used for bioassay experiments.

siphons from a single vacuum line, of precisely timing flow rates and the lag time between the synthesis of labile chlorine compounds and their delivery to the test aquaria. All of the "fail-safe" features of the system described by Mount and Brungs were retained in the modified design.

The heart of the modified diluter consisted of a repeat cycle timer run by a chronometrically governed 12 VDC motor. This timer sequentially opened and closed a series of switches, which in turn energized a series of seven 12 VDC solenoid valves. The entire system operated by gravity flow and a 12-volt automobile storage battery. The power supply was totally independent of external power sources and, hence, was not subject to interruption by external power failures. A fully charged 60-amp-hour battery was more than adequate to power the system for the duration of a 96-hour experiment.

Dilution water was collected from Lake Michigan at the City of Milwaukee Linnwood Avenue Water Purification Plant prior to any treatment or chemical additions. The water was transported to the laboratory in 15-gallon FDA food grade polyolefin drums. Water in the drums was filtered through a number 20 Nitex nylon net to remove zooplankton and then pumped into glass-lined steel storage tanks suspended from the ceiling of the laboratory. From the storage tanks, the water flowed into a temperature-controlled environmental chamber housing the bioassay dilution system. A constant head tank received the inflowing water where aeration and temperature equilibration occurred. The temperature of the environmental chamber was maintained at 15°C for all experiments. Light in the chamber was supplied from cool white fluorescent tubes on a 16-8 hour light-dark cycle. The light level at the surface of the test aquaria was between 40 and 60 foot candles.

Once per hour the repeat cycle timer opened a solenoid valve (S-1 Figure 17) allowing water to flow from the head tank through a 20 mesh Nitex filter into the dilution water chambers (W1-6 Figure 17) of the diluter. When the last chamber filled, water overflowed into a bottle which depressed and opened a microswitch which closed the solenoid valve in the inflow line. Water in the overflow bottle slowly drained through a capillary tube allowing the microswitch to close before the next cycle began. The microswitch did not close, however, until the timer switch opened, breaking the circuit to the solenoid valve.

The next valve to open drained dilution water from the W-1 cell through a vacuum venturi which drew the chemical to be tested into the chemical-mixing chamber (M Figure 17) where it was mixed with the dilution water. An overflow siphon in the mixing chamber started as the last portion of dilution water entered, drawing the mixed solution into the chemical cells (C1-5 Figure 17) and to the test aquaria having the greatest concentration of test chemical.

The chemicals were measured and delivered to the mixing chamber via a standpipe siphon tube arrangement described by McAllister, et al. 1972. When dechlorination required the addition of two chemical solutions simultaneously, two such delivery systems were connected to the diluter.

The next timer switch to close opened five valves, simultaneously draining dilution water cells W-2 through W-6. The water flowed through vacuum venturies which siphoned the chemical solution from the chemical cells into mixing chambers where dilution and mixing occurred. The volumes of the dilution water cells and the chemical cells were such that 400 ml of each test concentration was prepared with a 50% dilution between each concentration. The W-6 cell supplied 400 ml of unadultered dilution water for the control aquaria. The solutions flowed from the mixing chambers and were split in two, half the flow going to each of the two test aquaria.

The test aquaria were 2000 ml beakers with central overflow standpipes screened with number 20 Nitex netting (Figure 18). Incoming water entered the beaker at the bottom and flowed out at the surface to enhance mixing. The volume of water in the test aquaria was maintained at 1600 ml for the Cyclops experiments and at 1200 ml for the rotifer tests. With 200 ml of fresh water entering each test aquaria per hour, the flushing time for the test aquaria was once every eight hours for Cyclops runs and once per 6 hours for the rotifer experiments.

The entire dilution system was constructed of glass, silicon rubber, teflon and a small amount of nylon for screening. Double distilled water, reagent grade 5% sodium hypochlorite solution, ammonium hydroxide and sodium sulfite were used to prepare all test solutions. Water chemistry data on the dilution water from Lake Michigan was monitored by the City of Milwaukee at the Linnwood Water Purification Plant.

All of the test organisms used in these experiments were collected from Lake Michigan near Milwaukee. The rotifers for the bioassay experiments were obtained by filtering them

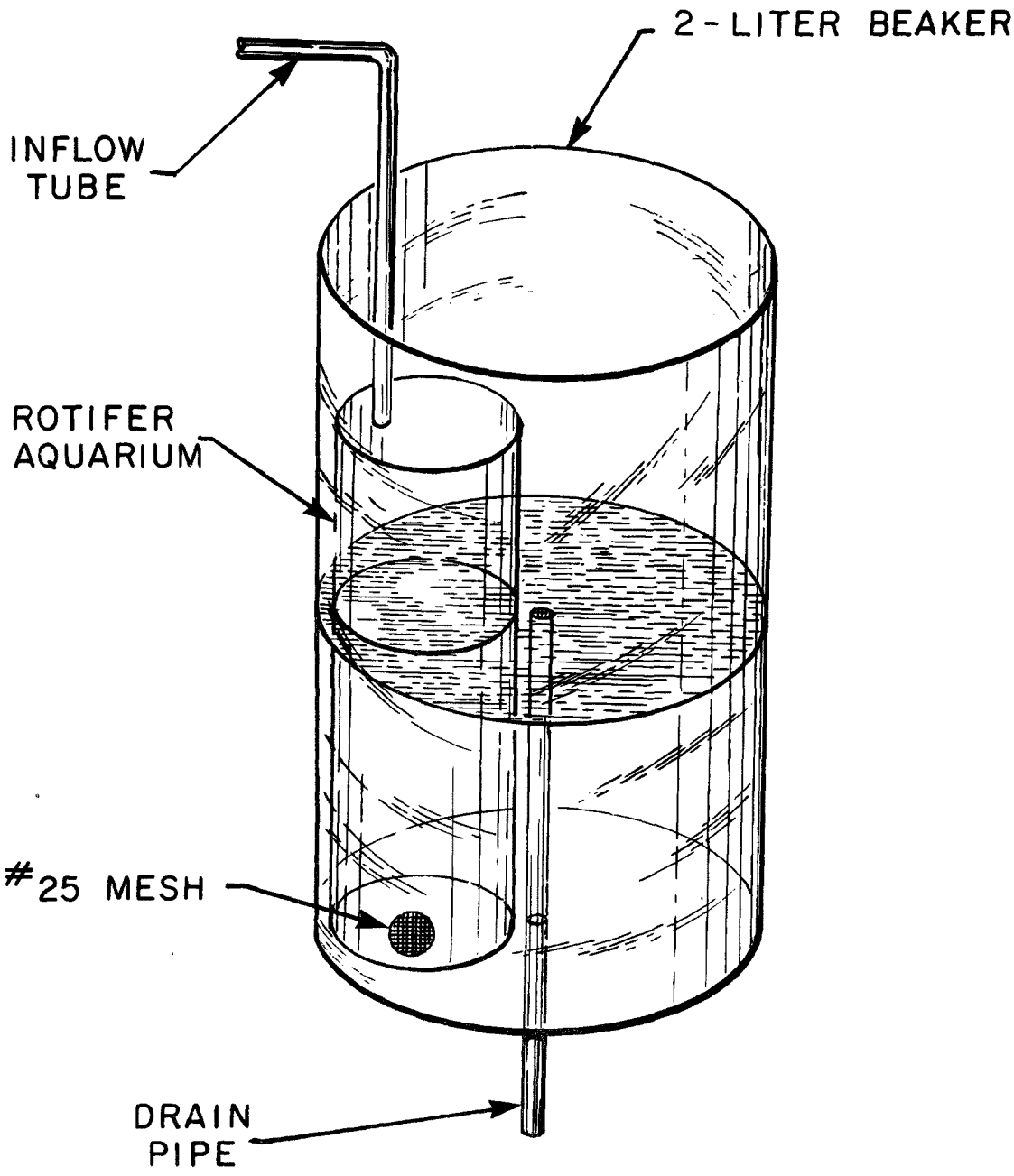

Figure 18. Test aquarium used in bioassay experiments with insert jar used for rotifer tests.

with a number 20 Nitex net from untreated Lake Michigan water taken from the Linnwood Avenue Water Purification Plant. Lake Michigan water entered the purification plant from an intake crib 17 meters below the surface of the lake approximately 1.6 km offshore 6 to 7 km north of Milwaukee Harbor. The filtered water remaining from this filtration process was used as the dilution water in the rotifer bioassay experiments. The rotifers were returned to the laboratory and acclimated overnight in a 4-liter container at the same temperature and lighting conditions which were to be employed during the bioassay exposures.

The copepods used in the bioassay studies were either collected in a manner similar to that described for the rotifers or obtained from a vertical plankton tow taken from the R/V NEESKAY at a point in Lake Michigan 5 km northeast of Milwaukee Harbor. The net used for vertical tows was a one-half meter number 20 mesh Nitex net. The organisms collected from both the vertical tow and from the purification plant were returned to the laboratory and acclimated in the environmental chamber in a 20-liter aquarium for at least 24 hours before the start of a bioassay run.

Prior to the start of each bioassay experiment, 20 actively swimming organisms were pipetted into twelve vials, one for each test aquaria. The distribution of the organisms to the vials was randomized to avoid the aggregation of easily caught individuals in any one test chamber. After all the test organisms were in the vials, they were transferred to their respective test aquaria.

Test organisms were removed from the aquaria at the end of each bioassay by one of two methods. Rotifers were removed and concentrated for observation simply by lifting the small test chamber shown in Figure 18 from the 2000 ml beaker allowing the water to drain through the screened opening near the bottom. The water and organisms remaining in the container were then transferred to a microscope counting chamber for mortality assessment. Copepods were removed from the 2000 ml beakers by gently pouring the contents through a screened jar similar to that used for the rotifer exposure chambers. The organisms concentrated in this manner were then transferred to a microscope counting chamber for mortality assessment. Test organisms were considered dead if they did not swim or exhibit any internal or external movement when examined under 100 X magnification. Occasionally a few animals would be unaccounted for at the time

of examination. It was assumed that these losses occurred in transferring the organisms to or from the test aquaria. The percent mortalities reported were calculated on the basis of the actual number of living and dead organisms observed at the end of each experiment.

Observations of test organism mortality were only made at the termination of each experiment. More frequent observations of mortality would have been desirable but since mortality was determined under the microscope, considerable handling was involved in transferring the organisms from the test aquaria to the observation chamber. It was felt that once the test organisms were removed from the test aquaria they would no longer be fit for further study due to the rigors of handling.

Measurements of total residual chlorine, pH and temperature were made at the start and finish of each 4-hour bioassay and at 24-hour intervals for the duration of the 96-hour bioassay exposures. The dissolved oxygen in the test aquaria was measured periodically to assure that the aerated dilution water was at or near the saturation valve.

Bioassay experiments with the rotifer Keratella cochlearis were terminated at 1, 4, and 24 hours. The 4-hour exposure period was chosen as the best compromise between sufficient exposure time and minimal control mortality which increased with longer exposures. All experiments with Cyclops bicuspidatus thomasi were conducted over a 96-hour exposure period.

The results of the bioassay experiments are reported as the median tolerance limit, or TL_{50}. This value is the estimated concentration at which 50 percent of the test organisms survived for the exposure period indicated. The TL_{50} values for each experiment where mortality was observed were determined by plotting the toxicant concentration on a log scale versus percent mortality on a log-probit scale (American Public Health Assn. 1971). A regression line was then drawn through the points on the graph. The toxicant concentration at which the regression line intersected the 50 percent mortality level was taken to be the TL_{50} value for the experiment.

Chlorine concentrations cited are expressed as total residual chlorine. This includes both free and combined chlorine species which were detectable using an amperometric titration procedure described in the chemistry section of this report. It was not possible to accurately differentiate between free chlorine and combined forms using this technique. Based on the calculated equilibrium concentrations of Draley (1972), it was assumed that monochloramine was the

dominant chlorine constituent present when monochloramine was added as the test chemical. Because of the presence of ammonia in the dilution water, some chloramines were undoubtedly formed when sodium hypochlorite was added as the test chemical. Hence, experiments run adding hypochlorite reflect the effects of the hypochlorite ion and monochloramine. This would be the situation in most natural waters where added chlorine would react with ammonia to yield chloramines. The relative amounts of chlorine and chloramine present would be determined by the pH, ammonia content of the water and the amount of chlorine added (Draley 1972). Since the bioassay experiments were designed to simulate the natural chemical conditions in Milwaukee Harbor and Lake Michigan, it was deemed unsuitable to remove ammonia from the dilution water or to substitute ammonia free water for dilution water obtained from Lake Michigan.

RESULTS

Seventeen duplicate bioassay experiments were performed during the course of this study. Nine 96-hour bioassay experiments were conducted with the copepod Cyclops bicuspidatus thomasi. Three of these were undertaken using sodium monochloramine. One Cyclops bioassay was conducted using sodium sulfite alone, and two experiments using sodium sulfite to reduce monochloramine were completed. Seven 4-hour bioassay experiments were performed with the rotifer Keratella cochlearis. Three of these experiments were run exposing the organism to monochloramine. In addition, one bioassay was undertaken adding sodium sulfite alone, while three dechlorination bioassays were completed using sodium sulfite to reduce monochloramine.

As discussed previously, chlorine concentrations measured in conjunction with the bioassay experiments will be presented as total residual chlorine. The dominant combined chlorine consituent present in the test solutions was monochloramine. No dichloramine or trichloramine was ever detected. Although free chlorine could not be measured accurately, the hypochlorite ion was undoubtedly present in the test aquaria when sodium hypochlorite was added as the test chemical. No attempt was made to calculate the amount of free chlorine present using published equilibrium constants. The uncertainties involved in applying laboratory-derived equilibrium conditions to natural waters overshadowed the value of making such determinations.

Other parameters which could have influenced the toxicity tests, namely dissolved oxygen, pH and temperature, were held relatively constant for all bioassay experiments. The

temperature of the test aquaria was maintained at 15 + 0.5°C for all runs. The pH ranged between 8.1 and 8.5 over the course of all experiments but did not vary more than 0.2 pH unit during any one individual test. Dissolved oxygen was maintained at or near saturation by constant aeration of the incoming dilution water. The saturated value for dissolved oxygen at 15°C is 10.2 mg/l. Frequent measurements of dissolved oxygen in the test aquaria were always within a few tenths of this value. Specific values for pH measurements are presented in Appendix C in conjunction with the results of the individual bioassay experiments. A summary of the water quality characteristics of the dilution water obtained from the Linnwood Avenue Water Purification Plant is contained in Table 4.

The results of the three bioassay experiments exposing Cyclops bicuspidatus thomasi to residual chlorine added as sodium hypochlorite are contained in Tables 1-3 Appendix C. The 96-hour TL_{50} value derived by pooling the results of the three experiments was 0.069 mg/l total residual chlorine (Figure 19). No mortalities were observed after exposures of 96 hours at total residual chlorine concentrations below 0.01 mg/l. Nearly complete mortality was observed at concentrations above 0.20 mg/l total residual chlorine after 96 hours of exposure. The scatter of points observed on Figure 19 may be due in part to changing chlorine equilibrium conditions between individual bioassay experiments. Test solutions with varying proportions of free and combined chlorine species could possibly account for the variability observed. Under more defined chemical conditions where monochloramine predominated, much less variability was seen.

Tables 4-7 Appendix C contain the results of the four Cyclops bioassay experiments run adding monochloramine as the test chemical. The 96-hour TL_{50} value calculated from the pooled data from the three experiments with monochloramine is 0.084 mg/l total residual chlorine (Figure 20). No mortality was observed below 0.01 mg/l total residual chlorine while nearly complete mortality would be expected to occur at concentrations above 0.30 mg/l total residual chlorine following exposures of 96-hour duration.

Bioassay experiments to determine the toxicity of sodium sulfite to Cyclops bicuspidatus thomasi indicated that sodium sulfite was not toxic at maximal concentrations ranging between 0.510 and 0.637 mg/l for a 96-hour exposure period. Mortalities observed within this concentration range and lower were equal to or less than mortalities

Table 4. AVERAGE CHEMICAL AND PHYSICAL ANALYSES OF UNTREATED LAKE MICHIGAN WATER FROM THE LINNWOOD AVENUE WATER PURIFICATION PLANT, MILWAUKEE, FOR 1973.

Parameter	Concentration (mg/l)
Dissolved Solids	160
Suspended Solids	12
Total Solids	172
Turbidity - J. T. U.	5.5
Bicarbonate Alkalinity - as $CaCO_3$	108
Carbonate Alkalinity - as $CaCO_3$	0
Non-carbonate Hardness - as $CaCO_3$	28
Total Hardness - as $CaCO_3$	136
Chlorides (Cl^-)	9.1
Free Ammonia (as N)	0.029
Sulfate (SO_4)	21.1
Chemical Oxygen Demand	7
pH	8.17

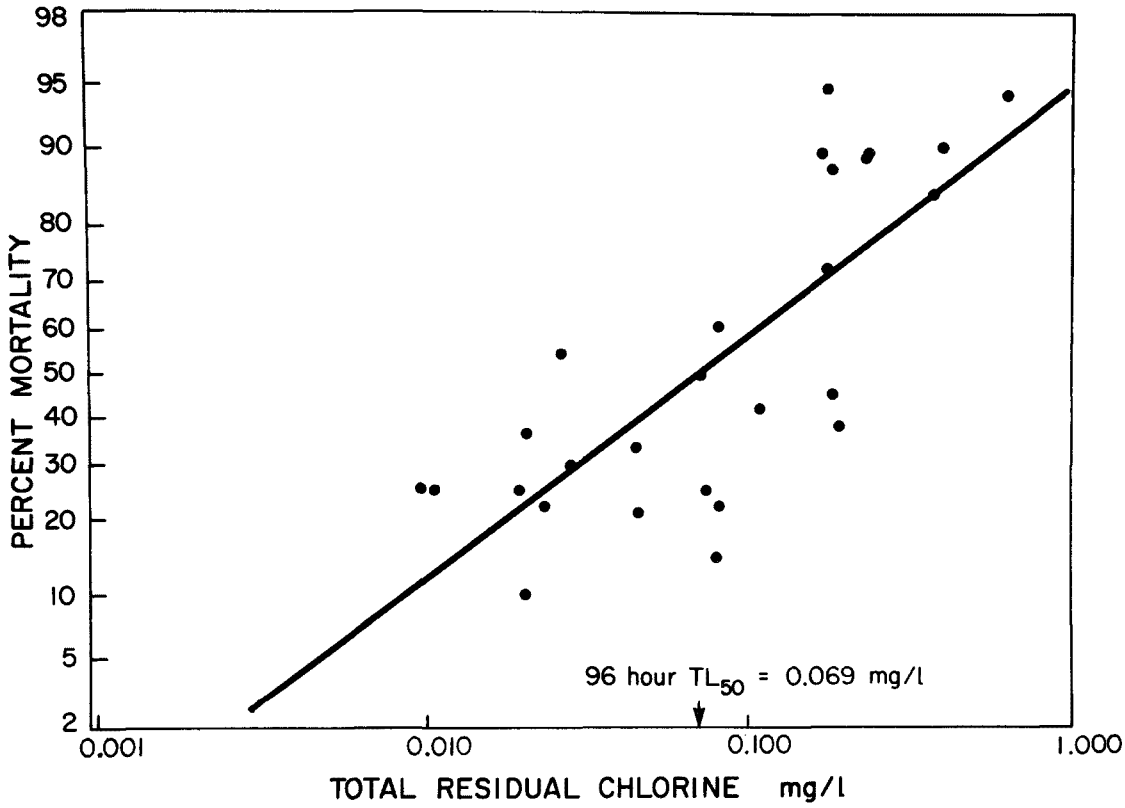

Figure 19. The toxicity of residual chlorine, as sodium hypochlorite, to Cyclops bicuspidatus thomasi during 96-hour exposures at 15°C.

observed in control organisms (Table 8 Appendix C). The highest concentration of sulfite used for this bioassay was adequate to reduce chlorine residual levels commonly observed at sewage plant outfalls.

Preliminary Cyclops bioassay experiments using sodium sulfite to reduce chlorine residuals failed to protect the organisms over the entire 96-hour exposure period although some reduction in mortality was noted. It was later found that the strength of stock sulfite solution added to the bioassay system degraded quite rapidly, thereby losing its ability to reduce chlorine. If fresh sulfite solutions

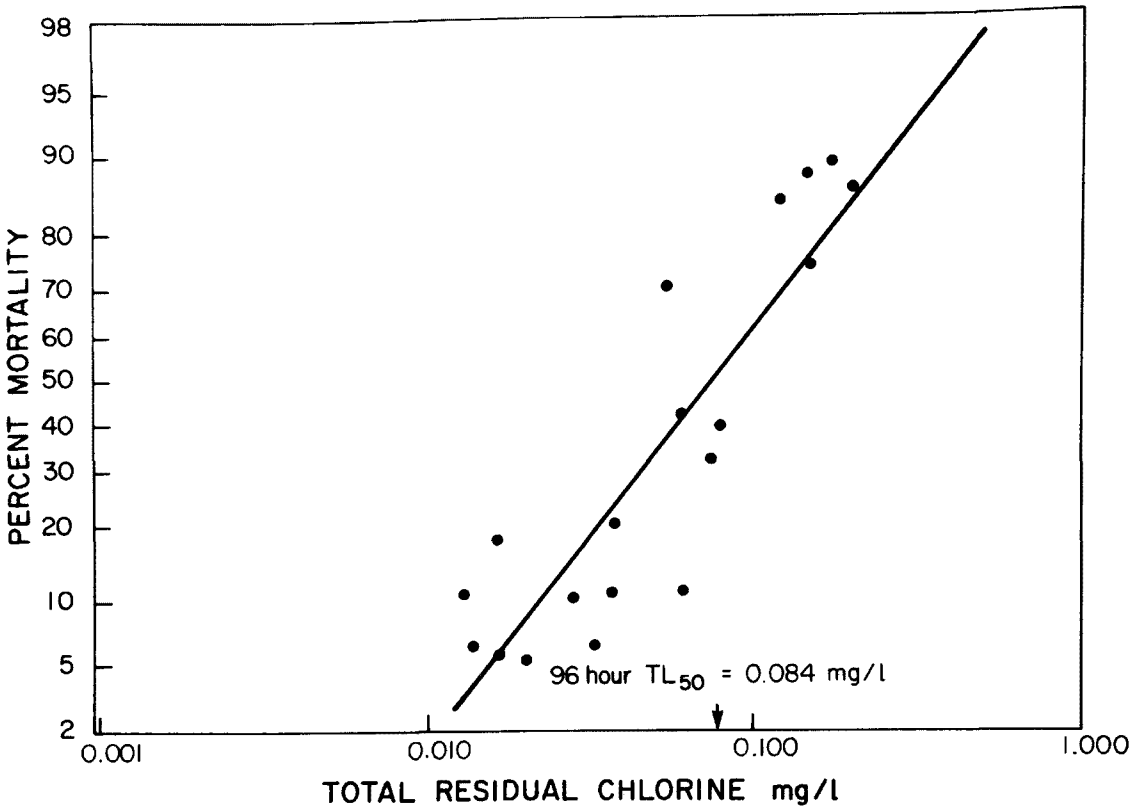

Figure 20. The toxicity of residual chlorine, as monochloramine, to Cyclops bicuspidatus thomasi during 96-hour exposures at 15°C.

were prepared daily, chlorine residuals were reduced or eliminated and the test organisms survived the 96-hour exposure.

Tables 9 and 10 Appendix C contain the results of the successful sodium sulfite dechlorination bioassay experiments. Monochloramine was added to the chemical-mixing chamber, as was done for experiments previously described, at levels sufficient to produce a range of concentrations in the test aquaria between 0 and 1.0 mg/l had dechlorination not been employed. Simultaneously, an equimolar concentration of sodium sulfite was added to the chemical-mixing chamber which completely reduced the monochloramine. After an exposure of 96 hours to the sulfite dechlorinated water the mortalities of Cyclops observed in the test aquaria were equal to or less than those experienced by the control organisms.

Results of the three 4-hour Keratella cochlearis bioassay experiments with monochloramine are contained in Tables 11-13 Appendix C. The 4-hour TL_{50} value derived by pooling the results of the three experiments was 0.019 mg/l total residual chlorine (Figure 21). Residual chlorine concentrations above 0.05 mg/l produced nearly complete mortality after a 4-hour exposure. No mortalities were recorded below 0.003 mg/l total residual chlorine following a 4-hour exposure period.

Bioassay experiments exposing Keratella to sodium sulfite concentrations as high as 0.821 mg/l for 4 hours did not result in any significant mortalities in the test aquaria in comparison to the controls (Table 14 Appendix C).

Having established that sodium sulfite by itself was not toxic to Keratella at levels sufficient to reduce chlorine residuals observed at sewage outfalls, three 4-hour dechlorination bioassays were undertaken. Monochloramine was added to the chemical-mixing chamber of the diluter at a level sufficient to produce a range of concentrations in the test aquaria between 0 and 1.0 mg/l total residual chlorine had dechlorination not been employed. An equimolar amount of sodium sulfite was added to the mixing chamber simultaneously with the monochloramine to reduce the chlorine residual. The results of the dechlorination bioassay experiments with Keratella are contained in Tables 15-17 Appendix C. Sodium sulfite effectively eliminated the residual chlorine toxicity to Keratella. The highest mortality observed in any test aquaria over the entire series of experiments was only 11.8%.

DISCUSSION

The 96-hour TL_{50} values calculated for Cyclops bicuspidatus thomasi and Keratella cochlearis indicate that these invertebrates are among the more sensitive organisms with respect to the acute toxicity of chlorine residuals. Table 5 compares these tolerance limits with those for several other aquatic species. The 0.084 mg/l 96-hour TL_{50} value reported here for Cyclops exposed to monochloramine is within the lower range of TL_{50} values reported by Arthur (1972) as cited in Brungs 1973 for the scud Gammarus pseudolimnaeus and the stonefly Acroneuria lycorias exposed to chlorinated sewage for seven days. The 0.019 mg/l 4-hour TL_{50} value reported here for Keratella exposed to monochloramine is well below any TL_{50} values reported in the literature for aquatic organisms.

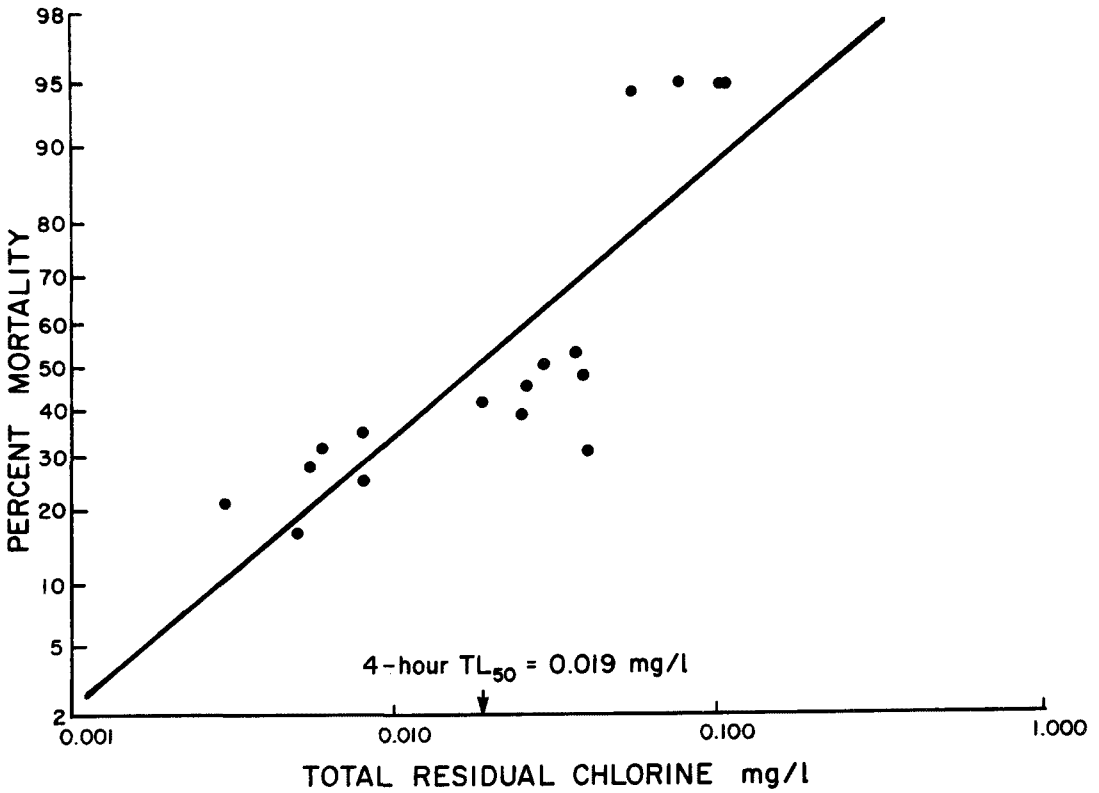

Figure 21. The toxicity of residual chlorine, as monochloramine, to Keratella cochlearis during 4-hour exposures at 15°C.

The lower 96-hour TL_{50} value of 0.069 mg/l calculated for Cyclops exposed to hypochlorite together with monochloramine suggests that free chlorine may be somewhat more toxic to this species than monochloramine alone. It is difficult to make a more definitive statement in this situation without knowing the exact quantities of the various active chlorine species which were present during the tests. This observation is consistent with findings summarized by Brungs (1973). Brungs (1973) notes that "the toxicities of the principal components of residual chlorine (free chlorine, dichloramine and monochloramine) are not sufficiently different to preclude using a measure of residual chlorine to define acute toxicity." He goes on to state: "When a high percentage of residual chlorine exists as free chlorine, toxicity will be greater and its effect will occur more quickly."

Table 5. SUMMARY OF EFFECTS OF RESIDUAL CHLORINE ON AQUATIC LIFE (ADAPTED FROM BRUNGS 1973).

Species	Effect endpoint	Time	Residual chlorine conc. (mg/l)	Reference
Brook trout	TL_{50}	7-day	0.083	Arthur, 1972
Rainbow trout	TL_{50}	7-day	0.08	Merkens, 1958
Coho salmon	TL_{50}	7-day	0.083	Arthur, 1972
Fathead minnow	TL_{50}	1-hour	0.79	" "
Fathead minnow	TL_{50}	12-hour	0.26	" "
Fathead minnow	TL_{50}	7-day	0.082-0.115	" "
Largemouth bass	TL_{50}	1-hour	0.74	" "
Largemouth bass	TL_{50}	12-hour	0.365	" "
Largemouth bass	TL_{50}	7-day	0.261	" "
Yellow perch	TL_{50}	1-hour	0.88	" "
Yellow perch	TL_{50}	12-hour	0.494	" "
Yellow perch	TL_{50}	7-day	0.205	" "
Daphnia magna	Safe Concentration	--	0.003	Arthur, 1972
Gammarus pseudolimnaeus	TL_{50}	96-hour	0.220	Arthur and Eaton, 1971
Cyclops bicuspidatus thomasi	TL_{50}	96-hour	0.069-0.084	This report
Keratella cochlearis	TL_{50}	4-hour	0.019	" "

The criteria proposed by Brungs (1973) and The National Academy of Sciences (1972) for continuous chlorine application are supported by the results of the bioassay experiments reported here. A total residual chlorine concentration of 0.01 mg/l would appear to protect most adult Cyclops bicuspidatus thomasi while producing some mortalities of Keratella cochlearis. A 0.002 mg/l total residual chlorine concentration applied continuously would appear to be adequate to protect the adults of both species tested although the safety factor for Keratella would be minimal. Additional research will be required to determine if the TL_{50} values calculated for the adults of these species would permit reproduction to occur and the subsequent development of pre-adult life stages.

The reduction of chlorine residuals with sodium sulfite appears to be a viable means of protecting aquatic life from the adverse effects of chlorinated effluents. Sodium sulfite itself was not toxic to either of the test organisms used in the bioassay experiments reported here when applied at levels sufficient to reduce chlorine residuals observed in the field. Sodium sulfite preferentially reacts with residual chlorine constituents over dissolved oxygen and hence would not increase the oxygen demand of an effluent if added in concentrations equivalent to the residual chlorine present. The reaction products resulting from residual chlorine reduction are common constituents in most freshwaters which should not pose a serious threat to the aquatic environment. Additional research should be undertaken to document this under varying field conditions.

SUMMARY

The 96-hour TL_{50} values calculated for Cyclops bicuspidatus thomasi exposed to chlorine residuals added as monochloramine was 0.084 mg/l total residual chlorine. The 96-hour TL_{50} value for this species exposed to a combination of free chlorine and monochloramine was 0.069 mg/l total residual chlorine.

The 4-hour TL_{50} value calculated for Keratella cochlearis exposed to chlorine residuals added as monochloramine was 0.019 mg/l total residual chlorine.

Sodium sulfite was not toxic to either Cyclops bicuspidatus thomasi when exposed to concentrations as high as 0.637 mg/l for 96 hours or to Keratella cochlearis exposed to levels up to 0.821 mg/l for four hours.

The addition of sodium sulfite to solutions containing up to 1.0 mg/l total residual chlorine effectively reduced the chlorine residual and eliminated its toxicity to both test organisms.

SECTION VII

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SECTION VIII

PUBLICATIONS RESULTING FROM THIS PROJECT

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3. Brooks, A. S., N. E. Grossnickle and A. M. Beeton. 1975. The acute toxicity of residual chlorine to the copepod Cyclops bicuspidatus thomasi and the rotifer Keratella cochlearis. Submitted to Journal of the Fisheries Research Board of Canada.

4. Grossnickle, N. E., A. S. Brooks and A. M. Beeton. 1975. Reduction of residual chlorine toxicity to the copepod Cyclops bicuspidatus thomasi and the rotifer Keratella cochlearis with sodium sulfite. Submitted to Journal of the Water Pollution Control Federation.

SECTION IX

APPENDICES

APPENDIX A

FIELD STUDIES

Table A-1. ABUNDANCE OF BENTHIC INVERTEBRATES, INDIVIDUALS/m^2, AT STATIONS SAMPLED 7 JUNE 1971, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRAB SAMPLES, FOLLOWED BY THE STANDARD ERROR OF THE MEAN. Presence (+) or absence (0) of nematodes is indicated.

Station	Oligochaeta	Hirundinea	Gastropoda	Pelycpoda	Amphipoda	Isopoda	Chironomidae	Nematoda
1	146357±4660							+
2	164242±7037							+
3	84949±7766	6±6						+
4	68590±4200	6±6	13±13	1621±83			82±35	+
5	203±44			57±22				+
6	70±17							0
7	38±25							0
8	101±28				6±6			0
9	57±22							0
10	0							0
11	1545±290	6±6		19±11		114±105	424±89	+
12	0							0
13	138244±5089		63±39	38±29			95±22	+
14	70±6			32±32				+
15	33662±1662	6±6		281±45				+
16	133285±12191			722±140				+

Table A-2. ABUNDANCE OF BENTHIC INVERTEBRATES, INDIVIDUALS/m^2, AT STATIONS SAMPLED 6 AND 7 JULY 1971, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRAB SAMPLES, FOLLOWED BY THE STANDARD ERROR OF THE MEAN. Presence (+) or absence (0) of nematodes is indicated.

Station	Oligochaeta	Hirundinea	Gastropoda	Pelycpoda	Amphipoda	Isopoda	Chironomidae	Nematoda
1	150258±18950							+
2	160233±15134							+
3	101732±7487	19±11		51±28			13±13	+
4	68400±6349	19±11		1336±158			139±61	+
5	329±63			19±11				0
6	89±63				25±17			0
7	25±25							0
8					108±90	38±19	25±17	0
9	76±11					127±72	82±13	0
10	1697±199		6±6	13±6	32±13	203±102	323±29	0
11	1672±48			44±25	32±17	6±6	462±77	+
12	139±10	19±11			38±11			0
13	127186±14776		25±6	513±134			72±146	+
14	89±42	25±17		19±11	6±6		82±23	+
15	34953±11166			450±125				+
16	136813±9789	25±11		773±121				+

Table A-3. ABUNDANCE OF BENTHIC INVERTEBRATES, INDIVIDUALS/m^2, AT STATIONS SAMPLED 1 DECEMBER 1971, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRAB SAMPLES, FOLLOWED BY THE STANDARD ERROR OF THE MEAN. Presence (+) or absence (0) of nematodes is indicated.

Station	Oligochaeta	Hirundinea	Gastropoda	Pelycpoda	Amphipoda	Isopoda	Chironomidae	Nematoda
1	165414±9589							+
2	173008±4748							+
3	86178±10519							+
4	73251±6974			2014±177			38±11	+
5								+
6	215±94				6±6			0
7	19±11							0
8	196±45						6±6	0
9	6±6							0
10	1596±900				6±6			+
11	1418±130			44±28			139±28	+
12	63±35							0
13	143874±4749		13±13	184±34			42±19	+
14	89±39							+
15	31812±3078			329±96				+
16	145280±6418	13±6		773±45				+

Table A-4. ABUNDANCE OF BENTHIC INVERTEBRATES, INDIVIDUALS/m^2, AT STATIONS SAMPLED 17 APRIL 1972, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRAB SAMPLES, FOLLOWED BY THE STANDARD ERROR OF THE MEAN. Presence (+) or absence (0) of nematodes is indicated.

Station	Oligochaeta	Hirundinea	Gastropoda	Pelycpoda	Amphipoda	Isopoda	Chironomidae	Nematoda
1	147731±5665							+
2	119314±4880							+
3	83726±13321						6±6	+
4	52022±5194		32±32	1659±221			171±58	+
5	127±63						19±19	0
6	51±17						19±11	0
7	6±6							0
8	19±19							0
9	101±35						51±28	0
10	2128±320						6±6	0
11	1393±139				13±13	9±6	432±116	+
12								0
13	11046±8031	6±6		386±121			51±6	+
14								0
15	12103±2141		6±6	108±56				+
16	33662±1662		6±6	609±134				+

Table A-5. ABUNDANCE OF BENTHIC INVERTEBRATES, INDIVIDUALS/m^2, AT STATIONS SAMPLED 20 JUNE 1972, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRAB SAMPLES, FOLLOWED BY THE STANDARD ERROR OF THE MEAN. Presence (+) or absence (0) of nematodes is indicated.

Station	Oligochaeta	Hirundinea	Gastropoda	Pelycpoda	Amphipoda	Isopoda	Chironomidae	Nematoda
1	151145±8873							+
2	112480±3923							+
3	72783±7378							+
4	55436±5648			1545±204			203±110	+
5	228±94				6±6			0
6	19±11				6±6	44±44	13±13	0
7	38±29					6±6	6±6	0
8	89±50				13±13	25±19	6±6	0
9	158±61					44±44	95±22	0
10	2261±378				6±6	32±17	19±19	0
11	1995±295						241±84	+
12	25±17				6±6			+
13	99649±5090		6±6	336±92			19±11	+
14	19±11	6±6					25±17	+
15	13072±1889	19±11	19±19	133±58				+
16	31863±7422			558±155				+

Table A-6. ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 7 JUNE 1971, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 1	2	3	4	5
Stylodrilus heringianus					89 (44)
Dero digitata				686 (1)	
Limnodrilus cervix					
L. cervix-claparedeanus		4927 (3)	5097 (6)	10289 (15)	
L. hoffmeisteri	23417 (16)	52557 (32)	22936 (27)	15090 (22)	51 (25)
Peloscolex multisetosus multisetosus			38159 (45)	20577 (30)	
Tubifex tubifex	54152 (37)	26279 (16)	5946 (7)	4801 (7)	
Undetermined immature					
With hair chaetae	54152 (37)	11497 (7)	1757 (2)	4116 (2)	
Without hair chaetae	13172 (9)	68982 (42)	14441 (17)	13032 (19)	63 (31)
Total	146357±4660	164242±7037	84949±7766	68590±4200	203±44
Probable L. hoffmeisteri	25%	74%	44%	41%	56%
Probable T. tubifex	74%	23%	9%	9%	
Probable No. species	2	3	4	5	2
No. specimens examined	86	74	86	114	32

Table A-6. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 7 JUNE 1971, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, and the total number of specimens examined.

Taxon	Station 6	7	8	9	10	11
Stylodrilus heringianus	57 (82)	38 (100)	88 (87)	57 (100)		325 (11)
Dero digitata						
Limnodrilus cervix						
L. cervix-claparedeanus						
L. hoffmeisteri	13 (18)					371 (24)
Peloscolex multisetosus multisetosus						
Tubifex tubifex						201 (13)
Undetermined immature						
With hair chaetae						77 (5)
Without hair chaetae			13 (13)			572 (37)
Total	70±17	38±25	101±28	57±22		1545±290
Probable *L. hoffmeisteri*			13%			61%
Probable *T. tubifex*						18%
Probable No. species	2	1	2	1		3
No. specimens examined	11	6	15	9		62

Table A-6. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 7 JUNE 1971, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 12	13	14	15	16
Stylodrilus heringianus			15 (22)		
Dero digitata					
Limnodrilus cervix					
L. cervix-claparedeanus		12442 (9)		2693 (8)	17327 (13)
L. hoffmeisteri		20091 (29)	31 (44)	12792 (38)	49316 (37)
Peloscolex multisetosus multisetosus		42860 (31)		6745 (20)	17327 (13)
Tubifex tubifex		9677 (7)		1347 (4)	6664 (5)
Undetermined immature					
With hair chaetae		6908 (5)		1333 (4)	1533 (1)
Without hair chaetae		27649 (20)	32 (33)	8752 (26)	42651 (32)
Total		138244±5089	70±6	33662±1162	133285±12191
Probable L. hoffmeisteri		43%	77%	58%	58%
Probable T. tubifex		12%		8%	6%
Probable No. species		4	2	4	4
No. specimens examined		123	9	91	126

Table A-7. ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 6 AND 7 JULY 1971, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, and the total number of specimens examined.

Taxon	Station 1	2	3	4	5
Stylodrilus heringianus					145 (44)
Dero digitata					
Limnodrilus cervix					
L. cervix-claparedeanus		1602 (1)	7121 (7)	10944 (16)	
L. hoffmeisteri	22539 (15)	43263 (27)	28485 (28)	14364 (21)	82 (25)
Peloscolex multisetosus multisetosus			35586 (35)	27360 (40)	
Tubifex tubifex	60103 (40)	40058 (25)	9156 (9)	1368 (2)	
Undetermined immature					
With hair chaetae	40570 (27)	25637 (16)	6124 (6)	1368 (2)	
Without hair chaetae	27046 (18)	49672 (31)	14243 (14)	12996 (19)	69 (21)
Total	150258±18950	160233±15134	101732±7487	68400±6349	329±179
Probable *L. hoffmeisteri*	33%	57%	38%	32%	46%
Probable *T. tubifex*	67%	41%	15%	4%	
Probable No. species	2	3	4	4	2
No. specimens examined	95	77	116	128	52

Table A-7. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 6 AND 7 JULY 1971, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 6	7	8	9	10	11
Stylodrilus heringianus	82 (92)	25 (100)		76 (100)	458 (27)	318 (19)
Dero digitata						
Limnodrilus cervix					102 (6)	
L. cervix-claparedeanus						
L. hoffmeisteri					526 (31)	518 (31)
Peloscolex multisetosus multisetosus						
Tubifex tubifex						217 (13)
Undetermined immature						
With hair chaetae					34 (2)	184 (11)
Without hair chaetae	7 (8)				560 (33)	451 (27)
Total	89±63	25±25		76±11	1697±199	1672±48
Probable L. hoffmeisteri	8%				58%	58%
Probable T. tubifex						24%
Probable No. species	2	1		1	3-4	3
No. specimens examined	13	4		12	51	75

Table A-7. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 6 AND 7 JULY 1971, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 12	13	14	15	16
Stylodrilus heringianus	70 (50)		13 (14)		
Dero digitata		3816 (3)			
Limnodrilus cervix					
L. cervix-claparedeanus		12719 (10)		2796 (8)	10945 (8)
L. hoffmeisteri	20 (14)	30525 (24)	63 (71)	11884 (34)	47885 (35)
Peloscolex multisetosus multisetosus		35612 (28)		9437 (27)	36940 (27)
Tubifex tubifex		8903 (7)		2097 (6)	2736 (2)
Undetermined immature					
With hair chaetae		6360 (5)		1049 (3)	4104 (3)
Without hair chaetae	50 (36)	29253 (23)	13 (14)	8039 (23)	34203 (25)
Total	139±10	127186±14776	89±42	34952±1166	136813±9789
Probable L. hoffmeisteri	50%	38%	85%	50%	58%
Probable T. tubifex		12%		9%	4%
Probable No. species	2	5	2	4	4
No. specimens examined	22	92	14	113	121

Table A-8. ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 1 DECEMBER 1971 BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, and the total number of specimens examined.

Taxon	Station 1	2	3	4	5
Stylodrilus heringianus					
Dero digitata					
Limnodrilus cervix					
L. cervix-claparedeanus			862 (1)	733 (10)	
L. hoffmeisteri	13233 (8)	32872 (19)	15512 (18)	16848 (23)	
Peloscolex multisetosus multisetosus			29300 (34)	28001 (38)	
Tubifex tubifex	43008 (26)	20761 (12)	4308 (5)	1300 (2)	
Undetermined immature					
With hair chaetae	81053 (49)	39792 (23)	22406 (26)	13918 (19)	
Without hair chaetae	28120 (17)	77854 (45)	31024 (36)	19778 (27)	
Total	165414±9589	173008±4748	86178±10519	73251±6974	
Probable *L. hoffmeisteri*	25%	65%	52%	38%	
Probable *T. tubifex*	75%	35%	8%		
Probable No. species	2	2	4	3	
No. specimens examined	104	98	90	121	

Table A-8. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 1 DECEMBER 1971 BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, and the total number of specimens examined.

Taxon	Station 6	7	8	9	10	11
Stylodrilus heringianus	163 (76)	19 (100)	129 (66)	6 (100)	798 (50)	199 (14)
Dero digitata						
Limnodrilus cervix					32 (2)	
L. cervix-claparedeanus						
L. hoffmeisteri	26 (12)		10 (5)		192 (12)	355 (25)
Peloscolex multisetosus multisetosus						
Tubifex tubifex						
Undetermined immature						
With hair chaetae					80 (5)	326 (23)
Without hair chaetae	26 (12)		57 (29)		511 (32)	539 (38)
Total	215±94	19±11	196±45	6±6	1596±900	1418±130
Probable *L. hoffmeisteri*	24%		34%		40%	63%
Probable *T. tubifex*						
Probable No. species	2	1	2	1	3	2
No. specimens examined	34	3	41	1	66	108

Table A-8. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 1 DECEMBER 1971 BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 12	13	14	15	16
Stylodrilus heringianus	46 (73)		32 (36)		
Dero digitata					
Limnodrilus cervix					
L. cervix-claparedeanus		12949 (9)		954 (3)	1453 (1)
L. hoffmeisteri		33091 (23)	32 (36)	8589 (27)	34867 (24)
Peloscolex multisetosus multisetosus		35970 (25)		8589 (27)	40678 (28)
Tubifex tubifex		5755 (4)		1591 (5)	5811 (4)
Undetermined immature					
With hair chaetae		5753 (4)		2227 (7)	3075 (2)
Without hair chaetae	17 (27)	48917 (34)	26 (29)	10182 (32)	49395 (34)
Total	63±35	143874±4749	89±39	31812±3078	145280±6418
Probable L. hoffmeisteri	27%	44%	65%	55%	57%
Probable T. tubifex		8%		11%	13%
Probable No. species	2	4	2	4	4
No. specimens examined	11	112	14	107	145

Table A-9. ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 17 APRIL 1972 BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 1	2	3	4	5
Stylodrilus heringianus					70 (55)
Dero digitata				1040 (2)	
Limnodrilus cervix		1193 (1)			
L. cervix-claparedeanus				4162 (8)	
L. hoffmeisteri	20682 (14)	29829 (25)	12559 (15)	9884 (19)	19 (15)
Peloscolex multisetosus multisetosus			36002 (43)		19 (15)
Tubifex tubifex	39887 (27)	10738 (9)	2512 (3)	2601 (5)	
Undetermined immature					
With hair chaetae	56138 (38)	26249 (22)	8374 (10)		
Without hair chaetae	31024 (21)	51305 (43)	24281 (29)	10404 (20)	19 (15)
Total	147731±5665	119314±4880	83726±3321	52022±5194	127±63
Probable L. hoffmeisteri	35%	67%	44%	31%	20%
Probable T. tubifex	65%	31%	13%	10%	
Probable No. species	2	3	3	5	2
No. specimens examined	117	103	91	122	20

Table A-9. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 17 APRIL 1972 BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 6	7	8	9	10	11
Stylodrilus heringianus	51 (100)	6 (100)		19 (100)	101 (5)	320 (23)
Dero digitata						
Limnodrilus cervix						
L. cervix-claparedeanus						
L. hoffmeisteri					511 (24)	223 (16)
Peloscolex multisetosus multisetosus					277 (13)	
Tubifex tubifex					213 (10)	56 (4)
Undetermined immature						
With hair chaetae					106 (5)	153 (11)
Without hair chaetae					979 (46)	655 (47)
Total	51±17	6±6	19±19	101±35	2128±320	1393±139
Probable L. hoffmeisteri					70%	63%
Probable T. tubifex					15%	15%
Probable No. species		1	1	1	4	3
No. specimens examined		1	3	16	63	75

Table A-9. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 17 APRIL 1972 BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 12	13	14	15	16
Stylodrilus heringianus					
Dero digitata					
Limnodrilus cervix					
L. cervix-claparedeanus		5523 (2)		605 (5)	2356 (7)
L. hoffmeisteri		20987 (19)		5026 (25)	8752 (26)
Peloscolex multisetosus multisetosus		36452 (33)		3147 (26)	5050 (15)
Tubifex tubifex		6628 (6)		726 (6)	1683 (5)
Undetermined immature					
With hair chaetae		8636 (16)		969 (8)	2692 (8)
Without hair chaetae		32033 (29)		3752 (31)	12792 (38)
Total		110460±8031		12103±2141	33662±1662
Probable L. hoffmeisteri		45%		45%	54%
Probable T. tubifex		14%		14%	13%
Probable No. species		4		4	4
No. specimens examined		118		81	84

Table A-10. ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 20 JUNE 1972, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, and the total number of specimens examined.

Taxon	Station 1	2	3	4	5
Stylodrilus heringianus				554 (1)	139 (61)
Dero digitata				554 (1)	
Limnodrilus cervix					
L. cervix-claparedeanus				2772 (5)	7 (3)
L. hoffmeisteri	30229 (20)	37118 (33)	18924 (26)	15522 (28)	50 (22)
Peloscolex multisetosus multisetosus			32024 (44)	19402 (35)	
Tubifex tubifex	54412 (36)	23621(21)	3639 (5)	3098 (11)	
Undetermined immature					
With hair chaetae	43832 (29)	22496 (20)	4267 (6)	3326 (6)	7 (3)
Without hair chaetae	22672 (15)	30370 (27)	13829 (19)	7207 (13)	25 (11)
Total	151145±8873	112480±3923	72783±7378	55436±5648	228±94
Probable *L. hoffmeisteri*	35%	60%	45%	39%	32%
Probable *T. tubifex*	65%	40%	11%	17%	
Probable No. species	2	2	3	6	4
No. specimens examined	116	101	93	128	36

Table A-10. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 20 JUNE 1972, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 6	7	8	9	10	11
Stylodrilus heringianus	19 (100)	32 (83)	89 (100)	158 (100)	68 (3)	519 (26)
Dero digitata						
Limnodrilus cervix						
L. cervix-claparedeanus						
L. hoffmeisteri					588 (26)	778 (39)
Peloscolex multisetosus multisetosus					588 (26)	
Tubifex tubifex					68 (3)	
Undetermined immature						
With hair chaetae					68 (3)	219 (11)
Without hair chaetae		16 (17)			904 (40)	459 (23)
Total	19±11	48±29	89±50	158±61	2261±378	1995±295
Probable L. hoffmeisteri					66%	62%
Probable T. tubifex					6%	
Probable No. species	1	2	1	1		3
No. specimens examined	3	6	14	25		99

Table A-10. (continued) ABUNDANCE OF OLIGOCHAETE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 20 JUNE 1972, BASED ON THE MEAN OF COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS; AND IN PARENTHESES, THE RELATIVE ABUNDANCE OF EACH TAXON AS A PERCENTAGE OF THE TOTAL. The lower portion of the table lists probable number of each species in each collection, probable number (%) of Limnodrilus hoffmeisteri, Tubifex tubifex, and the total number of specimens examined.

Taxon	Station 12	13	14	15	16
Stylodrilus heringianus	9 (75)				
Dero digitata					
Limnodrilus cervix					
L. cervix-claparedeanus		8968 (9)		1176 (9)	956 (3)
L. hoffmeisteri		26905 (27)		3529 (27)	9878 (31)
Peloscolex multisetosus multisetosus		28898 (29)		4445 (34)	6691 (21)
Tubifex tubifex		7972 (8)		392 (3)	2868 (9)
Undetermined immature					
With hair chaetae		6975 (7)		261 (3)	2231 (7)
Without hair chaetae	6 (25)	20926 (21)	19 (100)	3399 (26)	9240 (29)
Total	27±17	99649±5090	19±11	13072±1889	31863±7422
Probable L. hoffmeisteri	25%	41%	100%	47%	57%
Probable T. tubifex		25%		6%	16%
Probable No. species	2	4	1	4	4
No. specimens examined	4	116	3	90	98

Table A-11. ABUNDANCE OF INVERTEBRATE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 7 JUNE 1971, BASED ON THE MEAN COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS.

	Station															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Chironomids																
Heterotrissocladius sp.								82			399		89			
Procladius sp.													6			
Chironomus sp.											25					
Hirudinea																
Helobdella stagnalis			6	6							6				6	
Gastropoda																
Vivaparus sp.				13									57			
Valvata sp.													6			
Isopods																
Asellus intermedius											114					
Amphipods																
Gammarus fasciatus																
Hyalella azteca																
Pontoporeia affinis								6								

Table A-12. ABUNDANCE OF INVERTEBRATE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 6 AND 7 JULY 1971, BASED ON THE MEAN COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS.

	Station															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Chironomids																
Heterotrissocladius sp.			13	139				25	82	323	253		21	82		
Procladius sp.											127		51			
Chironomus sp.											82					
Hirudinea																
Helobdella stagnalis			19	19								19		25		25
Gastropoda																
Vivaparus sp.													25			
Valvata sp.										6						
Isopods																
Asellus intermedius								38	127	203	6					
Amphipods																
Gammarus fasciatus								13		4						
Hyalella azteca											11					
Pontoporeia affinis						25		95		28	21	38		6		

Table A-13. ABUNDANCE OF INVERTEBRATE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 1 DECEMBER 1971, BASED ON THE MEAN COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS.

	Station															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Chironomids																
Heterotrissocladius sp.				38				6			139		42			
Procladius sp.																
Chironomus sp.																
Hirudinea																
Helobdella stagnalis																13
Gastropoda																
Vivaparus sp.													13			
Valvata sp.																
Isopods																
Asellus intermedius																
Amphipods																
Gammarus fasciatus																
Hyalella azteca																
Pontoporeia affinis						6					6					

Table A-14. ABUNDANCE OF INVERTEBRATE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 17 APRIL 1972, BASED ON THE MEAN COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS.

	Station															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Chironomids																
Heterotrissocladius sp.			6	171	19	19			51	6	142		51			
Procladius sp.											108					
Chironomus sp.											82					
Hirudinea																
Helobdella stagnalis													6			
Gastropoda																
Vivaparus sp.				26											6	6
Valvata sp.				6												
Isopods																
Asellus intermedius											9					
Amphipods																
Gammarus fasciatus																
Hyalella azteca											3					
Pontoporeia affinis											10					

Table A-15. ABUNDANCE OF INVERTEBRATE TAXA, INDIVIDUALS/m^2, AT STATIONS SAMPLED 20 JUNE 1972, BASED ON THE MEAN COUNTS FROM THREE 23 cm x 23 cm PONAR GRABS.

	Station															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Chironomids																
Heterotrissocladius sp.				203		13	6	6	95	19	154		19	25		
Procladius sp.											87					
Chironomus sp.																
Hirudinea																
Helobdella stagnalis														6	19	
Gastropoda																
Vivaparus sp.													6		13	
Valvata sp.															6	
Isopods																
Asellus intermedius																
Amphipods																
Gammarus fasciatus																
Hyalella azteca																
Pontoporeia affinis					6	6		13		6						

APPENDIX B

CHEMISTRY OF N-CHLORAMINES

1. Amination of Toluene, Adamantane, and tert-Butyl Chloride with Monochloramine-Aluminum Chloride

The reaction of monochloramine with toluene in the presence of aluminum chloride at -35°C yielded 13-15% of m-toluidine, whereas trichloramine gave 39-43% yields. To rationalize the meta substitution, a mechanistic scheme entailing addition-elimination is proposed. Amination of adamantane with monochloramine under Friedel-Crafts conditions gave 1-aminoadamantane in 40% yield. An analogous reaction with tri-chloramine-aluminum chloride provided 1-aminoadamantane in 85% yield with no detectable 2-aminoadamantane. The reaction pathway presumably involves formation of the 1-adamantyl cation followed by attack by the nitrogen-containing nucleophile. Reaction of tert-butyl chloric monochloramine, and aluminum chloride yielded tert-butylamine (7-20%). Similarly, trichloramine generated tert-butylamine in 50-56% yield and 2, 2-dimethylaziridine in 7-12% yield. Mechanistically, the tert-butyl cation is thought to participate as an intermediate. Possible reasons are discussed for the lower yields in all cases with monochloramine, as compared to trichloramine.

2. Preferred Route for vic-Dichloride Formation from Alkenes. Comparison of Cyclohexene Chlorination with Chlorine, Sulfuryl Chloride, and Trichloramine

The best method for generating vic-dichlorides was investigated by comparing chlorination of cyclohexene with chlorine, sulfuryl chloride, and trichloramine. In relation to cleanness of reaction, trichloramine is the reagent of choice (97% yield of trans-1,2-dichlorocyclohexane). If some sacrifice in yield is not critical, sulguryl chloride or chlorine would be preferred on the basis of simpler operating procedures.

3. Rearrangement of o-Hydroxyaldehydes and Ketones to o-Hydroxyanilides by Monochloramine

o-Hydroxyaldehydes and ketones are converted in good yield to o-hydroxyanilides by reaction with monochloramine in base. The reaction was carried out with benzene nuclei containing alkyl, methoxyl, chlorine, and nitro substituents, as well as with a naphthalene nucleus. The overall transformation is similar to the Beckmann, Schmidt, Theilacker, and Pearson rearrangement. There appears to be mechanistic similarity to the Dakin oxidation.

4. A Novel, Directed Synthesis of Unsymmetrical Azoxyalkanes and Azoxyaralkanes from N,N-Dihaloamine and Nitroso Precursors

A novel, directed synthesis of unsymmetrical azoxyalkanes and azoxyaralkanes from nitroso compounds (RNO) and N,N-dichloramines ($R'NCl_2$) in the presence of methanolic caustic is described. An investigation of the scope of the reaction revealed that the highest yields of azoxy compounds were produced when R is tert-alkyl or aryl and R' is tert-alkyl. This method possesses advantages not offered by prior techniques. Possible mechanistic pathways are also discussed.

5. Dealkylation of N,N-Dichloroalkylamines with Silver Salts

Reaction of N,N-dichloro-t-butylamine in refluxing acetonitrile with silver (I) fluoride gave isobutylene in 80-85% yield (Sharts 1969). Investigation of N,N-dichloro-1-amino-1-methylcyclohexane in dimethyl sulfoxide with silver acetate at room temperature afforded 50-70% alkene (45% exo: 55% endo). This reaction is being studied further.

APPENDIX C

BIOASSAY TESTS

Table C-1. TOXICITY OF RESIDUAL CHLORINE ADDED AS SODIUM HYPOCHLORITE TO CYCLOPS BICUSPIDATUS THOMASI.

Date: May 19-23, 1973. Exposure Period: 96 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	15.8	8.15
1B	20	0.000	10.0	8.15
2A	20	0.011	25.0	8.10
2B	20	0.010	25.0	8.05
3A	20	0.024	22.0	8.10
3B	20	0.021	36.8	8.10
4A	20	0.081	61.1	8.15
4B	20	0.072	50.0	8.10
5A	20	0.170	90.0	8.10
5B	20	0.178	95.0	8.10
6A	20	0.264	100.0	8.15
6B	20	0.269	100.0	8.15

Table C-2. TOXICITY OF RESIDUAL CHLORINE ADDED AS SODIUM HYPOCHLORITE TO CYCLOPS BICUSPIDATUS THOMASI.

Date: June 1-5, 1973. Exposure Period: 96 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	0.0	8.20
1B	20	0.000	10.0	8.15
2A	20	0.020	25.0	8.18
2B	20	0.021	10.0	8.18
3A	20	0.046	33.3	8.20
3B	20	0.047	21.1	8.18
4A	20	0.083	22.2	8.20
4B	20	0.109	42.1	8.18
5A	20	0.185	45.5	8.23
5B	20	0.191	38.5	8.28
6A	20	0.401	90.5	8.25
6B	20	0.376	85.0	8.25

Table C-3. TOXICITY OF RESIDUAL CHLORINE ADDED AS SODIUM HYPOCHLORITE TO CYCLOPS BICUSPIDATUS THOMASI.

Date: June 14-18, 1973. Exposure Period: 96 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	20.0	8.20
1B	20	0.000	65.0	-
2A	20	0.029	30.0	8.20
2B	20	0.027	55.0	8.20
3A	20	0.075	25.0	8.22
3B	20	0.081	14.3	8.21
4A	20	0.184	88.2	8.23
4B	20	0.178	73.0	8.20
5A	20	0.234	89.5	8.22
5B	20	0.238	90.0	8.18
6A	20	0.630	94.7	8.20
6B	20	0.660	100.0	8.21

Table C-4. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONO-CHLORAMINE TO CYCLOPS BICUSPIDATUS THOMASI.

Date: July 13-17, 1973. Exposure Period: 96 hrs.
Temperature 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	5.6	-
1B	20	0.000	6.7	-
2A	20	0.018	5.3	8.15
2B	20	0.015	5.9	8.20
3A	20	0.042	20.0	8.21
3B	20	0.036	5.9	8.22
4A	20	0.136	84.2	8.19
4B	20	0.165	87.5	8.19
5A	20	0.236	100.0	8.21
5B	20	0.312	100.0	8.25
6A	20	1.025	100.0	8.29
6B	20	0.982	100.0	8.20

Tabe C-5. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONO-CHLORAMINE TO CYCLOPS BICUSPIDATUS THOMASI.

Date: July 19-23, 1973. Exposure Period: 96 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	5.0	8.37
1B	20	0.000	5.9	8.39
2A	20	0.012	0.0	8.39
2B	20	0.024	0.0	8.36
3A	20	0.067	10.5	8.37
3B	20	0.041	10.5	8.36
4A	20	0.169	73.7	8.35
4B	20	0.168	100.0	8.36
5A	20	0.308	100.0	8.34
5B	20	0.398	100.0	8.35
6A	20	0.979	100.0	8.37
6B	20	0.990	100.0	8.37

Table C-6. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONO-CHLORAMINE TO CYCLOPS BICUSPIDATUS THOMASI.

Date: Aug. 9-13, 1973. Exposure Period: 96 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	15.0	8.33
1B	20	0.000	0.0	8.30
2A	20	0.014	10.5	8.40
2B	20	0.018	17.6	8.34
3A	20	0.067	41.2	8.45
3B	20	0.061	70.0	8.37
4A	20	0.147	90.0	8.49
4B	20	0.156	90.0	8.42
5A	20	0.323	100.0	8.50
5B	20	0.359	100.0	8.45
6A	20	0.838	100.0	8.53
6B	20	0.868	100.0	8.48

Table C-7. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONO-CHLORAMINE TO CYCLOPS BICUSPIDATUS THOMASI.

Date: Aug. 16-20, 1973. Exposure Period: 96 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	0.0	8.39
1B	20	0.000	0.0	8.42
2A	20	0.022	5.0	8.32
2B	20	0.031	10.0	8.39
3A	20	0.089	38.9	8.38
3B	20	0.083	31.6	8.45
4A	20	0.227	85.7	8.39
4B	20	0.195	88.9	8.40
5A	20	0.447	100.0	8.42
5B	20	0.507	100.0	8.40
6A	20	1.110	100.0	8.40
6B	20	1.194	100.0	8.32

Table C-8. TOXICITY OF SODIUM SULFITE TO CYCLOPS BICUSPIDATUS THOMASI.

Date: Jan. 11-15, 1974. Exposure Period: 96 hrs. Temperature 15°C

Aquarium	No. of organisms	Average $SO_3^{=}$ as SO_2 (mg/l)	Mortality %	Average pH
1A	20	0.000	25.0	8.27
1B	20	0.000	12.5	8.32
2A	20	0.011	20.0	8.32
2B	20	0.015	17.6	8.30
3A	20	0.030	10.5	8.30
3B	20	0.035	23.5	8.30
4A	20	0.050	5.9	8.30
4B	20	0.062	20.0	8.32
5A	20	0.130	22.2	8.30
5B	20	0.137	11.1	8.33
6A	20	0.477	12.5	8.32
6B	20	0.563	15.8	8.29

Table C-9. TOXICITY OF RESIDUAL CHLORINE TO CYCLOPS BICUSPIDATUS THOMASI. DECHLORINATING WITH SULFITE.

Date: April 16-20, 1974. Exposure Period: 96 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Average $SO_3^=$ as SO_2 (mg/l)	Mortality %	Average pH
1A	20	0.000	0.000	5.0	8.08
1B	20	0.000	0.000	11.1	8.20
2A	20	0.000	0.000	5.0	8.19
2B	20	0.000	0.005	10.5	8.20
3A	20	0.000	0.024	5.0	8.24
3B	20	0.000	0.018	5.0	8.19
4A	20	0.000	0.028	10.0	8.24
4B	20	0.000	0.026	0.0	8.20
5A	20	0.000	0.000	5.0	8.29
5B	20	0.000	0.019	0.0	8.26
6A	20	0.000	0.014	10.5	8.30
6B	20	0.000	0.069	5.0	8.32

Table C-10. TOXICITY OF RESIDUAL CHLORINE TO CYCLOPS BICUSPIDATUS THOMASI. DECHLORINATING WITH SODIUM SULFITE.

Date: June 3-7, 1974. Exposure Period: 96 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Average $SO_3^{=}$ as SO_2 (mg/l)	Mortality %	Average pH
1A	20	0.000	0.000	22.2	8.08
1B	20	0.000	0.000	5.0	8.16
2A	20	0.000	0.006	14.3	8.35
2B	20	0.000	0.004	10.0	8.18
3A	20	0.000	0.006	5.0	8.31
3B	20	0.000	0.011	5.0	8.39
4A	20	0.000	0.026	11.1	8.15
4B	20	0.000	0.022	18.8	8.27
5A	20	0.000	0.042	5.3	8.32
5B	20	0.000	0.039	11.8	8.30
6A	20	0.000	0.099	15.0	8.36
6B	20	0.000	0.098	15.8	8.38

Table C-11. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONOCHLORAMINE TO KERATELLA COCHLEARIS.

Date: Sept. 27, 1973. Exposure Period: 4 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	5.0	8.10
1B	20	0.000	19.0	8.10
2A	20	0.000	12.5	8.10
2B	20	0.003	21.1	8.10
3A	20	0.006	31.6	8.10
3B	20	0.004	30.0	8.10
4A	20	0.039	47.4	8.10
4B	20	0.019	41.2	8.10
5A	20	0.055	94.4	8.10
5B	20	0.062	100.0	8.10
6A	20	0.135	100.0	8.15
6B	20	0.156	100.0	8.15

Table C-12. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONOCHLORAMINE TO KERATELLA COCHLEARIS.

Date: Oct. 11, 1973. Exposure Period: 4 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	5.0	8.20
1B	20	0.000	0.0	8.20
2A	20	0.000	29.4	8.20
2B	20	0.000	5.9	8.20
3A	20	0.000	16.7	8.20
3B	20	0.000	15.8	8.20
4A	20	0.008	35.0	8.20
4B	20	0.005	27.8	8.20
5A	20	0.026	45.0	8.20
5B	20	0.037	52.4	8.20
6A	20	0.077	95.0	8.20
6B	20	0.107	94.7	8.25

Table C-13. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONOCHLORAMINE TO KERATELLA COCHLEARIS.

Date: Oct. 18, 1973. Exposure Period: 4 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Mortality %	Average pH
1A	20	0.000	5.0	8.15
1B	20	0.000	14.3	8.15
2A	20	0.000	16.7	8.15
2B	20	0.000	5.9	8.15
3A	20	0.000	15.8	8.15
3B	20	0.000	11.1	8.15
4A	20	0.008	25.0	8.15
4B	20	0.005	16.7	8.15
5A	20	0.029	50.0	8.15
5B	20	0.025	42.1	8.15
6A	20	0.103	94.7	8.20
6B	20	0.124	100.0	8.20

Table C-14. TOXICITY OF SODIUM SULFITE ALONE ON KERATELLA COCHLEARIS.

Date: Nov. 29, 1973. Exposure Period: 4 hrs.
Temperature: 15°C

Aquarium	No. of organisms	$SO_3^{=}$ as SO_2 (mg/l) Start	Finish	Mortality %	Average pH
1A	20	0.000	0.000	14.3	8.20
1B	20	0.000	0.000	5.0	8.20
2A	20	0.692	0.750	5.0	8.20
2B	20	0.750	0.861	9.5	8.20

Table C-15. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONOCHLORAMINE TO KERATELLA COCHLEARIS. DECHLORINATING WITH SODIUM SULFITE.

Date: Jan. 26, 1974. Exposure Period: 4 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Average $SO_3^{=}$ as SO_2 (mg/l)	Mortality %	Average pH
1A	20	0	0.000	11.1	8.40
1B	20	0	0.000	0.0	8.40
2A	20	0	0.000	10.5	8.40
2B	20	0	0.000	6.3	8.40
3A	20	0	0.000	0.0	8.40
3B	20	0	0.000	5.9	8.40
4A	20	0	0.000	0.0	8.40
4B	20	0	0.000	11.1	8.40
5A	20	2.5	0.000	5.9	8.40
5B	20	3.0	0.000	0.0	8.40
6A	20	14.0	0.000	11.1	8.40
6B	20	13.5	0.000	10.5	8.40

Table C-16. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONOCHLORAMINE TO KERATELLA COCHLEARIS. DECHLORINATING WITH SODIUM SULFITE.

Date: Jan. 31, 1974. Exposure Period: 4 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Average $SO_3^=$ as SO_2 (mg/l)	Mortality %	Average pH
1A	20	0	0.000	0.0	8.40
1B	20	0	0.000	0.0	8.40
2A	20	0	0.000	0.0	8.40
2B	20	0	0.069	5.9	8.40
3A	20	0	0.023	10.0	8.40
3B	20	0	0.000	11.8	8.40
4A	20	0	0.000	0.0	8.40
4B	20	0	0.000	6.7	8.40
5A	20	0	0.000	6.3	8.40
5B	20	0	0.000	0.0	8.40
6A	20	6	0.000	5.9	8.40
6B	20	6	0.000	0.0	8.40

Table C-17. TOXICITY OF RESIDUAL CHLORINE ADDED AS MONOCHLORAMINE TO KERATELLA COCHLEARIS. DECHLORINATING WITH SODIUM SULFITE.

Date: Feb. 9, 1974. Exposure Period: 4 hrs.
Temperature: 15°C

Aquarium	No. of organisms	Average total residual chlorine (mg/l)	Average $SO_3^{=}$ as SO_2 (mg/l)	Mortality %	Average pH
1A	20	0	0.000	6.3	8.40
1B	20	0	0.000	0.0	8.40
2A	20	0	0.000	0.0	8.40
2B	20	0	0.034	0.0	8.40
3A	20	0	0.000	5.0	8.40
3B	20	0	0.080	0.0	8.40
4A	20	0	0.069	6.3	8.40
4B	20	0	0.242	6.7	8.40
5A	20	0	0.196	0.0	8.40
5B	20	0	0.161	5.3	8.40
6A	20	0	0.161	5.9	8.40
6B	20	0	0.253	5.3	8.40

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16. ABSTRACT

The acute toxicity of residual chlorine was determined for the copepod Cyclops bicuspidatus thomasi and the rotifer Keratella cochlearis. The 96-hour TL_{50} value for Cyclops was 0.084 mg/l total residual chlorine added as monochloramine. When Cyclops was exposed to sodium hypochlorite the 96-hour TL_{50} was 0.069 mg/l total residual chlorine. The 4-hour TL_{50} value for Keratella was 0.019 mg/l total residual chlorine added as monochloramine.

Chemical studies determined that sodium sulfite was an efficient, inexpensive chemical agent for reducing chlorine residuals which did not produce undesirable by-products. Complete reduction was accomplished in less than 20 seconds with a calculated k_{min} of 43 sec^{-1}. Bioassay studies indicated that sodium sulfite added to chlorinated water completely eliminated the acute toxicity of residual chlorine to both Cyclops bicuspidatus thomasi and Keratella cochlearis.

Field studies in Milwaukee Harbor and adjacent Lake Michigan indicated that measurable chlorine residuals were confined to a very small area surrounding the effluent from the Jones Island Sewage Treatment Plant. Significant reductions in the populations of benthic organisms were observed in the effluent plume area after the start of chlorination.

17. KEY WORDS AND DOCUMENT ANALYSIS

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