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**SECOND REPORT ON THE  
OAK RIDGE Y-12 PLANT  
FISH KILL FOR  
UPPER EAST FORK POPLAR CREEK**

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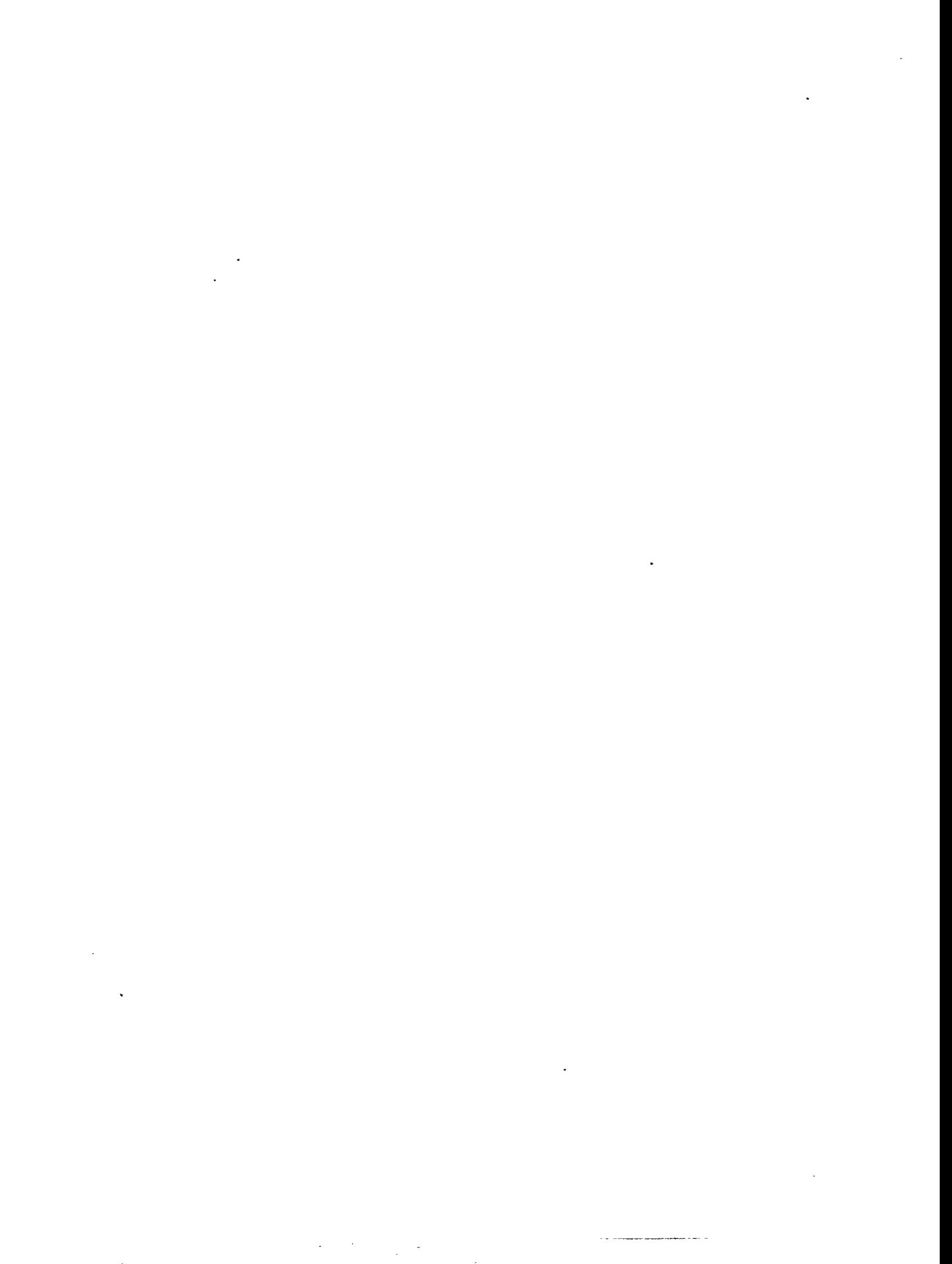
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## ACRONYMS

ACD	Analytical Chemistry Division
ANOVA	analysis of variance
AS-8	Area Source Study Site 8
BF	Brushy Fork
BFK	Brushy Fork kilometer
BMAP	Biological Monitoring and Abatement Program
BMP	best management practice
BVK	Beaver Creek kilometer
CCC	criterion continuous concentration
CMC	criterion maximum concentration
CPI	chemical perturbation index
CV	coefficient of variation
DOE	U.S. Department of Energy
EDTA	ethylenediaminetetraacetic acid
EFK	East Fork kilometer
EFPC	East Fork Poplar Creek
EPA	U.S. Environmental Protection Agency
EPT	Ephemeroptera Plecoptera Tricoptera
ESD	Environmental Sciences Division
GC/MS	gas chromatography/mass spectrometry
HCK	Hinds Creek kilometer
ICP	inductively coupled plasma
IWC	instream waste concentration
LR	Lake Reality
LR-i	Lake Reality inlet
LR-o	Lake Reality outlet
NAWQC	National Ambient Water Quality Criteria
NHP	New Hope Pond
NHP <sub>i</sub>	New Hope Pond inlet
NHP <sub>o</sub>	New Hope Pond outlet
NOEC	no-observed-effect concentration
NPDES	National Pollutant Discharge Elimination System
NSP	north-south pipe
NTU	nephelometric turbidity units
ORNL	Oak Ridge National Laboratory
ORR	Oak Ridge Reservation
ORWTF	Oak Ridge Wastewater Treatment Facility
ppm	parts per million
PVC	polyvinyl chloride
RCRA	Resource Conservation and Recovery Act
RIA	randomized intervention analysis

## ACRONYMS (continued)

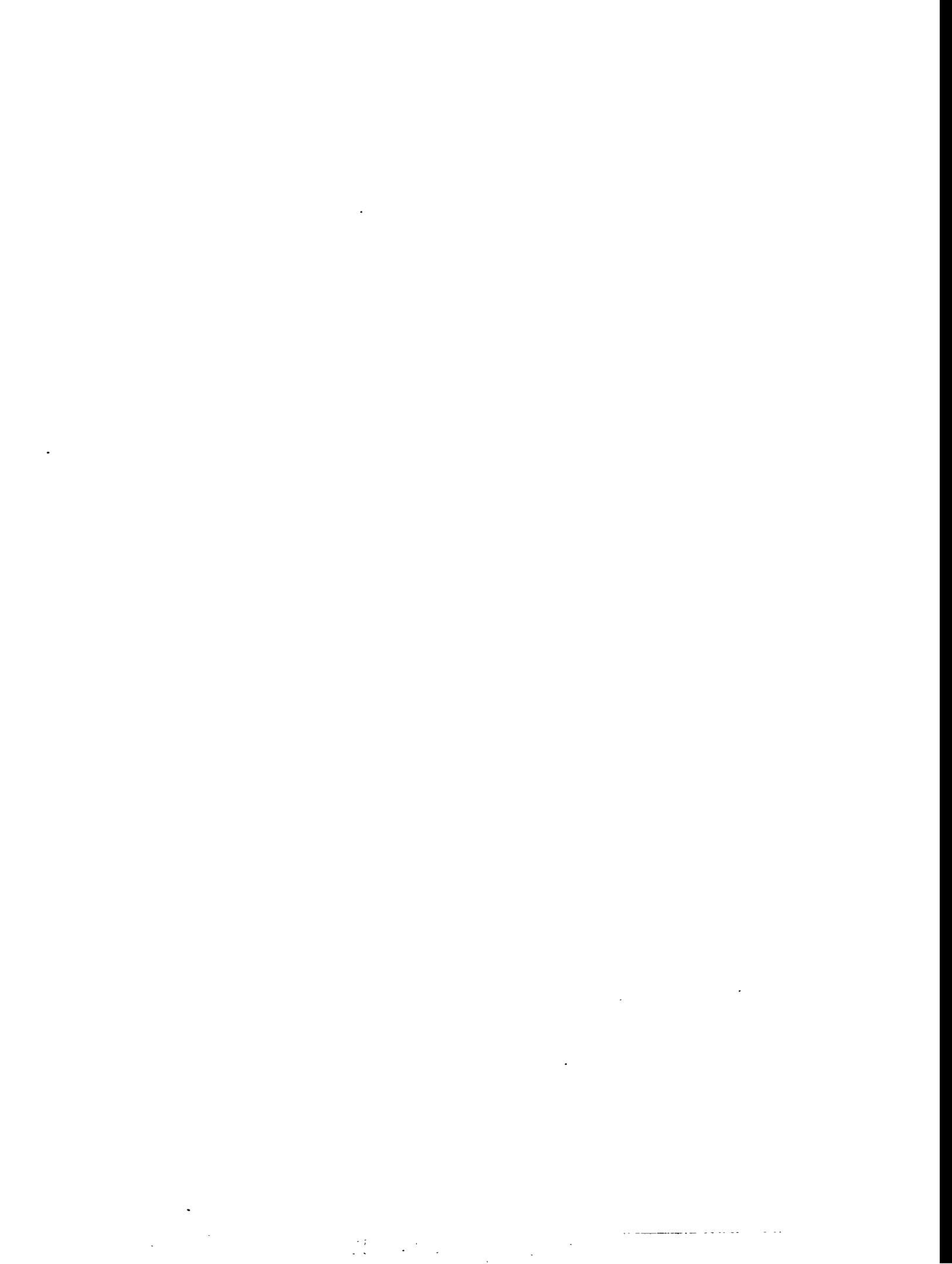
SAS	statistical analysis system
SOP	standard operating procedure
TDEC	Tennessee Department of Environment and Conservation
TDHE	Tennessee Department of Health and Environment (name changed in 1991 to Tennessee Department of Environment and Conservation, see TDEC)
TDS	total dissolved solids
TRC	total residual chlorine
TSS	total suspended solids
TTD	time to death
TU	toxicity unit
TWRA	Tennessee Wildlife Resources Agency
WCK	White Oak Creek kilometer
WOC	White Oak Creek

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## ABSTRACT

This report summarizes the monitoring of fish kills in upper East Fork Poplar Creek (EFPC) from July 1990 to June 1993. Since the opening of Lake Reality (LR) in 1988, total numbers of fish inhabiting upper EFPC have increased. However, species diversity has remained poor. Water quality data have been collected in upper EFPC during the time period covered in this report. Total residual chlorine (TRC) levels have exceeded federal and state water quality criteria over the years. However, with the installation of two dechlorination systems in late 1992, TRC levels have been substantially lowered in most portions of upper EFPC. By June 1993, concentrations of TRC were 0.04 to 0.06 mg/L at the north-south pipes (NSP) and below detection limits at sampling station AS-8 and were 0 to 0.01 mg/L at the inlet and outlet of LR.

The daily chronic fish mortality in upper EFPC has been attributed to background stress resulting from the continuous discharge of chlorine into upper EFPC. Chronic mortality rates generally ranged from 1 to 5 fish per day; however, the data suggest that mean chronic mortality rates have decreased in successive years to 1 to 2 fish per day during 1992-93. Chronic mortality continued during the first 3 months of 1993, following installation of the dechlorination systems; TRC data indicate that chlorine levels were still greatly fluctuating during that time period. In the summer of 1993, TRC levels appeared to have stabilized in upper EFPC at relatively low to nondetectable levels; consequently, fish kill monitoring was suspended in June 1993. Additional daily surveys may be required to confirm that reduced TRC levels in EFPC have indeed reduced chronic fish kills to negligible levels.

Mean daily mortality rates for 22 acute fish kills were three fold or more above background and usually exceeded ten fish per day. Total number of dead fish collected per acute kill event ranged from 30 to over 1000 fish; predominant species killed were central stonerollers (*Camptostoma anomalum*) and striped shiners (*Luxilus chrysocephalus*). Spills or elevated releases of toxic chemicals, such as acids, organophosphates, aluminum nitrate, ammonia, or chlorine, were identified as possible causative agents; however, a definitive cause-effect relationship was rarely established for any acute kills.

Ambient toxicity testing, in situ chemical monitoring, and streamside experiments were used to examine TRC dynamics and ambient toxicity in EFPC. The results of the studies provide clear and consistent evidence of toxicity in EFPC upstream from LR and inconclusive evidence for toxicity downstream from LR. Field and laboratory studies were conducted on various phyla to further evaluate the ambient toxicity test results. These studies showed physiological adaptations, as well as detection and avoidance mechanisms, in fish. Snail survival in EFPC near the NSP was significantly affected. Mortality and growth of clams placed in EFPC was affected, as was valve-closure response of clams treated streamside with EFPC water. Taxonomic and physiological changes were noted in periphyton communities exposed to changes in water quality in EFPC, and microbial activity was found to be lower at the NSP than at sites further downstream.



## 1. INTRODUCTION

The East Fork Poplar Creek (EFPC) watershed drains the south slope of Pine Ridge and the north slope of Chestnut Ridge. The headwaters of EFPC originate within the U.S. Department of Energy (DOE) Oak Ridge Y-12 Plant, one of three plants on the Oak Ridge Reservation (ORR) managed by Martin Marietta Energy Systems, Inc. Structural changes in EFPC and its tributaries have resulted in changes in fish fauna and community dynamics within the creek. The opening of Lake Reality (LR) in 1988 following the closure of New Hope Pond (NHP) has allowed fish to migrate into the upper reaches of EFPC, where they have been exposed to higher concentrations of contaminants, including total residual chlorine (TRC). Fish kills have been monitored in upper EFPC since 1986. Attempts have been made since that time to document both chronic and acute fish kill events in what has become known as upper EFPC and to determine the impact of effluents entering EFPC from the Y-12 Plant. The Biological Monitoring and Abatement Program (BMAP) was designed to evaluate the effects of Y-12 Plant effluents through toxicity testing, bioaccumulation studies, biological indicator studies, and instream monitoring of the benthic invertebrate and fish communities; results of the most recent BMAP studies have been summarized in Hinzman et al. 1995 and cover the time period October 1988–December 1990. The report presented here summarizes data on both chronic and acute fish kills in upper EFPC as well as the changes in community characteristics. Laboratory and field studies performed in an attempt to define ecotoxicological conditions in EFPC are also presented;

several of these studies are completely described in the Third BMAP report (Hinzman et al. 1995) and are only summarized here.

### 1.1 DESCRIPTION OF SITE

The headwaters of EFPC consist of springs that originate on the northwest slope of Chestnut Ridge. Since construction of the Y-12 Plant in the early 1940s, EFPC and its tributaries have been significantly altered. All of the north–south tributaries were eliminated and either replaced by storm sewers or buried under fill material. EFPC itself was straightened and replaced by storm sewers in some areas. The stream is now contained in culverts through much of the west end of the Y-12 Plant [the north–south pipes (NSP)] before entering a rip-rap channel. Figure 1.1 shows EFPC as it flows from the NSP to Bear Creek Road. In 1963, NHP was constructed approximately at East Fork kilometer (EFK) 24.0. This created what is now termed upper EFPC, above NHP, and lower EFPC, extending from NHP to the confluence with Poplar Creek (see Fig. 1.2). The purpose of NHP was to provide for pH equalization and neutralization of plant effluents, sediment retention, and spill control. Later construction of a bypass channel around NHP allowed for long-term retention of hazardous chemicals and sediments within the pond. Historically, upper EFPC has received a wide variety of discharges from more than 200 individual National Pollutant Discharge Elimination System (NPDES) outfalls as it flows ~1.5 km through the plant.

# Y-12 Plant

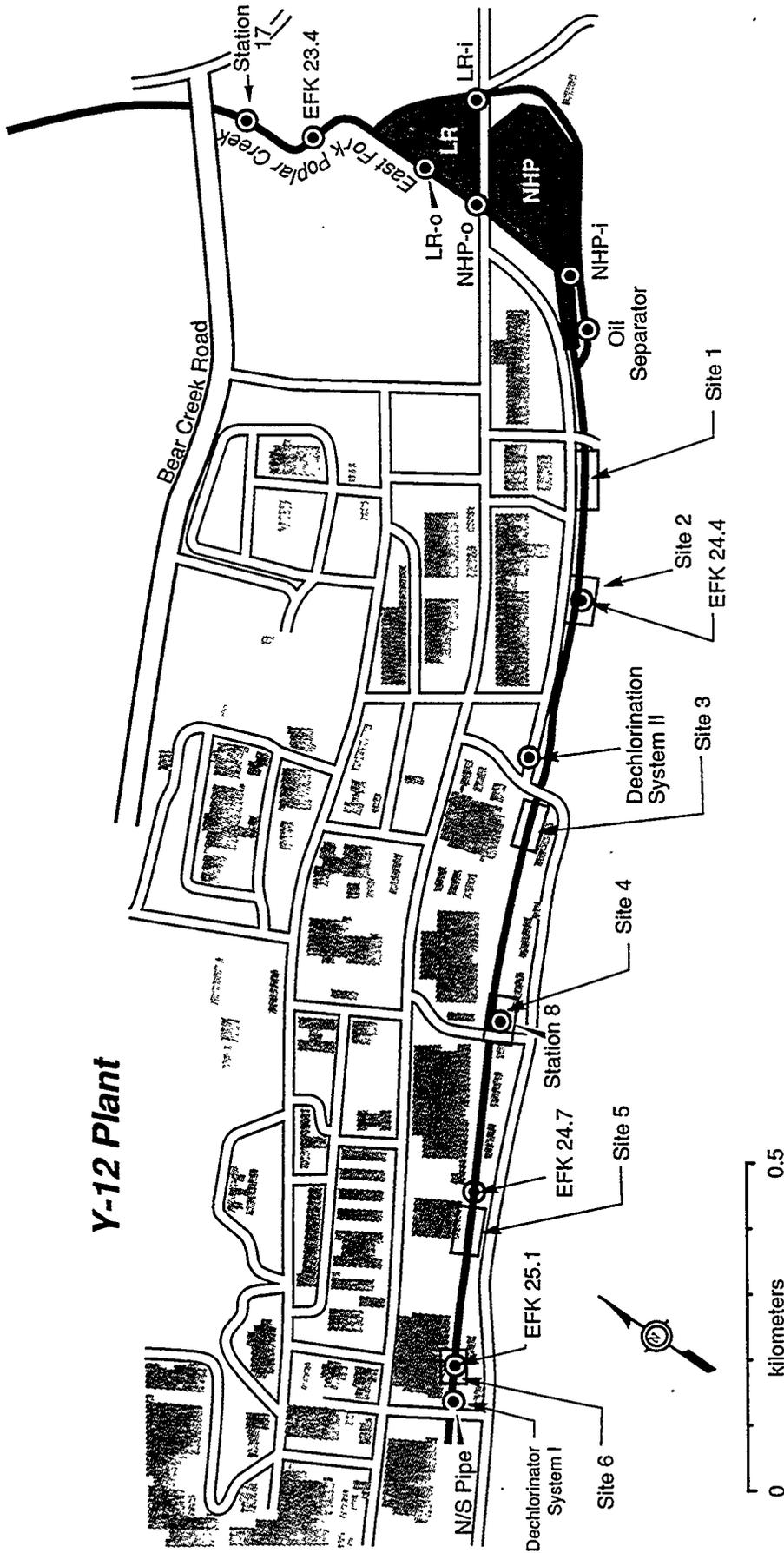


Fig. 1.1. Location of study sites, monitoring stations, and various reference points on East Fork Poplar Creek from the north-south pipes to Bear Creek Road. N/S pipe = north-south pipe; EFK = East Fork kilometer; Station 8 = Area Source Study Site 8 (AS8); NHP = New Hope Pond; NHP-i = NHP inlet; NHP-o = NHP outlet; LR = Lake Reality; LR-i = LR inlet; LR-o = LR outlet (Note: EFK markings are estimates and are not to scale).

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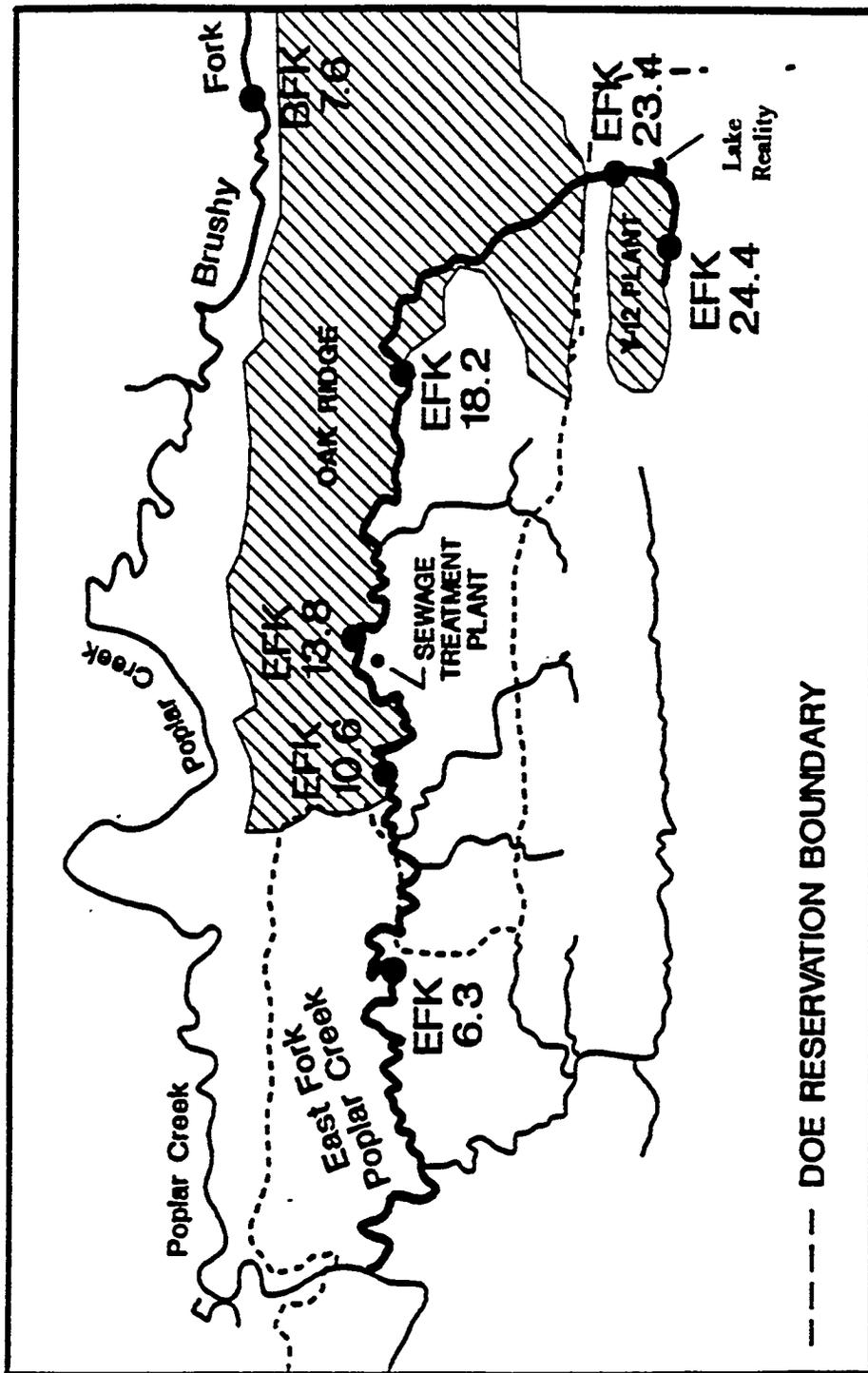


Fig. 1.2. Reference sites for lower East Fork Poplar Creek and Brushy Fork.

On November 7, 1988, water was diverted away from NHP and into the newly constructed LR, immediately downstream from NHP (see Fig. 1.1). NHP was backfilled and closed under the Resource Conservation and Recovery Act (RCRA). Water enters LR from an extension of the NHP diversion ditch and is released through a weir in the west berm into the existing channel of EFPC. An emergency weir is located at the north end of the LR distribution ditch. LR is a 1-ha impoundment that has a smaller surface area than NHP, is slightly deeper, and has a synthetic liner. The third BMAP report describes LR in more detail (Hinzman et al. 1995).

The weir at the outfall of NHP provided an effective physical barrier to the movement of fish into upper EFPC. However, the outfall from LR is lower in elevation than that of NHP, allowing the free passage of fish into upper EFPC. Fish community data indicate a rapid natural reintroduction of fish into LR and upper EFPC since the opening of LR (see Sect. 2.3). Figure 1.1 indicates the location of NHP, LR, and the diversion channel. Also noted are sampling stations discussed throughout this report as the LR outlet and inlet (LR-o and LR-i, respectively), with similar notations for NHP.

Sampling stations utilized for various types of studies on upper EFPC are indicated on Fig. 1.1. Traditional designations for EFK points used for the BMAP studies are noted on the map; however, they are only approximations of the actual kilometer distances and have not been field measured. Six sites have been used for observations on fish kills, Sites 1 through 6 (approximate locations noted on Fig. 1.1). These sites were selected because of the presence of pool areas and ease of observing the pools for dead fish. A description of these sites is found in Sect. 1.3.3.

Figure 1.2 shows upper and lower EFPC, along with various study sites. Brushy Fork (BF), located in a watershed adjacent to the EFPC watershed, was selected as a reference study area for many of the studies summarized in this report. Although BF does not represent a pristine reference area, it is probably representative of the conditions occurring in EFPC before industrial development. BF is in a rural setting with a moderately open canopy and cover provided by narrow strips of mixed hardwoods and shrubs. Riffles and deep pools alternate throughout the reach selected as a reference site (BFK 7.6). Little alteration of instream habitat or cover has occurred at Brushy Fork in contrast to that found at EFPC.

A complete description of the EFPC watershed and the study sites appears in the third BMAP report, including a summary of the geohydrology of the area, characteristics of stream flow, and land use (Hinzman et al. 1995).

## 1.2 PREVIOUS FISH KILLS

Two fish kills occurred on November 21, 1986, and July 9, 1987, in the reach between NHP and EFK 22.3. These fish kills extended over a period of 2 to 3 weeks and have been documented in a report by Ryon et al. (1990). Central stoneroller (*Campostoma anomalum*) was the predominant species affected, comprising 99.8% (1148 fish) and 97.6% (747 fish) of the total in 1986 and 1987, respectively. Affected fish showed extensive scale loss, fin rot, and hemorrhaging from the gills, fins, and anus. Necropsy investigations of diseased fish revealed the cause of death to be bacterial hemorrhagic septicemia, a stress-mediated disease caused by *Aeromonas hydrophila*. Although the specific cause of the disease was not determined, several possible stressors have

been suggested. These include documented elevated concentrations of mercury and chlorine in EFPC just prior to the fish kills. Two other hypothetical stress-related causes are (1) the use of intensive electroshocking practices in EFPC prior to the kills and (2) the potential for elevated levels of the pathogen *A. hydrophila* in NHP. A combination of these causes, coupled with overcrowding and temperature fluctuations, may have resulted in a cumulative effect. However, a positive correlation between environmental conditions in EFPC and either of the fish kills was not evident, given the available data. There were no fish kills observed above EFK 23.4 (above NHP) during the period July 1987–July 1990. This was undoubtedly a function of the barrier to upstream movement of fish provided by NHP. With the opening of LR, fish were able to gradually colonize LR and the diversion channel and, subsequently, move into upper EFPC.

### 1.3 FISH KILL INVESTIGATION TECHNIQUES

Staff from the Oak Ridge National Laboratory (ORNL) Environmental Sciences Division (ESD) and the Y-12 Plant recognized the need to (1) understand the causes for historical fish kill events, both chronic and acute, and (2) develop action plans to deal with future fish kills. A BMAP Quality Assurance Plan including a Standard Operating Procedure (SOP) was developed to establish investigation procedures for fish kill incidents at DOE facilities. In addition, a self-directed work team was established to investigate Y-12 Plant fish kills.

#### 1.3.1. Standard Operating Procedures for Routine or Nonroutine Sampling Events

The SOP for the BMAP Fish Community Studies Quality Assurance Program is presented in Appendix A. The purpose of this SOP (SOP-10) is to establish investigative procedures for fish kill incidents at DOE facilities. Following notification of a fish kill, established procedures are implemented for recovery of field data and investigation of possible causes. These include a determination of the extent of the kill, measurement of water quality parameters in the affected reach(es) of stream, and implementation of control measures as necessary to reduce the impact of the kill.

A removal census of all injured or dead fish is established, which continues until no fatalities are observed for a series of successive census counts. The progress and results of field investigations are recorded in a numbered, regulated, field book. Section 2 of SOP-10 lists data analysis requirements for census data, water quality data, and field observations.

Section 3 of the SOP establishes the protocol for reporting fish kills and resulting data among BMAP and DOE facilities. Notification must be made to the Tennessee Wildlife Resources Agency (TWRA) and the Tennessee Department of Environment and Conservation (TDEC) of the relative size and impact of the kill. Throughout the duration of the kill, a continued exchange of information among the field investigators, ESD staff, and DOE and ESD managers is implemented. TWRA and TDEC are also continually updated. When enough information is gathered, a press release is

issued. After the fish kill, a complete documentation of all findings is made in a draft report by BMAP staff to the associated DOE facility.

### 1.3.2. Self-Directed Work Team

The self-directed work effort on Y-12 Plant fish kills grew out of meetings called by James Loar, manager of the BMAP for EFPC, with Y-12 Plant personnel. The meetings, which were initiated on November 26, 1991, addressed the need to understand the causes of the fish kills and ways to predict and develop action plans to deal with future fish kills. The group, consisting of Sigurd Christensen, Roxanna Hinzman, James Loar, Michael Ryon, Elizabeth Schilling, George Southworth, and Arthur Stewart of ESD at ORNL and Stanton Duke, R. S. Leete, Jr., Richard Mowery, Jennifer Seagraves, and Paul Stumb of the Y-12 Plant, became the Informal Team to Investigate Y-12 Fish Kills. The project was based on the concept of Total Quality Management.

#### 1.3.2.1 Informal team activities

The group identified sources of information and information needs for fish kill investigations. Data sources included maps of the Y-12 Plant area, daily fish kill reports, chlorine concentrations along EFPC, NPDES data, the Statistical Analysis Systems (SAS) Institute data base of physicochemical properties and other information, counts of dead fish, fish population sizes, models for fish kill predictions, and narrative analyses/ compendia of each fish kill event. Discussions or logs of Y-12 Plant operations that may have impacted EFPC were also discussed. James Loar led the interaction

between Y-12 Plant management and ESD; he contributed knowledge about past EFPC fish kills and fish ecology and coordinated the BMAP response. Roxanna Hinzman served as a logistical interface between Y-12 Plant data groups and the BMAP team. In addition, she contributed information about environmental variables and special toxicity studies. Michael Ryon and Elizabeth Schilling identified information needs related to the fish community. These included additional population data, migration of fish into pipes, species differences in chlorine tolerance, death rates in reference streams, carrying capacity of upper EFPC, drift rates and decomposition rates of dead fish, chlorine monitoring, and reliability of fish survey data. George Southworth contributed knowledge about past EFPC fish kills, bioaccumulation potentials, general water chemistry, and toxicant availability. Arthur Stewart was a source of information about past EFPC fish kills, toxicity tests, fish ecology, and specific toxicity potentials of select Y-12 Plant operations. R. S. Leete and Richard Mowery provided statistical analysis, use of a modeling approach to problem solving, and details on Y-12 Plant operations. In addition to plant operations, Paul Stumb, Jennifer Seagraves, and Stanton Duke provided knowledge about environmental monitoring at the Y-12 Plant, in particular water chemistry and NPDES data; they coordinated the on-site response to fish kills.

The Kepner-Tregoe problem analysis technique, which can be applied to phenomena that are not understood was applied to the fish kill problem. This technique is used to apply rational processes—situation appraisal, problem analysis, decision analysis, and potential problem analysis—related to specific concerns or to integrating processes into systems and activities. The Informal Team

applied this process to the fish kill of April 27–29, 1992, a fish kill of unknown cause(s). Although factual data were solicited from all members, a most probable cause of this fish kill did not emerge from the known data.

### 1.3.2.2 Fish kill action plans

The Fish Kill Investigation Committee was formed during the investigation of chronic fish kills. This committee developed action plans to direct the response of Y-12 Plant and ESD personnel in the event of unusually high fish mortality in upper EFPC. The Y-12 Plant action plans detail various responses to kill events, including water sampling, spill containment, and emergency notifications. The ESD action plan details levels of action by BMAP staff to determine the severity of a fish kill and to provide all possible “fish related” information needed to determine a possible cause. This plan consists of surveys, behavioral observations, specimen collection, and logistical considerations. The emphasis on each part escalates depending on the strength of the trigger observation. Trigger observations are defined as low (<18 dead fish for 1 d), high (18 to 100 dead fish on first day or >20 fish on two consecutive days), or crisis (>100 on first day). Details of the ESD surveys include search areas, frequencies, intensity, observations, and target organisms. Behavioral observations focus on detection of stress reactions and changes in normal behavior patterns that may provide clues to the cause of the fish kill. Specimen collections include all dead or dying fish to provide an estimate of the degree of damage. In some circumstances these collections may also include slightly stressed or nonstressed fish from upper EFPC and reference streams to be analyzed for comparative histopathological information. Finally, the plans detail

logistical considerations to improve crew response performance under varying conditions. These plans were originally designed to fit the unique situations of the chronic fish kill in upper EFPC. However, since being developed, they have evolved and become broader in scope. They no longer represent a fixed response but have become a flexible working guide to increase response efficiency under varying fish kill situations. These plans will be applied to future fish kill investigations conducted by ESD in other ORR streams as well as upper EFPC.

### 1.3.3. Sampling Technique

Implementation of the SOP and the Y-12 Plant and ESD Action Plans has resulted in a standard method of sampling in EFPC. Initially, fish surveys were conducted only at Sites 1 through 6 (see Fig. 1.1); if any dead fish were found, the entire stream within the Y-12 Plant was surveyed. Site 1 spans 150 m upstream from the pipes that exit the east side of the Y-12 Plant and includes several riffles and two large pools. Site 2 spans 125 m of pool upstream from a low check dam next to the Y-12 Plant truck scales; this site corresponds with the BMAP sampling site EFK 24.4. Site 3 covers 50 m of deep pool just upstream from the bridge near Y-12 Plant Building 9404-5. Site 4 also spans 50 m of pool associated with the Y-12 Plant instream water sampling site, Area Source Study Area 8 (AS-8; also referred to as Station 8). Site 5 covers 50 m of pool associated with NPDES Outfall 109 and the bridge near Y-12 Plant Building 9720-1. Site 6 spans 25 m of pool immediately downstream of the NSP.

For reporting purposes, the location of dead fish was recorded as being within a site or between two sites. However, for compilation into tables, all dead fish found below a site were added to the next downstream site (i.e., dead fish found

between Sites 3 and 4 were tabularized for Site 3). In addition to the sites within the Y-12 Plant, spot checks were also made at the road crossing of the diversion channel. If it was found that more extensive surveys were required, EFPC was surveyed from Bear Creek Road through the diversion channel up to the most upstream, aboveground point inside the plant. This extended survey also included a bank observation of LR. In a few cases, a small boat was launched on LR to search and recover dead fish within the lake.

#### 1.4 DOCUMENT ORGANIZATION

The following chapters of this report summarize Y-12 Plant fish kills from July 18, 1990, to March 31, 1993. Further data have been gathered on specific fish kill events after March 1993. These data will be summarized in subsequent BMAP reports. Chapter 2 of this report summarizes water quality data collected from several sites in upper EFPC and from the Y-12 Plant instream monitoring site, Station 17, just below LR. Also included in this chapter are the results of a toxicity loading analysis and a summary of fish community data from

October 1988 through March 1993. Several approaches, including ambient toxicity testing, in situ chemical monitoring, and streamside experiments, that investigated total residual chlorine (TRC) dynamics and ambient toxicity in EFPC are also summarized in this chapter. Chapter 3 summarizes the intensive Y-12 Plant fish kill monitoring efforts of 1990–93, which included investigation of both chronic (continuous, low level of mortality) and acute fish kill events (intermittent, high levels of mortality).

Many special studies and experiments have been performed since 1989 to evaluate specific ecotoxicological conditions at EFPC. Such studies were performed on fish, snails, clams, periphyton, and microbes, both as field studies and laboratory studies. Chapter 4 summarizes the findings of these studies. Chapter 5 describes the installation of two major upper EFPC dechlorination systems in November and December of 1992 and implementation of individual treatment processes for specific outfalls. Chapter 6 describes further pollution control activities to be undertaken in upper EFPC as well as ongoing or future BMAP and non-BMAP studies. A summary and conclusion appear in Chap. 7.

## 2. BACKGROUND CONDITIONS

The information contained in this chapter provides a general overview of the water quality of EFPC in terms of physical and chemical parameters, toxicity loading from Y-12 Plant outfalls, fish community structure, and ambient toxicity to test organisms. Much of the data presented here has been extracted from the Third BMAP Report for EFPC (Hinzman et al. 1995).

### 2.1 WATER QUALITY SUMMARY

Water quality information pertinent to this study was obtained from several sources. The most comprehensive set of data was that recorded at a Y-12 Plant monitoring site located downstream of NHP/LR (Station 17, at approximately EFK 23.2). Yearly mean and maximum values for 28 parameters measured in 1989-92 are listed in Table 2.1. Also included in this table are the U.S. Environmental Protection Agency's (EPA's) National Ambient Water Quality Criteria (NAWQC). Criteria maximum concentrations (CMC) are the highest concentrations of a pollutant to which aquatic life can be exposed for a short period of time (1-h average) without deleterious effects. Criteria continuous concentrations (CCC) are the highest concentrations of a pollutant to which aquatic life can be exposed for an extended period of time (4 d) without deleterious effects (EPA 1992).

Water quality data for upper EFPC were also available from several monitoring sites within the Y-12 Plant: at the NSP, at AS-8 (approximately halfway between NSP

and LR), at the oil-water separator upstream of LR, and at the inlet and outlet of LR (LR-i and LR-o). Locations of these sampling points are indicated in Fig. 1.1. The following sections provide a brief summary of the major water quality parameters evaluated for upper EFPC over the past several years. Additional information on water quality in upper EFPC can be found in the *Oak Ridge Reservation Environmental Report for 1992* (Kornegay et al. 1993) and in the *Y-12 Third Report on the Biological Monitoring and Pollution Abatement Program for East Fork Poplar Creek* (Hinzman et al. 1995).

#### 2.1.1. Total Residual Chlorine

Historically, during periods of dry weather, as much as 70% of the flow in upper EFPC consisted of chlorinated water; consequently, concentrations of chlorine in upper EFPC (expressed as TRC) were quite high. Data obtained at Station 17 (downstream of LR) and those obtained during BMAP studies conducted along upper EFPC provide some indication of the changes in TRC over the past 6 years. In the BMAP studies, TRC concentrations were monitored either by grab samples or by in situ monitoring. Grab samples were analyzed for TRC amperometrically with a Wallace and Tiernan titrator. In situ monitoring was accomplished by using a Xertex<sup>R</sup> model DC925 amperometric chlorine analyzer (Delta, Inc., Hauppauge, N.Y.), equipped with a Li-Cor<sup>R</sup> LI-100 data logger (LI-COR, Inc., Lincoln, Nebr).

Table 2.1. Mean (maximum) concentrations of water quality parameters measured at Station 17 from November 1988 to December 1992 compared with EPA Water Quality Criteria (concentrations in mg/L unless otherwise noted)

Parameter	1988					1989					1990					1991					1992					Water quality criteria		
	1988	1988	1988	1988	1988	1989	1989	1989	1989	1989	1990	1990	1990	1990	1990	1991	1991	1991	1991	1991	1992	1992	1992	1992	1992	CMC	CCC	
Alkalinity	116(130)					113(170)					110.5(150.0)				108(140)					108(200)							20 <sup>b</sup>	
Cadmium	<0.004(0.006)					<0.0035(<0.006)					<0.004(<0.004)				<0.0006(<0.004)					<0.004(<0.02)						0.0081 <sup>c</sup>	0.0019 <sup>e</sup>	
Calcium	56.9(66.9)					59.3(77.4)					62.2(79.6)				54.37(73.6)					57(100)								
Chloride	25(28)					23(86)					22.3(210.0)				19(150)					18.4(33)								
Chromium	<0.007(0.013)					<0.006(0.008)					<0.006(<0.006)				<0.006(0.030)					<0.006(0.03)								0.011 <sup>e</sup>
Conductivity, mhos/cm	NA <sup>a</sup>					NA <sup>a</sup>					55.6(97.0)				80(334)					6.3(471.2)								
Copper	0.012(0.047)					<0.008(0.013)					<0.008(0.014)				<0.007(0.030)				<0.008(0.032)									0.021 <sup>c</sup>
Dissolved oxygen	8.1(9.4)					6.7(11)					5.4(9.2)				7.6(10.2)					7.4(10.3)								5.0 <sup>b</sup>
Fluoride	1.23(2.0)					0.92(1.8)					0.99(7.7)				1.0(5.8)					1.3(72)								
Lead	<0.01(0.02)					<0.02(<0.02)					<0.02(0.02)				<0.002(0.02)				<0.004(0.061)									0.007 <sup>c</sup>
Lithium	0.067(0.451)					0.037(0.567)					<0.033(0.184)				<0.03(0.41)				<0.03(0.2)									
Magnesium	11.3(15.0)					11.02(13.1)					11.7(14.5)				10.86(14.40)					11(21.7)								
Mercury	<0.0019(0.0037)					0.0017(0.013)					0.0017(0.0057)				0.0014(0.0076)				0.0017(0.0089)									0.000012
Molybdenum	0.128(0.173)					0.195(0.481)					<0.042(0.179)				<0.007(0.110)				<0.007(0.03)									
Nickel	<0.011(0.02)					<0.008(0.109)					<0.01(0.2)				<0.037(<0.050)				<0.008(0.04)									0.273 <sup>c</sup>
Nitrate-N	4.3(10.0)					3.9(6.2)					4.8(6.9)				5.61(130.0)				<5.2(16)									
pH, standard units	NA(8.3)					NA(8.3)					NA(8.6)				NA(8.8)				8.0(8.8)									6.5-8.5
Phosphorus-total	0.34(0.4)					0.263(0.84)					0.33(0.82)				0.27(0.47)				<0.33(0.74)									
Potassium	2.9(3.9)					2.6(16.6)					2.51(3.8)				2.3(3.3)				2.2(4.7)									
Selenium	<0.002(0.002)					<0.002(<0.002)					<0.002(<0.002)				<0.002(<0.007)				<0.002(0.002)									
Sodium	23.6(55.3)					20.1(56.7)					27.6(81.4)				17.6(43.0)				16.9(43.9)									
Sulfate	334(1900)					<96(1400)					92.0(220.0)				68(220)				<63(150)									
Temperature, °C	16.5(20.5)					20.7(22.2)					21.2(22.2)				20.0(27.8)				20(35.6)									
Total suspended solids	21(90)					<19(170)					<12.4(41.0)				<11(62)				<13(310)									
Total organic carbons	26(110)					<11(29)					<16.4(305.0)				14.9(27.0)				<17.2(29)									
Total dissolved solids	317(440)					294.3(470)					320.0(600.0)				278(520)				421(32000)									
Total residual chlorine total	<0.13(0.2)					<0.11(0.48)					<0.1(<0.1)				<0.1(<0.1)				0.09(7.9)									0.011
Zinc	0.08(0.139)					0.063(0.109)					0.055(0.1)				0.044(0.170)				0.06(0.31)									0.183 <sup>c</sup>

<sup>a</sup>No EPA water quality criterion established for this parameter.

<sup>b</sup>Value is a minimum.

<sup>c</sup>Value given based on instream hardness of 191 mg/L at EFK 22.8 (see Table 2.6) and a default water effect ratio (WER) of 1.0 (see EPA 1992).

<sup>d</sup>Value shown is for hexavalent chromium.

<sup>e</sup>NA = not available.

<sup>f</sup>Suspended solids should not reduce the depth of the compensation point for photosynthetic activity by more than 10% from the seasonally established norm for aquatic life.

Source: EPA (U.S. Environmental Protection Agency). 1992. Water quality standards: Establishment of numeric criteria for priority toxic pollutants; states compliance. Fed. Regist. 57:60848-60917.

In 1988 and 1989, maximum TRC concentrations at Station 17 exceeded EPA's acute NAWQC (0.019 mg/L) and chronic NAWQC (0.011 mg/L) for the protection of aquatic organisms (see Table 2.1). In 1990, the maximum TRC concentrations at Station 17 were reported only as "< 0.1 mg/L"; therefore, some or all values may have exceeded the NAWQC. More-precise measurements of TRC concentrations at Station 17, as well as at four sites in upper EFPC (Outfall 135, Building 9204-1, AS-8, and in the diversion channel) were made on September 5 and 6, 1990 (Table 2.2). These measurements, made at different times of the day, revealed that TRC concentrations at Station 17 (0.03 to 0.10 mg/L) and in upper EFPC exceeded the NAWQC for TRC during this time period. These data, as well as those from other BMAP studies, revealed that a downstream gradient in TRC conditions occurred within upper EFPC, with the highest concentrations (0.98 to 1.43 mg/L) occurring furthest upstream at Outfall 135 located near NSP.

In 1991, the mean and maximum TRC concentrations at Station 17 were reported only as "< 0.1 mg/L"; therefore, some or all values may have exceeded the NAWQC. TRC concentrations at NSP from December 10-31, 1991, ranged from 0.19 to 0.42 mg/L (mean 0.31 mg/L,  $n = 11$ ), and those at AS-8 ranged from 0.06 to 0.19 mg/L (mean 0.13 mg/L,  $n = 11$ ).

As part of the Y-12 Plant BMAP studies (Hinzman et al. 1995), TRC concentrations were monitored in situ over 7-d periods at AS-8 (October 26-November 2, 1990) and at the oil-water separator (April 4-10, 1991). The monitoring data revealed strong daily cycles in TRC concentrations (Fig. 2.1). At each of these sites, TRC concentrations were about three times greater at night than those measured during midday, even though TRC loading rates were constant. Photolysis experiments suggested that these oscillations were driven by sunlight and periphyton. During the day, sunlight decreased the concentration of TRC through photolysis reactions, and exposure to periphyton decreased the concentration

**Table 2.2. Total residual chlorine concentrations (mg/L) in East Fork Poplar Creek, September 5-6, 1990**

Date	Time of day	Location				
		Outfall 135	Building 9204-1	AS-8	Diversion Channel	Station 17 <sup>a</sup>
09/05/90	9:47-10:52 a.m.	1.3	0.31	0.18	0.18	0.05
	2:50-3:48 p.m.	1.13	0.40	0.15	0.12	0.10
	7:37-9:05 p.m.	1.40	0.43	0.14	0.10	0.06
09/06/90	7:01-8:03 a.m.	1.43	0.48	0.12	0.09	0.03
	12:14-13:35 p.m.	0.98		0.13	0.21	0.10
	5:02-5:53 p.m.	1.09				

<sup>a</sup>Station 17 is located below Lake Reality, just upstream of Bear Creek Road.

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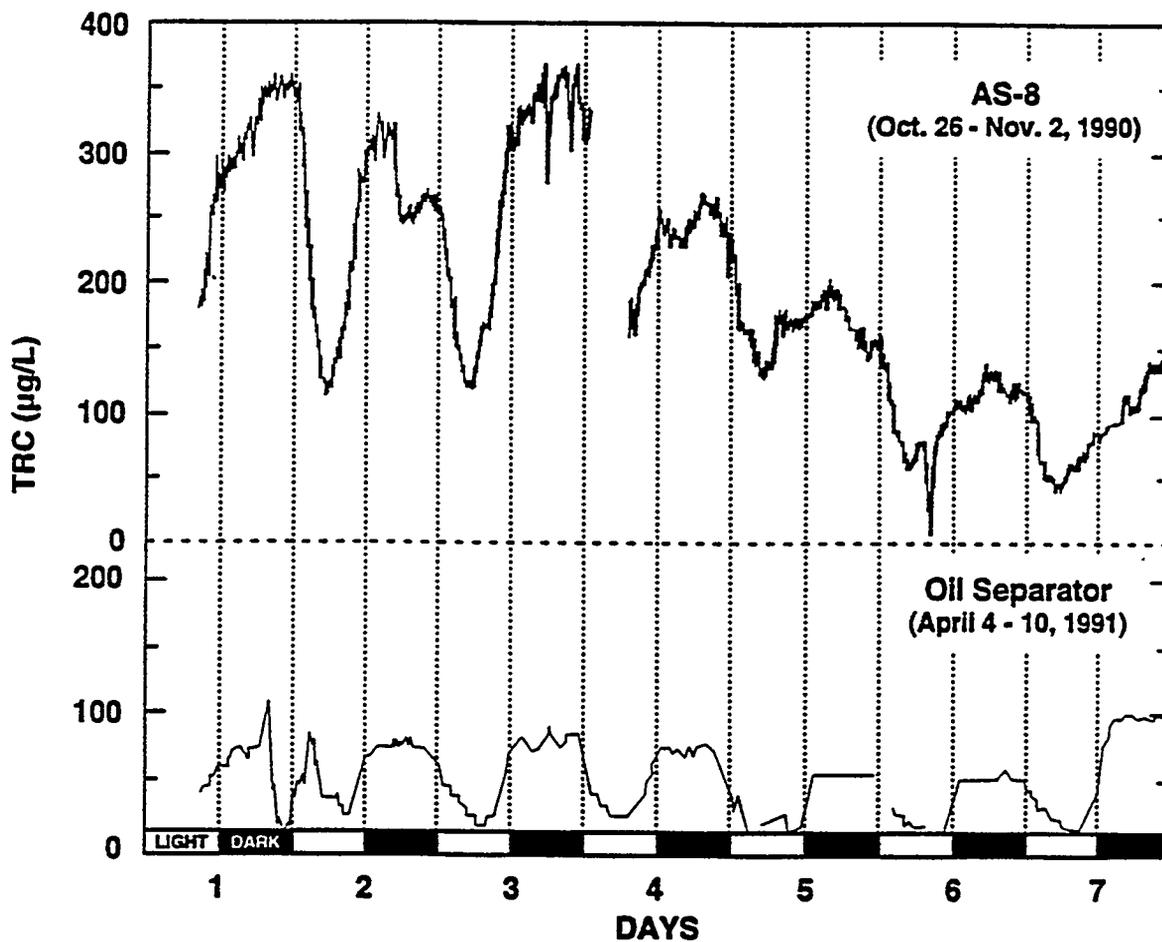


Fig. 2.1. Total residual chlorine concentrations at AS-8 and the oil separator site, measured by in situ monitoring.

by providing organic substrates that can combine with TRC constituents. Furthermore, high rates of algal photosynthesis during the day can increase the pH of the water through the assimilation of CO<sub>2</sub>, while at night photosynthesis stops and algal and microbial respiration continue, which lowers the pH. This cyclic change in pH results in a proportional increase in the more toxic hypochlorous ions at night. Increases in stream flows during storm periods can also lower TRC concentration by dilution. However, severe floods might also eliminate much of the periphyton by scouring (see Power and Stewart 1987), reducing the amount of organic matter available for binding with chlorine and thereby allowing the TRC concentrations to increase. The theoretical relationship between TRC and periphyton is illustrated in Fig. 2.2.

In 1992, the mean TRC concentration at Station 17 was 0.09 mg/L and the maximum recorded concentration was 7.9 mg/L (Table 2.1). From March 30–April 5, 1992, TRC measurements were made each day from 6 a.m. to 7 p.m. at six sites along upper EFPC (Table 2.3). As shown in previous studies, a downstream gradient in TRC concentration occurred in upper EFPC, with the highest concentrations near the NSP at Outfall 135. The TRC concentrations at this outfall (0.10 to 0.41 mg/L) were about 25% of the maximum levels that had been recorded in September of 1990. The lowest recorded TRC concentration at any of the sampling sites was 0.06 mg/L.

TRC measurements made in 1992 at NSP, AS-8, and LR-i are summarized in Table 2.4. A dechlorinator, installed at NSP, became operational on November 30, 1992. As a result, the TRC concentrations at all three sampling sites showed a substantial decrease in December 1992. A second dechlorinator was installed on Outfall 21 downstream of Site 3 (see Fig. 1.1) and became operational on December 29, 1992.

As a result of the installation of the two dechlorinators in upper EFPC, TRC concentrations in the first 3 months of 1993 were substantially lower than those recorded prior to December 1992 (Table 2.5), and by June 1993 concentrations were 0.04 to 0.06 mg/L at NSP, below detection limits at AS-8, and 0 (below detection limits) to 0.01 mg/L at the inlet and outlet of LR (Table 2.6).

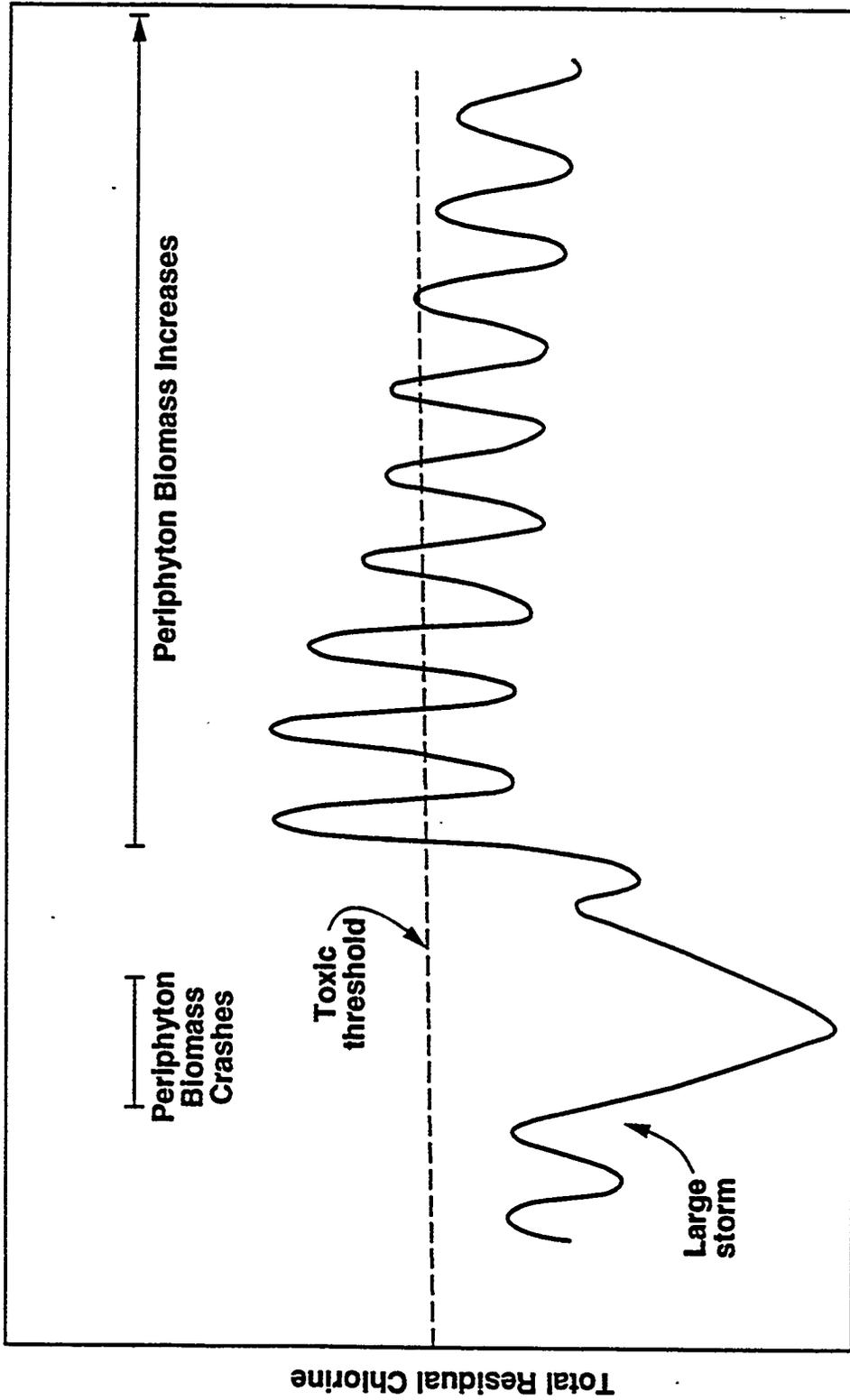
### 2.1.2. pH, Conductivity, Alkalinity, and Hardness

As part of the BMAP studies, pH, conductivity, alkalinity, and water hardness were measured routinely at four upper EFPC sites (NSP, AS-8, LR-i, and LR-o), as well as at six sites below LR (EFKs 22.8, 21.9, 20.5, 18.2, 13.8, and 10.9). Mean values of these parameters for the period November 1988 through August 1992 are summarized in Table 2.7.

The data indicate that there is a downstream gradient in conductivity in upper EFPC. This reduction in conductivity downstream may be the result of inputs of drinking water, seepage from groundwater or springs, or loss of ionic calcium through formation of insoluble salts. At NSP and AS-8, water hardness and conductivity were closely correlated, suggesting that carbonate formation may be an important stream process. As noted in the Third BMAP Report (Hinzman et al. 1995), the formation of carbonates in upper EFPC could have an important impact on the overall flux of contaminants downstream. Metals and organics that bind with these particles can be deposited in the stream bed and then washed downstream during storm events (Stewart and Wetzel 1981).

For the period January 1993 through June 1993, measurements of these parameters were made at NSP, AS-8, LR-i, and LR-o during six 7-d periods. The results of this monitoring (means and standard

ORNL DWG 91M-1846R



Time (d)

Fig. 2.2. Predicted effect of floods, sunlight, and periphyton on TRC oscillations in TRC-contaminated streams.

Table 2.3. Total residual chlorine concentrations (mg/L) in upper East Fork Poplar Creek, March 30–April 5, 1992

Date	Time of day	Location					
		NSP	Outfall 135	Outfall 109	AS-8	Outfall 36/9404-5	Outfall 21&22
March 30	6:00-7:00 a.m.	0.15	0.18	0.12	0.06	0.06	0.06
	10:00-11:00 a.m.	0.15	0.22	0.12	0.09	0.08	0.09
	12:00-1:00 p.m.	0.13	0.27	0.13	0.14	0.09	0.10
	2:00-3:00 p.m.	0.21	0.30	0.13	0.07	0.08	0.11
	6:00-7:00 p.m.	0.08	0.10	0.10	0.05	0.06	0.10
March 31	6:00-7:00 a.m.	0.17	0.21	0.09	0.09	0.09	0.10
	10:00-11:00 a.m.	0.20	0.25	0.16	0.09	0.07	0.11
	12:00-1:00 p.m.	0.22	0.25	0.14	0.09	0.07	0.10
	2:00-3:00 p.m.	0.13	0.17	0.10	0.11	0.09	0.12
	6:00-7:00 p.m.	0.12	0.13	0.08	0.08	0.10	0.06
April 1	6:00-7:00 a.m.	0.13	0.22	0.11	0.09	0.09	0.12
	10:00-11:00 a.m.	0.25	0.25	0.09	0.04	0.10	0.11
	12:00-1:00 p.m.	0.14	0.20	0.11	0.09	0.09	0.11
	2:00-3:00 p.m.	0.17	0.21	0.10	0.08	0.08	0.10
	6:00-7:00 p.m.	0.15	0.23	0.12	0.08	0.08	0.12
April 2	6:00-7:00 a.m.	0.12	0.22	0.11	0.10	0.10	0.12
	10:00-11:00 a.m.	0.16	0.24	0.11	0.07	0.08	0.10
	12:00-1:00 p.m.	0.13	0.21	0.10	0.10	0.08	0.08
	2:00-3:00 p.m.	0.16	0.24	0.13	0.09	0.07	0.11
	6:00-7:00 p.m.	0.14	0.23	0.13	0.10	0.08	0.13
April 3	6:00-7:00 a.m.	0.19	0.28	0.10	0.10	0.10	0.11
	10:00-11:00 a.m.	0.17	0.34	0.14	0.12	0.11	0.11
	12:00-1:00 p.m.	0.14	0.32	0.20	0.08	0.11	0.11
	2:00-3:00 p.m.	0.19	0.34	0.12	0.10	0.10	0.13
	6:00-7:00 p.m.	0.15	0.30	0.11	0.10	0.08	0.15
April 4	6:00-7:00 a.m.	0.25	0.41	0.18	0.14	0.12	0.14
	10:00-11:00 a.m.	0.23	0.34	0.14	0.11	0.08	0.14
	12:00-1:00 p.m.	0.22	0.34	0.13	0.09	0.10	0.12
	2:00-3:00 p.m.	0.19	0.32	0.11	0.09	0.08	0.12
	6:00-7:00 p.m.	0.22	0.31	0.14	0.06	0.07	0.10
April 5	6:00-7:00 a.m.	0.20	0.31	0.14	0.10	0.10	0.12
	10:00-11:00 a.m.	0.21	0.31	0.12	0.07	0.10	0.10
	12:00-1:00 p.m.	0.23	0.31	0.12	0.06	0.11	0.09
	2:00-3:00 p.m.	0.21	0.30	0.13	0.07	0.11	0.12
	6:00-7:00 p.m.	0.19	0.34	0.15	0.09	0.09	0.12

Table 2.4. Total residual chlorine concentrations (mg/L)<sup>a</sup> at NSP, AS-8 and LR-i, January 1–December 31, 1992

Month	Number of sampling days	NSP	AS-8	LR-i
January	22	0.29 (0.03–0.43)	0.13 (0.02–0.19)	0.04 <sup>b</sup>
February	19	0.24 (0–0.41)	0.09 (0–0.14)	0.01 (0–0.3) <sup>d</sup>
March	21	0.22 (0.11–0.44)	0.09 (0.03–0.17)	0.06 (0–0.24)
April	17	0.16 (0.10–0.25)	0.08 (0.04–0.11)	0.01 (0–0.05)
May	20	0.13 (0.07–0.25)	0.07 (0.01–0.10)	0.02 (0–0.12)
June	22	0.14 (0.06–0.26)	0.07 (0.02–0.10)	0.02 (0–0.05)
July	21	0.16 (0.07–0.30)	0.07 (0.03–0.24)	0.02 (0–0.06)
August	21	0.17 (0.02–0.31)	0.06 (0.02–0.12)	0.02 (0.01–0.03)
September	21	0.18 (0.07–0.31)	0.07 (0.02–0.11)	0.03 (0.02–0.06)
October	20	0.26 (0.06–0.68)	0.08 (0.04–0.13)	0.03 (0.01–0.06)
November	20	0.26 (0–0.43)	0.14 (0–0.28)	0.05 (0–0.12)
December	21	0.06 (0–0.30)	0.05 (0–0.21)	0.03 (0.01–0.07)

<sup>a</sup>Mean and range.

<sup>b</sup>One sampling day.

<sup>c</sup>0 = below detection limits.

<sup>d</sup>Fourteen sampling days.

Table 2.5. Mean total residual chlorine concentrations (mg/L) at NSP, AS-8, and LR-i, January–March 1993

Month	Number of sampling days	NSP	AS-8	LR-i
January	20	0.06 (0–0.17) <sup>a</sup>	0.06 (0–0.12)	0.03 (0–0.04)
February	16	0.03 (0–0.15)	0.03 (0–0.06)	0.01 (0–0.3)
March	11	0.03 (0.01–0.09)	0.04 (0.01–0.07)	0.02 (0–0.05)

<sup>a</sup>Range, 0 = below detection limits.

Table 2.6. Total residual chlorine concentrations (mg/L) in upper East Fork Poplar Creek, January 28–June 30, 1993

Date	Location			
	NSP	AS-8	LR-i	LR-o
01/28/93	0.09	0.03	0.02	0.01
01/29/93	0.00	0.04	0.02	0.02
01/30/93	0.14	0.10	0.02	0.01
01/31/93	0.26	0.13	0.02	0.01
02/01/93	0.18	0.12	0.02	0.02
02/02/93	0.06	0.04	0.01	0.00
02/03/93	0.12	0.04	0.02	0.01
02/25/93	0.00	0.00	0.00	0.01
02/26/93	0.00	0.00	0.00	0.01
02/27/93	0.10	0.05	0.01	0.01
02/28/93	0.00	0.00	0.00	0.01
03/01/93	0.12	0.04	0.02	0.00
03/02/93	0.11	0.16	0.06	0.04
03/03/93	0.09	0.06	0.01	–
03/11/93	0.10	0.06	0.01	0.02
03/12/93	0.08	0.09	0.02	0.01
03/13/93	0.10	0.08	0.02	0.01
03/14/93	0.10	0.08	0.02	0.01
03/15/93	0.08	0.04	0.00	0.01
03/16/93	0.02	0.02	0.01	0.01
03/17/93	0.00	0.00	0.00	0.00
04/29/93	0.07	0.01	0.00	0.00
04/30/93	0.07	0.01	0.00	0.01
05/01/93	0.09	0.02	0.01	0.01
05/02/93	0.00	0.01	0.00	0.01
05/03/93	0.04	0.00	0.00	0.01
05/04/93	0.00	0.00	0.00	0.00
05/05/93	0.05	0.02	0.00	0.00
05/20/93	0.09	0.08	0.02	0.01
05/21/93	0.07	0.03	0.00	0.03
05/22/93	0.07	0.04	0.01	0.01
05/23/93	0.12	0.04	0.01	0.01
05/24/93	0.06	0.05	0.01	0.01
05/25/93	0.05	0.02	0.01	0.02
05/26/93	0.03	0.02	0.01	0.02
06/24/93	0.04	0.00	0.00	0.00
06/25/93	0.00 <sup>a</sup>	0.00	0.00	0.00
06/26/93	0.00	0.00	0.00	0.00
06/27/93	0.04	0.00	0.01	0.00
06/28/93	0.06	0.00	0.01	0.01
06/29/93	0.04	0.00	0.01	0.01
06/30/93	0.04	0.00	0.01	0.01

Table 2.7. Mean ( $\pm$ SE) of 7-d means for pH, conductivity, alkalinity, and hardness for grab samples of water collected in East Fork Poplar Creek from November 1988 through August 1992

Site	N <sup>a</sup>	pH	Conductivity ( $\mu$ mho/cm)	Alkalinity (mg/L)	Hardness (mg/L)
NSP	24	7.96 $\pm$ 0.03	554 $\pm$ 20	105.9 $\pm$ 1.4	227 $\pm$ 5
AS-8	43	8.03 $\pm$ 0.02	468 $\pm$ 14	105.8 $\pm$ 0.9	194 $\pm$ 3
LR-i	43	8.02 $\pm$ 0.02	435 $\pm$ 9	112.6 $\pm$ 1.0	184 $\pm$ 2
LR-o	44	8.06 $\pm$ 0.02	457 $\pm$ 8	110.6 $\pm$ 1.1	193 $\pm$ 2
EFK 22.8	14	7.97 $\pm$ 0.05	439 $\pm$ 9	111.3 $\pm$ 1.6	191 $\pm$ 4
EFK 21.9	14	7.90 $\pm$ 0.04	430 $\pm$ 8	114.4 $\pm$ 1.3	193 $\pm$ 3
EFK 20.5	14	7.96 $\pm$ 0.04	430 $\pm$ 8	115.1 $\pm$ 1.4	193 $\pm$ 3
EFK 18.2	14	7.92 $\pm$ 0.04	407 $\pm$ 8	122.0 $\pm$ 1.5	188 $\pm$ 4
EFK 13.8	14	7.95 $\pm$ 0.04	376 $\pm$ 8	122.1 $\pm$ 1.8	179 $\pm$ 4
EFK 10.9	14	7.91 $\pm$ 0.04	408 $\pm$ 10	123.7 $\pm$ 1.6	175 $\pm$ 4

<sup>a</sup>N is the number of 7-d means used to compute the final mean value for each water quality factor.

errors) are shown in Table 2.8. These values are similar to those recorded in 1992.

Conductivity, alkalinity, and water hardness can be used to derive a chemical perturbation index (CPI), which is a measure of the degree that a stream has been chemically altered from a natural condition (Hinzman et al. 1993). In this procedure, statistical methods are used to compute the Spearman (rank) correlation coefficient between each pair of the three parameters and the three correlation values are summed to derive the CPI. A CPI of 3.0 is the theoretical upper limit for an undisturbed stream. In the reporting period from November 1988 through August 1992, the lowest CPI values in EFPC were found furthest upstream; they ranged from 0.89 at AS-8 to 2.05 at EFK 13.8. The conductivity, alkalinity, and water hardness recorded for upper EFPC in the first 6 months of 1993 suggest a similar low CPI for this section of EFPC.

### 2.1.3. Water Temperature

During the BMAP studies, water temperatures were monitored at five sampling sites in EFPC below LR, at one site just above LR (EFK 24.4), and in a reference stream, Brushy Fork (BF). Mean water temperatures in EFPC just below LR (EFK 23.4) were generally 4 to 7°C higher than those in BF. Although temperatures below LR frequently exceeded 25°C (maximum, 40°C), the maximum temperature in BF seldom exceeded 25°C. Water temperatures at EFK 24.4, inside the Y-12 Plant, were generally similar to those at EFK 23.4 in the summer but were 2 to 4°C warmer in the winter. Warmer winter temperatures may be attributed to the proximity of the site to several effluent discharges.

Table 2.8. Means ( $\pm$ SE) of 7-d composite samples for pH, conductivity, alkalinity, and hardness for water collected from East Fork Poplar Creek from January 1993 through June 1993

Site	1993 Date	<i>N</i> <sup>a</sup>	pH (standard units)	Conductivity ( $\mu$ mho/cm)	Alkalinity (mg/L)	Hardness (mg/L)
NSP	1/28-2/03	7	8.08 $\pm$ 0.04	562.8 $\pm$ 121.5	89.2 $\pm$ 13.6	261.7 $\pm$ 47.9
NSP	2/25-3/03	7	7.97 $\pm$ 0.06	566.6 $\pm$ 93.1	99.3 $\pm$ 2.6	245.7 $\pm$ 37.4
NSP	3/11-3/17	7	8.12 $\pm$ 0.05	544.6 $\pm$ 90.6	106.6 $\pm$ 5.2	212.9 $\pm$ 26.1
NSP	4/29-5/05	7	8.17 $\pm$ 0.03	531.1 $\pm$ 72.2	104.1 $\pm$ 4.4	212.6 $\pm$ 23.4
NSP	5/20-5/26	7	8.22 $\pm$ 0.07	639.4 $\pm$ 86.8	106.6 $\pm$ 3.6	256.3 $\pm$ 21.1
NSP	6/24-6/30	7	8.01 $\pm$ 0.02	498.0 $\pm$ 49.6	104.0 $\pm$ 2.9	216.3 $\pm$ 23.9
AS-8	1/28-2/03	7	8.15 $\pm$ 0.04	477.6 $\pm$ 74.6	103.7 $\pm$ 1.9	222.0 $\pm$ 28.5
AS-8	2/25-3/03	7	8.01 $\pm$ 0.05	460.7 $\pm$ 64.8	111.9 $\pm$ 13.0	200.9 $\pm$ 24.1
AS-8	3/11-3/17	7	8.19 $\pm$ 0.04	527.0 $\pm$ 88.4	105.3 $\pm$ 4.1	208.0 $\pm$ 23.6
AS-8	4/29-5/05	7	8.26 $\pm$ 0.04	388.3 $\pm$ 24.2	104.0 $\pm$ 5.1	169.7 $\pm$ 7.0
AS-8	5/20-5/26	7	8.28 $\pm$ 0.05	532.7 $\pm$ 79.0	105.4 $\pm$ 2.1	237.4 $\pm$ 21.2
AS-8	6/24-6/30	7	8.23 $\pm$ 0.05	429.3 $\pm$ 32.4	98.3 $\pm$ 1.5	191.1 $\pm$ 11.2
LR-i	1/28-2/03	7	8.06 $\pm$ 0.02	446.4 $\pm$ 29.5	117.3 $\pm$ 2.0	201.7 $\pm$ 11.3
LR-i	2/25-3/03	7	8.00 $\pm$ 0.08	541.9 $\pm$ 41.4	119.6 $\pm$ 4.0	201.7 $\pm$ 14.1
LR-i	3/11-3/17	7	8.16 $\pm$ 0.04	522.0 $\pm$ 23.1	114.6 $\pm$ 5.0	210.0 $\pm$ 10.7
LR-i	4/29-5/05	7	8.20 $\pm$ 0.05	395.3 $\pm$ 38.5	106.6 $\pm$ 8.5	171.1 $\pm$ 14.0
LR-i	5/20-5/26	7	8.39 $\pm$ 0.05	429.4 $\pm$ 27.1	117.0 $\pm$ 1.4	187.1 $\pm$ 2.3
LR-i	6/24-6/30	7	8.31 $\pm$ 0.07	452.4 $\pm$ 38.1	104.6 $\pm$ 2.0	182.6 $\pm$ 8.3
LR-o	1/28-2/03	7	8.08 $\pm$ 0.03	524.7 $\pm$ 13.4	118.7 $\pm$ 0.6	225.4 $\pm$ 5.9
LR-o	2/25-3/03	7	7.95 $\pm$ 0.08	626.6 $\pm$ 52.3	119.4 $\pm$ 4.3	263.7 $\pm$ 39.8
LR-o	3/11-3/17	7	8.15 $\pm$ 0.03	617.4 $\pm$ 89.4	114.0 $\pm$ 6.1	219.7 $\pm$ 11.5
LR-o	4/29-5/05	7	8.29 $\pm$ 0.06	428.6 $\pm$ 32.8	109.1 $\pm$ 4.7	186.6 $\pm$ 10.2
LR-o	5/20-5/26	7	8.26 $\pm$ 0.07	451.9 $\pm$ 11.7	109.7 $\pm$ 2.9	199.7 $\pm$ 6.1
LR-o	6/24-6/30	7	8.56 $\pm$ 0.05	474.0 $\pm$ 21.4	108.0 $\pm$ 1.1	198.9 $\pm$ 5.0

<sup>a</sup>*N* is the number of 7-d means used to compute the final mean value for each water quality factor.

#### 2.1.4. Ammonia

During June 1992, water samples collected at various sites in upper EFPC were analyzed for ammonia by the salicylate method described by Verdouw et al. (1978). The results of this analysis showed low concentrations of ammonia at NSP and AS-8 (25 and 16  $\mu\text{g/L}$ , respectively) and a high concentration in the diversion channel near LR-i (230 to 280  $\mu\text{g/L}$ ). Outfall 017 (near EFK 24.4 and located just upstream of the diversion channel) was identified as the major source of ammonia. The concentration of ammonia in Outfall 017 water was  $\sim 83$  mg/L on June 25 and  $\sim 95.2$  mg/L on July 9, 1992 (concentrations upstream of Outfall 017 were  $< 80$   $\mu\text{g/L}$ ). The maximum concentration of ammonia in a sample of water taken 60 m downstream from Outfall 017 was 2040  $\mu\text{g/L}$ . Water samples collected on July 14, 1992, and the absence of any intervening rain events suggest that ammonia concentrations in excess of 1000  $\mu\text{g/L}$  may have been present in upper EFPC below Outfall 017 for a week or more.

#### 2.1.5. Metals

The most complete data on concentrations of metals in EFPC are those obtained at Station 17 downstream of LR (Table 2.1). These concentrations were compared with EPA acute and chronic water quality criteria (CMC and CCC, respectively). Water quality criteria for heavy metals are a function of water hardness, and they can also vary with intrinsic stream characteristics defined as a water effect ratio (EPA 1992). For this report, the appropriate criteria were calculated by using a water hardness of 191 mg/L (as measured at EFK 22.8, see Table 2.7) and a water effect ratio of 1.0. For the 1991 and 1992 reporting periods, the mean concentrations of chromium,

copper, lead, zinc, and nickel at Station 17 were all below their corresponding CCCs. Maximum concentrations of the first four of these metals exceeded their CMCs in some cases. The mean and maximum concentrations for cadmium in 1992 were reported as  $< 0.004$  and  $< 0.02$  mg/L, respectively, and the CCC and CMC were 0.0019 and 0.0081 mg/L, respectively, suggesting that the criteria might have been exceeded at some time during this period. Mean mercury concentrations for both 1991 and 1992 (0.0014 and 0.0017 mg/L, respectively) were above the CCC of 0.000012 mg/L and maximum concentrations were above the CMC of 0.0024 mg/L. A more detailed discussion of mercury contamination in upper EFPC can be found in the Third BMAP Report (Hinzman et al. 1995).

## 2.2 TOXICITY LOADING ANALYSIS

The upper 1.5-km reach of EFPC lies entirely within the boundaries of the Y-12 Plant and historically has received effluents from more than 200 NDPES-permitted outfalls (Loar et al. 1992). Pollutants in these effluents may be toxic to aquatic organisms. The NDPES permit for the Y-12 Plant requires the periodic monitoring of some of these effluents for acute and chronic toxicity. Two EPA-approved toxicity tests are used for this purpose: (1) the 7-d fathead minnow (*Pimephales promelas*) larval survival and growth test and (2) the *Ceriodaphnia* 7-d survival and reproduction test (see Weber et al. 1989 for a description of the test methods). These tests are used to estimate a wastewaters' no-observed-effect concentration (NOEC). The NOEC is calculated by comparing the responses of the animals exposed to control water with those of animals exposed to various dilutions of the test water. The NOEC is the highest concentration (for toxicity loading

analysis, this is a percentage of full-strength effluent) that does not adversely affect the test species. If a test wastewater's NOEC is <100%, the potential effects on the stream are estimated from the toxicity loading rate. The latter is expressed in terms of toxicity units (TUs), which are calculated by dividing the mean NOEC for the effluent into the effluent's mean instream waste concentration (IWC), which is expressed as a percentage of total stream flow. The lower the NOEC and the higher the effluent discharge rate, the greater the number of TUs released into the stream and the greater the potential for environmental impact. A summary of the toxicity loading of upper EFPC for selected outfalls is shown in Table 2.9.

During the assessment period of October 1988 to August 1992, a total of 0.238 TUs entered upper EFPC (total wastewater discharge rate of  $801.3 \times 10^6$  L per year), substantially below the 4.07 TU that entered upper EFPC during the period from January 1986 to October 1988 (Hinzman et al. 1995). The reduction in toxicity loading to upper EFPC since 1988 has resulted in improved water quality in upper EFPC.

### 2.3 FISH COMMUNITY DATA

Beginning in October of 1988 and continuing intermittently through March and April of 1993, surveys were conducted at four sites along EFPC to determine the size and composition of the fish communities. Three of the sampling sites were located upstream of NHP/LR at stream kilometer (EFK) markers 25.1, 24.7, and 24.4, and one site was located downstream at EFK 23.4. Over the same time periods, sampling was also conducted at a reference stream, BF. The sampling data for each site were analyzed for species richness (number of species of fish present), fish density (number of fish per square meter of

surface), and total fish biomass (total weight of fish per square meter of surface). These data are summarized in Table 2.10.

Over the 4.5-year study period, the number of species found just downstream of NHP/LR at EFK 23.4 ranged from 7 to 14 (17 species have been collected from this stretch of EFPC during the daily dead fish surveys, see Sect. 3). In comparison, 14 to 23 species of fish have been found in BF over the same time period. Prior to the opening of LR, few fish were found in upper EFPC because the weir at the outfall of NHP was an effective barrier to upstream movement of fish. With the opening of LR in November 1988, fish were able to colonize LR, the diversion channel, and then the reach of stream above the channel. Since 1988, the number of species in upper EFPC has gradually increased, and by spring 1993, four species [striped shiners (*Luxilus chrysocephalus*), central stonerollers (*Campostoma anomalum*), blacknose dace (*Rhinichthys atratulus*), and redbreast sunfish (*Lepomis auritus*)] were found at each of the upper EFPC sampling sites. Evidence from the daily dead fish surveys conducted along EFPC (see Sect. 3) indicates that these four species are now present as far upstream as the NSP. The daily surveys have also revealed that as many as eight species of fish can now be found in LR and in the diversion channel.

In the March–April 1993 survey, striped shiners and stonerollers constituted 83 to 99% of the total fish population in upper EFPC and 93% of the population downstream of LR at EFK 23.4 (Table 2.11). In contrast, stonerollers in BF accounted for only 1.3% of the fish population and striped shiners accounted for 11.4%.

The increase in fish species in upper EFPC since the opening of LR has been accompanied by an increase in the fish population (Fig. 2.3). In the first 2 years of the study, most of this increase was probably the result of colonization by

Table 2.9. Toxicity loading analysis of upper East Fork Poplar Creek, October 1988 through August 1992

Facility, NPDES outfall	Toxicity test date	NOEC <sup>a</sup> (%)	Discharge (10 <sup>6</sup> L/yr) <sup>b</sup>	IWC <sup>c</sup> (%)	Toxic units <sup>d</sup>
Cooling tower, 613	05/11/89	25			
Cooling tower, 622	08/09/90	100			
Cooling tower, 613	01/18/90	100	354.40	4.35	0.051
Cooling tower, 622	10/10/90	100			
Cooling tower, 602	08/06/92	100 (85)			
Catch Basin, 408	08/16/90	25			
	03/12/92	50 (38)	17.94	0.22	0.006
Sump Pump Oil Separator, 506	08/09/90	100			
	02/07/92	100 (100)	99.47	1.22	0.012
Ground Water Treatment Facility, 512	12/05/91	<3			
	01/09/92	25 (14)	79.58	0.97	0.069
Central Pollution Control Facility, 501	08/19/90	100			
	10/24/91	1	15.98	0.20	0.005
	02/20/92	12 (38)			
West End Treatment Facility, 502	10/12/89	5			
	10/11/90	3			
	05/28/92	5	24.10	0.30	0.050
	06/25/92	11 (6)			
Steam Plant Wastewater Treatment Facility, 503	06/08/89	100			
	01/17/91	12 (56)	181.64	2.23	0.040
Plating Rinsewater Treatment Facility, 504	09/13/90	100			
	09/05/91	30 (65)	28.15	0.35	0.005

<sup>a</sup>NOEC is the highest tested concentration of wastewater (%) causing no adverse affect to either fathead minnows or *Ceriodaphnia*. The mean NOEC for each set of tests is given in parentheses.

<sup>b</sup>Discharge rates for facilities are from Komegay, F. C., D. C. West, L. W. McMahon, J. B. Murphy, L. G. Shipe, and W. S. Koncinski, 1993. Oak Ridge Reservation Environmental Report for 1992. ES/ESH-31. Oak Ridge National Laboratory, Oak Ridge, Tenn., pp. 79-86.

<sup>c</sup>IWC is the instream waste concentration of each wastewater, computed relative to a 4-year mean discharge at EFPC Station 17 (1988 through 1991, 258.5 L/s).

<sup>d</sup>Mean instream waste concentration (IWC) divided by the mean NOEC.

Table 2.10. Species richness (total number of species), fish density (fish per square meter), and total biomass (g/m<sup>2</sup>) in East Fork Poplar Creek and a reference stream, Brushy Fork

Sampling period	Parameters	Location				
		EFK <sup>a</sup> 25.1	EFK <sup>a</sup> 24.7	EFK <sup>a</sup> 24.4	EFK <sup>a</sup> 23.4	BFK <sup>b</sup> 7.6
1988 October	Species richness	NS <sup>c</sup>	NS	0	10	23
	Density				3.52	1.18
	Biomass				24.87	7.95
1989 March–April	Species richness	NS	NS	1	7	17
	Density			<0.01	1.98	0.65
	Biomass			0.03	11.05	4.50
October	Species richness	2	NS	1	11	22
	Density	0.10		<0.01	3.21	0.76
	Biomass	2.38		0.03	20.26	6.85
1990 March	Species richness	0	NS	4	10	17
	Density			0.55	3.75	0.41
	Biomass			19.40	16.20	6.40
Sept.–Nov.	Species richness	1	NS	5	11	22
	Density	0.01		0.73	10.24	0.83
	Biomass	0.19		9.62	51.84	10.00
1991 March–May	Species richness	2	NS	4	10	14
	Density	0.06		0.85	6.08	0.37
	Biomass	1.03		11.97	24.61	4.08
October	Species richness	1	NS	5	14	22
	Density	0.01		2.68	10.34	1.72
	Biomass	0.07		31.20	32.78	8.57
1992 March–April	Species richness	0	4	4	11	18
	Density		2.21	2.22	9.03	0.95
	Biomass		34.73	34.10	37.90	12.04
October	Species richness	0	4	4	11	23
	Density		4.87	4.87	10.13	1.25
	Biomass		50.73	36.13	48.05	6.39
1993 March–April	Species richness	4	4	4	12	21
	Density	2.91	3.19	2.95	7.65	0.70
	Biomass	14.82	30.60	33.36	32.44	9.79

<sup>a</sup>EFK = East Fork kilometer marker.

<sup>b</sup>BFK = Brushy Fork kilometer marker.

<sup>c</sup>NS = not sampled during this sampling period.

Table 2.11. Percentage of central stoneroller and striped shiner densities (fish per square meter) of total fish density in East Fork Poplar Creek and a reference stream, Brushy Fork

Sampling period	Species	Location					
		EFK <sup>a</sup> 25.1	EFK <sup>a</sup> 24.7	EFK <sup>a</sup> 24.4	EFK <sup>a</sup> 23.4	BFK <sup>b</sup> 7.6	
1988	October	Central stoneroller	NS <sup>c</sup>	NS	NF <sup>d</sup>	39.0	4.0
		Striped shiner				43.0	33.1
1989	March–April	Central stoneroller	NS	NS		53.0	6.2
		Striped shiner			100	34.4	26.2
	October	Central stoneroller		NS		30.2	3.9
		Striped shiner	97		100	56.1	22.4
1990	March	Central stoneroller	NF	NS	0.7	32.3	2.4
		Striped shiner			98.2	60.0	29.3
	Sept.–Nov.	Central stoneroller			17.8	27.5	6.0
		Striped shiner	100	NS	71.2	40.3	38.6
1991	March–May	Central stoneroller	0	NS	23.5	60.9	2.7
		Striped shiner	66.7		74.1	32.1	21.6
	October	Central stoneroller	100	NS	42.5	28.6	7.0
		Striped shiner			50.0	60.8	46.5
1992	March–April	Central stoneroller	NF	28.5	69.3	36.4	5.2
		Striped shiner		68.8	23.4	58.5	7.3
	October	Central stoneroller	NF	25.3	41.9	48.8	1.6
		Striped shiner		69.0	34.5	34.2	20.8
1993	March–April	Central stoneroller	19.9	34.8	58.0	56.9	1.3
		Striped shiner	78.0	57.4	25.1	36.3	11.4

<sup>a</sup>EFK = East Fork Poplar Creek kilometer marker.

<sup>b</sup>BFK = Brushy Fork kilometer marker.

<sup>c</sup>NS = not sampled during this sampling period.

<sup>d</sup>NF = no fish collected during sampling period.

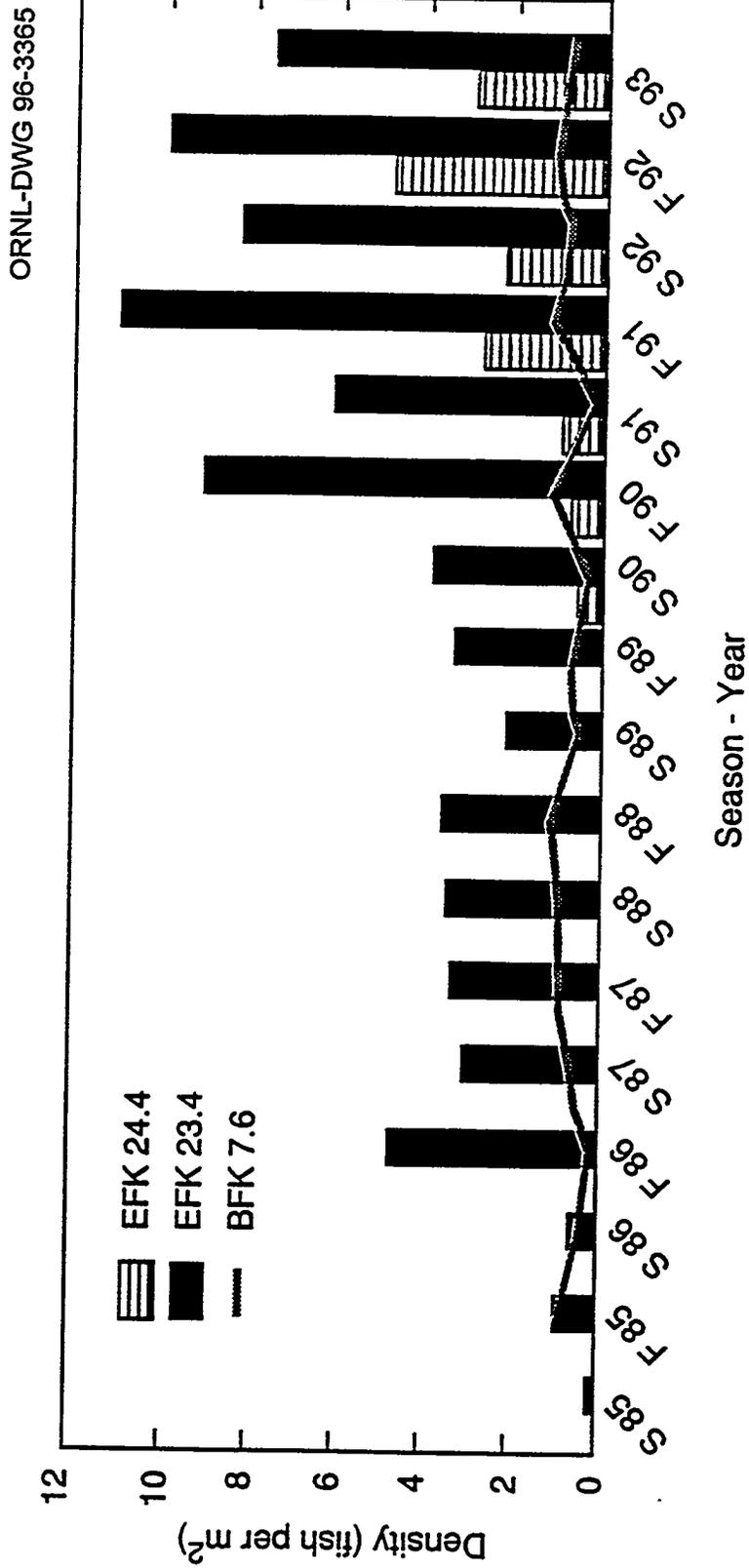


Fig. 2.3. Fish density trends in upper East Fork Poplar Creek, 1985-1993.

upstream movement. Eventually, enough fish inhabited upper EFPC under suitable conditions to successfully spawn. By spring of 1991, reproduction rather than colonization by upstream movement became the most probable mechanism of population growth, and from spring 1991 to fall 1992, the population density at EFK 24.4 increased fivefold. From 1991 to 1993, fish densities and biomass at EFK 24.4 (0.85 to 4.87 fish per square meter and 11.97 to 36.13 g/m<sup>2</sup>, respectively) have exceeded levels in BF. For the March–April 1993 sampling period, densities and biomass values at all three upper EFPC sites were higher than those for BF. Over the 4.5-year study period, fish densities below LR at EFK 23.4 (1.98 to 10.34 fish per square meter) have been consistently higher than those for samples taken at BF (0.37 to 1.72 fish per square meter), and biomass values have followed a similar pattern (11.05 to 51.84 g/m<sup>2</sup> at EFK 23.4 and 4.08 to 12.04 g/m<sup>2</sup> at BF).

The differences in fish community structure between upper EFPC and lower EFPC (at EFK 23.4) are probably indicative of the relatively short time that upper EFPC has become available for fish colonization; however, some species might be excluded from upper EFPC because of their lower tolerance to Y-12 Plant effluents. The differences in fish community structure between both upper and lower EFPC and BF are probably indicative of different levels of stress and environmental alteration at each of the sites. The smaller number of species at EFK 23.4 and in upper EFPC suggests greater environmental and pollutant stresses, which would eliminate species of low tolerance; however, the higher density and biomass values suggest higher productivity resulting from increased inputs of nutrients.

## 2.4 AMBIENT TOXICITY DATA

A combination of approaches, including ambient toxicity testing, in situ chemical monitoring, and streamside experiments were used to examine TRC dynamics and ambient toxicity in EFPC. Ambient toxicity was determined by 7-d static-renewal tests that measured the survival and growth of fathead minnow (*Pimephales promelas*) larvae and the survival and reproduction of a microcrustacean (*Ceriodaphnia dubia*). Between November 1988 and August 1992, 238 7-d static-renewal tests were conducted to characterize the biological quality of ambient stream water at 10 sites in EFPC (Table 2.12). Grab samples of full-strength water from each of the sites were tested. The samples were also analyzed for pH, conductivity, alkalinity, hardness, and TRC. The studies focused on toxicity tests used to characterize water quality conditions at four sites located upstream from LR (Fig. 1.1) and six sites located downstream from LR (Fig. 1.2). For the four upstream sites, the importance of TRC as a toxicant in water was evaluated directly by comparing survival and growth of fathead minnow larvae and survival and fecundity of *Ceriodaphnia* in untreated water with that in water treated with sodium thiosulfate. Streamside toxicity experiments that used untreated and dechlorinated stream water that were conducted on adult fathead minnows and stonerollers (*Campostoma anomalum*), also directly tested the importance of TRC as a toxicant. The results of the studies provided clear and consistent evidence of toxicity in EFPC at the four sites located upstream of LR and little clear or consistent evidence for toxicity in EFPC at six sites located downstream from LR. For the tests conducted with water

Table 2.12. Toxicity test schedule for East Fork Poplar Creek, November 1988–July 1991

Date <sup>a</sup>	NSP	AS-8	LR-i	LR-o	22.8	21.9	20.5	18.2	13.8	10.9
11/03/88		X								
12/08/88		X	X	X						
01/05/89		X	X	X						
02/09/89		X		X	X	X	X	X	X	X
03/02/89		X	X	X						
04/13/89		X	X	X						
05/04/89		X	X	X	X	X	X	X	X	X
06/01/89		X	X	X						
07/07/89		X	X	X						
08/10/89		X	X	X						
09/07/89		X	X	X	X	X	X	X	X	X
10/19/89		X	X	X						
11/15/89		X	X	X						
12/07/89		X	X	X						
01/11/90			X	X						
03/08/90		X	X	X	X	X	X	X	X	X
04/19/90		X	X	X						
05/10/90		X	X	X						
06/07/90	X	X	X	X	X	X	X	X	X	X
07/12/90	X	X	X							
08/02/90	X	X	X	X						
09/06/90	X	X	X	X	X	X	X	X	X	X
10/18/90	X	X	X	X	X	X	X	X	X	X
11/14/90	X	X	X	X						
12/06/90	X	X	X	X						
01/03/91	X	X	X	X	X	X	X	X	X	X
02/07/91	X	X	X	X						
03/07/91			X	X						
04/25/91	X	X	X	X	X	X	X	X	X	X
05/30/91	X	X	X	X						
06/26/91	X	X	X	X						
07/25/91	X	X	X	X	X	X	X	X	X	X
08/15/91	X	X	X							
09/26/91	X	X	X	X						
10/17/91	X	X	X	X	X	X	X	X	X	X
11/14/91	X	X	X	X						
02/06/92	X	X	X	X	X	X	X	X	X	X
03/12/92	X									
04/30/92	X	X	X	X	X	X	X	X	X	X
05/28/92	X	X	X	X						
06/25/92	X	X	X	X						
07/16/92	X	X	X	X	X	X	X	X	X	X

<sup>a</sup>Date refers to the day on which a 7-d chronic toxicity test started.

from upper EFPC, a frequency-of-occurrence-analysis framework (logistic regression of ambient toxicity test outcomes) was used to quantitatively relate chemical conditions (TRC data) and ambient toxicity test outcomes.

#### 2.4.1. Lower East Fork Poplar Creek

##### 2.4.1.1 Methods

Water from six sites in EFPC downstream from LR-i was tested for acute and chronic toxicity to larvae of the fathead minnow and daphnia from 1988 to 1992. Water from each of the six sites was tested fourteen times, at approximately quarterly intervals (84 tests). During each test period, water from all six sites was tested concurrently on both species. Controls, consisting of fathead minnow larvae or daphnia reared in diluted mineral water, were included in every test period. Each fathead minnow test used four replicates; a replicate consisted of a 500-mL beaker containing about 250 mL of water and 10 larvae. Each daphnia test used 10 replicates; a replicate consisted of a 20-mL beaker containing 15 to 17 mL of water and a single daphnid. The procedures used for conducting the tests are described by Weber et al. (1989).

A *Ceriodaphnia* test was considered to have failed (i.e., provided evidence for toxicity) if survival was  $\leq 60\%$  by the end of the 7-d test, provided that the survival of *Ceriodaphnia* in the control was satisfactory (i.e.,  $\geq 80\%$ ). A statistical validation of this 60%-survival criterion for use in ambient toxicity testing situations was developed earlier (Kszos and Stewart 1992). That analysis compared the results of *Ceriodaphnia* controls and tests of water from noncontaminated headwater streams near the Oak Ridge National Laboratory (ORNL) and showed that the probability of obtaining a *Ceriodaphnia* survival value of  $\leq 60\%$  in tests of noncontaminated streams

was less than 0.01 ( $n = 98$  ambient tests vs  $n = 77$  control tests).

An SAS Institute program (SAS PROC GLM; SAS 1985a, 1985b) was used to assess the relative influence of site vs test period on minnow survival and growth by two-way analysis of variance (ANOVA). Site, date, and the interaction between site and date were used as explanatory variables in the analysis of the reproduction of *Ceriodaphnia* by two-way ANOVA. TRC measurements that were below the detection limit (about 10  $\mu\text{g/L}$ , by amperometric titration) were set equal to one-half the detection limit.

##### 2.4.1.2 Results

A test-by-test summary of the results of the fathead minnow assessments of the six sites is provided in Table 2.13. For all test periods combined, the mean survival of the minnows was 88.2% at EFK 22.8; 87.3% at EFK 21.9; 86.3% at EFK 20.5; 84.6% at EFK 18.2; 79.5% at EFK 13.8; and 78.3% at EFK 10.9. Thus, the overall tendency was for slightly higher survival of the minnows in sites closer to the Y-12 Plant than farther downstream.

The two-way ANOVA test showed that site, test period, and the interaction between site and test period collectively explained about 58.7% of the variation in minnow survival and about 78.8% of the variance in minnow growth. For minnow survival, the three factors (site, test period, and the interaction term) were each statistically significant ( $p = 0.0005$ ,  $p = 0.0001$ , and  $p = 0.029$ , respectively). For minnow growth, only test period was significant ( $p$  values were 0.239 for site, 0.0001 for test period and 0.754 for the interaction between site and test period). Thus, minnow growth did not yield any evidence for "toxic sites" in EFPC below LR, and minnow survival indicated that sites in EFPC nearer to LR were "better" than those farther downstream.

Table 2.13. Mean survival (%) and mean growth ( $\pm$  SD; mg dry wt per fish) of fathead minnow larvae in ambient toxicity tests of water from six sites in lower East Fork Poplar Creek

Date	EFK 22.8		EFK 21.9		EFK 20.5		EFK 18.2		EFK 13.8		EFK 10.9	
	Survival	Growth										
02/09/89	92.5	0.70 $\pm$ 0.05	87.5	0.71 $\pm$ 0.06	100.0	0.66 $\pm$ 0.04	87.5	0.64 $\pm$ 0.08	80.0	0.69 $\pm$ 0.14	90.0	0.63 $\pm$ 0.06
05/04/89	82.5	0.40 $\pm$ 0.07	85.0	0.40 $\pm$ 0.11	70.0	0.42 $\pm$ 0.10	87.5	0.36 $\pm$ 0.11	70.0	0.46 $\pm$ 0.09	77.5	0.43 $\pm$ 0.08
09/07/89	97.5	0.53 $\pm$ 0.01	97.5	0.52 $\pm$ 0.04	97.5	0.51 $\pm$ 0.04	97.5	0.53 $\pm$ 0.06	87.5	0.57 $\pm$ 0.09	95.0	0.54 $\pm$ 0.11
03/08/90	62.5	0.49 $\pm$ 0.07	62.5	0.54 $\pm$ 0.09	62.5	0.49 $\pm$ 0.07	72.5	0.46 $\pm$ 0.12	62.5	0.49 $\pm$ 0.16	45.0	0.50 $\pm$ 0.16
06/07/90	95.0	0.56 $\pm$ 0.07	92.5	0.55 $\pm$ 0.03	97.5	0.54 $\pm$ 0.11	100.0	0.52 $\pm$ 0.03	92.5	0.63 $\pm$ 0.10	100.0	0.48 $\pm$ 0.06
09/06/90	92.5	0.67 $\pm$ 0.01	90.0	0.65 $\pm$ 0.07	95.0	0.69 $\pm$ 0.05	87.5	0.70 $\pm$ 0.04	97.5	0.63 $\pm$ 0.06	90.0	0.66 $\pm$ 0.06
10/18/90	75.0	0.48 $\pm$ 0.03	77.5	0.53 $\pm$ 0.03	82.5	0.50 $\pm$ 0.04	80.0	0.49 $\pm$ 0.09	85.0	0.60 $\pm$ 0.06	75.0	0.53 $\pm$ 0.04
01/03/91	97.5	0.45 $\pm$ 0.07	72.5	0.45 $\pm$ 0.02	75.0	0.49 $\pm$ 0.06	72.5	0.52 $\pm$ 0.06	50.0	0.50 $\pm$ 0.03	47.5	0.50 $\pm$ 0.11
04/25/91	92.5	0.53 $\pm$ 0.07	92.5	0.54 $\pm$ 0.13	87.0	0.48 $\pm$ 0.10	90.0	0.56 $\pm$ 0.07	57.5	0.55 $\pm$ 0.08	75.0	0.53 $\pm$ 0.09
07/25/91	97.5	0.79 $\pm$ 0.04	97.5	0.84 $\pm$ 0.03	100.0	0.80 $\pm$ 0.04	90.0	0.71 $\pm$ 0.12	95.0	0.76 $\pm$ 0.08	95.0	0.72 $\pm$ 0.08
10/17/91	95.0	0.75 $\pm$ 0.09	97.5	0.82 $\pm$ 0.03	95.0	0.77 $\pm$ 0.08	97.5	0.81 $\pm$ 0.03	92.5	0.82 $\pm$ 0.09	90.0	0.75 $\pm$ 0.09
02/06/92	77.5	0.58 $\pm$ 0.08	92.5	0.59 $\pm$ 0.03	85.0	0.61 $\pm$ 0.09	67.5	0.63 $\pm$ 0.13	62.5	0.67 $\pm$ 0.04	55.0	0.60 $\pm$ 0.08
04/30/92	77.5	0.54 $\pm$ 0.06	80.0	0.60 $\pm$ 0.07	65.0	0.64 $\pm$ 0.15	72.5	0.61 $\pm$ 0.06	85.0	0.54 $\pm$ 0.02	65.0	0.65 $\pm$ 0.08
07/16/92	100.0	0.29 $\pm$ 0.05	97.5	0.29 $\pm$ 0.06	95.0	0.33 $\pm$ 0.05	82.5	0.36 $\pm$ 0.02	95.0	0.35 $\pm$ 0.02	97.5	0.35 $\pm$ 0.03

A test-by-test summary of the results of the *Ceriodaphnia* assessments of the six sites is provided in Table 2.14. For all test periods combined, mean survival of *Ceriodaphnia* ranged from 84.3% (at EFK 10.9) to 95% (at EFKs 22.8 and 20.5). Mean survival was lower than the 60% survival criterion in only three instances—once at EFK 22.8 and twice at EFK 10.9. Thus, no consistent or strong evidence for acute toxicity to *Ceriodaphnia* was noted at any site in EFPC downstream from LR.

Analysis of *Ceriodaphnia* reproduction by two-way ANOVA, in which site, date, and the interaction between site and date were used as explanatory variables, showed that these three factors together accounted for 48.9% of the total variance in reproduction ( $p = 0.0001$ ;  $DF_{82,770}$ ). However, date and the site-date interaction term accounted for the model's overall significance ( $p = 0.0001$  in each case); the effect of site alone was not significant ( $p = 0.538$ ). Thus, no strong, consistent evidence of differences in chronic toxicity among the sites was detected based on *Ceriodaphnia* reproduction. Pooled temporally, the mean reproduction of the daphnids ranged from 27.6 offspring per female at EFK 10.9 to 29.1 offspring per female at EFK 20.5. The mean reproduction of *Ceriodaphnia* in 44 control tests conducted for EFPC assessments over the October 1988–August 1992 period was  $24.6 \pm 0.8$  ( $\pm SE$ ) offspring per female. This value was about 10% lower than the overall mean reproduction of *Ceriodaphnia*, even at EFK 20.5. Because reproduction in the controls tended to be lower than reproduction in even the “worst” of the six EFPC sites, site-to-control comparisons would not provide additional insight.

## 2.4.2. Upper East Fork Poplar Creek

### 2.4.2.1 Methods

**Ambient toxicity tests with fathead minnows and *Ceriodaphnia*.** During November 1988–July 1992, four sites in upper EFPC (NSP, AS-8, LR-i, and LR-o) were tested for acute and chronic toxicity to *Ceriodaphnia* and larvae of *P. promelas* as described in Sect. 2.4.1.1. During each test period, three to four sites were tested concurrently with both species. The relationship between TRC concentration and *Ceriodaphnia* survival in ambient toxicity tests was analyzed by examining data from 169 7-d chronic toxicity tests, conducted during Nov. 1988 and March 1993. *Ceriodaphnia* proved to be more sensitive than the minnow larvae to TRC. Thus, the emphasis of the studies described in this section is on *Ceriodaphnia* toxicity test results. The outcomes of fathead minnow tests at the four upstream sites are provided, but in less detail. The procedures used for conducting the tests are described by Weber et al. (1989). The results of the statistical analyses for the *Ceriodaphnia* tests are described in detail in Stewart et al. (1995).

**Toxicity tests with fathead minnows and *Ceriodaphnia* in dechlorinated water.** In addition to the ambient tests, the importance of TRC as a toxicant in water samples from NSP, AS-8, LR-i, and LR-o was evaluated directly by comparing survival and growth of fathead minnow larvae and survival and fecundity of *Ceriodaphnia* in untreated water with that in water treated with sodium thiosulfate. A stock solution of reagent-grade sodium thiosulfate (250 mg of sodium thiosulfate per 25 mL of deionized distilled water) was prepared daily. The quantity of thiosulfate

Table 2.14. Survival (%) and mean reproduction ( $\pm$ SD; offspring per surviving female) of *Ceriodaphnia* in ambient toxicity tests of water from six sites in lower East Fork Poplar Creek

Date	EFK 22.8		EFK 21.9		EFK 20.5		EFK 18.2		EFK 13.8		EFK 10.9	
	Survival	Offspring	Survival	Offspring								
02/09/89	100	16.6 $\pm$ 4.1	90	18.1 $\pm$ 2.1	100	16.0 $\pm$ 3.4	90	18.1 $\pm$ 5.4	100	13.9 $\pm$ 2.4	90	22.0 $\pm$ 1.7
05/04/89	100	22.7 $\pm$ 3.5	100	20.6 $\pm$ 2.7	100	25.8 $\pm$ 4.4	100	21.5 $\pm$ 4.4	100	23.4 $\pm$ 1.8	100	22.7 $\pm$ 2.8
09/07/89	100	26.8 $\pm$ 6.9	90	30.4 $\pm$ 2.1	100	29.8 $\pm$ 2.3	80	27.6 $\pm$ 2.0	80	24.3 $\pm$ 3.7	<sup>a</sup>	<sup>a</sup>
03/08/90	90	30.9 $\pm$ 3.4	100	26.7 $\pm$ 2.5	100	28.1 $\pm$ 2.2	100	27.8 $\pm$ 2.9	100	27.6 $\pm$ 3.2	100	27.2 $\pm$ 1.9
06/07/90	100	28.8 $\pm$ 5.1	100	27.9 $\pm$ 9.0	90	29.1 $\pm$ 9.5	80	26.8 $\pm$ 7.4	90	23.0 $\pm$ 9.2	80	19.3 $\pm$ 9.2
09/06/90	100	34.0 $\pm$ 6.4	90	36.0 $\pm$ 8.8	90	36.1 $\pm$ 7.8	100	31.5 $\pm$ 9.3	100	34.1 $\pm$ 8.7	100	32.2 $\pm$ 5.7
10/18/90	100	42.2 $\pm$ 8.8	100	40.0 $\pm$ 9.9	100	38.9 $\pm$ 11.0	100	32.7 $\pm$ 6.2	100	35.9 $\pm$ 8.4	100	35.6 $\pm$ 9.0
01/03/91	100	25.8 $\pm$ 7.6	90	28.4 $\pm$ 8.5	100	23.7 $\pm$ 2.1	100	24.0 $\pm$ 2.4	100	24.8 $\pm$ 3.0	100	31.2 $\pm$ 8.2
04/25/91	50	31.6 $\pm$ 14.0	70	29.6 $\pm$ 7.1	90	32.2 $\pm$ 12.9	90	32.2 $\pm$ 11.9	90	33.3 $\pm$ 8.5	40	43.5 $\pm$ 1.3
07/25/91	100	19.3 $\pm$ 7.5	90	26.8 $\pm$ 3.4	90	29.7 $\pm$ 6.1	100	27.3 $\pm$ 8.6	100	30.1 $\pm$ 4.5	100	21.3 $\pm$ 7.9
10/17/91	100	26.5 $\pm$ 9.0	100	26.0 $\pm$ 8.0	90	30.0 $\pm$ 5.2	70	34.3 $\pm$ 4.9	90	28.8 $\pm$ 7.4	90	27.6 $\pm$ 3.8
02/06/92	100	24.8 $\pm$ 7.9	100	26.1 $\pm$ 4.8	100	23.2 $\pm$ 7.5	90	30.7 $\pm$ 3.6	90	26.1 $\pm$ 7.1	100	28.8 $\pm$ 3.8
04/30/92	90	46.6 $\pm$ 9.4	80	31.6 $\pm$ 14.4	80	45.4 $\pm$ 10.9	100	33.9 $\pm$ 9.5	100	41.3 $\pm$ 10.6	90	26.4 $\pm$ 10.4
07/16/92	100	21.0 $\pm$ 3.4	80	25.0 $\pm$ 5.5	100	24.1 $\pm$ 5.5	80	23.8 $\pm$ 5.1	70	27.1 $\pm$ 4.6	90	28.2 $\pm$ 4.9

added to a sample to achieve dechlorination varied daily, depending on the amount of TRC measured in the sample; an empirically derived relationship between TRC concentration and the minimum amount of thiosulfate stock solution needed to achieve dechlorination was used to prevent thiosulfate from being added to excess.

On four dates, a positive control was also included with each dechlorination test. Each of these controls consisted of *Ceriodaphnia* reared in diluted mineral water containing sodium thiosulfate. The volume of thiosulfate solution added to this control varied daily: it was equal to that added to dechlorinate the EFPC water sample that contained the greatest concentration of TRC.

#### **Streamside toxicity tests with fathead minnows and central stonerollers.**

Additionally, streamside experiments at two sites in upper EFPC—AS-8 and the oil-water separator—were used to estimate the relationship of TRC to fish survival. These studies utilized streamside dechlorination to test the toxicity of TRC to juvenile and adult fathead minnows (*P. promelas*) and central stonerollers (*C. anomalum*). Tests were conducted with stream water and lasted 7 or 21 d.

In October and November 1990, streamside fish toxicity experiments were conducted at AS-8 and the oil-water separator. The experimental apparatus consisted of 10-L flow-through aquaria provided with stream water at the rate of 1 L/min by a submersible pump. Water was either untreated or treated (dechlorinated) with sodium thiosulfate at a rate of 3.7 mg/L. This rate was several times the amount needed to eliminate the highest measured concentration of TRC (about 350 µg/L) based on in situ measurements. The studies were conducted with adult fathead minnows from ORNL laboratory stock or stonerollers that had been captured by electroshocking from a downstream area.

The aquaria containing fathead minnows were provided with polyvinyl chloride (PVC) pipe shelters. The fathead minnows were not fed during the experiment. Periphyton-covered rocks collected from EFPC were added as food and cover to aquaria that contained stonerollers. The top of each aquarium was covered with screen to prevent the fish from escaping. The aquaria were inspected daily for fish mortality.

In the first experiment, conducted at upstream site AS-8, 11 adult fathead minnows were placed in each of four aquaria for 7 d; two aquaria received untreated water, and two aquaria received dechlorinated water. The second experiment consisted of 21-d tests conducted at the oil-water separator site. Ten juvenile fathead minnows (5 cm in length) or 10 stonerollers (5 cm in length) were placed in each of four streamside aquaria as in the first experiment. For each species tested, two of the aquaria were treated with sodium thiosulfate and two were untreated.

**Data analysis.** For the toxicity tests, ANOVA was used to test differences in survival of fathead minnows among sites. Correlations between growth and survival were tested with Pearson's correlation coefficient. A two-way ANOVA was used to test for effects of dechlorination on minnow survival and growth for toxicity tests of water from NSP and AS-8.

Because the basic response variables are binary (mortality or survival) for the *Ceriodaphnia* survival study, logistic regression (SAS PROC LOGISTIC) was used to quantify the relationship between TRC and *Ceriodaphnia* survival. For this analysis, it is assumed that the sites are similar with respect to toxicity because of the presence of toxicants other than TRC, including possible interactions of these substances with chlorine. To increase the generality of the analysis and increase the precision of parameter estimates, TRC data

and toxicity test results from the four sites were pooled prior to analysis.

For statistical analysis, TRC measurements that were below the detection limit were set at one-half the detection limit. At the other extreme, TRC concentrations at the NSP were sometimes as high as 600 µg/L. Because the range in TRC concentration was large (more than 100-fold), the TRC data were transformed (logarithm base 10) before analysis.

The results of one test (LR-o; April 23–30, 1991) were excluded because (1) *Ceriodaphnia* survival and fecundity were both unusually low, (2) no TRC was detected in the water during this test period, and (3) *Ceriodaphnia* survival and reproduction in filtered (0.5-µm-pore-size glass fiber filter) LR-o water on this date had high survival and reproduction. The responses of the daphnids to the filtration treatment in this test suggested that particulate matter in the water accounted for the toxicity.

It was hypothesized that the 7-d mean TRC concentration and some measure of the day-to-day variation in TRC concentration could each be important determinants of *Ceriodaphnia* survival. Two considerations led us to use the TRC semirange (defined here as the 7-d maximum transformed TRC concentration minus the 7-d mean transformed TRC concentration) as an estimate of TRC variability in the regression model. First, after data have been transformed and analyzed, semirange values can be back-transformed. In contrast, it is difficult to interpret the back-transformation of the sample variance values. Second, whereas variance provides a measure of spread both above and below the mean, the semirange quantifies excursions of TRC above the mean and ignores excursions that are lower than the mean. Thus, the semirange seemed likely to be of greater toxicological interest than variance, which gives equal weight to values that are above or below the mean.

The logistic regression model provided estimates for five parameters (the intercept,

the 7-d mean concentration of TRC, the 7-d mean pH, and the TRC and pH semiranges). Of these parameters, only mean TRC and the TRC semirange proved to be significantly related to *Ceriodaphnia* mortality. Accordingly, only TRC and its variation were inspected more closely to evaluate the relationship between EFPC water quality and *Ceriodaphnia* mortality. To explore the effect of TRC variation on the probability of *Ceriodaphnia* mortality, an analysis that evaluated TRC in relation to the probability of *Ceriodaphnia* mortality ( $P^*$ ) was used. This analysis consisted of selecting a particular value for  $P^*$  and iteratively solving an equation for combinations of mean TRC and TRC semirange values that permitted  $P^*$ . The generalized equation used for this analysis follows:

$$\underline{X}_2 = (1/b_2)[\ln(P^*/[1-P^*]) - b_0 - b_1 \underline{X}_1] \quad (1)$$

where  $\underline{X}_2$  is the 7-d maximum ( $\log_{10}$ -transformed) TRC concentration minus the 7-d mean ( $\log_{10}$ -transformed) TRC concentration;  $\underline{X}_1$  is the mean ( $\log_{10}$ -transformed) 7-d mean TRC concentration; and  $b_0$ ,  $b_1$  and  $b_2$  are the parameter estimates for the intercept, mean TRC concentration, and TRC semirange, respectively, as obtained from the logistic regression. Procedures available in SAS® (SAS 1985a,b) were used in these analyses.

#### 2.4.2.2 Results

**Analysis of fathead minnow ambient toxicity tests.** Mean survival of the minnow larvae at the four upstream sites was  $\geq 93\%$  at each site. An ANOVA of the transformed (arc sine square root) survival proportions showed that the differences in survival among the four sites were not significant ( $p = 0.917$ ), with site explaining  $< 1\%$  of the total variation. Mean survival of the minnows in the controls was 82.9%.

Mean growth of the minnow larvae at the four upstream sites was highest at the NSP (0.569 mg per fish), and declined steadily and slightly with distance downstream (0.531 mg per fish at AS-8, 0.527 mg per fish at LR-i, and 0.524 mg per fish at LR-o). The differences in minnow growth among the sites was not significant ( $p = 0.549$ ), with site explaining only about 1.6% of the total variation. Mean growth of the minnows in the controls was 0.510 mg/fish.

Pearson correlations between growth and survival were positive and nearly significant at each site ( $p = 0.076, 0.059, 0.091, \text{ and } 0.113$  at NSP, AS-8, LR-i, and LR-o, respectively). Because test-to-test variation in minnow growth was large relative to the site-to-site variation in growth, the most parsimonious explanation for the correlation is that in some tests, food quality problems or smaller initial larvae translated into lesser growth and slightly greater risk of mortality.

**Analysis of fathead minnow toxicity tests in dechlorinated water.** A two-way ANOVA was used to test for effects of dechlorination on minnow survival and growth for toxicity tests of water from NSP and AS-8. This analysis showed that the two factors together (dechlorination, yes or

no; and site, NSP or AS-8) and the interaction term between the two factors explained only about 5% of the variation in mean survival and about 3% of the variation in mean growth. The amount of variance explained by the model was not significant either for survival ( $p = 0.094$ ) or growth ( $p = 0.286$ ). Within the model for survival, though, the dechlorination treatment was considered to be significant ( $p = 0.014$ ); the  $p$  values for site and the site-treatment interaction term were much greater (0.631 and 0.493, respectively). This outcome suggests that the minnow larvae did benefit slightly, in terms of survival, from the addition of sodium thiosulfate to the water at both sites. The effect of the dechlorination treatment raised mean survival from 94.6 to 98.7% (back-transformed data).

**Analysis of *Ceriodaphnia dubia* ambient toxicity tests.** A longitudinal gradient in TRC concentrations existed within EFPC between NSP and LR-o, with TRC conditions upstream being far more severe than those at sites farther downstream (Table 2.15). For example, only 3.2% of the 7-d mean TRC concentrations at LR-i were  $\geq 200 \mu\text{g/L}$ , but 15.6% of the TRC concentrations for AS-8 and 29.8% of those for NSP were  $\geq 200 \mu\text{g/L}$ .

**Table 2.15. Relationship between total residual chlorine (TRC) and *Ceriodaphnia* ambient toxicity test outcomes for sites in upper East Fork Poplar Creek**

Site	Number of Tests	Failure frequency ( $\leq 60\%$ survival)	TRC exceedance frequency (%) <sup>a</sup>	Anomalous passes (%)	Anomalous failures (%)	Predicted correctly (%)	TRC concentration ( $\mu\text{g/L}$ ; mean $\pm$ SD; N)
NSP	15	0.733	55.2	7.1	14.3	78.6	167.0 $\pm$ 10.4 (203)
AS-8	41	0.634	31.9	2.6	5.1	92.3	125.9 $\pm$ 80.6 (329)
LR <sub>i</sub>	36	0.139	8.3	6.1	6.1	87.8	34.7 $\pm$ 5.0 (329)
LR <sub>o</sub>	35	0.028	0.0	0	0	100	5.1 $\pm$ 0.9 (322)
Overall	117			20.5	6.8	88.0	

<sup>a</sup>Percentage of 7-d test periods in which mean TRC concentration equaled or exceeded 200  $\mu\text{g/L}$ .

The toxicity test failure frequency (defined as the percentage of tests having a survival value that was lower than 60%) was greatest at NSP and declined with distance downstream, revealing a longitudinal gradient in test outcomes (Table 2.15). In most cases, survival of *Ceriodaphnia* in untreated water from NSP or AS-8 was either high (90 to 100%) or zero. Survival at LR-o was almost always  $\geq 80\%$ , and the number of test failures tended to increase with distance upstream (Fig. 2.4). At AS-8, most of the tests early in the reporting period had low survival, and the majority of the tests conducted later in the reporting period had high survival.

**Logistic regression of *Ceriodaphnia dubia* toxicity tests in relation to TRC.** The stepwise logistic regression procedure included both the 7-d mean TRC concentration ( $\chi^2 = 282.8$ ;  $p = 0.0001$ ) and the TRC semirange ( $\chi^2 = 93.6$ ;  $p = 0.0001$ ) as significant factors in explaining *Ceriodaphnia* survival in tests of water from the sites in upper EFPC. In the 169 7-d tests, 1228 animals lived and 462 animals died. With both factors included, the regression model correctly predicted mortality in 89.3% of the cases. The model's false positive rate (i.e., no mortality was expected based on TRC conditions, but mortality occurred) was 20.0%; the model's false negative rate (i.e., a mortality was predicted by the model, but the animal lived) was 7.0%. False positives may be expected to be more common than false negatives if toxicants other than chlorine were sometimes present.

The relationships between mean TRC concentration and day-to-day variation in TRC needed to generate mortality probabilities ( $P^*$ ) of 0.95, 0.85, and 0.50 [computed by using Eq. (1)], and the proportions of test periods with various ranges of maximum TRC to median TRC are shown in Fig. 2.5. The influence of TRC variation on the predicted mortality of *Ceriodaphnia* is clearly evident. For

example, the probability of mortality was 0.95 either when the median TRC concentration of 300  $\mu\text{g/L}$  and the ratio of maximum TRC to median TRC was 2 or when the median TRC concentration was 100  $\mu\text{g/L}$  and the ratio of maximum TRC to median TRC was 8.

The distribution of within-test variation values in TRC for the four EFPC sites (variation being expressed as the 7-d maximum TRC concentration divided by the 7-d median TRC concentration, in which detection-limit censored data was used as described previously) is shown in Fig. 2.5, above the three  $g P^*$  curves. About 45% of the variation values were  $\geq 1.5$ , indicating that day-to-day variation in TRC concentrations in the daily grab-sample sampling regime (a regime typically used to provide water for ambient toxicity testing) was great enough to substantially affect the probability of *Ceriodaphnia* mortality in 7-d toxicity tests.

**Analysis of *Ceriodaphnia* toxicity tests in dechlorinated water.** At NSP, AS-8, and LR-i, the sodium thiosulfate additions to water that was toxic to *Ceriodaphnia* almost always eliminated the toxicity. This situation is summarized for AS-8, where the record of toxicity testing of untreated and dechlorinated samples was most detailed (Fig. 2.6).

In the control series involving either untreated or thiosulfate-treated diluted mineral water, survival and mean reproduction of *Ceriodaphnia* in the four thiosulfate-treated controls was 94.8% and  $28.0 \pm 1.1$  offspring per surviving female ( $\pm\text{SE}$ ). Survival and reproduction values for the corresponding untreated controls were 90.0% and  $29.4 \pm 2.9$  offspring per surviving female. Thus, in the absence of TRC, neither *Ceriodaphnia* survival nor reproduction was significantly affected by the thiosulfate additions (one-way ANOVA;  $p = 0.66$  and  $p = 0.37$  for reproduction and survival respectively).

ORNL-DWG 94-12681

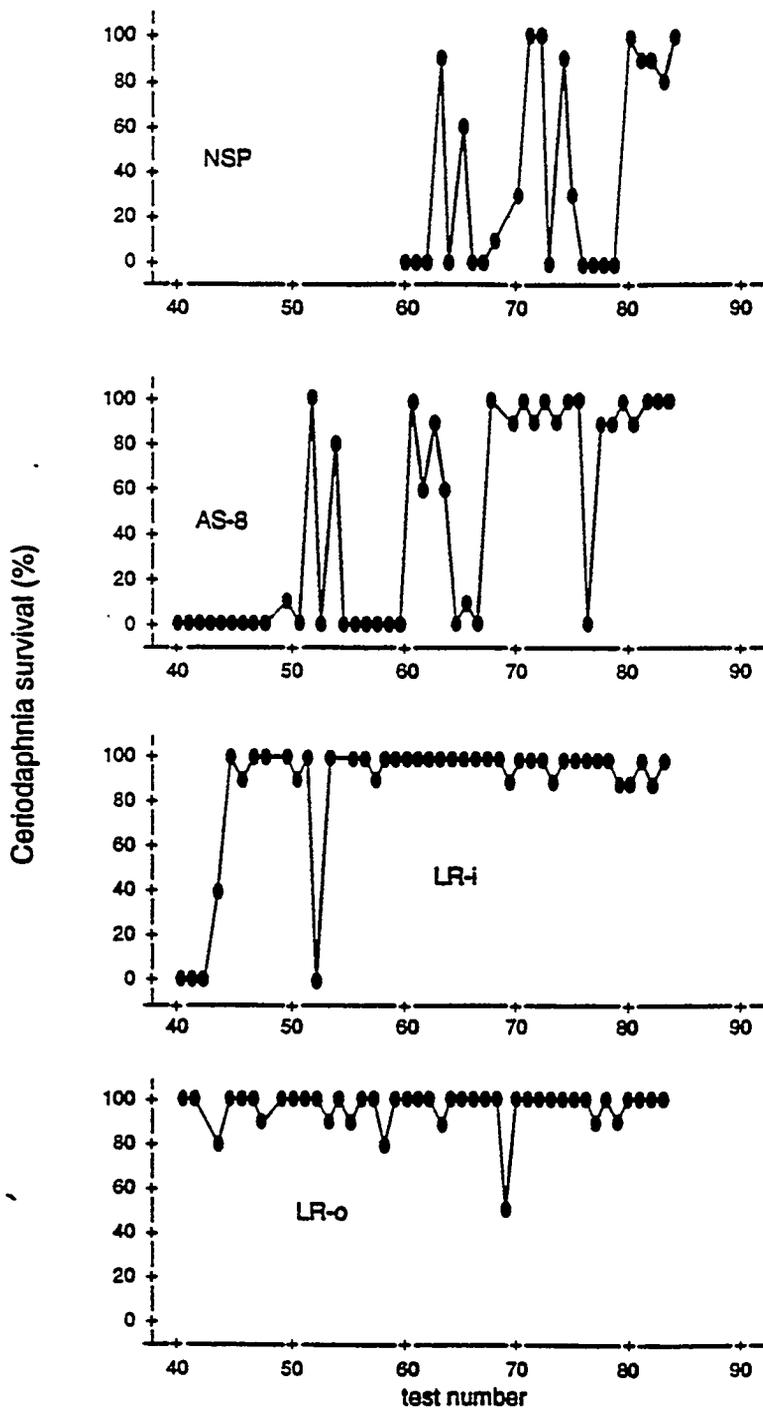


Fig. 2.4. Distribution of survival values (%) for *Ceriodaphnia* toxicity tests of water from the NSP, AS-8, LR-i, and LR-o. Test numbers are coded test dates; test numbers 40 and 60, for example, were initiated in December 1988 and April 1990, respectively.

ORNL-DWG 94-12682

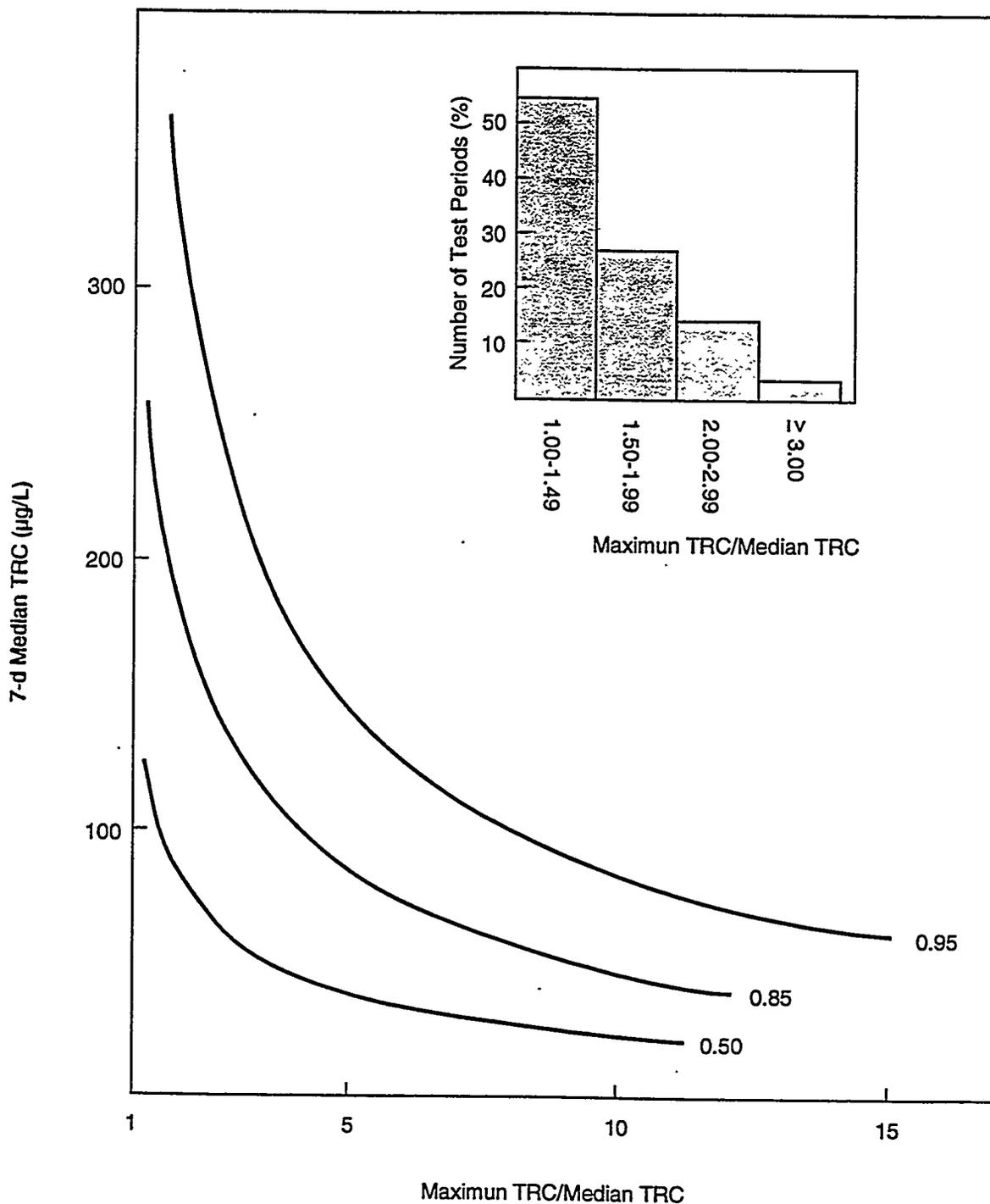


Fig. 2.5. Relationship between median concentration of TRC and day-to-day variation in TRC concentration (maximum 7-d concentration minus 7-d median concentration) for various probabilities ( $p$  values) of lethality to *Ceriodaphnia* in 7-d tests. Insert in upper right corner shows the proportion of tests (%) with various ranges in ratios of maximum TRC to median TRC.

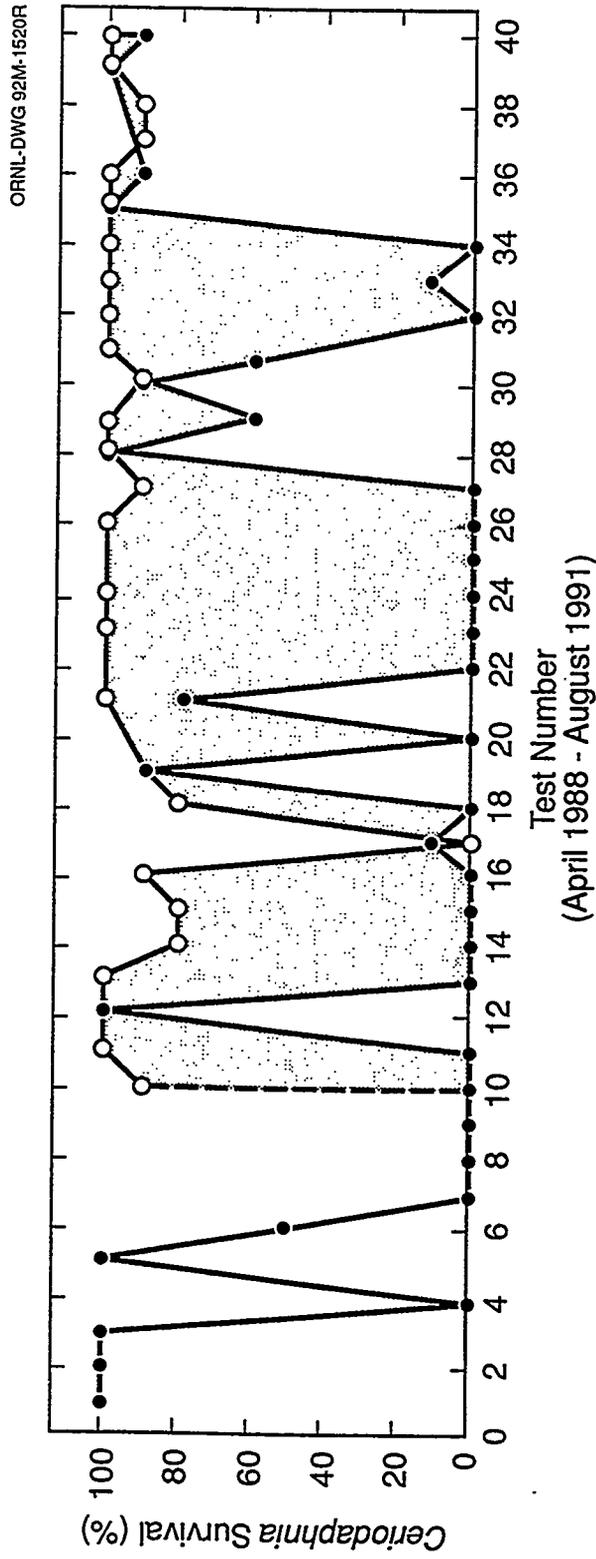


Fig. 2.6. *Ceriodaphnia* survival (%) in 7-d toxicity tests of water from AS-8. Solid circles show survival values for untreated water; open circles show survival values for tests that used water samples experimentally treated with sodium thiosulfate to dechlorinate the water. Shaded area shows the degree of improvement in toxicity test outcomes that might be expected if upper East Fork Poplar Creek was dechlorinated.

**Streamside experiments.** In the first experiment, concentrations of chlorine at AS-8 resulted in mortality of adult fathead minnows in the aquaria receiving untreated stream water. By the end of the second day, mortality in the untreated aquaria was 68% (15/22); mortality increased to 86% (19/22) by the end of day 7 (Table 2.16). At this time, the three remaining fish were swimming erratically and appeared to be seriously distressed. Survival in the treated aquaria was 100%.

In the second experiment, where TRC concentrations ranged from 10 to 80  $\mu\text{g/L}$ , mortalities of juvenile fathead minnows in both replicates of both the treated and untreated aquaria were 0%. Mortality of

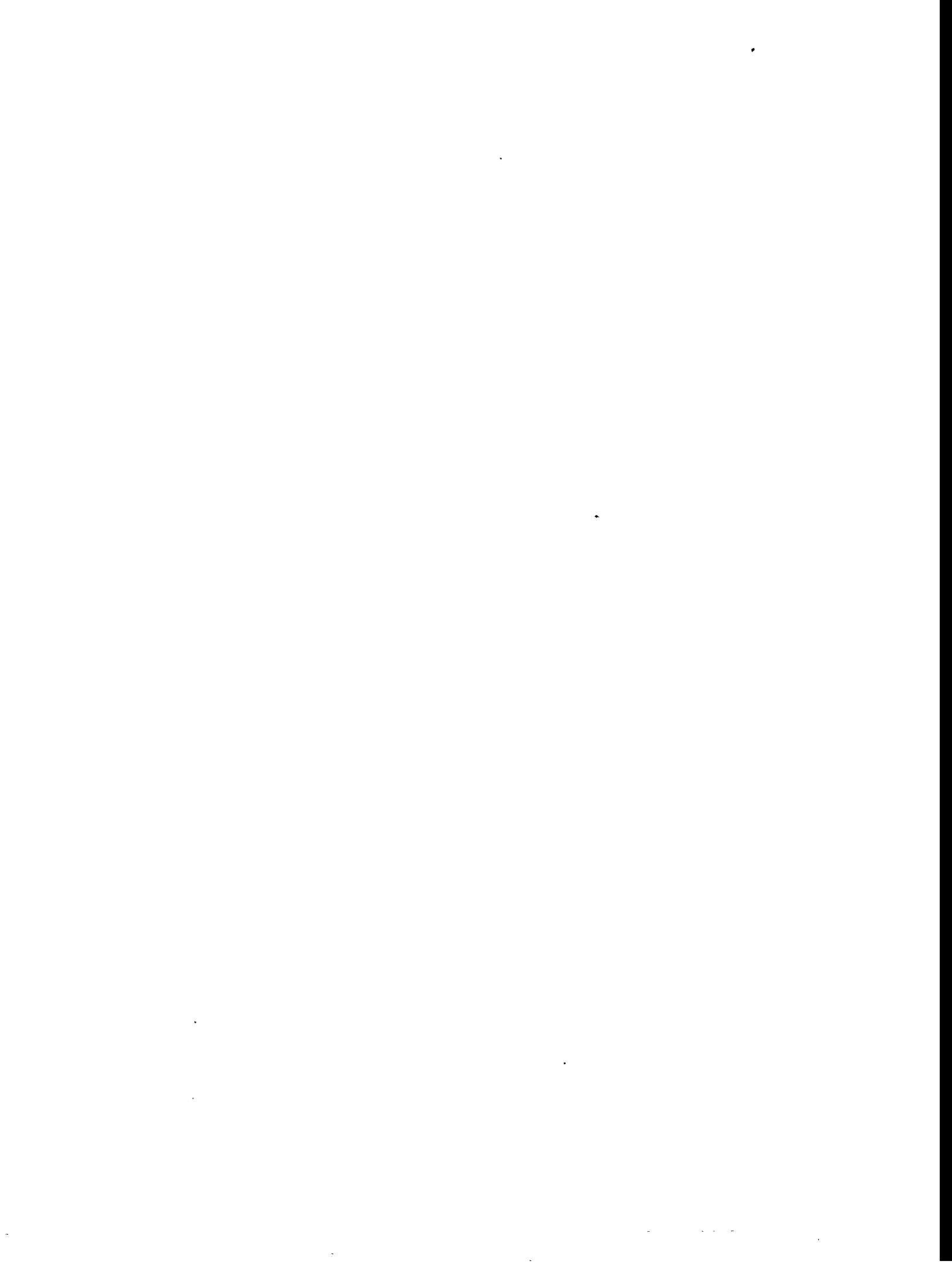
stonerollers was 15% in aquaria receiving thiosulfate-treated water and 20% in aquaria receiving untreated water.

The streamside toxicity experiments conducted with fathead minnows showed that TRC at AS-8 was acutely toxic to fish. However, TRC concentrations further downstream at the oil-water separator were not great enough to kill fathead minnows in 21-d experiments. Comparison of these data with ambient toxicity testing with *Ceriodaphnia dubia* indicate that *Ceriodaphnia dubia* are more sensitive than fish to elevated TRC.

**Table 2.16. Streamside toxicity experiments with fathead minnow (*Pimephales promelas*) and central stoneroller (*Campostoma anomalum*) at AS-8 and the oil-water separator site<sup>a</sup>**

Site/Species	Test duration (d)	Water treatment	Mortality (%)
AS-8			
Fathead minnow	7	None	86
		Sodium thiosulfate	0
Oil-water separator			
Fathead minnow	21	None	0
		Sodium thiosulfate	0
Central stoneroller	21	None	20
		Sodium thiosulfate	15

<sup>a</sup>Results of two replicates



### 3. FISH KILLS

The intense monitoring of the fish kills that began in July 1990 indicated that two types of fish mortality were occurring in upper EFPC. An almost continuous, but relatively low level of mortality appeared to be the result of chronic stresses caused by one or more pollutants present in the stream, whereas, intermittent, but relatively high levels of mortality (i.e., acute fish kills) were viewed as being the result of spills or sudden releases of pollutants. The cause of the chronic mortality was hypothesized to be background stresses resulting from the continuous discharge of chlorine into upper EFPC. During most of 1990 to 1992, TRC concentrations in upper EFPC were usually above 0.01 mg/L (Sect. 2.1.1); concentrations potentially lethal to fish and other aquatic organisms. Environmental factors such as sunlight, water flow, suspended particulate matter, water temperature and pH, and potential interactions with other contaminants, such as ammonia, may have made the effects of TRC vary both temporally and spatially within the stream. Following installation of two dechlorination systems in upper EFPC in November and December of 1992, TRC concentrations have been substantially reduced, and in June 1993, TRC levels measured over a 7-d period were  $\leq 0.06$  mg/L in upper EFPC (see Table 2.6).

Historically, the weir at the outfall of NHP was an effective physical barrier to the movement of fish into the upper reaches of EFPC inside the Y-12 Plant. With the opening of LR in November of 1988, upstream movement was no longer impeded and fish rapidly colonized LR, the diversion

channel, and the reach of stream above the channel. The increase in the fish population upstream of LR was dominated by a colonization phase in the first 2 years when most of the increase in population size was probably the result of the upstream movement of fish. Eventually, enough fish inhabited upper EFPC under suitable conditions to successfully spawn. At this time (spring 1991), when the original occurrence reporting criteria were established, reproduction rather than colonization by upstream movement became the primary mechanism of population growth. From spring 1991 to fall 1992, the fish population density upstream of LR at EFK 24.4 increased five-fold (see Sect. 2.3 and Fig. 2.3).

The fish kills that occurred in upper EFPC from July to December 1990 were evaluated by DeGraeve and Clement (1991, see Appendix B) of Battelle Great Lakes Environmental Center, who concluded that the increase in fish mortality was primarily the result of the significant increase in the fish population following the opening of LR (Appendix B). Because of the inherent physiological variability within natural populations of organisms, there is, within most natural populations, a small percentage of individuals that are extremely sensitive to an environmental or pollutant stress (as there is an equally small percentage that is extremely resistant to the same stress). When the size of the population exposed to that stress increases, the percentage affected (and killed) may not change; however, the total number dying would increase. Consequently, as the number of fish

exposed to the ambient conditions in upper EFPC increased, the number of dying fish also increased.

### 3.1 CHRONIC KILLS

#### 3.1.1. Daily Stream Surveys

The results of the daily dead fish surveys conducted along EFPC from July 18, 1990, to March 31, 1993, are presented in Appendix C. Over most of this time period, the surveys were made once per day (during the morning hours) and 5 d per week (Monday to Friday). The surveys were extended through the weekends and, in some cases, additional afternoon surveys were made when there was evidence that an acute kill was occurring. The stretch of EFPC surveyed extended from NSP to just downstream of LR. Six sampling sites were selected in upper EFPC, one was located in the diversion channel, another in LR, and a third just downstream of LR (see Sect. 1.1 for a description of these sites). The dead fish collected during each survey were identified to species whenever possible, and their location when found was recorded (see Appendix C). In addition, the general condition of the collected dead fish was noted and was used to provide a rough estimate of how long the fish had been dead. Information was also recorded on any unusual behavior patterns observed in stressed fish.

Altogether, 4487 fish were collected over the 32-month period from July 1990 to April 1993. Forty-three percent were central stonerollers (*Campostoma anomalum*) and 24% were striped shiners (*Luxilus chrysocephalus*). These two species were the first to colonize upper EFPC and remained the dominant component of the fish fauna throughout the study period (see Sect. 2.3).

#### 3.1.2. Patterns of Chronic Mortality

Fish kill events in upper EFPC are listed in Table 3.1 and presented graphically in Figs. 3.1–3.3. The horizontal bars in the figures represent the mean daily mortality rates for the time periods indicated, and the dotted line suggests a likely dividing point between the chronic and acute kills (see Sect. 3.1.3). The total number collected during each time period, number of collection days, maximum daily mortality, and the number of fish found at each collecting site (expressed as a percentage of the total collected) are shown in Table 3.1. The averaging periods (number of survey days) were selected on the basis of the number of dead fish and on the location where the fish were found. A pattern consistent over time or showing a downstream gradient over several days was assumed to have a common cause. Studies that used tagged dead fish (see Sect. 4.1.1) suggested that under normal stream flow conditions dead fish would not be carried very far downstream from where they had died and that most would disappear from the stream (probably as a result of predation) within 1 to 2 d.

Because of the considerable variability in the daily mortality and because acute kills of varying intensity or duration could have been superimposed over the chronic mortality, the distinction between chronic and acute kills is not always clearcut. Arrangement of the data in Table 3.1 and Figs. 3.1–3.3 is intended only to provide an overview of the general pattern of fish mortality in upper EFPC from July 1990 to April 1993. Selection of different starting- or endpoints for specific kill events could be made based on different interpretations of the data; however, the overall pattern would not be expected to change appreciably. As presented in Figs. 3.1–3.3, the mean daily mortality rates for the background or

Table 3.1. Pattern of fish mortality in upper East Fork Poplar Creek, July 18, 1990 to March 31, 1993

Date	No./d <sup>b</sup>	Max	Mean	Below		In LR	Division Channel	Percentage of total number collected <sup>c</sup>									
				LR	LR			Site 1	Site 2	Site 3	Site 4	Site 5	Site 6				
07/18-09/14/90	41/40	4	1.0	49			41										
09/17-10/04/90	116/14	15	8.3	81		8	9										
10/05/90	70/1	70	70	100													
10/06-10/12/90	31/6	9	5.2	65		19	16										
10/15-10/16/90	34/2	21	17				18		18								
10/17-11/06/90	53/14	11	3.2	34		15	19				35	5					
11/07-11/08/90	39/2	29	19.5	95						5							
11/09-11/21/90	27/9	5	3.0	67			11					11		7			
11/26-12/21/90	59/18	9	3.3				37					8		14		17	
12/24/90	33/1	33	33				100										
12/26/90-02/01/91	14/23	6	0.6				79										
02/04-02/13/91	42/8	11	5.2											14		21	48
02/14-02/28/91	11/10	6	1.1				30										
03/01-03/03/91	304/2	293	152														
03/03-03/15/91	19/10	5	1.9	16		21											
03/18-03/28/91	1057/10	370	105.7	17		79	4										
04/01-05/10/91	124/29	15	4.3	10		8	65			8							
05/13-06/14/91	21/24	5	0.88	43			43										
06/15/91	41/1	41	41							100							
06/17-08/14/91	39/40	6	0.95	44			28			8		5					
08/15-08/16/91	39/2	23	19.5	100													
08/19-09/09/91	19/14	4	1.4	68			11			11							
09/10/91	137/1	137	137				3			3		39				21	
09/11-10/15/91	43/23	8	1.9	16			23			14				72		16	
10/16-10/18/91	36/3	15	12	13										19		16	
10/21-11/04/91	19/11	6	1.9	26			16							15		18	39
11/05-11/08/91	34/4	12	8.5	12										16		21	
														15		21	47

Table 3.1 (continued)

Date	No./d	Max	Mean	Below		Percentage of total number collected <sup>a</sup>									
				LR	In LR	Division Channel	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6			
11/11-11/22/91	20/9	5	2.2			15		15		40		20			
11/25-11/29/91	135/5	22	27							8	36	33	16		
12/04-12/06/91	42/3	22	14			14	36			19		7	12		
12/09/91-01/14/92	22/21	9	1.0	9		59				14	29	11	8		
01/15-01/19/92	78/5	28	15.6			6				23	15	23			
01/20-01/24/92	13/5	5	2.6			15				14		30	70		
01/27/92	23/1	23	23							19		24	24		
01/28-02/14/92	21/14	4	1.5								16	34	41		
02/18-02/21/92	44/4	19	11									8			
02/24-04/10/92	118/32	14	3.7			55				20		10	10		
04/13-04/16/92	59/4	23	14.7	10		46	7			15		10			
04/20-07/06/92	102/52	6	2.0	12		55	9		4	12		12			
07/07-07/10/92	41/4	16	10.2	24		29	7			17	10	12			
07/13-10/06/92	97/59	8	1.6	11		42				8		9	19		
10/07-10/09/92	69/3	38	23									7	86		
10/10-11/09/92	34/19	6	1.8	21		15				15		18	18		
11/10-11/30/92	772/18	264	42.9			3	18			33	6	14	11		
12/01-12/23/92	18/17	4	1.1			50						14	17		
12/27-12/31/92	155/5	112	31			87				12					
01/04-01/18/93	13/10	3	1.3			15	15			15	15	15			
01/19-01/21/93	90/3	61	30							90					
01/22-02/22/93	15/17	2	0.9			27				47					
02/24/93	19/1	19	19			5	14								
02/25-03/31/93	22/11	5	2.0			50	27								

<sup>a</sup>Numbers in bold indicate sites with highest mortality.

<sup>b</sup>Number of dead fish/number of survey days.

ORNL DWG 94-12390

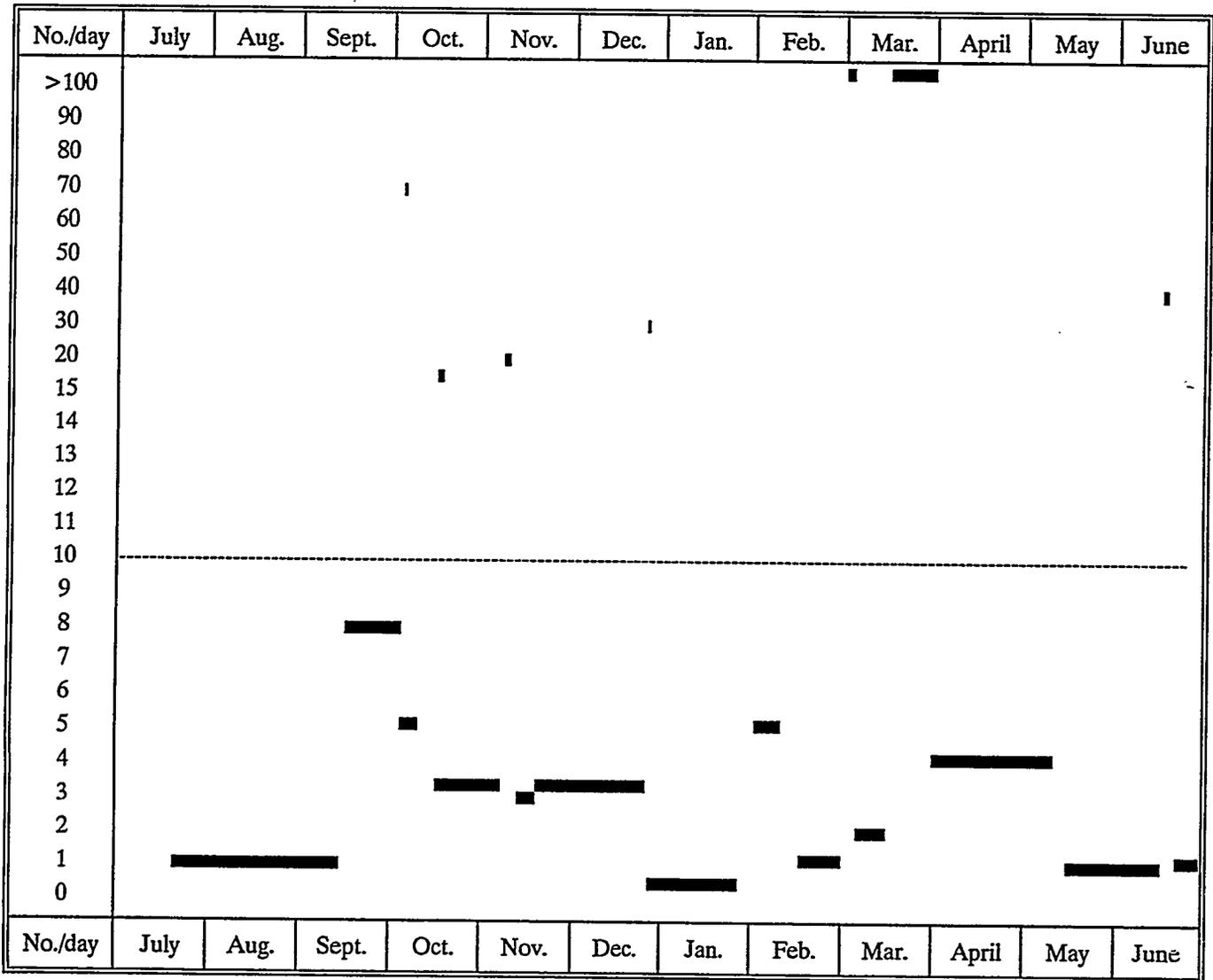


Fig. 3.1. Mean daily fish mortality for upper East Fork Poplar Creek, 1990-1991.

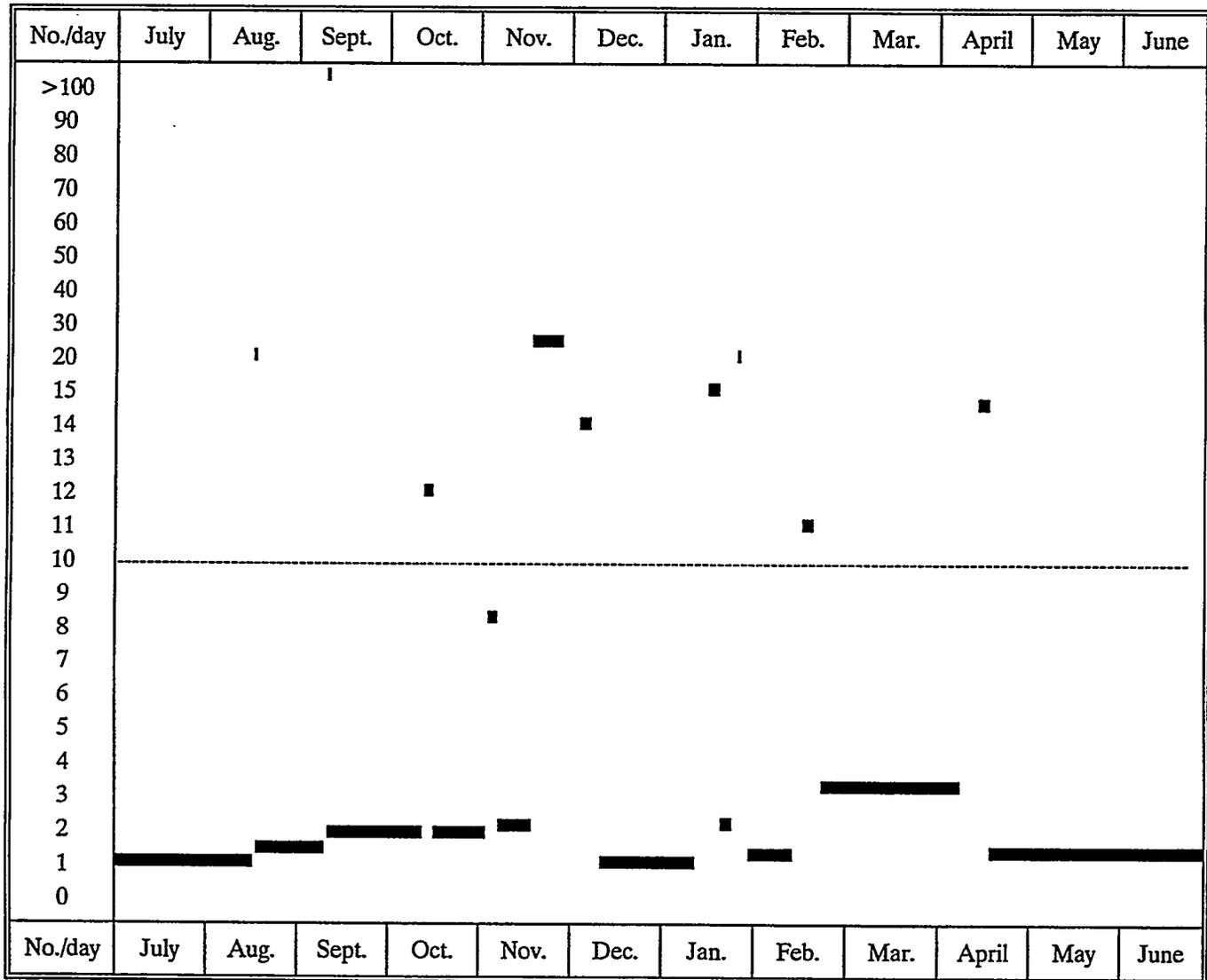


Fig. 3.2. Mean daily fish mortality for upper East Fork Poplar Creek, 1991-1992.

ORNL DWG 94-12392

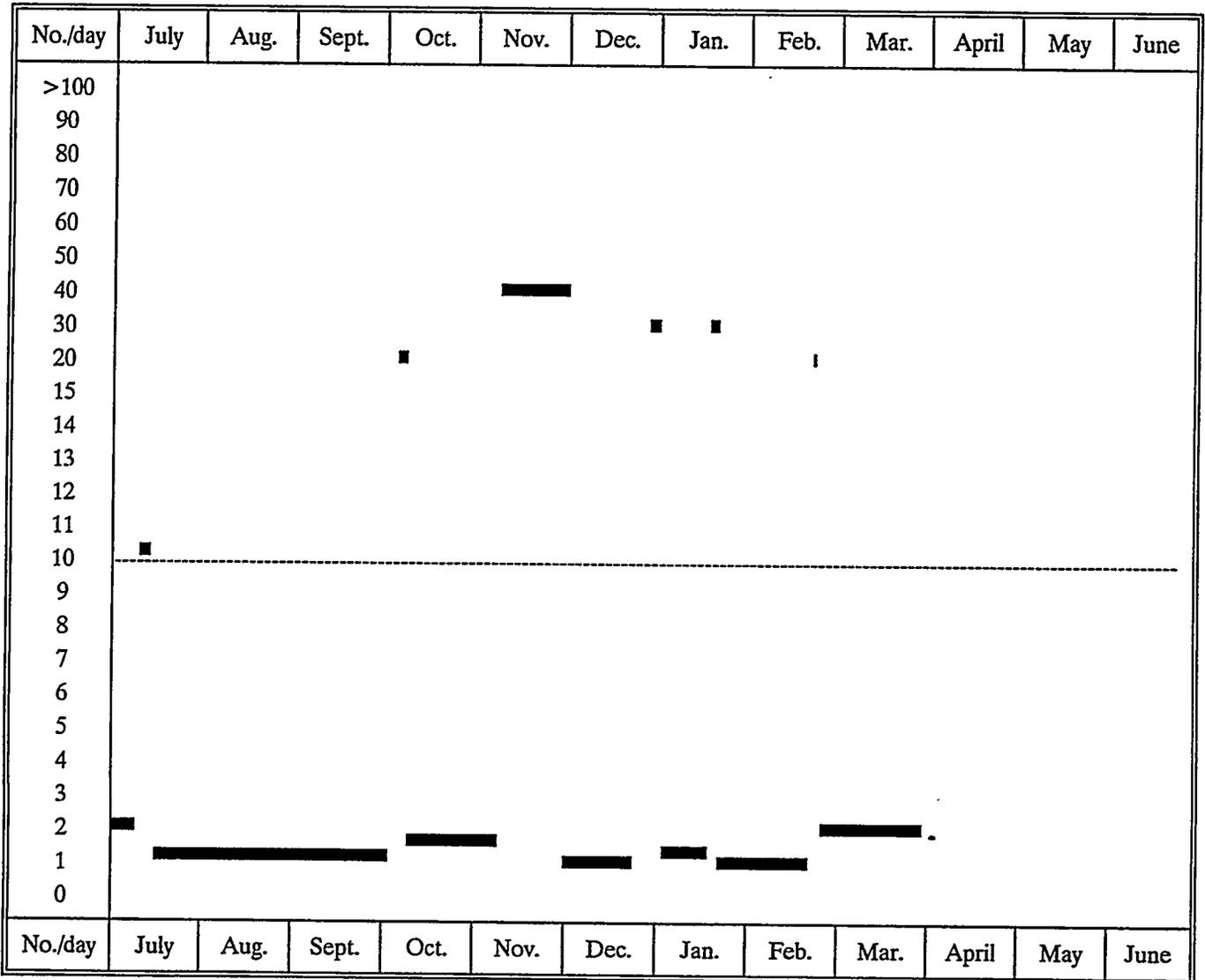


Fig. 3.3. Mean daily fish mortality for upper East Fork Poplar Creek, 1992-1993.

chronic conditions generally ranged from 1 to 5 fish per day, with the exception of two events in which the rates were 8 to 9 per day. The data suggest that the mean chronic mortality rates have generally decreased in successive years from 1 to 5 fish per day in 1990–91 to 1 to 2 fish per day in 1992–1993. Chronic mortality continued during the first 3 months after the two dechlorinators were installed along upper EFPC. TRC data for that time period (January–March, 1993, see Table 2.6) indicate that there were still considerable fluctuations in TRC that might have accounted for the continuing mortality. It was not until the summer of 1993 that TRC concentrations stabilized at very low levels in EFPC (see Table 2.6). Additional daily surveys would be needed to fully evaluate the impact of dechlorination on chronic fish mortality in upper EFPC.

### 3.1.3. Statistical Analysis of the Data and Development of a Predictive Model

The fish kills in upper EFPC for the time period July 1990 to May 1992 were evaluated statistically. The general approach and results of this effort are discussed in Appendix D. Because of the variability of the daily survey data, it was decided that the most appropriate statistical approach would be one based on cumulative weekly mortality data. Control charts were used to distinguish between acute and chronic mortality. The average weekly fish mortality was 14, and the upper control limit was 48 per week. Therefore, any values greater than 48 per week were considered as statistical evidence of a special-cause event. Mortality fluctuations of  $\leq 48$  per week were attributed to normal variation. These results generally support the graphical evidence presented in Figs 3.1–3.3 that the chronic daily mortality rates averaged 1 to 5 fish per day.

Control chart methods were also applied to the available environmental and

water chemistry data for EFPC to determine the normal range and upper control limits for these parameters (Appendix E). Finally, both the weekly chronic fish mortality data (i.e.,  $\leq 48$  per week) and the available environmental and water chemistry data were used to conduct a basic stepwise regression analysis. The resulting model explained about 33% of the results when acute mortality events were excluded from the analysis. One model correlated weekly fish mortality with pH, cloud cover, and temperature. Fish mortality was predicted to be lower at higher pH values and on cloudy days and higher for weeks with higher fluctuations in water temperature and pH (see Appendix D).

## 3.2 ACUTE KILLS

In addition to the chronic, chlorine-related fish mortality observed in EFPC during the past several years, there have also been a number of instances in which fish mortality increased substantially above background levels for periods lasting from 1 d to several weeks. Spills or elevated releases of toxic chemicals, such as acids, organophosphate-type insecticides, aluminum nitrate, ammonia, surfactants, or chlorine were identified as possible causative agents of some of the kills; however, in many cases, the cause could not be determined despite intensive chemical and biological monitoring. Changes in environmental conditions, such as pH or water temperature, may have also contributed to acute kills by increasing the susceptibility of the fish to the toxic effects of contaminants already present in the stream.

The combination of the acute kills related to spills and the chronic kills linked to the almost continuous release of chlorine into EFPC produced a complex and occasionally conflicting pattern of fish mortality that made definitive investigations

very difficult. As noted in Sect. 3.1, the effects of small spills of limited duration may have been obscured by the considerable variability in the daily chronic mortality. In general, however, mean daily mortality rates for acute kills were threefold or more above background and usually exceeded ten fish per day (see Figs. 3.1–3.3). The total number of dead fish collected during these acute kills ranged from about 30 to over 1000. Central stonerollers and striped shiners were the two species most commonly associated with acute kills occurring in upper EFPC at Sites 1 through 6 (see Fig. 1.1). Small numbers of blacknose dace and redbreast sunfish were also found during some of these events. In addition, redbreast sunfish and bluegill (*Lepomis macrochirus*) were the two species most affected in several acute kills occurring in or downstream of LR.

The following section lists the acute fish kills that occurred in upper EFPC from July 18, 1990, to March 31, 1993. The data are also summarized in Table 3.1 and Figs. 3.1–3.3. The acute kills were documented in the same reaches of EFPC as those used during the daily fish surveys (see Sect. 1.1 for description of sites). The species collected and the collection sites are recorded in Appendix C.

### 3.2.1. Chronological Listing of Acute Kills

#### 1. October 15–16, 1990:

**fish mortality**—Over a 2-d period, 34 dead fish were collected: 21 fish were found on the 15th and 13 on the 16th, mainly from the diversion channel and Sites 2 and 3. Thirty-eight percent of the dead fish were central stonerollers, and 62% were striped shiners.

**field observations**—The condition of the fish collected on the 15th suggested that the kill had occurred sometime over

the weekend and that the fish were still dying during the survey.

**histological/pathological data**—Many of the fish that had been collected for examination were too decomposed for evaluation. Of the 20 fish examined that were in reasonably good condition, there was no evidence of pathology or parasitic infestation. Several of the fish had a slight-to-heavy coating of mucous on their gills.

**water quality analysis**—A heavy foam was observed on the surface of EFPC on October 15–17. No other data were available.

**conclusions**—Cause unknown.

#### 2. December 24 1990:

**fish mortality**—Thirty-three dead fish were collected, of which 31 (94%) were central stonerollers. All of the dead fish were found within the diversion channel.

**field observations**—Dying fish exhibited a “whirling” behavior that has been associated with ammonia toxicity. Stream flow over the previous several days was quite high, suggesting that the fish may have died farther upstream. Many of the fish collected were large (about 18 cm), and all appeared to be in the same state of decomposition. Many were covered by silt. It was estimated that they had been dead for 24 to 48 h.

**histological/pathological data**—None.

**water quality analysis**—No data.

**conclusions**—Cause unknown. The kill was probably an acute phenomenon similar to what was observed periodically the previous winter (November 1989 to February 1990).

**3. March 1, 1991:**

**fish mortality**—Two hundred ninety-three dead fish were collected in a morning and afternoon survey; 243 (84%) of the fish were striped shiners, and 45 (15%) were central stonerollers. Most of the fish were found at Sites 2 (80), 3 (34), and 4 (110). Twelve additional fish were collected on March 2, mainly from Sites 3 through 6.

**field observations**—Most of the fish collected had been dead less than 24 h. Several of the fish had blood near the gills and fins, and distressed fish were seen in the stream.

**histological/pathological data**—None.

**water quality analysis**—On February 28, the pH of the water at AS-8 decreased from 8.0 to <2.0 between 9 p.m. and 10 p.m.; it returned to approximately 8.0 by midnight. The decrease in pH was associated with a twofold increase in conductivity during the same time period.

**conclusions**—The kill probably was caused by an acid spill occurring on February 28. However, no acid spill or leak could be documented by Y-12 Plant staff who investigated the incident. Later investigation revealed that from February 20–27 there had been a leak of cooling tower water into EFPC at a rate of about 76,000 gal/d (53 gpm).

**4. March 18–28, 1991:**

**fish mortality**—Over a 10-d period, 1,057 fish were collected. Ninety-eight percent were sunfish [redbreast sunfish, bluegill, and green sunfish (*Lepomis cyanellus*)]. Most of the fish were found in LR, and a smaller number in the diversion channel or below LR. Only 6 fish were found upstream of the diversion channel. Heavy rains occurred

on March 22 and 23, and no survey was conducted on March 24. High flows in the creek interfered with the dead fish surveys; consequently, only one dead fish was found on March 23; however, the number increased to 56 and then to 161 over the next 2 d.

**field observations**—On March 12, 1991, a cooling tower had malfunctioned for several hours, causing a spill of  $1.26 \times 10^5$  gal of cooling water. It was reported that the spill washed over an excavated area and resulted in an increase in the turbidity of EFPC. Many stressed fish were observed in the stream. Hemorrhages were not seen around the gills, fins, or anus of the stressed or dying fish; however, some had a dark, burn-like patch several centimeters long on the anterior dorsal part of the body.

**histological/pathological data**—A fish disease specialist from the U.S. Fish and Wildlife Service tested samples of the dead fish for bacterial and viral pathogens, but none were found. Some of the fish exhibited an abnormal pectoral fin position, suggesting that death was caused by a neurotoxin. Gross pathological evaluation revealed gill damage, and acetylcholinesterase activity in brain tissue was found to be elevated, compared to that found in fish from a reference stream (Table 3.2). Toxicity tests on cooling water constituents did not reveal the same type of toxic response as seen in the dead fish (i.e., splayed fins).

**water quality data**—A review of the water quality data for EFPC did not reveal anything unusual; chlorine concentrations, measured at the oil/water separator in the diversion channel, were normal for this reach of stream, ranging from <0.01 to 0.10 mg/L.

**Table 3.2. Acetylcholinesterase activity ( $\mu$ moles/mg of protein per min) in the brain tissue of sunfish collected from Lake Reality and a reference stream (Hinds Creek), March 20, 1991**

Species	Site	
	Lake Reality	Hinds Creek
Redbreast sunfish	0.053	0.342
Redbreast sunfish	0.032	0.184
Redbreast sunfish	0.032	0.148
Bluegill	0.036	0.264
Mean $\pm$ SD	0.038 <sup>a</sup> $\pm$ 0.010	0.235 $\pm$ 0.087

<sup>a</sup>Difference is statistically significant ( $0.001 < P < 0.01$ ).

**conclusion**—Histochemical evidence suggests that the kill may have been attributable to a pesticide, possibly an organophosphate compound; however, no pesticide spill could be documented.

#### 5. June 15, 1991:

**fish mortality**—Forty-one dead fish were found at Site 1, but none at any other site; 37 were unidentified cyprinids, and 4 were sunfish. No follow-up survey was made on the 16th, and only 6 dead fish were found during the survey conducted on June 17.

**field observations**—The survey was made by Y-12 Plant staff as requested by ORNL personnel. Collected field data resulted in inconclusive species identifications and location (“inside Y-12,” mostly upstream of AS-8).

**histological/pathological data**—None.

**water quality analysis**—Control chart analysis revealed that during the week of June 6–15 there was a significant change in the range of daily pH values at Station 17 downstream of LR (see Appendix E, Fig. E.2, data for week 47). For the week, the range among daily mean pH values was 2.7. Ranges within a week (for daily means) are

normally 0.5, with an upper control limit (99%) of 1. The average low pH at Station 17 for the same week was 6.5. The following week the average low pH was 6.4. For comparative purposes, the average daily pH, measured over 1.5 years, was 7.7 and the previous lowest weekly average was about 7.1. This pH excursion was not noticed at AS-8. During the same week, total suspended solids (TSS) averaged 21 mg/L (range 55 mg/L) compared with normal average of 12 mg/L (range 18 mg/L), and total dissolved solids (TDS) averaged 175 mg/L, compared with a normal average of  $290 \pm 60$  mg/L, based on control chart analysis. The upper control limit for TSS was 21 mg/L (range 26 mg/L).

**conclusions**—Cause unknown.

#### 6. September 10, 1991:

**fish mortality**—One hundred thirty-seven dead fish were collected in a morning and afternoon survey. The dead fish were found primarily at Site 2 (39), Site 4 (71), and Site 5 (21). Most of the dead fish (97%) were central stonerollers. Only five dead fish were found the following day.

**field observations**—None of the dead fish exhibited hemorrhages or other signs of damage. Live fish did not exhibit any patterns of behavior indicative of stress.

**histological/pathological**

**data**—Twenty-two central stonerollers collected on September 10 and 11 were examined. There was no evidence of pathogens or disease or of internal or external parasites. Of the four or five relatively fresh dead fish, none showed evidence of gill damage. The fish appeared to be in good overall condition, and in seventeen fish the guts were full.

**water quality analysis**—Monitoring data for chlorine, pH, dissolved oxygen, conductivity, and temperature indicated that measured values were within normal ranges. The 24-h composite sample taken at Station 17 on September 12 showed a nitrate concentration of 26 mg/L. The highest previous record over the preceding 1.5 years was 8.8 mg/L.

**conclusions**—Cause unknown. The high nitrate concentration at Station 17 suggests a spill of a nitrate-rich chemical such as aluminum nitrate or uranyl nitrate.

**7. October 16–18, 1991:**

**fish mortality**—Thirty-six dead fish were collected, of which 27 were found at Sites 4, 5, and 6. All but two of the fish were central stonerollers. Live fish were seen at Sites 1 through 5. No surveys were made on October 19 and 20.

**field observations**—Live fish seen in the stream did not show any behavioral signs indicative of stress.

**histological/pathological data**—None.

**water quality analysis**—High turbidity [32 NTU (nephelometric turbidity units)] was reported downstream of LR on October 15.

**conclusions**—Cause unknown.

**8. November 5–8, 1991:**

**fish mortality**—Twenty-eight of 34 dead fish collected were found at Sites 4, 5, and 6; 26 were central stonerollers, and 8 were striped shiners.

**field observations**—None recorded.

**histological/pathological data**—None.

**water quality analysis**—No data.

**conclusions**—Cause unknown.

**9. November 25–29, 1991:**

**fish mortality**—Over a 5-d period, 135 fish were collected. On the first day, 84% were found at Sites 5 and 6. On the following days the majority of the fish (79/86, 86%) were collected from Sites 3 and 4. Almost all of the dead fish (94%) were central stonerollers (131/140). Dead fish were observed only in upper EFPC and only in locations associated with outfalls; however, none were found within about 75 m of the N-S pipes.

**field observations**—A spill of machine coolant into the storm drains occurred on November 19, 1991; however, information on the size, location, and chemical composition of the spill has not been found. A brine leak of 1.5 gal/h for 8 h was also reported, but the time and location of the leak was not recorded.

**histological/pathological data**—Based on the absence of parasites and disease, evidence of recent feeding, and adequate levels of lipid stores in the examined fish, the deaths appeared to be the result of a sudden episodic event in the stream. No obvious signs of gill damage, such as erosion, fraying, or hemorrhaging, were observed. No external signs of hemorrhaging, indicative of bacterial hemorrhagic septicemia, were seen.

**water quality analysis**—A rainfall event totalling 1.5 in. occurred 3 d before the kill.

**conclusions**—The Kepner-Tregoe problem analysis procedure was applied to this kill. No determination could be made about the cause of this event.

#### 10. December 4—6, 1991:

**fish mortality**—Over a 3-d period, 42 fish were collected: 8 were found on the first day, 22 on the second and 12 on the third. Fifteen fish we found at Site 1 and 8 at Site 2. Sixty-seven percent of the fish were central stonerollers.

**field observations**—Total rainfall over the preceding 4 d was 7 in. No surveys were made on November 30 or on December 1, and rain prevented surveys from being conducted on December 2 and 3.

**histological/pathological data**—None.

**water quality analysis**—No data.

**conclusions**—Cause unknown.

#### 11. January 15—19, 1992:

**fish mortality**—Seventy-eight dead fish were collected over 5 d: 30 fish were found at Site 4, and smaller numbers at

the other upper EFPC sites. Only one fish was found in LR. Eighty-six percent of the dead fish were striped shiners.

**field observations**—At 8:56 p.m. on Jan. 14, it was reported that the section of EFPC just south of Building 9201-2 (beside AS-8) had a very milky color. Examination of the creek upstream at Building 9767-8 several minutes later revealed that the color was fading, and no discoloration was seen in the stream on the west side of Building 9201-2. The milky discoloration was present in the stream near the Truck Scales at 9:30 p.m., and a whitish residue was seen on rocks and the retaining wall near the NSP. Fish were not seen near Outfall 109, where they normally congregate. Stream flow was routed through the diversion channel from January 15 to 18, and water was held in LR to determine its potential toxicity before being released downstream.

A spill of floor wax occurred on January 14; however, information on the size and location of the spill was not available. A 1000-gal spill of a bioreactor slurry containing aluminum was reported at Building 9818.

**histological/pathological data**—Some of the dead fish had extreme gill damage with excessive mucous on the surface; this may have resulted in asphyxiation. Analysis of the gill surface by scanning electron microscopy revealed an abnormal amount of aluminum compared with samples taken from dead fish collected from EFPC several days later.

**water quality analysis**—At AS-8, the pH decreased by 1 unit from 8:30 to 10:00 p.m. A white sheen was observed on the surface of the water.

**conclusions**—The drop in pH and the white sheen on the surface of the water are consistent with a spill of aluminum nitrate. The gill damage seen in the affected fish is consistent with the effects of a caustic agent.

**12. January 27, 1992:**

**fish mortality**—Twelve central stonerollers and 11 striped shiners were collected. Sixteen fish were found at Site 6, and the remaining ones at Site 5.

**field observations**—Live fish were seen at Sites 1 through 4 and 6, but not at Site 5. Most of the fish collected appeared to have been dead less than 24 h.

**histological/pathological data**—None.

**water quality analysis**—The TRC concentration at NSP on January 27 was 0.13 mg/L, a value relatively low compared with others recorded at NSP during January.

**conclusions**—Cause unknown.

**13. February 18–21, 1992:**

**fish mortality**—Forty-four fish were collected over 4 d, all but four from Sites 4, 5, and 6.

**field observations**—An oily sheen was reported on the surface of EFPC inside the Y-12 Plant on February 18–20. Live fish were seen at Sites 5 and 6 only infrequently and not on all survey days.

**histological/pathological data**—None.

**water quality analysis**—The TRC concentration at NSP on during the 4-d period ranged from 0.22 to 0.41 mg/L.

**conclusions**—Cause unknown.

**14. April 13–16, 1992:**

**fish mortality**—Fifty-nine dead fish were collected over 4 d: 17 on the first day and 23 on the second day. Forty-six percent were found in the diversion channel; 64% were central stonerollers, and 24% striped shiners.

**field observations**—None.

**histological/pathological data**—Based on the absence of parasites and disease and evidence of recent feeding in the examined fish, the deaths appeared to be relatively rapid and caused by a sudden change in water quality. Gill damage could not be evaluated in the central stonerollers or shiners because of the poor condition of the specimens. Several of the fish showed signs of external hemorrhaging, but this was not thought to be caused by bacterial hemorrhagic septicemia.

**water quality analysis**—No data.

**conclusions**—Cause unknown.

**15. July 7–10, 1992:**

**fish mortality**—Forty-one fish were collected over 4 d: 12 from the diversion channel, 10 from below LR, and the remainder almost equally distributed between Sites 1, 2, 4, and 5.

**field observations**—In addition to the morning surveys, afternoon surveys were made on July 7 and 8 in conjunction with the dead tagged fish distribution study (see Sect. 4.1.1). If only the morning survey results are considered, the mean mortality rate for the 4-d period would have been only five fish per day.

**histological/pathological data**—None.

**water quality analysis**—The TRC concentration at NSP during the 4-d period ranged from 0.17 to 0.25 mg/L, and those at AS-8 from 0.07 to 0.08 mg/L.

**conclusions**—This was probably just part of the chronic kill that appeared to be more severe because of the multiple surveys conducted on July 7 and 8. The morning surveys may underestimate the total daily mortality by 80 to 90% (see Sect. 4.1.1.).

#### 16. October 7–9, 1992:

**fish mortality**—Over a 3-d period, 69 dead fish were collected (mean 23/d), mainly from Site 6 (59/69). Ninety-seven percent of the fish were central stonerollers.

**field observations**—Dead fish were first seen at 8:15 a.m. on October 7, near the NSP. Observations made at 9:50 a.m. included a distinct oil sheen on the surface of the water, an odor of kerosene, and an above-normal amount of foam. Live fish in the stream exhibited avoidance behavior by burying under rocks in an eddy of the stream that appeared to receive seepage (presumably from uncontaminated water). Stressed fish were also seen upstream of the grate at the NSP.

#### **histological/pathological**

**data**—Examinations were conducted on 17 central stonerollers; there was no evidence of parasites, disease, external hemorrhaging, gill damage, or any other anomalies. There was little food in the digestive tract, and mesenteric lipid levels were low, indicating long-term metabolic stress or limited food availability. The deaths appeared to occur relatively rapid, possibly as a result of a sudden change in water quality.

**water quality analysis**—Colorimetric measurements of TRC taken at the time were 0.28 and 0.26 mg/L. An atypically high TRC concentration of 0.68 mg/L was measured at NSP in the early evening of October 9. By 9:30 p.m., the TRC concentration at NSP had decreased to 0.26 mg/L.

**conclusions**—The kill very probably was caused by elevated TRC concentrations at the NSP.

#### 17. November 10–12, 1992:

**fish mortality**—Over a 3 d period, 457 dead fish were collected, primarily from Sites 1 and 2 in upper EFPC (134 from Site 1 and 124 from Site 2). Fifty-eight percent of the fish were collected on the first day, and 34% on the second day. Striped shiners (46%) and central stonerollers (45%) were the species most affected.

**field observations**—None.

**histological/pathological data**—None.

**water quality analysis**—On November 10 (at 1:15 pm) water samples were collected from four locations in upper EFPC: NSP, AS-8, LR-i, and LR-o. Portions of each sample were analyzed for TRC, pH, conductivity, alkalinity and hardness. The results of these analyses are shown in Table 3.3. TRC concentrations were slightly higher than usual, but the other parameters were within the ranges typical for those measured in upper EFPC. On November 11, the TRC concentration was 0.42 mg/L in a water sample from NSP, 0.20 mg/L for AS-8, and nondetectable in samples from LR-i. Ammonia concentrations for NSP and AS-8 were very low, and those for LR-i were high. Water samples taken at the NSP were

Table 3.3. Water quality analyses, East Fork Poplar Creek, November 11, 1992

Site	TRC (mg/L)	pH	Conductivity ( $\mu$ mhos/cm)	Alkalinity (mg/L)	Hardness (mg/L)
NSP	0.31	8.03	406	111	188
AS-8	0.17	8.16	402	115	182
LR-i	0.05	8.29	480	116	196
LR-o	0.03	8.06	541	119	230

found to be toxic to *Ceriodaphnia*, whereas dechlorinated water was much less toxic (Table 3.4). Additional tests were conducted in which AS-8 water was used with and without a dechlorinating agent and/or a metal chelator [ethylenediaminetetraacetic acid (EDTA)]. The results are shown in Table 3.5.

**conclusions**—The fish kill was most probably the result, at least in part, of elevated chlorine concentrations in EFPC. The results of the toxicity tests suggest that a metal may have also contributed to the fish kill.

#### 18. November 16–20, 1992:

**fish mortality**—In a 5-d period, 216 dead fish were collected, most being from Sites 4, 5, 2, and 3 (81, 42, 41 and 35, respectively). No dead fish were found in or downstream from LR, and only four were found in the diversion channel. Seventy percent of the dead fish (152/216) were central stonerollers.

**field observations**—None.

#### histological/pathological

**data**—Examinations were conducted mainly on central stonerollers and sunfish. There was no evidence of parasites or disease. The gills of the central stonerollers appeared normal, but those of the sunfish appeared frayed or had abnormal accumulations of mucous

on the surface, possibly indicative of waterborne irritants such as heavy metals, chlorine, or excessive amounts of suspended solids. Evidence of recent feeding and relatively large amounts of mesenteric lipids indicated that the fish were well nourished. The deaths appeared to have occurred relatively rapidly, probably in response to a sudden change in water quality.

**water quality analysis**—TRC concentrations of 0.28 to 0.43 mg/L were recorded at NSP, 0.15 to 0.26 mg/L at AS-8, and 0.03 to 0.08 mg/L at LR-i during this time period. These values were about two times higher than the typical TRC concentrations at these three stations.

**conclusions**—It is likely that this kill was associated with elevated TRC concentrations in upper EFPC.

#### 19. November 24–30, 1992:

**fish mortality**—Over a 7-d period 99 dead fish were collected: 35 from Sites 5 and 6 on the first day and 40 from Site 2 over the next six days. Fifty-eight percent of the dead fish were central stonerollers.

**field observations**—Rain interrupted surveys between November 20 and 24. Stressed fish were seen “huddled” along the margins of the stream on November 24.

**Table 3.4. Testing of the toxicity of NSP water collected November 10, 1992, on *Ceriodaphnia***

Water sample	No. alive/No. tested	Percentage Survival (16-h)	Percentage Survival (24-h)
Control	36/36	100	100
NSP (nontreated)	0/36	0	0
NSP (dechlorinated) <sup>a</sup>	34/36	94	47

<sup>a</sup>Water was dechlorinated with sodium thiosulfate.

**Table 3.5. Testing of the toxicity of AS-8 water collected November 10, 1992, on *Ceriodaphnia***

Water sample	No. alive at start	Survival (%)		
		21-h	29-h	44-h
Not treated	24	96	67	67
EDTA <sup>a</sup> added	24	100	92	88
Dechlorinated	25	100	96	96
Dechlorin. + EDTA	23	100	100	100
Candle wax added	24	100	100	100
Daphnid food added	24	100	100	100

<sup>a</sup>EDTA = ethylenediaminetetraacetic acid.

**histological/pathological data**—Fish collected by electroshocking on November 24 were examined and found to have no evidence of infectious disease (bacterial or viral) and no indication of parasite infestation. The fish appeared healthy and had food in the gut.

**water quality analysis**—No data.

**conclusions**—This kill probably was a continuation of the one occurring on November 16–20, 1992 and may have been caused by elevated TRC concentrations at the upper end of EFPC.

#### 20. December 27, 1992:

**fish mortality**—One hundred twelve dead fish were found in the diversion channel, but none in upper EFPC, in LR, or downstream of LR. Ninety of the fish were central stonerollers, and 20 were blacknose dace. Thirteen fish were found the following day (3 in the diversion channel and 10 at Site 2), and 17 on the 29th (15 in the diversion channel and 2 at Site 2).

**field observations**—The initial survey was conducted at 5:30 p.m. in the dark (with flashlights). Many of the fish seen in the diversion channel were exhibiting stress behavior (spinning, swimming in

circles, and flashing on side), but none were seen to be "gasping" at the water's surface or hiding along the margin of the stream. Signs of stress behavior were seen in fish in upper EFPC for 3 d following the fish kill.

**histological/pathological data**—None.

**water quality analysis**—Ammonia concentrations measured in water samples taken at NSP, AS-8, and LR-i on December 29 were 58, 7, and 1356  $\mu\text{g/L}$ , respectively. The ammonia concentration in the effluent from Outfall 017 was estimated to be 102 mg/L. Additional water sampling on December 30 revealed high concentrations of ammonia (up to 5.84 mg/L) along the south side of the stream in and just upstream of the diversion channel. Water from Outfall 017 diluted to an ammonia concentration of 764  $\mu\text{g/L}$  was lethal to fathead minnow larvae within 1 h, and a sample that had an ammonia concentration of 551  $\mu\text{g/L}$  caused disequilibrium in 1 h (Table 3.6).

**conclusions**—The kill probably was the result of the elevated ammonia concentrations.

#### 21. January 19–21, 1993:

**fish mortality**—Ninety dead fish were collected over 3 d: 12 on the first day, 61 on the second, and 17 on the third. All but three of the fish were found at Site 2. Eighty-three percent of the fish were striped shiners. No dead fish were found during a morning stream survey (9:00–10:00 a.m.) conducted on January 19.

**field observations**—At 1:30 p.m. on January 19, foam was observed discharging from Outfall 021. The dead fish were found to have hemorrhages.

**histological/pathological data**—None.

**water quality analysis**—Water samples taken from the foamy discharge were found to contain two disinfectants, a fragrance, a germicide, and a sulfonated surfactant.

**conclusions**—The available data suggests that the kill was a result of the spill from Outfall 021; however, no toxicity studies were undertaken to identify the specific cause.

#### 22. February 24, 1993:

**fish mortality**—Fourteen dead fish were collected from Site 1 and 5 from the diversion channel. Eleven of the fish were central stonerollers, and eight were striped shiners.

**field observations**—None.

**histological/pathological data**—None.

**water quality analysis**—TRC concentrations at NSP, AS-8, and LR-i were below detection limits.

**conclusions**—Cause unknown.

#### 3.2.2. Fish Mortality Not Associated with Toxic Chemicals

In three instances, fish mortality in EFPC was attributed to causes other than toxic contaminants in the stream; these involved entrapment of fish on block nets, electroshocking, or stranding of fish in the diversion channel.

On October 5, 1990, 70 dead fish were found in block nets downstream of LR. The most probable cause of death was entrapment of small fish (<5 cm) on the block nets during heavy stream flows.

**Table 3.6. Toxicity of Outfall 017 effluent to fathead minnow larvae**

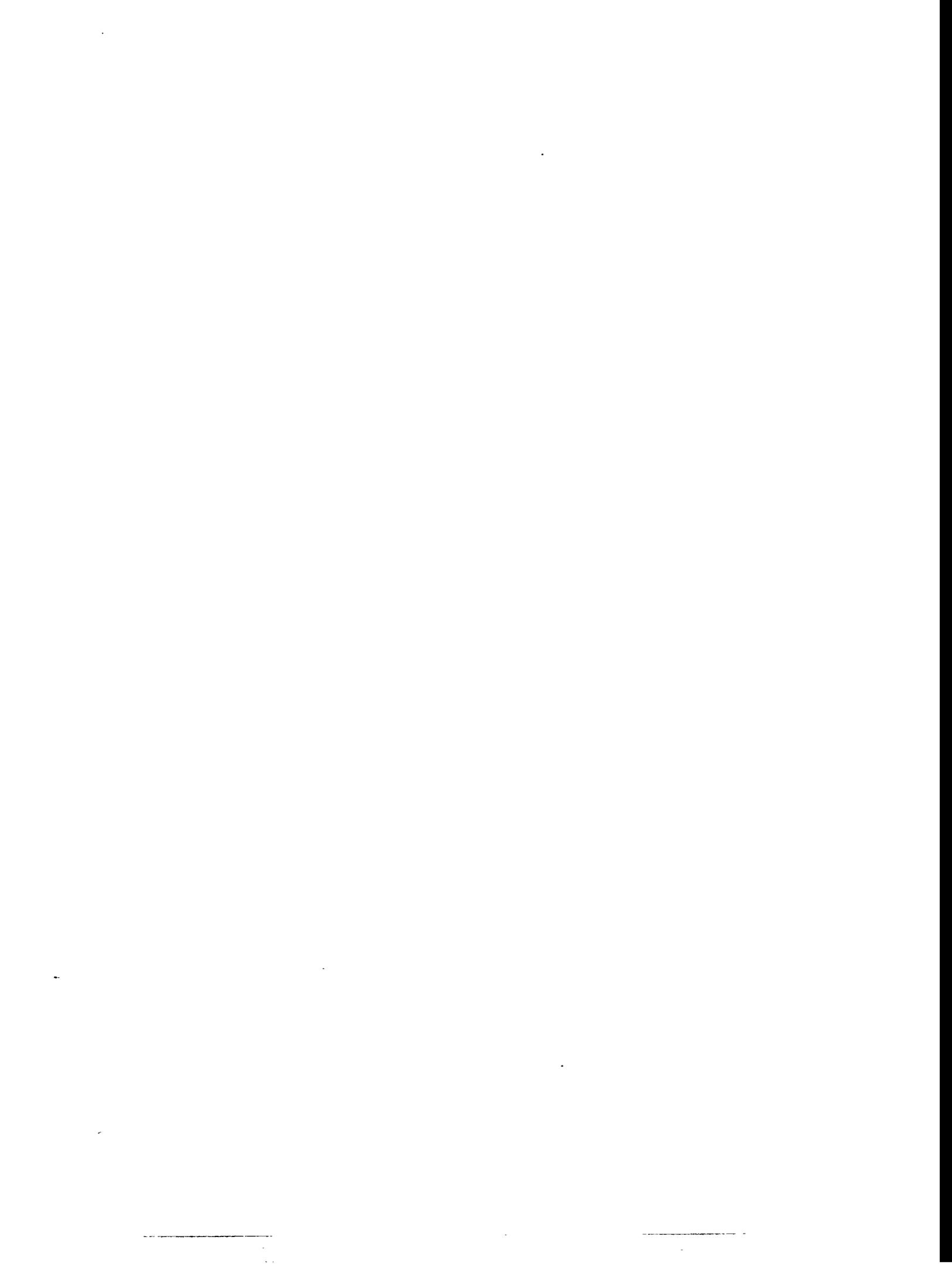
Sample No.	Ammonia conc. ( $\mu\text{g/L}$ )	pH	Response (1 h)
1	11	7.60	No effect
2	75	7.66	No effect
3	338	7.92	No effect
4	487	7.94	No effect
5	551	7.95	Disequilibrium
6	764	7.98	Total death

Note: During the period September–December 1990, six block nets were used in EFPC to identify areas where fish were dying. On September 11, 1990, block nets were placed at three locations: No.1 at the culvert below EFK 24.4 (at Site 1), No. 2 in the diversion channel above the inlet to LR, and No. 3 5 m below the outfall of LR. On September 25, block net No. 4 was placed across the concrete apron just below the outfall of LR. On October 2, block net No. 5 was placed across EFPC below LR. On October 5, nets Nos. 1 and 3 through 5 were washed out and removed. Net No. 2 had been removed prior to this time. On October 8, a new block net, No. 6, was placed across EFPC just above

Station 17; this was the only net that extended across the entire stream. This net was washed out and removed on December 24, 1990.

On November 7–8, 1990, 39 dead fish were found in EFPC. From 9:30 a.m.–2:00 p.m. on November 7, fish populations near Station 17 below LR had been sampled by electroshocking, and the fish mortality appeared to be a delayed response to the electroshocking.

On August 15–16, 1991, 35 dead fish were found between the outfall of the diversion channel and EFPC. The flow of EFPC had been restricted to the diversion channel while liner repairs were made on LR. After the liner repair, restoration of flow into LR resulted in a sudden drop in water level in the downstream end of the diversion channel. As a result, fish were trapped in isolated areas.



## 4. EXPERIMENTAL FIELD AND LABORATORY STUDIES

Special studies and experiments were conducted to explore specific questions or test hypotheses about ecotoxicological conditions in EFPC. Such studies can augment the biological monitoring data in that they can identify factors or conditions that are causally related to phenomena of concern (i.e., fish kills, toxicity test failures, and taxonomically depauperate invertebrate communities). Such studies can also establish a framework that can be used to correctly interpret routinely obtained monitoring data. Studies of the drift of dead fish can help to identify the location/source of toxicity following fish kill events.

The following field and laboratory studies were conducted to further evaluate the results of ambient toxicity tests conducted in upper EFPC: (1) distribution of tagged dead fish; (2) potential acclimation of fish to TRC in EFPC; (3) avoidance behavior of fish to chlorine in the laboratory; (4) survival and growth of snails in EFPC; (5) feeding of snails in relation to water quality; (6) survival and growth of clams in EFPC; (7) valve movement response of clams to fluctuating, low-level concentrations of chlorine in EFPC; (8) taxonomic/physiological adaptations of periphyton in EFPC; and (9) microbial enzyme activities in EFPC.

### 4.1 FISH

#### 4.1.1 Distribution Studies of Tagged Dead Fish

A study of the downstream distribution of tagged dead fish in upper EFPC was

undertaken to determine whether the location of dead fish found during the regular stream surveys could be used to indicate where and when the fish died and thereby provide some insight into the cause of death. The study was also designed to evaluate: (1) the effectiveness of the daily fish surveys, (2) the impact of scavengers in removing dead fish from the stream, (3) the likelihood that fish dying in upper EFPC might be dispersed as far downstream as LR, and (4) whether the decomposition state of the dead fish could be correlated with the time of death.

One hundred twenty dead golden shiners (*Notemigonus crysoleucas*) were tagged and released at three sites along upper EFPC: at the north-south pipes (NSP), at Site 5 (Outfall 109), and at Site 2 (truck scales). The location of each fish in the stream was monitored over the following 3 d; the fish were not removed until the afternoon of the third day. Stream flow, pH, and temperature were monitored at Site 1 (Building 9720-4), Site 4 (AS-8), and Site 6 (just downstream of NSP) during the first day of the experiment. Water temperature ranged from 24.2 to 28.7°C, with the most variation at the NSP; pH ranged from 7.5 to 8.0; and water flow was highest at Site 1 (0.19 to 0.22 m/s) and lowest at NSP (0.12 to 0.15 m/s). Discharge rate was also consistent at each site, indicating that there were no surges in flow that might affect dispersal patterns.

A complete description of the study design, protocol, and results are given in Appendix F. The following paragraphs summarize the major results and conclusions of the 3-d study.

Of the 120 tagged dead fish, 60 to 79% were found during each of four surveys conducted at 1.5-h intervals during the first day. Over the entire 3-d study period, 94% of the released fish or tags were observed. Dispersal of the tagged dead fish was highly dependent on the location of the release point. At the NSP, the majority of the fish remained within 5 m of the release point (effluent from pipe on north bank) during the entire first day; 18 to 26% moved more than 25 m, with the maximum distance traveled varying from 210 to 345 m. Most fish that moved >25 m were lodged on rocks within the middle of the stream. At Site 5, none of the tagged fish were dispersed more than 10 m during the first day; most were concentrated in a deep pool at the end of the culvert, where they were released. At Site 2, 64 (95%) of the tagged fish moved a distance >25 m; the maximum distance traveled ranged from 225 to 228 m. The fish at this site were equally distributed between rocks in the middle of the stream and bank vegetation that drooped into the water. No tagged dead fish were found in LR or in the diversion channel; however, three were recovered from the oil/water separator at the top of the diversion channel during the regular stream survey conducted the day after the 3-d experiment was concluded.

Only 26 fish (in some cases only the tag) were found during a morning stream survey conducted on the second day of the experiment (8 at NSP, 11 at Site 5, and 7 at Site 2), and only 20 were found during an afternoon survey. This suggests that overnight removal of the dead fish by scavengers such as crayfish, muskrats, rats, raccoons, turtles, and snakes was quite high. Decomposition of the tagged fish was relatively rapid; almost every fish collected in the morning of the second day was bleached-out and bloated, and many were very soft or mushy.

Results of this study indicate that dead fish found at a specific location in upper

EFPC probably died in that general area, although it is possible that stressed or dying fish might be dispersed prior to dying (this factor was not evaluated in this study). Some dispersal over longer distances is likely to occur as dead fish decompose and become bloated. Rapid decomposition, however, prevents using the condition of the fish to estimate the specific time of death, other than to indicate that the fish died less or more than 24 h prior to collection. The absence of dead tagged fish in the diversion channel or downstream LR suggests that dead fish found in these areas during the regular daily stream surveys probably died in the same areas and not further upstream.

If the efficiency of the daily surveys is similar to that for the tagged fish study, it is likely that daily surveys are finding only 50 to 80% of the dead fish in the creek at a given time. Peripheral data on dead nontagged fish gathered during the first day of the experiment also indicate that the daily surveys may be finding only a fraction of the total number of fish dying per day. Before the experiment began, only 1 dead, nontagged stoneroller was found in upper EFPC; however, on succeeding surveys conducted on the same day (during the tagged fish experiment), an additional 11 dead or dying nontagged fish were found, 5 of which were in a location where they could not have been previously overlooked (in the diversion channel). Therefore, the daily surveys conducted in the morning may be finding only 50 to 80% of the dead fish present at the time, and this may represent only 10 to 50% of the fish dying during the day. However, of the dead fish not collected, probably only a small portion (10 to 30%) escape overnight scavengers and are collected during the regular stream survey conducted the next day. Overall, the daily surveys may underestimate the actual fish mortality by 50 to 90%.

#### 4.1.2 Instream Fish Acclimation and Toxicity Studies

TRC was suspected to cause or contribute to the fish kills in upper EFPC. Data collected from TRC analyzers positioned at bankside sites in the creek revealed that fish are frequently exposed to lethal concentrations of TRC, based on 96-h  $LC_{50}$  values of 0.08 to 0.19 mg/L reported for *P. promelas* (Giattina et al. 1981). In situ experiments were conducted with two indigenous species of fish, striped shiners (*Luxilus chrysocephalus*) and central stonerollers (*C. anomalum*) to test the hypothesis that the fish had become acclimated to TRC.

##### 4.1.2.1 Methods

Two experiments were conducted to determine if minnows found in a TRC-contaminated environment could acclimate to TRC. On August 3, 1992, 10 striped shiners and 4 central stonerollers were collected by seining in EFPC near AS-8, an area where chlorine is almost always detectable; 25 striped shiners and 17 stonerollers were collected near EFK 21.8, where TRC is rarely detectable. The lower or upper caudal fin of each fish was clipped

to indicate the location of capture. The marked fish were immediately placed in coolers containing aerated water from the collection site. The fish were then placed in mesh cages at each of two sites: 3 m downstream of the NSP and within a stream pool near EFK 21.8. During the next 2 d, the fish were checked at 30 min to 2-h intervals and dead fish, if present, were removed from the cages. The species, total length, time to death, and source of the dead fish were noted. Incident light, TRC, pH, and temperature were monitored at the NSP; TRC and pH were monitored at AS-8. In the second experiment, striped shiners collected at AS-8 (16 fish) and EFK 21.8 (13 fish) were fin clipped and placed in a cage near the NSP as in experiment one.

##### 4.1.2.2 Results

The results of the two experiments are summarized in Table 4.1. Both species collected from the TRC-contaminated site (AS-8) had a longer time to death than fish collected near EFK 21.8 (Table 4.1). An analysis of variance indicated that differences in the time to death were affected strongly by where fish were collected.

**Table 4.1. Summary of results for field experiments using stoneroller (SR) and striped shiner (SS) minnows from a TRC-free and a TRC-contaminated site in East Fork Poplar Creek, placed in a cage in upper East Fork Poplar Creek at a site where TRC concentrations were great enough to be lethal**

Experiment (date) and source of fish	Minnow species	No. of fish tested	Time of death (h; mean $\pm$ SE)	Time to 50% mortality (h)
First (8/3/92)				
TRC-free site	SR	17	6.1 $\pm$ 0.9	4.3
TRC-contaminated site	SR	5	15.8 $\pm$ 2.0	15.9
TRC-free site	SS	25	3.7 $\pm$ 0.4	2.1
TRC-contaminated site	SS	10	14.1 $\pm$ 1.9	13.8
Second (8/18/92)				
TRC-free site	SS	13	3.1 $\pm$ 0.4	2.3
TRC-contaminated site	SS	16	5.5 $\pm$ 0.5	4.7

#### 4.1.2.3 Conclusions

The results suggest that both central stonerollers and striped shiners can partially acclimate physiologically to TRC. Laboratory studies with appropriate replicates were conducted; the results of these studies confirmed this supposition, as described in Lotts and Stewart (1995).

#### 4.1.3 Laboratory Study of Fish Avoidance Behavior to Chlorine

A laboratory study was conducted to determine if striped shiners and central stonerollers can detect and avoid low concentrations of TRC.

##### 4.1.3.1 Methods

Striped shiners and central stonerollers were placed, one at a time, in a plexiglass chamber, 72 × 19 × 19 cm high, and tested for their ability to detect and avoid chlorine. The chambers were fed at both ends with dechlorinated tap water piped from tygon tubing. Near the middle of each chamber four tygon tubes siphoned excess water. The depth of the water was maintained at 10.6 cm by a gravity-induced siphon. The water removal process resulted in a distinct boundary (at the center of the chamber) between water supplied from either end. Chlorine [sodium hypochlorite, Na(OCl)<sub>2</sub>] was delivered to either end of each chamber by means of a peristaltic pump. A food color tracer was used to ensure a distinct boundary between the TRC-treated and TRC-free sides of the chambers. The chambers were enclosed in cardboard and black plastic to prevent visual disturbances; in addition, tests were conducted late at night to eliminate noise disturbances. The movements of fish were monitored with a closed-circuit video camera.

Three striped shiners and two central stonerollers that had been acclimated to the laboratory for 2 weeks were used in the experiments. The fish had been acclimated to either dechlorinated water or water with a TRC concentration of 0.04 mg/L. At the start of each experiment, a single fish was placed in the chamber and allowed to acclimate for 30 min. Chlorine at a concentration of 0.07 mg/L was slowly pumped into the side of the chamber in which the fish was residing. An equilibrium distribution of 0.07 mg/L was achieved in one side of the chamber in 12 min. The time at which an avoidance response, defined as an abrupt twisting movement followed by swimming to the opposite side of the chamber, occurred was observed, and the time at which the fish crossed the boundary line near the middle of the chamber was recorded. If the fish remained calm after moving to the TRC-free end of the chamber, the test was repeated 15 min later. An additional fish was tested at an input rate of 0.05 mg TRC per liter. Negative controls in which fish were observed for an additional 20 min in chlorine-free water following the 30-min acclimation period or following the first chlorine avoidance behavior were also used. None of these fish showed an avoidance response unless chlorine had been added to the water.

##### 4.1.3.2 Results

The avoidance response of all fish was similar (i.e., an abrupt twisting movement followed by swimming to the other side of the chamber). The time to elicit this response depended primarily on the distance of the fish in the chamber from the source of the chlorine. Although fish were observed for 20 min during each trial, all of the fish moved to the TRC-free side of the chamber in ≤7 min (Table 4.2).

Table 4.2. Laboratory studies of chlorine-avoidance with striped shiners (*L. chrysocephalus*) and central stonerollers (*C. anomalum*)<sup>a</sup>

Species tested	Acclimation water	Time to avoidance (min)		
		Trial 1 <sup>b</sup>	Trial 2	Trial 3
<i>L. chrysocephalus</i>	Dechlorinated	3	NT <sup>c</sup>	NT
<i>L. chrysocephalus</i>	Dechlorinated	7	NT	NT
<i>L. chrysocephalus</i>	0.04 mg/L TRC	7	NT	NT
<i>C. anomalum</i>	Dechlorinated	3	3	3
<i>C. anomalum</i>	0.04 mg/L TRC	4	NT	NT

<sup>a</sup>Data show the number of minutes a fish remained in the TRC treated end of the chamber after the addition of chlorinated water at a concentration of 0.07 mg/L had been started.

<sup>b</sup>15- min interval between trials.

<sup>c</sup>NT indicates no test.

Concentrations of TRC at these times were <0.07 mg/L (i.e., the time to reach equilibrium distribution had not been reached). The number of animals tested and number of trials were insufficient to draw conclusions regarding source of acclimation water and time to response. A single fish tested at an equilibrium concentration of 0.05 mg/L responded by moving to the chlorine-free side of the chamber in <3 min (data not shown).

#### 4.1.3.3 Conclusions

TRC at concentrations of  $\leq 0.07$  mg/L was used to confirm the avoidance of chlorine by fish. Two species of fish, striped shiners and central stonerollers, were able to detect and avoid these concentrations within 7 min.

#### 4.1.4 Laboratory Study of Chlorine Toxicity

Central stonerollers and striped shiners are the dominant fish taxa in upper EFPC. Biannual quantitative sampling of fish populations since 1990 showed that striped

shiners occurred in densities up to four times greater than the central stoneroller (Table 2.11); however, daily surveys during fishkills revealed that 42% of the dead fish collected during the survey periods were central stonerollers, whereas only 24% were striped shiners. To test the hypothesis that central stonerollers are more sensitive than striped shiners to TRC, flow-through tests were conducted in which chlorinated tap water was used at concentrations that have been observed in the stream.

#### 4.1.4.1 Methods

Central stonerollers and striped shiners were collected from pools in EFPC (just downstream of LR) by electrofishing. Fish were returned to the laboratory, placed in holding tanks containing dechlorinated tap water, and acclimated to 19°C by stages.

Three flow-through tests were conducted in which chlorinated tap water diluted to approximate concentrations of 0.8, 0.4, and 0.2 mg TRC/L (concentrations measured to the nearest tenth) was used. Dechlorinated tap water was used as a control (mean  $0.013 \pm 0.0047$  mg TRC/L). The desired concentration of TRC was

achieved by mixing chlorinated and dechlorinated tap water in a head tank. Water from the head tank was gravity-siphoned into 12-L glass aquaria. Four replicate aquaria containing ten fish of the same species were used for each treatment. The time-to-death (TTD), the measured endpoint, was determined by the absence of observable gill or body movement. Fish were monitored continuously for the tests at 0.8 and 0.4 mg TRC/L and at about 30-min intervals for the test at 0.2 mg TRC/L. Water temperature ( $^{\circ}\text{C}$ ) and TRC concentrations (determined amperometrically with a Wallace and Tiernan titrator) were measured hourly. To test the hypothesis that fish size (e.g., weight of the fish) was positively correlated with TTD, individual fish were weighed (to the nearest 0.1 g) and measured (total length to the nearest 0.1 cm) after death had occurred. Both TTD (log transformed) and weight data (including interaction terms) were analyzed by using ANOVA by PROC GLM in SAS (1985b).

#### 4.1.4.2 Results

Because the range of values for TTD differed across treatments by orders of magnitude and the general positive skewness of the TTD values, a log transformation was performed on the TTD data. A general analysis of variance model was used to evaluate TRC and species effects, using the individual observations. The TRC-species interaction was significant ( $P = 0.049$ ), as was the TRC effect ( $P < 0.0001$ ) and the species effect ( $P = 0.036$ ). A plot of the mean log TTD estimates (Fig. 4.1) showed that the TRC effect was highly significant. A general decrease in TTD as TRC increased was observed. The significance of the species effect, in the presence of the potential interaction, is seen at the TRC = 0.2 mg/L level, where the

mean log TTD for the striped shiner is less than the mean log TTD for the central stoneroller, whereas at the other two TRC levels, very little difference was observed between the two mean log TTD values.

An attempt to collect fish in similar size classes for each test was made; however, in general, central stonerollers were larger than striped shiners (Table 4.3). To test the hypothesis that there was an association between weight and the log TTD, we used an analysis of covariance. We tested for homogeneity of slopes across TRC levels for both species. For each species, no significant difference in the estimated slope of the regression, across the three TRC levels, of log TTD on weight was detected ( $P = 0.85$  for central stonerollers and  $P = 0.14$  for striped shiners). However, for central stonerollers, the association of weight with TTD was not significant ( $P = 0.62$ ) and for striped shiners this association was significant ( $P < 0.0001$ ), where the association of the covariate (weight) was measured by the slope of the regression line of log TTD on weight.

As an additional analysis, the tank effect was also evaluated for each species-TRC combination by comparing the individual regression of log TTD on weight using analysis of covariance. For the central stonerollers, the covariate (weight) over the range considered in the data set was not significantly associated with the TTD over all TRC levels ( $P > 0.3$ ). However, in the TRC = 0.2 mg/L treatment, there was a significant tank effect ( $P < 0.001$ ); whereas, in the TRC = 0.4 and 0.8 mg/L treatments, no significant tank effect was seen ( $P > 0.15$ ). For the striped shiner, the covariate (weight) over each TRC value was significantly positively associated with TTD ( $P < 0.02$ ). At the TRC = 0.2 mg/L treatment, we also saw a significant tank effect ( $P = 0.022$ ). The tank effect was not significant at the TRC = 0.4 and 0.8 mg/L

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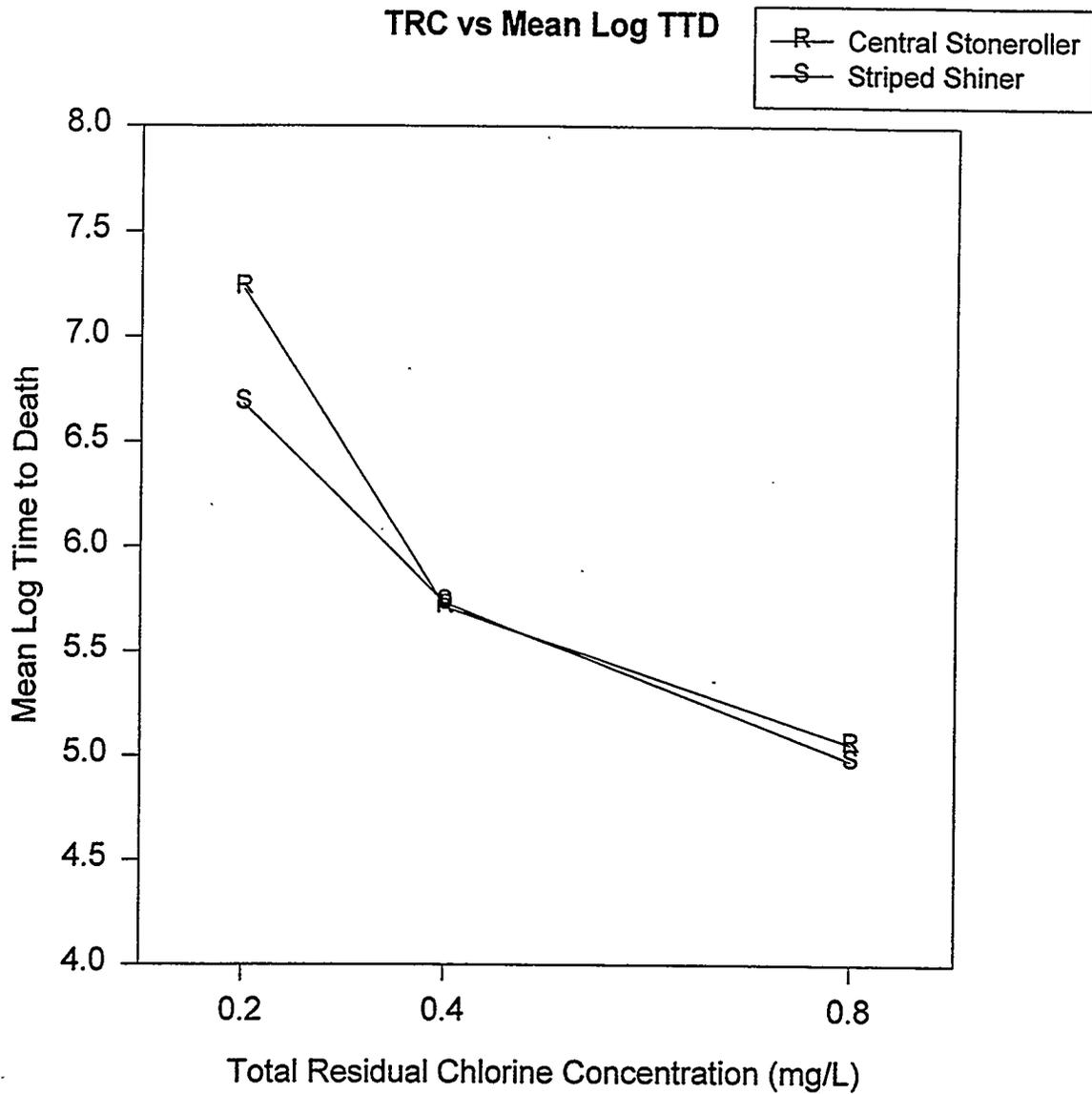


Fig. 4.1. Plot of the mean of the log time to death (TTD) for the central stoneroller (R) and striped shiner (S) at total residual chlorine (TRC) concentrations of 0.2, 0.4, and 0.8 mg/L.

Table 4.3. Mean ( $\pm$ SE) temperature ( $^{\circ}$ C), total residual chlorine (mg TRC/L) (measured hourly) and fish weight (g) in chlorine toxicity tests

Test No.	Temperature ( $^{\circ}$ C)	TRC (mg/L)	Fish weight (g)	
			Central stoneroller	Striped shiner
1	18.89 $\pm$ 0.15	0.77 $\pm$ 0.05	6.89 $\pm$ 0.28	3.01 $\pm$ 0.09
2	19.79 $\pm$ 0.06	0.39 $\pm$ 0.03	7.14 $\pm$ 0.37	3.13 $\pm$ 0.13
3	20.44 $\pm$ 0.05	0.19 $\pm$ 0.01	7.98 $\pm$ 0.36	3.11 $\pm$ 0.13

treatments ( $P > 0.45$ ). The tank effect seen at the 0.2 mg TRC/L treatments for both species did not appear to be related to a single observation. Survival in the control fish was 100% for each test.

#### 4.1.4.3 Conclusions

A significant species-TRC interaction was seen, and was explained by the increased difference in the mean log TTD between the two species at the 0.2 mg/L treatment level. A plot of the data showed that the mean log TTD for the striped shiners was less than that of the central stonerollers at the TRC = 0.2 mg/L level. It is likely, therefore, that some factor other than relative sensitivity to TRC (e.g., behavior, increased sensitivity to fluctuating TRC concentrations, or some other toxicant) causes the greater mortality of central stonerollers in the stream. A test of fish movement away from a chlorine source showed that both species were able to detect and avoid TRC concentrations of  $< 0.07$  mg/L. For striped shiners, the covariate weight was significantly positively associated with TTD. An explanation of the effect of the tank variable at the 0.2 mg TRC/L treatment was not forthcoming from statistical analyses of the data. With further testing, a biological explanation of the effect may emerge. In toxicity tests where

pathogens are suspected to be the cause of mortality to fathead minnows, testing individual fish in separate beakers (e.g., forty beakers with one fish per beaker) reduces overall mortality compared with multiple fish tested in fewer beakers (e.g., four beakers with ten fish per beaker) (L. A. Kszos, Environmental Sciences Division, unpublished data). A similar phenomenon may have occurred in the 0.2 mg/L treatment, where fish lived for several days.

## 4.2 SNAILS

### 4.2.1 In Situ Snail Toxicity Experiments

Pleurocerid (gill breathing) snails are considered key grazers and influential competitors in many streams, where they may reach densities in which they comprise  $>90\%$  of the biomass. The pleurocerid snail *Elimia clavaeformis* is found in the lower reaches of EFPC but is absent from upper EFPC in the vicinity of the Y-12 Plant. This species was used as an in situ monitor of water quality at several sites in EFPC. Tests were conducted before and after installation of dechlorination equipment. Additional tests involving the effect of water quality on the feeding rate of *E. clavaeformis* were conducted in the laboratory.

#### 4.2.1.1 Methods

Snails, *E. clavaeformis*, were collected from an unnamed tributary of EFPC ~100 m upstream of the intersection of Scarboro and Lafayette roads. The snails were placed in cages (10 snails per cage), and sets of cages (four cages per set) were placed at five sites in EFPC (NSP, AS-8, LR-i, EFKs 23.4 and 24.4, [Fig. 1.1]). A reference set of four cages was placed in the tributary from which the snails were collected. Four rounds of tests, each lasting 14 d, were conducted between February and April 1992. Survival among sites was analyzed by ANOVA. Following installation of the dechlorination facilities in December 1992 (see Sect. 5), four additional tests were conducted at three sites (NSP, AS-8, and the reference site) between January and March 1993.

#### 4.2.1.2 Results

Before dechlorination, survival was similar among the four test dates, and test date was not significant in the analysis of variance ( $p = 0.807$ ,  $t$  test,  $\alpha = 0.05$ ). Survival varied among sites ( $p = 0.0001$ ) and ranged from 0% near the NSP on all dates to up to 100% in the reference tributary (Table 4.4, Fig 4.2). After dechlorination, survival was  $\geq 90\%$  at all sites, including the NSP.

#### 4.2.1.3 Conclusions

Prior to installation of the dechlorination equipment, the water at the NSP was toxic to *E. clavaeformis*. The data show that snails are sensitive indicators of water quality differences among sites in upper EFPC. The high survival at the NSP following the installation of dechlorination equipment indicates a distinct improvement of water quality near the NSP.

#### 4.2.2 Feeding of Snails in Relation to Water Quality

Studies were also conducted in the laboratory with *E. clavaeformis* to evaluate the biological quality of water from various sites in EFPC. *Elimia clavaeformis* is a key grazer and competitor in many streams; this species is common and abundant in most of the White Oak Creek (WOC) drainage basin and is found in the lower reaches of EFPC. It is absent from upper EFPC. Past studies (see Hinzman et al. 1995) have shown that snail activity and behavior, including feeding, may be related to water quality. When exposed to toxicants, snails may cease feeding and actively try to avoid contaminated water. Feeding rates of *E. clavaeformis* were used to assess water quality from the inlet and outlet of LR (LR-i and LR-o).

#### 4.2.2.1 Methods

*Elimia clavaeformis* was collected from minimally disturbed areas in upper WOC (near WCK 6.8). Water from the site of snail collection was used as reference water. Grab samples of water were collected daily from LR-i and LR-o for use in the tests.

In the laboratory, snails were placed in 12-L aquaria having a constant flow of dechlorinated tap water; they were fed lettuce and acclimated to a temperature of 25°C and a photoperiod of 16 h light and 8 h dark. At the start of each test, 12 randomly selected snails were placed in a 600-mL beaker containing 250 mL of test water; four replicate beakers were used to assess each treatment. Water was replaced daily. Three green lettuce leaf discs (3 cm<sup>2</sup>) were supplied as food each day. Initial and final wet weights of the discs were recorded daily. Feeding rate (expressed as mean grams of wet weight of lettuce eaten) of the snails was used as the response variable.

**Table 4.4. Survival of snails (*Elimia clavaeformis*) in in situ tests at sites in upper East Fork Poplar Creek**

Initiation date	Termination date	Site	Survival <sup>a</sup> (%)
<i>Predechlorination</i>			
02/18/92	03/03/92	Tributary <sup>b</sup>	100
		EFK 23.4	100
		LR-i	100
		EFK 24.4	87
		AS-8	85 <sup>c</sup>
		NSP	0 <sup>c</sup>
03/03/92	03/17/92	Tributary	97.5
		EFK 23.4	90
		LR-i	100
		EFK 24.4	100
		AS-8	82.5 <sup>c</sup>
		NSP	0 <sup>c</sup>
03/17/92	03/31/92	Tributary	100
		EFK 23.4	97.5
		LR-i	90 <sup>c</sup>
		EFK 24.4	97.5
		AS-8	95
		NSP	0 <sup>c</sup>
03/31/92	04/14/92	Tributary	100
		EFK 23.4	92.5 <sup>c</sup>
		LR-i	100
		EFK 24.4	95
		AS-8	97.5
		NSP	0 <sup>c</sup>
<i>Postdechlorination</i>			
01/05/93	01/18/93	Tributary	100
		AS-8	95
		NSP	90
01/19/93	02/01/93	Tributary	94.4
		AS-8	100
		NSP	92.5
02/02/93	02/15/93	Tributary	94.4
		AS-8	100
		NSP	94.4
03/04/93	03/17/93	Tributary	97.7
		AS-8	100
		NSP	100

<sup>a</sup>Based on percentage survival of the animals recovered in each replicate.

<sup>b</sup>Tributary = an unnamed tributary to EFPC, located about 100 m upstream of the intersection of Scarboro and Lafayette roads.

<sup>c</sup>Designates sites in which survival was significantly different from the reference tributary ( $p = 0.0001$ ).

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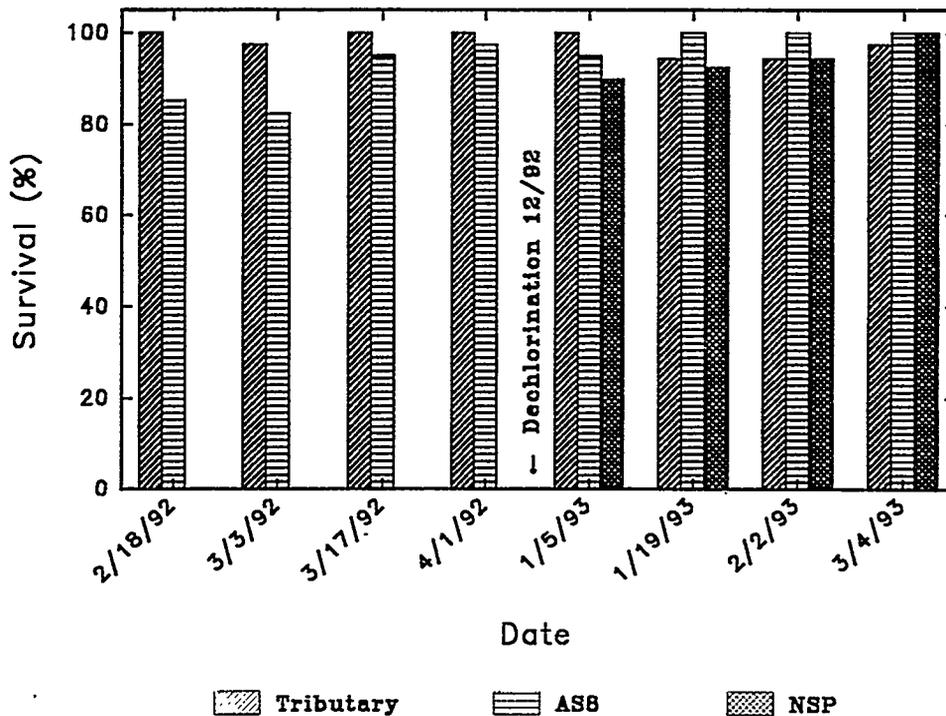


Fig. 4.2. Snail survival in East Fork Poplar Creek before and after dechlorination. There was 0% survival at NSP before dechlorination;  $\geq 90\%$  survival after dechlorination.

The data were analyzed by using SAS GLM (SAS 1985b).

A series of 3-d tests was conducted to (1) evaluate the toxicity of water from LR-o and (2) identify the component(s) of the water that caused the toxicity. Whenever possible, the tests were conducted concurrently with toxicity tests performed with fathead minnow larvae (*P. promelas*) and daphnids (*C. dubia*).

The water samples from LR-i and LR-o were treated in various ways, depending on the objectives of the particular tests. The treatment combinations that were evaluated follow:

1. Whole (= nontreated) water from LR-i and LR-o.
2. Whole water from LR-i and LR-o plus
  - (a) LR-o water filtered through a 0.7- $\mu\text{m}$  pore size filter, (b) LR-o water treated with 1 ppm EDTA, and (c) LR-o water that had been aerated for 1 h.
3. Whole LR-i and LR-o water; LR-o water diluted to 75, 50, and 25% of full-strength with 20% diluted mineral water; and LR-o water and WCK 6.8 water filtered through a 0.7- $\mu\text{m}$  filter.
4. Whole LR-o water, LR-o water filtered through 53-, 26-, 8-, and 0.7- $\mu\text{m}$  filters, in which water was passed through each of the filter(s) with larger pore size(s) before being filtered through the smallest pore-size filter.
5. Particles in LR-o water were counted, and their sizes measured to obtain the water's particle-size distribution. The sample was preserved in 2% Lugol's

solution. A test was then conducted in which chemically inert polymer microbeads of defined sizes (66  $\mu\text{L}$  of 1.03- $\mu\text{m}$  bead-diameter solution, 21  $\mu\text{L}$  of 3.4- $\mu\text{m}$  bead-diameter solution, and 23  $\mu\text{L}$  of 6.4- $\mu\text{m}$  bead diameter solution in 1 L of water) were used. This mixture of beads mimicked the size distribution of particles present in the LR-o water sample. In this test, the snails were exposed either to whole LR-o water, LR-o water filtered through a 0.7- $\mu\text{m}$  pore-size filter, filtered LR-o water spiked with the bead mixture, or filtered WCK 6.8 water spiked with the bead mixture.

6. Thiourea (in the same molar concentration as was used for the EDTA test), a chelator that binds preferentially to Hg, was added to LR-o water that had been pH adjusted (pH dropped to about 4.5 with HCl, then raised back to about 7.2 with NaOH), filtered, or some combination of pH adjustment and filtration. Snails were exposed to whole WCK 6.8 water and LR-o water; WCK 6.8 water (pH adjusted); LR-o water (pH adjusted); LR-o water in which the pH was lowered to 4.5 before it was filtered through a 0.7  $\mu\text{m}$  pore-size filter and thiourea was added after which the pH was readjusted to 7.2; WCK 6.8 water plus thiourea; LR-o water in which the pH was adjusted to 4.5 before the thiourea was added and the pH was adjusted back to 7.2; and LR-o water in which the pH was adjusted to 4.5 before it was filtered and thiourea was added and the pH readjusted back to 7.2.

Finally, the water was analyzed chemically in an attempt to identify the material(s) in LR-o water that inhibited feeding of *E. clavaeformis*. Two liters of nontreated LR-o water was centrifuged at 1000 rpm for 15 min, and the supernatant was decanted. The particle-laden water was

centrifuged again to reduce the total particle-laden volume to 150 mL; this was then separated into three 50-mL subsamples. The same procedure was used for treating 2 L of water from LR-o, after the water had been pH adjusted to 4.3. One 50-mL sample of deionized distilled water was passed through a 0.7- $\mu\text{m}$ -pore-size filter. Each 50-mL sample was then preserved with 100  $\mu\text{L}$  of Ultrex nitric acid and refrigerated until shipped to the University of Georgia for analysis of metals by inductively coupled plasma (ICP) spectroscopy.

#### 4.2.2.2 Results

The outcomes of the snail feeding tests in relation to the various treatments are summarized in Table 4.5. A brief discussion of the results of each test follows.

1. The snail feeding rate (mean for four replicates, summed over the 3-d test period, grams of wet weight eaten) in LR-i water was not significantly different from controls, but the feeding rate of the snails in the water from LR-o was reduced by more than 50%.
2. The feeding rates of the snails in WCK 6.8 water, LR-i water, and filtered LR-o water (0.7- $\mu\text{m}$  pore-size) were similar to one another. However, snails exposed to whole LR-o water, LR-o water that had been sparged with air, or LR-o water that had been treated with EDTA had low feeding rates.
3. The feeding rates of the snails exposed to LR-i water or to filtered (0.7- $\mu\text{m}$  pore-size) LR-o water were greater than those of snails in the reference water. The feeding rate of the snails in filtered water from WCK 6.8 was not significantly different from that of reference snails or snails in LR-i water or in filtered LR-o water. Snails in the

Table 4.5. Comparison of feeding rates of the snail (*Elimia clavaeformis*) in different water sources and treatments

Test No. <sup>a</sup>	Water source/treatment	Mean wet wt. eaten <sup>b</sup> (g)	SE	SAS GLM group <sup>c</sup>
1	WCK <sup>d</sup>	0.4118	0.021	A
	LR-i	0.3917	0.027	A
	LR-o	0.1554	0.007	B
2	WCK	0.4659	0.010	A
	LR-i	0.4578	0.032	A
	LR-o-Filter	0.4723	0.035	A
	LR-o	0.2641	0.019	B
	LR-o-Air	0.2086	0.028	B
	LR-o-EDTA	0.1980	0.028	B
	WCK	0.2963	0.029	B
3	WCK-Filter	0.3521	0.022	AB
	LR-i	0.3634	0.016	A
	LR-o-Filter	0.3636	0.030	A
	LR-o-25%	0.1858	0.028	C
	LR-o-50%	0.1300	0.011	CD
	LR-o-75%	0.1235	0.009	CD
	LR-o-100%	0.0731	0.023	D
	WCK	0.4492	0.048	A
4	LR-o-0.7µm	0.4146	0.029	A
	LR-o-8.0µm	0.2666	0.028	B
	LR-o-26.0µm	0.2518	0.025	B
	LR-o-53.0µm	0.2719	0.004	B
	LR-o-100%	0.2813	0.038	B
	WCK	0.4867	0.032	A
5	LR-o-Filter-Beads	0.4902	0.023	A
	LR-o-Filter	0.4795	0.014	A
	WCK-Filter-Beads	0.4479	0.027	A
	LR-o-100%	0.2989	0.019	B
	WCK	0.1804	0.004	A
6	WCK-pH	0.1788	0.008	AB
	WCK-Thiourea	0.1706	0.009	AB
	WCK	0.1684	0.007	ABC
	LR-o-pH-Filter	0.1656	0.005	ABC
	LR-o-Thiourea	0.1654	0.009	ABC
	LR-o-pH-Filter-Thiourea	0.1576	0.011	BCD
	LR-o-pH-Thiourea	0.1481	0.014	CD
	LR-o-100%	0.1397	0.004	D

<sup>a</sup>Test No. corresponds to water sample/treatment groups described in Sect. 4.2.2.1.

<sup>b</sup>Data for tests 1—5 are the mean wet weights eaten (g) summed over the three day test period; test 6 is the mean wet weight eaten per day.

<sup>c</sup>SAS GLM groups with the same letter are not significantly different (T test,  $p = 0.05$ ).

<sup>d</sup>WCK 6.8 is the reference site.

100, 75, 50, and 25% concentrations of LR-o water ate significantly less than the snails in the reference water; a dilution effect on feeding rate for the snails in the LR-o dilution series was evident.

4. Only the feeding rate of the snails exposed to water filtered through a final pore size of 0.7  $\mu\text{m}$  was not significantly lower than the feeding rate of snails in the reference water. Thus, the particles that were inhibitory to snail feeding were in the 0.7- to 8- $\mu\text{m}$  size range.
5. About 91% of the particles in the LR-o water sample were  $<8 \mu\text{m}$  in diameter, with a peak abundance occurring in the range of 1.5 to 3.75  $\mu\text{m}$ . The feeding rate of the snails exposed to the polystyrene bead mixture in water from WCK 6.8 or LR-o water did not differ significantly from the feeding rate of the snails in the reference water.
6. The mean wet weights of lettuce eaten for this test were summed over a 2-d test period (days one and three); on day two, the lettuce disks for three treatment combinations (the LR-o plus thiourea, LR-o plus pH treatment plus thiourea, and LR-o plus pH treatment plus filter treatment plus thiourea) were accidentally lost. The pH adjustment of LR water elevated the feeding rate back to that of the controls.

The ICP analyses did not reveal differences in concentrations of metals between treatments that could explain the toxic nature of the particles in LR-o water.

#### 4.2.2.3 Conclusions

The series of tests showed that the agent responsible for lowering the feeding rate of *E. clavaeformis* in LR-o water was associated with particles  $>0.7 \mu\text{m}$ , but

$<8 \mu\text{m}$  in diameter. The agent could be neutralized by pH adjustment or removed by filtration (through a 0.7- $\mu\text{m}$  pore-sized filter). Although the specific toxicant(s) in LR-o water that inhibited the feeding rate of *E. clavaeformis* was not identified, it is clear that the test method can be used to detect sublethal conditions that could influence the long-term survival of *E. clavaeformis*. Since its application to EFPC, the snail-feeding bioassay has been used successfully to assess water quality in Bear Creek and in stormwater runoff at ORNL. In these applications, the test was at least as sensitive as 7-d chronic toxicity tests with *C. dubia* (R. Hinzman, ESD/ORNL, unpublished data). Reference toxicant tests are currently under way to compare directly the sensitivity of the *E. clavaeformis* feeding-test endpoint to the most sensitive endpoints of other tests (e.g., *C. dubia* reproduction and *P. promelas* growth) commonly used for effluent and ambient toxicity assessments.

## 4.3 CLAMS

### 4.3.1 In Situ Clam Toxicity Investigations

From 1989 through 1991, in situ bioassays were conducted in EFPC, using the fingernail clam, *Sphaerium fabale*, as the test organism. In all 3 years, clams were placed at two sites in EFPC (EFKs 23.4 and 13.8) and in several relatively undisturbed reference streams, BF (BFK 7.6) and Hinds Creek (HCK 20.6). In 1989 and 1991, one site in Bull Run Creek (BRK 20.0) was used as an additional reference station; in 1990 and 1991, a reference site in Cox Creek, CXK 0.26 was used; and in 1990, an additional site in upper EFPC (EFK 24.4), above LR, was used. The sites on upper EFPC are shown in Fig. 1.1. Sites on lower EFPC and BF are shown in Fig. 1.2. Clams

used in the 1989 study were obtained from BF and those used in the 1990 and 1991 studies were obtained from Beaver Creek. The clams were monitored for survival and growth over time periods of 110 to 150 d, except for the fall 1990 study, which lasted ~75 d.

#### 4.3.1.1 Methods

For studies in 1989 and 1990, individually marked clams ranging in length from ~7.2 to 9.0 mm were placed into 20 cm by 25 cm white plastic photographic trays partially filled with fine gravel and covered with a piece of nitex netting (mesh size of 2 mm). Four trays, each containing 15 uniquely numbered clams (total of 60 clams per site), were anchored securely to the streambed with wire and rebar. For the 1991 study, individually marked clams ranging in length from ~9.3 to 10.9 mm were placed into individual clear, plexiglass chambers covered with netting on the ends (2-mm mesh on downstream end and 1-mm mesh on upstream end) and filled one-third full of fine gravel. Eight chambers, each containing one clam were attached side-by-side with panduit cable ties to each of four flat pieces of plexiglass (total of 32 clams/site). The plexiglass plates were anchored securely at each site with rebar and wire. At intervals of 2 (1991) to ~3 (1989 and 1990) weeks, clams were retrieved and checked for mortality; the length of live clams was measured and they were returned to their original containers.

#### 4.3.1.2 Results

Survival of clams in the reference streams and at EFK 13.8 just above the City of Oak Ridge Wastewater Treatment Facility was similar in all 3 years (Fig. 4.3). At EFK 24.4 (above LR), mortality was relatively rapid during the summer of 1990,

with all clams dead after ~60 d. When the study was repeated in the fall of 1990, similar results were obtained (i.e., 100% mortality at EFK 24.4 and >90% survival at all other sites at 75 d). Survival of clams at EFK 23.4, just below LR, was not consistent among years. In both 1989 and 1991, survival was significantly reduced ( $p < 0.05$ ) during the first 4 weeks. Thereafter the rate of mortality at this site was comparable to the reference site. In 1990, however, clam survival at EFK 23.4 was high in both the summer and fall studies and similar to survival at EFK 13.8 and the reference sites ( $p > 0.05$ ).

With one exception (fall of 1990), growth of the clams at all EFPC sites, as measured by increases in shell length, was significantly less ( $p < 0.05$ ) than that at all reference sites (Fig. 4.4). At EFK 24.4, clams did not grow during their brief survival periods in either the summer or fall 1990 studies. At EFKs 13.8 and 23.4 growth was very similar in all but the fall 1990 study. In the 1989, summer 1990, and 1991 studies, clams at EFKs 13.8 and 23.4 grew steadily for about the first 35 to 40 d of exposure but grew very little during the remainder of the study. In contrast, clams in the reference streams in all studies tended to grow steadily throughout each study. In the fall 1990 study, clams had grown significantly more ( $p < 0.05$ ) at EFK 23.4 than at EFK 13.8 by the end of the study. Interestingly, the amount they had grown at EFK 23.4 by the end of the study was significantly greater ( $p < 0.05$ ) than at CXK, the source stream of the clams.

#### 4.3.1.3 Conclusions

Similar patterns in growth of clams between years at EFKs 13.8 and 23.4 suggest that the factors controlling growth of this species at these sites remained relatively unchanged over the 3-year period. It is not known if the general suppression of

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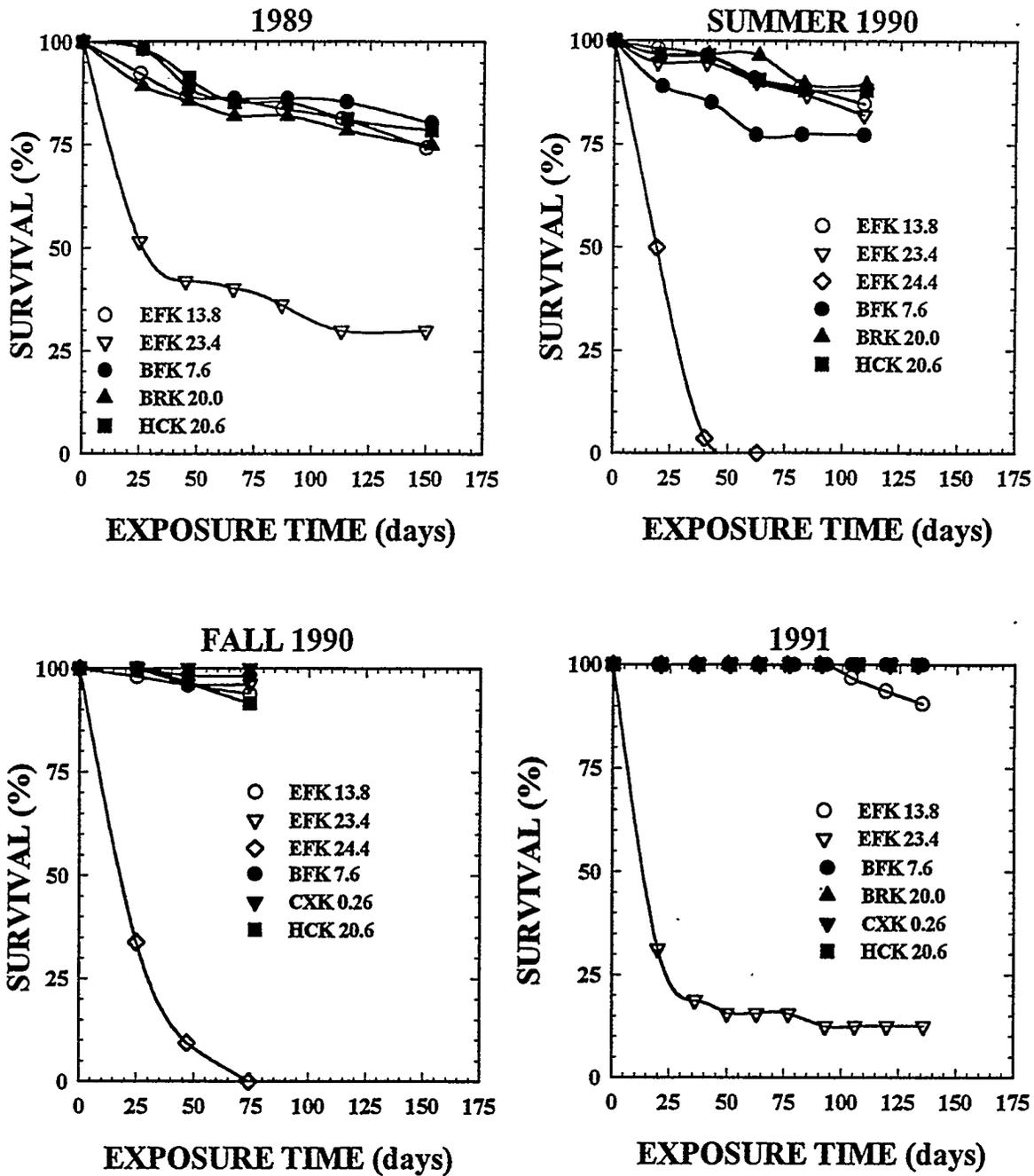


Fig. 4.3. Survival of sphaeriid clams (*Sphaerium fabale*) placed in situ at East Fork Poplar Creek sites and off-site reference streams, Cox Creek, Brushy Fork, Bull Run Creek, and Hinds Creek, 1989-1991. EFK = East Fork Poplar Creek kilometer; CXK = Cox Creek kilometer; BFK = Brushy Fork kilometer; BRK = Bull Run Creek kilometer; HCK = Hinds Creek kilometer.

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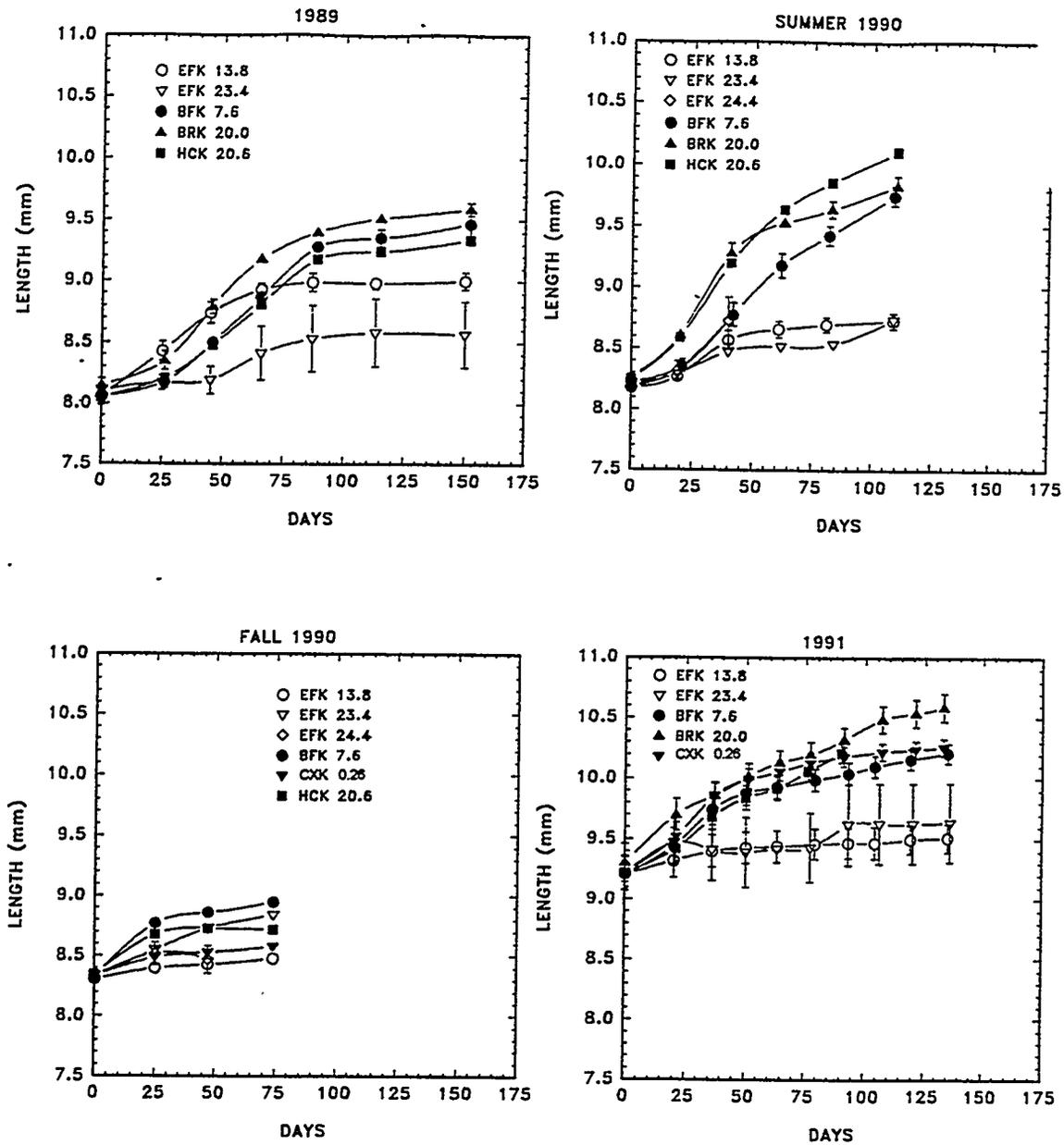


Fig. 4.4. Growth of sphaeriid clams (*Sphaerium fabale*) placed in situ at East Fork Poplar Creek sites and off-site reference streams, Cox Creek, Brushy Fork, Bull Run Creek, and Hinds Creek, 1989–1991. EFK = East Fork Poplar Creek kilometer; CXK = Cox Creek kilometer; BFK = Brushy Fork kilometer; BRK = Bull Run Creek kilometer; HCK = Hinds Creek kilometer.

growth was caused by some unnatural factor (e.g., industrial effluents), altered thermal regimes, nonpoint-source pollution (such as urban and agricultural runoff), or natural factors (e.g., insufficient quantities or quality of food). At EFK 23.4, the consistently low growth rate combined with occasionally high initial mortality followed by periods of minimal mortality suggests (1) the presence of a low-level chronic stress (either unnatural, natural, or a combination of both) and (2) the occurrence of infrequent periods when upper EFPC may be acutely toxic. The high mortality of clams at EFK 24.4 in the 1990 studies suggests that this site was acutely toxic to this species.

#### 4.3.2 Valve Movement of Clams in Response to Chlorine

Results of laboratory studies on responses of bivalves to exposure to contaminants including chlorine show that bivalves stop siphoning activity and close their valves (Hinzman et al. 1995). Valve closure is a response to stressful conditions and decreases the exposure time of tissues to potentially toxic substances. These laboratory studies suggest that valve closure in bivalves could be used to detect the onset of stressful conditions in the field. The study reported here is an attempt to apply the laboratory-tested principle of valve-movement monitoring in a field situation where only a minimal control of water conditions is possible. In the study, the valve movement response of the freshwater Asiatic clam, *Corbicula fluminea*, to fluctuating, low-level concentrations of chlorine was evaluated as a monitoring assay of chlorine levels in upper EFPC. The study compared valve closure behavior of clams exposed to untreated stream water to that of clams exposed to dechlorinated stream water; daily changes in valve movement were also noted.

#### 4.3.2.1 Methods

Clams were collected from Little Sewee Creek, Meigs County, Tennessee, during March 1991, at which time the stream temperature was 18°C. Clams were placed in a holding tank and acclimated to the EFPC temperature of 16°C over a 2-d period before beginning the experiment. Four large clams (23.6 to 25.6 mm shell length), were chosen as experimental subjects. The clams were not artificially fed during the experimental exposure period.

Experiments were conducted streamside in flow-through tanks housed in a mobile biomonitoring laboratory. During two monitoring periods, March 13–April 1, 1991 and April 1–19, 1991, pairs of clams were exposed to either untreated or dechlorinated water from EFPC. Water from upper EFPC was pumped into two 80-L experimental tanks (46 × 66 × 38 cm deep) at a rate of 5 L/min. One tank received unmodified water, and the other received water pretreated with sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) at a concentration of 0.22 mg/L, which was sufficient to reduce the TRC (up to 0.25 mg/L) to chloride. The TRC of the source water was measured amperometrically by a chlorine analyzer (Delta 925 TRC analyzer/transmitter) and recorded on a computer-linked data acquisition system or on a data-logger (Licor LI-1000). Clams were exposed to muted ambient light from windows in the mobile laboratory. Records of solar radiation intensity were available for sites nearby and were used to determine light and dark hours during the experiment.

Two clams were placed in each tank and monitored with an automated valve-movement monitoring apparatus (Fig. 4.5). Four monitoring units were connected to an interface that powered the sensors and sent the sensor information to the data acquisition equipment. During the first 18-d monitoring period, clams 1 and 2 were exposed to untreated water and clams

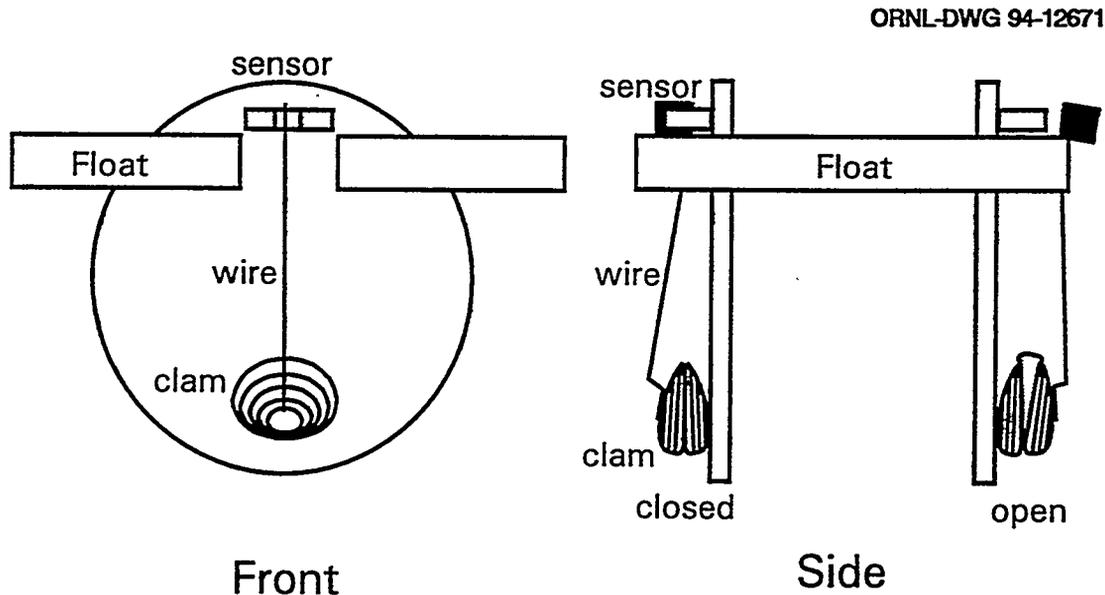


Fig. 4.5. Front and side view of one clam valve-movement monitoring unit. Side view illustrates valve movement and tripping of sensor.

3 and 4 were exposed to dechlorinated water. During the second 18-d monitoring period, the units were switched with the opposite tank so that clams 1 and 2 were exposed to dechlorinated water and clams 3 and 4 were exposed to untreated water. Mean TRC concentrations and closure status were recorded for 15-min intervals. Valve closure of clams exposed to untreated water was compared with that of clams exposed to dechlorinated water. The daily cycle of valve movement was also recorded.

Closure was expressed as the number of 15-min intervals during which an individual clam was recorded as closed divided by the total number of intervals monitored in each period. Differences in proportion of intervals closed between individuals were analyzed by means of a test for comparison of proportions in independent samples. The comparisons yield a  $z$  statistic that is used to determine a  $p$  value based on area of the normal curve.

For the purposes of this study, differences that produce  $p < 0.01$  were considered statistically significant. Closure differences between individuals exposed to different treatments were compared by means of a one-tailed test because dechlorination was expected to reduce closure. For comparison of individuals in the same treatment, a two-tailed test was used. Differences in daily valve closure timing in the two water types and the two monitoring periods were compared graphically.

#### 4.3.2.2 Results

Total residual chlorine concentrations in stream water exhibited a pronounced diurnal cycle. Data from the second monitoring period were used to construct a representative 24-h cycle by computing a mean TRC concentration for each 15-min interval of the day (Fig. 4.6). Mean TRC

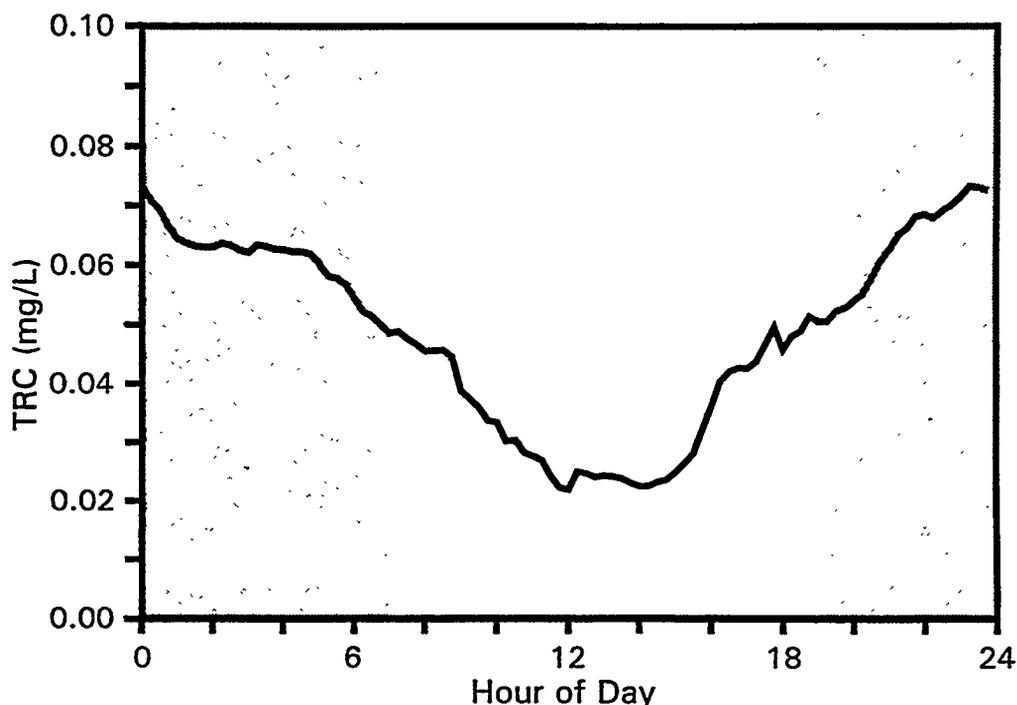


Fig. 4.6. Representative TRC cycle. Each data point represents a mean TRC concentration of each 15-min interval of the day computed for all data in the second monitoring period. Dark hours are indicated by shading; light hours are unshaded.

concentrations ranged between 0.02 mg/L in early afternoon to 0.07 mg/L during the night. The daily cycle was disrupted by rainfall and power outages but returned after conditions stabilized; valve closure data and TRC measurements for periods when flow to the exposure tanks was interrupted were discarded. Because the wire attached to clam 2 was lost, valve movements of this individual were not recorded during the second monitoring period. No mortality occurred in the monitored clams during exposure to untreated water or for the 2 months after the experiment when the clams were kept in dechlorinated tap water. The monitoring apparatus did not appear to impede valve opening and closing.

The proportion of monitored intervals during which individuals exposed to untreated water were closed was >0.55, but no individual exposed to dechlorinated

water was closed >0.35 of the intervals. In untreated water, the mean proportion of intervals closed was >0.70; in dechlorinated water the mean proportion of intervals closed was <0.30. Statistical comparison of the proportions produced a  $z$  statistic and corresponding  $p$  illustrated in Table 4.6. The high number of intervals monitored ( $n > 1100$  in each period) resulted in all but one comparison being statistically significant and  $p$  values too low to calculate exactly. In this case, however, statistical significance may not indicate that the differences are significant in a practical or biological sense. A ranking of comparisons by value of  $z$  provides a more practical assessment of which experimental factors were associated with the greatest differences. For both periods, the comparisons of clams in the same water type produced the lowest  $z$  values, as is shown in the rank order

Table 4.6. Comparison of individual valve closure proportions for *Corbicula fluminea*

Comparison/ treatment <sup>a</sup>	Proportion closure	z value	p value	Rank order
<i>Period 1<sup>b</sup></i>				
1 (U) vs 2 (U)	0.556 vs 0.603	2.24	0.025	1
1 (U) vs 3 (D)	0.556 vs 0.113	22.45	<0.000001	7
1 (U) vs 4 (D)	0.556 vs 0.200	17.53	<0.000001	4
2 (U) vs 3 (D)	0.603 vs 0.113	24.44	<0.000001	8
2 (U) vs 4 (D)	0.603 vs 0.200	19.64	<0.000001	5
3 (D) vs 4 (D)	0.113 vs 0.200	5.70	<0.000002	3
<i>Period 2<sup>c</sup></i>				
1 (D) vs 3 (U)	0.339 vs 0.849	25.23	<0.000001	9
1 (D) vs 4 (U)	0.339 vs 0.769	21.04	<0.000001	6
3 (U) vs 4 (U)	0.849 vs 0.769	4.86	<0.000002	2

<sup>a</sup>U = untreated water, D = dechlorinated water.

<sup>b</sup>n = 1146 intervals.

<sup>c</sup>n = 1184 intervals.

column in Table 4.6. In the first period, comparisons of individuals in different water types resulted in z values at least three times as large as comparisons of individuals in the same water type. In the second period, closure values were significantly higher for each treatment type. Comparisons of individuals in different water types resulted in z values at least four times greater than comparison of individuals in the same water type. These values are more striking because comparison of individuals in different water types is a one-tailed test, requiring a smaller z to produce a given p value than the two-tailed test for comparison of individuals in the same water type.

Comparisons of differences between monitoring periods was done on pooled data for both clams in a water type in each period. Comparisons resulted in high z and low p values illustrated in Table 4.7. The highest z values were from comparisons between different water types, with the exception of comparison of the single individual in dechlorinated water during the

second monitoring period versus the two in untreated water in the first period. The relatively low value of z for this comparison is partly the result of a lower value of n and the general increase in closure during the second monitoring period but might also indicate an effect of prior exposure to untreated water.

In the first monitoring period, closure in dechlorinated water was consistently low during day and night, but closure in untreated water remained high except near midday [Fig. 4.7(a)]. During the second period, closure in untreated water exhibited a similar pattern to that in the first period, but closure in dechlorinated water increased at night [Fig. 4.7(b)]. The curves of compiled closure data illustrate the differences between treatments and monitoring periods well, but the raw data reveal even more distinct differences.

Figure 4.8 illustrates a 36-h record of clam movement for one individual exposed to untreated water and one individual exposed to dechlorinated water during the second monitoring period. This selected

**Table 4.7. Comparison of pooled valve closure proportions for *Corbicula Fluminea***

Comparison/ treatment <sup>a</sup>	Proportion closure	z value	p value	Rank order
1 (U) vs 2 (U)	0.579 vs 0.809	17.05	<0.000002	3
1 (U) vs 1 (D)	0.579 vs 0.156	29.71	<0.000001	5
1 (U) vs 2 (D)	0.579 vs 0.339	13.45	<0.000001	2
2 (U) vs 1 (D)	0.809 vs 0.156	44.58	<0.000001	6
2 (U) vs 1 (D)	0.809 vs 0.156	27.75	<0.000001	4
1 (D) vs 2 (D)	0.156 vs 0.339	12.34	<0.000002	1

<sup>a</sup>U = untreated water, D = dechlorinated water. For pooled values  $n$  = sum of  $n$  for all individuals in Table 4.6 that fit pool criteria.

subset of the movement record illustrates the differences in reaction to light of clams in the two water types. The clam in dechlorinated water opened as light increased and closed when light decreased. In contrast, the clam exposed to untreated water opened only for a short time near midday.

#### 4.3.2.3 Conclusions

When exposed to TRC concentrations fluctuating daily in a range that included low but possibly long-term lethal concentrations, *C. fluminea* reduced tissue exposure to TRC by increasing valve closure time. Tissue exposure was further decreased by increasing valve closure during periods when TRC concentrations were highest. Reduction of tissue exposure could decrease the uptake or toxicity of many chemicals. The ability to remain closed is limited by the need to respire and feed, but tissue exposure may be reduced by increasing closure within physiological limits. Though it is difficult to judge the extent to which feeding or respiration were

compromised in this study, the valve closure behavior changes seemed particularly effective in minimizing tissue exposure to TRC.

Though TRC concentrations studied here were low compared with those used in most laboratory investigations or to control fouling, the effect on valve closure was large. Clams exposed to water containing residual chlorine were closed significantly more often than clams exposed to dechlorinated water. Valve closure timing further reduced TRC exposure by isolating tissues during highest concentrations.

Although physical and chemical factors of water quality such as temperature, light, pH, and dissolved oxygen also can affect the periodic activity of bivalves, the source water was the same for both tanks, and thus these factors were assumed to be identical except for dechlorination. The valve closure differences between untreated water and dechlorinated water is assumed to be caused by the presence of TRC because similar compounds that would be chemically reduced by thiosulfate addition were not likely to occur at significant levels in upper EFPC.

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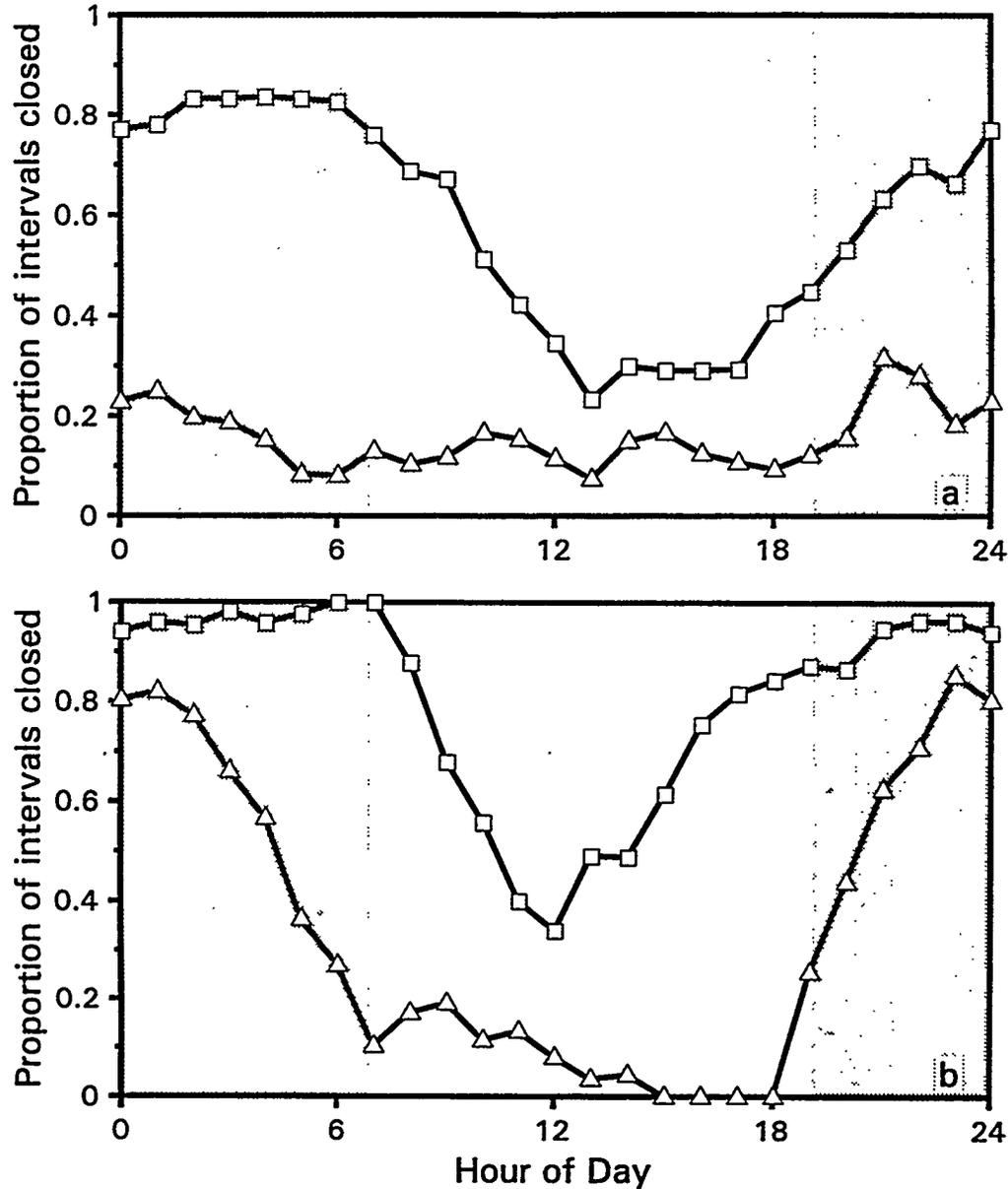


Fig. 4.7. Valve closure of clams in relation to time of day. Panel *a* includes values for the first monitoring period. Panel *b* includes values for the second monitoring period. Points represent proportion of intervals closed for clams pooled within a water type for each 15-min interval of the day. Squares indicate clams exposed to stream water containing chlorine; triangles indicate clams exposed to dechlorinated stream water. Dark hours are indicated by shading; light hours are unshaded. Note that in the second period (panel *b*) the dechlorinated curve is from one individual.

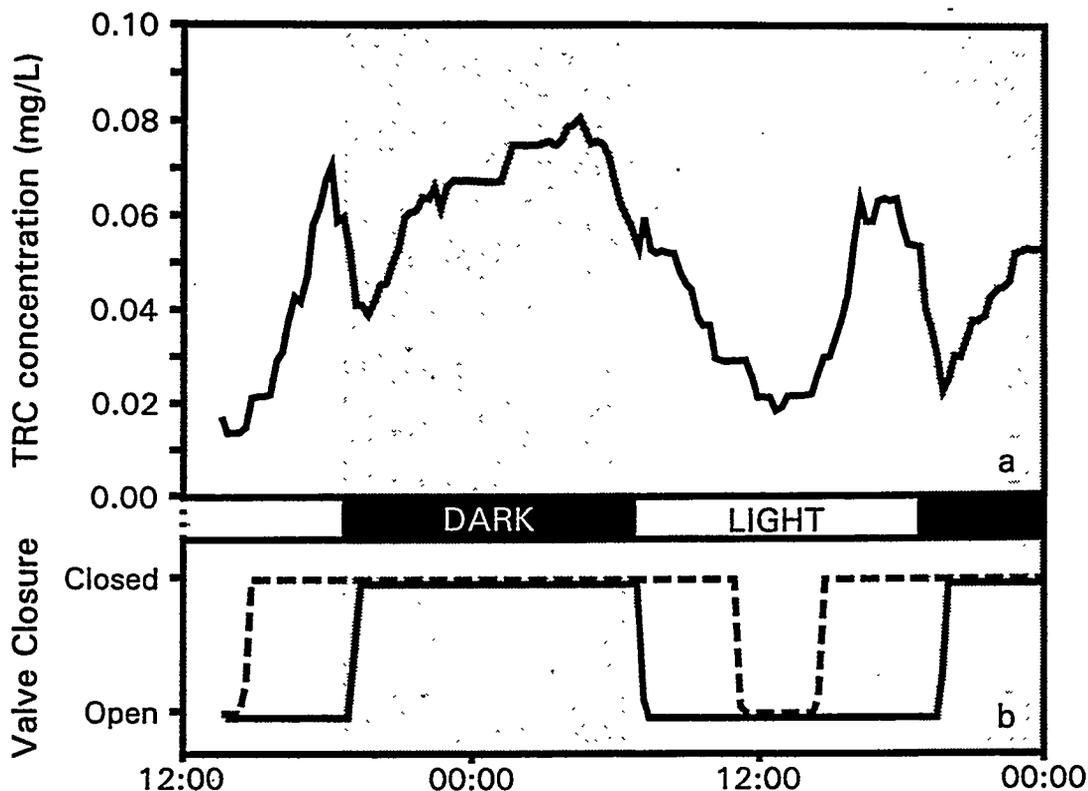


Fig. 4.8. Valve movement and TRC cycle in relation to light and dark periods. Panel *a* shows TRC concentrations during a 36-h portion of the second exposure period. Panel *b* shows the movement of two individual clams during the period. The dashed line indicates values movements of the clam exposed to untreated water; the solid line indicates the movements of the clam in dechlorinated water. Dark hours are indicated by shading; light hours are unshaded.

In summary, *C. fluminea* altered their valve closure behavior in a way that minimized exposure to waterborne residual chlorine. Similar behavioral changes could minimize exposure to many types of stressful conditions. This behavior can be monitored to detect toxic or stressful conditions and in some cases could measure relative severity.

#### 4.4 PERIPHYTON TAXONOMY, LIPID, AND FATTY ACID CONTENT

Periphyton is a complex matrix of algae, bacteria, fungi, and protozoans that attach to submersed surfaces. Because these organisms have rapid growth rates and short

life cycles, they can rapidly change taxonomically or physiologically in response to changing water quality. Taxonomic changes and the lipid content of periphyton were investigated as potential biomonitoring tools to assess water quality.

Lipids are a heterogenous group of biogenic and hydrophobic compounds that consist primarily of carbon and hydrogen; they are metabolically related to fatty acids. Lipids are intimately associated with two main functions: they serve as (1) structural components of cellular membranes and as (2) a compact and concentrated form of storable energy. Changes in the metabolism and composition of various lipid components in EFPC periphyton, as related to water quality, were investigated as a

biomonitoring tool. Changes in lipid and fatty acid composition were also related to taxonomic changes.

#### 4.4.1 Methods

The lipid and fatty acid compositions of periphyton were measured at two sites, EFKs 23.2 and 24.4. These sites are similar with respect to ambient light regimes and nutrient concentrations, but differ in other parameters such as water temperature and TRC. Water temperatures are elevated at EFK 24.4 compared with EFK 23.2. Concentrations of TRC at EFK 24.4 are detectable and often exceed 150  $\mu\text{g/L}$ , whereas concentrations of TRC at EFK 23.2 are below the limit of detection. The periphytic algae at EFK 24.4, located upstream of LR, is characterized by a low taxonomic diversity; EFK 23.2, located about 500 m downstream of LR, has a greater algal diversity (Boston et al. 1991). For the purposes of this study, EFK 23.2 was considered a reference site, and EFK 24.4 was considered an impacted site.

Unglazed ceramic tiles (6.25  $\text{cm}^2$ ) were placed in a reference stream, WOC, at WCK 4.9 (upstream of ORNL effluents), and periphyton were allowed to colonize the surfaces for 2 months. By the end of February 1992, a 0.5- to 1.5-mm-thick biofilm of periphyton was present on the tiles. Equal numbers of the tiles were transferred to EFKs 23.3 and 24.4.

Fifteen tiles were recovered from each of the two EFPC sites after 3, 17, or 36 d and transported to the laboratory for analysis. Four tiles from each site were extracted and analyzed for lipids, and one tile was analyzed for photosynthetic pigments. Other tiles were used for radiotracer uptake studies to determine photosynthetic activity. Additional tiles were used for algal taxonomic assessment. Lipids were extracted into a mixture of chloroform and methanol. Radiolabeled lipids were separated by column chromatography and

analyzed by liquid scintillation spectroscopy. Phospholipids were converted to their fatty acid methyl esters and separated and identified by capillary gas chromatography/mass spectrometry (GC/MS).

Radiolabel studies of the rate of synthesis of cell membrane lipids to stored lipids were used to determine and compare the physiological condition of periphyton at the monitoring sites. Data from the radiolabel incubation experiment were used to obtain a MEM:STO (membrane:storage lipid ratio) value for periphyton from each site-date combination. The MEM:STO ratio, as determined by the radiolabel incubation procedure, provides a nearly instantaneous estimate of the algae's physiological condition, determined by the presence of recently synthesized lipids. Estimates of photosynthetic activity were made from tiles incubated with  $\text{NaH}^{14}\text{CO}_3$ .

In the following discussion, the fatty acid nomenclature follows the form 18:2 $\omega$ 6, where 18 represents the total number of carbon atoms, 2 represents the number of double bonds, and 6 represents the position of the double bond closest to the aliphatic, or  $\omega$ , end of the molecule.

#### 4.4.2 Results

Periphyton on tiles colonized in WOC was dominated by diatoms, including species of *Gomphonema*, *Cymbella*, *Nitzschia*, *Eunotia*, and *Surirella*. A small number of filaments of a green alga (*Stigeoclonium*) were also present (Table 4.8). The taxonomic composition of the periphyton on the tiles changed greatly after they had been incubated in the two EFPC sites for 2 or 4 weeks. The changes involved recolonization of the tiles by more green algae, which gradually replaced the original diatom species. Changes in algal species composition in periphyton on tiles at EFK 24.4 were greater than those that

Table 4.8. Taxonomic composition of periphytic algae in the White Oak Creek (WCK 4.9) and East Fork Poplar Creek colonization sites<sup>a</sup>

Site	February 26, 1992	March 12, 1992	March 31, 1992
WCK 4.9	Diatoms <i>Gomphonema</i> sp. <i>Cymbella</i> sp. <i>Nitzschia</i> sp. <i>Emotia</i> sp. <i>Surirella</i> sp. Green algae <i>Stigeoclonium</i> sp.		
EFK 23.2		Green algae Unidentified unicellular forms Diatoms <i>Achanthes</i> sp. <i>Surirella</i> sp. <i>Gomphonema</i> sp.	Green algae <sup>b</sup> <i>Stigeoclonium</i> (basal cells) Blue-green algae <i>Phormidium</i> sp. Diatoms <i>Nitzschia</i> sp. <i>Achanthes</i> sp. <i>Surirella</i> sp. <i>Navicula</i> sp. <i>Gomphonema</i> sp. <i>Cymbella</i> sp.
EFK 24.4		Green algae <i>Stigeoclonium</i> sp. (basal cells)	Green algae <i>Stigeoclonium</i> sp. (basal cells and short filaments)

<sup>a</sup>Algal taxa are listed according to a semiquantitative estimation of abundance.

<sup>b</sup>Green algae, blue-green algae, and diatoms were present in approximately equal amounts.

occurred at EFK 23.2; at EFK 24.4 diatoms were no longer found after 2 weeks of exposure.

Algal biomass, as measured by chlorophyll *a*, changed little during the 3-week period after the tiles had been transferred to the EFPC sites. However, it increased greatly between March 12 and 31, 1992. Chlorophyll *a* per unit area was slightly greater at the impacted site on the first sampling date after the tiles transfer (February 27, 1992), but was 20 to 30% lower on each of the two later sampling days. A depression in carbon uptake at EFK 24.4 compared with that at EFK 23.2 and the WOC reference site indicated that

the periphyton community was metabolically stressed at the EFK 24.4 site.

At day three, algal biomass (estimated by chlorophyll *a*) in the reference site was similar to that of the corresponding impacted site ( $\sim 10 \mu\text{g}/\text{cm}^2$ ) (Fig. 4.9). At days 17 and 36, chlorophyll *a* concentrations at reference sites were typically greater than those at the impacted sites ( $p = 0.003$  to  $0.005$ ). For example, at day 36, chlorophyll *a* contents of periphyton in EFPC increased to  $37.0 \mu\text{g}/\text{cm}^2$ . Carbon uptake by periphyton in the EFPC reference site was greater than in any of the other reference sites. Carbon uptake rates per unit of chlorophyll *a* in reference sites were

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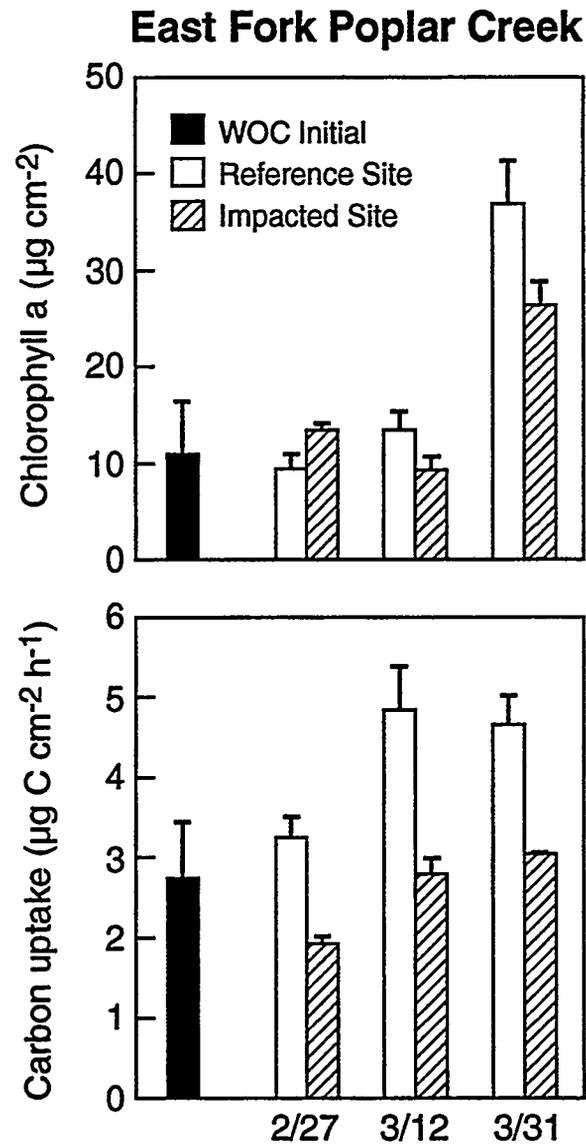


Fig. 4.9. Chlorophyll *a* and carbon uptake by periphyton from reference and impacted sites in White Oak Creek and East Fork Poplar Creek (means  $\pm$ SD,  $n = 3$ ).

greater than in the impacted sites (Fig. 4.10). Chlorophyll-specific carbon uptake rates for the two EFPC sites converged on the later sampling dates, possibly because the periphyton at the impacted site had changed taxonomically to include more stress-tolerant species. The analysis of chlorophyll-specific carbon uptake is complicated by a nonlinear relationship between carbon uptake and periphyton biomass (Boston and Hill 1991). One consequence of this relationship is that carbon uptake per unit chlorophyll tends to be lower for communities that contain larger quantities of chlorophyll per unit area. This situation probably explains why both EFPC sites had low chlorophyll-specific uptake values on March 31. The nonlinear relationship may also have contributed to the smaller-than-expected differences in photosynthesis between the two EFPC sites on March 12.

The lipid profile of the WOC assemblage resembled that reported for various microalgae (Volkman et al. 1989), with ~50% of the lipids consisting of about equal amounts of phospholipids and triglycerols; the remainder included minor amounts of glycolipids, hydrocarbons, sterols, diglycerols and free fatty acids.

The MEM:STO lipid ratios in the reference site of EFPC were typically lower than those in the impacted sites (Fig. 4.11). MEM:STO assessments revealed differences not only between reference and impacted sites, but between sampling dates within each site, as well. Fig. 4.11 compares the changes in MEM:STO values for all sites and sampling dates in EFPC.

Elevated MEM:STO values were previously reported for both the autotrophic and heterotrophic portions of periphyton from impacted sites in EFPC (Guckert et al. 1992). Changes in the MEM:STO ratio may occur as the periphyton switches from producing storage materials to cellular repair, in cases where repair may be needed

because of damage from exposure to toxicants.

Under some conditions, diatoms can produce large quantities of triglycerol (Parrish and Wangersky 1987). Therefore, a complementary interpretation of the high MEM:STO in the impacted sites could be based on the observed changes in the periphyton taxonomic composition (Table 4.8).

Periphyton phospholipids contained fatty acids with a carbon chain length ranging from 14 to 24 (i.e., C14-C24) and containing from zero to six double bonds. More than 50 different fatty acids were routinely separated and identified from their phospholipids by GC/MS. However, only about a dozen of these accounted for 90% of the total on a weight or mol-percent basis (Tables 4.9 and 4.10).

The C18 and C20 polyunsaturated fatty acids pair is a useful biomarker for diatoms and green algae (the two major algal classes present in the periphyton studied here). The fatty acid 20:5 $\omega$ 3 is a well established biomarker for diatoms (e.g., Ackman et al. 1968, Napolitano et al. 1990) and has been used in a number of geochemical (Prahl and Muehlhausen 1989) and food-web studies (reviewed by Sargent and Whittle 1981). Arachidonic acid (20:4 $\omega$ 6) is present in many species of diatom but can also be found in nondiatom algae (Kayama et al. 1990) and heterotrophs. Thus, it is less useful than 20:5 $\omega$ 3 as a diatom marker. The fatty acid composition of green algae, on the other hand, is quite different from that of diatoms. Green algae are phylogenetically related to vascular plants, and their lipids reflect, to a large extent, this phylogeny. Lipids in green algae are rich in fatty acids commonly found in vegetable oils, such as 18:2 $\omega$ 6 (linoleic acid) and 18:3 $\omega$ 3 (linolenic acid). Freshwater green algae in particular contain few fatty acids with more than three double bonds or 18 carbon atoms (Ahlgren et al. 1992).

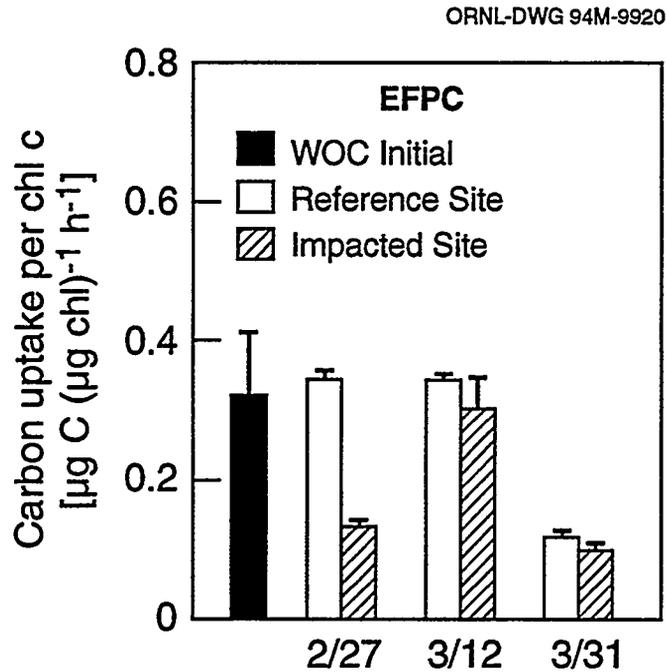


Fig. 4.10 Chlorophyll *a*-specific carbon uptake by periphyton from reference and impacted sites in White Oak Creek and East Fork Poplar Creek (means  $\pm$ SD,  $n = 3$ ).

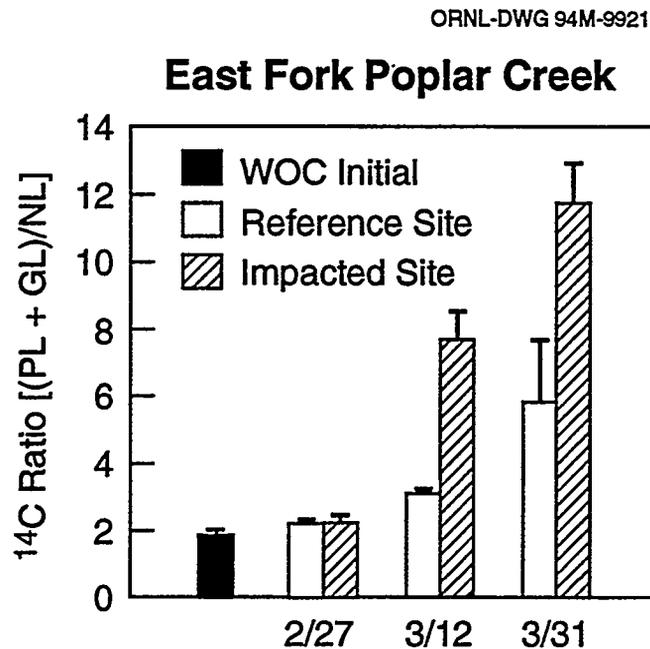


Fig. 4.11. Membrane (phospholipid and glycolipid) to storage (triglycerols) lipid ratios of periphyton from reference and impacted sites in White Oak Creek and East Fork Poplar Creek, as determined by  $^{14}\text{C-NaH}^{14}\text{CO}_2$  incorporation (means  $\pm$ SD,  $n = 3$ ).

**Table 4.9. Major phospholipid fatty acids (mol %) in periphyton from the White Oak Creek colonization site (WCK 4.9) pm February 24, 1992, and after 3 days of constant exposure to water at EFK 23.2 or EFK 24.4 (mean  $\pm$ SD,  $n = 3$ )**

Phospholipid fatty acids	WCK 4.9	EFK 23.2	EFK 24.4
14:0	3.59 $\pm$ 0.09	3.40 $\pm$ 0.41	2.81 $\pm$ 0.18
16:3 $\omega$ 4	1.50 $\pm$ 0.03	1.90 $\pm$ 0.30	1.33 $\pm$ 0.40
16:4 $\omega$ 1	1.62 $\pm$ 0.09	1.61 $\pm$ 0.16	1.24 $\pm$ 0.36
16:1 $\omega$ 7	16.0 $\pm$ 0.33	16.51 $\pm$ 1.40	17.91 $\pm$ 0.62
16:1 $\omega$ 13t	2.29 $\pm$ 0.27	2.44 $\pm$ 0.08	1.86 $\pm$ 0.19
16:0	22.12 $\pm$ 0.57	21.83 $\pm$ 0.81	20.35 $\pm$ 2.06
18:4 $\omega$ 3	2.06 $\pm$ 0.26	1.97 $\pm$ 0.13	1.72 $\pm$ 0.25
18:2 $\omega$ 6	4.82 $\pm$ 0.10	3.62 $\pm$ 0.28	6.09 $\pm$ 0.37
18:3 $\omega$ 3	10.61 $\pm$ 0.40	11.44 $\pm$ 1.06	11.32 $\pm$ 2.52
18:1 $\omega$ 9	3.79 $\pm$ 0.13	4.19 $\pm$ 0.05	4.44 $\pm$ 0.27
18:1 $\omega$ 7	3.99 $\pm$ 0.23	4.33 $\pm$ 0.23	3.90 $\pm$ 0.12
20:4 $\omega$ 6	2.10 $\pm$ 0.09	2.03 $\pm$ 0.16	2.02 $\pm$ 0.38
20:5 $\omega$ 3	14.06 $\pm$ 1.43	11.95 $\pm$ 1.43	13.48 $\pm$ 3.53

**Table 4.10. Major phospholipid fatty acids (mol %) in periphyton after 2 and 4 weeks of exposure at EFK 23.2 and EFK 24.4 in EFPC (mean  $\pm$ SD,  $n = 3$ )<sup>a</sup>**

Phospholipid fatty acids	March 12, 1992		March 31, 1992	
	EFK 23.2	EFK 24.4	EFK 23.2	EFK 24.4
14:0	2.84 $\pm$ 0.18	1.41 $\pm$ 0.04	3.30 $\pm$ 0.63	2.57 $\pm$ 0.26
16:3 $\omega$ 4	2.04 $\pm$ 0.23	2.27 $\pm$ 0.32	1.62 $\pm$ 0.08	1.97 $\pm$ 0.10
16:4 $\omega$ 1	2.33 $\pm$ 0.06	3.13 $\pm$ 0.42	2.73 $\pm$ 0.14	3.94 $\pm$ 0.07
16:1 $\omega$ 7	16.47 $\pm$ 1.02	14.30 $\pm$ 2.8	14.04 $\pm$ 1.65	5.95 $\pm$ 0.23
16:1 $\omega$ 13t	2.09 $\pm$ 0.15	3.84 $\pm$ 0.30	2.52 $\pm$ 0.06	4.04 $\pm$ 0.30
16:0	19.09 $\pm$ 0.13	17.99 $\pm$ 0.62	22.10 $\pm$ 1.50	24.68 $\pm$ 1.01
18:4 $\omega$ 3	2.06 $\pm$ 0.10	1.49 $\pm$ 0.22	1.79 $\pm$ 0.06	2.15 $\pm$ 0.17
18:2 $\omega$ 6	4.65 $\pm$ 0.07	8.15 $\pm$ 0.39	5.17 $\pm$ 0.25	7.21 $\pm$ 0.52
18:3 $\omega$ 3	15.99 $\pm$ 1.55	24.76 $\pm$ 2.99	18.59 $\pm$ 1.15	30.31 $\pm$ 1.61
18:1 $\omega$ 9	3.36 $\pm$ 0.37	5.08 $\pm$ 0.27	2.60 $\pm$ 0.06	3.78 $\pm$ 0.19
18:1 $\omega$ 7	3.63 $\pm$ 0.37	4.08 $\pm$ 0.33	3.10 $\pm$ 0.24	3.59 $\pm$ 0.24
20:4 $\omega$ 6	2.69 $\pm$ 0.15	0.80 $\pm$ 0.11	2.43 $\pm$ 0.35	0.53 $\pm$ 0.08
20:5 $\omega$ 3	11.12 $\pm$ 0.51	3.49 $\pm$ 0.16	9.48 $\pm$ 1.98	1.98 $\pm$ 0.22

<sup>a</sup>Tiles were transferred to the two EFPC sites from WCK 4.9 on February 24, 1992.

Phospholipid fatty acids in WOC periphyton at the start of the experiment showed a balanced fatty acid profile, with similar levels of C18 and C20 polyunsaturated fatty acids. This fatty acid profile remained reasonably intact in the periphyton transferred both to the reference and the impacted sites in EFPC, for tiles recovered 3 d after being moved (Table 4.9). The simultaneous presence of C18 (e.g., 18:2 $\omega$ 6, 18:3 $\omega$ 3) and C20 (e.g., 20:5 $\omega$ 3 and 20:4 $\omega$ 6) polyunsaturated fatty acids in WOC periphyton and periphyton in the two EFPC sites reflected the combined contribution of green algae and diatoms, respectively, to periphyton biomass. Nevertheless, a small increase in the proportion of 18:3 $\omega$ 3 and a corresponding depletion of 20:5 $\omega$ 3 was evident in periphyton from the two EFPC sites, relative to WOC. During the initial round of sampling, no significant differences ( $p > 0.05$ ) were found between the phospholipid fatty acid compositions of the EFPC reference and impacted sites. This finding suggests that both of the EFPC sites were relatively more polluted than WOC at site WCK 4.9, where the tiles were originally placed to be colonized.

Large changes occurred in the periphyton recovered from the two EFPC sites 2 and 4 weeks later. On these sampling dates, the quantities of some important phospholipid fatty acids in the periphyton from the EFK 24.4 impacted site were significantly greater or lesser ( $p < 0.05$ ) than at either the EFK 23.2 reference site or WCK 4.9 (Table 4.10). Linoleic acid (18:2 $\omega$ 6) was present at 4.65% in the EFPC reference site and at 8.15% in the impacted site in samples collected 2 weeks after deployment. This difference in the content of 18:2 $\omega$ 6 was still significant 2 weeks later. Even greater differences were noted in the amount of linolenic acid (18:3 $\omega$ 3). The amounts of 18:3 $\omega$ 3 in the phospholipids from periphyton at the reference and impacted sites were 15.99 and 24.76%,

respectively, for samples recovered on March 12, 1992. After 4 weeks of deployment, the concentration of 18:3 $\omega$ 3 in the reference site was 18.59% and in the impacted site was 30.31%.

Some C20 polyunsaturated fatty acids, such as arachidonic acid (20:4 $\omega$ 6) and especially eicosapentaenoic acid (20:5 $\omega$ 3) showed a trend opposite to that displayed by the C18 polyunsaturated fatty acids. The levels of 20:4 $\omega$ 6 and 20:5 $\omega$ 3, for example, decreased significantly at the impacted site (Table 4.10). The concentrations of 20:5 $\omega$ 3 in the phospholipids of periphyton from WCK 4.9 and EFK 23.2 were 14.06 and 11.12%, respectively. The amount of 20:5 $\omega$ 3 in periphyton from EFK 24.4 decreased to a value as low as 1.98%.

#### 4.4.3 Conclusions

Periphyton on tiles colonized in the reference creek (WOC) was dominated by several diatom species with a few species of green algae. These organisms seem to be sensitive to particular environmental insults, such as the high levels of TRC observed in the EFPC impacted sites. Certain species and morphological types of green algae, such as *Stigeoclonium* sp. basal cells and an unidentified unicellular alga, also seem to be well adapted to streams that contain elevated concentrations of TRC and rapidly (in few days) replace the original algal taxa. The examination of algal lipid components as described in this section provided an insight into the physiological and taxonomic changes that occurred in the periphyton communities in response to waterborne environmental conditions. Such changes can be detected and quantified easily by monitoring lipids and specific fatty acid biomarkers. Following placement in EFKs 23.2 and 24.4, the species assemblage changed from domination by diatoms to domination by green algae, with a greater and more rapid change at EFK 24.4 than at

EFK 23.2. Based on carbon uptake studies, the periphyton community appeared to be metabolically stressed at the EFK 24.4 site.

The examination of algal lipid components provided an insight into the physiological and taxonomic changes that occurred in the periphyton communities in response to waterborne environmental conditions. Changes in lipids reflected taxonomic changes as well as changes in the membrane:storage lipid ratio. The lipid content of periphyton is a potential biomonitoring tool of water quality.

#### 4.5 MICROBIAL ACTIVITIES

A method involving measurement of microbial exoenzyme activity was developed in an attempt to quantify microbial activity in upper EFPC. Cellobiosidase is a microbial exoenzyme that converts cellulose into disaccharides. Cellobiosidase activity was measured at six sites in upper EFPC to document the effect of TRC on microbial activity before dechlorination equipment was installed. Activities will also be measured after dechlorination equipment is in place to document changes that could be attributed to dechlorination of the effluent entering EFPC.

##### 4.5.1 Methods

Instream cellobiosidase activity was used to quantify microbial activities at six sites in upper EFPC (the NSP, AS-8, EFK 24.4, LR-i, LR-o, and EFK 22.8). Groups of six 20-mm disks cut from freshly fallen sycamore (*Platanus occidentalis*) leaves were dried, weighed, and placed in 2-mm mesh leaf disk cages. The marked cages were attached to a piece of rebar pounded into the stream bed; the cages were positioned halfway between the streambed and the surface of the water. The cages

remained in the stream for 7 or 14 d. Several thousand disks were tested in 13 experiments conducted between February 28 and August 22, 1992.

Cellobiosidase activity was measured in the laboratory by the release of *p*-nitrophenol from *p*-nitrophenyl- $\beta$ -D-cellobioside, an analogue of its natural substrate. *p*-Nitrophenol is an intensely-colored chemical that can be detected easily by spectrophotometry. Disks were placed in clean 20-mL glass scintillation vials containing 5 mL of filtered stream water collected from the disk's incubation site. The vials were incubated with a saturating concentration of the substrate in a shaking water bath at a constant temperature of 25°C for 60 min. Filtered water from the vials was measured spectrophotometrically at 410 nm; corrections were made for the absorbance of the filtered stream water and organic matter that had leached from the disks. The disks were then dried (65°C), weighed, ashed, and reweighed to measure organic content. The background-corrected absorbance value and the organic content of each disk were analyzed separately in analyses of variance tests in which site, date, and the interaction between site and date were used as the independent variables.

Seven-day incubation periods were used in the first two experiments; thereafter, each experiment used a 14-d incubation period. The 14-d period allowed more extensive colonization of the leaf disks by microbes and, therefore, yielded a greater rate of cellobiosidase activity.

##### 4.5.2 Results

When data for all sites and dates was combined, there was no significant correlation between cellobiosidase activity and leaf-disk organic content. For five of the six sites, the correlation between cellobiosidase activity and organic content was nonsignificant. At AS-8 the relationship

was negative and statistically significant. This overall weak relationship suggested that microbial activities on the disks were supported by a supply of organic carbon from the water rather than from the disk itself.

A summary of the analyses of variance for cellobiosidase activity and leaf-disk organic content is shown in Table 4.11. Site, date, and the site-date interaction, collectively, explained nearly 76% of the variance in cellobiosidase activity; the influence of site, date, and the site-date interaction term was each highly significant for each factor. Mean cellobiosidase activity at the six sites followed the sequence:

$$\text{LR-i} = \text{EFK 24.4} \gg \text{LR-o} = \text{EFK 22.8} = \text{AS-8} \gg \text{NSP}$$

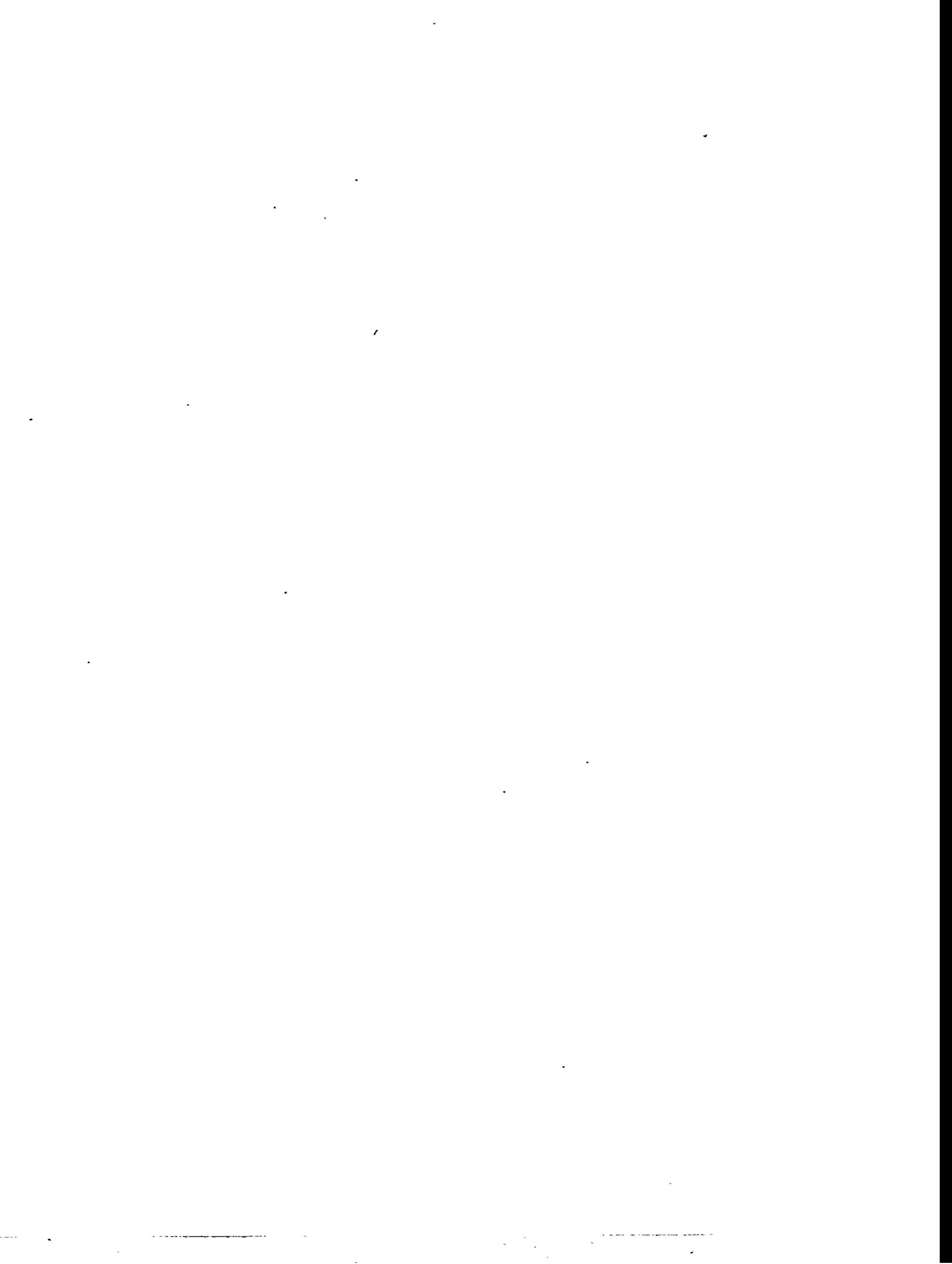
The data also showed a relationship between activity and date; activity was highest during the summer period, late June to early August.

### 4.5.3 Conclusions

Site, date, and the interaction of site and date were related to cellobiosidase activity, whereas, leaf-disk organic content had little relationship to activity. Low cellobiosidase activity at the NSP, five times less than that at LR-i and one-third that at AS-8, has several possible explanations: stream microbes colonized the disks much more slowly at the NSP compared with the other sites; microbial communities colonizing the disks were composed of organisms that produced less cellobiosidase per cell; or the communities produced cellobiosidase that was subsequently deactivated by constituents such as chlorine. Additional tests directed at studying seasonal differences and the affects of stream dechlorination are planned.

**Table 4.11. Analysis of variance results for cellobiosidase activity and organic content of leaf disks at six sites in East Fork Poplar Creek**

Source of variation	Sum of squares	Mean square	Degrees of Freedom	F ratio	Probability of F
<i>Cellobiosidase activity (<math>R^2 = 0.759</math>)</i>					
Site	0.1223	0.024455	5	25.52	0.0001
Date	0.2683	0.026826	10	27.99	0.0001
Site + date	0.1576	0.003581	44	3.74	0.0001
Error	0.1811	0.000958	189		
Total	0.7541		248	10.13	0.0001
<i>Organic carbon content (<math>R^2 = 0.351</math>)</i>					
Site	0.00021	0.000042	5	1.46	0.2044
Date	0.00087	0.000087	10	3.02	0.0014
Site + date	0.00188	0.000043	44	1.48	0.0395
Error	0.00546	0.000029	189		
Total	0.00842		248	1.73	0.0029



## 5. DECHLORINATION PROJECT

Elevated chlorine levels in EFPC have long been known to result from discharges of once-through cooling water and cooling tower blowdown. A dechlorination project was established in 1990 to reduce levels of chlorine. Figure 5.1 shows the results of a feasibility study for residual chlorine reduction in upper EFPC (CDM 1991). Residual chlorine levels were measured on September 5 and 6, 1990, at several sites along EFPC and ranged from about 0.08 to 0.51 mg/L. An estimate was made for expected TRC concentrations in upper EFPC after implementation of the dechlorination systems (see Fig. 5.1).

Two major systems have been designed to treat discharges of chlorine at two NPDES-permitted outfalls, which represent 74% of the total flow of EFPC. In addition, other process discharges are being treated with dechlorinator tablet units. These actions were initiated in anticipation of a new NPDES permit that will establish a chlorine discharge limit for outfalls on EFPC. This new permit is expected to be issued in 1995. TRC monitoring data since the dechlorination systems went on-line (December 1992—June 1993) indicate that TRC levels have been substantially lowered in most portions of upper EFPC. By June 1993, concentrations of TRC were 0.04 to 0.06 mg/L at the NSP, below detection limits at sampling station AS-8, and up to 0.01 mg/L at the inlet and outlet of LR (see Sect. 2.1).

### 5.1 MAJOR TREATMENT SYSTEMS

Two Dechlorination Systems (I and II) were designed in 1991 and placed in operation in late 1992. Figure 5.1 shows the location of the dechlorination systems. Systems I and II use sodium bisulfite (38% solution) to treat chlorine based on observed levels and a desired control level of 20 ppb. System I became operational on November 30, 1992, and System II on December 29, 1992. System I treats the water emerging from Outfall 200 (NSP) and Outfall 135, which together represent the beginning of the "waters of the state" and approximate 67% of the total flow in EFPC. System II treats Outfall 21 and represents about 7% of the total flow in EFPC.

Each System consists of a small prefabricated building (3.66 m by 3.66 m) that contains a small holding tank of sodium bisulfite, metering pumps, electronic controls, a chlorine analyzer, digital and strip chart recorders, and miscellaneous piping, heaters, fans, etc. Bulk tank storage of sodium bisulfite is adjacent to the building. Flow rates at Outfall 135 and Outfall 21 are monitored. Predechlorination levels are monitored and used as a control during the automatic mode in System II, which uses a split system (i.e., the postdechlorination analyzer is remotely located downstream from the main building). The systems can operate in either a manual or automatic mode. In the manual

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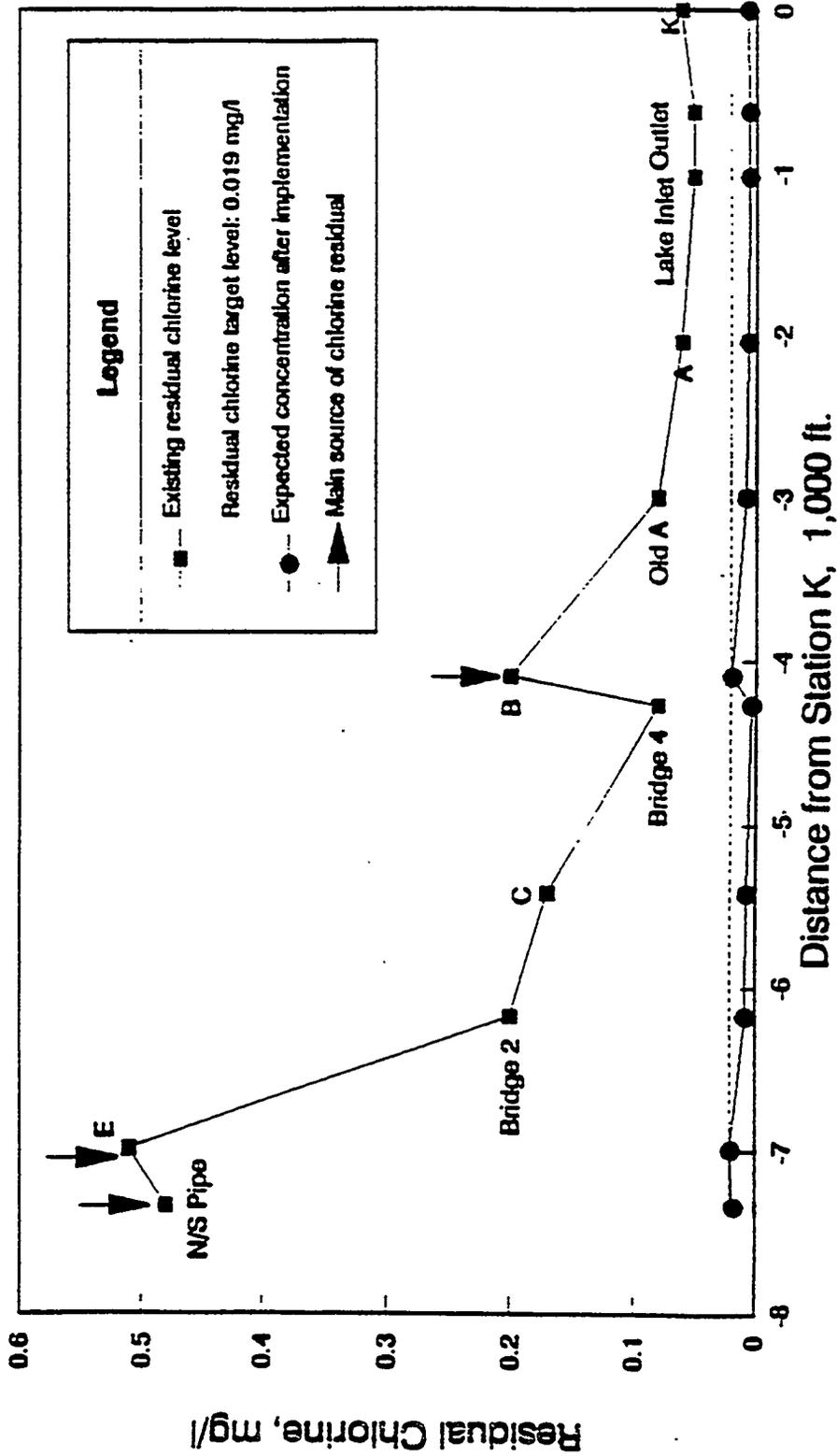


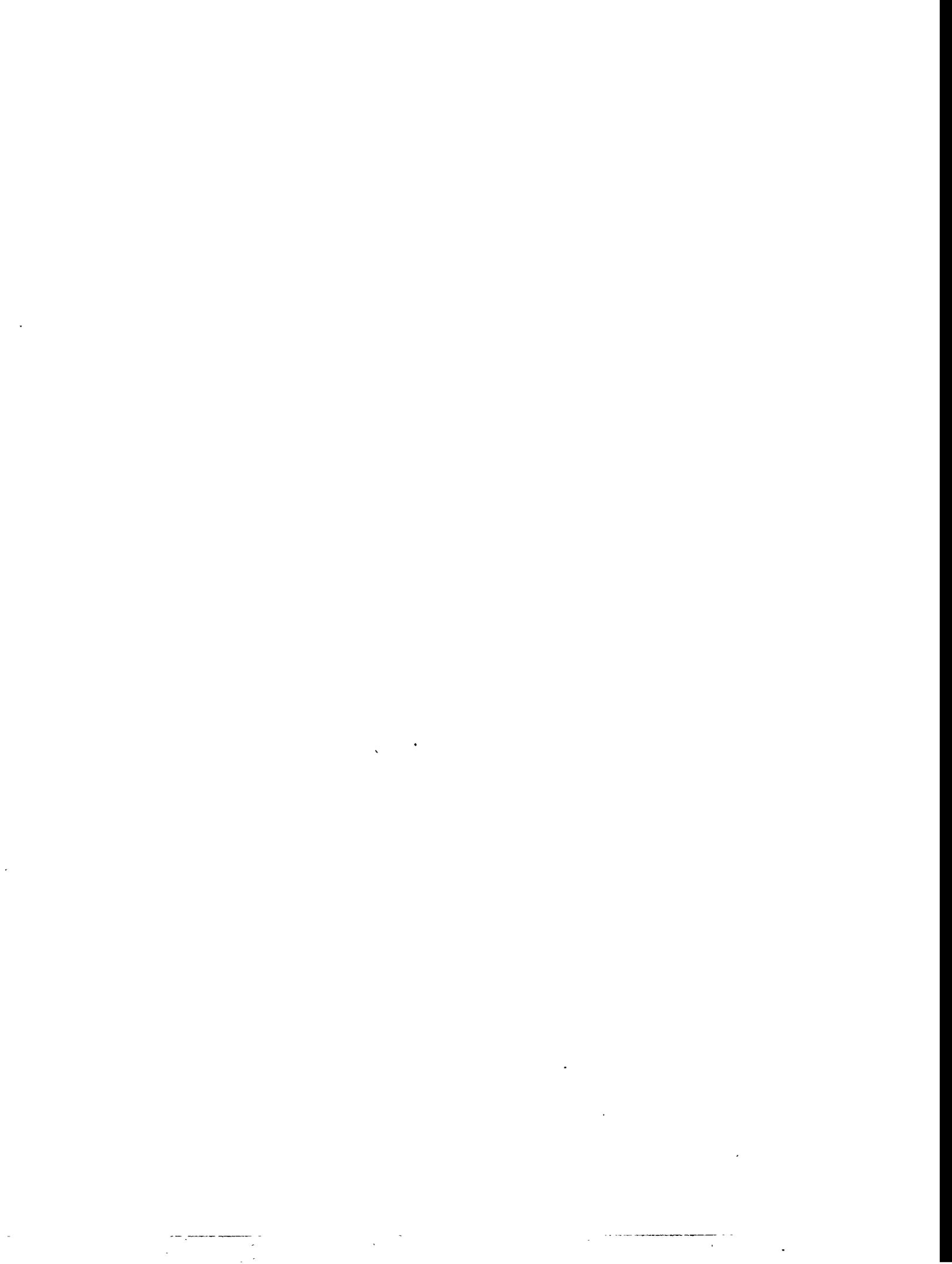
Fig. 5.1. Residual chlorine levels in East Fork Poplar Creek, September 5-6, 1990, and expected concentrations after implementation of dechlorination systems. Sampling stations are as follows: Station K = Station 17; A = the oil-water separator at NHP; old A = behind Bldg. 9720-4; B = the junction box east of 9201-3; Bridge 4 = Third Street bridge, south of Bldg. 9201-3; C = AS-8; Bridge 2 = south of Bldg. 9201-3; E = the bridge behind Bldg. 9204-1 (CDM 1991).

mode, a set amount of sodium bisulfite is discharged to the stream regardless of results of the monitoring data. In the automatic mode, the results of monitoring data (i.e., flow and chlorine levels) impact the amount of sodium bisulfite released to the stream. The systems predominantly operated under the manual mode in 1993; they went on automatic in March 1994.

Chlorine levels have significantly been reduced since Systems I and II began operation. Typical historical chlorine levels at Outfalls 200 and 135 (300 to 800 ppb) are being controlled to 20 ppb, except for some infrequent spiking. Chlorine levels exiting LR (and the Y-12 Plant) are below 50 ppb, considered the lower detection limit by the TDEC for reporting purposes. All values less than the TDEC level of detection are reported as zero. (Note: typical cooling water supplied to the Y-12 Plant from public water supplies has chlorine levels of 1400 to 1600 ppb).

## **5.2 INDIVIDUAL TREATMENT PROCESSES**

Individual processes that discharge significant quantities of cooling water and do not discharge through System I or II are being modified with tablet dechlorinators. These small units use compressed tablets of sodium bisulfate for treatment and are controlled based on flow rate. Thirty such units are planned for installation in late 1993 and early 1994. Four were installed in September 1993; additional units will be installed on significant cooling water sources as they become operational to ensure outfall compliance.



## 6. ACTIONS TO BE PERFORMED AND FUTURE STUDIES

The Y-12 Plant is developing plans for the development and implementation of non-point-source pollution control. An area source sampling plan will be prepared for execution in FY 1994. In support of the Y-12 Plant pollution control plan, the BMAP will continue to monitor ambient toxicity of EFPC and to conduct field and laboratory studies. Non-BMAP studies will focus on specific factors associated with fish kills. In addition, colonization studies initiated to determine the effects of dechlorination on the benthic macroinvertebrate community of EFPC will be completed.

### 6.1 NON-POINT-SOURCE POLLUTION CONTROL

The 1985 NPDES permit for the Y-12 Plant included requirements for the development and implementation of a Best Management Practices (BMP) Plan. An explicit requirement of the BMP Plan is to update the Y-12 Plant Area Source Management Control Proposal entitled "Evaluation and Interim Management of Area Source Discharges to East Fork Poplar Creek (EFPC)," dated February 15, 1984. Storm water event monitoring was conducted in 1988-89 and 1991-92 for the purpose of identifying contaminants and their concentrations. These measurement events were conducted at various outfalls on EFPC, and the list of chemical analytes was extensive.

These data have been analyzed, and two reports issued: *Feasibility Study of Best Management Practices for Non-Point*

*Source Pollution Control of Parking Lots at the Oak Ridge Y-12 Plant* (1992) and *Feasibility Study of Best Management Practices for Non-Point Source Pollution Control at the Oak Ridge Y-12 Plant* (February 1993). The data from these reports were used to develop and execute site-specific sampling exercises in 1993 and 1994 for parking lots, selected roofs, scrap yard(s), hillside runoff, and other sites of specific interest. A new area source sampling plan with limited, but targeted, analysis of contaminants has been implemented in FY 1994.

### 6.2 BMAP STUDIES

The third annual BMAP report (Hinzman et al. 1995) discusses future BMAP studies including the following:

1. Ambient toxicity tests with *Ceriodaphnia* and fathead minnow larvae will be conducted quarterly; these tests will use EFPC water collected at six sites downstream from Bear Creek Road.
2. Water quality data obtained in support of the ambient toxicity tests will be used to monitor stream conditions.
3. Ambient toxicity tests with *Ceriodaphnia* and fathead minnow larvae will be conducted bimonthly; these tests will use EFPC water collected at four sites upstream from Bear Creek Road.

4. Laboratory tests will be conducted to further evaluate the use of cello-biosidase activity as a microbial indicator of water quality.
5. The temporal stability of relationships between water hardness, alkalinity, and conductivity in wastewaters from various Y-12 Plant effluents will be evaluated to determine if the CPI can be used to assess improvements in water quality conditions in EFPC. The data also might be used to "chemically fingerprint" specific wastewaters and thereby aid in identifying spills or inadvertent chemical releases.
6. Studies will be initiated to better understand processes in LR that might help explain the absence of certain pollution-intolerant aquatic invertebrates (including *Elimia*) from downstream sites. These studies are likely to involve the deployment of data-logging instruments at the inlet and outlet of LR for continuous monitoring of pH, conductivity, and temperature. Water from these sites will be analyzed periodically for various metals, the concentration and biological availability of which are expected to show diel variations in response to changes in pH.
7. Periphyton studies will focus on the impact of contaminant sorption by periphyton on overall stream quality. The effects of environmental variables, such as sunlight on sorption and diel and seasonal changes in rates of sorption, will be studied to determine the role of EFPC periphyton in the fate and transport of toxicants.
8. Twenty-four-hour monitoring of pH, oxygen, and other parameters expected to vary with photosynthetic activity will be conducted at a number of

EFPC sites to determine the range of fluctuations and to provide data with which to estimate diel variability in toxicity.

9. Studies will be conducted to determine whether primary production in upper EFPC is nutrient-limited and saturated and whether reductions in nutrient loading will have a significant impact on overall stream quality.

### 6.3 NON-BMAP STUDIES

In addition to the regular BMAP fish community monitoring, some special studies will be implemented to try to determine specific factors associated with fish kills. These studies may include: (1) an expansion of the community characterization to cover the LR fish community, (2) analysis of environmental factors that could shape the community close to the Y-12 Plant, and (3) investigations of fish kill causes.

#### 6.3.1 Fish Community Analyses

Because of the growth of fish communities in LR since the fall of 1988, the fish community in the lake will be sampled annually. These data will provide a baseline for assessing impacts and will help define the lake fish community as a source for upstream colonization and recovery. The analysis of environmental factors and their influences on fish communities could be useful in assessing fish kills. For example, the role of elevated temperatures could be important in shaping the EFPC fish community and determining the physiological condition of individual fish. A study approach may involve comparisons of communities under similar conditions (e.g., flow, nutrient enrichment, and canopy conditions) but under different temperature regimes. These could be combined with literature or

laboratory assessments of temperature limitations for species found in upper EFPC to determine the role that temperature plays in the susceptibility of such species. Similarly, the role of habitat and limitations imposed by the existing habitat in upper EFPC could be a principal determinant of the fish communities and fish kill events. Finally, the investigation of fish kills could include studies investigating specific aspects of the kill environment similar to the study of the drift pattern of dead fish. One such study would evaluate seasonal differences in the sensitivity of central stonerollers and striped shiners. The goal of the special studies would be to address specific hypotheses associated with fish kill events.

### 6.3.2 Benthic Community Comparisons

Instream ecological monitoring results obtained during the second year of the BMAP studies suggested that effluents from the Y-12 Plant were affecting the benthic macroinvertebrate community in upper EFPC. Monitoring data collected June 1986 through May 1987 showed that community parameters were lowest at EFK 24.4, with gradual improvement occurring with increased distance from the Y-12 Plant. EFK 13.8 exhibited the greatest improvement; however, relative to a nearby reference stream, BF, impacts were still evident. The evidence from fish population studies also indicated that the upper reaches of EFPC were highly stressed, with slight improvements found at EFK 13.8 (Hinzman et al. 1995).

A benthic macroinvertebrate colonization study was designed in anticipation of the implementation of a dechlorination system to treat Y-12 Plant effluents discharging into upper EFPC. Two benthic macroinvertebrate colonization studies were initiated in EFPC to help evaluate the effects of effluent

dechlorination on the benthic macroinvertebrate community. The primary study was designed to use the Before-After-Control-Impact (BACI) design to compare the benthic macroinvertebrate community before and after dechlorination of two major effluent discharges into EFPC (see Sect 5. for a description of the dechlorination process). See comments on Appendix G. A secondary study was designed to examine the processes of colonization over 6-week colonization periods in the spring and summer, both before and after dechlorination. A complete description of the materials and methods used in these studies appears in Appendix G.

Three sites were chosen based on their proximity to the main sources of chlorine: EFKs 24.4 (nearest to the NSP), 23.4, and 13.8 (far site assumed to be unaffected by chlorine discharges) (see Fig. 5.2). EFK 13.8 was selected to serve as a reference site because no undisturbed upstream site existed and because it was felt that effluent dechlorination would have no effect this far downstream. Water quality parameters consisting of temperature, dissolved oxygen, conductivity, pH, and TRC were measured weekly during the studies.

#### 6.3.2.1 Primary study

For the primary study, the colonization of  $4 \times 8 \times 0.25$  in. ( $10.16 \times 20.32 \times 0.64$  cm) unglazed paving bricks by benthic macroinvertebrates was examined for a series of 20 3-week exposure periods, before and after dechlorination. Phase I began December 12, 1991, to establish conditions prior to dechlorination, and Phase II was run for the same series of 20-week exposure periods beginning December 29, 1992 (coincident with the day the second dechlorinator System came on line-Sect. 5). Running each phase during the same time period was based on the

assumption that the year-to-year variation among sites would be less than the season-to-season variation.

In the primary study, 3 sets of 10 bricks (30 bricks total) were placed randomly in a riffle at each of the 3 study sites and staggered in time so that (1) bricks that had been colonized for 3 weeks could be collected weekly and (2) data for at least 20 3-week exposure periods would be available. After an exposure period of 3 weeks, three bricks were chosen randomly within a set and removed for processing and subsequent identification in the laboratory. After a brick was removed and processed, it was cleaned and returned to the stream as part of a new set; the remaining five bricks in the set were also removed and replaced with clean bricks.

A stereoscopic dissecting microscope will be used to identify all organisms except chironomids, to the lowest taxonomic level

practical (genus in most cases). If necessary, chironomid larvae will be identified to genus by mounting them on a slide in CMC-10 Mounting Media for viewing with a compound scope.

#### 6.3.2.2 Secondary study

The secondary study was designed to run for 6-week periods in the spring and summer before and after dechlorination. At the beginning of each exposure period for this 6-week colonization experiment, 30 bricks were placed at each of the 3 sites following the same procedures as in the primary study (see Appendix B). Each week thereafter over a 6-week period, three bricks were chosen randomly and retrieved. After removal, each brick was taken to the laboratory for processing by following the same procedures used for the primary study.

## 7. SUMMARY AND CONCLUSIONS

This report summarizes the monitoring of fish kills in upper EFPC from July 1990 to March 1993. Since the opening of LR in 1988, the total numbers of fish inhabiting upper EFPC have increased. However, species diversity has remained poor. Water quality data have been collected in upper EFPC during the time period covered in this report. TRC levels have exceeded federal and state water quality criteria over the years. However, with the installation of two dechlorination systems in late 1992, TRC levels have been lowered substantially in most portions of upper EFPC.

Daily chronic fish mortality in upper EFPC from 1990-93 has been attributed to background stress resulting from the continuous discharge of chlorine into upper EFPC. TRC data indicate that there were still greatly fluctuating chlorine levels during the first few months that the dechlorinators were on line. In the summer of 1993, TRC levels appeared to have stabilized in upper EFPC at relatively low to nondetectable levels; consequently, fish kill monitoring was suspended in June 1993. Additional daily surveys may be required to confirm that reduced TRC levels in EFPC have indeed reduced chronic fish kills to negligible levels.

Acute mortality events have been recorded for upper EFPC. Spills or elevated releases of toxic chemicals, such as acids, organophosphates, aluminum nitrate, ammonia, or chlorine, were identified as possible causative agents; however, a definitive cause-effect relationship was rarely established for any acute kills.

Ambient toxicity testing, in situ chemical monitoring, and streamside

experiments were used to examine TRC dynamics and ambient toxicity in EFPC prior to installation of the dechlorination systems. The results of the studies provide clear and consistent evidence of toxicity in EFPC upstream from LR and inconclusive evidence for toxicity downstream from LR. Field and laboratory studies were conducted on various phyla to further evaluate the ambient toxicity test results.

### 7.1 WATER QUALITY SUMMARY

TRC concentrations in upper EFPC have exceeded EPA water quality criteria in past years. The highest TRC concentrations have been recorded upstream near the NSP. As a result of the installation of the two dechlorinators in upper EFPC, TRC concentrations in the first 3 months of 1993 were substantially lower than those recorded prior to December 1992. By June 1993, concentrations were 0.04 to 0.06 mg/L at NSP, below detection limits at AS-8, and 0 to 0.01 mg/L at the inlet and outlet of LR (Table 2.6).

Water temperatures in EFPC just below LR (EFK 23.4) were generally 4 to 7°C, higher than those in the reference stream, BF. Although temperatures frequently exceed 25°C (maximum, 40°C) below LR, the maximum temperature in BF seldom exceeds 25°C. Water temperatures at EFK 24.4, inside the Y-12 Plant, are generally similar to those at EFK 23.4 in the summer and 2 to 4°C warmer in the winter.

Ammonia concentrations in upper EFPC are generally low in the upper

reaches of the stream (25 µg/L at NSP and 16 µg/L at AS-8) and high near the diversion channel (230 to 280 µg/L). Outfall 017 (near EFK 24.4) was identified as the major source of ammonia. Monitoring data from Station 17 downstream of LR indicate that concentrations of some heavy metals, such as chromium, copper, lead, zinc, cadmium, and mercury, may at times exceed EPA NAWQC.

The overall water quality of EFPC can be estimated from the CPI, which is derived from conductivity, alkalinity, and water hardness measurements. The CPI is a measure of the degree to which a stream has been chemically altered from a natural condition (a CPI of 3.0 is the theoretical upper limit for an undisturbed stream). In the reporting period from November 1988 through August 1992, the lowest CPI values in EFPC were found furthest upstream; they ranged from 0.89 (at AS-8) to 2.05 (at EFK 13.8).

During the period from October 1988 to August 1992, 0.238 toxicity units (TUs) entered upper EFPC as a result of a total wastewater discharge rate of  $801.3 \times 10^6$  L per year. This was substantially below the 4.07 TUs that entered upper EFPC during the period from January 1986 to October 1988 (Hinzman et al. 1995), indicating an improvement in stream conditions.

Based on actual ambient water quality data and the two indices of perturbation (CPI and TU), it appears that overall water quality for upper EFPC has improved during the interval of this reporting period (July 1990–March 1993). TRC has been lowered appreciably since the installation of the dechlorination systems.

## 7.2 FISH COMMUNITY DATA

Analysis of fish community data for four locations in EFPC and in the reference stream, BF, over a 5-year period revealed substantial differences in the size and

composition of the fish fauna at the various sites. BF consistently had a greater species richness than any site in EFPC; however, fish density and total biomass were consistently lower than the values recorded at EFK 23.4 (downstream of LR). Since the opening of LR in 1988, the total number of fish inhabiting upper EFPC has increased. From spring 1991 to fall 1992, the fish population density upstream of LR at EFK 24.4 increased fivefold. By spring 1993, the fish density and total biomass at all sampling sites in upper EFPC (EFKs 25.1, 24.7, and 24.4) exceeded those for BF however, species richness was only about 20% of that at BF and only about 33% of that at EFK 23.4 downstream of LR. This would suggest that to date only the more tolerant species of fish have migrated upstream and that the environmental conditions in upper EFPC may not yet be suitable for less tolerant species.

## 7.3 AMBIENT TOXICITY TEST DATA

Between October 1988 and August 1992, a combination of approaches, including ambient toxicity testing, in situ chemical monitoring, and streamside experiments, were used to examine TRC dynamics and ambient toxicity in EFPC. Ambient toxicity was determined by 7-d static-renewal tests that measured the survival and growth of fathead minnow larvae and the survival and reproduction of *Ceriodaphnia*. Grab samples of full-strength water from each of ten sites—four sites located upstream from LR and six sites located downstream from LR—were tested. The samples were also analyzed for pH, conductivity, alkalinity, hardness, and TRC. For the four upstream sites, the importance of TRC as a toxicant in water was evaluated directly by comparing survival and growth of fathead minnow larvae and survival and fecundity of *Ceriodaphnia* in untreated water to that in water treated with sodium

thiosulfate. Streamside toxicity experiments that used untreated and dechlorinated stream water, were conducted with adult fathead minnows and central stonerollers. These experiments also directly tested the importance of TRC as a toxicant. The results of the studies provided clear and consistent evidence of toxicity in EFPC at the four sites located upstream of LR and inconclusive evidence for toxicity in EFPC at six sites located downstream from LR. A logistic regression of *Ceriodaphnia* toxicity tests in relation to TRC indicated that day-to-day variation in TRC concentrations was great enough to affect the survival of *Ceriodaphnia*.

#### 7.4 FISH KILLS

The monitoring of the fish kills in EFPC that began in July 1990 indicated that two types of fish mortality were occurring in upper EFPC. An almost continuous but relatively low level of mortality appeared to be the result of chronic stresses perhaps caused by one or more pollutants or ecological conditions present in the stream, whereas intermittent but relatively high levels of mortality (i.e., acute fish kills) were viewed as being the result of spills or sudden releases of pollutants. The cause of the chronic mortality was hypothesized to be background stresses resulting from the continuous discharge of chlorine into upper EFPC. Because of the considerable variability in the daily mortality and because acute kills of varying intensity or duration could have been superimposed over the chronic mortality, the distinction between chronic and acute kills was not always clearcut.

##### 7.4.1 Chronic Fish Kills

The mean daily chronic fish mortality in upper EFPC from 1990 to 1993 generally

ranged from one to five fish per day, with the exception of two periods during which the rates were eight to nine fish per day. The data suggest that the mean chronic mortality rates have decreased in successive years from one to five fish per day for 1990–91 to one to two fish per day for 1992–93. Chronic mortality continued during the first 3 months after the two dechlorinators were installed along upper EFPC. TRC data for that time period (January–March 1993, see Table 2.6) indicate that there were still considerable fluctuations in TRC that might have accounted for the continuing mortality. It was not until the summer of 1993 that TRC concentrations stabilized at very low levels in EFPC (see Table 2.6). Additional daily surveys would be needed to fully evaluate the impact of dechlorination on chronic fish mortality in upper EFPC.

A study of the downstream distribution of tagged dead fish in upper EFPC indicated that dead fish found at specific locations in upper EFPC probably died in those general areas and were not carried very far downstream by currents. Data obtained during this study also suggest that the daily surveys may have underestimated the actual fish mortality by 50 to 90%.

##### 7.4.2 Acute Fish Kills

In addition to the chronic, chlorine-related fish mortality observed in EFPC during the past several years, there have also been a number of instances in which fish mortality increased substantially above background levels for periods lasting from 1 d to several weeks. Spills or elevated releases of toxic chemicals, such as acids, organophosphate-type insecticides, aluminum nitrate, ammonia, surfactants, or chlorine, were identified as possible causative agents of some of the kills; however, in many cases, the cause could not be determined despite intensive chemical

and biological monitoring. Changes in environmental conditions, such as pH or water temperature, may have also contributed to acute kills by increasing the susceptibility of the fish to the toxic effects of contaminants already present in the stream.

The combination of the acute kills related to spills and the chronic kills linked to the almost continuous release of chlorine into EFPC produced a complex and occasionally conflicting pattern of fish mortality that made definitive investigations very difficult. As noted in Sect. 3.1, the effects of small spills of limited duration may have been obscured by the considerable variability in the daily chronic mortality. In general, however, mean daily mortality rates for acute kills were threefold or more above background and usually exceeded ten fish per day (see Figs. 3.1 through 3.3). The total number of dead fish collected during these acute kills ranged from about 30 to over 1000. Central stonerollers and striped shiners were the two species most commonly associated with acute kills occurring in upper EFPC at Sites 1 through 6 (see Fig. 1.1). Small numbers of blacknose dace and redbreast sunfish were also found during some of these events. In addition, redbreast sunfish and bluegill were the two species most affected in several acute kills occurring in or downstream of LR.

## 7.5 EXPERIMENTAL FIELD AND LABORATORY STUDIES

Field and laboratory studies involving the aquatic community were conducted to answer specific questions and further evaluate the ambient toxicity test results from upper EFPC. In situ experiments with central stonerollers and striped shiners collected from uncontaminated (EFK 21.8) and TRC-contaminated (near the NSP) areas of EFPC and placed in cages at the TRC-

contaminated site indicated that both species partially adapted physiologically to TRC in that times to death were longer for fish initially collected at the TRC-contaminated site. In the laboratory, striped shiners and central stonerollers were able to detect and avoid concentrations of TRC as low as 0.07 mg/L.

Prior to installation of dechlorination equipment, snails (*E. clavaeformis*) caged in situ for 14 d near the NSP died, whereas survival at sites downstream of the NSP ranged from 82.5 to 100%. Following installation of the dechlorination equipment at the NSP, 14-d survival at the NSP increased to 90 to 100%. Results of this study help explain the absence of this species from upper EFPC.

The feeding rate of *E. clavaeformis* in relation to water quality at LR-i and LR-o was studied in 3-d tests in the laboratory. Feeding rates of snails in water from a reference stream and LR-i did not differ, but feeding rates of snails maintained in LR-o water were significantly lower. A series of water treatments, including filtration, identified particles  $>0.7 \mu\text{m}$  but  $<8 \mu\text{m}$  in size as the agent(s) responsible for lowering the feeding rate. A chemical toxicant could not be identified.

All clams (*S. fabale*) placed in situ at EFK 24.4 steadily died over a period of 60 to 75 days; survival of clams at EFK 23.4 was variable among tests. During some tests, mortality was rapid and significant during the first 3-4 weeks. These periods of rapid mortality were followed by a pattern of survival similar to the reference sites and EFK 13.8. Growth of clams at all EFPC sites was generally less than that at reference sites.

In experiments conducted streamside at upper EFPC, the Asiatic clam (*C. fluminea*) closed its valves in response to sublethal concentrations of TRC, which ranged from 0.02 to 0.07 mg/L. Intervals of valve closure were significantly different between clams in untreated and dechlorinated water.

Valve closure minimizes the amount of time tissues are exposed to toxicant.

Taxonomic as well as physiological changes took place in periphyton communities exposed to changes in water quality. Placement of tiles colonized by periphyton from a reference site in EFPC resulted in taxonomic changes and changes in the membrane:storage lipid ratio.

Microbial activity, as quantified by cellobiosidase activity, was lower at the NSP than at sites downstream from the NSP. Site, date, and the interaction of site and date were related to cellobiosidase activity; the organic content of the leaf substrates had little relationship to activity.



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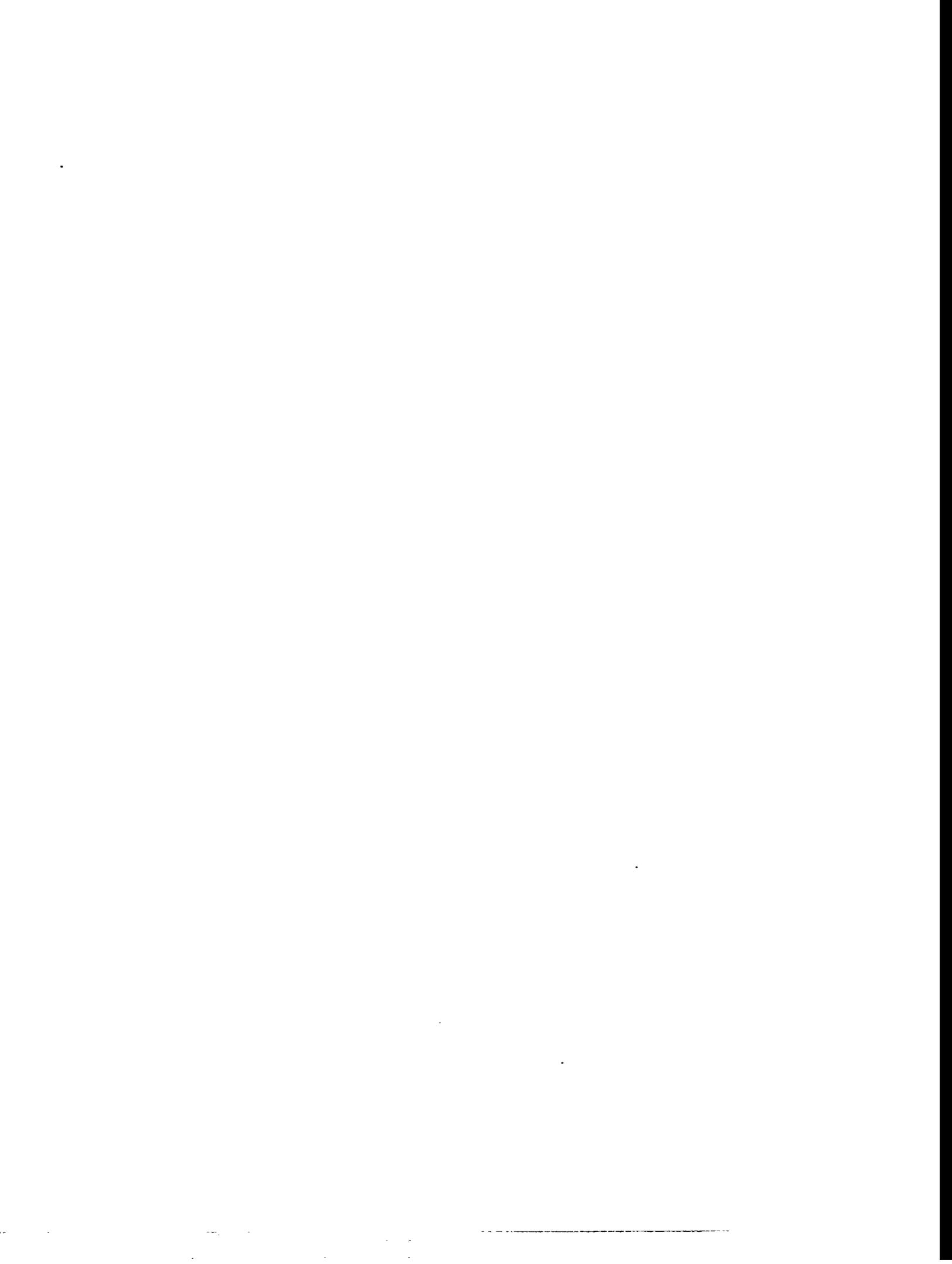
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**Appendix A**

**FISH COMMUNITY STUDIES BMAP QUALITY ASSURANCE  
PLAN STANDARD OPERATING PROCEDURE-10**



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**FISH COMMUNITY STUDIES**

SECTION

SOP-10

**BMAP QUALITY ASSURANCE PLAN**

PAGE

1 of 3

DATE

12/29/92

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**SUBJECT: INVESTIGATION PROCEDURES FOR FISH KILL INCIDENTS**

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**PURPOSE**

To investigate fish kills that occur in receiving streams or in aquatic areas associated with DOE facilities in Oak Ridge.

**SCOPE**

One of the responsibilities of the FCS project is to investigate any fish kills. This involves field procedures, data analysis, and reporting responsibilities. These procedures apply to all fish kills that are investigated by the FCS Group.

**EQUIPMENT**

Field equipment (buckets, dipnets, scales, measuring board, water chemistry collection vials) Field logbook (A104060)

**PROCEDURE****1. Field Procedures**

- a. Upon notification or discovery of a fish kill, established procedures are implemented for recovery of field data and investigation of possible causes.
- b. Initiate activities to determine the extent of the kill. These include determining the fish species affected, the range of acute responses (behavioral, physical, etc.) shown in the fish, the numbers of fish injured or dead, the range of stream kilometers (or area of pond or reservoir) in which the affected fish occur, and the presence of any visible clues to the cause of the kill.
- c. Simultaneously, implement procedures to assess water quality (e.g., collect water samples or review real-time monitoring data) in the affected stream reach (not all of these activities will be conducted by ESD staff) and to initiate control measures that may be necessary to limit the impact of the kill.

**FISH COMMUNITY STUDIES**

SECTION

SOP-10

PAGE

2 of 3

**BMAP QUALITY ASSURANCE PLAN**

DATE

12/29/92

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**SUBJECT: INVESTIGATION PROCEDURES FOR FISH KILL INCIDENTS**

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- e. Establish the most downstream extent of the kill and initiate a systematic census of the affected stream reach to collect all injured/dead fish is made.
- f. Repeat the census at intervals deemed appropriate by the field investigators (the interval, for example, will depend upon the mortality rate and whether the kill is an ongoing event). Continue the census until mortality is zero for survival successive stream surveys.
- g. As part of this stream census, estimate the time of death of all fish collected during the survey period. Use a method based on the relative degree of decomposition, rigor mortis, or life color of each fish. The basic categories are fish dying, dead <24 h, or dead >24 h.
- h. Determine if lengths or weights of the dead/injured fish are required to estimate the economic impact of the kill.
- i. As part of the stream census, collect and count other organisms affected by the kill event (e.g., crayfish, frogs).
- j. Document the date and findings of each survey in the numbered, registered field logbook used for all FCS project field work.
- k. As the fish kill event proceeds an understanding of the possible causes is reached, other procedures may be implemented that involve additional sampling and/or experiments.

**2. Data Analysis**

- a. As census data, water quality data, and observations of the fish kill become available. Evaluate the causes or the pattern of effects of the kill.
- b. In consultation with the Principal Investigators of BMAP projects that were involved in the fish kill investigation, as well as plant operations personnel, determine the need for additional analyses.
- c. Thoroughly evaluate all data regarding plant operations that may affect fish populations, weather conditions, BMAP field operations, water quality, and fish health assessments to identify possible correlations. This preliminary analysis is intended to guide the direction of ongoing investigations or possible follow-up experiments and will not always lead to definitive associations.

**FISH COMMUNITY STUDIES**

SECTION

SOP-10

**BMAP QUALITY ASSURANCE PLAN**

PAGE

3 of 3

DATE

12/29/92

**SUBJECT: INVESTIGATION PROCEDURES FOR FISH KILL INCIDENTS****3. Reporting Procedures**

- a. An established protocol exists for reporting fish kills and the resulting data among the BMAP and DOE facilities.
- b. Initial investigations of the fish kill involve both BMAP and the appropriate environmental compliance groups at the DOE Oak Ridge facilities.
- c. As more data on the kill are gathered, discussions among the BMAP Program Manager, ESD management, and the associated DOE facility management continue in order to provide support for additional investigations and actions.
- d. Throughout the duration of the kill, exchange information and submit regular status reports to the BMAP Program Manager for submittal to management of the DOE facility. This information may also be provided to TWRA and TDEC by DOE.
- e. After the event has concluded, document the results of the census surveys, water quality sampling, effluent discharges weather conditions, and the ecological impact assessment in a draft report that will be submitted to the DOE facility for review.

**RESULTS**

The results of this procedure are used for analyzing fish kills and for documenting the efforts of DOE to investigate fish kills.

**APPROVAL**

Principal Investigator \_\_\_\_\_ Date \_\_\_\_\_



**Appendix B**

**SUMMARY REPORT ON THE CHRONIC FISH KILL IN  
EAST FORK POPLAR CREEK JULY 1990-PRESENT**



**SUMMARY REPORT**  
**ON THE**  
**CHRONIC FISH KILL IN EAST FOR POPLAR CREEK, JULY 1990 - PRESENT**

**TO**

**OAK RIDGE NATIONAL LABORATORY**

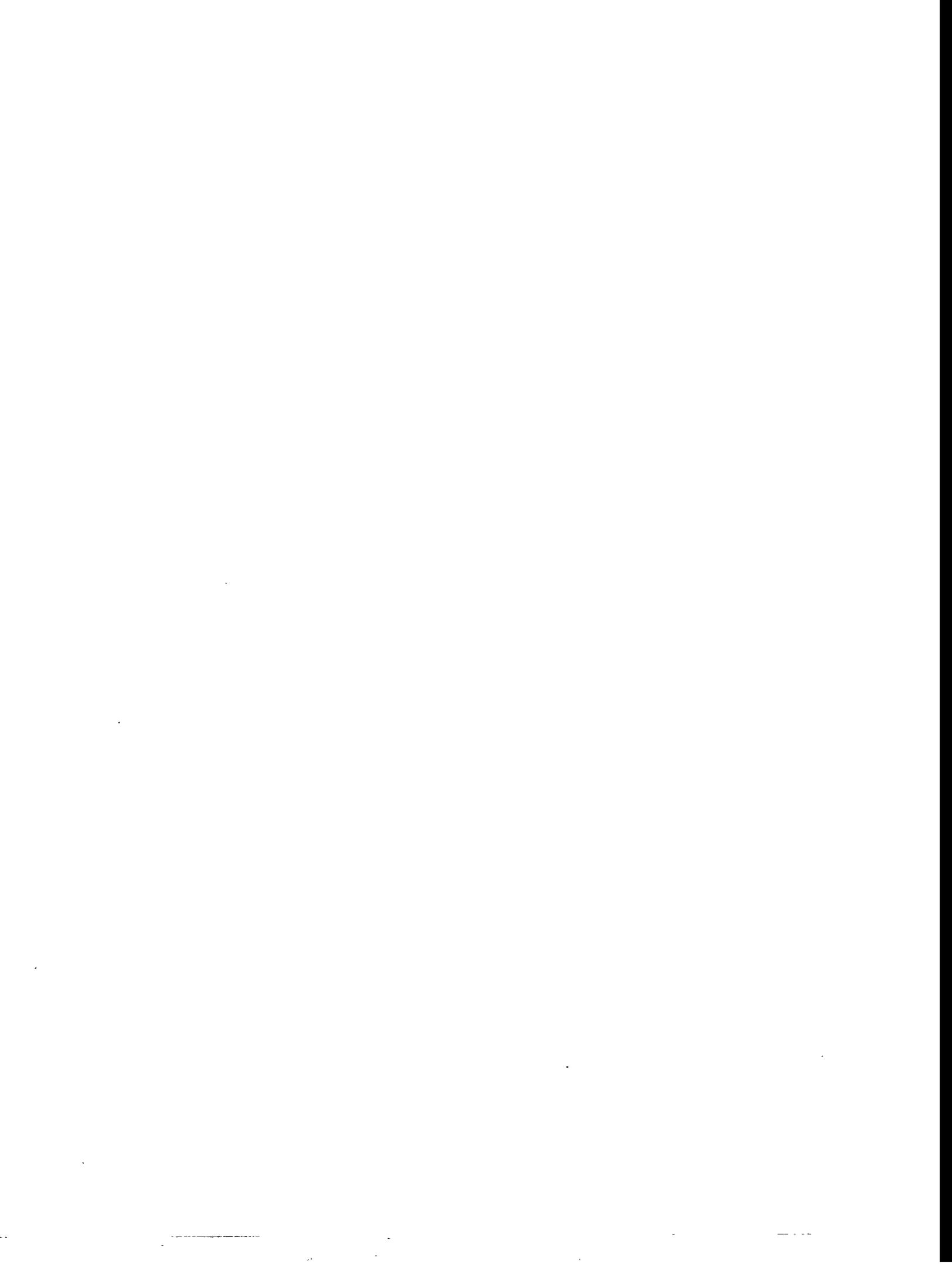
**BY**

**G. M. DEGRAEVE AND W. H. CLEMENT**

**BATELLE**  
**GREAT LAKES ENVIRONMENTAL CENTER**

**TRAVERSE CITY, MICHIGAN**

**MAY 1991**



## INTRODUCTION

In October 1990, Battelle's Great Lakes Environmental Center was asked by the Environmental Sciences Division (ESD) of Oak Ridge National Laboratory (ORNL) to perform an independent review of the available information pertaining to an ongoing fish kill at the Oak Ridge Y-12 Plant. This fish kill has been observed and documented in East Fork Poplar Creek (EFPC) at the Y-12 Plant, and both ESD and the Y-12 Plant are very serious about determining the cause of this problem. Therefore, they requested an independent perspective on their efforts to date and their plans for the future concerning this issue. This review consists of three related activities: (1) Review of the data package which was provided to Battelle by ORNL. (2) Summary of the principle topics covered and conclusions reached during a November 2, 1990 review meeting held at ORNL, and (3) Battelle's conclusions and recommendations regarding ESD's investigation of the fish kill and their plans for future work.

This type of review was requested by the ESD because they felt that it was necessary to have their approach and conclusions scrutinized before making final recommendations to the Y-12 Plant. This is an important consideration because the solution(s) to this problem may be expensive and difficult to implement, and because the Y-12 Plant needs to be as confident as possible that the cause for the fish kill has been correctly characterized prior to implementing remedial actions.

## DATA REVIEW

In advance of the November 2 meeting, Battelle was sent a data and information package to review and to use to prepare for the meeting. This package of information consisted of a map of the Oak Ridge Y-12 Plant, a summary of ESD's investigations of the fish kill, a summary of fish population surveys above and below Lake Reality, results of Ceriodaphnia toxicity tests with water from Area Source Study Site 8 (AS-8), and chemistry data on total residual chlorine (from a variety of locations over a range of time periods), nitrate, dissolved oxygen, ammonia, pH, metals, and selected conventional and organic pollutants.

This information was reviewed prior to the November 2 meeting, and the essence of this material can be summarized as follows: There have been a number of fish kills in EFPC since November 1988. The most recent fish kill (beginning July 1990) differs from the earlier kills because

it has lasted much longer (nine months as opposed to a few days) and because the affected area may include EFPC below Lake Reality. There are good reasons to suspect chlorine (measured as total residual chlorine) as the toxicant responsible for these fish kills, because there are multiple chlorinated discharges entering EFPC from the Y-12 Plant above Lake Reality, and because the chlorine concentrations in EFPC above Lake Reality are well within the range that we would expect to be acutely toxic to aquatic organisms (approximately 0.2-1.0 mg/L). However, there is some concern among the ESD and Y-12 Plant staff that chlorine may not account for all of the problem because the chlorine concentrations below Lake Reality are much lower (approximately 0.05-0.10 mg/L) than the chlorine concentrations above the Lake. It is also important to note that ESD staff have determined that the increased number of fish dying is correlated with measured increased fish abundance in and below Lake Reality, which has taken place since the filling of Lake Reality in early November 1988.

Much literature addresses the toxicity of chlorine to freshwater fish. We reviewed several of the major publications on this subject, and found sufficient data to suggest that chlorine concentrations as low as 0.05-0.10 mg/L can have adverse effects on freshwater fish when exposure is chronic (Brungs, 1973; Brungs, 1976; Basch and Truchan, 1976; Michigan Department of Natural Resources; 1971; Mattice and Zittel, 1976; Finlayson and Hinkelman, 1977; Ward and DeGraeve, 1978; DeGraeve et al., 1979). For example, in his review of the literature on the effects of residual chlorine on aquatic life, Brungs (1973) cited several studies which demonstrated effects at the levels of chlorine measured at Station 17 on EFPC about 200 m below Lake Reality. In addition, Ward and DeGraeve (1978) found chronic effects at total residual chlorine concentrations as low as 0.033 mg/L, which is lower than the typical residual chlorine concentrations measured at Station 17.

Of the other chemical parameters (including temperature, nitrate, dissolved oxygen, ammonia and a wide variety of other organic and inorganic constituents), none appeared to be high enough or low enough (relative to the acceptable range of tolerance for aquatic organisms) to be responsible for the observed fish mortalities. In summary, the data reviewed prior to the November 2 meeting did not provide any evidence of causes for the fish kill other than chlorine and chlorination by-products; based upon the available data these materials seem to be likely candidates responsible for the observed mortalities.

### MEETING SUMMARY

On November 2, 1990, a meeting was held at Oak Ridge to discuss the ongoing fish kill and ESD's plans for future studies. A number of individuals from ORNL/ESD, the Y-12 Plant, Environmental Sciences Division, the Tennessee Wildlife Resources Agency and Mick DeGraeve from Battelle attended the meeting. During the meeting, the ESD staff presented the historical information summarized above, and discussed the current status of the fish kill in EFPC. In addition, ESD staff presented ongoing studies and evaluations on the stream bank fish test at AS-8, the fish health investigations, and the experimental dechlorination of EFPC.

The stream bank fish test at AS-8 provided strong evidence of the toxicity of chlorine in EFPC. All of the organisms exposed to dechlorinated EFPC water survived after about 1½ days of exposure, but only 1/3 of the organisms exposed to untreated EFPC water survived after a similar exposure. During this exposure period, residual chlorine concentrations ranged from about 0.1 mg/L to about 0.35 mg/L, and were high enough to account for the observed mortality. A similar bankside dechlorination experiment was performed in the diversion channel at the oil/water separator site. In this case, four species (fish and snails) were exposed for 21 days to untreated (0.01 - 0.08 mg/L chlorine) and dechlorinated EFPC water. These results showed that survival of the aquatic organisms exposed to dechlorinated or untreated EFPC water was high. The fact that all of the fish in the dechlorinated EFPC water at AS-8 survived concurrent with significant mortalities in the organisms exposed to untreated water provides strong evidence that only chlorine (or another oxidant like chlorine) was responsible for the mortalities observed during the study period. This evidence also lends credibility to the concept that chlorine could be responsible for the chronic mortalities which have been observed about 0.5 km downstream in the diversion channel, since chlorine residuals (0.01 - 0.08 mg/L) persist for that distance. If there are no other toxics entering EFPC downstream of AS- 8, then the evidence supporting the chlorine toxicity hypothesis becomes even stronger. The diversion channel dechlorination experiment did not provide contradictory information, because 0.01 - 0.08 mg/L residual chlorine is not sufficient to cause acute mortality, but rather is more likely to be responsible for chronic effects.

Fish health information was presented on condition indicators, diseases and metabolic stress indicators. The condition indicator information illustrated that there were few parasites, that the organs were generally in good condition, and that the fish had not been eating significant amounts of food prior to their capture. Minimal food consumption is not unusual in the fall of the year,

although this condition could also be due to the fact that the organisms were stressed, and therefore less inclined to pursue food items. One interesting finding of the fish health investigation performed by Carlson (1990) was that gill aneurysms were present in greater than 50 percent of the fish examined from Lake Reality and the diversion channel, but none were found in fish collected from a nearby uncontaminated reference stream (Hinds Creek). This is valuable information, because it is well documented in the literature that chlorine is a gill irritant, which can cause excessive mucus secretion and gill lamella irritation, resulting in damaged gills.

Fish were collected for Carlson from a variety of locations and checked for parasites, bacteria and viruses. As a result of the disease analyses, there was no evidence of the presence of unusual types of, or quantities of parasites or bacterial/viral infections. These results indicate that the ongoing fish kill is not likely due to any external or internal pathogens, and therefore must be a result of an environmental factor(s). Finally, the metabolic stress indicator information suggested that the oxygen uptake rate of the study fish in EFPC water from Station 17 was similar to reference fish collected from Hinds Creek or upper EFPC, which neither supports nor refutes the chlorine toxicity hypothesis.

An experimental approach to definitively determine whether chlorine is the factor responsible for the ongoing fish kill was presented by ESD staff. In this study, ESD staff have proposed to experimentally dechlorinate EFPC at the North-South Pipe, adding sufficient dechlorinating agent at that point to reduce all of the chlorine in EFPC at the North-South Pipe, and adding excess dechlorinating agent to reduce all of the chlorine which enters EFPC downstream from the North-South Pipe. The ESD staff's rationale for this approach was that very large numbers of fish would have to be exposed in the laboratory to reproduce the instream conditions, making such a laboratory study impractical. Thus, if the entire stream is experimentally dechlorinated, the effects on the entire affected population can be determined. ESD staff proposed to dechlorinate with sulfite or sodium thiosulfate for a two-week period, and monitor fish mortality in EFPC during and after the dechlorination period. ESD staff considered the potential negative side effects, such as pH reduction, oxygen depletion, increased dissolved salts, mercury transport and transformation, and the possibility of induced fish mortalities as a result of the experimental manipulations. They concluded that the likelihood that experimental dechlorination would cause additional mortalities is very low, and not of sufficient concern to eliminate this approach from consideration.

### CONCLUSIONS/RECOMMENDATIONS

Based upon Battelle's review of the provided data, the information provided in the November 2 meeting and our familiarity with the published literature concerning chlorine chemistry and the effects of chlorine on aquatic organisms, we are in general agreement with the ORNL/ESD staff that chlorine or chlorination by-products are the likely cause for the observed mortalities in EFPC and in the vicinity of Lake Reality. Even though the measured chlorine residual values in Lake Reality and EFPC at Station 17 are relatively low (approximately 0.1 mg/L), that concentration of residual chlorine has been shown experimentally to cause chronic effects to fish, including death. Of course, if chlorine is responsible for the observed mortalities, the most obvious question to be asked is "Why did the number of fish dying increase so dramatically beginning in mid-September?" It certainly is possible that one reason for the increased numbers of dead fish observed at that time was higher total residual chlorine concentrations in EFPC beginning in September 1990. However, there is no chlorine monitoring data from EFPC to support or refute this hypothesis. Irrespective of whether that is the case or not, there has been a definite increase in fish mortalities during July-December 1990.

We feel that an important factor contributing to the increase in mortalities during the July-December timeframe is the dramatically increased fish population in and immediately below Lake Reality since the filling of Lake Reality, which has been documented by the fish abundance surveys conducted by the ESD staff. This increased fish abundance has likely increased the number of exposed individuals which are susceptible to the low chlorine concentrations found in and below Lake Reality, therefore resulting in increased numbers of fish dying. It is well-recognized among aquatic toxicologists that the sensitivity of individuals within a species to a given toxicant can be described by a bell-shaped curve. That is, there will be some individuals within a species which are quite tolerant to a toxicant (chlorination by-products in this case), some individuals which are marginally sensitive, and some individuals which are extremely sensitive. Consequently, when a toxicant is present at a low but potentially toxic concentration, the more susceptible members of the species will be affected. We feel that this is likely to be what is happening in Lake Reality and in EFPC just below Lake Reality. The measured chlorine residuals of 0.05 - 0.1 mg/L have been demonstrated in the laboratory to produce chronic effects (including mortality), but those concentrations are not high enough to be lethal to all of the members of the exposed populations of fish in EFPC and Lake Reality. Therefore, the more susceptible individuals in each population of each species are dying at a

slow rate. These mortalities may have been taking place historically, but the absolute numbers of fish dying may have been so low that the dead fish went unnoticed. However, when the fish abundance increased, the absolute numbers of exposed, susceptible organisms correspondingly increased, resulting in increased observed fish mortalities.

It seems to us that all of the data support this perspective, and that there are no conflicting data to confuse the issue. There are no other measured water quality parameters which deviate sufficiently from the acceptable range to cause this pattern of mortality, and the fish health investigation did not result in any suggestion that this mortality pattern is being caused by any type of fish disease. Consequently, of the parameters which were monitored, chlorine (measured as total residual chlorine) is most likely responsible for the mortalities observed to date. However, as is the case in any natural or manipulated ecosystem (such as EFPC), there may be a factor(s) responsible for this fish kill which has not been measured or even considered. It is virtually impossible to measure all parameters, or to understand all of the ecological/environmental factors which may be contributing to this situation. In spite of that fact, it still seems likely that chlorine must be contributing to the ongoing mortality pattern, and that chlorine is the parameter which deserves the most serious consideration at this point.

Because of the strong likelihood that chlorine is contributing to the ongoing fish kill, the idea of experimentally dechlorinating EFPC and observing the results during and after the dechlorination period is particularly appealing. It is an unusual situation to have the opportunity to experimentally test an affected population. This opportunity can be used to (1) definitively test the chlorine toxicity hypothesis, and (2) concurrently determine how reduced chlorine concentrations will affect the ecological conditions in EFPC. We feel this is a very worthwhile effort, but we have several comments and suggestions for ESD staff to consider when planning and implementing this study.

- We feel that the experimental period (two weeks) is too short. If the dechlorination is only two weeks, there may be already-stressed fish which will continue to die for a significant portion of the experiment (several days, or for perhaps for up to a week). If this were to happen, it could make interpretation of the results difficult. Since the most time-consuming and technically difficult component of the study will be setting up the chemical dosing system and optimizing the dose rate, it seems logical to us that the actual experimental timeframe should be increased to four weeks. This would provide

ESD staff sufficient time to gain a clear perspective on the effects of the experimental treatment.

- ESD staff have proposed to dechlorinate with either sulfite or thiosulfate. We feel that either approach will be effective, and that each has certain advantages and disadvantages. We agree with the ESD staff on their conclusion that neither dechlorination treatment is likely to have a meaningful impact on either the pH or dissolved oxygen in EFPC. Sulfite will react rapidly with oxygen and will therefore be depleted quickly. Consequently, much greater-than-expected amounts of sulfite may be required to provide a sulfite residual at the head of the diversion channel. Thiosulfate will not be as readily depleted, but it may complex and/or precipitate metals in EFPC. Thiosulfate could also potentially react with certain metal ions, and as a result the amounts of thiosulfate needed to maintain a thiosulfate residual at the boundary of the Y-12 Plant will also be difficult to determine. For both chemical reductants, we feel that the actual amount of either sulfite or thiosulfate required to accomplish the objective will need to be determined experimentally in EFPC. This can be accomplished by measuring either sulfite or thiosulfate residuals plus chlorine residuals at the boundary of the Y-12 Plant, and adjusting the dose of the dechlorinating agent until all of the chlorine has been reduced, and only a small residual of either sulfite or thiosulfate remains.
- After the proper amount of sulfite or thiosulfate has been determined during normal working hours, ESD staff will need to finalize the amount of reductant needed by measuring sulfite or thiosulfate residuals and chlorine residuals during the night. The chlorine residual data for EFPC clearly illustrate that nighttime chlorine residuals typically increase to nearly double the daytime residuals. Therefore, it will be very important to insure that sufficient reductant is available to reduce the highest normal chlorine residuals. After ESD staff have determined the maximum amount of reductant necessary to dechlorinate EFPC at night, we suggest that the reductant application rate be fixed at the nighttime levels for the duration of the experiment. This will help insure that all foreseeable chlorine residuals are reduced, and it will eliminate some mechanical/dose rate/human error problems that could develop if reductant feed rates are manipulated daily.
- Because there is no experimental control which can be run simultaneously with the proposed manipulation, the results will be open to alternate interpretations. For

example, a reduction of mortalities during the dechlorination period could be due to a coincidental change in some unidentified toxicant input. We feel that the odds of this happening are remote but, nonetheless, must be considered.

Overall, we think that the experimental dechlorination approach is a very good way to determine if chlorine is responsible for the ongoing fish kill in EFPC. Obviously, if this study demonstrates that the residual chlorine is causing this problem, the Y-12 Plant will be facing a difficult challenge: finding a way to remove or dramatically reduce the chlorine concentrations in the various discharges from the Y-12 Plant to EFPC. This can be accomplished by (1) reducing chlorine in the source water used by the Y-12 Plant, (2) recycling much of the water currently being discharged to EFPC or (3) dechlorinating some or all of the discharges to EFPC which contain significant concentrations of chlorine. Irrespective of which approach is taken, the solution to the problem is likely to be expensive and/or administratively difficult.

It is also important for all involved to recognize that EFPC is without question a manipulated stream ecosystem as it passes through the Y-12 Plant, and that it is being used as a conveyance of multiple industrial wastes from the Plant. For this reason, we should expect intermittent accidental releases of industrial wastes other than chlorine from the Y-12 Plant, and these releases could cause future fish kills. This is likely to be the case whether or not the chlorine has been removed. Thus, we should not view chlorine reduction or removal as the panacea which will completely eliminate fish kills in the future, even if chlorine is determined to be the only cause for the current fish kill.

**Appendix C**

**DEAD FISH SURVEYS OF UPPER  
EAST FORK POPLAR CREEK**









Appendix C continued

Sample date	Location						Total		
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3		Site 4	Site 5
<u>1990</u>									
10-4 (am)	1 <sup>BG</sup> 1 <sup>RB</sup> 1 <sup>SH</sup> 4 <sup>SR</sup>								
10-4 (pm)	2 <sup>BG</sup> 1 <sup>RB</sup> 2 <sup>SH</sup>	2 <sup>BG</sup> 1 <sup>SR</sup>							15
10-5 <sup>c</sup> (am)	30 <sup>BG</sup> 3 <sup>FH</sup> 1 <sup>MQ</sup> 5 <sup>RB</sup> 3 <sup>SH</sup> 10 <sup>SU</sup>								
10-5 (pm)	11 <sup>BG</sup> 2 <sup>RB</sup> 1 <sup>SH</sup> 4 <sup>SR</sup>								70
10-6	1 <sup>SU</sup>								1
10-8	1 <sup>FH</sup> 1 <sup>SR</sup>								2
10-9 (am)	1 <sup>MQ</sup> 1 <sup>SU</sup>		1 <sup>SH</sup> 1 <sup>SR</sup>						
10-9 (pm)	1 <sup>FH</sup> 1 <sup>SH</sup> 1 <sup>SR</sup> 1 <sup>UK</sup>	2 <sup>RB</sup> 1 <sup>SH</sup>							11
10-10	1 <sup>BG</sup> 3 <sup>SR</sup>	1 <sup>RB</sup> 1 <sup>UK</sup>	1 <sup>SH</sup> 2 <sup>SR</sup>						9
10-11	2 <sup>SR</sup> 1 <sup>SU</sup>								3
10-12	4 <sup>SR</sup>	1 <sup>UK</sup>							5
10-15	1 <sup>SR</sup>		2 <sup>SH</sup> 3 <sup>SR</sup>	1 <sup>SH</sup>	2 <sup>SH</sup> 1 <sup>SR</sup>	6 <sup>SH</sup> 1 <sup>SR</sup>	2 <sup>SH</sup> 2 <sup>SR</sup>		21
10-16	2 <sup>SR</sup>		1 <sup>SH</sup>		2 <sup>SH</sup>	3 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup> 2 <sup>SR</sup>	1 <sup>SH</sup>	13
10-17	1 <sup>SR</sup>	1 <sup>SR</sup>			1 <sup>SH</sup>	1 <sup>SR</sup>			4

## Appendix C continued

Sample date	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1990</u>										
10-18	NS									NS
10-19	1 <sup>SR</sup>		1 <sup>SR</sup>		1 <sup>RB</sup> 1 <sup>SR</sup>		1 <sup>SR</sup>			5
10-22	1 <sup>SR</sup>						1 <sup>SH</sup>			2
10-23	1 <sup>SR</sup>	1 <sup>SU</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>							4
10-24	1 <sup>SR</sup>									1
10-25	1 <sup>SR</sup> 1 <sup>UK</sup>	2 <sup>RB</sup>	1 <sup>RB</sup>							5
10-26	3 <sup>SR</sup>		1 <sup>BG</sup> 2 <sup>SH</sup>			2 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>			11
10-29					1 <sup>SH</sup>	3 <sup>SH</sup>	1 <sup>RB</sup> 1 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup>		8
10-30	1 <sup>FH</sup> 1 <sup>RB</sup>									2
10-31	1 <sup>SR</sup>	1 <sup>SR</sup> 1 <sup>SU</sup>								3
11-1	1 <sup>BG</sup> 1 <sup>RB</sup> 1 <sup>SR</sup>									3
11-2	1 <sup>SR</sup>									1
11-5		1 <sup>UK</sup>								1
11-6	1 <sup>SR</sup>	1 <sup>SR</sup>	2 <sup>SH</sup> 1 <sup>SR</sup>							5
11-7 <sup>c</sup>	1 <sup>FH</sup> 2 <sup>RB</sup> 5 <sup>SH</sup> 2 <sup>SR</sup>									10
11-8 <sup>c</sup>	2 <sup>BG</sup> 2 <sup>FH</sup> 5 <sup>RB</sup> 16 <sup>SH</sup> 2 <sup>SR</sup>			1 <sup>BN</sup> 1 <sup>SH</sup>						29

C-8 Y-12 Plant Fish Kill Report

Appendix C continued

Sample date	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1990</u>										
11-9	1 <sup>FH</sup> 2 <sup>SH</sup> 1 <sup>SR</sup>									4
11-12							1 <sup>SH</sup> 2 <sup>SR</sup>	2 <sup>SH</sup>		5
11-13	2 <sup>SH</sup> 1 <sup>SR</sup>		1 <sup>SR</sup>							4
11-14	1 <sup>CY</sup> 1 <sup>SH</sup>		1 <sup>SH</sup>							3
11-15	2 <sup>SH</sup> 1 <sup>SK</sup> 1 <sup>SR</sup> 1 <sup>UK</sup>									5
11-16	1 <sup>SR</sup>		1 <sup>SH</sup>	1 <sup>SH</sup>						3
11-19	1 <sup>RB</sup>									1
11-20	0									0
11-21	1 <sup>RB</sup> 1 <sup>SR</sup>									2
11-26			1 <sup>RB</sup> 1 <sup>SR</sup>							2
11-27	1 <sup>SR</sup>		1 <sup>SH</sup> 5 <sup>SR</sup> 1 <sup>SU</sup>							8
11-28	0									0
11-29	0									0
11-30							2 <sup>SR</sup>	1 <sup>SH</sup>	1 <sup>SR</sup>	4
12-3	NS									NS
12-4	0									0
12-5	1 <sup>SR</sup>								1 <sup>SR</sup>	2
12-6	2 <sup>SH</sup>							1 <sup>SR</sup>		3



C-10 Y-12 Plant Fish Kill Report

Appendix C continued

Sample date	Below LR	In LR	Div chn	Location						Total
				Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1991</u>										
1-9	0									0
1-10	0									0
1-14			1 <sup>SR</sup>							1
1-15	0									0
1-16	NS									NS
1-17	0									0
1-18	0									0
1-21	0									0
1-22	0									0
1-23	0									0
1-24	0									0
1-25			1 <sup>SR</sup>	1 <sup>SR</sup>						2
1-28	0									0
1-29	0									0
1-30	0									0
1-31	0									0
2-1	0									0
2-4								2 <sup>SH</sup>	1 <sup>SR</sup>	3
2-5			1 <sup>SH</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>		1 <sup>SH</sup>			2 <sup>SH</sup>	6
2-6									3 <sup>SH</sup>	3
2-7	0									0
2-8			1 <sup>SH</sup>							1
2-11					1 <sup>SR</sup>	1 <sup>SR</sup>		2 <sup>SH</sup> 1 <sup>SR</sup>	5 <sup>SH</sup> 1 <sup>SR</sup>	11

## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1991</u>										
2-12						2 <sup>SH</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup>	6 <sup>SH</sup>	11
2-13			1 <sup>SR</sup>				1 <sup>SH</sup>	2 <sup>SH</sup> 1 <sup>SR</sup>	2 <sup>SH</sup>	7
2-14			1 <sup>SH</sup>				1 <sup>SH</sup>			2
2-15			1 <sup>RB</sup>							1
2-19	NS									NS
2-20	NS									NS
2-21			1 <sup>SR</sup>		4 <sup>SH</sup> 1 <sup>SR</sup>					6
2-22	1 <sup>SR</sup>	1 <sup>UK</sup>								2
2-25	0									0
2-26	0									0
2-27	0									0
2-28	0									0
3-1 (am)	1 <sup>CP</sup> 2 <sup>SH</sup>		8 <sup>SH</sup>	17 <sup>SH</sup>	67 <sup>SH</sup> 2 <sup>SR</sup>	25 <sup>SH</sup> 2 <sup>SR</sup>	76 <sup>SH</sup> 30 <sup>SR</sup>	15 <sup>SH</sup> 8 <sup>SR</sup>	10 <sup>SH</sup>	
3-1 (pm)			4 <sup>SH</sup> 1 <sup>SR</sup>	2 <sup>SH</sup>	10 <sup>SH</sup> 1 <sup>RB</sup>	3 <sup>SH</sup> 1 <sup>SR</sup>	3 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup>	2 <sup>SH</sup>	292
3-2			1 <sup>BN</sup> 2 <sup>SH</sup>			3 <sup>SH</sup>	2 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup>	2 <sup>SH</sup>	12
3-3	NS									NS
3-4					2 <sup>SH</sup>				1 <sup>SH</sup>	3
3-5					1 <sup>SH</sup>		1 <sup>SH</sup>			2
3-6		2 <sup>SU</sup> 1 <sup>SK</sup>					1 <sup>SH</sup>			4

C-12 Y-12 Plant Fish Kill Report

Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1991</u>										
3-7		1 <sup>UK</sup>							1 <sup>SH</sup>	2
3-8				1 <sup>SH</sup> 2 <sup>SR</sup>						3
3-11	0									0
3-12	0									0
3-13	0									0
3-14 <sup>c</sup>				1 <sup>SR</sup>	4 <sup>SH</sup>					5
3-15	0									0
3-18	36 <sup>BG</sup> 2 <sup>GS</sup> 92 <sup>RB</sup>	19 <sup>BG</sup> 13 <sup>GS</sup> 185 <sup>RB</sup> 1 <sup>SH</sup>	19 <sup>RB</sup>	1 <sup>SH</sup>		1 <sup>RB</sup>			1 <sup>RB</sup>	370
3-19 (am)	3 <sup>BG</sup> 16 <sup>RB</sup> 1 <sup>RO</sup> 1 <sup>SR</sup> 7 <sup>SU</sup>	5 <sup>BG</sup> 6 <sup>RB</sup>	13 <sup>RB</sup>						1 <sup>RB</sup>	
3-19 (pm)	1 <sup>SH</sup> 3 <sup>SU</sup> 3 <sup>RB</sup> 1 <sup>UK</sup>	1 <sup>BG</sup> 7 <sup>RB</sup>	1 <sup>RB</sup>							70
3-20		37 <sup>BG</sup> 1 <sup>GS</sup> 15 <sup>RB</sup>								53
3-21		12 <sup>BG</sup> 106 <sup>RB</sup>	2 <sup>SU</sup>	1 <sup>RB</sup>						121
3-22	4 <sup>RB</sup>	23 <sup>BG</sup> 1 <sup>FH</sup> 70 <sup>RB</sup>	2 <sup>RB</sup>							100
3-23		1 <sup>BG</sup>								1
3-25	2 <sup>BG</sup> 1 <sup>UK</sup>	27 <sup>BG</sup> 27 <sup>RB</sup>	1 <sup>SR</sup>							58

## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1991</u>										
3-26	2 <sup>RB</sup>	48 <sup>BG</sup> 1 <sup>FH</sup> 107 <sup>RB</sup> 4 <sup>SU</sup>								162
3-27	1 <sup>SU</sup>	35 <sup>BG</sup> 50 <sup>RB</sup> 14 <sup>SU</sup>			1 <sup>SR</sup>					101
3-28		6 <sup>BG</sup> 8 <sup>RB</sup> 1 <sup>SH</sup>	3 <sup>SH</sup> 3 <sup>SR</sup>							21
4-1	1 <sup>BG</sup>	2 <sup>FH</sup> 1 <sup>RB</sup> 2 <sup>SH</sup>	2 <sup>BN</sup> 1 <sup>CY</sup> 1 <sup>SH</sup> 3 <sup>SR</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>						15
4-2	2 <sup>BN</sup>		5 <sup>SR</sup>		1 <sup>SH</sup>					8
4-3	1 <sup>SR</sup>		4 <sup>SR</sup>							5
4-4		1 <sup>CY</sup> 1 <sup>SK</sup>	1 <sup>SR</sup>			1 <sup>SR</sup>				4
4-5				1 <sup>SR</sup>						1
4-8			2 <sup>CY</sup> 6 <sup>SR</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>	2 <sup>SR</sup>					12
4-9	1 <sup>SR</sup>		1 <sup>SH</sup> 3 <sup>SR</sup>							5
4-10			5 <sup>SR</sup>	1 <sup>SR</sup>						6
4-11	1 <sup>SS</sup>	3 <sup>SR</sup>	1 <sup>SR</sup>							5
4-12	3 <sup>SR</sup>		2 <sup>SH</sup> 5 <sup>SR</sup>							10
4-15			1 <sup>SK</sup> 1 <sup>SR</sup>			1 <sup>SR</sup>				3
4-16	1 <sup>BG</sup>					1 <sup>SR</sup>				2
4-17	1 <sup>GZ</sup>		6 <sup>SR</sup>							7
4-18	1 <sup>SR</sup>		4 <sup>SR</sup>		1 <sup>SR</sup>					6



## Appendix C continued

Sample date <sup>d</sup>	Location									
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Total
<u>1991</u>										
5-21	0									0
5-22			1 <sup>RB</sup>							1
5-23	1 <sup>SH</sup> 1 <sup>SR</sup>									2
5-24	0									0
5-28			1 <sup>BN</sup>							1
5-29	1 <sup>SR</sup>									1
5-30	0									0
5-31	1 <sup>SR</sup>									1
6-3			1 <sup>SR</sup>							1
6-4			1 <sup>RB</sup>							1
6-5	0									0
6-6	1 <sup>SH</sup> 1 <sup>SR</sup>			1 <sup>SH</sup>	1 <sup>SR</sup>				1 <sup>RB</sup>	5
6-7			1 <sup>SR</sup>							1
6-10	2 <sup>SR</sup>		1 <sup>FH</sup> 1 <sup>UK</sup>							4
6-11	0									0
6-12			1 <sup>BG</sup>							1
6-13			1 <sup>SR</sup>							1
6-14	1 <sup>SH</sup>									1
6-15 <sup>f</sup>				37 <sup>CY</sup> 4 <sup>SU</sup>						41
6-17			1 <sup>BG</sup> 1 <sup>SH</sup>	2 <sup>SR</sup>	1 <sup>BG</sup>			1 <sup>UK</sup>		6
6-18						2 <sup>SR</sup>				2
6-19					1 <sup>SH</sup>					1



## Appendix C continued

Sample date <sup>d</sup>	Location						Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	
<u>1991</u>							
7-29	0						0
7-30	0						0
7-31			1 <sup>SH</sup>				1
8-1	1 <sup>SR</sup>		1 <sup>SR</sup>				2
8-2	1 <sup>SR</sup>						1
8-5	1 <sup>SR</sup>						1
8-6	0						0
8-7	1 <sup>SR</sup>		1 <sup>SR</sup>				2
8-8	1 <sup>SR</sup>						1
8-9	0						0
8-12	0						0
8-13	0						0
8-14	1 <sup>SR</sup>						1
8-15	2 <sup>SR</sup>						
(am)							
8-15	1 <sup>FH</sup>						
(pm)	4 <sup>SH</sup>						
	9 <sup>SR</sup>						16
8-16	21 <sup>SR</sup>						21
8-19	0						0
8-20	2 <sup>SR</sup>						
(am)							
8-20	1 <sup>SR</sup>						3
(pm)							
8-21	1 <sup>BN</sup>						
	1 <sup>SR</sup>						2
8-22	1 <sup>SR</sup>						1
8-23	3 <sup>SR</sup>						3

Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1991</u>										
8-26	NS									NS
8-27	0									0
8-28	0									0
8-29	0									0
8-30	0									0
9-3	1 <sup>SR</sup>									1
9-4	1 <sup>SH</sup>				1 <sup>SH</sup>					2
9-5	1 <sup>SR</sup>									1
9-6			1 <sup>SH</sup> 1 <sup>SR</sup>							2
9-9	1 <sup>SR</sup>			1 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup>					4
9-10 (am)			3 <sup>SR</sup>	3 <sup>SR</sup>	3 <sup>RB</sup> 3 <sup>SH</sup> 33 <sup>SR</sup>		2 <sup>RB</sup> 49 <sup>SR</sup>	21 <sup>SR</sup>		
9-10 (pm)							1 <sup>SH</sup> 19 <sup>SR</sup>			137
9-11							4 <sup>SR</sup> 1 <sup>UK</sup>			5
9-12			1 <sup>SR</sup>	2 <sup>SH</sup> 2 <sup>SR</sup>				3 <sup>SR</sup>		8
9-13						1 <sup>UK</sup>	1 <sup>UK</sup>			2
9-16	0									0
9-17	1 <sup>SR</sup>		1 <sup>SR</sup>							2
9-18	2 <sup>SR</sup>		2 <sup>SR</sup>							4
9-19		1 <sup>SH</sup>					1 <sup>SR</sup>			2
9-20								1 <sup>SR</sup>		1

## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1991</u>										
9-23	0									0
9-24	NS									NS
9-25	0									0
9-26	0									0
9-27		1 <sup>UK</sup>						1 <sup>SH</sup> 2 <sup>SR</sup>		4
9-30			2 <sup>SR</sup>		1 <sup>SH</sup>		1 <sup>SR</sup>			4
10-1			2 <sup>SR</sup>							2
10-2	NS									NS
10-3	1 <sup>SR</sup>		1 <sup>SR</sup>							2
10-4	0									0
10-7	1 <sup>SH</sup>									1
10-8	1 <sup>SR</sup>		1 <sup>SH</sup>							2
10-9				1 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>RB</sup>					3
10-10	0									0
10-11	1 <sup>SR</sup>									1
10-14	0									0
10-15	0									0
10-16							1 <sup>SH</sup> 1 <sup>SR</sup>	3 <sup>SR</sup>	1 <sup>SH</sup>	6
10-17	2 <sup>SR</sup>		1 <sup>SR</sup>			1 <sup>SR</sup>	3 <sup>SR</sup>	1 <sup>SR</sup>	7 <sup>SR</sup>	15
10-18	3 <sup>SR</sup>		1 <sup>SR</sup>		1 <sup>SR</sup>		1 <sup>SR</sup>	3 <sup>SR</sup>	6 <sup>SR</sup>	15
10-21	3 <sup>SR</sup>						1 <sup>SR</sup>	2 <sup>SR</sup>		6
10-22					1 <sup>SR</sup>		1 <sup>SR</sup>			2
10-23				1 <sup>SH</sup> 1 <sup>SR</sup>		1 <sup>SH</sup>	1 <sup>SR</sup>	1 <sup>SR</sup>		5



## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1991</u>										
11-25			1 <sup>SR</sup>		1 <sup>SR</sup>		3 <sup>SR</sup>	15 <sup>SR</sup>	12 <sup>SR</sup>	32
11-26			1 <sup>SR</sup>	1 <sup>SR</sup>	1 <sup>SH</sup>	4 <sup>SR</sup>	1 <sup>RB</sup> 2 <sup>SH</sup> 19 <sup>SR</sup>	1 <sup>SH</sup> 17 <sup>SR</sup>	6 <sup>SR</sup>	53
11-27			1 <sup>RB</sup>		1 <sup>SR</sup>	5 <sup>SR</sup>	9 <sup>SR</sup>	1 <sup>RB</sup> 5 <sup>SR</sup>	3 <sup>SR</sup>	25
11-28					1 <sup>SR</sup>	2 <sup>SR</sup>	1 <sup>SH</sup> 9 <sup>SR</sup>	5 <sup>SR</sup>		18
11-29					1 <sup>SR</sup>		1 <sup>RB</sup> 4 <sup>SR</sup>		1 <sup>SR</sup>	7
12-2	NS									NS
12-3	NS									NS
12-4	1 <sup>CY</sup> 1 <sup>SH</sup>		1 <sup>RB</sup> 1 <sup>SR</sup>	3 <sup>SR</sup>	1 <sup>SH</sup>					8
12-5			1 <sup>BN</sup> 1 <sup>SH</sup>	1 <sup>SH</sup> 9 <sup>SR</sup>	2 <sup>SH</sup> 3 <sup>SR</sup>		2 <sup>SR</sup>	2 <sup>SR</sup>	1 <sup>SR</sup>	22
12-6	1 <sup>SR</sup>		1 <sup>SH</sup> 1 <sup>SR</sup>	2 <sup>SR</sup>	2 <sup>SH</sup>			1 <sup>SR</sup>	2 <sup>SH</sup> 2 <sup>SR</sup>	12
12-9	NS									NS
12-10			7 <sup>SR</sup>		1 <sup>SR</sup>				1 <sup>SR</sup>	9
12-11								1 <sup>SR</sup>		1
12-12			1 <sup>CY</sup>					1 <sup>SH</sup>		2
12-13	0									0
12-16	0									0
12-17			1 <sup>SR</sup>							1
12-18	0									0
12-19			1 <sup>SR</sup>							1
12-20			1 <sup>SR</sup>							1

## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1991</u>										
12-23	NS									NS
12-26			1 <sup>SR</sup>							1
12-27	0									0
12-30			1 <sup>SR</sup>							1
12-31	0									0
<u>1992</u>										
1-2				1 <sup>SR</sup>		1 <sup>SR</sup>				2
1-3	NS									NS
1-6	0									0
1-7	1 <sup>LM</sup>									1
1-8	0									0
1-9	0									0
1-10	1 <sup>SR</sup>									1
1-13	1 <sup>SH</sup>									1
1-14	0									0
1-15 (am)			2 <sup>SH</sup>		8 <sup>SH</sup>		7 <sup>SH</sup>		1 <sup>SH</sup>	
1-15 (pm)						1 <sup>SH</sup>	4 <sup>SH</sup>		1 <sup>SR</sup> 4 <sup>SH</sup>	28
1-16	1 <sup>SR</sup>				3 <sup>SH</sup>		5 <sup>SH</sup>	3 <sup>SH</sup> 1 <sup>SR</sup>		13
1-17			1 <sup>SH</sup> 1 <sup>SR</sup>		1 <sup>SH</sup>	3 <sup>SH</sup>	4 <sup>SH</sup> 1 <sup>SR</sup>	4 <sup>SH</sup> 2 <sup>SR</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>	19
1-18			1 <sup>SH</sup>		1 <sup>SH</sup>	2 <sup>SH</sup>	5 <sup>SH</sup>	2 <sup>SH</sup> 1 <sup>SR</sup>		12
1-19 (am)			1 <sup>SR</sup>		1 <sup>SH</sup>	1 <sup>SH</sup>	1 <sup>SH</sup>		1 <sup>SH</sup> 1 <sup>SR</sup>	
1-19 (pm)	0									6
1-20							2 <sup>SH</sup>			2

## Appendix C continued

Sample date <sup>d</sup>	Location									Total	
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6		
<u>1992</u>											
1-21			1 <sup>BN</sup> 1 <sup>SR</sup>		1 <sup>SR</sup>						3
1-22					2 <sup>SH</sup>		1 <sup>SH</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>			5
1-23	NS										NS
1-24						1 <sup>SH</sup> 1 <sup>SR</sup>		1 <sup>SH</sup>			3
1-27								4 <sup>SH</sup> 3 <sup>SR</sup>	7 <sup>SH</sup> 9 <sup>SR</sup>		23
1-28	0										0
1-29			1 <sup>SR</sup>				1 <sup>SH</sup>	1 <sup>SH</sup>	1 <sup>SH</sup>		4
1-30	0										0
1-31					1 <sup>SR</sup>			1 <sup>SR</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>		4
2-3						1 <sup>SR</sup>					1
2-4	0										0
2-5			1 <sup>SH</sup>					1 <sup>SR</sup>			2
2-6			1 <sup>SR</sup>			2 <sup>SR</sup>	1 <sup>SR</sup>	1 <sup>SR</sup>			5
2-7	0										0
2-10	0										0
2-11	1 <sup>SH</sup>		1 <sup>SH</sup>								2
2-12	0										0
2-13	0										0
2-14								1 <sup>SR</sup>	2 <sup>SR</sup>		3
2-18			1 <sup>SR</sup>		1 <sup>SR</sup>		2 <sup>SR</sup>	1 <sup>SR</sup>	6 <sup>SR</sup>		11
2-19							1 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup> 2 <sup>SR</sup>	4 <sup>SH</sup>		9

## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1992</u>										
2-20			1 <sup>SH</sup> 1 <sup>SR</sup>				2 <sup>SR</sup>	1 <sup>SH</sup> 8 <sup>SR</sup>	4 <sup>SH</sup> 2 <sup>SR</sup>	19
2-21							1 <sup>SR</sup>	2 <sup>SR</sup>	2 <sup>SR</sup>	5
2-24			1 <sup>CY</sup> 2 <sup>SR</sup>		2 <sup>SR</sup>		1 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SR</sup>		8
2-25	NS									NS
2-26	NS									NS
2-27						1 <sup>SR</sup>				1
2-28						1 <sup>SR</sup>		1 <sup>SR</sup>		2
3-2			2 <sup>BN</sup> 1 <sup>SH</sup>					1 <sup>SR</sup>		4
3-3			1 <sup>SH</sup> 3 <sup>SR</sup>			1 <sup>SR</sup>	1 <sup>SH</sup>	2 <sup>SR</sup>		8
3-4			3 <sup>SR</sup>			1 <sup>SR</sup>			1 <sup>SR</sup>	5
3-5			1 <sup>SR</sup>					1 <sup>SR</sup>		2
3-6			2 <sup>SR</sup>							2
3-9			5 <sup>SR</sup>							5
3-10	0									0
3-11			10 <sup>SR</sup>		4 <sup>SR</sup>					14
3-12			6 <sup>SR</sup>							6
3-13			2 <sup>SR</sup>							2
3-16					1 <sup>SR</sup>					1
3-17			1 <sup>SR</sup>		1 <sup>SR</sup>		1 <sup>SR</sup>			3
3-18			2 <sup>SR</sup>							2
3-19	NS									NS
3-20			3 <sup>SR</sup>		1 <sup>SR</sup>					4
3-23			1 <sup>SR</sup>							1

## Appendix C continued

Sample date <sup>d</sup>	Below LR	In LR	Div chn	Location						Total
				Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1992</u>										
3-24			1 <sup>CY</sup>	1 <sup>SR</sup>					1 <sup>SR</sup>	3
3-25			2 <sup>SR</sup>							2
3-26			1 <sup>SH</sup>		2 <sup>SH</sup> 3 <sup>SR</sup>			1 <sup>SR</sup>		7
3-27	1 <sup>SR</sup>		3 <sup>SR</sup>	1 <sup>SR</sup>	1 <sup>SR</sup>					6
3-30			1 <sup>SR</sup>		2 <sup>SR</sup>					3
3-31			1 <sup>BN</sup>	1 <sup>BN</sup>						2
4-1								1 <sup>SR</sup>		1
4-2			2 <sup>BN</sup> 2 <sup>SR</sup>							4
4-3	1 <sup>CP</sup>		1 <sup>SR</sup>		2 <sup>SR</sup>					4
4-6					1 <sup>SR</sup>		1 <sup>SR</sup>			2
4-7					2 <sup>SR</sup>		1 <sup>SR</sup>	1 <sup>SR</sup>		4
4-8			4 <sup>SR</sup>		1 <sup>SR</sup>					5
4-9			1 <sup>SR</sup>							1
4-10					1 <sup>SR</sup>	1 <sup>SR</sup>	1 <sup>SR</sup>	1 <sup>SR</sup>		4
4-13			7 <sup>SH</sup> 4 <sup>SR</sup>	1 <sup>SR</sup>	2 <sup>SH</sup> 1 <sup>SR</sup>		1 <sup>SR</sup>	1 <sup>SR</sup>		17
4-14	2 <sup>SR</sup>		2 <sup>BN</sup> 3 <sup>SR</sup>	1 <sup>SR</sup>	5 <sup>SR</sup>			3 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>RB</sup> 2 <sup>SH</sup> 3 <sup>SR</sup>	23
4-15	1 <sup>SR</sup>		2 <sup>SR</sup>	2 <sup>SR</sup>				1 <sup>SR</sup>		6
4-16	1 <sup>GZ</sup> 1 <sup>SK</sup> 1 <sup>SR</sup>		1 <sup>BN</sup> 1 <sup>SH</sup> 7 <sup>SR</sup>		1 <sup>SR</sup>					13
4-20	2 <sup>SR</sup>		2 <sup>SR</sup>		1 <sup>SR</sup>					5
4-21					1 <sup>SR</sup>					1
4-22			2 <sup>SR</sup>	1 <sup>SR</sup>				1 <sup>SR</sup>	1 <sup>SH</sup>	5

Appendix C continued

Sample date <sup>d</sup>	Location						Total			
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3		Site 4	Site 5	Site 6
<u>1992</u>										
4-23			3 <sup>SR</sup>							3
4-24			1 <sup>CY</sup>							1
4-27	1 <sup>RB</sup>		1 <sup>CY</sup> 1 <sup>SR</sup>							3
4-28	0									0
4-29			1 <sup>CY</sup>					1 <sup>SR</sup>	1 <sup>SR</sup>	3
4-30				1 <sup>SR</sup>						1
5-1			1 <sup>SR</sup>					1 <sup>SH</sup>		2
5-4	1 <sup>SR</sup>		2 <sup>SR</sup>							3
5-5			1 <sup>SH</sup> 2 <sup>SR</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>						5
5-6	0									0
5-7	0									0
5-8			1 <sup>SH</sup>							1
5-11			1 <sup>SH</sup>		1 <sup>SH</sup>	1 <sup>SR</sup>				3
5-12			1 <sup>UK</sup> 2 <sup>SR</sup>				1 <sup>SR</sup>			4
5-13			5 <sup>SR</sup>				1 <sup>SR</sup>			6
5-14	0									0
5-15			1 <sup>BN</sup> 1 <sup>SR</sup>							2
5-18	0									0
5-19				1 <sup>SR</sup>						1
5-20	1 <sup>SR</sup>		2 <sup>SR</sup>							3
5-21			2 <sup>SR</sup>		1 <sup>SR</sup>					3
5-22		1 <sup>SH</sup>	2 <sup>SR</sup>		1 <sup>SR</sup>					4



## Appendix C continued

Sample date <sup>d</sup>	Location						Total			
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3		Site 4	Site 5	Site 6
<u>1992</u>										
6-29			1 <sup>SH</sup> 1 <sup>SR</sup>							2
6-30	3 <sup>SR</sup>									3
7-1			1 <sup>SR</sup>							1
7-6			2 <sup>SR</sup>							2
7-7 (am)			1 <sup>SR</sup>							
7-7 (pm)			1 <sup>BN</sup> 4 <sup>SR</sup>	1 <sup>SH</sup>	2 <sup>SH</sup> 2 <sup>SR</sup>			1 <sup>SH</sup>		12
7-8 (am)	1 <sup>BN</sup> 1 <sup>LM</sup> 3 <sup>SR</sup>		1 <sup>SH</sup>	1 <sup>SH</sup>						
7-8 (pm)	3 <sup>SH</sup> 1 <sup>SR</sup>		1 <sup>SH</sup>		1 <sup>BN</sup>		1 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup>		16
7-9			3 <sup>SR</sup>		1 <sup>SH</sup>		1 <sup>SR</sup>			5
7-10	1 <sup>SR</sup>		1 <sup>SR</sup>	1 <sup>BN</sup>	1 <sup>SH</sup>		1 <sup>SH</sup>	1 <sup>RB</sup> 2 <sup>SH</sup>		8
7-13			1 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SR</sup>						3
7-14			1 <sup>SH</sup>		2 <sup>SH</sup>					3
7-15	1 <sup>SR</sup>		1 <sup>SR</sup>							2
7-16			1 <sup>SH</sup> 1 <sup>SR</sup>							2
7-17 (am)			2 <sup>SR</sup>		1 <sup>SH</sup>	1 <sup>SH</sup>				
7-17 (pm)			1 <sup>SH</sup> 2 <sup>SR</sup>							7
7-20	0									0
7-21			1 <sup>SR</sup>							1
7-22			1 <sup>SH</sup> 3 <sup>SR</sup>							4
7-23			1 <sup>SR</sup>							1



Appendix C continued

Sample date <sup>d</sup>	Location						Total			
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3		Site 4	Site 5	Site 6
1992										
8-27								1 <sup>SR</sup>		1
8-28	0									0
8-31	1 <sup>SR</sup>									1
9-1			1 <sup>SH</sup>							1
9-2			1 <sup>BN</sup> 1 <sup>SR</sup>				1 <sup>SR</sup>			3
9-3			1 <sup>SR</sup>							1
9-4	0									0
9-8			1 <sup>SR</sup>							1
9-9			1 <sup>SH</sup>							1
9-10	2 <sup>SH</sup>									2
9-11									1 <sup>SH</sup> 1 <sup>SR</sup>	2
9-13							1 <sup>SH</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>	1 <sup>SH</sup> 4 <sup>SR</sup>	8
9-14			1 <sup>SR</sup>					2 <sup>SH</sup> 1 <sup>SR</sup>		4
9-15						1 <sup>CY</sup> 1 <sup>SR</sup>				2
9-16				1 <sup>SH</sup>						1
9-17	NS									NS
9-21			2 <sup>SR</sup>							2
9-22	1 <sup>SR</sup>		1 <sup>SH</sup> 1 <sup>SR</sup>							3
9-23			1 <sup>BN</sup>			1 <sup>SR</sup>				2
9-24			1 <sup>SR</sup>					2 <sup>SR</sup>	1 <sup>SR</sup>	4
9-25							1 <sup>SR</sup>			1
9-28			1 <sup>SR</sup>						2 <sup>SR</sup>	3

## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1992</u>										
9-29									1 <sup>SR</sup> 1 <sup>SU</sup>	2
9-30								1 <sup>SR</sup>		1
10-1	0									0
10-2	1 <sup>SR</sup>				1 <sup>SR</sup>				1 <sup>SH</sup> 2 <sup>SR</sup>	5
10-5	0									0
10-6			1 <sup>SR</sup>							1
10-7 (am)					1 <sup>SR</sup>			3 <sup>SR</sup>	1 <sup>SH</sup> 19 <sup>SR</sup>	
(pm)			1 <sup>SR</sup>	1 <sup>SR</sup>					12 <sup>SR</sup>	38
10-8			1 <sup>SR</sup>				1 <sup>SR</sup>	2 <sup>SR</sup>	13 <sup>SR</sup>	17
10-9									13 <sup>SR</sup> 1 <sup>SH</sup>	14
10-10									3 <sup>SR</sup>	3
10-12				1 <sup>SR</sup>	1 <sup>RB</sup> 1 <sup>SR</sup>			1 <sup>BN</sup>		4
10-13						1 <sup>SH</sup>				1
10-14							1 <sup>SH</sup>			1
10-15					3 <sup>SR</sup>	1 <sup>SH</sup>	1 <sup>SH</sup>		1 <sup>SH</sup>	6
10-16			1 <sup>SH</sup> 1 <sup>SR</sup>							2
10-19	0									0
10-20	1 <sup>SR</sup>									1
10-21	1 <sup>SH</sup> 1 <sup>SR</sup>								1 <sup>SH</sup>	3
10-22	1 <sup>SR</sup>		2 <sup>SR</sup>							3
10-23								3 <sup>SR</sup>		3

Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1992</u>										
10-26	0									0
10-27	1 <sup>SH</sup>									1
10-28	1 <sup>SH</sup>		1 <sup>SR</sup>							2
10-29									1 <sup>SH</sup>	1
10-30	1 <sup>MQ</sup>									1
11-2	NS									NS
11-3	0									0
11-4	NS									NS
11-5	NS									NS
11-6								1 <sup>SR</sup>		1
11-9								1 <sup>SR</sup>		1
11-10 (am)				113 <sup>SH</sup> 7 <sup>SR</sup> 7 <sup>BN</sup>	19 <sup>SH</sup> 7 <sup>SR</sup> 1 <sup>BN</sup>	1 <sup>SH</sup> 8 <sup>SR</sup>	1 <sup>SH</sup> 22 <sup>SR</sup> 1 <sup>BN</sup> 1 <sup>RB</sup>	9 <sup>SR</sup>	19 <sup>SR</sup>	
11-10 (pm)			1 <sup>SH</sup>	1 <sup>BN</sup> 5 <sup>SH</sup> 1 <sup>SR</sup>		1 <sup>BN</sup>		16 <sup>SR</sup>	1 <sup>BN</sup> 1 <sup>RB</sup> 1 <sup>SH</sup> 19 <sup>SR</sup>	263
11-11 (am)			2 <sup>SH</sup> 2 <sup>SR</sup> 1 <sup>UK</sup>		2 <sup>BN</sup> 1 <sup>RB</sup> 39 <sup>SH</sup> 14 <sup>SR</sup> 1 <sup>UK</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>	4 <sup>BN</sup> 1 <sup>SH</sup> 4 <sup>SR</sup>	2 <sup>BN</sup> 2 <sup>RB</sup> 10 <sup>SR</sup>	21 <sup>SR</sup>	
11-11 (pm)					3 <sup>BN</sup> 1 <sup>RB</sup> 10 <sup>SH</sup> 8 <sup>SR</sup>	1 <sup>RB</sup> 1 <sup>SH</sup> 1 <sup>SR</sup>	2 <sup>RB</sup> 5 <sup>SR</sup>	2 <sup>RB</sup> 12 <sup>SR</sup>	11 <sup>SR</sup>	165
11-12		1 <sup>SU</sup>	2 <sup>SH</sup>		2 <sup>RB</sup> 12 <sup>SH</sup> 4 <sup>SR</sup>		5 <sup>RB</sup> 3 <sup>SR</sup>			29

## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1992</u>										
11-13			1 <sup>SH</sup> 3 <sup>SR</sup>				1 <sup>RB</sup>		1 <sup>SR</sup>	6
11-16				4 <sup>SR</sup>		2 <sup>SR</sup>	6 <sup>SR</sup>	2 <sup>BN</sup> 13 <sup>SR</sup>	1 <sup>SH</sup> 2 <sup>SR</sup>	30
11-17 (am)					3 <sup>BN</sup> 4 <sup>SH</sup> 9 <sup>SR</sup>	1 <sup>BN</sup> 1 <sup>SR</sup>	1 <sup>BN</sup> 1 <sup>RB</sup> 1 <sup>SH</sup> 12 <sup>SR</sup>	1 <sup>RB</sup> 2 <sup>SH</sup> 7 <sup>SR</sup>	1 <sup>SH</sup> 1 <sup>SR</sup>	
11-17 (pm)			1 <sup>SR</sup>		3 <sup>SR</sup>	1 <sup>RB</sup>	2 <sup>SR</sup>	3 <sup>SR</sup>		55
11-18			1 <sup>BN</sup>		2 <sup>BN</sup> 6 <sup>SH</sup> 7 <sup>SR</sup>	1 <sup>BN</sup> 1 <sup>RB</sup> 7 <sup>SR</sup>	2 <sup>RB</sup> 1 <sup>SH</sup> 20 <sup>SR</sup>	1 <sup>BN</sup> 1 <sup>SH</sup> 5 <sup>SR</sup>		55
11-19			1 <sup>RB</sup> 1 <sup>CY</sup>		1 <sup>BN</sup> 1 <sup>RB</sup> 3 <sup>SR</sup>	1 <sup>BN</sup> 1 <sup>RB</sup> 5 <sup>SR</sup>	2 <sup>RB</sup> 2 <sup>SH</sup> 7 <sup>SR</sup>	1 <sup>SH</sup> 6 <sup>SR</sup>		32
11-20			1 <sup>SR</sup>	3 <sup>SR</sup>	2 <sup>SR</sup>	3 <sup>RB</sup> 1 <sup>SR</sup>	1 <sup>BN</sup> 4 <sup>RB</sup> 2 <sup>SH</sup> 17 <sup>SR</sup>			34
11-21			1 <sup>RB</sup> 1 <sup>SR</sup>							2
11-22										
11-23		1 <sup>RB</sup>				1 <sup>SR</sup>		1 <sup>SR</sup>		3
11-24					1 <sup>SR</sup>		1 <sup>SR</sup>	2 <sup>BN</sup> 1 <sup>SH</sup> 9 <sup>SR</sup>	2 <sup>BN</sup> 2 <sup>SH</sup> 19 <sup>SR</sup>	37
11-25	1 <sup>BG</sup>	1 <sup>SK</sup>	2 <sup>RB</sup>	1 <sup>SH</sup>	4 <sup>SH</sup> 3 <sup>SR</sup>					12
11-26	1 <sup>SK</sup>		1 <sup>SR</sup>		1 <sup>BN</sup> 4 <sup>SH</sup> 10 <sup>SR</sup>	1 <sup>SR</sup>				18
11-27			1 <sup>SH</sup>		3 <sup>SH</sup>			1 <sup>SH</sup>		5



## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1992</u>										
12-28			2 <sup>SH</sup> 1 <sup>SR</sup>		7 <sup>SH</sup> 3 <sup>SR</sup>					13
12-29			1 <sup>SH</sup> 3 <sup>SR</sup> 11 <sup>UK</sup>		2 <sup>SR</sup>					17
12-30	1 <sup>SR</sup>		4 <sup>UK</sup>		1 <sup>BN</sup> 1 <sup>SR</sup>	1 <sup>SR</sup>				8
12-31			1 <sup>SR</sup>		1 <sup>BN</sup> 3 <sup>SR</sup>					5
<u>1993</u>										
1-4-93								1 <sup>SR</sup>		1
1-5	0									0
1-6	1 <sup>SR</sup>		1 <sup>SR</sup>	1 <sup>SR</sup>						3
1-7			1 <sup>SR</sup>			1 <sup>SR</sup>	1 <sup>SR</sup>			3
1-8	0									0
1-11	NS									NS
1-12	0									0
1-13					1 <sup>SR</sup>	1 <sup>SR</sup>				2
1-14							1 <sup>SR</sup>		1 <sup>SR</sup>	2
1-15	0									0
1-18				1 <sup>SR</sup>	1 <sup>SR</sup>					2
1-19 (am)	0									
1-19 (pm)					1 <sup>BN</sup> 10 <sup>SH</sup> 1 <sup>SR</sup>					12
1-20 (am)					2 <sup>BN</sup> 31 <sup>SH</sup> 2 <sup>SR</sup>			2 <sup>SR</sup>		

## Appendix C continued

Sample date <sup>d</sup>	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<u>1993</u>										
1-20 (pm)					1 <sup>BN</sup> 22 <sup>SH</sup> 1 <sup>SR</sup>					61
1-21					1 <sup>BN</sup> 12 <sup>SH</sup> 4 <sup>SR</sup>					17
1-22					1 <sup>SR</sup>	1 <sup>SR</sup>				2
1-25	0									0
1-26	1 <sup>SR</sup>									1
1-27	1 <sup>SR</sup>								1 <sup>SH</sup>	2
1-29	0									0
2-1									1 <sup>SR</sup>	1
2-2									1 <sup>SR</sup>	1
2-3	0									0
2-4	0									0
2-5	0									0
2-8									2 <sup>SR</sup>	2
2-9									1 <sup>SR</sup>	1
2-10									2 <sup>SR</sup>	2
2-11	0									0
2-12									1 <sup>SR</sup> 1 <sup>SH</sup>	2
2-17*	0									0
2-19									1 <sup>SH</sup>	1
2-22	NS									NS
2-24									2 <sup>SH</sup> 3 <sup>SR</sup> 3 <sup>BN</sup> 6 <sup>SH</sup> 5 <sup>SR</sup>	19



## Appendix C continued

	Location									Total
	Below LR	In LR	Div chn	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<b>Totals:</b>										
BA	1									1
BN	9	1	40	16	22	4	7	9	4	112
BG	100	216	4		1					321
CY	2	3	11	37	1	1				55
FH	30	4	1							35
GZ	3									3
MQ	4									4
RB	147	588	53	2	12	8	24	8	7	849
SH	63	7	109	165	339	66	152	79	82	1062
SK	3	3	1							7
SR	234	11	486	78	211	77	309	250	261	1917
SU	26	23	3	4		1			1	58
UK	8	7	20		1	1	3	1		41
CP	2									2
GS	2	14								16
RO	1									1
SS	1									1
DR	1									1
LM	2									2

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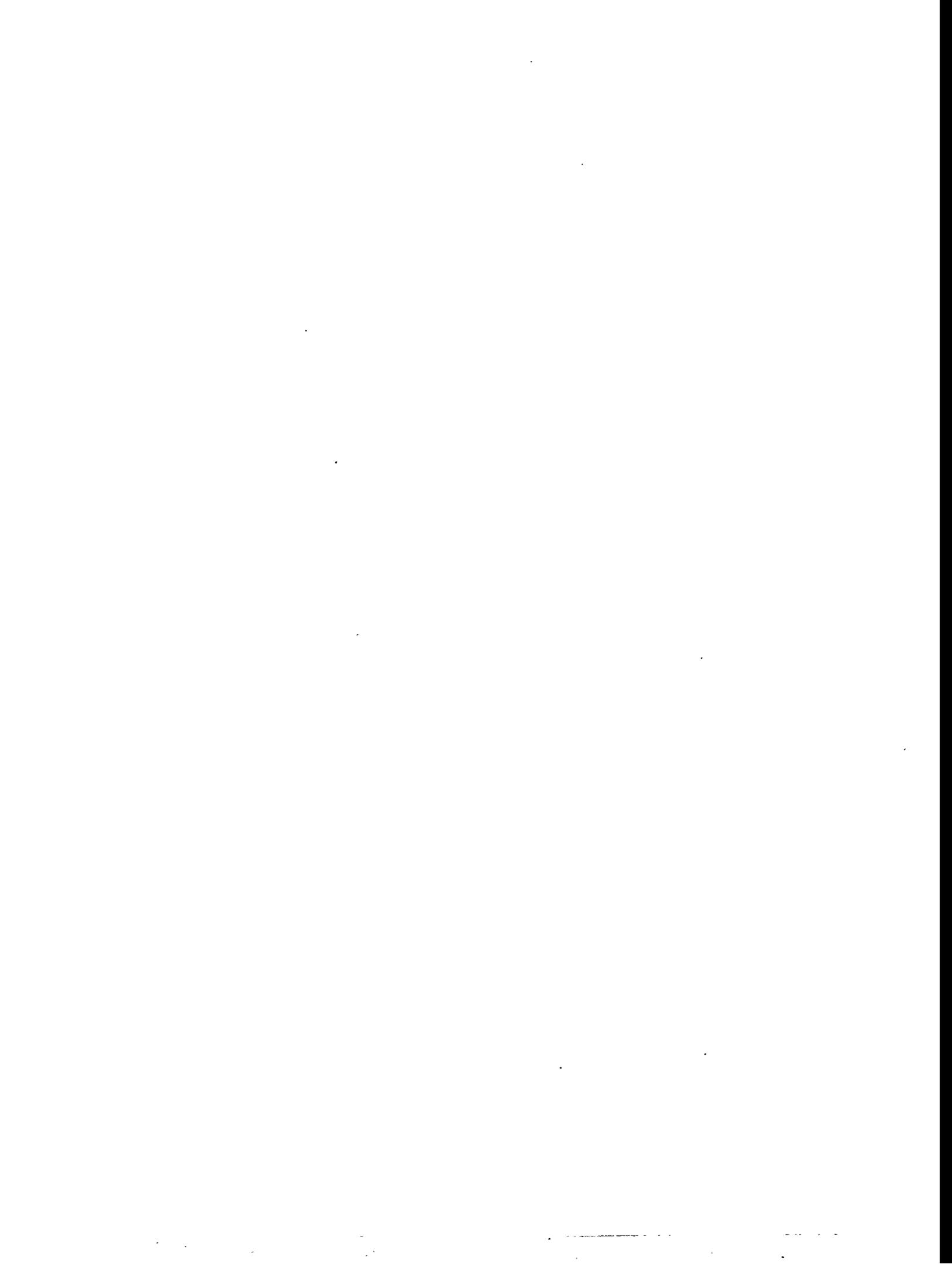
## Footnotes for Appendix C

- a. Abbreviations: BA = unidentified bass species (*Micropterus* sp.), BG = bluegill (*Lepomis macrochirus*), BN = blacknose dace (*Rhinichthys atratulus*), CP = common carp (*Cyprinus carpio*), CY = unidentified cyprinid species, DR = freshwater drum (*Aplodinotus grunniens*), FH = fathead minnow (*Pimephales promelas*), GS = green sunfish (*Lepomis cyanellus*), GZ = gizzard shad (*Dorosoma cepedianum*), LM = largemouth bass (*Micropterus salmoides*), MQ = western mosquitofish (*Gambusia affinis*), NS = site not sampled due to high water or turbidity, RB = redbreast sunfish (*Lepomis auritis*), RO = rock bass (*Ambloplites rupestris*), SH = striped shiner (*Luxilus chrysocephalus*), SK = white sucker (*Catostomus commersoni*), SR = central stoneroller (*Campostoma anomalum*), SS = spotted sucker (*Minytrema melanops*), SU = unidentified sunfish (*Lepomis* sp.), UK = unidentified fish species, and YB = yellow bullhead (*Ameiurus natalis*).
- b. Morning surveys were conducted by FCS staff and afternoon surveys were conducted by Y-12 Department of Environmental Management (DEM) staff.
- c. Mortality increased because of impingement of fish on blocknets.
- d. Y-12 DEM staff made daily surveys on October 8 to 12, March 7, March 21, April 2, April 5, April 12, April 18, April 19, April 25, April 26, May 14, June 10-14, June 17-21 and June 25.
- e. Mortality increased because of electroshocking activity in area.
- f. Survey was made by inexperienced Y-12 DEM staff, resulting in poor locality data (inside Y-12) and questionable species identification. A follow-up survey was not made on June 16.

**Appendix D**

**STATISTICAL WORK ON FISH KILLS IN  
EAST FORK POPLAR CREEK**

**AN OVERVIEW REPORT**



## APPENDIX D

### STATISTICAL WORK ON FISH KILLS IN EAST FORK POPLAR CREEK AN OVERVIEW REPORT

Bud Leete  
Y-12 Statistical Applications  
January 14, 1994

#### ABSTRACT

Statisticians participated in the study of fish kills in East Fork Poplar Creek for several years beginning in the fall of 1991. Two specific methods that we used may be of interest to others involved in similar studies. This report describes our use of control charts and our work to model fish kills by using basic stepwise regression.

#### INTRODUCTION

In the fall of 1991 the Y-12 Plant Manager remarked in a meeting that Y-12 Plant was spending a lot of time tracking occurrences and not enough time making things better. He suggested two specific areas for improvement. One was East Fork Poplar Creek. Statisticians from Y-12's Statistical Application Department were asked to participate with other people who were already working hard to identify and eliminate fish kill occurrences. In our first meeting with Clarence Hill of the Health, Safety, and Environmental Division, he commented "You'll find that this is a very complicated issue. There isn't one reason why the fish die. There are lots of independent reasons. There won't be a simple solution." We would come to appreciate how right he was.

#### EARLY DATA COLLECTION

One of the initial difficulties we experienced was with the data on fish kills. Although many people had data we needed, no person or group had worked to collect data for an organized study. We found that one group had the information on the fish kills, another group had chemistry results from the creek, and still another had data from the monitoring stations at Stations 8 and 17. Other organizations had information on events such as spills and observations made by walking the creek in daily surveillances. The process of learning who maintained what types of information was slow.

Y-12 Plant staff also contributed creek surveillances and reports on a regular basis, as requested by ORNL personnel. The idea for collecting and building a data base of daily observations gelled when we saw the information that Stan Duke, one of the environmental

engineers, maintained. He had a computer spreadsheet of daily observations showing how many fish were found, what types of fish were found, and where along the creek the dead or dying fish were discovered. We learned later that his work was made possible by the very thorough reports that environmental scientists at ORNL issued each day when they surveyed the creek.

We decided to collect all the information we could from the various sources and build a data base of daily observations to use for study. We placed the database on a Digital mainframe computer at the Y-12 Plant. Our hope was to be able to study fish kills to see if these kills could be statistically correlated with the measurements and observed events that were being taken routinely.

### GROWTH OF THE DATA BASE

Over the months that followed, we worked to build and enhance the data base. We put in the daily fish observations, the chemistry data from samples at Station 17 (which is located near where the water exits the Y-12 site), and creek real-time monitoring data from Station 8. As people saw what we were doing, they offered more ideas for things to add. What about the weather that day? What about the occurrences of spills, foam, and shock testing to learn of the fish population? What about the water usage at the Y-12 Plant that day? How about the data from Station 8, which is the monitoring station located much closer to the North-South pipes? We continued to add results.

Figure D.1 shows the final list of variables in the database we created.

General Information			
Date			
Total Fish Found			
Fish Found at Locations: A, B, C, D, E, F, G, H, I, J, K (if 3 or more fish found)			
Weather for the Date			
Max Temperature			
Min Temperature			
Inches of Precipitation			
Fraction of day covered by clouds			
Station 17 Conditions		Station 8 Condition	
pH		pH	
Temperature		Temperature	
Conductivity		Conductivity	
Dissolved Oxygen		Dissolved Oxygen	
Water Flow		Water Flow	
Station 17 Chemistry			
Alkalinity	Nitrate	Sulfate	Molybdenum
Cadmium	Organic Carbon	Calcium	Potassium
Chlorides	Phosphorous	Chromium	Sodium
Fluoride	Dissolved Solids	Copper	Zinc
Lead	Suspended Solids	Lithium	
Nickel	Selenium	Magnesium	
Other Additions:			
Chlorine Data (added later) in three locations			
Y-12 Water Usage			
Reason for Kill (if known)			
Notes (A place for observations)			

Fig. D.1. Items maintained in data base.

Figure D.2 shows a single observation from the data base. It is the observation for April 18, 1991. The bolder figures in the figure are the actual data results.

EAST FORK POPLAR CREEK DATA	
Date: <b>18APR91</b>	Fish Found: <b>6</b> Locations: A: ___ B: ___ C: ___ D: ___ E: ___ F: ___ G: <b>4</b> H: ___ I: ___ J: ___ K: ___
Weather: Max_T: <b>86</b>	Min_T: <b>49</b> Precip: <b>0</b> Cloud_cov: <b>0.8</b>
Station 17: pH: <b>8</b>	Temp: <b>68</b> Cond: <b>65</b> DisO: <b>6.8</b> CFS: <b>14</b>
Station 8: pH: <b>8.1</b>	Temp: <b>72</b> Cond: <b>68</b> DisO: <b>8.7</b> CFS: <b>3.8</b>
Station 17 Chemistry:	Alka: <b>110</b> Cd: <b>0.0005</b> Chrd: <b>22</b> Fl: <b>0.73</b>
	Pb: <b>0.001</b> Ni: <b>0.04</b> Nitrate: <b>6.6</b> TOC: <b>22</b> Phos: <b>0.27</b> TDS: <b>290</b>
	TSS: <b>5</b> Se: <b>0.002</b> Sulfate: <b>85</b> Ca: <b>56.3</b> Cr: <b>0.006</b> Cu: <b>0.006</b>
	Li: <b>0.03</b> Mg: <b>11.5</b> Mo: <b>0.006</b> Pot: <b>3</b> Na: ___ Zn: ___
ORNL Chlorine Data: N/S: ___	Sta_8: ___ Down: ___ Y-12 Water: <b>5.7</b>
Reason: _____	Notes: _____

Fig. D.2. Sample day from the data base.

## EARLY MODELING ATTEMPTS

Readers familiar with linear modeling can see how the data organization allows modeling to be done. Using SAS software on the mainframe computer, we made efforts to build a linear model. We tried to predict fish kills on a daily basis from the available process variables.

To explain the methods, the idea is to build a model of the type . . .

$$\text{Fish} = k + a(V_1) + b(V_2) + c(V_3) + \dots$$

where the lower case letters are constants and the  $V_i$  are various levels of observed process variables.

The computer chooses the variable that best correlates with fish findings. This variable is included in a model. The computer then looks at the unexplained variation, called residual variation, in findings and chooses the variable that best correlates with the residual variation. It incorporates this new variable into the model. This process continues until the terms added no longer make a statistically significant contribution to the model.

The early modeling efforts were not very successful. The more we studied the results, the more complex we realized the total system was. Many factors came into play. For example,

1. Some kill events were catastrophic events, killing hundreds of fish. Other kills were minor in comparison. In most cases, we could not explain the catastrophic events with correlations to variables in our data. This quickly resulted in a lack of fit to the modeling attempts.
2. Kill events could last for several days. One problem with lack of fit stemmed from the fact that fish could die for several days after measured water quality had seemingly returned to normal conditions.
3. Other problems arose with trying to model on a daily basis. On some days, no survey was taken. On others, the survey might not correspond very closely in time to the water quality sampling.
4. We realized that the ecological system was quite dynamic. Suppose a sudden outpouring of solution containing copper at some level into the creek would kill fish within 3 m of the point of entry. For the model to discover the relationship between fish and copper, the timing and circumstances would have to be consistently uniform. By nature, this wouldn't occur. On some days there might be no fish in the neighborhood; on others there might be many. On some days, the copper might be detected in the sample; on others, the sampling might have been done before the solution entered the water.
5. From the early efforts, we learned three things of significance: (1) there would not be a simple solution, (2) we needed a way to sort out the catastrophic kill days from the normal-mortality kill days, and (3) modeling could better be done on a weekly basis than on a daily basis.

## USE OF CONTROL CHARTS

Control chart methods came quickly to mind as we searched for a way to sort out the catastrophic kill days from the normal-mortality kill days. Control charts are special graphs in which observations are plotted in time order and assessed for statistical signals of special causes.

Attachment A shows step-by-step instructions for how to construct an Individuals Control Chart for this type of data. In our data, we computed how many fish were killed each calendar week and constructed the charts following the methods outlined.

These charts are described in statistical literature. They are useful for distinguishing unusual occurrences in results. Following Attachment A is a copy of the Individuals Control Chart for weekly kills in East Fork Poplar Creek is presented. The notes show what was learned as a result of investigations. As indicated by the chart, the average number of kills for the time frame was 14 and the upper control limit was 48. The chart thus becomes an accepted way to distinguish normal variation from abnormal.

## FURTHER EFFORTS TO MODEL

We continued with our efforts to build a model to help us in our studies. We were able to develop a model that explained about 33% of the results, which we felt represented the underlying system when catastrophic events were not present. To eliminate the catastrophic events, we excluded those weeks in which more than 48 fish died and attempted to model the remaining weeks with variables that we could measure. We used averages of chemical results, averages for temperatures and cloud cover, and in some cases, ranges of measures across the week.

One model of interest was . . .

$$\text{FISH} = 97 - 10.3 (\text{pH average at Station 8}) - 18.3 (\text{average fraction of cloud cover for the week}) + 1.1 (\text{range of the average daily temperature at Station 8 for the week}) + 1.4 (\text{range of the average daily pH readings at Station 8 for the week}).$$

This model gives the indication that fish kill rates are lower at higher pH values (within the range of our data) and on cloudy days. Kill rates are higher for weeks with higher fluctuations in water temperature and water pH during the week.

The final chart shows both the control chart and the predicted kills from the model.

## SUMMARY

In summary, statistical methods were useful in providing insight into when to respond to kills. They were useful in discussions with the Department of Energy and the state of Tennessee in deciding what magnitudes of fish kills should be reported at what organizational levels. They also produced statistical evidence that not all fish kills are the result of negligence or poor management practices. Some, though certainly not all, could be found to correlate with weather conditions such as temperature changes and cloud conditions.

As a final note, the data base is no longer being maintained. In the spring of 1993, the daily surveillances of East Fork Poplar Creek were discontinued. Without the key response variable of fish findings, the data base loses much of its value as a tool for study. The methods of analysis could be useful to others working with similar data.

ATTACHMENT A

CONTROL CHARTING WEEKLY FISH KILL DATA  
(A Step-by-Step Guide)

1. Here are the criteria for a "stable" process. Individuals chart: (1) Any result outside the control limits, (2) eight or more consecutive points on the same side of the central line, and (3) six or more points consecutively larger (or smaller) than the previous point. Range Chart: (1) Any moving range above the upper control limit.
2. Assemble your data. Charts will be constructed the number of fish killed each week. Use the results from at least 30 weeks.
3. List the results in time order.
4. Calculate the individual moving ranges of two. Let's say you have 30 numbers listed in time order. You will calculate 29 ranges. For example, assume you have these values for the number of fish found. . .

	<u>Value</u>	<u>Moving range</u>	
1	0	*	←No value of range . . .
2	4	4	
3	5	1	
4	2	3	

All the ranges will be positive numbers. For each value, find the smaller of the value and the value just before it. Subtract this smaller value from the larger value.

5. Average the ranges. This answer is  $\bar{R}$ .
6. Multiply  $\bar{R}$  by 3.27. This answer is the upper control limit for the R chart, since  $D_4 = 3.27$ .
7. Scan the list of moving ranges.
  - °°° If all the moving ranges fall below the upper control limit, go to Step 8. If not, do this:
    - A. Recalculate the average of the ranges, omitting any moving ranges that falls outside the upper limit. This new average is now  $\bar{R}$ . If two consecutive moving ranges fall outside the upper limit, note the weekly kill result that contributes to both of these consecutive moving ranges. Make a check mark by it on your list.
    - B. Using your new value of  $\bar{R}$ , go back to Step 6 and start the sequence again. Cycle through steps 6 and 7 until all the moving ranges fall below the upper control limit.
8. Plot the R chart. Draw the center line at  $\bar{R}$ . Plot all the individual moving range values, even those you did not use in computing the average range. Draw the upper control limit at the answer for step 6 (on your final time through the step). There is no lower control limit for the range chart. Are any moving ranges larger than the upper control limit? If so, find out why.
9. Compute the average of all the weekly fish kill results. Do not include any values that you marked in step 7A. This answer is  $\bar{x}$ .
10. Multiply  $\bar{R}$  by 2.66. This answer will be added to  $\bar{x}$  to get the upper control limit for the Individuals chart. This same answer will be subtracted from  $\bar{x}$  to get the lower control limit for the Individuals chart.
11. Plot the individuals chart. Plot all of the individual values, even those you did not use in computing the average. Draw the center line at  $\bar{x}$ . Draw the upper control limit and the lower control limit at the places determined by following step 10. Note: if the results are skewed significantly, draw the center line at the median value.
12. Study the individuals chart. Do the values meet the criteria in step 1 for a stable process? If not, find out why.
13. As new values come in, add them to the charts. If nonstability is indicated, find out why.
14. If you observe eight or more consecutive points on the same side of  $\bar{R}$  in the range chart, consider this as a signal that the control limits need revision.

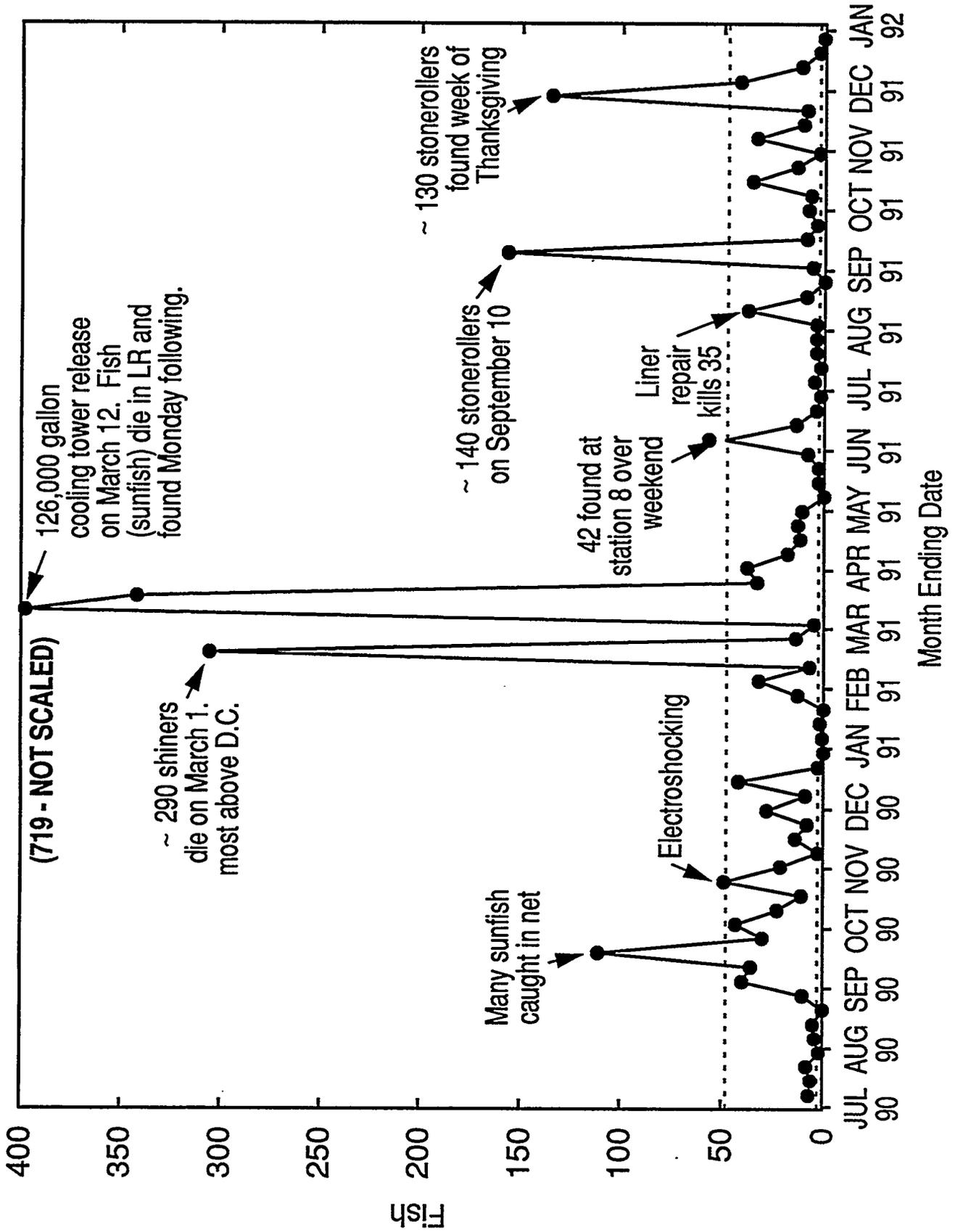


Fig. D.3. Weekly East Fork Poplar Creek fish kills.

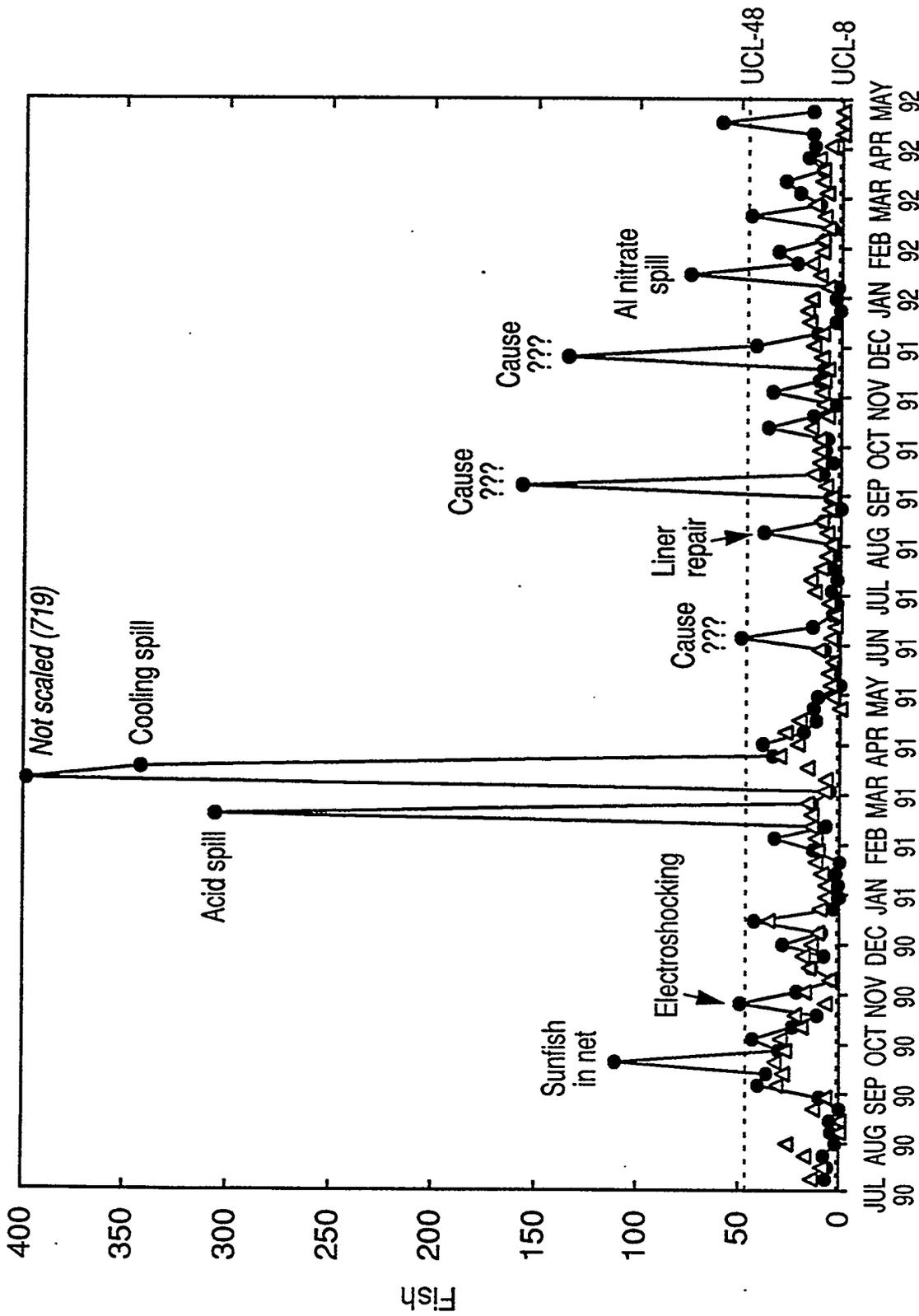
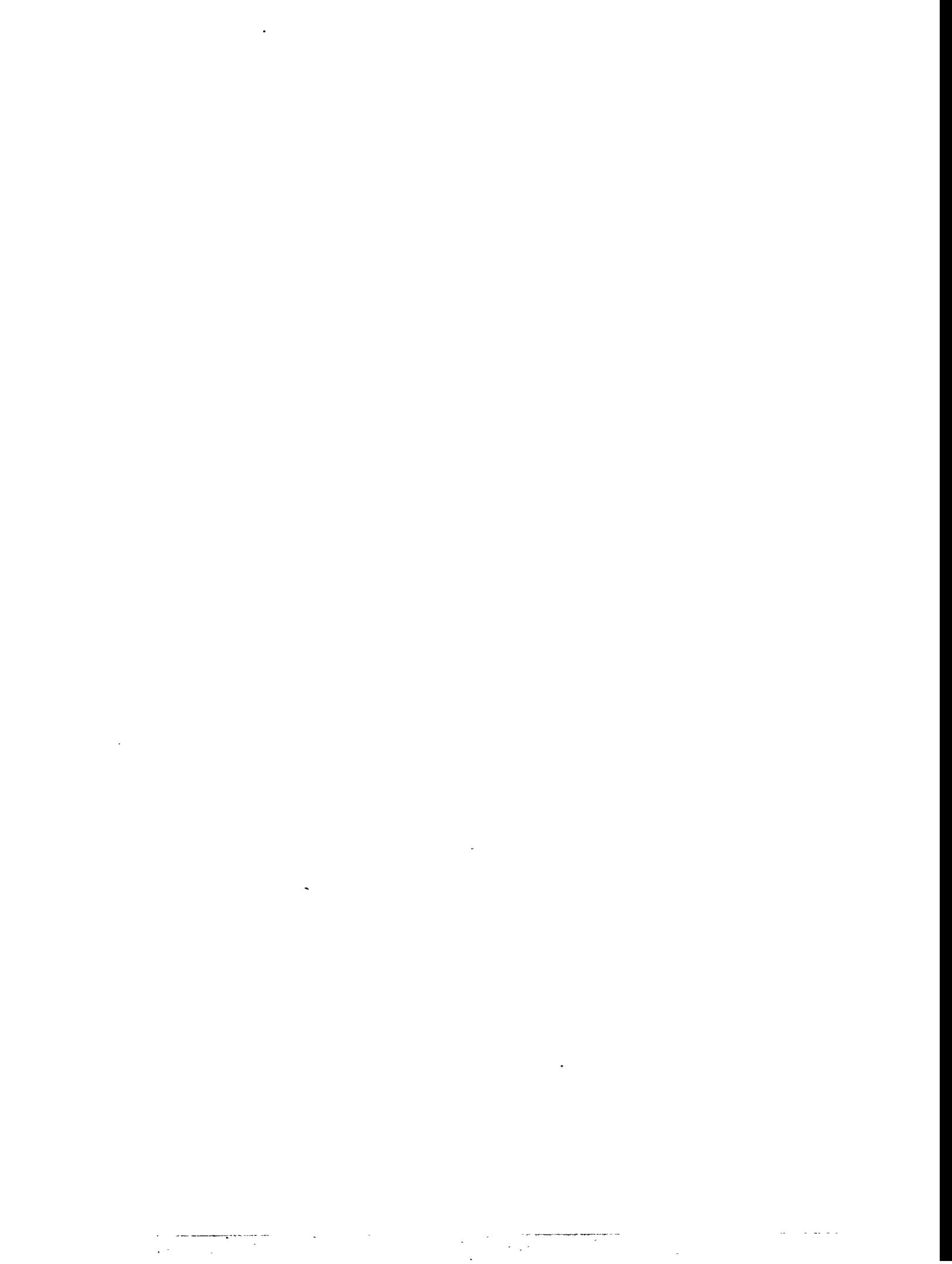


Fig. D.4. Weekly East Fork Poplar Creek fish kills compared with prediction model. Triangles show the predicted number of dead fish based on the following model: Dead fish = 97 minus 10.3 x average pH @ Station 8, plus 1.4 x range of the average fractional cloud cover for the week, plus 1.1 x average temperature @ Station 8, minus 18.3 x average fractional cloud cover for the week, plus 1.1 x average temperature @ Station 8, plus 1.4 x range of the average daily pH @ Station 8.

**Appendix E**

**WATER CHEMISTRY CONTROL CHARTS**



## APPENDIX E

### WATER CHEMISTRY CONTROL CHARTS

Moving range control charts were used as a statistical tool to evaluate environmental and water chemistry data for upper EFPC for the time period from July 1990 through December 1991. A list of the parameters used in the data base and their abbreviations are given in Table E.1. Most water chemistry data were obtained from monitoring stations located along EFPC (i.e., Station 17 and AS-8); a typical 1-d set of observations is shown in Fig. E.1. Values used in the data base were weekly averages or weekly ranges. Ranges were the absolute difference in the highest and lowest values observed during the week. A "week" was defined as the time period from Sunday through Saturday (see Table E.2).

Typical examples of water chemistry control charts are shown in Figs. E.2 and E.3 for pH; E.4 and E.5 for total suspended solids (TSS); and E.6 and E.7 for total dissolved solids (TDS). In each figure, the numbers plotted on the top chart are the averages of 1 to 7 values reported for the week. The values plotted on the bottom chart are the ranges used to make up the corresponding averages. Horizontal lines on both the top and bottom charts delineate the upper control limit ( $3\sigma$ ), average, and lower control limit ( $3\sigma$ ) for that parameter. Vertical reference lines denote weeks when a significant fish kill occurred (see Appendix D).

Signals of special causes of variation include: (1) an average value outside the control limits; (2) eight consecutive averages on the same side of the center line; and (3) a range value outside the limits. A special cause is an indication that the variation has resulted from some change in the underlying system. When special cause signals coincide with significant fish kill events, they may provide a clue as to the factors that produced the fish kill.

Table E.1. Parameter abbreviations in the data base

Abbreviation	Description
ALKA	Alkalinity: Station 17
CA	Calcium: Station 17
CD	Cadmium: Station 17
CHRIDE	Chloride: Station 17
CL_8	Chloride: Station 8
CL_DS	Chloride:
CL_NS	Chloride: NS Pipe
COND	Conductivity: Station 17
COND_8	Conductivity: Station 8
CR	Chromium: Station 17
CU	Copper: Station 17
DISO	Dissolved oxygen: Station 17
DISO_8	Dissolved oxygen: Station 8
FL	Fluoride: Station 17
LI	Lithium: Station 17
MAX_T	Maximum air temp
MIN_T	Minimum air temp
MO	Molybdenum: Station 17
NA	Sodium: Station 17
NI	Nickel: Station 17
N_NO3	Nitrates as nitrogen: Station 17
PB	Lead: Station 17
PH	pH - Station 17
PH_8	pH - Station 8
POT	Potassium: Station 17
SE	Selenium: Station 17
SULFATE	Sulfate: Station 17
TDS	Total dissolved solids: Station 17
TEMP	Water temp: Station 17
TEMP_8	Water temp: Station 8
TOC	Total organic carbon: Station 17
TOTP	Total phosphorous: Station 17
TSS	Total suspended solids: Station 17
ZI	Zinc: Station 17

## EAST FORK POPLAR CREEK DATA

Date: 09/09/91 Fish Found: 5 Locations: A: \_\_\_ B: \_\_\_ C: \_\_\_  
D: \_\_\_ E: 3 F: \_\_\_ G: \_\_\_ H: \_\_\_ I: \_\_\_ J: \_\_\_ K: \_\_\_

Weather: Max\_T: 86 Min\_T: 66 Precip: 0 Cloud\_cov: 0.5

Station 17: pH: 8 Temp: 79 Cond: 28 DisO: 7.5 CFS: 5

Station 8: pH: 7.9 Temp: 79 Cond: 39 DisO: 6.8 CFS: 4.2

Station 17 Chemistry: Alka: 98 Cd: 0.0005 Chrd: 15 Fl: 1  
Pb: 0.005 Ni: 0.04 Nitrate: 4 TOC: 12 Phos: 0.33 TDS: 280  
TSS: 21 Se: 0.002 Sulfate: 53 Ca: 53.2 Cr: 0.006 Cu: 0.008  
Li: 0.02 Mg: 10.1 Mo: 0.006 Pot: 2 Na: \_\_\_ Zn: \_\_\_

ORNL Chlorine Data: N/S: \_\_\_ Sta\_8: \_\_\_ Down: \_\_\_ Y-12 Water: 6.7

Reason: \_\_\_\_\_ Notes: WETF PUMPS 16000 GAL

Fig. E.1. Data input sheet for 1-d observation for environmental and water chemistry data used for control chart analysis.

Table E.2. Date Codes for Chemistry Control Charts

Week Number	Sunday through	Saturday	Week Number	Sunday through	Saturday
1	7/22/90	7/28/90	51	7/7/91	7/31/91
2	7/29/90	8/4/90	52	7/14/91	7/20/91
3	8/5/90	8/11/90	53	7/21/91	7/27/91
4	8/12/90	8/18/90	54	7/28/91	8/3/91
5	8/19/90	8/25/90	55	8/4/91	8/10/91
6	8/26/90	9/1/90	56	8/11/91	8/17/91
7	9/2/90	9/8/90	57	8/18/91	8/24/91
8	9/9/90	9/15/90	58	8/25/91	8/31/91
9	9/16/90	9/22/90	59	9/1/91	9/7/91
10	9/23/90	9/29/90	60	9/8/91	9/14/91
11	9/30/90	10/6/90	61	9/15/91	9/21/91
12	10/7/90	10/13/90	62	9/22/91	9/28/91
13	10/14/90	10/20/90	63	9/29/91	10/5/91
14	10/21/90	10/27/90	64	10/6/91	10/12/91
15	10/28/90	11/3/90	65	10/13/91	10/19/91
16	11/4/90	11/10/90	66	10/20/91	10/26/91
17	11/11/90	11/17/90	67	10/27/91	11/2/91
18	11/18/90	11/24/90	68	11/3/91	11/9/91
19	11/25/90	12/1/90	69	11/10/91	11/16/91
20	12/2/90	12/8/90	70	11/17/91	11/23/91
21	12/9/90	12/15/90	71	11/24/91	11/30/91
22	12/16/90	12/22/90	72	12/1/91	12/7/91
23	12/23/90	12/29/90	73	12/8/91	12/14/91
24	12/30/90	1/5/91	74	12/15/91	12/21/91
25	1/6/91	1/12/91	75	12/22/91	12/28/91
26	1/13/91	1/19/91	76	12/29/91	1/4/92
27	1/29/91	1/26/91	77	1/5/92	1/11/92
28	1/27/91	2/2/91	78	1/12/92	1/18/92
29	2/3/91	2/9/91	79	1/19/92	1/25/92
30	2/10/91	2/16/91	80	1/26/92	2/1/92
31	2/17/91	2/23/91	81	2/2/92	2/8/92
32	2/24/91	3/2/91	82	2/9/92	2/15/92
33	3/3/91	3/9/91	83	2/16/92	2/22/92
34	3/10/91	3/16/91	84	2/23/92	2/29/92
35	3/17/91	3/23/91	85	3/1/92	3/7/92
36	3/24/91	3/30/91	86	3/8/92	3/14/92
37	3/31/91	4/6/91	87	3/15/92	3/21/92
38	4/7/91	4/13/91	88	3/22/92	3/28/92
39	4/14/91	4/20/91	89	3/29/92	4/4/92
40	4/21/91	4/27/91	90	4/5/92	4/11/92
41	4/28/91	5/4/91	91	4/12/92	4/18/92
42	5/5/91	5/11/91	92	4/19/92	4/25/92
43	5/12/91	5/18/91	93	4/26/92	5/2/92
44	5/19/91	5/25/91	94	5/3/92	5/9/92
45	5/26/91	6/1/91	95	5/10/92	5/16/92
46	6/2/91	6/8/91	96	5/17/92	5/23/92
47	6/9/91	6/15/91	97	5/24/92	5/20/92
48	6/16/91	6/22/91	98	5/31/92	6/6/92
49	6/23/91	6/29/91	99	6/7/92	6/13/92
50	6/30/91	7/6/91	100	6/14/92	6/20/92

ORNL DWG 94-12397

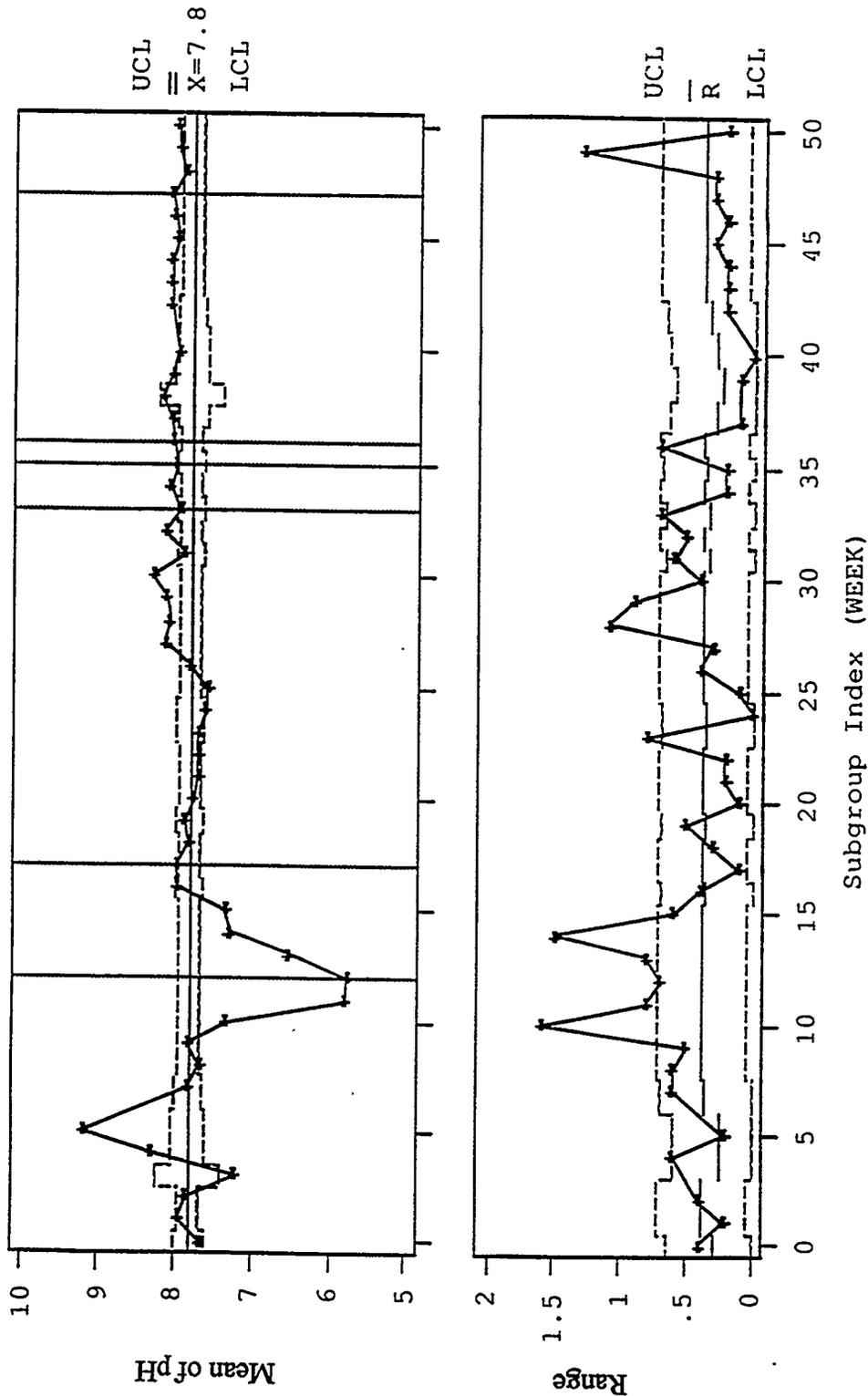


Fig. E.2. Control charts for weekly pH values for East Fork Poplar Creek for weeks 0-50.

ORNL DWG 94-12398

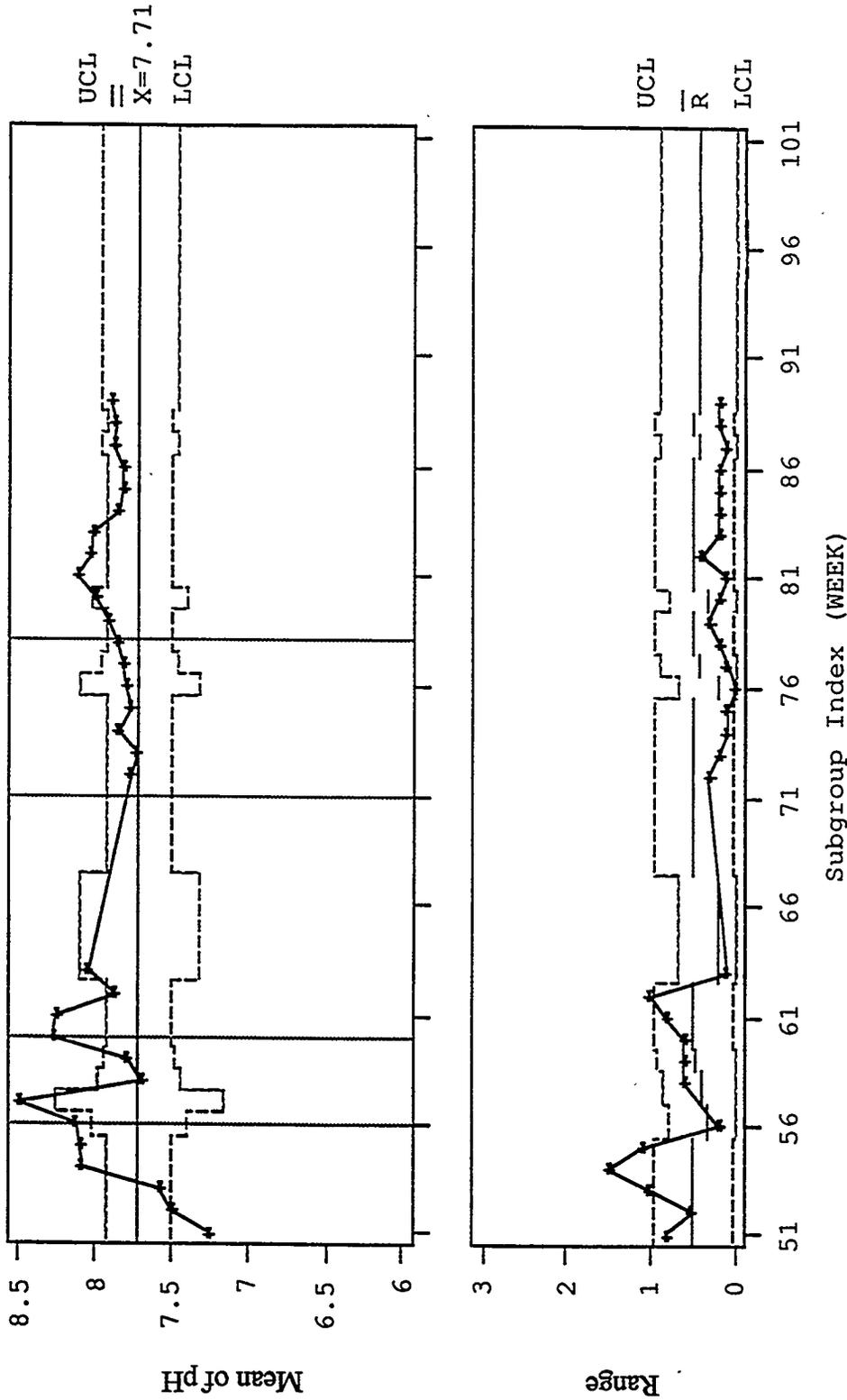


Fig. E.3. Control charts for weekly pH values for East Fork Poplar Creek for weeks 51-101.

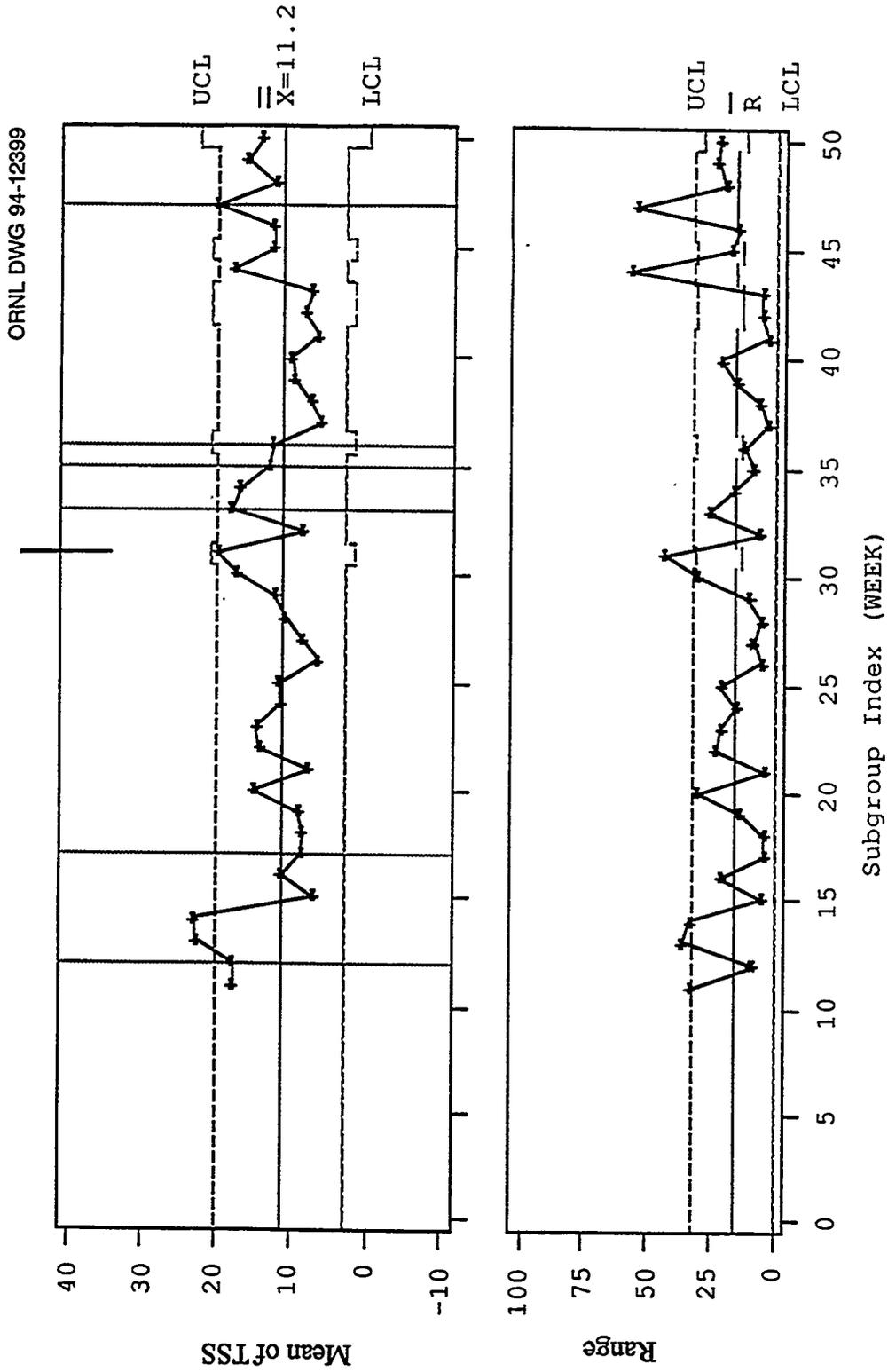


Fig. E.4. Control charts for weekly total suspended solids values for East Fork Poplar Creek for weeks 0-50.

ORNL DWG 94-12400

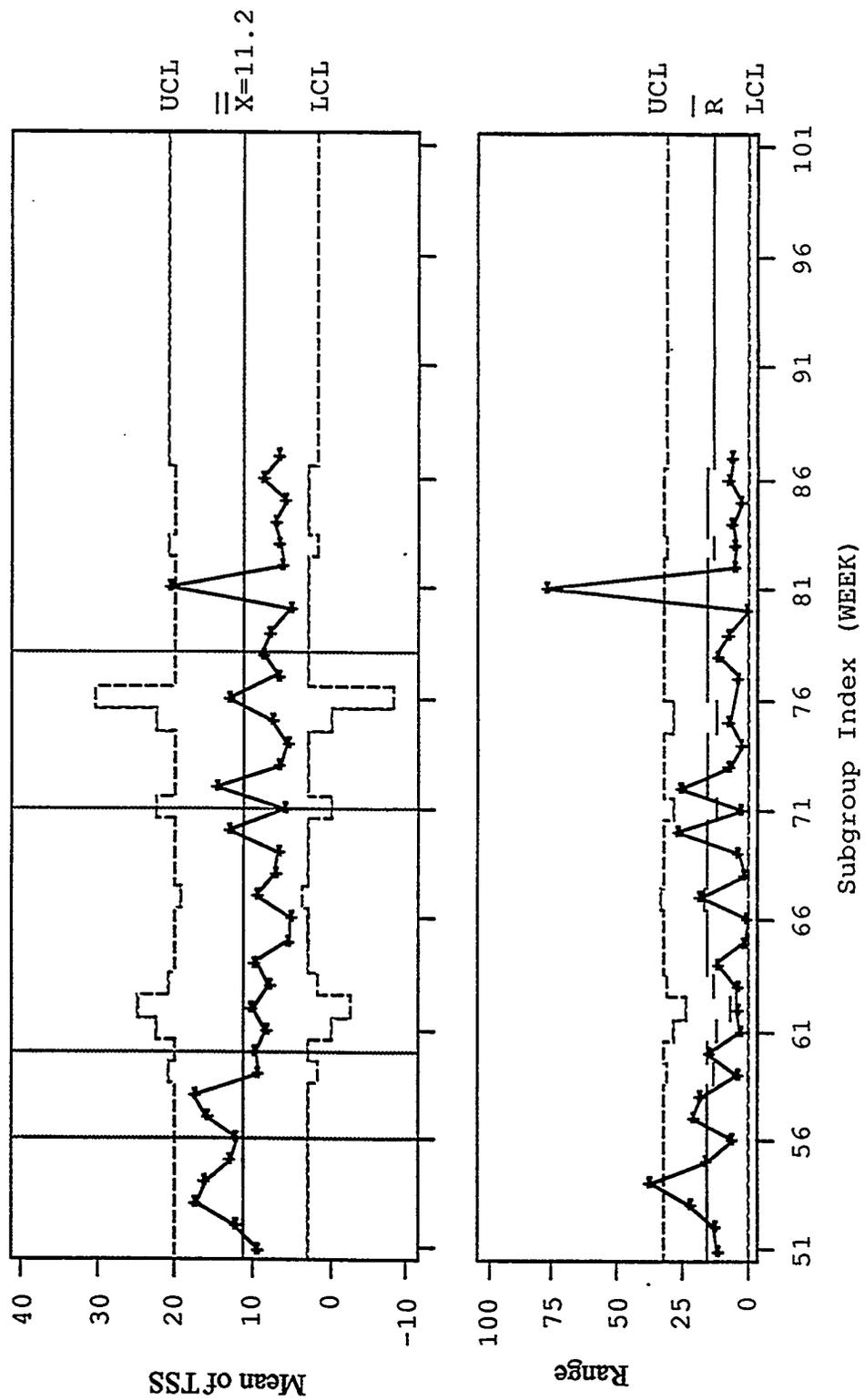


Fig. E.5. Control charts for weekly total suspended solids values for East Fork Poplar Creek for weeks 51-101.

ORNL DWG 94-12401

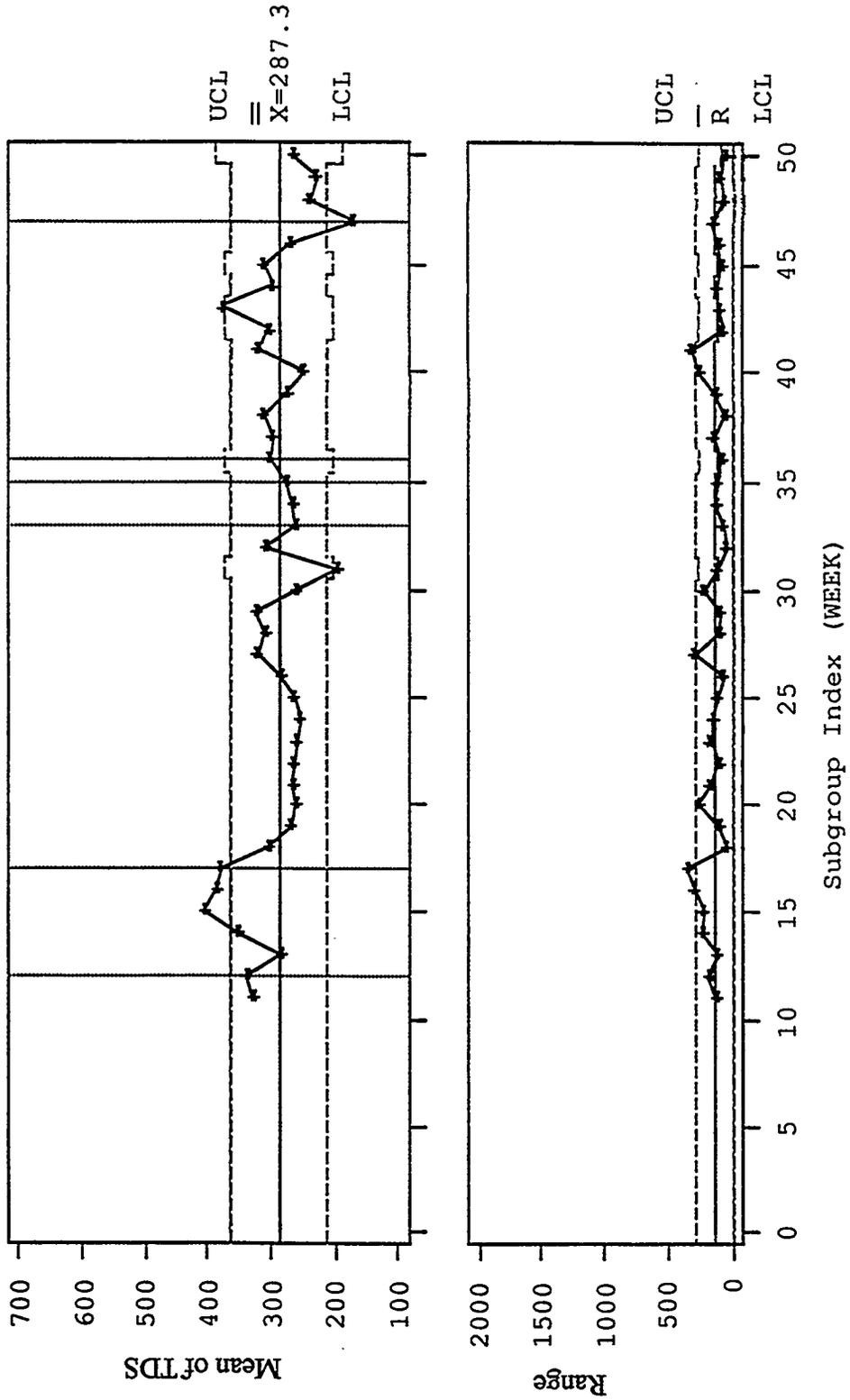


Fig. E.6. Control charts for weekly total dissolved solids values for East Fork Poplar Creek for weeks 0-50.

ORNL DWG 94-12402

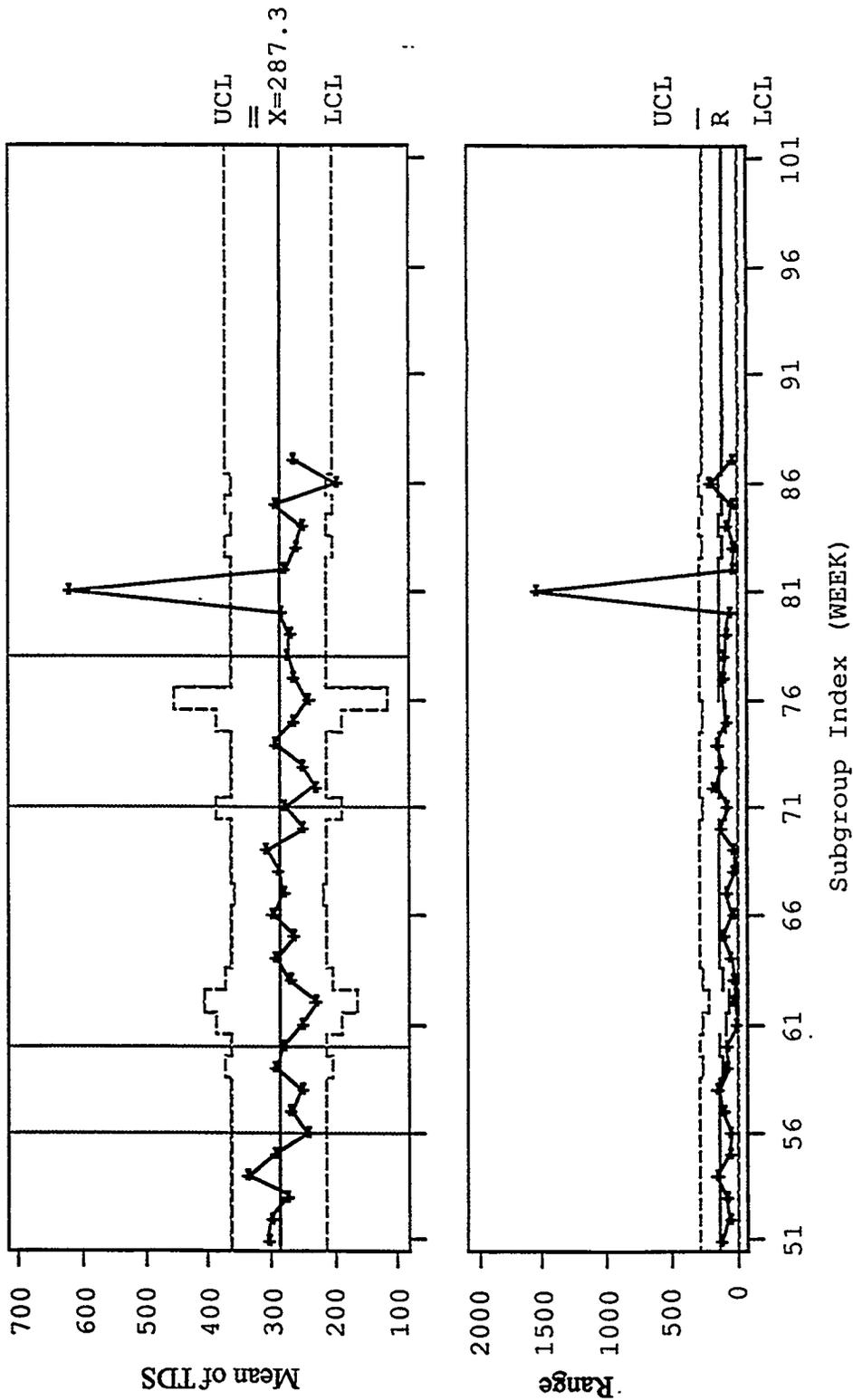
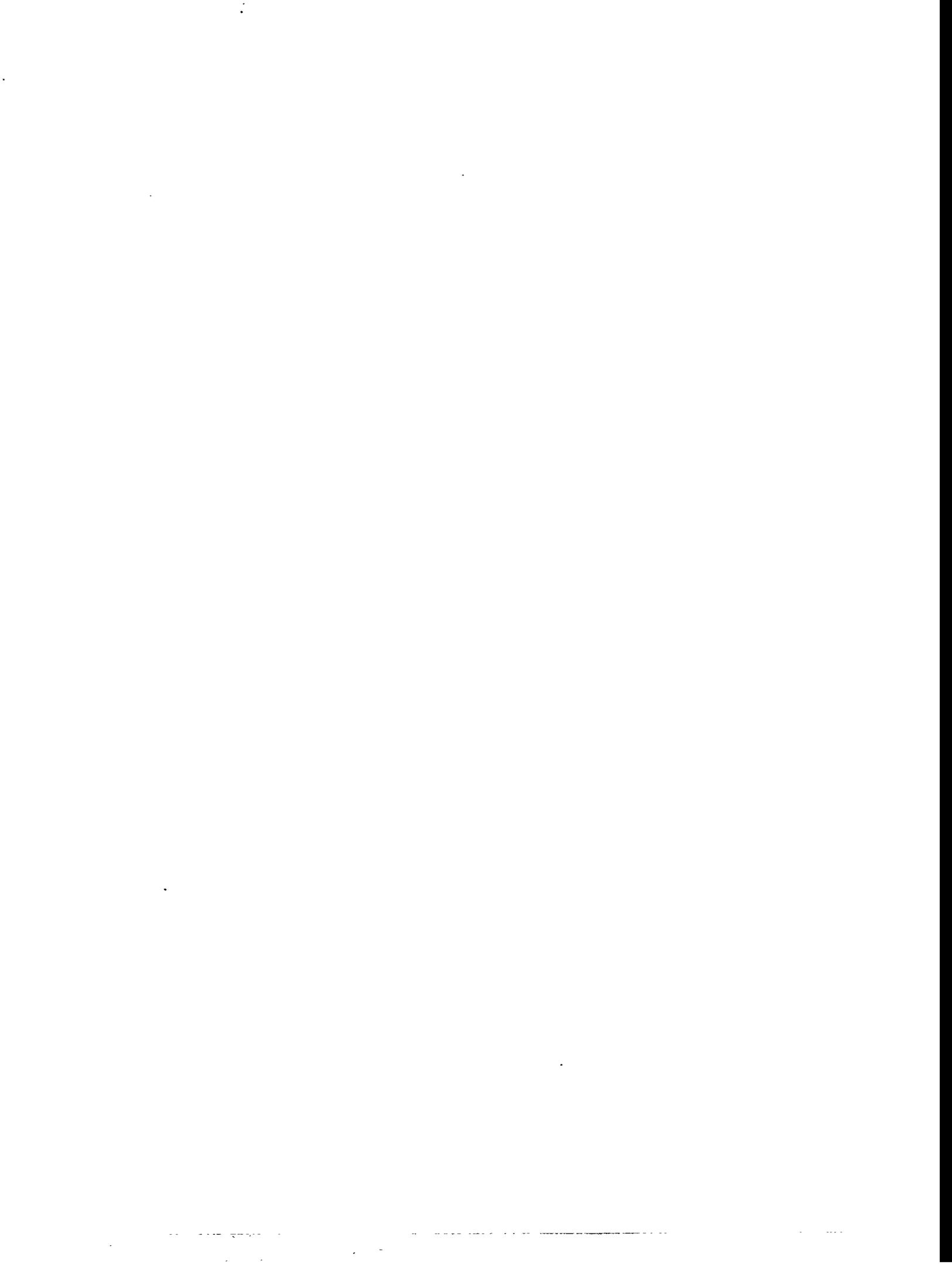


Fig. E.7. Control charts for weekly total dissolved solids values for East Fork Poplar Creek for weeks 51-101.

**Appendix F**

**EXPERIMENT TO STUDY DISTRIBUTION PATTERN FOR  
DEAD FISH IN EAST FORK POPLAR CREEK**

**AN OVERVIEW REPORT**



## APPENDIX F

### EXPERIMENT TO STUDY DISTRIBUTION PATTERN FOR DEAD FISH IN UPPER EAST FORK POPLAR CREEK

*M. G. Ryon, B. A. Carrico, R. L. Hinzman, W. K. Roy, and E. M. Schilling*

#### OBJECTIVES

##### Specific

To evaluate the downstream travel time and distribution pattern for fish dying at the North-South pipes (NSP) in upper East Fork Poplar Creek (EFPC).

reach the lower sections (e.g., diversion channel)?

These factors will help assess whether the location of fish found in the daily fish surveys has any relationship to where they died (and therefore what may have killed them).

##### General

1. Obtain information on dispersal patterns that would be applicable to daily fish survey data collected in 1990–1992.
  - Distribution pattern: Are dead fish aggregated at certain structural features, flow areas, or regions of the stream? How frequent is movement within a limited time period?
  - Dispersal distance: How far can dead fish be dispersed? Is there a maximum distance a dead fish can be moved? After fish have begun to decompose, can they still be dispersed a significant distance?
  - Dispersal rate: How fast are dead fish dispersed through upper EFPC? What is the average dispersal rate? How fast do fish

2. To determine the effectiveness of daily fish surveys. What percentage of fish actually dying are found by our surveys?
3. To determine the impact that scavengers (e.g., turtles, crayfish, birds, and mammals) have on the number of fish we report as dying. How many fish are eaten before we can count them?
4. To determine if a fish dying in upper EFPC could float through Lake Reality (LR) and be counted in survey of EFPC below the lake.
5. To get some idea of the rate of decomposition. Currently we assign a dead fish as being dead <24 h or >24 h based on our general impression of the rate at which fish discolor, decompose etc. By following tagged fish over several days, we should get a better feel for the accuracy of such time assignments.

## PROCEDURES

Tagged dead fish were released at the NSP, Site 5 (outfall 109), and Site 2 (truck scales). They were observed for the following 3 d. Fifty fish were released at NSP, 40 at Site 5, and 30 at Site 2. The distribution of fish was monitored intensively for the first day and twice a day for the following day. All fish were retrieved on the afternoon survey of the second day. During the experiment, the location of individual fish was recorded for each survey. The resulting information on distribution and travel time down EFPC to LR was used to assess the current fish kill in this section of EFPC. These data helped evaluate the hypothesis that some of the peaks in dead fish numbers are a result of a small but highly toxic release that kills a limited number of fish (e.g., in a pipe), which then float downstream. The information also helped evaluate general conditions associated with the nearly 2 years of daily fish survey data.

## Fish

The test species was the golden shiner (*Notemigonus crysoleucas*). This species was selected because (1) it is not found in upper EFPC; (2) it has distinctive features (down curved lateral line behind head, anal fin ray counts, and strongly keeled venter between tail and midbody) that would aid in identification, even if it were dismembered; and (3) the body size is similar to adult striped shiners and stonerollers. Because golden shiners are so distinct from stonerollers and striped shiners, the chance for including dead fish from this experiment in the regular dead fish survey was very minimal.

One hundred twenty fish obtained from local bait shops were used. Immediately prior to their release, live

fish were placed in MS-222 (an anesthetic) until dead and then tagged.

## Tags

Tags were applied to all dead fish released in EFPC. A combination of tag color, number, and location on the fish was used to identify individual specimens. There were five colors (blue, red, orange, green, and white), and every tag had a unique number. The tags were attached with wire through the body of the fish at one of four locations (anterior dorsal, posterior dorsal, anterior ventral, and posterior ventral). The goal was to identify fish specimens without having to remove the specimen from the stream, thereby avoiding disturbance that could influence the downstream movement of the fish.

## Monitoring Plan

Before release of the fish, a system of markers was placed on the stream from the inlet to LR upstream of the NSP. Flags were placed every 25 m along the bank to allow the locations of dispersing fish to be pinpointed. Fish locations were recorded on a general map of the stream for each survey period. Thus, downstream movement could be followed for individual fish.

The stream was divided into 6 survey areas: (1) diversion channel from the inlet of LR to oil/water separator, (2) EFPC from Site 1 (Building 9720-4) to Site 2 (Building 9720-21), (3) EFPC from Site 2 (Building 9720-21) to Site 3 (Building 9720-29), (4) EFPC from Site 3 (Building 9720-29) to Site 4 (AS8), (5) EFPC from Site 4 (AS8) to Site 5 (Building 9720-1), and (6) EFPC from Site 5 (Building 9720-1) to NSP. One person was assigned to each area and made scheduled surveys upstream, recording numbers and locations of fish in

their section. One-half hour after release of the tagged fish, the surveys of each area were initiated and continued at 1.5-h intervals for four surveys.

To prevent accidental escape from the survey area, a large net was placed below the LR outfall. The net spanned the stream bank to bank to trap any fish that managed to float downstream through LR.

During the first day, additional factors that might affect the dispersal pattern were monitored. Stream flow, pH, and temperature were monitored at Site 1 (Building 9720-4), Site 4 (AS8) and Site 6 (just below NSP).

### Schedule

July 6

Verify that markers are all in place  
Setup large blocknet downstream of LR

July 7

Perform a regular dead fish survey  
(830-930)—3 people  
Tag fish (830-1000)—3 people  
Release fish (1030)  
Monitor downstream progress of group of  
fish (1030-1100)  
Perform first area survey (1100)  
Perform second area survey (1230)  
Perform third area survey (1400)  
Perform last area survey (1530)

July 8

Check net (a.m.)  
Perform regular dead fish survey (a.m.) in  
conjunction with fish release  
survey (a.m.)  
Remove net (p.m.)  
Perform fish release survey and begin to  
retrieve fish/tags (p.m.)

July 9

Perform regular dead fish survey (a.m.) in  
conjunction with fish release  
survey (a.m.)

July 10

Perform regular dead fish survey (a.m.)  
and remove any additional tagged fish

### RESULTS

The experiment provided information on the specific objective and the five general objectives of the study. In general, the study confirmed many suspicions regarding the distribution patterns found in the fish kill, but results for other objectives were more surprising.

The results of the flow, pH, and temperature data did not show much variation at the three monitoring sites. Temperature ranged from 24.2 to 28.7°C, the most variation occurring at the NSP. Similarly, pH ranged from 7.5 to 8.0 over the course of the day. Flow measurements were consistent within sites. The velocities (m/s) were highest at Site 1 (0.19 to 0.22) and lowest at the NSP (0.12 to 0.15). Discharge was also consistent at each site, indicating there were no surges in flow that might affect dispersal patterns.

### Distribution patterns

The fish released at the three different sites had three different dispersal patterns. At the NSP, 18 to 26% of the fish moved >25 m in the first day. The average distance moved was 9 to 35 m for the four surveys on July 7, with the maximum distance traveled varying from 210 to 345 m. The majority of the fish remained within 5 m of the release point (effluent from pipe on north bank) during the entire first day. Most fish that moved >25 m were lodged on rocks within the middle of the stream.

The fish released at Site 5 remained at the site; no fish (that was seen on the surveys) moved >25 m on the first day. The fish were concentrated in the deep pool at the end of the culvert where they were released. The maximum distance traveled on the first day was 10 m.

The fish released at Site 2 demonstrated the greatest dispersal. On the first day, 64 to 95% of the fish seen in the surveys moved >25 m. The average distance moved during the four surveys was 41 to 68 m, and the maximum distance moved ranged from 225 to 228 m. The fish at this site were equally distributed between rocks in the middle of the stream and bank vegetation that drooped into the water.

#### **Survey effectiveness**

The effectiveness of the daily fish surveys can be inferred from the effectiveness of the surveys used in the tagged dead fish study. On the first day of the study, 60 to 79% of the 120 tagged fish released were seen during each of the four surveys. Over the total 3 d of surveys, 94% of the released fish were seen at some point. The surveys done during this study were a little more thorough than the daily dead fish survey, but the results should be similar.

#### **Scavenger impact**

The impact of scavengers on the daily fish kill survey numbers was surprisingly high. On the first day of the survey, a majority of fish were located in the stream with roughly half of the fish concentrated in two pools. The tagged fish in these pools had moved <10 m over the course of the first day. However, the morning survey on the second day located only 27 of the 120 released fish. Only 8 of the 42 fish at NSP were found; only 11 of the 33 fish at Site 5 were found; and

only 7 of the 20 fish at Site 2 were found. Included in this total were tags without fish, partially eaten fish, and one tag left on the bank adjacent to one of the pools previously containing high numbers of dead fish. By the afternoon of the second day, only 20 tags or fish could be located. Although scavenging was not observed directly during the study, the impact of scavenging can be inferred from the rapid, overnight disappearance of a majority of fish. Potential scavengers include crayfish, muskrats, rats, raccoons, turtles, and snakes.

Additional circumstantial evidence for the effect of scavenging on the daily fish kill totals was obtained from the four surveys made on the first day. Surveyors were instructed to retrieve any nontagged dead fish in the course of making the surveys. Before the experiment began, only one dead stoneroller was found in the daily dead fish survey for July 7. However on succeeding surveys, an additional 11 dead or dying fish were found on July 7. Of these 11, 5 were found in the diversion channel, where they could not have been previously missed. Because the daily counts had remained fairly constant for the week prior to the study (one to three fish per day), it may be that the daily surveys are finding only 10 to 50% of the total fish dying per day.

Finally, more circumstantial evidence for scavenging was found on July 22. Three tags were found in a rock crevice below Site 5. These tags came from fish that were released at the NSP and had been tracked downstream roughly 100 m on the first day. All three tagged fish were last seen in the same general area on the last survey of the first day and were missing on the second day of surveys. The tags were found downstream another 275 m from this location, and it is improbable that they all drifted there together and were missed in subsequent surveys.

## Dispersal through Lake Reality

One objective of the study was to determine the feasibility of fish traveling to and or through LR. This would help define the toxic zone as only above LR or including EFPC below the LR outfall. No tagged fish were observed to travel to LR. No tagged fish made it to the diversion channel during the first day and only three tagged fish were recovered from the oil/water separator at the top of the diversion channel after 4 d of observation. Thus, it is highly unlikely that fish found below LR, or even in the diversion channel, died in upstream regions of EFPC.

## Decomposition rate

During the daily fish kill surveys, time since death is routinely assigned to any fish found. These assignments were based on previous experience with fish kills, but a controlled monitoring had not been made. During the tagged dead fish study, the surveyors were instructed to examine the fish and assign a time since death. On the first day, fish were generally rated as appearing to have died within the past 24 h. However, several specimens (based on their appearance) would have been assigned a time since death of >24 h. On the morning survey of the second day, almost every fish showed extreme signs of decomposition, consistent with an assignment of a time since death of >48 h. These fish were bleached-out, bloated, and often very soft or mushy. Thus, the decomposition rate appeared to be greater than we had previously realized. For the daily fish surveys, the most prudent approach may be to assign time since death as simply >24 h or <24 h and not try to get more specific.

## DISCUSSION

In evaluating the data from the tagged dead fish study, several conclusions can be made. First, dispersal is dependent on location, with greater dispersal in downstream areas. In general, dead fish found in an area probably died in that area. Some dispersal over long distances does occur, particularly as the fish decomposes and bloats as a result of accumulation of gases. Dead fish found in the diversion channel or below LR almost certainly died in these regions. The one dispersal factor not determined in this study is the amount of downstream dispersal for a stressed or dying fish.

Daily fish kill surveys are probably finding 50 to 80% of the dead fish in the creek during a survey, suggesting that numbers are a consistent gauge of mortality over time. However, losses of dead fish to scavengers is very high, particularly overnight. Thus, the numbers found in daily fish kill surveys may be consistent but probably underestimate the actual mortality by 50 to 90%. Also, appearance of the dead fish can be deceiving. The assignment of time since death occurred, should be restricted to one of two designating: >24 h or <24 h.

## RECOMMENDED STUDIES

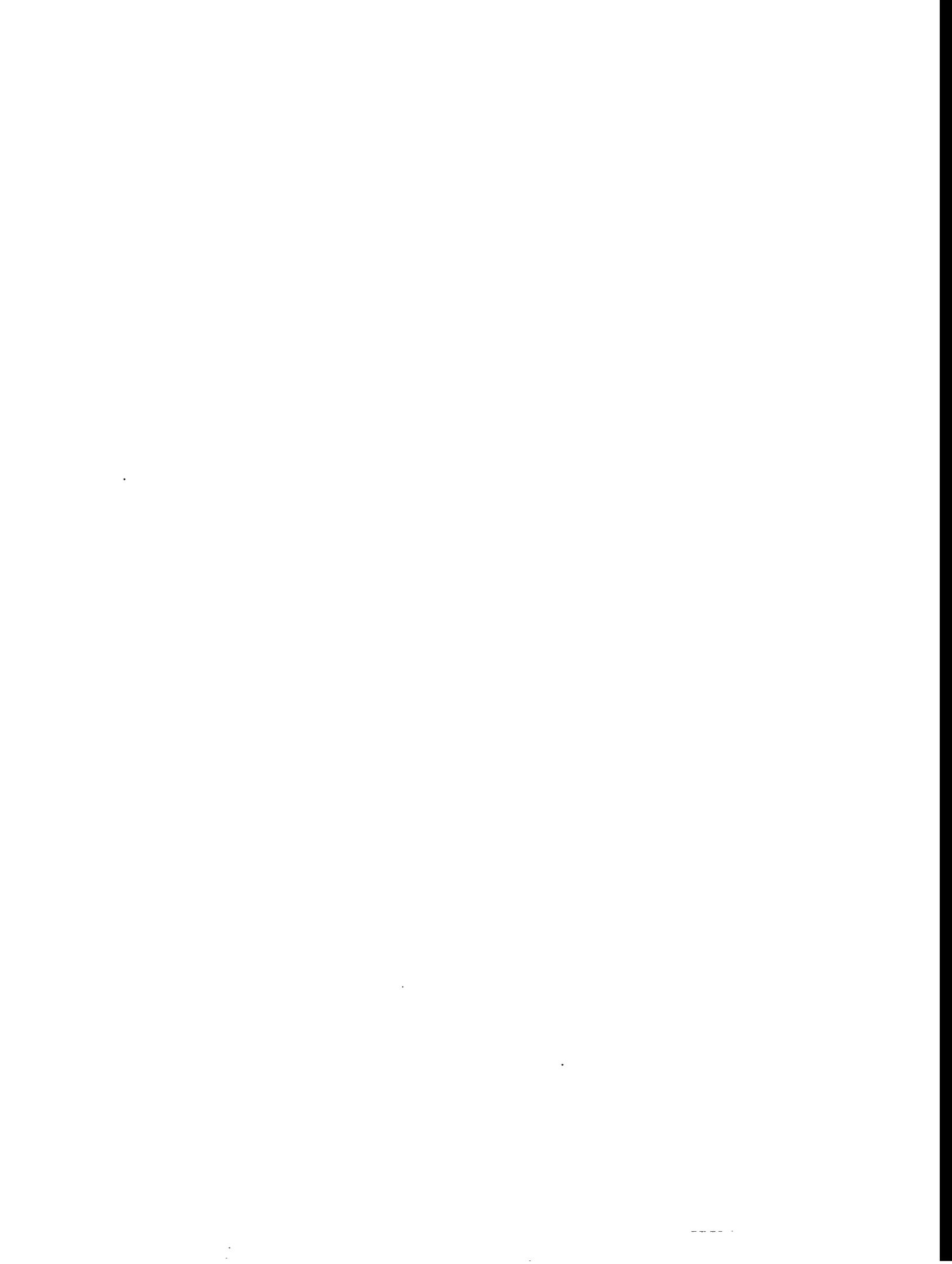
Follow-up studies are recommended to complete this phase of the fish kill investigation. First, some measure of dispersal without tags should be made. This would eliminate any biases that the tags imparted to the fish movement. A limited release of 10 to 20 golden shiners without tags could be made at one site. These released fish would be followed continuously for up to 4 h and then retrieved. The goal would not be to follow

individual movements as much as total dispersal distance. By limiting the study to a small number and a short duration, losses would be minimized. The second study would be to determine the types of scavengers operating in upper EFPC. This could be measured by placing a small number (10 to 20) of dead golden shiners

in a pool in the late afternoon and monitoring for scavengers drawn to the pool. Some dead shiners could also be put in minnow traps to determine the role that crayfish play in the disappearance of fish in upper EFPC. Minnow traps would prevent larger scavengers from removing these fish.

**Appendix G**

**BENTHIC COMMUNITY COMPARISONS  
MATERIALS AND METHODS**



## APPENDIX G

### BENTHIC COMMUNITY COMPARISONS MATERIALS AND METHODS

#### INTRODUCTION

The Oak Ridge Y-12 Plant, a nuclear weapons component production facility operated by Martin Marietta Energy Systems, Inc. for the U.S. Department of Energy, discharges once-through cooling water, cooling water blowdown, and process wastewaters from more than 200 outfalls into upper East Fork Poplar Creek (EFPC) (Loar et al. 1992). Previous studies of EFPC concluded that the benthic macroinvertebrate and fish communities were significantly affected by Y-12 Plant operations: these communities showed signs of recovery downstream from the plant (Smith 1992). The primary stressor to the aquatic biota in upper EFPC was suspected to be chlorine.

Chlorine is a very effective biocide, making it the most commonly used chemical to control undesirable organisms in cooling tower operations (Mattice 1984, Pratt et al. 1988). Unfortunately, its biocidal properties are not restricted to undesirable organisms. Many studies have shown the harmful effects of chlorine on stream organisms, often at levels far below those needed to control biofouling (e.g., Brooks et al. 1975, Dickson et al. 1974, Jolley 1984, Cumbie et al. 1984, and Stewart et al. 1995). Dickson et al. (1974) placed caged bluegill sunfish (*Lepomis macrochirus*) and snails (*Anculosa* sp.) downstream of cooling

tower blowdown discharged to the Clinch River. No fish deaths occurred, however the blowdown was acutely toxic in 72 hr to 50% of the snails exposed to less than 0.04 ppm total residual chlorine (TRC) for less than 2 hr/day.

Avoidance of chlorine by fish has been observed in both lab and field studies. Sprague and Drury (1969, as cited by Brooks et al. 1975) observed that rainbow trout (*Oncorhynchus mykiss*) placed in a tank avoided chlorinated waters at TRC concentrations of 0.001, 0.01, and 1.0 mg/l. In a field study, Fava et al. (1984) determined that the Atlantic Silverside (*Menidia menidia*) and White Perch (*Morone americana*) could detect and avoid chlorinated effluents containing TRC at levels as low as 0.02 mg/l. The ability of fish to detect and avoid low levels of chlorine make them less desirable as test organisms.

Many studies show the detrimental effects of chlorine on benthic macroinvertebrates (e.g., Osborne 1984, Murrery et al. 1984, Camargo 1991, Ham et al. 1994, and Petersen et al. 1994). Osborne (1984) concluded that following chlorination of a municipal sewage plant on the Sheep River, Alberta, macroinvertebrate community structure was significantly reduced. His results showed that species diversity dropped after chlorination, and that less than 1% of the total number of individuals collected were represented by the orders

Ephemeroptera, Plecoptera, and Trichoptera. Murray et al. (1984) did a study on the effects of chlorine on early life stages of a mayfly (*Hexagenia bilineata*). They found that freshly hatched nymphs were very sensitive to intermittent chlorine treatment, with only a 34% survival rate at a concentration of 0.10 mg/l. The effects of the chlorine on survival was attributed to gill damage. This was also seen in a study by Camargo (1991) in which *Hydropsyche pellucidula*, collected downstream of a hydroelectric plant, exhibited corrosive injury to tracheal gills.

A preliminary study revealed that the average concentration of total residual chlorine exceeded the U.S. Environmental Protection Agency's ambient water quality criteria (AWQC; 0.019 and 0.011 mg/L, for acute and chronic exposure scenarios, respectively), at 75% of all outfall found on upper EFPC, often by more than one order of magnitude (unpublished MMES technical memorandum, 1991). To improve water quality and reduce instream toxicity in upper EFPC, a chlorine abatement plan was developed and implemented by the Y-12 Plant. In 1991, dechlorination systems were installed at two major outfalls near the headwaters of EFPC. Together, these two outfalls accounted for approximately 80 percent of the TRC loading to EFPC (unpublished MMES tech memorandum, 1991).

The dechlorination systems use sodium metabisulfite as the dechlorinating agent and are considered to be very reliable (unpublished MMES tech memorandum, 1991). Powdered sodium metabisulfite is dissolved in water to form an aqueous solution which is stored in a solution tank connected to a metering pump. This pump injects a precise amount of the solution into a pipeline insuring rapid mixing of the solution with the water, which hastens dechlorination. The two dechlorination systems went "on line"

on December 29, 1992; they reduced the amount of total residual chlorine at the study sites to zero (personal observation). To determine the effects of dechlorination on the biota in EFPC, a colonization study of the benthic macroinvertebrate community was initiated in December, 1991. Colonization has been defined by Sheldon (1984) as "the sequence of events that leads to the establishment of individuals, populations, species, or higher taxa in places from which they were, however temporarily, absent." The rate at which species accumulate in uncolonized areas can be described by the species equilibrium model of MacArthur and Wilson (1967). This model states that the number of species found in the area undergoing colonization depends on the equilibrium between the rate at which new species are added, (immigration), and the rate at which species are lost (extinction). The immigration of new species depends on the colonizers' vagility and the distance between the area being colonized and the source of the immigrating organisms. The colonization rate should be high initially, then decrease geometrically with time as the extinction of established species slowly increases. Equilibrium is reached when the immigration rate equals the extinction rate. The time required to reach equilibrium depends upon the distance from the source of recolonizers, the taxonomic richness of that source, the size and type of the habitat being recolonized, the vagility of the colonizers, and time of year. For stream ecosystems, the time needed to achieve taxonomic equilibrium has been reported to range from 14 to 21 days (Sheldon 1977, Roby 1978, Peckarsky 1986, Clements 1991) to 28 to 64 (Lamberti et al. 1985, Meier et al. 1979, Shaw et al. 1980). In more extreme situations, longer times are noted (Gore 1982, Minshall et al. 1983).

The model first was developed to describe the colonization of oceanic islands by birds. Subsequently, it has been applied with some success to the recolonization of oceanic islands by terrestrial insects (Simberloff et al. 1968). They studied the recolonization of six mangrove islands in Florida Bay which had been fumigated with a strong insecticide. After 250 days, species composition on all of the islands, except the most distant one, had returned to near predisturbance levels following MacArthur and Wilson's model. Other studies have shown its applicability to aquatic systems with the colonization of denuded natural substrates after severe disturbance, or in colonization studies using artificial substrates (eg., Cairns et al. 1969, Minshall et al. 1983, Peckarsky 1986, Ciborowski et al. 1984). Cairns et al. (1969) further tested the model by studying the colonization of polyurethane substrates by freshwater protozoans. They found that the colonization of these organisms on artificial substrates also followed the predictions made by MacArthur and Wilson. More recently, Minshall et al. (1983) assessed the recovery rate of stream benthic macroinvertebrate communities after the failure of the Teton Dam in Idaho. In this study, recovery adhered to MacArthur and Wilson's model, with the sites closest to sources of recolonizers recovering more rapidly than more distant sites. The species-equilibrium time was estimated to be 625 days.

Several other studies have shown that the rate of colonization can be affected by taxon and season, and questioned whether species equilibrium could ever be ever achieved in aquatic systems (Hutchinson 1961, Williams et al. 1977, Shaw et al. 1980, Ciborowski et al. 1984).

A common problem with ecological studies is finding a design which can accurately test for perturbation effects on

communities. Pseudoreplication, as defined by Hurlbert (1984), is "the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (though samples may be) or replicates are not statistically independent." Use of the Before-After Control-Impact (BACI) design could help to eliminate the problem of pseudoreplication. The BACI design involves the collection of samples of a biologically significant parameter, paired in time, at a control and an impacted site or sites both before and after the event of interest (Stewart-Oaten et al. 1986, 1992, Smith et al. 1993). Stewart-Oaten et al. (1986) described this design and recommended using a t-test to determine if the mean of the Impact-Control differences had changed from Before to After the perturbation. Schroeter et al. (1993) used this design to determine the effects of once-through cooling water from the San Onofre Nuclear Generating Station on kelp forest invertebrates. They determined that this was design was an important tool for assessing environmental impacts, but difficulties in the collection of marine invertebrates lowered the power of the test in their study.

In the present study, three sites were selected in EFPC based on their proximity to the main sources of chlorine. These were EFK 24.4 (near-site), EFK 23.4 (far-site), and EFK 13.8 (far-site unaffected by chlorine discharges). The sites were identified as EFK, or East Fork Popular Creek Kilometer, giving the distance of the site from the stream's confluence with Popular Creek. EFK 24.4 and EFK 23.4 served as the impacted sites, and EFK 13.8 served as the "control" site: no undisturbed upstream site existed (Fig. 1). "Control" as used in this study refers to a reference point represented by a location unaffected by the event of interest (i.e., no chlorine or dechlorination impacts). This site is used to determine natural

fluctuations in the system. It cannot be considered to be a "true control," since not all other factors were held constant throughout the experiment.

Various smaller scale studies were conducted during the course of the study. A six-week colonization experiment was initiated at each of the three sampling sites to examine processes of benthic macroinvertebrate colonization, during the spring and summer, both before and after dechlorination. This study also allowed insight into the role of seasonality on the recovery process. Water temperature, dissolved oxygen concentration, conductivity, pH, and total residual chlorine (TRC) were measured weekly at all three sites throughout the study as well.

## MATERIALS AND METHODS

### Study Site

East Fork Popular Creek (EFPC), located near Oak Ridge, Tennessee (35°56'N, 84°18'W), receives effluents from the Y-12 Plant near its headwaters (Loar et al. 1992). Upper EFPC is contained in culverts through much of the plant before entering a rip-rap channel approximately 2.4 m wide and 2.6 m high (Kasten 1986). The stream runs approximately 1.5 km through the plant site before entering Lake Reality, a 2.2-ha pond constructed for neutralization, sediment retention and spill control (Loar et al. 1992). Downstream of Lake Reality, EFPC runs through the city of Oak Ridge, where it is subject to municipal and agricultural runoff. Water and sediment downstream of the Y-12 Plant contain metals, organic chemicals, and radionuclides discharged over many years of plant operation (Loar et al. 1992).

The near-site, or the site most affected by chlorine in this study, was

EFK 24.4. EFK 24.4 was located approximately 1375 m from the two major outfalls of chlorine, and within the Y-12 Plant boundary; here, the stream ran through a rip-rap channel (Fig. 1). For security reasons, trees had been removed from the stream banks. Only small shrubs and grass remained, so no canopy cover existed. The channel was bordered on both sides by a road and a parking lot, therefore stormwater runoff highly affected streamflow. The stream at this site was of uniform depth and current velocity throughout the reach, and was basically one giant riffle. The substrate consisted mostly of small pebbles and sand that was subject to substantial shifting during spates. Y-12 Plant discharges contributed to elevated water temperature that was generally 4-6°C higher than at Brushy Fork, a nearby stream that receives no industrial discharges (Loar et al. 1992). The channel at EFK 24.4 was bordered on both sides by roads and parking lots. Thus, stormwater runoff strongly affected streamflow.

The far-site, EFK 23.4, was located approximately 0.4 kilometers downstream from Lake Reality and though probably not affected by chlorine, it was still under the influences of Y-12 Plant discharges. This reach of stream was contained in a rip-rap channel with little or no canopy. It contained several pools and a long riffle. Predominate substrates included large rubble, gravel and sand. The riffle area was of uniform depth and current velocity, and water temperatures at EFK 23.4 were slightly higher than Brushy Fork (Loar et al. 1992). This section of the stream was bordered by open fields and so was less affected by stormwater runoff than EFK 24.4.

The control site, or the site not affected by chlorine, EFK 13.8, consisted of alternating large, deep pools and riffles. The predominate substrate of the riffles

was composed of large slabs of bedrock and a heterogeneous mixture of large and small rubble, gravel and sand. Riparian vegetation was well developed and provided almost complete canopy cover during periods when leaves were present. Water depth and current velocity were more variable than the other sites, but areas similar to EFK 24.4 and EFK 23.4 were available. Water temperatures were similar to Brushy Fork and exhibited seasonal variability. A large pasture for cattle grazing existed upstream of this site, therefore this site was subject to agricultural runoff.

### Experimental Design

For the primary study, benthic macroinvertebrates were allowed to colonize non-glazed paving bricks (4" x 8" x 0.25") during 20, 3-week exposure periods before and after dechlorination. The "before" period was initiated in late December, 1991, to establish conditions prior to dechlorination; the "after" period was initiated in late December, 1992.

Initially, a set of ten bricks was placed in a shallow riffle. At these sites, the water depth was 10 to 30 cm deep and the water velocity was 0.76 to 1.1 m/s. This was followed with additional sets of ten bricks placed one and two weeks after the initial set, thus giving three sets of ten staggered at one-week intervals. Each brick was secured to the stream bottom by attaching wire from holes that had been drilled in the front two corners of the brick to a piece of rebar driven into the stream bottom. The position of the rebar was selected randomly. Two bricks were attached to each piece of rebar; the bricks were oriented lengthwise in a downstream position. Each brick was divided in half by a horizontal line and labelled with an unique set number and letter. After a set had been colonized for three weeks, three

bricks within that set were selected randomly for collection and processing. Collection consisted of removing the chosen brick, scrubbing the front half with a stiff brush to remove macroinvertebrates and algae, then placing it into a plastic whirlpack with a label identifying the site, date and brick identification. The samples were preserved in approximately 80% ethanol and taken to the laboratory for processing. Two of the remaining bricks were collected for the measurement of periphyton biomass. These bricks were placed in zip-lock bags, filled with stream water and taken to the laboratory. The remaining five bricks were removed, and replaced with a fresh set of ten bricks. A fresh brick was defined as one which had been scrubbed and placed in a drying oven at 100°C for at least 24 hrs.

Supplemental data were obtained at the time of sampling. Water quality parameters (temperature, conductivity, pH, and dissolved oxygen) were measured with a Horiba meter and recorded in a logbook. A single grab sample of water was also taken at each site on each sampling date for analysis of total residual chlorine (TRC). Each TRC sample was collected in a 250-mL dark bottle and placed into a cooler containing ice until analyzed, which usually occurred <30 min after collection. This procedure reduced the dissipation of chlorine due to higher temperatures and sunlight (Nowell et al. 1991).

In the laboratory, the invertebrate and periphyton on the bricks were washed into a 250- $\mu$ m sieve. The sieve's contents then were placed into an 8" x 10" white plastic photographic tray. The contents within the tray were examined with the aid of a 2X illuminated magnifying lamp, and the macroinvertebrates were removed with forceps and placed into labelled vials containing 80% ethanol. All organisms were identified to the lowest taxonomic level practical (genus in most cases) using

a stereoscopic dissecting microscope. When a lower level of identification was needed on Chironomidae larvae, they were mounted in CMC-10 Mounting Media on a slide and viewed at 100-400X with a compound microscope.

Water samples were analyzed for TRC in the laboratory using a Wallace and Tiernan titrator. Approximately 200 mL of each water sample was mixed with 1 mL of pH 4 buffer solution and aqueous potassium iodine. This mixture was then titrated with an aqueous solution of phenylarsine oxide, usually in 0.01 mL increments, until the meter remained steady. At this point, the amount of titrant added (in mL) was equal to the concentration of TRC (in mg/L) in the sample.

The six-week colonization studies were conducted in the spring (March-April) and summer (June-July), both before and after dechlorination. In these studies, 30 bricks were placed at each site following the same procedure as in the primary study. Each brick was divided in half by a horizontal line and labelled with a number (1 through 30). Each week over a six-week period, three bricks were randomly selected and removed for processing. Collection, preservation, processing, and identification of the macroinvertebrate samples followed the same procedures as described for the primary study.

### Statistical Methods

After the organisms had been identified and counted, the data were entered into a computer data base using Lotus software. Two ecologically significant parameters, the number of organisms per unit area and taxonomic richness, were used to help evaluate the effectiveness of the Y-12 Plant's dechlorination efforts. All statistical manipulations were performed using

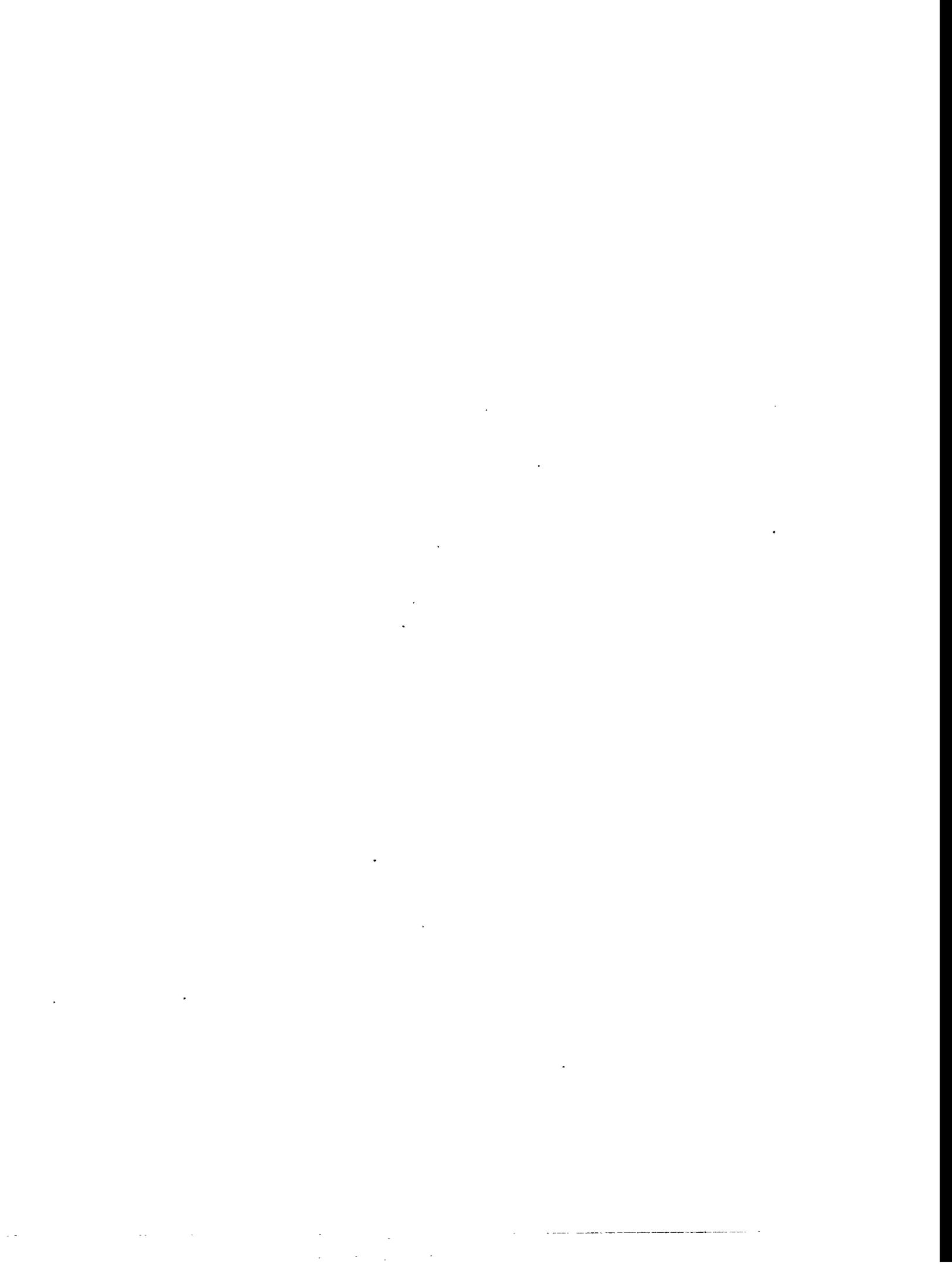
Statistical Analysis System (SAS 1985) software. The original density and richness data were initially checked for adherence to basic statistical assumptions (namely, that the data were normally distributed and that the samples had equal variances) using a program recommended by D'Agostino et al. (1990). The results of the normality test showed the data for density needed to be log-transformed to more nearly approximate a normal distribution, whereas richness values did not differ significantly from being normally distributed and therefore needed no transformation. The Levene's test for homoscedasticity showed that both density and richness exhibited variances that did not differ significantly between data collected before and after dechlorination.

The standard BACI approach (as described in Stewart-Oaten et al. 1986) was designed to look at data taken at controlled and impacted sites sampled simultaneously both before and after an event of interest. Stewart-Oaten's recommended approach was to use a two-sample t-test or a nonparametric test to assess change. However, Smith et al. (1993) suggested a split-plot model, where whole plots were the sampling times, and the split plots were the locations within the times. Further discussion of these two models and their advantages and disadvantages can be found in Smith et al. (1993). Based on the data in this experiment (i.e., multiple impacted sites), the split plot model was used in an analysis of variance (ANOVA). Using this model, there were three test of interest corresponding to two main effects, before-after (treatment), and control-impact (site), and the interaction between treatment and site. The test for the interaction term was of primary interest in that significance at the predetermined alpha value ( $\alpha=0.05$ ), would mean the intervention could be responsible for the changes seen in the test parameters. In the event that the

interaction term was found to be significant, an ANOVA on pairs of sites was performed to further investigate the changes.

The alpha value,  $\alpha$ , should be chosen based on the consequences of making a Type I error, or the rejection of a true null hypothesis. In this case, the null hypothesis is no change in benthic macroinvertebrate density or taxonomic richness will occur before and after dechlorination. The probability of making a Type II error, or the acceptance of a false null hypothesis, is denoted as  $\beta$ . It is related to the power of the statistical test

(power =  $1 - \beta$ ), which is dependent on sample size. Since the number of samples collected was small, the power of the analysis was reduced. Therefore, a conservative  $\alpha = 0.05$  was chosen to reduce the chance of stating improvement was detected when indeed it was not, which would keep other plants from spending a considerable amount of money implementing dechlorination based on a misinterpretation of the data. A good discussion on the specification of  $\alpha$  and  $\beta$  can be found in Sokal and Rohlf (1981, Chapter 7).



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