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OAK RIDGE Y-12 PLANT

MARTIN MARIETTA

Second Report on the Oak Ridge Y-12 Plant Biological Monitoring and Abatement Program for East Fork Poplar Creek

R. L. Hinzman, editor

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ENVIRONMENTAL SCIENCES DIVISION

SECOND REPORT ON THE OAK RIDGE Y-12 PLANT BIOLOGICAL
MONITORING AND ABATEMENT PROGRAM FOR EAST FORK
POPLAR CREEK

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LIST OF ACRONYMS

ACD	Analytical Chemistry Division
AFDM	ash-free dry mass
ANOVA	analysis of variance
ANCOVA	analysis of covariance
AS8	Area Source Study Site 8
ATDL	Atmospheric Turbulence and Diffusion Laboratory
ATP	adenosine triphosphate
B[a]P	benzo[a]pyrene
BCF	bioconcentration factor
BCK	Bear Creek kilometer
BF	Brushy Fork
BFK	Brushy Fork kilometer
BHS	bacterial hemorrhagic septicemia
BMAP	Biological Monitoring and Abatement Program
BRK	Bull Run Creek kilometer
BTRI	blood triglycerides
Chl <i>a</i>	chlorophyll <i>a</i>
CPCF	Central Pollution Control Facility
CPI	cohort production interval
CPI	chemical perturbation index
CPM	coarse particulate matter
CPOM	coarse particulate organic matter
C.V.	coefficient of variation
DDT	1,1'-(2,2,2-trichloroethylidene)bis[4-chlorobenzene]; 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
DOE	U.S. Department of Energy
DOM	dissolved organic matter
EDTA	ethylene diamine tetracetic acid
EFK	East Fork Poplar Creek kilometer
EFPC	East Fork Poplar Creek
EPA	U.S. Environmental Protection Agency
EPT	Ephemeroptera, Plecoptera, and Trichoptera
EROD	7-ethoxyresorufin <i>O</i> -deethylase
ESD	Environmental Sciences Division
FC	free chlorine
FDA	U.S. Department of Agriculture Food and Drug Administration
FHM	fathead minnow
GC/ECD	gas chromatography with electron capture detection
GC/MS	gas chromatography/mass spectrometry
GSI	gonadal-somatic index
HBA	hydrophobic acid fraction

HCK	Hinds Creek kilometer
HBN	hydrophobic neutral fraction
HL	hydrophilic acid fraction
HOC	hydrophobic compounds
HPLC	high performance liquid chromatography
IBI	index of biotic integrity
ICP	inductively coupled plasma
IGR	instantaneous growth rate
IWC	instream waste concentration
LFPMA	functional parenchyma in liver
LMACA	macrophage aggregates in liver
LNPMA	necrotic liver parenchyma
LOEC	lowest-observed-effect concentration
LPARS	liver parasites
LR	Lake Reality
LSI	liver-somatic index
LTF	liquid treatment facility
MFO	mixed function oxidase
MHR	Melton Hill Reservoir
m.p.	microsomal protein
MS-222	tricain methanesulfonate
<i>N</i>	normal
NADH	nicotinamide adenine dinucleotide, reduced form
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
NBS	National Bureau of Standards
NHP	New Hope Pond
NHP-i	New Hope Pond inlet
NHP-o	New Hope Pond outlet
NOEC	no-observed-effect concentration
NPDES	National Pollutant Discharge Elimination System
NPH	naphthalene
OC	organic content
ORNL	Oak Ridge National Laboratory
ORR	Oak Ridge Reservation
ORTF	Oak Ridge Task Force
ORWTF	Oak Ridge Wastewater Treatment Facility
PAH	polycyclic aromatic hydrocarbon
PAR	photosynthetically active radiation
P/B	ratio of production to biomass
PC	phosphatidylcholine
PCB	polychlorinated biphenyl
PCK	Poplar Creek kilometer
PE	phosphatidylethanolamine
PGV	preliminary guidance values
POC	particulate organic carbon
POM	particulate organic matter
PPM	parts per million

RNA	ribonucleic acid
SAS	Statistical Analysis System
SD	standard deviation
SE	standard error
SGOT	serum glutamate oxaloacetate transaminase
SPARS	spleen parasites
SRP	soluble reactive phosphorus
SUP	soluble unreactive phosphorus
TCB	2,2',5,5'-tetrachlorobiphenyl
TCMP	Toxicity Control and Monitoring Program
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 above)
TDPH	Tennessee Department of Public Health
TLIP	total body lipid
TOC	total organic carbon
TRC	total residual chlorine
TRI	total body triglycerides
TSS	total suspended solids
TU	toxicity unit
TVA	Tennessee Valley Authority
UGCAL	University of Georgia Chemical Analysis Laboratory
USGS	U.S. Geological Survey
VSI	visceral-somatic index
WBR	Watts Bar Reservoir
WOC	White Oak Creek
WPCP	Water Pollution Control Program

PREFACE

On May 24, 1985, a National Pollutant Discharge Elimination System permit was issued for the Oak Ridge Y-12 Plant, a nuclear weapon components production facility managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy. As required in Part III(C): Special Condition No. 7 of the permit, a plan for the biological monitoring of the receiving stream, East Fork Poplar Creek (EFPC), was prepared and submitted for approval to the U.S. Environmental Protection Agency (Region IV) and Tennessee Department of Environment and Conservation (formerly the Department of Health and Environment) in August 1985 (Loar et al. 1989). Because it was anticipated that the chemical composition of several effluent streams could be altered when construction was completed on the new Central Pollution Control Facility soon after the permit was issued, some biomonitoring studies were initiated in May 1985 before formal approval of the plan was received from the regulatory agencies.

This document is the second volume of a series of reports on the results of the Y-12 Plant Biological Monitoring and Abatement Program. This report describes studies that were conducted between July 1986 and July 1988, although additional data collected outside this time period are included as appropriate. The studies conducted during the first year were directed toward an ecological characterization of EFPC (Loar et al. 1992b). The studies conducted during the second and third years continued the ecological characterization of EFPC, with emphasis on testing various hypotheses regarding the causal factors and underlying mechanisms associated with the effects documented in the initial studies. Significant modifications in the parameters that were monitored or the frequency and location of monitoring are addressed in this report.

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EXECUTIVE SUMMARY

As stipulated in the National Pollutant Discharge Elimination System (NDPES) permit issued to the Oak Ridge Y-12 Plant on May 24, 1986, a Biological Monitoring and Abatement Program (BMAP) was developed for the receiving stream, East Fork Poplar Creek (EFPC). The objectives of BMAP are (1) to demonstrate that the current effluent limitations established for the Y-12 Plant protect the classified uses of EFPC (e.g., the growth and propagation of fish and aquatic life), as designated by the Tennessee Department of Environment and Conservation (TDEC) and (2) to document the ecological effects resulting from implementation of a Water Pollution Control Program that includes construction of several large wastewater treatment facilities. BMAP consists of four major tasks: (1) ambient toxicity testing; (2) bioaccumulation studies; (3) biological indicator studies; and (4) ecological surveys of stream communities, including periphyton (attached algae), benthic (bottom-dwelling) macroinvertebrates, and fish. This document, the second in a series of reports on the results of the Y-12 Plant BMAP, describes studies that were conducted between July 1986 and July 1988, although additional data collected outside this time period are included, as appropriate.

BACKGROUND

Effluent discharges from the Y-12 Plant entered the headwaters of EFPC above New Hope Pond (NHP), a 2.2-ha impoundment that was located just east of the plant boundary. In November 1988, the flow of EFPC was redirected from NHP to a new, lined impoundment (Lake Reality) adjacent to the old one. New Hope Pond was eventually drained, the sediment was removed, and the impoundment was filled and capped in 1989.

From the outfall of NHP to the confluence with Poplar Creek above the Oak Ridge K-25 Site, the stream was 23.7 km in length. Effluent discharges of 388 L/s from the Y-12 Plant at East Fork kilometer (EFK) 23.7 and 227 L/s from the Oak Ridge Wastewater Treatment Facility (ORWTF) at EFK 13.4 together constituted 39% of the mean annual flow (1456 L/s, 1960–85) in EFPC at EFK 5.3. Between EFK 22.7 and EFK 7.7 [the reach of EFPC located off the DOE Oak Ridge Reservation (ORR)], the stream also receives agricultural runoff. The BMAP studies were conducted at six primary sites on EFPC (EFK 24.4 above NHP, EFK 23.4, EFK 18.2, EFK 13.8, EFK 10.6, and EFK 6.3). These sites were compared with a site on Brushy Fork (BF), Brushy Fork kilometer 7.6, an off-site reference stream located just north of Oak Ridge. Other sampling sites on EFPC and several reference streams were also used, depending on the specific objectives of the various BMAP tasks.

Water and sediment in EFPC downstream from the Y-12 Plant contained metals, organic chemicals, and radionuclides discharged over many years of operation. Water quality parameters of particular interest were ammonia, copper, mercury, nitrogen, oil and grease, perchloroethylene, and residual chlorine. Of these, ammonia, copper, mercury, perchloroethylene, and total residual chlorine (TRC) could have been toxic at maximum concentrations, depending on the length of exposure. Water temperatures in EFPC just below NHP were generally 4–7°C higher than in BF.

TOXICITY TESTING

Ambient (instream) toxicity was determined by 7-d static-renewal tests that measured the survival and growth of fathead minnow (FHM) (*Pimephales promelas*) larvae and the survival and reproduction of a small crustacean (*Ceriodaphnia dubia/affinis*). Full-strength water from Area Source Study Site 8 (AS8) was found to be toxic to FHM in only one of eight tests but was toxic to *Ceriodaphnia* in three of seven tests. In the test initiated on November 3, 1988, water from AS8 that was toxic to both species contained TRC levels of 0.6 mg/L, a concentration high enough to have caused the observed mortality.

Data for toxicity tests using water from NHP outlet (NHP-o) and NHP inlet (NHP-i) are available for the period between September 1986 and October 1988. Collectively, the results of the toxicity tests with both species were in good general agreement; chronic and acute toxicity were at least intermittently evident at the inlet to NHP, but both chronic and acute toxicity of the water declined as it flowed from NHP-i to NHP-o.

Water samples from six sites in EFPC downstream from the outfall of NHP were tested for toxicity eight times with both species from October 1986 through October 1988. These sites were ranked with the number of times they were "best" or "worst" for each species. The results of the ranking procedure showed no longitudinal pattern to water quality in EFPC based on either FHM growth or *Ceriodaphnia* fecundity in 7-d tests.

SPECIAL STUDIES TOXICITY TESTS

Several special studies were started during fall 1986. These included (1) export of aquatic plants from NHP, (2) effects of aquatic plants from NHP on biota, (3) amphipod food preference test, (4) snail food preference transition test, (5) snail food preference "beaker" test, (6) instream snail food preference test, and (7) *Ceriodaphnia* test of *Potamogeton* leachates. Results of the special studies showed that (1) large amounts of plant matter produced in NHP, largely *Potamogeton*, filamentous algae, and *Najas*, were exported from the pond during the growing season; (2) the exported material was enriched, relative to the plants from a noncontaminated pond, with polychlorinated biphenyls (PCBs) and various metals; and (3) very little of the particulate matter exported from the pond continued intact (i.e., in recognizable form) for more than several kilometers once it entered EFPC. In short-term laboratory and field tests, snails, amphipods, and *Ceriodaphnia* were able to discriminate between *Potamogeton foliosus* from NHP and *P. foliosus* from a noncontaminated pond. Plants from NHP were either preferred less than those from the noncontaminated pond or yielded leachates that were toxic to *Ceriodaphnia*.

INSTREAM MONITORING OF THE PERIPHYTON COMMUNITY

The periphyton monitoring sites were characterized during September 1987 (when leaves were present on the riparian vegetation) and in March 1988 (when the riparian vegetation was leafless). The major conclusions of the first two years' efforts were that

1. Both the Y-12 Plant and the ORWTF discharged nutrients that may have stimulated algal growth in EFPC.
2. Algal biomass was generally high in EFPC, particularly at sites that were not shaded by riparian vegetation.
3. The algal periphyton at EFK 13.8 and EFK 6.3 (which is far enough downstream from ORWTF that its effects were no longer evident) had high chlorophyll-adjusted rates of primary production, suggesting that they were in good physiological condition, while algal periphyton at EFK 10.6 (below ORWTF) had low rates of chlorophyll-adjusted production.
4. Short-term bioassays of water quality for algal photosynthesis and field studies of algal colonization/development indicated little difference among sites downstream of NHP that related to the observed differences in algal biomass or production.
5. Occasional releases of toxicants from the Y-12 Plant probably resulted in the highly variable spatial and temporal distribution of periphyton at EFK 24.4, above NHP (activities at the Y-12 Plant may also have had an occasional adverse impact on the algal periphyton directly below NHP; however, those effects likely did not extend far downstream).
6. Periphyton just downstream of NHP accumulated metals and may have transferred these metals to higher trophic levels.

BIOACCUMULATION STUDIES

Contaminant monitoring was conducted from December 1986 through May 1988. Fish were collected from five sites in EFPC and Hinds Creek, a reference stream. Concentrations of mercury in fish from EFPC were elevated above those found in fish from Hinds Creek. Thirty percent of the fish collected from EFPC exceeded the U.S. U.S. Department of Agriculture Food and Drug Administration (FDA) tolerance limit of 1 $\mu\text{g/g}$ (FDA 1984a). Mean mercury levels in sunfish [bluegill (*Lepomis macrochirus*) and redbreast sunfish (*L. auritus*)] were highest just below NHP and decreased steadily downstream from the site. Mean concentrations in carp (*Cyprinus carpio*) did not appear to follow any consistent pattern among sites. No significant relationship between mercury concentrations and fish weight existed in the fish collected. Linear regressions of mercury concentrations in redbreast vs time indicated that mercury contamination in this species increased a small but statistically significant amount since the BMAP monitoring was started in May 1985. The slopes of mercury vs time were statistically significant for all sites on EFPC except EFK 2.1 and corresponded to average increases in mercury of 0.2 to 0.8 ppm in fish.

Sunfish collected from EFK 23.4 in 1987 and 1988 contained concentrations of metals (other than mercury) that were similar to those found in fish from Hinds Creek. Copper and selenium tended to be higher in EFPC fish than in Hinds Creek fish in January 1987. PCB contamination detected in fish from EFPC in the 1985/1986 sampling was also observed in the 1987/1988 collections. The pattern of highest PCB concentrations in fish nearest NHP with decreasing levels at sites farther downstream continued as it did for mercury. Analysis of covariance performed on data for the period December 1986 through May 1988 found no significant relationship between PCB concentrations and weight in bluegill and carp (nor site-weight or season-weight interactions) but did indicate

a significant relationship and season-weight interaction for redbreast sunfish. No significant differences were observed in the mean total PCB concentration between bluegill and redbreast sunfish on six of eight date-site combinations where both species were abundant in December 1986–May 1988. PCB concentrations in carp significantly exceeded those in sunfish on eight of ten site-date combinations.

Studies using caged Asiatic clams (*Corbicula fluminea*) suggested that NHP was a source of much of the PCB contamination in EFPC; however, the downstream pattern of PCB accumulation in clams did not show the consistent decrease with distance that was noted in fish. Acute and chronic toxicity tests were conducted to assess the adverse effects of concentrations of PCB congeners to *D. magna* and FHM. Both 48-h *D. magna* and 96-h FHM static and static/renewal toxicity tests showed that the LC₅₀ values for both species were greater than the aqueous solubility limit for all congeners except 2,2',5-trichlorobiphenyl (IUPAC No. 18) for FHM. Results from the chronic toxicity test indicated that individual PCB congeners did not produce adverse effects on any aspect of the reproductive competence of *D. magna*. In addition to high levels of PCBs, concentrations of polycyclic aromatic hydrocarbons were markedly higher in clams after 4 weeks residence in EFPC, although the concentrations of individual compounds were not high. In clams exposed to EFPC water (EFK 23.4) for 4 weeks, mean concentrations of benzo[*a*]anthracene, benzo[*b*]fluoranthene, and pyrene increased by more than a factor of 4.

All concentrations of ¹³⁷Cs in fish were well below the screening preliminary guidance value (PGV) of 100 Bq/kg used previously to assess human health concerns (Hoffman et al. 1984). It does not appear that the Y-12 Plant was a significant source of ¹³⁷Cs contamination in fish.

To understand and eventually predict the accumulation of organic contaminants, especially PCBs, by fish in EFPC, several laboratory experiments were conducted. The effect of dissolved organic material (DOM) on the uptake of benzo[*a*]pyrene (B[*a*]P) and 2,2',5,5'-tetrachlorobiphenyl (TCB) by trout gills was measured by using a fish metabolic chamber. There were no significant changes in trout respiratory functions with DOM concentration increases in either the B[*a*]P or TCB exposures. DOM did reduce the apparent uptake efficiency of B[*a*]P and TCB by trout gills. As DOM concentrations increased, B[*a*]P and TCB uptake efficiencies decreased. Reductions in uptake are equal to reduction in the freely dissolved compound and can be predicted from determinations of the binding coefficient, K_p, and the concentration of the DOM.

Binding of PAHs or PCBs to DOM, or to fractions of DOM, reduced the bioavailability of B[*a*]P and TCB to *D. magna*. The reduction in accumulation was directly related to the amount of the contaminant bound to the DOM or to a fraction of the DOM.

Laboratory studies were conducted to determine physiological factors affecting contaminant uptake. Exposure to chlorine caused gill tissue damage resulting in changes in gill membrane diffusional properties. Oxygen and PCB uptake efficiencies were reduced to an equivalent extent. Ventilatory functions compensated for the gill damage and permitted oxygen consumption to remain constant, but these compensatory adjustments maintained a constant PCB dosage throughout the chlorine exposure.

Manipulation of trout respiration by acute temperature changes resulted in similar changes in oxygen and compound uptake efficiencies. Data from this experiment and the chlorine experiment suggest that toxicant uptake can be estimated by using oxygen uptake data.

BIOLOGICAL INDICATORS OF CONTAMINANT-RELATED STRESS

The biological indicators task was designed to address three concerns relative to the effects of operation of the Y-12 Plant on the biota of EFPC: (1) the health status of fish in various areas of EFPC compared with that of fish in nonaffected areas, (2) temporal effects or changes in the health status of fish in EFPC resulting from clean-up or remedial actions, and (3) evaluation of the causative agents or mechanisms responsible for any effects observed on the fish populations in EFPC.

Bioindicators representative of several levels of organismal function are necessary to evaluate the effects of chronic stress on fish. Studies indicated a downstream gradient in fish health; fish in the poorest health were found below NHP. The health of fish in the lower sections of EFPC improved over the study period. While the health of fish in the upper reaches of EFPC (EFK 23–EFK 19) did not improve in this time period, the condition of fish is expected to improve in the future with continued remedial actions. The condition of fish in EFPC was due primarily to toxicological exposure, evidenced by high levels of detoxification enzymes, metallothioneins, deoxyribonucleic acid (DNA) damage, and liver-somatic indices observed in EFPC fish. The reproductive potential of female redbreast sunfish collected directly below NHP was compromised at the outset of the 1988 breeding season while fish collected 4 km or further downstream exhibited few, if any, indications of reproductive dysfunction. Metabolic stress due to toxicant exposure (1) reduces the amount of energy available for growth, gonadal maturation, and repair of damaged tissues and (2) compromises the integrity of the reproductive and immune systems.

INSTREAM ECOLOGICAL MONITORING

Results obtained during the second year of BMAP indicated that effluents from the Y-12 Plant were still adversely affecting the benthic macroinvertebrate community in upper EFPC; however, the extent of the influence of the Y-12 Plant is not yet known. Richness, diversity, density, biomass, and production were lowest at EFK 24.4 through May 1987. With increasing distance downstream from the Y-12 Plant, the benthic community exhibited gradual improvement, with greatest improvement occurring at EFK 13.8. At the two sites downstream of EFK 13.8, the benthic community exhibited signs of additional stress, most likely resulting from ORWTF effluent discharges. Results from instream clam studies during the summer and fall of 1988 suggested continued impact through this period. Although the causes of impact have not yet been identified, impact was most likely the result of a combination of several factors, such as elevated temperatures, sublethal concentrations of toxicants, periodic releases of toxicants, and siltation.

Results of the fish population studies during 1986–88 indicated that the population in EFPC was in poor, stressed condition, although some recovery was suggested. Index of biotic integrity (IBI) ratings for the upper reaches (EFK 24.4–EFK 18.2) of EFPC continued to be poor, with little improvement. Even where improvements were noted (e.g., an increase in species richness at EFK 23.4), the levels were still far below the expected level for a stream with the available amount of water and habitat in EFPC. Downstream sites (EFK 13.8–EFK 6.3) showed steady improvement over the four

sampling periods in 1986–88. The IBI reflected improvements with ratings going from very poor to poor, with increasing values for metrics on numbers of darters, numbers of intolerant species, and percentage of lithophilic spawners. The improvements at these sites indicated that recovery of the lower portion of EFPC was occurring and should continue to become more evident with further monitoring.

FUTURE STUDIES

The results of studies conducted to date will be used to direct further monitoring efforts. Sampling strategy and frequency will remain the same for the effluent and toxicity studies, although new site(s) will be added upstream of NHP/Lake Reality. Beginning in the third year, the macroinvertebrate sampling will be reduced from monthly to quarterly. A single qualitative sample will also continue to be taken annually at each site during the spring. Instream clam studies will continue, but with increased replication. Regular, quantitative monitoring of the density, biomass, and richness of fish populations will continue on a spring-fall sampling regime.

Several new toxicity studies will be initiated, including *in situ* snail tests, snail feeding tests, and instream monitoring of chlorine. Periphyton studies will be expanded to include investigations to determine whether microbial activity is impacted in a manner similar to algal periphyton activity and to determine to what extent the composition or contaminant content of the periphyton in EFPC influences the composition, numbers, and production of grazing organisms. Future bioaccumulation studies will focus on efforts that could lead to development of quantitative structure-property relationships that can be used to predict partition coefficients and binding mechanisms for a variety of compounds and humic materials. Bioindicator studies will incorporate manipulative caging experiments as well as field and laboratory studies to evaluate the reproductive competence of sunfish in EFPC. Benthic macroinvertebrate studies will focus on the more sensitive parameters [e.g., density, taxonomic richness, and Ephemeroptera, Plecoptera, Trichoptera (EPT) richness], and analyses will emphasize longitudinal trends rather than seasonal trends, unless important seasonal trends are observed. In addition to routine sampling of fish populations, procedures to assess the role of habitat differences in explaining community differences will be evaluated. As part of the growth evaluation study, a program will be developed to validate the use of scale analysis for age determination.

In addition to continuing the monitoring phase of BMAP, future studies will place greater emphasis on development and testing of various hypotheses regarding the causal factors and underlying mechanisms documented in this report. In some cases, the impacts characterized by the initial studies are complex with several contributing causes. Ultimately, the rate of recovery of the stream communities, the elimination of toxicity above NHP, and the reduction in contaminant residues (e.g., mercury and PCBs) of fish below NHP will all depend upon accurate identification of the causal factor(s).

1. INTRODUCTION

As a condition of the National Pollutant Discharge Elimination System (NPDES) permit issued to the Y-12 Plant on May 24, 1985, a Biological Monitoring and Abatement Program (BMAP) was developed for East Fork Poplar Creek (EFPC), the receiving stream (Loar et al. 1992b).

The proposed BMAP was developed to meet two major objectives. First, studies were designed to provide sufficient data to determine whether the effluent limitations established for the Y-12 Plant protect and maintain the classified uses of EFPC, as identified in the State of Tennessee Water Quality Management Plan for the Clinch River Basin (TDPH 1978). The two most significant uses of EFPC are (1) growth and propagation of fish and aquatic life and (2) recreation, including fishing and swimming. Primarily because of elevated levels of mercury in fish, fishing (and swimming) in EFPC have been prohibited by the Tennessee Department of Environment and Conservation (TDEC) [formerly the Tennessee Department of Health and Environment (TDHE)] since November 1982.

A second major objective of BMAP for EFPC is to document the effects on stream biota resulting from implementation of a Water Pollution Control Program (WPCP) at the Y-12 Plant. The WPCP consists of strategies to (1) eliminate direct discharges of wastewaters to EFPC and (2) minimize the inadvertent release of pollutants to the environment. Significant elements of the WPCP include (1) construction of several collection/storage facilities and nine new wastewater treatment facilities (Table 1-1 in Loar et al. 1992b) and (2) development of numerous countermeasures to reduce or preclude the release of pollutants to EFPC, including Best Management Practices Plan; Area Source Pollution Control Management Plan; a Spill Prevention, Control, Countermeasures, and Contingency Plan; and various spill prevention projects.

The BMAP consists of four major tasks that reflect different but complementary approaches to evaluating the effects of Y-12 Plant effluent on the ecological integrity of EFPC. These tasks include (1) toxicity testing, (2) bioaccumulation studies, (3) biological indicator studies, and (4) instream monitoring of the benthic invertebrate and fish communities. Ecological effects are evaluated at different levels of biological organization—from tissues and organs of individuals to populations and communities. The BMAP also utilizes a variety of approaches, including laboratory studies, manipulative field experiments, and direct instream sampling of biota to identify causal mechanisms underlying the observed effects.

It is particularly important to identify adverse effects of new wastewater treatment facilities during their initial operation so that corrective measures can be identified and alternatives for plant operation can be evaluated. As a condition of the NPDES permit for the Y-12 Plant, a Toxicity Control and Monitoring Program (TCMP) was established to evaluate the toxicity of the effluent from those facilities. Both the TCMP and BMAP, in turn, will be used to establish the final effluent limitations for each of the new wastewater treatment plants (Fig. 1-1).

WASTEWATER TREATMENT FACILITIES EFFLUENT LIMITATIONS

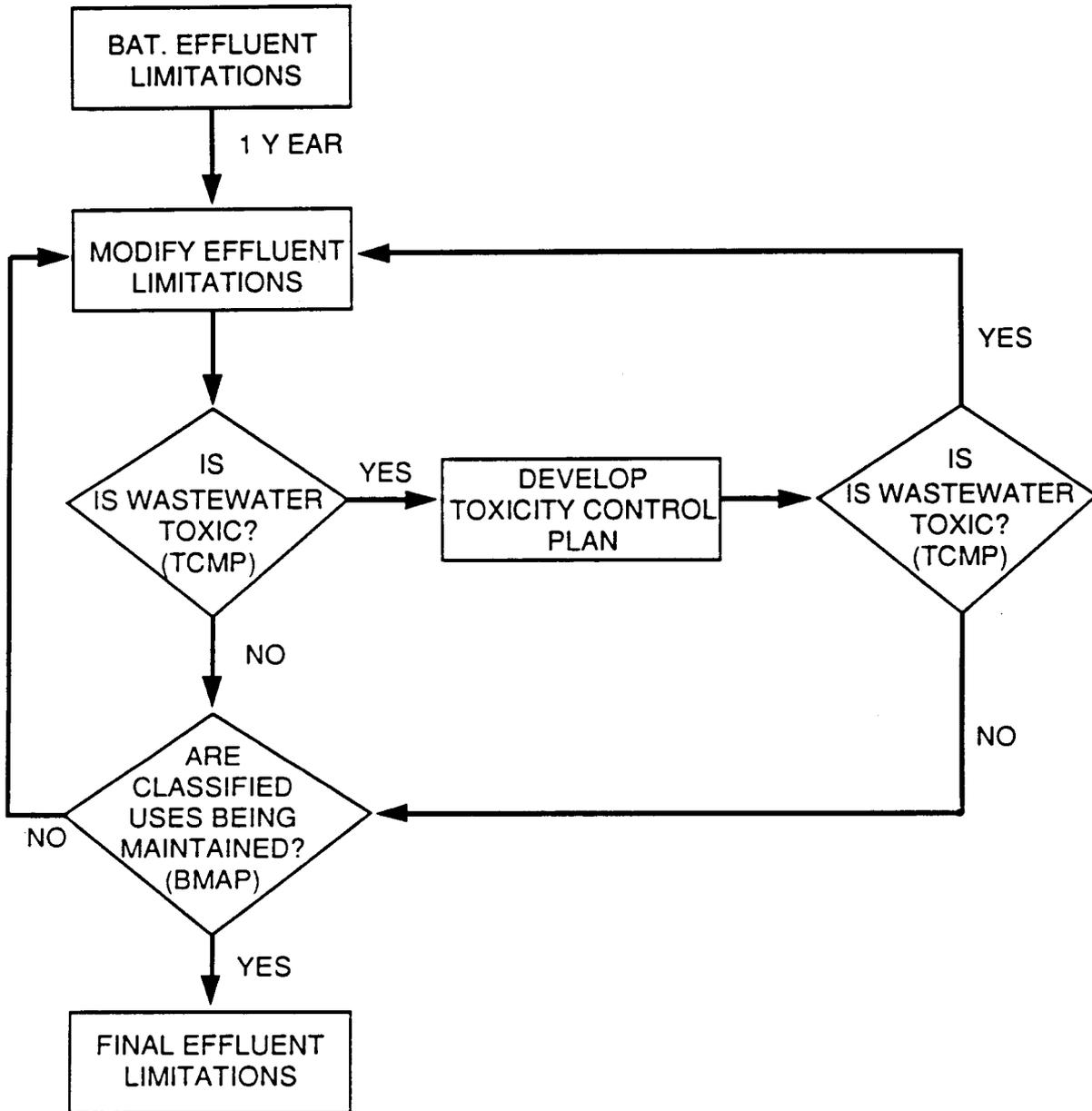


Fig. 1-1. Decision tree for establishing effluent limitations for new wastewater treatment facilities at the Y-12 Plant. BAT = Best Available Technology; TCMP = Toxicity Control and Monitoring Program; BMAP = Biological Monitoring and Abatement Program. *Source:* R. Kingrea. *Toxicity Control and Monitoring Program for Category IV Discharges at the Y-12 Plant, Y/TS-187, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1986.*

2. DESCRIPTION OF STUDY AREA

*H. L. Boston, R. L. Hinzman, J. M. Loar, M. J. Peterson, and J. G. Smith**

The EFPC drainage basin is located near the northern boundary of the U.S. Department of Energy (DOE) Oak Ridge Reservation (ORR) and has an area of 77.2 km² from the headwaters to the mouth at Poplar Creek kilometer (PCK) 8.7**. Parallel northeast-tending ridges constitute the northern (Black Oak Ridge) and southern (Chestnut Ridge) boundaries of the watershed. Elevations in the basin range from 226 m to 390 m. The largest tributary is Bear Creek, which has a drainage area of 19.1 km² and joins EFPC at EFK 2.4*. The Y-12 Plant is located near the watershed divide of Bear Creek and EFPC which flow to the west and east, respectively, of the plant (Fig. 2-1).

Because the BMAP addresses only the effects of the Y-12 Plant discharges on the biotic communities in EFPC, Bear Creek is excluded from the description of the EFPC watershed that follows. Extensive information on the Bear Creek watershed is available, however, from the numerous studies conducted in association with the development of a remedial action plan for the Bear Creek Valley waste disposal area (e.g., Evaldi 1984, 1986; Turner and Kamp 1984; Geraghty and Miller, Inc. 1985; Loar et al. 1985; Pulliam 1985a, 1985b; TVA 1985a, 1985b, 1985c, 1985d, 1985e, 1986; Turner et al. 1988; Southworth et al. 1992).

2.1 STUDY SITES (*R. L. Hinzman and J. M. Loar*)

Six primary study sites were selected on EFPC. Criteria used in the selection of these sites included (1) location of sampling sites utilized in other studies, (2) known or suspected sources of downstream pollution, (3) proximity to DOE ORR boundaries, (4) concentration of mercury in adjacent floodplain, (5) proper combination of habitat requirements, and (6) access. The sampling sites included East Fork Poplar Creek kilometer (EFK) 24.4 and EFK 23.4 [above and below New Hope Pond (NHP) respectively]; EFK 18.2, located below an area of intensive commercial and limited light industrial development and just above the area of highest mercury contamination (Table 11 in TVA 1986); EFK 13.8, located approximately 400 m above the outfall of the Oak Ridge Wastewater Treatment Facility (ORWTF); EFK 10.0 located approximately 900 m below Gum Hollow Road bridge and 3.4 km below the ORWTF; and EFK 6.3 located approximately 1.4 km below the ORR boundary and 1.0 km above the U.S. Geological Survey (USGS) gaging station (Fig. 2-1). These sites were all routinely sampled for fish and benthic invertebrates as part of the instream monitoring task (Sect. 6.0). In some cases, however, sites were excluded and/or others added depending

*Throughout this document, authors of each chapter will be listed alphabetically; authors of specific sections will be identified according to their level of primary research responsibility.

**Poplar Creek kilometer (PCK) 0.0 and East Fork Poplar Creek kilometer (EFK 0.0) are located at the confluence of Poplar Creek with the Clinch River and at the confluence of East Fork Poplar Creek with Poplar Creek respectively. All discharges are based on the list of key features described in Table I-1 in TVA 1986.

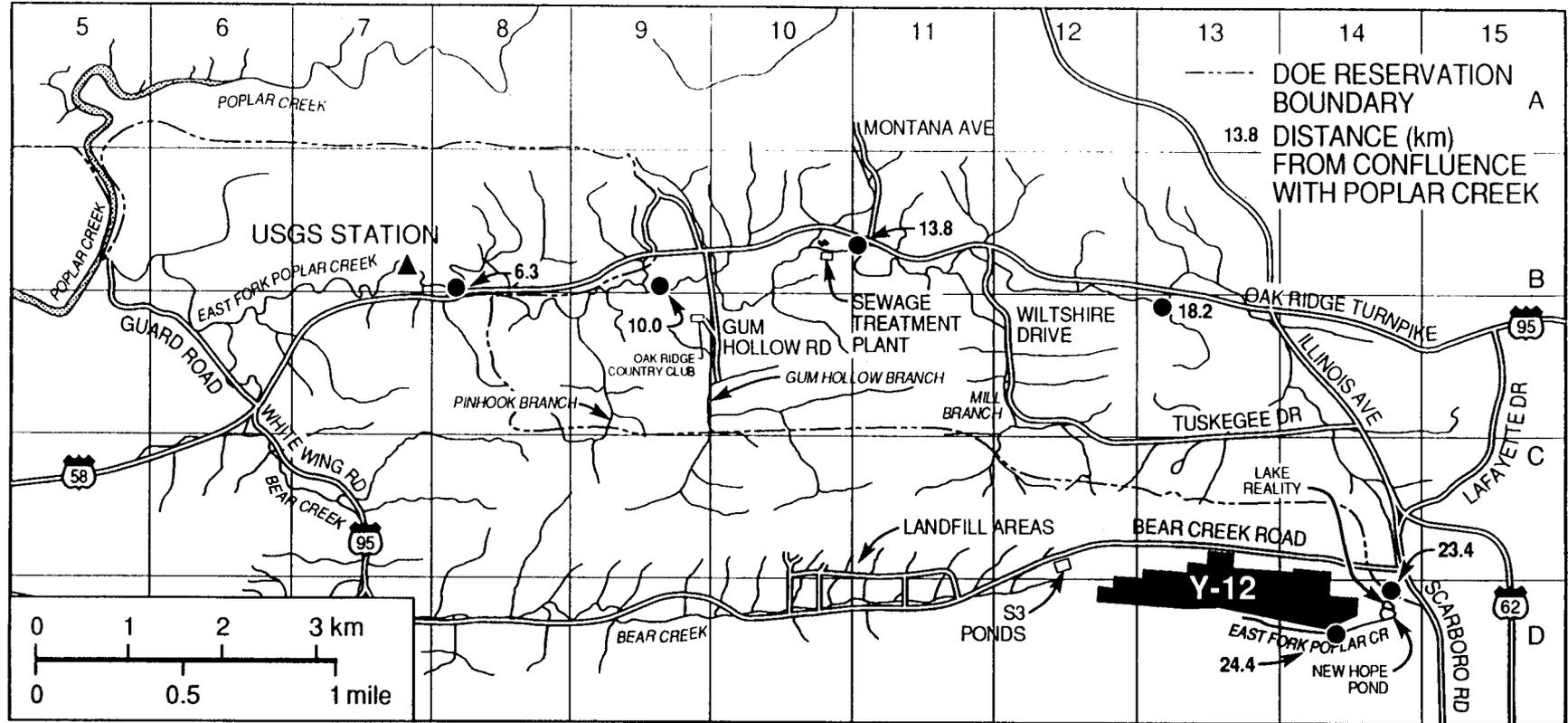


Fig. 2-1. East Fork Poplar Creek watershed showing the locations of the six primary study sites. EFK = East Fork Poplar Creek kilometer. Numerals indicate distance (in kilometers) from the confluence of East Fork Poplar Creek and Poplar Creek.

upon the specific objectives of the various subtasks included in the BMAP. Abundances of the target fish species were also a consideration at some sites (e.g., EFK 18.2 and EFK 10.0; see Sect. 6.2).

Brushy Fork (BF) at Brushy Fork kilometer (BFK) 7.6 was used as a reference stream in all four tasks of BMAP. Additional streams off the ORR were also utilized as reference sites, including Beaver Creek, Hinds Creek, and the Emory River in Watts Bar Reservoir (Fig. 2-2). Extensive sampling of BF was delayed until late 1985 when preliminary results of contaminant analyses and bioindicator studies were available for all four reference sites.

2.2 GEOHYDROLOGY

The study area is located in the Valley and Ridge physiographic province of the Southern Appalachians. The ridges are composed primarily of sandstones and dolostones and the valleys are underlain by shales, limy shales, and limestones (Geraghty and Miller, Inc. 1985). The principal groundwater-bearing formation in the Oak Ridge area is the Knox Dolomite which comprises 25% of the surface area of the EFPC drainage basin; another 32% of the area consists of Chickamauga Limestone (Table 2-1). One of the largest springs in the basin (Crystal Spring located near EFK 9.8) is the contact between the Knox and the Chickamauga Limestone and has a mean discharge of 48 L/s with a range of 29 to 91 L/s, based on monthly measurements over a two-year period (McMaster 1967). Several smaller springs also occur along the Knox-Chickamauga contact in the EFPC basin.

The primary reference area used in the BMAP is the BF watershed located just north of Oak Ridge and adjacent to the EFPC watershed (Fig. 2-2). The two drainages, which are separated by Black Oak Ridge, have a similar geologic composition (Table 2-1). The Knox Dolomite that underlies Black Oak Ridge is the source of three large springs which are tributaries to BF above the study site (BFK 7.6). These springs are Bacon Spring (mean discharge of 108 L/s), Smith Spring (52 L/s), and Burress Spring (63 L/s); Bacon Spring provides 8.5 L/s to the town of Oliver Springs for domestic use (McMaster 1967). The almost identical unit-area low-flow discharges of EFPC at EFK 5.3 and BF at BFK 10.1 (Table 2-1) provides additional evidence of the similarity in their geologic composition.

The headwaters of EFPC consist of springs that originate on the northwest slope of Chestnut Ridge. The stream is contained in culverts through much of the west end of the Y-12 Plant before entering a rip-rap channel approximately 2.4 m wide and 2.6 m high (Kasten 1986). In November 1988, the creek received discharges from 226 individual outfalls as it flowed approximately 1.5 km through the plant site (M. J. Wiest, Y-12 Plant Department of Environmental Management, personal communication). These discharges included once-through cooling water and cooling tower blowdown (approximately 74% of discharge volume), process treatment (4%), with the remainder derived from groundwater. Process treatment includes effluents from wastewater treatment facilities and other treated effluents.

Prior to November 8, 1988, EFPC flowed into NHP, a 2.2-ha impoundment constructed in 1963 to equalize the pH of the effluent from the Y-12 Plant (Pritz and Sanders 1982). The pond was also used for neutralization, sediment retention, and spill control (including provision for oil recovery by means of skimmers or chemical treatment).

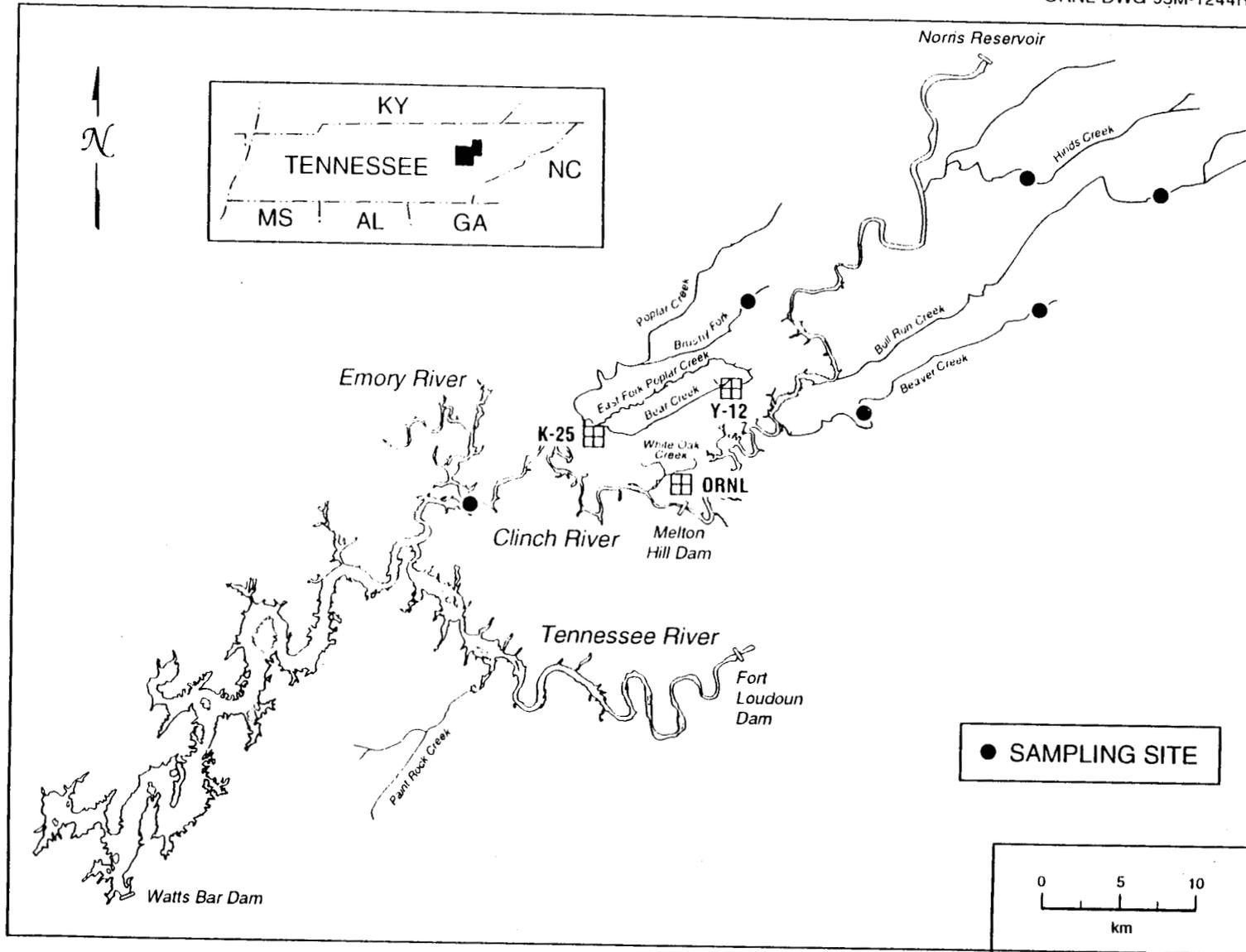


Fig. 2-2. Map of the Oak Ridge area showing locations of the reference (control) sites.

Table 2-1. General description of the geohydrology of East Fork Poplar Creek and Brushy Fork, a reference stream

Both streams are tributaries of Poplar Creek

Parameter	East Fork Poplar Creek	Brushy Fork
Confluence with Poplar Creek	PCK 8.7 ^a	PCK 29.3 ^a
Station location	USGS gage	Dossett
Distance above confluence with Poplar Creek (km)	5.3	10.1
Drainage area (km ²)	50.5	26.4 ^b
Distance/direction to nearest biological sampling site	1.0 km upstream	-2.5 km downstream
Mean annual streamflow ^c (L/s)		
1936-60 (estimated) ^b	878 (31) ^d	538 (19)
1960-85	1456 (51.4) ^e	603 (213) ^f
Low-flow per unit area (7Q10/drainage area) ^g	0.95 (0.09)	0.95 (0.09)
Geologic composition (percentage of surface area of watershed) ^b		
Rome Formation & Conasauga Group	26	49
Knox Dolomite	25	18
Chickamauga Limestone	32	31
Late Ordovician to Mississippian age	16	

^aPCK = Poplar Creek kilometer; PCK 0.0 is the confluence of Poplar Creek with the Clinch River.

^bFrom W. M. McMaster, *Hydrologic Data for the Oak Ridge Area, Tennessee*, U.S. Geological Survey, Tables 5, 8-10, Water Supply Paper No. 1838-N, U.S. Government Printing Office, Washington, D.C., 1967.

^cFlow in cubic feet per second in parentheses.

^dExcluding discharges from the Y-12 Plant and the City of Oak Ridge Wastewater Treatment Facility (ORWTF).

^eIncluding discharges from the Y-12 Plant and the ORWTF.

^fEstimated as $Q_{bf} = A_{bf}/A_{pc} (Q_{pc})$ where A_{bf} and A_{pc} are the areas of the Brushy Fork and Poplar Creek watersheds and Q_{pc} is the stream flow at the U.S. Geological Survey gaging station on Poplar Creek at PCK 22.2.

^gExpressed as cubic liters per second per square kilometer (cubic feet per second per square mile).

Construction of a bypass channel around NHP permitted long-term retention of hazardous chemical spills within the pond and thus provided a "last line of defense." On November 7, 1988, the pond was replaced with Lake Reality (LR), a 1-ha impoundment that serves essentially the same function as NHP, but has a smaller surface area, is slightly deeper than NHP, and has a synthetic liner. In addition, the outfall from LR is lower in elevation than that of NHP, allowing the free passage of fish into upper EFPC. From the outfall of NHP, EFPC flows a distance of 23.7 km to the confluence with Poplar Creek, a tributary of the Clinch River. The average gradient between the upstream limits of the reservoir backwater area and NHP is approximately 1.7 m/km (TVA 1985d).

Effluent discharges from the Y-12 Plant above NHP and from ORWTF at EFK 13.4 augment streamflow of EFPC. The mean discharge at the outfall of NHP was 388 L/s for the period 1980–85 (TVA 1985d, NPDES quarterly reports for CY 1985). Daily flows from January 1986 through June 1988 averaged 383 L/s. Mean daily flow seldom fell below 280 L/s prior to 1987, but often fell below 200 L/s in November and December 1987. The average daily discharge of ORWTF was 227 L/s between January 1983 and May 1985 (TVA 1985d). Using an estimated average discharge of 59 L/s to represent the contribution from the 3.24-km² drainage area above NHP (TVA 1986d), streamflow in EFPC is augmented by approximately 555 L/s.

Mean annual flow of EFPC at the USGS gaging station (EFK 5.3) was 1424 L/s for the period 1960–87; the station was discontinued in June 1988. The maximum and minimum daily flows over the same period were 1.16×10^5 and 340 L/s respectively (Lowery et al. 1988). The mean annual flow in 1986 and 1987 was 1002 L/s and 991 L/s respectively, and the minimum and maximum during these two years were within the range for the 1960–86 period. The adjusted mean annual flow (i.e., without the 555 L/s contributed by the Y-12 Plant and ORWTF) is 881 L/s, the same value as that estimated by McMaster (1967) based on limited flow records for the period 1961–64 (Table 2-1). The maximum flow occurred on November 28, 1973; this storm resulted in 22 cm of rainfall in 48 h (1-in-25 year flood), based on studies conducted by Edgar (1978) in nearby White Oak Creek watershed. The largest known flood occurred on September 29, 1944; the peak discharge was 1.30×10^5 L/s with a recurrence of 50 years (TVA 1985d).

The minimum daily flow of 340 L/s in EFPC is higher than that observed in much larger watersheds with little flow augmentation. Poplar Creek at the USGS gaging station (PCK 22.2), for example, has a drainage area of 213.7 km² (compared to 50.5 km² at EFK 5.3), yet the minimum discharge for the period of record (1960–87) is 105 L/s, which occurred on July 31 and August 5 and 6, 1986, and August 25 and 26, 1987 (Lowery et al. 1988). Although the creek receives discharges from the wastewater treatment facility in Oliver Springs (above PCK 22.2), the volume of these releases is probably insignificant relative to the average annual flow of 4878 L/s. Based on water yield for Poplar Creek (discharge per unit area at PCK 22.2), the minimum flow of 340 L/s in EFPC is at least an order of magnitude higher than the minimum flow would be without the contributions to streamflow from the Y-12 Plant and ORWTF.

In addition to the higher minimum flows, another characteristic of streamflow in EFPC is decreased temporal variability due to the near constant average daily discharge at the outfall of NHP (Fig. 2-3). This effect is especially evident from a comparison of the hydrographs for lower EFPC (EFK 5.3) and BF, a stream with no flow enhancement from industrial or municipal sources (Fig. 2-4). For example, the coefficient of variability (CV) based on the mean weekly discharge values plotted in Fig. 2-4 was 107.6 and 56.3%, respectively for the 1985–86 data, and 13.7 and 21.8% in 1987 and 1988 for EFPC. Although the increased

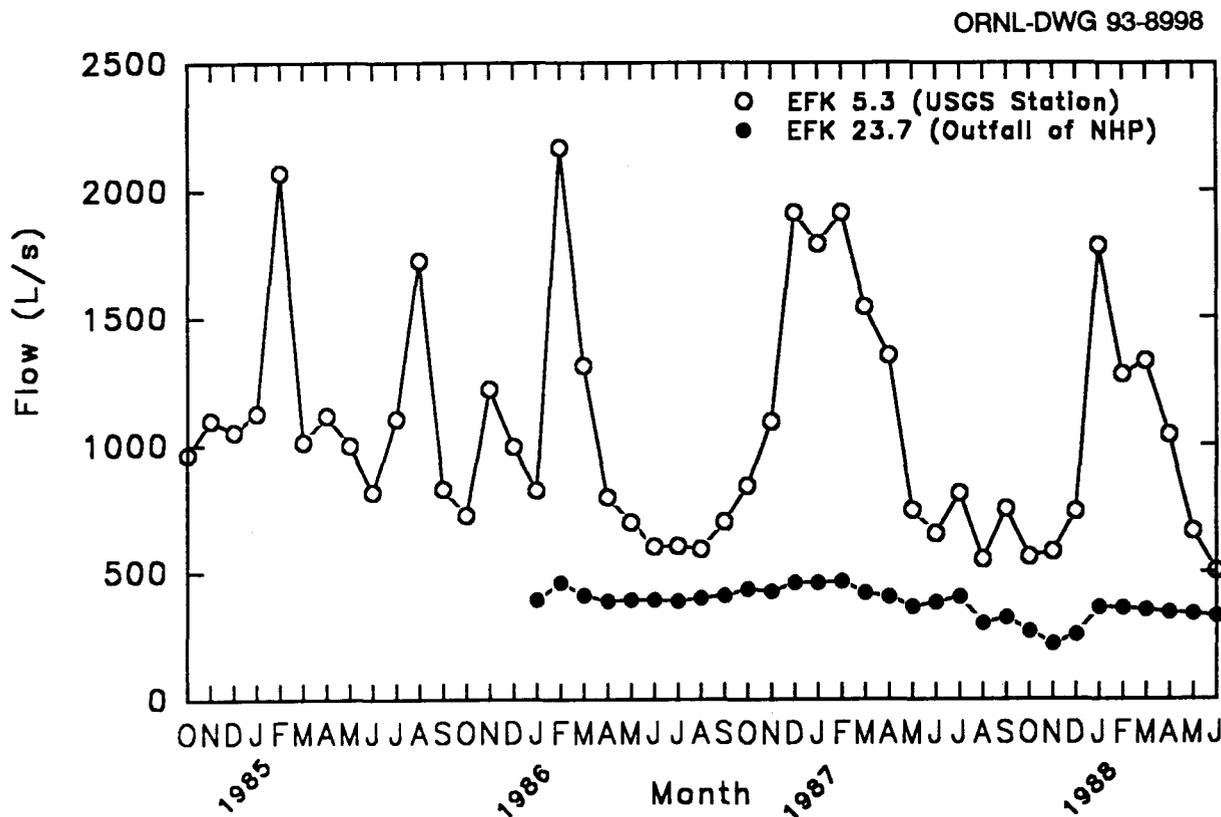


Fig. 2-3. Average monthly flows at the outfall of New Hope Pond (NHP) at East Fork Poplar Creek kilometer (EFK) 23.7 and at the U.S. Geological Survey (USGS) gaging station on lower East Fork Poplar Creek (EFK 5.3). *Source:* For EFK 23.7, NPDES quarterly reports. For EFK 5.3, J. F. Lowery, P. H. Counts, H. L. Edmiston, and F. D. Edwards, *Water Resources Data for Tennessee, Water Year 1985*, Report No. USGS/WRD/HD-86/216, 1986; *Water Resources Data for Tennessee, Water Year 1986*, Report No. USGS, WRD/HD-88/236, 1988; *Water Resources Data for Tennessee, Water Year 1988*, Report No. USGS/WRD/HD-89/258, 1989, U.S. Geological Survey, Nashville.

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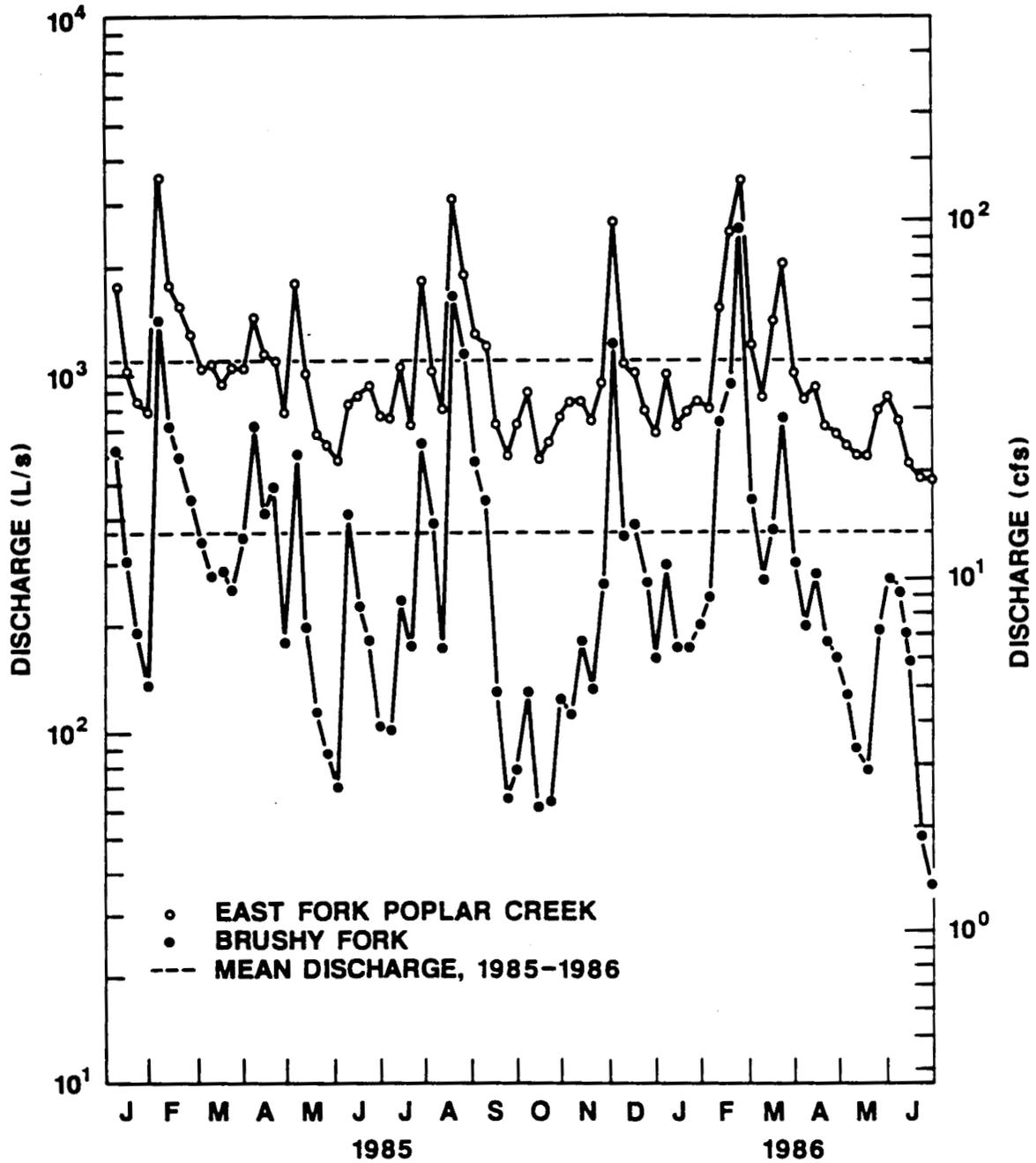


Fig. 2-4. Average weekly flows at East Fork Poplar Creek kilometer (EFK) 5.3 and Brushy Fork (BF), a reference stream, at BFK 10.1. Values represent weekly means obtained from the average daily flows at the U.S. Geological Survey gaging stations on lower EFPC (EFK 5.3) and on Poplar Creek (PCK 22.2) approximately 7 km below the confluence with BF. Estimates of flow in BF are based on extrapolation of water yield (discharge per unit area) at the gaged site on Poplar Creek (see also Table 2-1, footnote f of this document).

minimum flow benefits aquatic biota by reducing streambed dewatering and thus minimizing the loss of habitat, increased flow stability reduces environmental heterogeneity (i.e. habitat diversity) which can adversely affect species richness and/or density.

2.3 LAND USE

Land use in the EFPC watershed reflects the public and private ownership of property in the basin. The creek flows less than 1 km below NHP before leaving the DOE Oak Ridge Reservation at EFK 22.7. For the next 15 km, EFPC flows through the city of Oak Ridge, which had a population of 26,920 in 1986 (U.S. Department of Commerce 1988), before crossing the Reservation boundary again at EFK 7.7 for the remainder of its course. The lower portion of the watershed within the ORR is undeveloped, consisting mostly of pine plantations and mixed hardwood stands.

Land use in Oak Ridge consists mostly of commercial and residential developments, some light industry and agriculture, and forested areas. Most of the industrial development is limited to the northeastern part of the basin (Fig. 2-1, grids C-15, D-15). Drainage from this area enters EFPC between EFK 22.5 and EFK 21.5. Commercial development occupies much of the floodplain and adjacent areas of the creek from EFK 22.5 to EFK 18.0. Farther downstream to the Reservation boundary, residential and some agricultural development (primarily livestock grazing) occur. Construction of single and multifamily homes between EFPC and Route 95 (Oak Ridge Turnpike), which parallels EFPC west of Oak Ridge (Fig. 2-1), has increased recently.

Approximate land use in the watershed above the USGS gaging station at EFK 5.3 is 15% urban, 28% grass (pasture, lawn, etc.), and 57% forest (TVA 1985d). Although urban development accounts for a relatively small proportion of the land use, it has apparently had a significant impact on sediment transport in EFPC. TVA (1985d) estimated sediment transport in EFPC to be 352 t/km². This value exceeds the predicted sediment transport for a rural watershed the size of EFPC (50 km²) by a factor of ~4 and is more than two orders of magnitude greater than the sediment yield from a 50-km² forested watershed (based on data for the Central Atlantic States, Manning et al. 1977). It is also relatively high compared to other, mostly rural watersheds in the Tennessee Valley and is probably associated with urban development and channel realignment within Oak Ridge (TVA 1985d).

2.4 WATER QUALITY

Water and sediments in EFPC downstream from the Y-12 Plant contain metals, organic chemicals, and radionuclides that have been discharged over many years of operation. Most of the information on these contaminants was obtained in studies conducted by TVA for the Oak Ridge Task Force (ORTF), a multiagency group established in November 1983 to evaluate potential off-site contamination problems associated with the DOE facilities near Oak Ridge, Tennessee. Prior to that time, the only surveys of ambient water quality in EFPC below NHP were those conducted in 1961–64 (McMaster 1967) and 1974–75 (ERDA 1975).

The ORTF survey involved extensive sampling throughout the ORR and off-site. Water samples were taken at EFK 23.1 during the baseflow survey and analyzed for conventional parameters, priority pollutants (organics and metals), and radionuclides (TVA 1985a); only lithium and mercury exceeded background levels (Table 3 in TVA 1986). Sediment samples

were collected from EFPC and the floodplain near EFK 21.7 and EFK 2.7 and from the western and eastern ends of NHP; samples were analyzed for 114 organics, 14 metals and cyanide, and 12 radionuclides (Table I in Hoffman et al. 1984). Of these, 10 priority pollutants [7 polycyclic aromatic hydrocarbons or PAHs, bis (2-ethyl hexyl) phthalate, total polychlorinated biphenyls (PCBs) and total phenols] and 7 metals (As, Cd, Pb, Hg, Ni, Ag, and Zr) were found in EFPC at concentrations above background levels and/or above the analytical detection limit (Table 4 in TVA 1986). Additional and more extensive sampling of sediments in EFPC was conducted to estimate the quantity of mercury-contaminated sediment and floodplain deposits and to assess the transport and/or stability of mercury-contaminated sediment in the EFPC watershed (TVA 1985b, 1985d).

A review of EFPC water quality for this report is based on an analysis of NPDES data collected at the outfall of NHP from January 1, 1985, through June 30, 1988. Additional information is provided from supplemental analyses conducted in various subtasks of BMAP, including (1) routine measurements of several conventional parameters as part of the toxicity testing protocol (Sects. 3.1 and 3.2), and (2) nonroutine water and sediment sampling as part of periphyton and bioaccumulation studies (Sects. 3.3 and 4.1 respectively). More extensive ambient water quality sampling was initiated in EFPC and BF in 1986. This program will be modified, as appropriate, based on the results of future toxicity and ecological monitoring.

2.4.1 NPDES Monitoring at the Outfall of New Hope Pond

Mean and maximum values of the 25 parameters monitored at NPDES station 303 (outfall of NHP) are listed in Table 2-2. In the first Y-12 BMAP Report (Table 2-2 in Loar et al. 1992b) the potential toxicity of the maximum observed concentrations as well as the variance in the mean concentration, as indicated by the standard deviation (SD), were evaluated by screening the data to identify possible causal links with observed ecological effects downstream. On the basis of that review, ammonia, copper, nitrogen, oil and grease, perchloroethylene, and residual chlorine were identified for further evaluation.

Of the six, ammonia, copper, perchloroethylene, and residual chlorine could have been toxic at the maximum concentration reported, depending upon the length of exposure. Nitrogen is often associated with the adverse effects of nutrient enrichment. The oil and grease component was included because of the uncertainty regarding its composition and hence toxicity. All six parameters had a relatively high SD. The SD of total suspended solids concentration was also high, but a review of hourly precipitation data collected at the Oak Ridge Atmospheric Turbulence and Diffusion Laboratory (ATDL) showed that the high levels were associated with rainfall events.

The elevated levels of nitrogen and ammonia observed at the plant in February 1985 (Fig. 2-5 in Loar et al. 1992b) were most likely caused by the plant-wide use of urea, which is approximately 46% nitrogen, for snow removal after the supplies of bulk salt had been exhausted (Personal communication from T. R. Butz to G. H. Winebarger, Y-12 Plant, October 29, 1985). The source of other episodic increases in nitrogen concentration that occurred in November 1985 and March and June 1986 are unknown. A review of ATDL climatological data showed no snowfall or below-freezing temperatures prior to or on the date the samples were collected. Mean and maximum values for nitrogen in 1987 and 1988 were lower than values recorded for 1986 (Table 2-2). The source of the high perchloroethylene values in June 1985 (3.8 mg/L) and May 1988 (22.0 mg/L) are not known. Because it would volatilize during residence in NHP, perchloroethylene concentrations measured above NHP were probably even greater than the concentrations measured at the outfall of the pond.

Table 2-2. Mean (maximum value in parenthesis) concentrations of the 27 National Pollutant Discharge Elimination System parameters monitored at the outfall of New Hope Pond from June 1, 1985, through June 30, 1988, compared with U.S. Environmental Protection Agency water quality criteria

Concentrations are in milligrams per liter unless otherwise noted

Parameter	Concentration by monitoring period			EPA water quality criteria	
	1985-86	1986-87	1987-88	Acute	Chronic
Ammonia	<0.25 (3)	0.42 (2.4)	0.4 (3.2)	NC	NC
Beryllium ($\mu\text{g/L}$)	<0.5 (0.6)	<0.1 (0.7)	<0.1 (0.4)	130	5.3
Biochemical oxygen demand	<5.2 (8)	<5 (9)	<6 (67)	NC	NC
Cadmium ($\mu\text{g/L}$)	<2.2 (14)	<3 (12)	<3 (6)	7.4 ^a	1.8 ^a
Chemical oxygen demand	22 (560)	15 (320)	13 (110)	NC	NC
Copper ($\mu\text{g/L}$)	14 (190)	9 (29)	8 (53)	30	19
Chromium ($\mu\text{g/L}$) (as CrIII)	<10 (<12)	<6 (13)	<6 (21)	2746	327
Dissolved oxygen	8.7 (4.1 ^b)	8.6 (1.4 ^b)	8.6 (6.0 ^b)	5.0 ^b	
Dissolved solids	264 (830)	286 (3300)	273 (640)	NC	NC
Flow (L/s)	421 (1605)	417 (1370)	320 (1179)	NC	NC
Fluoride	1.1 (1.6)	1.0 (1.3)	1.0 (2.5)	NC	NC
Lead	<10 (10)	0.2 (30)	0.02 (0.04)	16.6	6.5
Lithium ($\mu\text{g/L}$)	<30 (400)	36 (460)	20 (85)	NC	NC
Mercury ($\mu\text{g/L}$)	2.2 (8.6)	3.0 (46)	2.3 (37)	2.4	0.012
Nickel ($\mu\text{g/L}$)	<10 (40)	<10 (204)	<8 (23)	2277	253
Nitrogen, total	13.6 (410)	4.0 (12)	3.7 (6.6)	NC	NC
Oil and grease	<3.1 (24)	<2.2 (4.0)	<2.2 (4.5)	NC	NC
Perchloroethylene	<0.1 (3.8)	6.9 (10)	10.2 (22)	NC	NC
pH	-(9.5)	7.7 (8.8)	7.7 (9.9)	-	6.5-8.5
Residual chlorine	0.3 (1.4)	0.2 (1.2)	0.2 (0.7)	0.019	0.011
Settleable solids	<0.1 (<0.1)	0.1 (0.1)	0.1 (0.5)	NC	NC
Surfactants as MBAS ^c	<0.05 (0.06)	<0.05 (0.06)	<0.05 (<0.05)	NC	NC
Uranium-235 (%)	-	0.99 (4.0) ^d	1.24 (19.4)	NC	NC
Uranium	-	0.02 (0.06) ^d	0.05 (7.0) ^d	NC	NC
Zinc ($\mu\text{g/L}$)	50 (110)	62 (162)	47 (111)	190	170

^aValue based on instream hardness of 175 mg/L (Table 3-7, in J. M. Loar (ed), *First report on the Oak Ridge Y-12 Plant Biological Monitoring and Abatement Program for East Fork Poplar Creek*. Y/TS-886, Oak Ridge National Laboratory, Oak Ridge, Tennessee. 1992).

^bValue in parentheses is a minimum value.

^cMethylene-Blue Active Substances.

^dMay and June only.

Note: NC = no EPA water quality criterion established for this parameter.

Source: Environmental Protection Agency (EPA), *Quality Criteria for Water, 1986*; EPA 440/5-86-001, U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, D.C., 1986.

The levels of residual chlorine measured in upper EFPC were undoubtedly toxic to biota (Mattice and Zittel 1976). Unlike the other NPDES parameters which were measured at the outfall of NHP, residual chlorine was measured at the inlet to the pond.

Mean mercury levels in NHP were slightly lower than the EPA acute water quality criterion for protection of freshwater aquatic life ($2.4 \mu\text{g/L}$) for the 1985–86 and 1987–88 periods, and higher than the criterion for 1986–87. Both the mean and maximum values for the entire study period exceeded the EPA chronic value.

2.4.2 Periphyton-Related Discrete Water Quality Sampling Program (*H. L. Boston*)

Water quality parameters such as pH, the concentrations of dissolved organic and inorganic carbon, plant nutrients, and suspended solids influence the structure and functioning of attached algal and microbial communities on submersed surfaces (components of the "periphyton"). As an example, the rates of algal photosynthesis and accrual of primary producer biomass are affected by the availability of nutrients such as phosphorus and nitrogen and by the rate of sediment deposition on benthic surfaces. Therefore, information on water quality is important for evaluating environmental conditions for biotic communities, and for predicting the responses of those communities to remedial actions.

As part of the periphyton component of the instream monitoring studies, a water quality monitoring program was initiated in mid-1986 to provide information on water quality for selected reaches of EFPC. This program consisted of the monthly collection of water samples (grab samples) from the five locations in EFPC where the periphyton community is monitored (Fig. 2-5), from EFK 17.0, and from the reference site on Brushy Fork (BFK 7.6). This program was intended to provide information for water quality parameters of interest for the periphyton component of the instream monitoring task and to augment the continuous water quality monitoring at NHP being conducted as part of the NPDES program.

2.4.3 Methods

Two 1-L grab samples of stream water were collected from each of the five periphyton community monitoring sites (Fig. 2-5), from EFK 17.0, and from BFK 7.6, at the time of collection of the monthly periphyton samples. The water samples were collected in acid washed polyethylene bottles and taken to the laboratory within one hour of collection. Samples for the determination of dissolved organic carbon (DOC) were collected in organic-free glass bottles with teflon sealed lids. The total amount of DOC was determined for samples collected each month. Quarterly, a subsample of this water was partitioned into hydrophilic and hydrophobic DOC fractions, which were again partitioned by molecular weight (discussed in Sect. 4.2). For several basic water quality parameters (pH, alkalinity, conductivity, hardness, and soluble metals) one sample was analyzed for each site. Analyses of the other parameters were performed on replicate samples. The determination of the pH, alkalinity, hardness and conductivity were conducted within two hours of collection. Samples for other analyses were preserved and/or frozen, according to approved methods (USEPA 1983), until they could be analyzed. The methods for the parameters included in the discrete water sampling program are listed in Table 2-3.

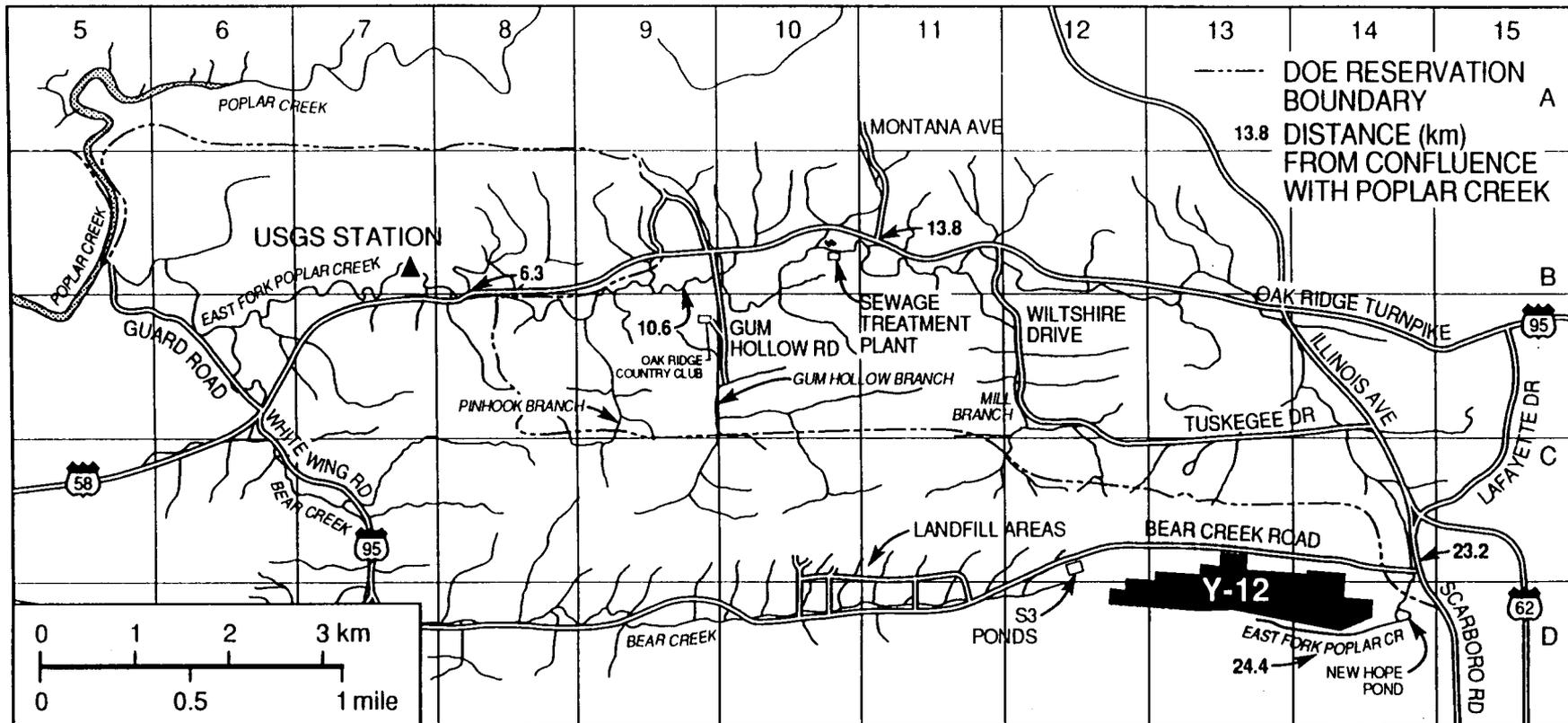


Fig. 2-5. Periphyton monitoring sites. Rocks collected from riffles (10- to 20-cm water depth) in 50- to 100-m area.

Table 2-3. Water quality parameters determined for discrete samples collected monthly at six sites in East Fork Poplar Creek and at Brushy Fork kilometer 7.6

Parameter	Method	Reference ^a
pH	Glass electrode	APHA 1985
Alkalinity	Acid titration to ~pH 4.7	APHA 1985
Conductivity	Conductivity bridge	APHA 1985
Hardness	EDTA titration	APHA 1985
Phosphorus	Ascorbic acid method	APHA 1985
Total P	Persulfate digestion	
Total soluble P	Filter & digestion	
Soluble reactive P	Filter only	
Nitrate & nitrite	Cadmium reduction	U.S. EPA 1983
Ammonia (ammonium)	Phenate method	U.S. EPA 1983
Suspended solids	Total filterable (105°C)	APHA 1985
Dissolved metals	Filter & induction coupled plasma emission spectros	APHA 1985
Dissolved organic C	Total carbon analyzer	APHA 1985

^aSources: American Public Health Association (APHA), *Standard Methods for the Evaluation of Water and Wastewater*, Sixteenth Edition, APHA, Washington, D.C., 1985; U.S. Environmental Protection Agency (EPA), *Extraction and Analysis of Priority Pollutants in Biological Tissue, Method PPB 12/83*, Mimeograph, U.S. Environmental Protection Agency, Environmental Services Division, Region IV, Analytical Support Branch, Athens, GA., 1983.

2.4.4 Results

The data from the discrete water quality sampling program are presented as data for year one (October 1986–September 1987), and year two (October 1987–September 1988) as was done for the data from the periphyton monitoring component (Sect. 3-8). The water quality at all of the sites [moderately well buffered and having slightly alkaline pH (Table 2-4)] reflect the calcareous geology of this area. The pH, alkalinity, and hardness were fairly similar for all sites. Conductivity, however, was substantially higher for the upstream sites on EFPC as a result of discharges from the Y-12 Plant. On January 22, 1987, the conductivities at EFK 24.4 and EFK 23.2 were 2460 and 809 $\mu\text{S}/\text{cm}$ respectively. When these data were excluded, the average conductivities at EFK 24.4 and EFK 23.2 for year one were 375 and 385 $\mu\text{S}/\text{cm}$ respectively. Similarly during year two, with the exception of an occasional high conductivity value, the average conductivity was similar at all EFPC sites. The utility of the correlations among the conductivity, alkalinity, and hardness data for evaluating anthropogenic changes in water quality is discussed in Sect. 3-8.

The concentrations of total suspended solids (TSS) were similar at all sites, with averages of about 13 to 20 mg dry wt/L during year one, and 2 to 11 mg/L during year two. A comparison with the data for year one showed that the concentrations of TSS decreased at all sites during year two partly due to a change in the timing of sample collection. During year one we found that the collection of periphyton samples was

Table 2-4. Water quality parameters based on samples collected monthly for two 1-year periods at six periphyton monitoring sites in East Fork Poplar Creek and Brushy Fork

Parameter	BFK 7.6	EFK 24.4	EFK 23.2	EFK 17.0	EFK 13.8	EFK 10.9	EFK 6.3
pH							
10/86-9/87							
Mean	7.99	7.94	7.94	7.97	7.99	7.89	7.92
SD	0.12	0.10	0.08	0.13	0.10	0.12	0.11
Range	7.82-8.21	7.80-8.12	7.84-8.15	7.75-8.13	7.83-8.24	7.69-8.12	7.77-8.17
pH							
10/87-9/88							
Mean	7.92	8.02	7.93	8.03	7.99	7.90	7.98
SD	0.16	0.15	0.09	0.12	0.11	0.08	0.12
Range	7.64-8.25	7.60-8.25	7.79-8.11	7.85-8.32	7.83-8.23	7.77-8.03	7.79-8.26
Alkalinity (meq/L)							
10/86-9/87							
Mean	2.57	2.12	2.18	2.41	2.34	2.48	2.43
SD	0.53	0.17	0.15	0.23	0.16	0.22	0.20
Range	1.62-3.26	1.84-2.40	1.82-2.42	1.90-2.88	2.06-2.66	1.93-2.82	1.92-2.72
Alkalinity (meq/L)							
10/87-9/88							
Mean	2.72	2.24	2.25	2.46	2.46	2.60	2.57
SD	0.38	0.23	0.20	0.25	0.11	0.17	0.16
Range	2.12-3.42	1.72-2.66	1.84-2.48	2.18-2.62	2.30-2.72	2.36-2.96	2.38-2.98
Hardness (mg/L as CaCO₃)							
10/86-9/87							
Mean	146	182	175	179	170	174	169
SD	24	21	12	12	12	16	17
Range	106-178	154-212	140-190	154-198	154-190	140-200	126-190
Hardness (mg/L as CaCO₃)							
10/87-9/88							
Mean	150	175	178	174	177	171	171
SD	26	25	19	11	9	6	13
Range	112-184	142-210	146-208	156-198	160-196	164-184	142-192
Conductivity (μS/cm)							
10/86-9/87							
Mean	244	549	421	366	363	374	347
SD	48	579	126	25	39	46	47
Range	144-313	325-2460	327-809	313-395	274-412	304-436	247-399
Conductivity (μS/cm)							
10/87-9/88							
Mean	290	447	431	411	413	445	411
SD	26	117	51	49	50	39	38
Range	238-322	342-796	358-543	365-533	372-541	402-541	306-447

Table 2-4 (continued)

Parameter	BFK 7.6	EFK 24.4	EFK 23.2	EFK 17.0	EFK 13.8	EFK 10.9	EFK 6.3
Total P ($\mu\text{g P/L}$)							
10/86-9/87							
Mean	81	556	365	234	199	1384	1322
SD	75	276	119	94	117	584	688
Range	33-321	201-1370	206-607	54-381	35-427	654-2598	382-2446
Total P ($\mu\text{g P/L}$)							
10/87-9/88							
Mean	41	349	281	216	198	1110	1244
SD	14	135	95	71	69	542	467
Range	16-60	37-576	45-400	58-296	43-292	426-1700	366-2100
Total soluble P ($\mu\text{g P/L}$)							
10/86-9/87							
Mean	31	271	218	151	155	952	1005
SD	27	160	107	84	80	440	456
Range	6-111	28-939	26-385	30-322	25-283	231-1588	142-1591
Total soluble P ($\mu\text{g P/L}$)							
10/87-9/88							
Mean	24	307	233	170	141	956	1021
SD	10	39	45	54	43	506	414
Range	0-43	229-397	179-310	98-313	94-221	117-2142	492-2070
Soluble reactive P ($\mu\text{g P/L}$)							
10/86-9/87							
Mean	15	115	83	105	103	769	722
SD	7	121	35	46	53	262	722
Range	6-24	5-456	8-143	14-194	11-201	308-1195	251-1214
Soluble reactive P ($\mu\text{g P/L}$)							
10/87-9/88							
Mean	10	54	61	91	90	662	687
SD	6	26	17	28	43	225	262
Range	0-21	26-130	32-89	54-130	38-173	309-919	311-1452
$\text{NO}_2^- + \text{NO}_3^-$ ($\mu\text{g N/L}$)							
10/86-9/87							
Mean	500	4020	3440	2780	2530	3310	3190
SD	100	910	700	250	340	800	880
Range	340-650	3590-6000	2920-4810	2640-2970	1960-3060	2000-4450	1720-4600
$\text{NO}_2^- + \text{NO}_3^-$ ($\mu\text{g N/L}$)							
10/87-9/88							
Mean	450	3090	3470	2790	2620	3450	3370
SD	190	540	1130	400	510	820	830
Range	150-840	2430-4480	1860-6630	2180-3400	1930-3390	2190-5040	1860-4930
NH_4^+ ($\mu\text{g N/L}$)							
10/86-9/87							
Mean	13	51	121	36	24	107	80
SD	6	36	70	38	25	84	99
Range	4-26	5-112	17-249	5-124	3-52	11-246	6-250

Table 2-4 (continued)

Parameter	BFK 7.6	EFK 24.4	EFK 23.2	EFK 17.0	EFK 13.8	EFK 10.9	EFK 6.3
NH₄⁺ (µg N/L)							
10/87-9/88							
Mean	27	150	334	44	25	249	179
SD	19	211	232	51	27	489	360
Range	1-59	0-685	45-706	2-195	0-105	0-1693	0-1192
Dissolved organic carbon (mg C/L)							
10/86-9/87							
Mean	1.8	3.7	2.9	2.3	2.4	3.0	2.8
SD	0.6	2.7	0.6	0.6	0.6	0.8	0.6
Range	1.0-3.0	2.0-11.0	2.2-4.2	1.6-2.8	1.5-3.1	1.9-4.8	2.1-4.1
Dissolved organic carbon (mg C/L)							
10/87-9/88							
Mean	2.0	2.8	2.8	2.5	2.6	3.3	3.2
SD	0.6	0.4	0.7	0.8	0.7	0.3	0.3
Range	1.0-3.0	1.9-3.4	1.8-4.5	1.7-4.7	1.7-4.4	2.7-4.1	2.5-3.6
Dissolved inorganic carbon (mg C/L)							
10/86-9/87							
Mean	31	25	25	28	28	29	25
SD	3	2	2	3	3	3	3
Range	27-35	21-27	21-26	23-31	23-32	25-33	24-33
Dissolved inorganic carbon (mg C/L)							
10/87-9/88							
Mean	31	25	26	28	29	30	30
SD	5	4	4	3	3	2	2
Range	23-38	19-30	21-31	24-32	26-34	26-34	26-33
Total suspended solids (mg dry wt/L)							
10/86-9/87							
Mean	14.3	13.1	13.9	14.0	16.3	20.3	16.4
SD	8.4	9.5	11.2	15.8	12.7	20.3	16.4
Range	3.4-31.0	3.7-34.3	4.5-45.3	3.4-62.8	4.7-44.7	4.3-79.2	2.8-65.8
Total suspended solids (mg dry wt/L)							
10/87-9/88							
Mean	9.3	1.9	7.7	7.5	9.2	9.5	11.1
SD	3.9	1.0	1.1	4.4	6.0	4.9	7.4
Range	2.7-14	1.0-3.8	3.6-15.6	3.0-16.4	3.1-21.4	2.5-17.3	2.6-26.4
Total N (µg N/L)							
10/88							
	470	4001	3083	2490	2327	3954	4090

Note: BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

difficult following precipitation events; therefore, during year two we usually waited several days following any precipitation event before sampling. Because TSS concentrations increase substantially during high discharge events, the data for year two tend to more closely reflect base flow conditions. During year two the average concentrations of TSS were positively correlated with drainage area (i.e., average TSS increased with distance downstream in EFPC and was moderate at BFK 7.6). High concentrations of TSS during storm discharge events influence the attached biotic communities by scouring and burial.

Concentrations of DOC in EFPC were greater than at the reference site (BFK 7.6). A single high DOC concentration (11.0 mg carbon per liter) at EFK 24.4 on January 22, 1987, elevated the average DOC at that site for year one. This high DOC concentration coincided with increased conductivity at that site, and was likely the result of a Y-12 discharge, as discussed earlier. Excluding that value, the average DOC at EFK 24.4 would be 2.76 mg carbon per liter, which is similar to the average for year two.

The concentrations of DOC were similar for EFK 24.4, EFK 23.2, EFK 17.0 and EFK 13.8. The concentrations at EFK 10.6 (below the ORWTF) and farther downstream at EFK 7.6, were consistently higher. These data suggest an influence of the sewage treatment plant on DOC concentrations.

Concentrations of dissolved nitrogen ($\text{NO}_2^- + \text{NO}_3^-$ and NH_4^+) and phosphorus were greatly enriched at all sites on EFPC, compared with data from the Brushy Fork site or other local streams not influenced by agriculture or industry (Loar et al. 1992b). The concentrations of these elements are of particular interest because they can regulate algae production and microbial production, and so can greatly influence the character of freshwater systems. Phosphorus is usually the mineral most limiting the growth of primary producers and microbes in aquatic systems. Soluble reactive phosphorus (SRP), an estimate of phosphorus available to algae and microbes, is typically $<10 \mu\text{g P/L}$ in unenriched systems. In Brushy Fork, the pastures adjacent to the stream likely contribute to the slightly elevated SRP concentrations (10 to $15 \mu\text{g P/L}$). Activities at the Y-12 Plant enriched SRP to about 50 to $115 \mu\text{g P/L}$ at EFK 24.4 and EFK 23.2. The concentrations of SRP at EFK 17.0 and EFK 13.8 were typically slightly greater than at the two upstream sites; however, examination of the data for total phosphorus suggests that the Y-12 Plant is the source of much of the phosphorus input to EFPC above EFK 13.8. All forms of phosphorus were increased to concentrations several orders of magnitude greater than typically found in natural systems at the sites below the ORWTF, which discharges at EFK 13.4. Data for total soluble phosphorus are included for the calculation of soluble unreactive phosphorus (SUP—soluble phosphorus not reacting with the molybdate reagents). The SUP provides an indication of other pools of soluble phosphorus, some of which may be available for biotic uptake. Data for total soluble phosphorus also allow the calculation of particulate phosphorus as total phosphorus minus total soluble phosphorus.

In unenriched local streams, ammonia typically averages $<30 \mu\text{g N/L}$ (Loar et al. 1992b). The annual average concentrations at BFK 7.6, EFK 17.0, and EFK 13.8 were typical of unenriched systems. Ammonia concentrations were greater than background above NHP at EFK 24.4 (averaging 51 and $150 \mu\text{g nitrogen per liter}$), and were increased below NHP at EFK 23.2 (averaging 120 and $334 \mu\text{g nitrogen per liter}$) as a result of biological activity in the pond. Ammonia concentrations were relatively high at EFK 10.6 and EFK 6.3 (averaging 80 to $249 \mu\text{g nitrogen per liter}$), reflecting the influence of the ORWTF. Concentrations of ammonia were somewhat higher at all sites (except EFK 17.0

and EFK 13.8) during year two compared with year one. Several high values at all sites during year two are partly responsible for the increased averages of ammonia concentrations.

Concentrations of $\text{NO}_2^- + \text{NO}_3^-$ nitrogen were typically less than 0.5 mg nitrogen per liter in local streams (e.g., BFK 7.6 averaged 0.5 mg nitrogen per liter during both years). The discharge of water from the Y-12 Plant that was rich in $\text{NO}_2^- + \text{NO}_3^-$ resulted in high concentrations of these ions upstream (averaging 3 to 4 mg nitrogen per liter at EFK 24.4) that persisted at the downstream sites. ORWTF is an additional source of $\text{NO}_2^- + \text{NO}_3^-$ to EFPC, as evidenced by the increased concentrations at EFK 10.6 compared with concentrations at the site upstream of the sewage treatment plant.

Water samples were analyzed for soluble metals by inductively-coupled plasma (ICP) spectroscopy on six dates during year one and four dates during year two. The basic cations Ca, Mg, and Na, were the only elements consistently detectable by ICP (Table 2-5). Other elements (Al, Ba, Be, Fe, Mn, Mo, Si, Sr, and Zn) were frequently detectable; however, their concentrations in EFPC were generally similar to those at BFK 7.6. We found no soluble metals at concentrations that are known to have direct toxic effects on the biota. However, data for the concentrations of metals in the periphyton matrix at the EFPC sites (see Sect. 3-8) show the accumulation of some metals, particularly at the sites nearest the Y-12 Plant.

2.4.5 Summary

Water samples collected monthly in conjunction with the periphyton component of the instream monitoring program provide useful snap-shots of water quality at the periphyton monitoring sites, and EFK 17.0. These data show that EFPC is greatly enriched in nutrients (N and P) that stimulate biological activity and that the Y-12 Plant and ORWTF were the major sources of these constituents. The Y-12 Plant and the sewage treatment plant are also sources of DOC that may serve as an energy source for biotic activity. We found no evidence of high or potentially toxic concentrations of soluble inorganic constituents.

2.5 AMBIENT TEMPERATURE REGIMES (*R. L. Hinzman*)

Water temperatures were monitored continuously at the five sampling sites in EFPC below NHP (Fig. 2-1), one site above NHP, and BF*. Annual temperature regimes at the seven sites are shown in Figs. 2-6 to 2-8; data on monthly means, standard deviations, and ranges are provided in Appendix A.

Water temperatures were generally 4–7°C higher in EFPC just below NHP (EFK 23.4) than in BF. Although temperatures reached 31°C below NHP, the maximum temperature in BF was 25°C. Seasonal trends in water temperatures at EFK 18.2 and EFK 13.8 were similar (Fig. 2-7). A longitudinal gradient of decreasing temperature was

*Water temperatures were collected through April 1987 at 2-h intervals using a Peabody Ryan Model J90 thermograph. Data were keypunched into data files on the IBM 3330 system as SAS data sets. Temperature data were collected with a Ryan Tempmentor from April to July 1987 at 20-min intervals and at 1-h intervals after July 1, 1987.

Table 2-5. Concentrations of dissolved elements at six periphyton monitoring sites in East Fork Poplar Creek watershed and in Brushy Fork (BF)^a

	BFK 7.6	EFK 24.4	EFK 23.2	EFK 17.0	EFK 13.8	EFK 10.9	EFK 6.3
Al ($\mu\text{g/L}$)							
8/86–9/87							
Mean	<20	<20	<20	<20	<20	<20	<20
Al ($\mu\text{g/L}$)							
2/88–8/88							
Mean	265	247	227	215	234	280	295
SD	5	91	83	86	108	20	15
Range	260–270	120–330	110–290	97–300	82–300	260–300	280–310
Ba ($\mu\text{g/L}$)							
8/86–7/87							
Mean	55	43	47	44	43	34	31
SD	28	14	7	14	11	7	7
Range	20–89	20–57	42–51	20–52	22–56	20–39	20–39
Ba ($\mu\text{g/L}$)							
10/87–8/88							
Mean	80	55	80	64	55	53	48
SD	11	5	26	17	9	15	9
Range	66–96	47–61	55–120	47–85	43–66	35–76	34–57
Be ($\mu\text{g/L}$)							
2/88–10/88							
Mean	1.6	2.5	3.5	2.7	2.6	2.5	2.4
SD	0.6	0.8	2.2	1.0	1.0	0.9	0.8
Range	1.2–2.5	1.5–3.5	1.5–6.5	1.5–4.0	1.4–3.8	1.4–3.5	1.4–3.2
Ca (mg/L)							
10/86–9/87							
Mean	24	47	37	43	43	44	43
SD	12	8	11	13	11	11	7
Range	21–48	34–55	25–52	43–59	43–56	42–56	43–53
Ca (mg/L)							
10/87–9/88							
Mean	45	56	41	57	53	42	55
SD	3	6	24	10	4	20	5
Range	41–49	50–64	0.75–60	47–73	49–56	6.6–57	50–62
Fe ($\mu\text{g/L}$)							
10/87–9/88							
Mean	61	37	35	28	34	29	40
SD	1	1	11	5	8	8	4
Range	50–78	23–45	24–46	23–34	24–44	23–40	36–43

Table 2-5 (continued)

	BFK 7.6	EFK 24.4	EFK 23.2	EFK 17.0	EFK 13.8	EFK 10.9	EFK 6.3
Mg (mg/L) 10/86-9/87							
Mean	10.7	10.5	12.0	11.1	11.5	10.8	10.9
SD	2.3	2.1	0.08	2.8	1.5	1.4	1.8
Range	6.8-13	7.1-13	11-13	10-14	9.2-14	9.1-13	8.1-12
Mg (mg/L) 10/87-9/88							
Mean	11.4	13.3	14.3	13.3	11.8	12.5	11.5
SD	2.5	0.8	0.4	0.4	1.6	0.9	1.6
Range	7.6-14	12-14	14-15	13-14	9.2-13	11-13	9.7-13
Mn (μ g/L) 10/86-9/87							
Mean	28.4	37.8	27	22.0	21.1	18.6	19.4
SD	26.0	22.8	18	11.7	13.7	13.0	13.6
Range	9.4-78	18-76	26-53	15-44	14-48	12-43	10-46
Mn (μ g/L) 10/87-9/88							
Mean	70.5	94.5	64.5	26.0	26.8	32.0	36.0
SD	24.1	102.4	38.3	10.2	7.2	22.2	25.4
Range	51-71	16-270	19-110	13-36	17-35	16-70	15-78
Mo (μ g/L) 2/88-9/88							
Mean	<40	167	89	73	94	74	54
SD	ND	52	52	45	15	10	9
Range	<40	130-240	16-130	10-110	74-110	63-87	47-66
Na (mg/L) 10/86-9/87							
Mean	2.1	90.0	110.2	17.8	21.3	23.5	21.8
SD	0	132.8	201.3	9.2	8.2	8.1	9.0
Range	1.7-2.7	13-320	14-560	14-19	11-29	13-27	9.8-24
Na (mg/L) 10/87-9/88							
Mean	1.9	14.3	14.7	13.1	10.8	18.8	18.0
SD	0.04	3.0	2.3	2.7	1.5	2.2	2.5
Range	1.8-1.9	10-18	12-17	9.3-17	9-13	16-22	14-20

Table 2-5 (continued)

	BFK 7.6	EFK 24.4	EFK 23.2	EFK 17.0	EFK 13.8	EFK 10.9	EFK 6.3
P ($\mu\text{g/L}$)							
10/86-9/87							
Mean	<300	343	<300	<300	<300	1132	1080
SD	ND	33	ND	ND	ND	340	423
Range	<300	300-380	<300	<300	<300	560-1600	490-1800
P ($\mu\text{g/L}$)							
10/87-9/88							
Mean	<300	323	<300	<300	<300	1275	1395
SD	ND	19	ND	ND	ND	249	373
Range	<300	310-350	<300	<300	<300	1100-1700	980-2000
Si (mg/L)							
10/86-9/87							
Mean	3.4	1.8	2.2	2.6	2.5	2.8	3.0
SD	0.3	0.7	0.5	0.2	0.2	0.2	0.3
Range	2.9-3.7	0.6-2.5	1.2-2.5	2.2-3.0	2.2-2.8	2.6-3.0	2.5-3.3
Si (mg/L)							
10/87-9/88							
Mean	3.3	2.1	2.1	2.4	2.0	2.4	2.7
SD	0.6	0.9	0.9	0.7	0.8	0.9	0.9
Range	2.7-4.3	0.9-3.3	0.8-3.2	1.3-3.3	0.8-2.8	0.9-3.3	1.1-3.5
Sr ($\mu\text{g/L}$)							
10/86-9/87							
Mean	52	143	109	114	112	101	101
SD	15	22	17	26	20	21	14
Range	47-78	130-180	90-130	97-140	100-140	73-130	82-120
Sr ($\mu\text{g/L}$)							
10/87-9/88							
Mean	88	147	160	157	143	150	137
SD	8	17	10	17	26	14	24
Range	82-100	130-170	140-180	140-180	120-180	140-170	120-170
Zn ($\mu\text{g/L}$)							
10/87-9/88							
Mean	27	62	39	16	24	19	17
SD	12	6	8	5	5	8	6
Range	18-44	57-71	31-51	11-21	19-28	13-30	12-25

Table 2-5 (continued)

ICP metals that were below detection limits (mg/L)							
AG	AL	AS	B	BA	BE	CD	CO
<0.05	<0.02	<0.10	<0.80	<0.020	<0.002	<0.005	<0.01
LI	MN	MO	NI	P	PB	SB	SE
<0.20	<0.005	<0.040	<0.06	<0.30	<0.20	<0.20	<0.20
ZR							
<0.02							

^aValues are based on inductively-coupled plasma (ICP) analysis of discrete water samples collected quarterly. Values below the ICP limit of detection were either presented as such or were assumed to be that concentration in the calculation of the mean.

Note: BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer. ND = no data available.

characteristic of EFPC; the only exceptions to this trend were the higher mean monthly temperatures in the winter at EFK 10.0 compared with EFK 13.8 and lower maximum temperatures in the summer at EFK 10.0 compared with EFK 6.3. Such trends at EFK 10.0 may be indicative of inputs from springs, which are known to occur in this region (Sect. 2.1) and/or discharges from the ORWTF at EFK 13.4 (Fig. 2-1). Water temperatures at EFK 24.4, inside the Y-12 Plant, are generally similar to those at EFK 23.4 in the summer and 2–4°C warmer in the winter. Warmer winter temperatures may be attributed to the proximity of the site to several effluent discharges (e.g., North-South Pipe).

2.6 SUBSTRATE AND COVER (*M. G. Ryon, J. G. Smith, and M. J. Peterson*)

The biological monitoring of EFPC involved analysis of the fish and benthic invertebrate communities at selected study sites distributed along the length of the stream. These sites were chosen after initial surveys so as to minimize community differences due to differences in habitat. In the summer of 1988, a complete analysis of the assumption that only minimal differences in habitat were present was begun, focusing on stream flow, stream substrate, bank cover and canopy, and the pool to riffle ratio. The data generated and briefly reported in this analysis represent the habitat conditions in EFPC during low flow months and will be followed by future surveys during other times of the year.

2.6.1 Methods

The analytical techniques used for the habitat survey were based on methods described in Platts et al. (1983). Two types of habitat data were included in this report. The first surveys were performed at each site after the fish populations were sampled.

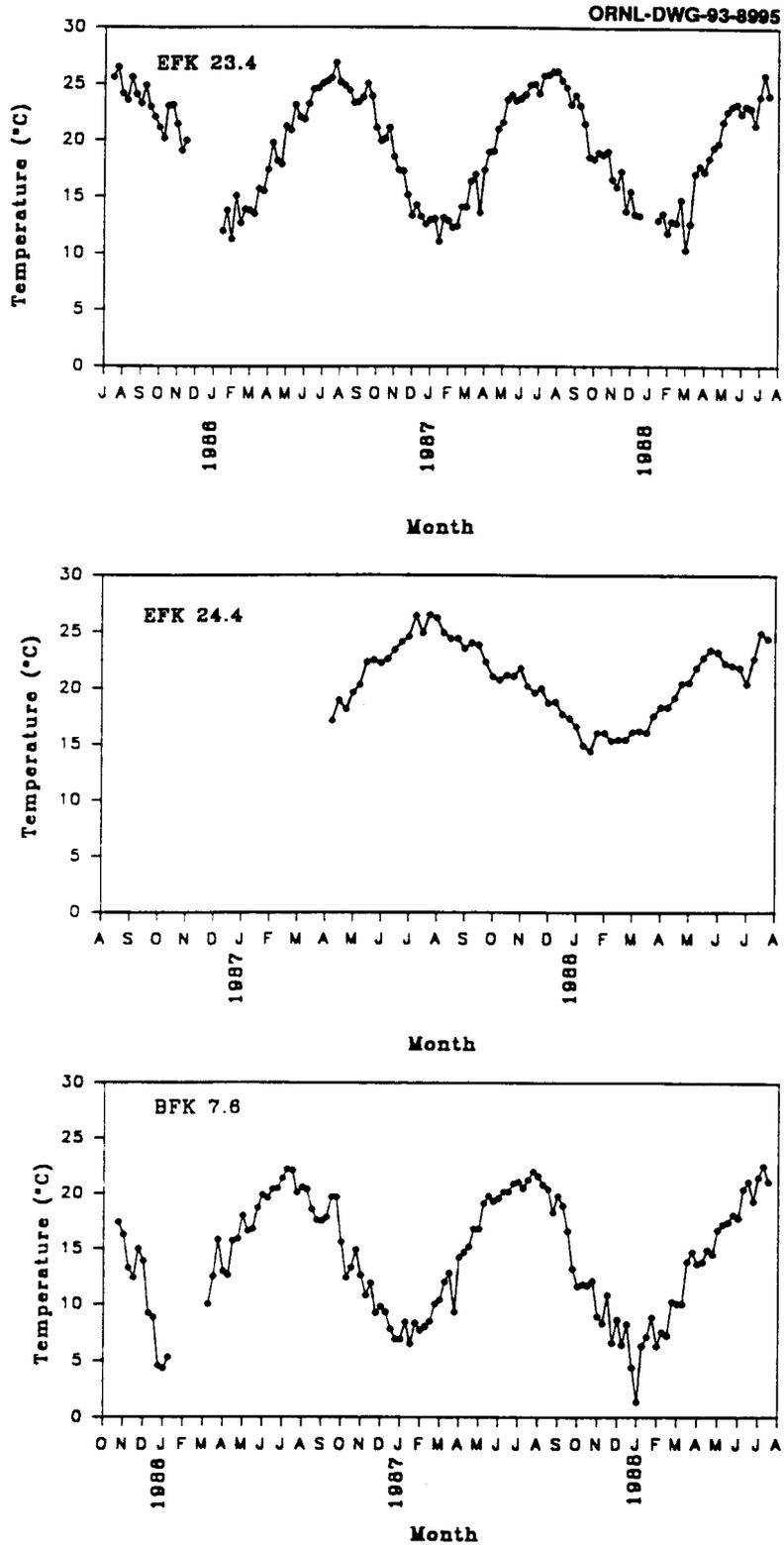


Fig. 2-6. Average weekly water temperatures (degrees Celsius) in upper East Fork Poplar Creek at East Fork Poplar Creek kilometer (EFK) 24.4 and EFK 23.4, and in Brushy Fork (at Brushy Fork kilometer 7.6), a reference stream.

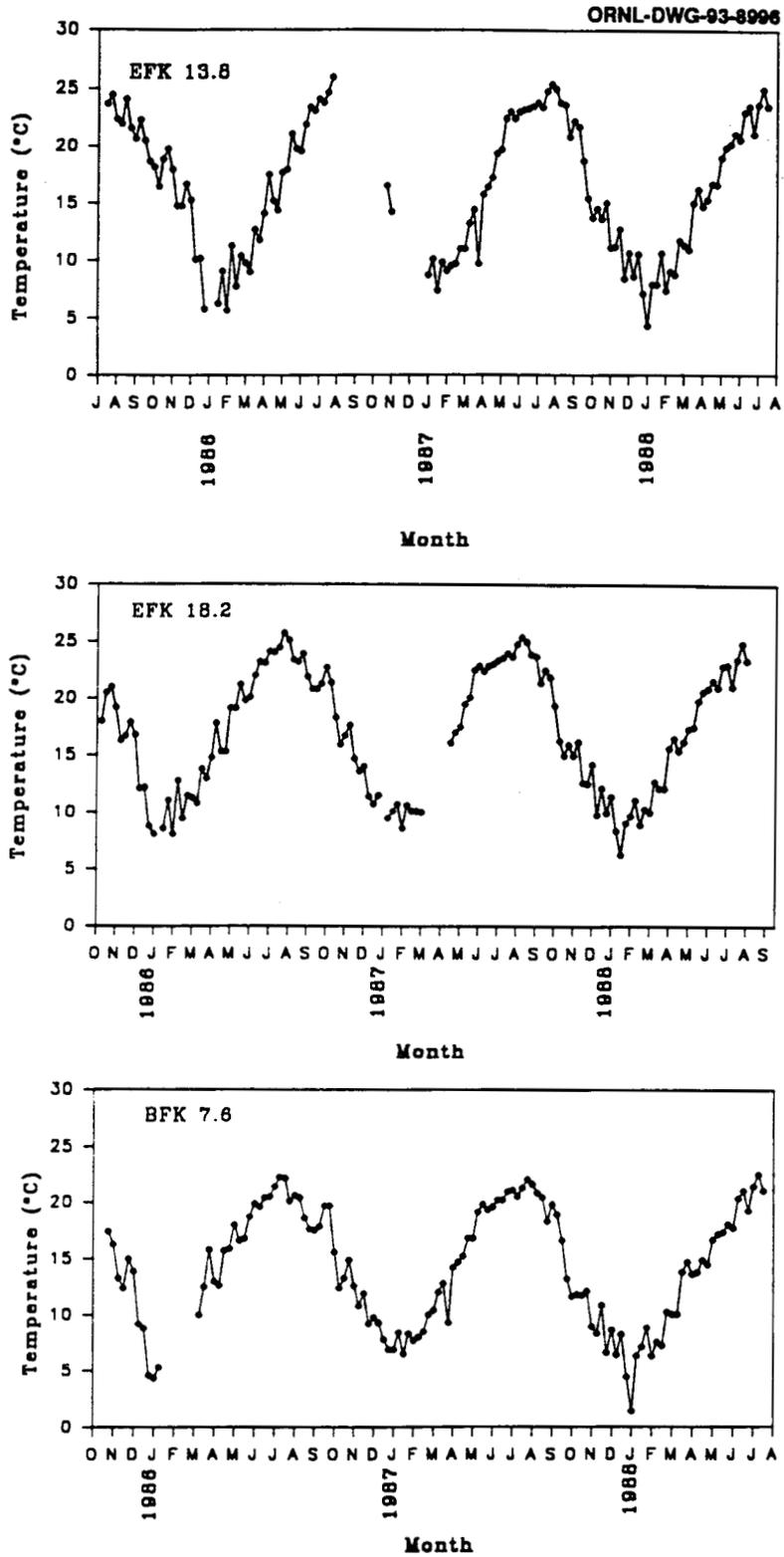


Fig. 2-7. Average weekly water temperatures (degrees Celsius) in the middle reaches of East Fork Poplar Creek at East Fork Poplar Creek kilometer (EFK) 18.2 and EFK 13.8 and in Brushy Fork (at Brushy Fork kilometer 7.6), a reference stream.

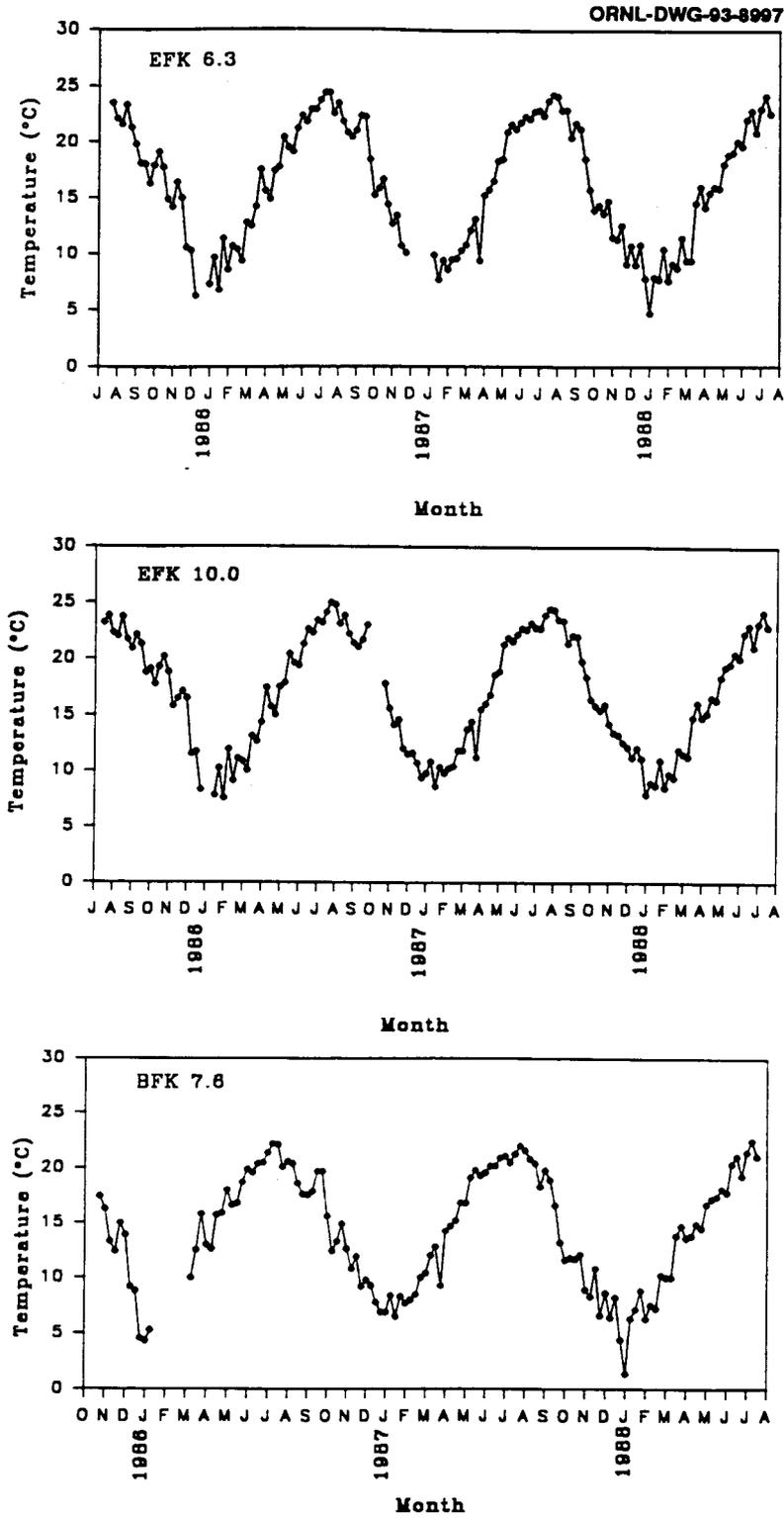


Fig. 2-8. Average weekly water temperatures (degrees Celsius) in lower East Fork Poplar Creek at East Fork Poplar Creek kilometer (EFK) 10.0 and EFK 6.3, and in Brushy Fork (kilometer 7.6), a reference stream.

These surveys included the length of the reach sampled, the stream width (taken at 5-m intervals), and the depth of the stream (taken at one-quarter, one-half, and three-quarters of the stream width for every width estimate). Data for these surveys cover the period from the fall of 1986 to the spring of 1988.

The second group of surveys were more comprehensive estimates of several habitat characteristics. The surveys of each study site were done using a nonrandom transect system, with transects at intervals of 5 to 15 m (depending on length of site and substrate variety) and samples taken at 3 to 5 positions on each transect. Some clustering of transects was added to the system to ensure that all habitat types were adequately covered. Such an approach to transect selection is acceptable when prior knowledge of site conditions exists (Platts et al. 1983).

Flow velocity was recorded using a Marsh McBirney Model 210D portable electronic water current meter with readings taken at the left bank, one-quarter width, one-half width, three-quarters width, and the right bank on every transect. Stream depths and width were also recorded for use in calculating discharge values. Measurements were taken during the May 19 to May 23 period without occurrence of a significant rainfall event.

Substrate analysis included a description of principal cover size, degree of embeddedness, and associated aquatic plants. The substrate was analyzed at the one-quarter and three-quarters width and/or one-half width positions (depending on stream width) on the transect with a 0.5- to 1-m zone of bottom described at each position. A weighted rope painted every 10 cm in alternating colors provided zones for which the dominant substrate type was identified (Bain et al. 1985). A rating system based on codes for certain rock/debris types and size (Table 2-6) was used to classify the dominant substrate type in each zone. The codes reflected a general trend toward increasing substrate coarseness and complexity with increasing code number. By using the zones, 5 to 10 codes were generated for each transect position. The mean and standard deviation of the codes could then provide information on coarseness and degree of uniformity of the substrate (Bain et al. 1985). A similar approach was used to grade the embeddedness or degree that the dominant particles were covered by fine sediments in each zone (Table 2-7). Aquatic plants were also identified and an estimate made of the percentage of coverage for the entire transect.

The stream bank cover was described for each transect based on three zones, with species listed and dominant species identified. The vegetation overhanging the stream constituted one zone, the herbaceous cover on the bank slope was the second zone, and the general forest type covered the third zone from the stream bank back roughly 10 meters. This qualitative description will only be briefly discussed in this report. A canopy measure was made at the mid-point of each transect, which consisted of a percentage reading from a convex mirror with a 10 by 10 cm² engraved grid. The canopy percentage represented the number of grids covered by the overhanging vegetation and was used as a comparative measure of available sunlight.

2.6.2 Results

The primary purpose of the habitat analysis was to provide data for the fish and benthic invertebrate community analysis; therefore, the study site descriptions are divided in that manner. Because most of the benthos sites are included within or adjacent to the

Table 2-6. Substrate codes used in the physical habitat analysis of East Fork Poplar Creek watershed

Code	Substrate index	Particle size range (mm)
1	Bedrock, smooth	<2.0
2	Clay	<0.004
3	Silt	<0.004-0.062
4	Sand/fine sediment	0.062-2.0
5	Gravel	2.0-64.0
6	Cobble/rubble	64.0-250.0
7	Small boulder	250.0-610.0
8	Large boulder	610.0-2000.0
9	Bedrock, rough	>2000.0
10	Plant detritus	NA ^a
11	Woody debris	NA
12	Root wads	NA
13	Trash, human origin	NA

^aNA = not applicable.

Source: Adapted from M. B. Bain et al., *Quantifying Stream Substrate for Habitat Analysis Studies*, N. Amer. Fish. Manag. 5:499-500, 1985; W. S. Platts, W. F. Megahan, and G. W. Minshall, *Methods for Evaluating Stream, Riparian, and Biotic Conditions*, Gen. Tech. Rep. INT-138, U.S. Department of Agriculture, Ogden, Utah, 1983.

Table 2-7. Embeddedness rating for substrate material

Rating	Rating description
5	Predominant particles have <5% of their surface covered by fine sediment
4	Predominant particles have 5-25% of their surface covered by fine sediment
3	Predominant particles have 25-50% of their surface covered by fine sediment
2	Predominant particles have 50-75% of their surface covered by fine sediment
1	Predominant particles have >75% of their surface covered by fine sediment

Source: Adapted from W. S. Platts, W. F. Megahan, and G. W. Minshall, *Methods for Evaluating Stream, Riparian, and Biotic Conditions*, Gen. Tech. Rep. INT-138, U.S. Department of Agriculture, Ogden, Utah, 1983.

fish sites, discussions in the fish section represent the general site conditions. Any differences or conditions especially applicable to the benthos will be identified in that section.

2.6.2.1 Fish study sites

The location of the six fish study sites in EFPC and the reference site in BF are shown in Figs. 2-1 and 2-2; and the stream order, length, average depth, and average width at sampling dates are given in Table 2-8.

The lowermost site, EFK 6.3, was the most undisturbed site in EFPC. In June 1988, it consisted of a wide, deep stretch dominated by pools (Table 2-9). The substrate was predominantly a coarse mixture of gravel, rough bedrock, cobble and rubble, and silt with an embeddedness between 50 and 75%. The high SD of the mean substrate codes indicated a heterogeneous substrate. Aquatic vegetation was absent. The May stream flow was typical for EFPC and fairly uniform across the site (Table 2-10). The surrounding vegetation was a young floodplain forest with mature trees closely bordering the stream. The bank ground cover was sparse with occasional monotypic colonies of violet (prob. *Viola cucullata*), honeysuckle (*Lonicera japonica*), clearweed (*Pilea pumila*), poison ivy (*Toxicodendron radicans*), and foam flower (*Tiarella cordifolia*). The large trees near the stream consisted of sycamore (*Platanus occidentalis*), box elder (*Acer negundo*), slippery elm (*Ulmus rubra*), ash (*Fraxinus* sp.), hackberry (*Celtis occidentalis*), and tulip poplar (*Liriodendron tulipifera*). The resulting canopy covered roughly 78% of the stream with most transects showing similar coverage (Table 2-9).

The next upstream site, EFK 10.0, was longer and narrower with more riffles. The substrate was a mixture of gravel, smooth bedrock, and clay with a high standard deviation indicating an extremely heterogeneous substrate. The embeddedness was moderate ranging from 25 to 50% (Table 2-9). Low levels of algae (1–5%) occurred on most transects. The flow rates were similar to EFK 6.3 with a little more variability. A large, deep pool was created below a waterfall at the top of the section and the right bank was bordered by a condominium complex. The surrounding vegetation was a young to mature floodplain forest with residential back yards encroaching upon the north bank. Many areas on the north bank were devoid of cover; only mowed grasses and occasional trees were present. On the south bank, herbaceous cover included marsh violet, rye (*Elymus* sp.), clearweed, false nettle (*Boehmeria cylindrica*), trumpet creeper (*Campsis radicans*), touch-me-not (*Impatiens capensis*), composites (*Aster* and *Bidens* spp.) and elderberry (*Sambucus canadensis*). The overhanging vegetation provided the densest canopy (80.3%) of any EFPC site, and included box elder, white ash (*Fraxinus americana*), slippery elm, sycamore, hackberry, and honey locust (*Gleditsia triacanthos*).

Continuing upstream, EFK 13.8 was a riffle-dominated stretch. Pools in this section occurred with undercut banks providing excellent cover areas. The substrate of the complete section was heterogeneous, dominated by gravel and smooth bedrock. Embeddedness was moderate to high (between 50 and 75%) with high variability. Mean flow rates in EFPC were the lowest in May (Table 2-10). A mature floodplain forest dominated the surrounding area, with the exception of a cleared, open area at the head of the reach. At many transects, the banks were comprised of extensive tree root systems, piles of woody debris, and protruding bedrock that left little room for herbaceous growth. Occasional bank vegetation included touch-me-not, clearweed, marsh violet, and honeysuckle. The overhanging vegetation was composed of mature sycamore, green ash (*Fraxinus pennsylvanica*), slippery elm, and walnut (*Juglans nigra*), as well as small trees and shrubs such as box elder, dogwood (*Cornus ammomum*), bluebeech (*Carpinus caroliniana*), and buckeye (*Aesculus* sp.) resulting in moderate canopy cover.

Table 2-8. Stream order, length, mean width, mean depth, and surface area of fish sampling sites for November 1986 to October 1988 in East Fork Poplar Creek and the reference stream, Brushy Fork

Parameters by sampling period	Site ^a						
	BFK 7.6	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3
Stream order	4	1	1	4	4	4	4
November-December 1986							
Length (m)	116	230	116	122	101	114	103
Width (m)	9.2	4.2	4.7	9.7	8.0	6.6	9.3
Depth (cm)	46.2	18.7	19.6	28.8	30.5	40.4	37.3
Area (m ²)	1063	958	542	1179	811	752	962
March 1987							
Length (m)	115	224	116	132	99	114	103
Width (m)	8.5	4.3	5.0	9.7	7.8	7.3	10.0
Depth (cm)	36.9	17.2	21.6	26.5	31.7	40.4	44.7
Area (m ²)	981	972	580	1285	770	834	1031
October 1987							
Length (m)	113	227	90 ^b	132	99	114	101
Width (m)	9.1	4.2	4.7	7.7	7.8	6.5	8.2
Depth (cm)	23.9	18.6	18.4	12.3	30.7	35.4	32.9
Area (m ²)	1024	947	421	1020	770	740	833
March 1988							
Length (m)	118	226	93	124	99	121	102
Width (m)	8.2	4.2	5.0	8.8	7.8	6.9	9.3
Depth (cm)	30.9	19.6	21.1	16.3	31.1	39.6	41.2
Area (m ²)	963	951	466	1091	767	835	951
Means for 1986-88							
Length (m)	116	227	104	128	100	116	102
Width (m)	8.8	4.2	4.9	9.0	7.9	6.8	9.2
Depth (cm)	34.4	18.5	20.2	21.0	31.0	39.0	39.0
Area (m ²)	1008	957	502	1144	780	790	944

^aBFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

^bSite was reduced in length because of high fish densities.

Table 2-9. Mean substrate rating, embeddedness rating, canopy percentage, and pool to riffle ratio (P/R) for fish and benthos sampling sites on East Fork Poplar Creek and Brushy Fork for June–July 1988

Site ^a	Substrate (± SD)	Embeddedness (± SD)	Canopy (± SD)	P/R ratio
BFK 7.6				
Fish	6.3 ± 2.5	2.1 ± 1.5	77.8 ± 10.0	3.27
Benthos	5.9 ± 2.1	4.5 ± 0.8	74.0	
EFK 24.4				
Fish	5.4 ± 0.7	4.1 ± 1.2	0.7 ± 1.6	0.61
Benthos	5.0 ± 0	4.3 ± 0.8	0	
EFK 23.4				
Fish	5.6 ± 1.7	3.1 ± 1.5	61.4 ± 26.9	0.58
Benthos	5.7 ± 0.5	3.6 ± 1.3	0	
EFK 18.2				
Fish	5.2 ± 1.6	3.3 ± 1.5	45.9 ± 37.9	0.12
Benthos	5.0 ± 0	2.4 ± 1.5	4.0	
EFK 13.8				
Fish	4.3 ± 2.5	2.5 ± 1.7	71.3 ± 29.9	0.88
Benthos	6.3 ± 1.7	3.7 ± 1.6	0	
EFK 10.0				
Fish	5.0 ± 2.8	3.6 ± 1.6	80.3 ± 7.4	1.49
EFK 10.9				
Benthos	5.1 ± 0.5	4.2 ± 0.8	75.0 ± 5.1	
EFK 6.3				
Fish	5.8 ± 2.1	2.6 ± 1.6	78.2 ± 11.7	1.90
Benthos	5.3 ± 1.0	3.1 ± 1.7	75.5 ± 16.0	

^aBFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

The next site, EFK 18.2, was much wider and shallower with many disturbance characteristics. The site was dominated by a shallow pool at the lower end of the reach and a long shallow riffle at the upper end. Undercut banks were a feature on one side of the pool structure. The substrate was less variable being dominated by loose gravel (Table 2-9). Embeddedness was moderate (5 to 50%) reflecting the importance of the riffle area. Aquatic vegetation was limited to patches of green algae (up to 33% of a transect) and scattered *Potamogeton* (1%). The site was influenced at the upper end by a bridge crossing of the Oak Ridge Turnpike and often contained discarded trash. The composition of the surrounding vegetation was very different on each side of the stream.

Table 2-10. Mean, standard deviation, minimum and maximum stream velocity, and mean depth for each fish and benthos sampling site on East Fork Poplar Creek and Brushy Fork for May 1988

Site ^a	Mean velocity (m/s)	SD	Minimum velocity (m/s)	Maximum velocity (m/s)	Mean depth (cm)
BFK 7.6					
Benthos	0.21	0.15	0	0.37	7.4
Fish	0.07	0.11	-0.02 ^a	0.64	21.0
EFK 24.4					
Benthos	0.36	0.28	0	0.67	14.2
Fish	0.35	0.25	0	0.79	15.7
EFK 23.4					
Benthos	0.30	0.29	0	0.60	13.6
Fish	0.22	0.25	0	0.88	18.8
EFK 18.2					
Benthos	0.17	0.15	0	0.30	18.0
Fish	0.20	0.19	0	0.72	12.1
EFK 13.8					
Benthos	0.30	0.28	0	0.69	10.9
Fish	0.14	0.20	-0.10	0.69	21.7
EFK 10.9					
Benthos	0.32	0.29	-0.02	0.75	11.9
EFK 10.0					
Fish	0.17	0.24	-0.06	0.94	31.4
EFK 6.3					
Benthos 1	0.27	0.30	0	0.70	12.1
Benthos 2	0.16	0.20	0	0.59	13.6
Fish	0.16	0.19	0	0.70	24.9

^aBFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

^bNegative values indicate upstream flow typical of eddy movements in pools.

One side was an open, early successional field with occasional shrubs; the other side was a young to mature forest. Very little bank herbaceous vegetation was present due to the nearly vertical banks and dense shade on the forest side of the stream. In contrast, the gradual banks and open canopy on the field side provided ideal conditions for many herbaceous species, including barnyard grass (*Echinochloa crus galli*), clearweed, horsenettle (*Solanum carolinianae*), touch-me-not, giant ragweed (*Ambrosia trifida*),

common ragweed (*Ambrosia artemisiifolia*), curly dock (*Rumex crispus*), rye, and smartweeds (*Polygonum* spp.). The dense canopy on the forest side provided only shade for approximately half of the stream width. The dominant overhanging trees were box elder, sycamore, and black willow (*Salix nigra*).

EFK 23.4 continued the shift toward areas with greater disturbance features. The site was narrower and shallower than EFK 18.2, had previously been channelized, and was dominated by riprap banks. The May stream flow emphasized the flow augmentation from Y-12 with a higher mean rate (Table 2-10). The substrate was moderately heterogeneous, dominated by gravel and cobble (Table 2-9), with an embeddedness of close to 25%. More aquatic vegetation (2–5% *Potamogeton* sp.) and algae (2–10%) were evident, perhaps due to downstream movement of plant material from NHP. Surrounding vegetation also reflected disturbance with few mature trees and a small tree/shrub [sycamore, pine, and smooth sumac, (*Rhus glabra*)] composition. The bank vegetation was dominated by viny species, including honeysuckle, poison ivy, trumpet creeper, Virginia creeper (*Parthenocissus quinquefolia*) and grape (*Vitis* sp.). Overhanging vegetation (and canopy) was sparse and variable and consisted of sycamore, box elder, redbud (*Cercis canadensis*), black willow (*Salix nigra*), sweetgum (*Liquidambar styraciflua*), and tulip poplar.

The uppermost site on EFPC, EFK 24.4, was located entirely within Y-12 and has been extremely altered by human activity. The site was the longest, narrowest, and shallowest site in EFPC (Table 2-8), with a low pool to riffle ratio. The mean flow rate in May was the highest in EFPC (Table 2-10). The substrate was homogeneous—primarily gravel with some cobble and smooth bedrock. The embeddedness was close to 5%. The surrounding vegetation was principally limited to a narrow band of disturbance-adapted herbs near the stream edge. Dominating plants included a number of graminoid species: fescue (*Festuca pratensis*), rice cutgrass (*Leersia oryzoides*), barnyard grass, and broomsedge (*Andropogon virginicus*). A few young trees [smooth sumac (*Rhus glabra*), redbud, and box elder] provided limited cover at the upper part of the reach, however most of the section lacked canopy.

The reference site in Brushy Fork, BFK 7.6, was similar to the lower areas of EFPC. Mean width and depth were intermediate between values for EFK 6.3 and EFK 13.8. The pool to riffle ratio was higher than any EFPC site, but closest to EFK 6.3. The substrate was a coarse mixture of gravel, cobble and rubble, woody debris, rough bedrock, and silt (Table 2-9), with an embeddedness of close to 50%. Aquatic vegetation was limited to green algae covering 1% of several transects. In contrast to EFPC, the mean velocity was low, indicating the effects of the drought on a nonflow augmented stream during the 1988 summer. The surrounding vegetation consisted of an open, heavily grazed field with young to mature trees closely bordering the stream. The resulting dense canopy (77.8%) was composed of sycamore, sugar maple (*Acer saccharum*), green ash, hackberry, box elder, and spicebush (*Lindera benzoin*). The nearly vertical, heavily eroded banks provided little habitat for ground cover vegetation; only poison ivy and touch-me-not were occasionally present at some transects.

2.6.2.2 Benthos study sites

A description of the vegetation at the EFPC and BF study sites has already been presented in Sect. 2.6.2.1. Most benthic study sites have the same surrounding plant

composition as the corresponding fish study sites. However, two sites (BFK 7.6 and EFK 13.8) have a different plant composition. At BFK 7.6 a steep hill forms the south bank of the benthic site and is dominated by an upland forest. At EFK 13.8 the benthic collecting site is surrounded by cow pastures with no trees, in contrast to the heavily shaded floodplain forest described for the fish site.

2.6.3 Discussion

The initial characterization survey of EFPC provided data on substrate and cover variables for the low flow periods of summer. In this regard the comparisons are limited, and some conclusions concerning the role of habitat differences may change under greater flow conditions.

The relationship of fish populations to available habitat has been examined from many perspectives. Gorman and Karr (1978) helped establish the relationship of fish community complexity to the variables of stream depth, bottom type, and current. Angermeier and Karr (1984) examined the correlation between the abundance of fish populations and the abundance of woody debris in streams. The role of large substrate, undercut banks, and aquatic vegetation in determining population characteristics of smallmouth bass and rock bass was examined by McClendon and Rabeni (1987). The influence of other environmental variables, such as temperature (Baltz et al. 1987) and regulated streamflows (Bain et al. 1988), on the microhabitat choices and fish community structure has been demonstrated as significant. Thus, it is important in any study examining the impact of remedial actions that the role of habitat differences on fish community structure be addressed.

For the fish study sites, there are noticeable differences in habitat structure between sites. The initial selection of sites was aimed at equal representation of pools and riffles at all sites. These data show that this is not the case in low flow situations, with more pool structure occurring at lower sites. The substrate and embeddedness data show no strong trends in dominant types of substrate between lower and upper sites. Current velocity in May showed a trend toward higher mean flows at the two upper sites than at lower sites and significantly more flow in EFPC than in BF. Lower EFPC sites had more mature forest with less disturbance and a higher percentage of canopy cover than did the upper sites.

Despite efforts to match similar habitat variables at all study sites, there were substantial differences among sites due to longitudinal changes in habitat characteristics. Substantial differences exist between upper and lower EFPC, with selected sites merely reflecting conditions typical of the changes occurring with distance. Fish species diversity and abundance have been demonstrated to vary as functions of some of the habitat characteristics that differ between upper and lower sites, such as pool structure (Foltz 1982) and riparian cover (Wesche et al. 1987). The flow augmentation from Y-12 results in substantial differences between EFPC sites and the reference site in BF. The influence on fish populations of the more stable flows in EFPC compared with fluctuating flows in BF must be considered (Hynes 1970). Also, as remedial actions further improve water quality in EFPC, the importance of understanding differences due to the longitudinal gradients in habitat characteristics becomes greater.

3. TOXICITY MONITORING

H. L. Boston, W. R. Hill, and A. J. Stewart

3.1 OVERVIEW

Seven-day static-renewal tests with the freshwater microcrustacean *Ceriodaphnia dubia* and larvae of the fathead minnow (*Pimephales promelas*) can be used to estimate acute and chronic toxicity of wastewaters and receiving waters (Horning and Weber 1985). From October 1986 through October 1988, we performed about 80 tests with these 2 species to (1) estimate the loading rates of toxicity from NPDES-permitted discharges of treated and nontreated wastewaters to EFPC, and (2) characterize the biotic quality of EFPC water at various sites. The information obtained from the toxicity loading analysis and the stream characterizations are detailed in Sects. 3.2 and 3.3 respectively.

Chemical analyses made in support of the ambient toxicity tests included measurements of total residual chlorine (TRC), conductivity, alkalinity and hardness. TRC is a dynamic toxicant (it typically degrades to chloride, which is not toxic), while conductivity, alkalinity, and hardness are conservative properties of all natural waters. The results of the analyses for these chemical parameters are summarized and discussed in Sect. 3.4.

Studies started in September 1986 showed that filamentous algae and two species of submersed aquatic vascular plants (*Potamogeton foliosus* and *Najas flexilis*) were seasonally abundant in NHP, and that these plants were exported from the pond to the stream. Chemical analyses showed that the exported plant matter was enriched with metals of toxicological interest, including Zn, Ni, Cd, Cu, and Cr. During 1987 and 1988, additional studies were conducted to more accurately determine (1) the loading rates of filamentous algae and *Potamogeton* to EFPC, (2) seasonal changes in the amounts or types of toxic metals in the exported plant matter, and (3) the possible effects of the plant material on aquatic biota. The information obtained from these special studies is given in Sect. 3.5.

3.2 TOXICITY LOADING ANALYSIS (*A. J. Stewart*)

3.2.1 Introduction and Methods

This analysis used the results of 9 toxicity tests of cooling tower blowdown, 22 tests of nontreated (Category IV) waste streams, and 11 tests of effluents from 3 different wastewater treatment systems (Table 3-1). We first calculated the no-observed-effect concentration (NOEC) for each tested wastewater. The procedure for calculating an NOEC, as outlined by Horning and Weber (1985), is as follows: First, various concentrations of the wastewater are tested, usually with both species (fathead minnows and *Ceriodaphnia*). After the 7-d test period, responses of the animals in the wastewater dilutions are compared statistically to those of animals in controls (i.e., fathead minnow larvae and *Ceriodaphnia* reared in water lacking toxicants at toxic concentrations). The comparisons involve an analysis of variance (ANOVA) followed by either Dunnett's t-test

Table 3-1. Summary of chronic toxicity tests conducted of cooling tower blowdown, nontreated waste streams, and treated wastewaters at the Y-12 Plant

Source	Toxicity test date	NOEC (%)	IWC (%)	Discharge (10 ⁴ L/year)	Toxic units
Cooling towers					
9409-12	Jan 09-16, 1986	24			
28-29	Aug 07-14, 1986	50			
32	Aug 07-14, 1986	25			
12	Oct 23-30, 1986	25			
17	Oct 23-30, 1986	≥75			
9409-10	Oct 01-08, 1987	80			
9409-2	Nov 12-19, 1987	100			
9409-30	Dec 03-10, 1987	<20			
9409-13	Aug 25-Sep 1, 1988	20			
Mean		46.6	5.4	56,600	0.116
Nontreated (Category IV) waste streams					
Dye penetrant/emulsifier					
	Apr 17-24, 1986	0.01			
	Apr 09-16, 1987	0.05			
Mean		0.03	0.074	775	2.467
Overhead still					
	Jan 24-31, 1986	2.0			
	Apr 30-May 7, 1987	5.0			
	Aug 04-11, 1988	5.0			
Mean		4.0	0.52	5,482	0.130
Photographic rinse waters					
	Feb 20-27, 1985	0.07			
	Sep 17-24, 1985	0.28			
	Apr 09-16, 1987	0.07			
	May 05-12, 1988	1.00			
	Jul 07-14, 1988	0.01			
	Jul 14-21, 1988	0.03			
Mean		0.24	0.28	2,906	1.167

Table 3-1 (continued)

Source	Toxicity test date	NOEC (%)	IWC (%)	Discharge (10 ⁴ L/year)	Toxic units
Category IV waste streams					
Steam condensate					
	Nov 19-26, 1985	0.5	0.021	225	0.042
Plasma torch burn table rinse waters					
	Jan 09-16, 1985	>20.0			
	Mar 17-24, 1988	≥30.0			
	Oct 20-27, 1988	≥30.0			
Mean		26.7	0.00047	1.00	<0.001
Building 9202 catch basin					
	Dec 03-10, 1985	40.0			
	Apr 30-May 7, 1987	≤10.0			
	Sep 08-15, 1988	10.0			
	Oct 13-20, 1988	25.0			
Mean		21.3	0.19	1,938	0.009
Circuit board rinse waters					
	Jan 11-21, 1988	5.0			
	Nov 03-10, 1988	20.0			
	Dec 01-08, 1988	25.0			
Mean		16.7	0.0022	22.5	<0.001
Treated waste streams					
Central pollution control facility					
	Jan 22-29, 1987	>20.0	0.20		
	Oct 23-30, 1987	1.0	0.33		
	Feb 04-11, 1988	<10.0	0.14		
Mean		10.3	0.22	121	0.021

Table 3-1 (continued)

Source	Toxicity test date	NOEC (%)	IWC (%)	Discharge (10 ⁴ L/year)	Toxic units
Plating rinse water treatment facility					
	Jun 11-18, 1987	10.0			
	Feb 18-25, 1988	25.0			
Mean		17.5	0.85	2,300	0.049
West end treatment facility					
	Mar 03-10, 1988	0.3	0.48		
	Apr 07-14, 1988	3.0	0.48		
	Apr 14-21, 1988	5.0	0.63		
	Apr 28-May 5, 1988	1.0	0.49		
	Nov 03-10, 1988	5.0	0.16		
Mean		2.9	0.45	1,126	0.157

Note: The instream waste concentration (IWC) for each wastewater is expressed as a percentage of the base flow of EFPC at outfall of New Hope Pond (2.87×10^7 L/d). Toxic units are computed by dividing a wastewater's average IWC by its average no-observed-effect concentration (NOEC).

(for growth or survival of the minnow larvae, and fecundity of *Ceriodaphnia*) or Fisher's Exact test (for *Ceriodaphnia* survival). In each test the most sensitive endpoint of the most sensitive species was used to calculate a wastewater's overall NOEC. When the results of more than one toxicity test for a particular wastewater category were available, the average NOEC for that wastewater was computed.

The average annual instream waste concentration (IWC) of the nontreated category IV wastewaters was taken from published data (Kingrea 1986). The IWC for blowdown from all Y-12 Plant cooling tower operations (5.4%) was a "best guess" estimate made by dividing the amount of blowdown released to EFPC ($\sim 566 \times 10^6$ L/year; A. Prosser, Y-12 Utilities, personal communication) by the low-flow (${}_3Q_{20}$) annual volume of water flowing into EFPC from NHP (~ 28.0 million L/d, or 10.5×10^9 L/year; Kingrea 1986). IWC values for the various treated waste streams were estimated using discharge records obtained from Y-12 Waste Treatment Operations (H. Boyd, N. McRae, I. Jeter, and P. Sadler; personal communication).

A wastewater's maximum likely contribution of toxicity to EFPC was computed by dividing the wastewater's average IWC (in percent) by its average NOEC (in percent). Dividing a wastewater's IWC by its NOEC conveniently weights a wastewater's intrinsic toxicity according to its volume contribution to the stream, and yields a dimensionless number referred to here as a toxicity unit (TU). The TU for a wastewater can range from

zero to infinity. A high-volume, intrinsically toxic wastewater (e.g., IWC = 20%, NOEC = 0.2%), for example, would have 100 TU, while a lower-volume discharge of lower toxicity (e.g., IWC = 0.3%, NOEC = 70%) would have only 0.004 TU. An estimate of TU loading to EFPC was then computed by summing the TU values for all tested waste streams.

3.2.2 Results and Discussion

The total number of TUs discharged to EFPC from all tested sources was 4.17 (Table 3-2). A TU sum equal to or exceeding 1.0 indicates that water in EFPC would adversely affect aquatic biota that are as sensitive or more sensitive than fathead minnow larvae or *Ceriodaphnia*. Fish and invertebrates live in EFPC despite the apparently high total TU loading rate, however; and *Ceriodaphnia* in particular is a sensitive test species. Thus, the TU load computation procedure used here appears to overestimate toxicity loading to EFPC. The assumptions underlying the concepts and computations involved in determining toxicity loading must be considered in determining the kinds of corrections that are most appropriate.

Table 3-2. Annual discharge volumes and contributions of toxicity to East Fork Poplar Creek by various types of wastewaters from the Y-12 Plant

Wastewater source	Annual flow (L/year)	Toxic units
Cooling towers	556×10^6	0.12
Category IV streams ^a	113.2×10^6	3.82
Treated waste streams ^b	137.6×10^6	0.23

^aIncludes toxicity test results for all permitted nontreated (Category IV) wastewaters except for those from the Sanitary Landfill, which is nontoxic and does not discharge to EFPC.

^bIncludes tests of effluents from the Plating Rinse Water Facility, the West End Treatment Facility, and the Central Pollution Control Facility.

The approach used to compute TU values first assumed that no changes in toxicity occurred as wastewaters flowed from their point of discharge through NHP to the pond's outfall. This assumption is not valid for most toxicants. Oxidants such as chlorine, for example, enter EFPC at various locations upstream from NHP. These materials were often detected at the inlet to NHP at concentrations high enough to be acutely toxic to fish (≥ 0.20 mg/L). At the pond's outfall, however, chlorine concentrations were much lower (typically ≤ 0.05 mg/L). Similarly, wastewaters that contain toxic volatile compounds

become less toxic with time if the volatile materials escape to the atmosphere, and organic toxicants may be destroyed or altered by oxidants such as chlorine. Toxicity of an effluent that contains toxic metals will also decline with distance downstream. Compelling evidence for this argument exists in the sediments of NHP: the concentrations of many metals are lower at the outfall of NHP than they are at the pond's inlet because the metals tend to (1) adsorb to sediment particles; (2) become insoluble due to changes in oxidation state; (3) are incorporated into, or adsorbed onto the surfaces of submersed aquatic plants; or (4) precipitate as insoluble metal sulfides as sulfate is reduced to hydrogen sulfide by microbiota.

Nearly 85% of the uncorrected TU loading to EFPC (3.64 of the 4.17 TU) was due to just two untreated waste streams (dye penetrant/emulsifier and photographic rinsewaters; Table 3-1). The toxic constituents in these two wastewaters are more likely to be organic than inorganic. The toxicity (and fluoranthene dye concentration) of the dye penetrant/emulsifier rinsewaters, for example, is virtually eliminated by passing the wastewater through a bed of activated carbon. The toxicant(s) in photographic rinsewaters have not yet been identified, but these waters contain hydroquinone, phenyl-substituted heterocycle, aldehyde bisulfite, and alkyl aldehyde (Kingrea 1986); silver may also be present. Organic compounds such as phenyl-substituted heterocycle are relatively labile so that toxicity of even untreated photographic and dye penetrant/emulsifier rinsewaters is likely to decline rapidly after being discharged to EFPC. Additionally, treatment systems are now being developed that will markedly reduce or eliminate the TU contributions by these wastewaters to EFPC. For these reasons the photographic and dye penetrant/emulsifier rinsewaters were excluded in developing a more realistic toxicity loading budget. Their exclusion radically reduces the TU loading value to a more plausible 0.63.

Other aspects of the TU-loading approach that may be contributing to the apparent overestimate are related to the amount of uncertainty associated both with the IWC and NOEC terminology. The IWC value used to calculate TU values for most wastewaters tested, for example, was an annual mean. However, some nontreated wastewaters are generated and released intermittently by facilities that operate on an "as needed" basis (e.g., circuit board rinsewaters). Other category IV wastewater generating facilities (e.g., Building 9818 overhead still) sometimes do not operate at all for extended periods of time. Some facilities operate routinely but discharge wastewater "batches" one to three times weekly (e.g., Building 9202 catch basin and Building 9201-1 plasma torch slag basin cooling water); and others, such as the West End Treatment Facility, operate continuously for extended periods. Similarly, cooling tower blowdown has an annual average discharge rate of about 556×10^6 liters, but cooling tower blowdown rates are much higher during the summer than during the winter. The net result of this heterogeneous discharge pattern is a considerable amount of uncertainty about the degree of accuracy one should expect when trying to convert the outcomes of toxicity tests that use 7-d, constant-exposure periods to estimates of instream conditions.

The NOEC used to calculate a wastewater's TU contribution is also straightforward conceptually but varies temporally and is ambiguous. The intrinsic toxicity of photographic rinsewaters, for example, varied by a factor of 33 based on six tests, while NOECs for cooling tower blowdown tests and for Central Pollution Control Facility tests varied by factors of 5 and 20 respectively. A wastewater's NOEC can also be influenced by the selection of effluent concentrations that are tested. If an effluent is tested at concentrations of 30% and 60% and is shown statistically to affect the test organisms at

the higher concentration but not the lower concentration, the effluent's NOEC would be 30%. The true NOEC for that effluent, however, is clearly somewhere between 60 and 30%. The quantitative ambiguity inherent in the use of a NOEC may be advantageous environmentally because it imparts a "safety bias," which influences regulatory decisions towards more a stringent control of toxicity. The bias, however, is also incorporated into a wastewater's computed TU value and inflates it accordingly. The size of the NOEC bias depends on the difference between the NOEC (as determined statistically) and the next highest tested concentration (the lowest-observed-effect concentration, or LOEC). For most of the tests reported here, the bias is on the order of 30 to 50%. Thus, the TUs computed using statistically determined NOECs for toxicity tests of effluents may be too high by 30 to 50 percent. An across-the-board 40% reductive correction in the computed TU values to compensate for the NOEC bias yields a TU loading value of about 0.38.

When categorized according to wastewater type (cooling tower blowdown and nontreated and treated waste streams), the corrected TU loading computations revealed several interesting situations (Table 3-2). First, although cooling tower blowdown has a high average annual flow and IWC (556×10^6 L/year and 5.4% respectively; Tables 3-1 and 3-2), it is on average not very toxic. Second, although the annual average discharge volume of the treated waste streams is only about 21% higher than of the nontreated waste streams (137.6×10^6 versus 113.2×10^6 L/year respectively; Table 3-2), they are distinctly more toxic than the nontreated waste streams: 52% of the total corrected TU loading to EFPC was attributable to discharges of treated wastewaters, but only 28% of the total was attributable to the nontreated wastewater streams.

Two caveats are needed when considering the conclusions from the toxicity loading approach described above. First, the analysis was based only on an incomplete suite of point-source waste streams. It does not include major instream toxicity contributions attributable to chlorine in process water, possible area-source inputs of toxic substances, or wastewaters for which no toxicity data are yet available. Second, although the procedures and approaches used to establish a "corrected" TU loading rate may appear reasonable, their application radically alters both the magnitudes of the TU values and the distribution of TU values among wastewater treatment categories. However, due to the procedures used in its computation, the final corrected loading value (about 0.38 TU) is likely to be an underestimate rather than an overestimate. Thus, the computation implies that a significant amount of EFPC's capacity to assimilate toxicants has been compromised. Regardless of how it is calculated, therefore, toxicity loading to EFPC is high. The "corrected" total TU loading value may not be directly meaningful in an ecological context, but it can still serve as a benchmark in toxicity reduction efforts. The relative distribution of TU values among wastewater types—and the differences among TU values for different waste streams within wastewater categories—may help identify where toxicity reduction efforts can be directed most cost effectively.

3.3 AMBIENT TOXICITY PATTERNS

3.3.1 Introduction and Methods

The conclusions drawn in this section are based on the results of 32 7-d chronic toxicity test periods; the test periods occurred between September 18, 1986, and October 10, 1988. In a given test period, the number of EFPC sites that were evaluated for

toxicity ranged from one to nine (Table 3-3). In each case, water samples from a site being evaluated for toxicity were tested concurrently with fathead minnow (*Pimephales promelas*) larvae and a microcrustacean (*Ceriodaphnia dubia*) using procedures described in Sect. 3.2.1. Almost all evaluations used daily renewal of the water. In most cases, the water samples were collected daily as grab samples. In a few instances, 24-h composite samples were used instead. The type of sample method used to collect water for testing depended on the specific question being addressed. When New Hope Pond was closed on Monday, November 7, 1988, a test of water from the pond's inlet and outfall was in progress. Samples collected from the pond's inlet and outfall on this day were refrigerated, and subsamples of these samples were withdrawn and warmed to the test temperature daily for the rest of the test. Sites downstream from the outfall of NHP were evaluated by testing only full-strength water. Tests of water from the outfall and inlet of NHP, and of water from the Y-12 Plant's Area Source Study Site 8 (AS-8) were tested at full strength and at various dilutions thereof (typically 80%, 75%, or 50%). Measurements of pH, alkalinity, hardness, TRC, free chlorine (FC), and conductivity were made for all water samples that were brought to the laboratory for testing.

If a sample that is tested only at full strength is found to be toxic, it is not possible to quantify the sample's NOEC: one can say only that the NOEC is <100%. This difficulty is especially troublesome in field studies where the need to assess ambient toxicity at many sites often exceeds the need to more accurately estimate toxicity at any given site. An alternative approach, in which one quantifies the *frequency* with which toxicity is evident at a particular site rather than the *degree* of toxicity at a particular time (Stewart et al. 1989) may be useful in such cases. In this study the toxicity-frequency approach was used because (1) most samples downstream from NHP-o were tested at only at full strength, and (2) enough tests of each site were conducted to make the toxicity-frequency approach feasible. Water from AS-8, for example, was tested 7 times, water from the inlet and outfall of NHP was tested 19 and 31 times respectively, and water from EFPC at each site downstream from NHP-o was tested 8 times (Table 3-3).

3.3.2 Results and Discussion

3.3.2.1 Area Source Study Site 8

Water from AS-8 was tested eight times with both species (Table 3-3). Full-strength water from AS-8 was toxic to fathead minnow larvae in only one test, but was toxic to *Ceriodaphnia* in three tests. For the seven tests in which water from AS-8 was not toxic to the minnows, average survival of the fish was 88.9% in full-strength AS-8 water and 93.2% in control water. For the same seven tests, average growth of the minnows was 0.54 mg per fish in water from AS-8 and 0.50 mg per fish in control water. Low fecundity of *Ceriodaphnia* in controls compromised test interpretation in two tests (those starting on September 15 and October 6, 1988); thus, poor survival of the *Ceriodaphnia* in AS-8 water on these two dates may have been due to inadequate food rather than to toxicants. In five of eight tests, *Ceriodaphnia* tested in pure AS-8 water had fecundity values that were at least as high as those of the controls.

Water from AS-8 was toxic to both species only in the test that started on November 3, 1988. In this test, all animals died within 24 h after the test had started.

Table 3-3. Toxicity test schedule for EFPC sites

East Fork Poplar Creek site ^a									
Date	AS-8	NHP-i	NHP-o	22.8	21.9	20.5	18.2	13.8	10.9
Sep. 18, 1986	--	--	X	--	--	--	--	--	--
Sep. 24, 1986	--	--	X	--	--	--	--	--	--
Oct. 7, 1986	--	X	X	X	X	X	X	X	X
Nov. 4, 1986	--	X	X	--	--	--	--	--	--
Nov. 18, 1986	--	--	X	--	--	--	--	--	--
Dec. 3, 1986	--	X	X	--	--	--	--	--	--
Dec. 9, 1986	--	X	X	--	--	--	--	--	--
Jan. 15, 1987	--	X	X	--	--	--	--	--	--
Feb. 5, 1987	--	--	X	X	X	X	X	X	X
Mar. 5, 1987	--	X	X	--	--	--	--	--	--
Apr. 9, 1987	--	--	X	--	--	--	--	--	--
May. 7, 1987	--	X	X	X	X	X	X	X	X
May. 21, 1987	--	--	X	--	--	--	--	--	--
Jun. 11, 1987	--	--	X	--	--	--	--	--	--
Jul. 9, 1987	--	X	X	--	--	--	--	--	--
Aug. 27, 1987	--	X	X	X	X	X	X	X	X
Sep. 10, 1987	--	X	X	--	--	--	--	--	--
Oct. 29, 1987	--	X	X	X	X	X	X	X	X
Nov. 17, 1987	--	--	X ^b	--	--	--	--	--	--
Dec. 3, 1987	--	X	X	--	--	--	--	--	--
Jan. 28, 1988	--	--	X	--	--	--	--	--	--
Feb. 18, 1988	--	X	X	X	X	X	X	X	X
Mar. 10, 1988	--	--	X	--	--	--	--	--	--
Apr. 7, 1988	X	--	--	--	--	--	--	--	--
Apr. 14, 1988	X	X	X	--	--	--	--	--	--
Apr. 28, 1988	X	--	--	--	--	--	--	--	--
May 5, 1988	--	X	X	--	--	--	--	--	--
Jun. 16, 1988	--	--	X	--	--	--	--	--	--
Jun. 23, 1988	X	X	X	X	X	X	X	X	X
Jul. 28, 1988	X	--	X	--	--	--	--	--	--
Aug. 11, 1988	--	X	X	--	--	--	--	--	--
Sep. 15, 1988	X	X	X	--	--	--	--	--	--
Oct. 6, 1988	X	X	X	X	X	X	X	X	X

^aAS-8 is Area Source Study Site 8, about halfway between the north-south pipes and the inlet to New Hope Pond. NHP-i and NHP-o designate New Hope Pond's inlet and outfall respectively. The values designating the remaining sites indicate the distance, in kilometers, upstream from EFPC's confluence with Poplar Creek. Date refers to the day on which a 7-d chronic toxicity test was started.

^b96-hr acute test using both species (*Pimephales promelas* and *Ceriodaphnia dubia*).

The concentration of TRC in AS-8 water on the day the test started was 0.60 mg/L. This concentration of TRC is high enough to have accounted for the observed mortality.

3.3.2.2 New Hope Pond inlet and outfall

Data for fathead minnow tests using water from NHP-o were available for 29 test periods between September 18, 1986, and October 13, 1988. During the same period, water from NHP-i was tested 19 times (Table 3-3). Survival of the minnow larvae averaged 95.3% in the controls ($n = 29$), 81.1% in water from NHP-i, and 86.0% in water from NHP-o. Growth of fish in NHP-o water was 0.44 ± 0.14 mg per fish (mean \pm 1 SD) for the 29 tests; growth of fish in the controls for these tests was 0.47 ± 0.12 mg per fish. Growth of fish in water from NHP-i was 0.41 ± 0.17 mg per fish, based on 19 tests; growth of fish in the controls for these tests was 0.49 ± 0.11 mg per fish. Normalized to their respective sets of controls, then, results of the fathead minnow survival and growth tests indicated that water from NHP-i had lower biological quality than water from NHP-o. Thus, based on ambient toxicity assessments with fathead minnow larvae, NHP appeared beneficial.

Ceriodaphnia tests were conducted concurrently with fathead minnow tests during 17 of the 19 periods when samples from NHP-i and NHP-o were both evaluated (Table 3-3). In all 17 tests, mean fecundity of the *Ceriodaphnia* tested in water from NHP-i was lower than that of mean fecundity of *Ceriodaphnia* tested in water from NHP-o. The magnitude of these reductions ranged from 3.2% to 100%. In 8 of the 17 tests, the average number of offspring produced per female *Ceriodaphnia* in the controls was ≤ 15 , suggesting that either the food or the control water was inadequate. Even when only the results from paired tests (NHP-i and NHP-o) having satisfactory controls were compared, fecundity of *Ceriodaphnia* tested with water from NHP-i was lower than that of *Ceriodaphnia* tested with water from NHP-o; the average reduction in mean fecundity was 44.4%. The distributions of the differences in average fecundity of *Ceriodaphnia* used to evaluate water from NHP-i and NHP-o, based on the 9 best tests and on all 17 tests, were nearly uniform (Fig. 3-1). These data show that the fecundity differences of the animals tested with water from the two sites were not controlled exclusively by "all-or-none" test outcomes. Collectively, then, the results of the toxicity tests with both species were in good general agreement: chronic and acute toxicity was at least intermittently evident at the inlet to NHP, and both chronic and acute toxicity of the water declined as it flowed from NHP-i to NHP-o.

3.3.2.3 East Fork Poplar Creek sites downstream from New Hope Pond

Water samples from six sites in EFPC downstream from the outfall of NHP were tested eight times with both species during October 1986 through October 1988 (Table 3-3). When possible, these tests were scheduled to coincide with tests used to assess toxicity at NHP-i, NHP-o, and AS-8 to provide more complete assessments of downstream changes in stream water quality. In each case, only full-strength water was tested. The results of the *Ceriodaphnia* and fathead minnow tests for these sites are shown in Tables 3-4 and 3-5 respectively.

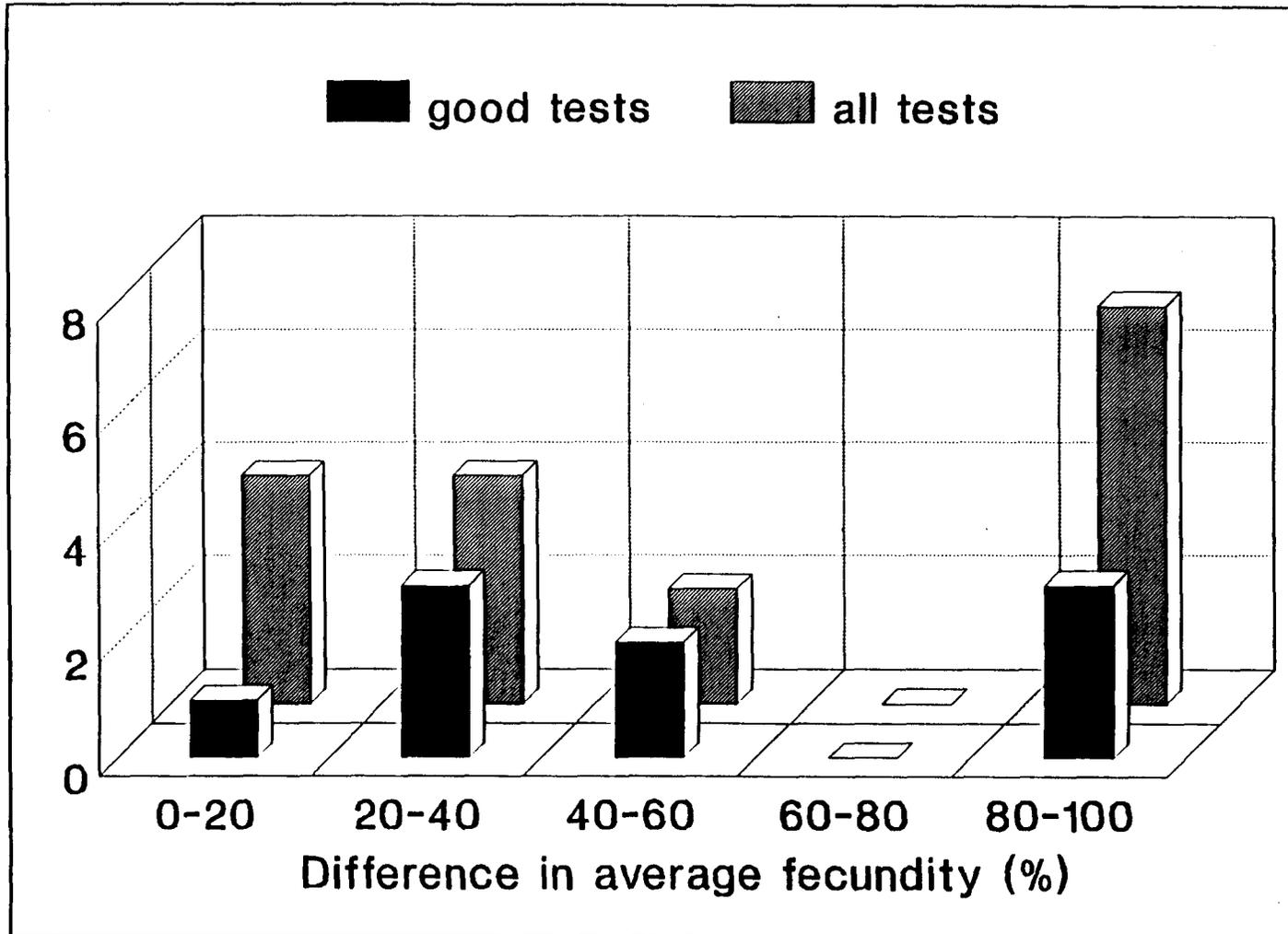


Fig. 3-1. Frequency distribution of differences in *Ceriodaphnia* mean fecundity for tests of water from the outfall and inlet to New Hope Pond. The fecundity difference for each temporally paired test of water from the pond's inlet and outfall was calculated as a percentage relative to the fecundity of animals tested in water from the pond's outfall. The histograms show the distribution of these percentage values (horizontal axis); the vertical axis shows the number of toxicity test pairs in each difference-magnitude category.

Table 3-4. Results of eight *Ceriodaphnia* toxicity tests of water from six sites downstream from the outfall of New Hope Pond in East Fork Poplar Creek

Date	Site (East Fork Poplar Creek kilometer)					
	22.8	21.9	20.5	18.2	13.8	10.9
Oct. 7, 1986	22.0 ± 3.8 (70) 2	25.0 ± 6.4 (90) 1	24.2 ± 3.7 (90) 1	22.0 ± 5.3 (90) 0	25.5 ± 3.0 (100) 0	24.7 ± 3.6 (80) 0
Feb. 5, 1987	26.8 ± 5.6 (40) 0	21.4 ± 7.7 (70) 0	22.0 ± 8.5 (80) 0	18.4 ± 4.3 (80) 0	25.7 ± 8.6 (100) 0	22.9 ± 3.2 (90) 0
May 7, 1987	20.9 ± 4.3 (90) 0	20.3 ± 7.2 (80) 0	20.9 ± 3.0 (80) 0	23.6 ± 3.0 (90) 0	24.5 ± 3.3 (100) 0	26.2 ± 9.1 (100) 0
Aug. 27, 1987	17.3 ± 5.4 (100) 0	15.9 ± 5.2 (100) 0	17.6 ± 4.9 (90) 0	16.2 ± 4.2 (100) 0	19.3 ± 4.7 (90) 0	14.9 ± 4.9 (80) 0
Oct. 29., 1987	29.3 ± 1.3 (90) 0	29.7 ± 3.3 (90) 0	29.5 ± 2.2 (100) 0	29.3 ± 2.1 (90) 0	31.6 ± 2.3 (80) 0	31.0 ± 2.8 (100) 0
Feb. 18, 1988	14.3 ± 5.1 (70) 0	12.9 ± 4.5 (100) 0	11.9 ± 5.7 (100) 0	14.9 ± 4.9 (80) 0	15.6 ± 4.6 (80) 0	14.2 ± 6.2 (100) 0
June 23, 1988	21.0 ± 2.8 (100) 0	22.8 ± 5.7 (100) 0	22.3 ± 4.7 (80) 1	20.3 ± 6.0 (100) 0	21.5 ± 7.3 (100) 0	21.7 ± 4.1 (100) 0
Oct. 6, 1988	14.0 ± 3.1 (100) 0	14.8 ± 3.0 (100) 0	14.1 ± 3.7 (90) 0	17.4 ± 4.1 (100) 0	16.5 ± 5.9 (100) 0	11.8 ± 5.9 (80) 1

Note: Values in the table are fecundity (mean number of offspring per surviving female ± 1 SD), percentage survival (in parenthesis), and the number of males. Ten replicates were used in each test. In each case, therefore, the number of replicates used to compute fecundity was (% survival/10) minus the number of males.

Table 3-5. Results of eight fathead minnow toxicity tests of water from six sites downstream from the outfall of New Hope Pond in East Fork Poplar Creek

Date	Site					
	EFK 22.8	EFK 21.9	EFK 20.5	EFK 18.2	EFK 13.8	EFK 10.9
Oct. 7, 1986	0.49 ± 0.05 (92.5)	0.49 ± 0.11 (90.0)	0.47 ± 0.07 (90.0)	0.55 ± 0.08 (87.5)	0.55 ± 0.15 (92.5)	0.54 ± 0.10 (95.0)
Feb. 5, 1987	0.24 ± 0.05 (90.0)	0.30 ± 0.08 (87.5)	0.38 ± 0.07 (77.5)	0.27 ± 0.04 (67.5)	0.29 ± 0.08 (45.0)	0.32 ± 0.07 (40.0)
May 7, 1987	0.34 ± 0.06 (85.0)	0.28 ± 0.03 (77.5)	0.34 ± 0.04 (71.3)	0.28 ± 0.07 (90.0)	0.33 ± 0.13 (55.0)	0.30 ± 0.07 (55.0)
Aug. 27, 1987	0.71 ± 0.10 (92.5)	0.64 ± 0.04 (92.5)	0.68 ± 0.09 (90.0)	0.71 ± 0.03 (87.5)	0.68 ± 0.10 (92.5)	0.73 ± 0.10 (90.0)
Oct. 29, 1987	0.40 ± 0.03 (72.5)	0.38 ± 0.10 (55.0)	0.41 ± 0.08 (85.0)	0.39 ± 0.02 (95.0)	0.42 ± 0.09 (75.0)	0.37 ± 0.04 (92.5)
Feb. 18, 1988	0.29 ± 0.02 (100.0)	0.24 ± 0.07 (90.0)	0.30 ± 0.03 (100.0)	0.29 ± 0.07 (75.0)	0.26 ± 0.01 (97.5)	0.27 ± 0.03 (97.5)
June 23, 1988	0.61 ± 0.03 (90.0)	0.64 ± 0.07 (95.0)	0.48 ± 0.40 (97.5)	0.50 ± 0.04 (95.0)	0.58 ± 0.07 (95.0)	0.53 ± 0.61 (95.0)
Oct. 6, 1988	0.57 ± 0.05 (92.5)	0.53 ± 0.07 (95.0)	0.54 ± 0.04 (90.0)	0.51 ± 0.03 (95.0)	0.46 ± 0.04 (90.0)	0.43 ± 0.10 (95.0)

Note: Values in the table are mean growth (milligrams of dry weight per fish, ± 1 SD) and, in parentheses, the percentage survival. Four replicates, each containing 10 larvae, were used in each test. EFK = East Fork Poplar Creek kilometer.

In general, the tests results provided little evidence for systematic changes in water quality with distance downstream from NHP. Within tests, for example, site-to-site maximum differences in minnow growth (compared to the greatest growth at any site) ranged from a low of 11.9% (October 29, 1987) to a high of only 36.8% (February 5, 1987). These differences are not great compared to the amount of variability in growth of fish among replicates within a test, which was typically on the order of 10–20% (Table 3-5). Similarly, site-to-site maximum differences in *Ceriodaphnia* fecundity within tests ranged from only 11.0% (June 23, 1988) to 32.2% (October 6, 1988), while within-test variability in *Ceriodaphnia* fecundity at a site was typically 10–40% (Table 3-4). Longitudinal differences in water quality that resulted in growth or fecundity changes smaller than ~20 to 30% (relative to the maximum value for the parameter at any site) therefore could not be detected statistically.

Concordances in the response patterns of one or more species at difference sites through time are intuitively more meaningful than a high degree of statistical significance associated with responses of one species reared in water from any one site in any one test. However, concordance patterns generated by comparing the response of one species (e.g., fecundity of *Ceriodaphnia*) to that of another (e.g., growth of fathead minnow larvae) can be tantamount to analyzing apples and oranges unless the response parameters are similar. To determine whether site-to-site concordance patterns for the two species could be detected, between-test variance was eliminated, and the response ranges of the two species were rendered more similar by expressing, for each test, fish growth and *Ceriodaphnia* fecundity as percentages of the highest value of growth or fecundity, respectively, for any site. This procedure established a sliding scale wherein any site could serve as a reference point for either species. The sites were then ranked with respect to the number of times that they were the "best" or the "worst" for each species. In some tests, the "best" or "worst" site with respect to fish growth or *Ceriodaphnia* fecundity was no better or worse than that at some other site. For example, *Ceriodaphnia* fecundity in test 1 was equally low at EFK 22.8 and EFK 18.2; these two sites were therefore each scored as "worst" in test 1 (Table 3-4). Similarly, growth of the minnows was equally high in water from EFK 18.2 and EFK 13.8 in test 1, and at EFK 22.8 and EFK 20.5 in test 3 (Table 3-5); these four sites were therefore each scored as "best" in their respective test periods.

The results of the ranking procedure, summarized in Table 3-6, showed no detectable longitudinal pattern to water quality in EFPC based either on fathead minnow growth or *Ceriodaphnia* fecundity in 7-d tests. Water from EFK 13.8, however, appeared to be consistently better than water from the other sites; it was never the worst for either species, and was the best for one or the other of the two species in six of the eight tests.

Table 3-6. Results of ambient toxicity tests of water from six sites on East Fork Poplar Creek

Water quality	Incidence per site					
	EFK 22.8	EFK 21.9	EFK 20.5	EFK 18.2	EFK 13.8	EFK 10.9
Best for minnow growth	2	1	3	1	2	1
Best for <i>Ceriodaphnia</i>	1	1	0	1	4	1
Best for both species	0	1	0	0	2	0
Best for either species	3	2	3	2	6	2
Worst for minnow growth	1	3	2	0	0	2
Worst for <i>Ceriodaphnia</i>	2	1	1	3	0	2
Worst for both species	0	1	0	0	0	1
Worst for either species	3	3	3	3	0	3

Note: The summary is based on eight 7-d static-renewal tests of each site. In each test period, the sites were ranked with respect to water quality based on relative growth rates of fathead minnow larvae and fecundity of *Ceriodaphnia*. EFK = East Fork Poplar Creek kilometer.

3.4 CHEMICAL ANALYSES OF WATER QUALITY

3.4.1 Introduction and Methods

Paradoxically, waste treatment systems can simultaneously improve wastewater quality and increase loading rates of chemicals to receiving waters. For example, treatment systems that remove uranium use metal-precipitating reactions that require additions of lime, acids, and/or bases. These operations lower the wastewater's concentration of heavy metals and can reduce its toxicity, but the same operations increase the concentration of soluble salts, as well. Ion-exchange systems remove highly toxic metals from dilute solutions but must be regenerated by using acids, bases, or salts; ion-exchange column regeneration, in turn, creates a strongly saline aqueous waste. Even toxic waste incinerators that efficiently destroy PCBs leave residues that are later neutralized chemically, creating a saline aqueous waste. Finally, some kinds of noncontaminated wastewaters that are not treated can also increase the salt load to a receiving stream. Cooling tower blowdown, for example, is enriched in conductivity, alkalinity and hardness partly because salts that occur naturally in the water are concentrated as the water is lost through evaporation.

Measurements of conductivity, hardness, and alkalinity provide different but related kinds of information about the types and amounts of dissolved salts that are present. These three water quality factors are highly correlated in most natural waters: conductivity provides information about the total quantity of dissolved salts; hardness provides a measure of the concentration of divalent cations (primarily calcium and magnesium); and alkalinity provides an estimate of the water's acid neutralizing capacity, which is controlled by the types and concentrations of soluble carbonates and bicarbonates. Conductivity, hardness, and alkalinity are also conservative, in that they are usually controlled more by weathering processes and dilution events than by biota (cf. Cole and Batchelder 1969, Cole 1983, Stewart 1988). For these reasons, it was thought that measurements of conductivity, hardness, and alkalinity made in association with the ambient toxicity tests could be used to provide insight into the degree to which operations at Y-12 chemically perturb EFPC.

Unlike the salt-linked factors described above, TRC and FC are both highly toxic (the chronic exposure limit for freshwater biota to TRC is only 0.011 mg/L) and nonconservative (TRC and FC are largely converted into nontoxic materials in the presence of sunlight and/or oxidizable organic compounds). TRC and FC are present at relatively high concentrations (> 1 mg/L) in the chlorinated drinking water which enters upper reaches of EFPC from its use as a once-through coolant at Y-12. Compounds that contain chlorine and/or bromine are also used as microbiocides in many cooling tower operations and can be released to EFPC during cooling tower blowdown. Such considerations suggested that measurements of chlorine—in addition to measurements of conductivity, alkalinity, and hardness—could provide information relevant to toxicological assessments of upper EFPC.

To evaluate these possibilities, water samples collected for use in the ambient toxicity tests (cf. Table 3-3) were routinely analyzed for conductivity, Ph, alkalinity, hardness, total residual and free chlorine, and temperature. Samples from AS-8, NHP-i, and NHP-o were usually collected as daily grabs, but sometimes as 24-h composites; samples from sites in EFPC downstream from NHP-o were grab samples in all cases.

The temperature of the water at each site was recorded as the sample was collected; all other measurements were made in the laboratory within 3 h after the samples had been collected. Conductivity of the samples was measured with a YSI model 32 temperature-correcting conductance meter equipped with a glass cell ($K = 0.1/\text{cm}$) and a temperature probe (YSI model 3220). The pH was measured with an Orion model 811 meter equipped with a combination probe; the probe was calibrated daily to pH 4.0 and 10.0 with standard buffers. Alkalinity of each sample was estimated by titrating samples with 0.01 N HCl to pH 4.5. Hardness measurements were made by titrating water samples with Betz hardness titrating solution to a colorimetric endpoint (Eriochrome black T). Concentrations of TRC and FC were measured amperometrically with a Wallace and Tiernen titrator.

The chemical data were analyzed using the PC-version of the Statistical Analysis System (PC SAS). The statistical procedures used for the analyses were selected for their capacity to summarize and describe conditions and to provide insight into possible linkages between water quality factors. Two areas were explored. First, an attempt was made to determine the degree of bias in estimates of TRC concentrations at AS-8, NHP-i, and NHP-o attributable to sampling method. This issue was addressed by (1) comparing results obtained for daily grab samples to those obtained for 24-h composite samples, and (2) examining frequency-of-occurrence distributions of TRC concentrations at NHP-o. This area seemed important because TRC is quite toxic to aquatic biota and because it was often detected at NHP-o. Second, changes in correlations among alkalinity, hardness, and conductivity were explored in relation to distance downstream from the Y-12 Plant. These changes were evaluated because (1) factors such as conductivity, alkalinity, and hardness are conservative and directly traceable to wastewater treatment operations at Y-12, and (2) an evaluation of relationships among these parameters in streams at ORNL suggested that changes in the correlation structure of these three factors could be used as a general index of chemical perturbation.

3.4.2 Results and Discussion

Summary statistics of the chemical analyses for each site, computed using all available data (i.e., the pooled results of analyses for grab and composite samples), are given in Table 3-7. Because this summary did not segregate data based on sampling method, results for AS-8, NHP-i, and NHP-o (where both sampling methods were used) as given in Table 3-7 should be interpreted cautiously. Sites that were downstream of NHP-o were always sampled by daily grabs, which makes direct comparisons among sites more meaningful. The summary analysis was not very informative but did show that effluent from ORWTF was easily detected based on measurements of conductivity, alkalinity, and TRC (cf. EFPC sites EFK 10.9 vs EFK 13.8, just upstream and downstream, respectively, from ORWTF; Table 3-7).

3.4.2.1 Analysis of the importance of sampling method

A summary of the results of the analyses for sites AS-8, NHP-i, and NHP-o, segregated by sampling method, is given in Table 3-8. Three main patterns were evident from this analysis.

Table 3-7. Summary statistics (range, mean \pm 1 SD, and number of observations) for pH, conductivity, alkalinity, hardness, free chlorine, and total residual chlorine at Area Study Site 8, the inlet and outfall of New Hope Pond, and at six sites in East Fork Poplar Creek downstream from New Hope Pond outfall

Data are for all sampling dates and include samples collected either as daily grabs or 24-h composites

Parameter	Site								
	AS-8 ^a	NHP-i ^b	NHP-o ^b	EFK 22.8 ^c	EFK 21.9	EFK 20.5	EFK 18.2	EFK 13.8	EFK 10.9
pH	7.51–8.87	7.75–8.39	7.74–9.00	7.84–8.36	7.89–8.30	7.88–8.32	7.85–8.30	7.79–8.31	7.79–8.19
standard units	8.09 \pm 0.18 (49)	8.10 \pm 0.10 (140)	8.20 \pm 0.23 (235)	8.07 \pm 0.10 (56)	8.05 \pm 0.10 (56)	8.09 \pm 0.10 (55)	8.08 \pm 0.11 (55)	8.10 \pm 0.09 (56)	7.98 \pm 0.10 (56)
Conductivity	250–1004	263–682	212–764	325–542	298–507	284–551	233–561	291–551	260–555
(μ S/cm)	552 \pm 187 (49)	406 \pm 82 (140)	412 \pm 75 (235)	412 \pm 52 (56)	409 \pm 50 (56)	410 \pm 52 (56)	411 \pm 56 (55)	405 \pm 48 (56)	435 \pm 46 (56)
Alkalinity	35–125	67–136	12–130	86–128	79–127	82–129	78–138	86–132	84–192
(mg CaCO ₃ /L)	106.7 \pm 14.8 (49)	109.1 \pm 9.6 (140)	108.2 \pm 11.8 (235)	112.6 \pm 8.2 (56)	113.4 \pm 9.1 (56)	114.9 \pm 8.0 (56)	119.3 \pm 9.2 (55)	120.9 \pm 8.9 (56)	128.9 \pm 12.8 (56)
Hardness	48–334	32–242	84–254	141–232	130–232	120–244	96–246	112–242	106–232
(mg CaCO ₃ /L)	198.9 \pm 43.1 (49)	170.0 \pm 23.9 (140)	170.7 \pm 20.6 (235)	173.5 \pm 19.0 (56)	175.6 \pm 18.6 (56)	175.8 \pm 18.6 (56)	178.1 \pm 20.9 (55)	175.2 \pm 21.9 (56)	173.9 \pm 19.6 (56)
Free chlorine	0–300	0–800	0–90	0–30	--	--	--	--	0–30
(μ g/L)	100 \pm 110 (28)	70 \pm 120 (128)	10 \pm 20 (87)	3 \pm 9 (10)	0 (2)	0 (2)	-- (1)	0 (1)	4 \pm 10 (17)
Total residual chlorine	0–480	0–1700	0–200	0–50	0–20	0–10	--	0–20	0–50
(μ g/L)	120 \pm 130 (49)	170 \pm 170 (142)	20 \pm 30 (238)	5 \pm 12 (50)	1 \pm 3 (50)	<1 \pm 1 (50)	0 (49)	<1 \pm 3 (50)	11 \pm 17 (50)

^aAS-8 = Area Source Study Site 8.

^bNHP-i = New Hope Pond's inlet; NHP-o = New Hope Pond's outfall.

^cEFK = East Fork Poplar Creek kilometer.

Table 3-8. Summary statistics for alkalinity, hardness, conductivity, and total residual chlorine (TRC) contents of grab and 24-h composite samples from Area Source Study Site 8 (AS-8), New Hope Pond inlet (NHP-i), and New Hope Pond outfall (NHP-o)

	Alkalinity (mg/L)		Hardness (mg/L)		Conductivity (μ S/cm)		TRC (mg/L)	
	Grab	Comp.	Grab	Comp.	Grab	Comp.	Grab	Comp.
AS-8								
Mean	112.6	102.3	206.4	193.4	446	633	0.214	0.056
<i>n</i> ^a	21	28	21	28	21	28	21	28
C.V. ^b (%)	5.9	17.2	20.8	22.3	27.9	29.8	44.4	181.8
NHP-i								
Mean	111.9	106.7	176.5	168.5	397	413	0.189	0.148
<i>n</i>	62	78	62	78	62	78	66	76
C.V. (%)	6.9	9.6	11.5	15.9	18.2	21.4	44.0	143.1
NHP-o								
Mean	109.1	107.5	173.6	168.9	397	424	0.027	0.020
<i>n</i>	98	138	98	138	98	138	104	135
CV (%)	10.8	11.0	13.7	10.7	13.5	20.4	143.7	235.9

^aNumber of observations used to compute the mean.

^bCoefficient of variation.

First, alkalinity and hardness were each slightly but consistently higher in grab samples than in composite samples at all three sites. The magnitude of this difference ranged from about 3% to 10%, depending upon the parameter and the site; the difference tended to be larger at AS-8 than at the other two sites. In contrast, conductivity was higher in composite samples than it was in grab samples. The grab- vs composite-sample discrepancy for this water quality factor ranged from about 4% at NHP-i to more than 40% at AS-8. This finding suggests that loading rates of neutral salts (e.g., those such as sodium chloride or sodium sulfate, which contribute to conductivity but not to alkalinity or hardness) to upper EFPC vary substantially over 24-h periods. The size of the conductivity discrepancy between grab and composite samples at AS-8 also suggests that pulses of conductivity were large enough to be traceable downstream into, and probably through, the new settling basin if continuous conductivity records were available.

Second, changes in alkalinity, hardness, and conductivity between NHP-i and NHP-o were negligible within each sample-type series. Thus, as expected, these three parameters behaved conservatively despite the high level of biological activity within the pond (as

evidenced by the pond's tendency to produce large quantities of algae and submersed rooted vegetation). TRC content of the water, in contrast, dropped by a factor of ~7 by passage through the pond (Table 3-8). The size of this decline in TRC highlights both the dynamic nature of TRC and the effectiveness with which a settling basin such as NHP can reduce the toxicity of water that would otherwise be discharged directly to the stream.

The average TRC concentration for the grab-sample series was substantially higher than for the composite-sample series at each site (Table 3-8). The size of this difference was much larger at AS-8 (a factor of about 3.8) than at the other two sites (1.28 at NHP-i, and 1.35 at NHP-o). The large disparity in TRC concentration between the grab- and composite-sample series at AS-8 relative to the other two sites could be attributed to (1) site-dependent differences in materials contributing to TRC, (2) site-dependent differences in concentration of dissolved materials capable of consuming TRC, or (3) a difference in pattern of TRC release upstream of AS-8 relative to that occurring between NHP-i and AS-8 (i.e., higher amplitude, in terms of TRC concentration, but with a lower frequency of occurrence). The first possibility is the most likely. Microbiocides used in cooling tower operations at Y-12 contain both chlorine and bromine (J. M. Napier, Y-12 Development, personal communication), and bromine titrates amperometrically like chlorine and is somewhat more toxic than chlorine but has a shorter half-life (Liden et al. 1980, Wilde et al. 1983). No data are available to strongly support or refute any of the three possibilities, however.

Finally, one or more fairly large sources of TRC must be present between AS-8 and NHP-i, because TRC concentrations at NHP-i were generally not much lower than they were at AS-8. The source of TRC between AS-8 and NHP-i is unlikely to be from cooling tower operations, because the grab- vs composite-sample series disparity in TRC content at NHP-i was lower than it was for AS-8. Regardless of source, however, the concentrations of oxidants such as chlorine or bromine titrating as TRC were high enough (i.e., 10 to 15 times the chronic exposure limit; Pratt et al. 1988) to be of concern both at AS-8 and NHP-i.

At NHP-o, the mean concentration of TRC in 24-h composite samples was only 30% of that detected in grab samples. Thus, although the bias against using grab samples to obtain estimates of conservative parameters such as conductivity, alkalinity, and hardness was small, the bias in using composite samples to estimate TRC concentrations was substantial. A sampling program based on grab samples collected in a temporally randomized fashion probably could provide about as much, or more, useful information about wastewater variability and toxicity loading than a similar sampling regime based on 24-h composites. However, composite samples or continuous measurements still would be needed to develop accurate mass-balance estimates of some materials, such as uranium and mercury, that are discharged to EFPC.

Frequency distribution plots of the concentration of TRC in grab samples of water from NHP-i and NHP-o showed that the TRC content of the water declined markedly as it passed through the pond (Figs. 3-2 and 3-3).

3.4.2.2 Correlation analysis of conservative properties

Ambient toxicity analyses conducted under the Oak Ridge National Laboratory's BMAP suggested that correlations between conductivity, alkalinity, and hardness could be used as an index of the degree to which a stream was chemically perturbed (Loar 1992a).

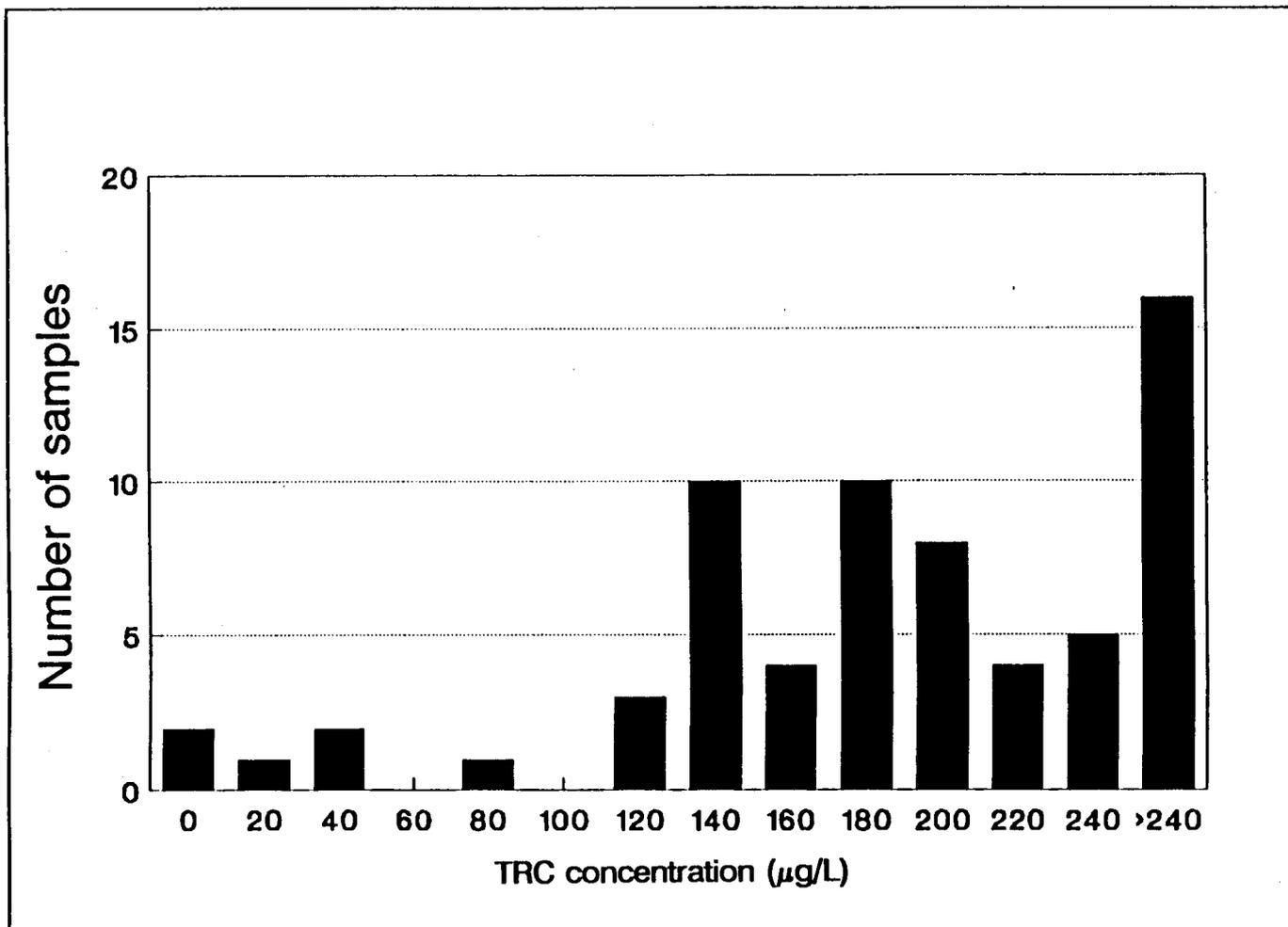


Fig. 3-2. Frequency distribution of total residual chlorine concentrations measured in samples from the inlet to New Hope Pond.

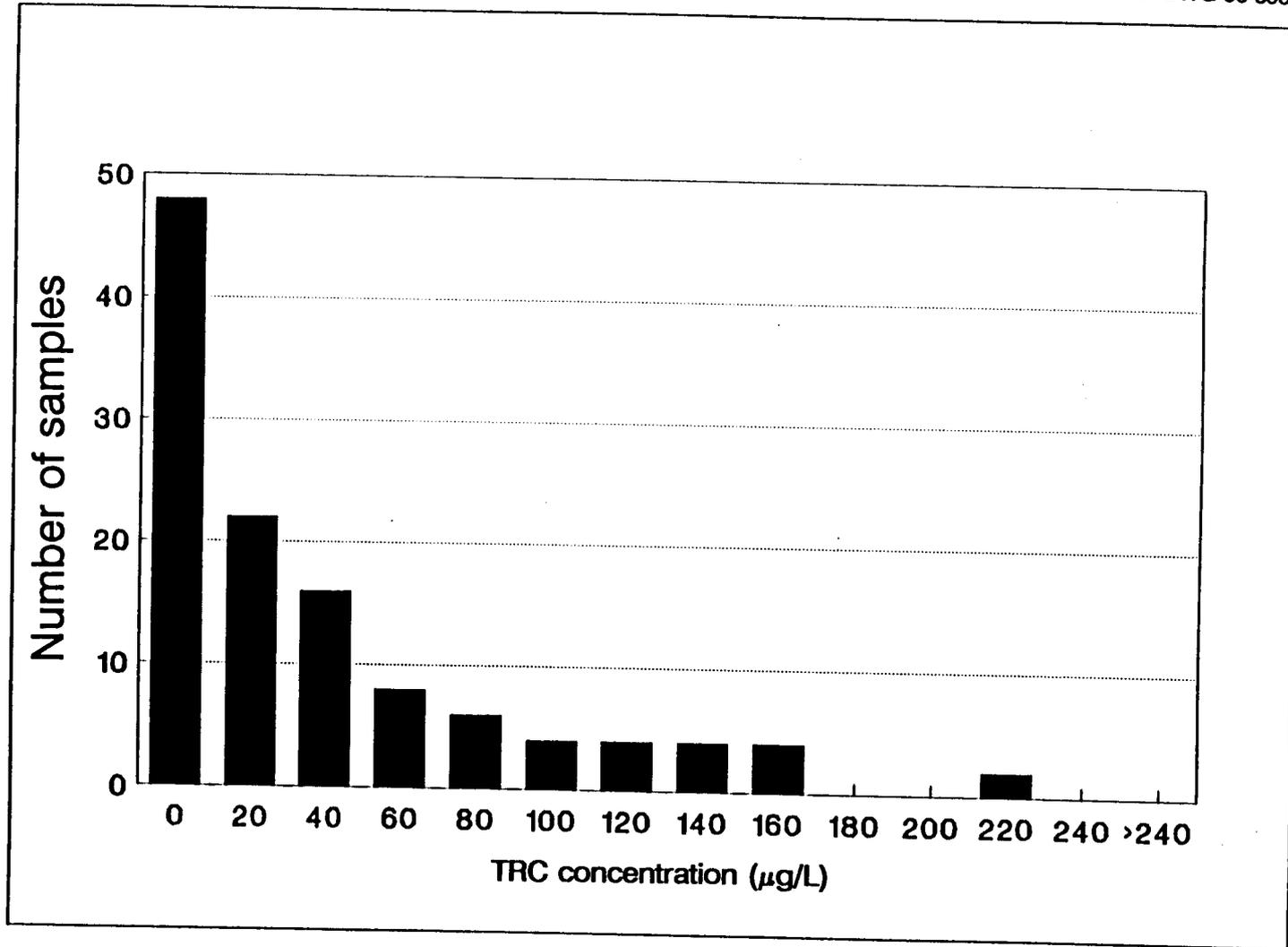


Fig. 3-3. Frequency distribution of total residual chlorine concentrations measured in samples from the outfall of New Hope Pond.

For pristine streams on the DOE's Oak Ridge Reservation, for example, these three water quality factors are highly correlated and the sum of the correlations (rank, Pearson r) ranges from about 2.6 to 2.8 (Stewart, unpublished data). The sum of the Pearson correlations between rank values for conductivity, alkalinity, and hardness, subtracted from 3.0, is referred to here as the chemical perturbation index (CPI).

The CPI was not less than 1.50 at any site in EFPC, and exceeded 2.0 at AS-8, NHP-i, and EFK 10.9, downstream from the ORWTF. These CPI values are high compared even with the highest values found for White Oak Creek (1.78, at a site just downstream from ORNL's sewage treatment plant) (Fig. 3-4).

Although presently there is no theoretical basis for suggesting that a stream site's CPI should be linked directly to the site's biotic condition, it is likely to be more than coincidental that the longitudinal changes in CPI values in EFPC are in good general agreement with those noted for the status of periphyton and benthic macroinvertebrates. Bioactive materials such as nutrients and toxicants, for example, enter streams largely through discharges of treated or nontreated wastewaters, and such wastewaters also tend to be enriched in salts that affect the CPI.

Using measurements of conductivity, alkalinity, and hardness to compute CPIs for receiving streams may be advantageous for three reasons. First, the analyses are simple and each of the three parameters can be measured quickly, accurately, and with high precision. Automated analysis systems for each of the three parameters are also available, which can reduce analytical costs, improve QA/QC aspects, and allow for the possibility of continuous monitoring if desired. Second, most of the variance in correlations between conservative properties of water can be attributed directly to flood events and changes in wastewater discharge patterns. Seasonally-linked conditions such as water temperature or photoperiod (which can radically alter biotic conditions such as fecundity status of fish or growth of invertebrates) are in most cases minor and do not affect the CPI (but see Stewart 1992). Third, the CPI provides an integrated evaluation of relationships between linked properties rather than a measurement of the properties per se. The integrative aspect of the CPI is analogous to (but much simpler than) that of stream-dwelling biota, which integrate all water quality factors in the habitat.

To ensure that a stream site's CPI is in fact an accurate reflection of the water's chemical conditions, the correlations should be based on a sufficient number of observations made through time. The fewest number of observations necessary to define statistical sufficiency depends upon the intrinsic variability of relationships among the parameters at a site and can be computed using a statistical boot-strap procedure after data from a dozen or so observation periods are available (Efron and Tibshirani 1986, Johnson 1988; A. R. Johnson, ORNL Environmental Sciences Division, personal communication). This procedure for determining the most appropriate sampling regime suffers the disadvantage of requiring considerable statistical expertise and a fairly substantial amount of data. More pragmatically, one can use an arbitrarily large number of observation periods (e.g., >40) selected to include flood and non-flood conditions, and the statistical power available to detect site-to-site differences in the CPI can, if desired, be computed from the data later.

The CPI values for EFPC sites reported here were not evaluated to determine whether sites should be considered to be statistically distinct from one another. The number of observations used to compute a site's CPI ranged from 48 (for AS-8) to 229 (for NHP-o), and so was probably large enough to correctly reflect actual conditions at a site. Noncontaminated sites on headwater streams at ORNL and the Oak Ridge K-25

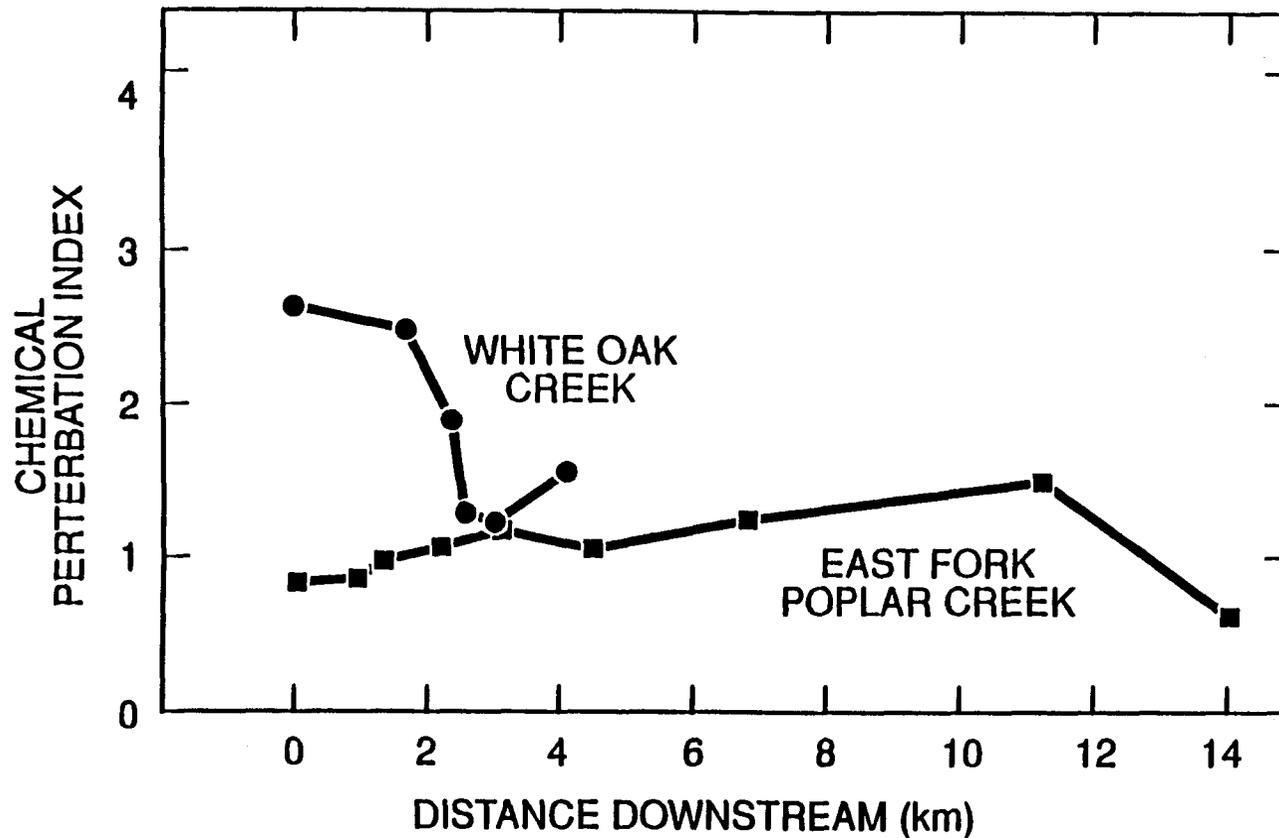


Fig. 3-4. Longitudinal distribution of chemical perturbation index (CPI) values in White Oak Creek (WOC) (circles) and East Fork Poplar Creek (squares). Distance downstream in EFPC is relative to Area Source Study Site 8, about halfway between the north-south pipes and the inlet to New Hope Pond; distance downstream in WOC is relative to White Oak Creek kilometer 6.8, a biological monitoring station north of Bethel Valley Road. Low values indicate little correlation between alkalinity, hardness, and conductivity; higher values indicate that these three parameters are more closely correlated. Values can range from zero (no correlation between any of the three factors) to 3.0 (perfect correlations between all three factors). Each triad of correlations was calculated using at least 48 observations.

Site had CPI values that differed from one another by less than 18% [2.46 for upper First Creek ($n = 81-84$), 2.26 for upper Fifth Creek ($n = 81-84$), 2.65 for upper White Oak Creek ($n = 80-84$), and 2.65 for upper Mitchell Branch ($n = 42$), respectively, calculated for a 12-month period]. These data substantiate the idea that the CPI method is a reasonably sensitive procedure for detecting factors that disturb innate relationships among conductivity, alkalinity and hardness. They also indicate that EFPC sites that differ in CPI by more than about 15% would probably be considered to be statistically distinct. Using this criterion, the change in CPI for EFPC between Tulsa Road and route 95 at Jefferson Road (from 1.21 to 1.09; a 9.9% decline) was probably not significant, whereas the change in CPI downstream from AS-8 to Tulsa Road (from 0.87 to 1.21; an increase in 39%) probably was significant. Additional studies are needed to more firmly establish the utility of the CPI approach in assessing streamwater quality.

3.5 SPECIAL STUDIES: EXPORT OF AQUATIC PLANTS FROM NEW HOPE POND

Studies started in the autumn of 1986 showed that various filamentous algae and two species of submersed vascular aquatic plants (*Potamogeton foliosus* and *Najas flexilis*) were seasonally abundant in NHP and that these plants were exported from the pond to the stream. Because neither vascular plant species grows within EFPC per se, *P. foliosus* and/or *N. flexilis* found at any site in EFPC downstream from NHP could only have been exported to the stream from NHP. Chemical analyses showed that the exported plant matter was enriched with metals of toxicological interest, including Zn, Ni, Cu, and Cr. During 1987 and 1988, additional studies were conducted to more accurately determine (1) the loading rates of aquatic plant matter to EFPC, (2) seasonal changes in the amounts or types of toxic metals in the exported plant matter, and (3) the possible effects of the plant material on aquatic biota. The information obtained from these studies follows.

3.5.1 Introduction

Many investigations have shown that submersed aquatic vegetation in lakes, rivers, ponds, and estuaries can alter the oxygen content of the water, can cycle nutrients from the sediments to the overlying water column, and can enhance the deposition of sediment (cf. Carpenter 1981, Gregg and Rose 1982, Carpenter and Lodge 1986, Carter et al. 1988). From more applied perspectives, submersed aquatic plants are also known for their ability to sequester metals, organic contaminants, and nutrients both from the sediments and the overlying water in which they grow. The common submersed plant *Ceratophyllum demersum*, for example, has an enrichment factor for arsenic of more than 10^3 (Hutchinson 1975). If contaminants are assimilated into plant tissues, they can be transported in particulate phase by moving water. Contaminated plant matter can also be consumed by herbivorous or detritivorous aquatic biota. Alternatively, plant-bound contaminants may be (1) released back to the water in dissolved form as the plants decompose, or (2) permanently interred within the sediments in a form that is largely resistant to microbial degradation. These considerations suggest that in certain habitats

submersed aquatic vegetation can drastically influence the fates, cycling patterns, and biotic affects of persistent contaminants.

3.5.2 Materials and Methods

During 1987, replicate samples of coarse particulate matter (CPM) in EFPC were collected at NHP-o, EFK 22.8, EFK 21.9 and EFK 18.2 seven times between March and August. These samples were used to provide an initial assessment of the loading rates of plant-bound contaminants to the stream. On each date, samples were collected using a pair of drift nets (363- μm mesh) positioned near the middle of the stream channel. The amount of time the nets were held in position to collect a sample varied with the rate at which CPM accumulated in a net, and ranged from 1 to 6 minutes. After sampling, the CPM was rinsed from the net into a clean labelled plastic container and taken to the laboratory. Each sample was transferred to a separate clean glass beaker before being dried (65°C) and weighed to the nearest milligram.

The dry weight of the sample, the average depth of the water flowing through the net where the sample was being collected, the width of the drift net, the length of time the net was left in position to collect the sample, and the flow rate of water at the sampling site were used to calculate the amount of CPM (in milligrams per cubic meter) being "instantaneously" transported by the water. The instantaneous rate of transport of CPM was then multiplied by the 24-h discharge from NHP for that date to provide an estimate of the gross loading rate of particulate matter to the stream.

Visual inspections showed that CPM samples collected from EFK 18.2 was consistently dominated by litter from terrestrial plants (i.e., twigs, tree leaves, fragments of riparian herbaceous vegetation), while those collected from EFK 21.9 contained primarily litter from terrestrial plants mixed with only small amounts of *P. foliosus*. Thus, although substantial quantities of plant matter were exported from NHP, little of this material was usually transported intact as far as EFK 21.9. These observations were used to design a more rigorous sampling plan for 1988.

During 1988, CPM exported from NHP-o was sampled with drift nets 17 times between March 28 and September 12. On each sampling date, the rate of transport of particulate matter at a site 1.4 km downstream from NHP-o (behind Dean Stalling's Ford in Oak Ridge; EFK 22.3) was also determined using procedures described above. In the laboratory, the material in each sample was inspected and sorted into one of five categories: *Potamogeton*, *Najas*, snails, algae, or miscellaneous. The miscellaneous category included all plant and animal matter (e.g. leaf fragments, feathers, twigs, damselfly larvae, aquatic insect exuvia, particulate detritus) that could not be obviously included into one of the other categories.

The area of streambed between NHP-o and EFK 22.3 was estimated by multiplying the length of the study reach by the reach's average width. The average width of the stream reach between NHP-o and EFK 22.3 (5.50 ± 1.05 m; mean \pm 1 SD) was calculated from width measurements made at nine locations; these locations were roughly equidistant from one another and were selected haphazardly by a surveying crew that walked upstream from EFK 22.3 to NHP-o. The distance between NHP-o and EFK 22.8 (1.4 km) was estimated from a 1:24,000 topographic map of the Oak Ridge area (S-16A, December 1987). The width and length measurements described above indicated that the area of streambed between NHP-o and EFK 22.3 was about 7.7×10^3 m².

After being sorted, the material in each category of every sample was dried (65°C) and weighed to the nearest 0.1 mg. Subsamples of material from selected categories (e.g., *Potamogeton*, *Najas*, and miscellaneous) for various sampling dates (Table 3-9) were then digested with aqua regia and the digestates were analyzed for metals. PCB content of *Potamogeton* was determined by ORNL's Analytical Chemistry Division in August 1987 from 10-g replicate samples of plants taken from NHP-o and from a noncontaminated pond south of Building 1504. These data were used to calculate loading rates of CPM-bound contaminants exported from NHP.

Snails collected in drift nets positioned at the outfall of NHP were presumed to be *Physella* sp. based on this genus's presence in other sites in EFPC (J. G. Smith, Environmental Sciences Division, personal communication), but no effort was made to positively identify these animals. Filamentous algae sometimes made up a large proportion of the material exported from the pond. Little effort was made to identify algae that was collected in the drift nets either, though seasonal changes in species composition were apparent.

3.5.3 Results and Discussion

The rate at which CPM was exported from NHP and the types of materials contributing to the CPM both varied seasonally. Export rates of CPM from NHP were low in spring, increased to a maximum during May-June, and declined to low levels again in middle to late August (Fig. 3-5). On May 9, when discharge from NHP was high due to a rain event, the rate of CPM export was very high (81.6 kg/d; 4.2 times greater than the next highest observed export rate). These findings suggest that loading rates of CPM to EFPC were very strongly affected by both the developmental state of the plant populations in the pond and rainfall events.

Potamogeton foliosus and algae were the major constituents of the CPM exported from NHP throughout the growing season (Fig. 3-6). The average contribution of *P. foliosus* to the total CPM was 17.2% in April, 20.0% in May, 49.1% in June, and 11.6% in July; both the total amount of CPM exported and the proportion of *P. foliosus* in the CPM declined again in August. Negligible amounts of algae were present in CPM samples collected during March and early April, but from April 11 through September 12 the contribution of algae to the CPM was high (on average, 54.4%; $n = 15$). *Najas flexilis* was present in the CPM at low levels (0.4% to 3% on a dry weight basis) only during late summer (mid-August through mid-September).

Seasonal differences occurred in the types of algae present in the CPM. On April 26, 1988, for example, when the export rate of algae was low (4.1 kg/d), microscopic examination showed that *Spirogyra* predominated. On August 8, 1988, when the export rate of algae was higher (8.4 kg/d), *Oedogonium* and *Spirogyra* were about equally abundant. *Oedogonium* is a coarse-textured alga, whereas *Spirogyra* is slimy and has thinner cell walls; both genera grow as unbranched filaments. Algae are important as food for many stream invertebrates (Fuller et al. 1986, Mayer and Likens 1987, Richards and Minshall 1988). Additionally, some kinds of stream invertebrates are quite selective about the types of food that they consume, and the development rates of stream invertebrates can be strongly affected by food quality (cf. Anderson and Cummins 1979, Cummins and Klug 1979, Arsuffi and Suberkropp 1986, and references therein). Thus, seasonal changes

Table 3-9. Concentration of contaminants in *Potamogeton foliosus* and filamentous algae from New Hope Pond and a noncontaminated pond (1504 Pond)

Contaminant	Concentration in <i>Potamogeton</i> ($\mu\text{g/g}$ dry wt)		Concentration in algae ($\mu\text{g/g}$ dry wt)	
	1504 Pond	New Hope Pond	1504 Pond	New Hope Pond
PCB (1254)	0.05 \pm 0.00	1.30 \pm 0.10	--	--
PCB (1260)	<0.01 \pm --	0.56 \pm 0.08	--	--
Cd	<0.55	9.6 \pm 3.9	0.27	9.5 \pm 3.1
Co	<0.56	6.7 \pm 2.8	<0.05	6.2 \pm 2.8
Cr	1.95	12.6 \pm 4.0	2.17	36.5 \pm 23.7
Cu	3.95	50.6 \pm 14.	11.6	67.2 \pm 33.7
Mn	412	2310 \pm 1150	1189	1538 \pm 1128
Ni	2.9	25.8 \pm 7.6	1.7	23.2 \pm 9.6
U	<2.5	8.0 \pm 3.4	<2.5	16.0 \pm 10.2
Zn	278	1269 \pm 274	54.5	1591 \pm 1034

Note: PCB values are means based on three replicates collected on one date. The number of samples used to determine mean values for the other samples are algae from 1504 Pond, $n = 1$; algae from New Hope Pond, $n = 11$; *Potamogeton* from 1504 Pond, $n = 2$; and *Potamogeton* from New Hope Pond, $n = 8$. Plant digestates were analyzed for metals by inductively coupled plasma spectroscopy.

in the types or amounts of algae contributing to CPM exported to EFPC could be important biologically.

The contribution of snails to CPM at the outfall of NHP was surprisingly large (0.35 kg/d, on average). However, much of this mass was due to shell material only, for many of the "snails" in the samples were actually only empty shells. *Physella* is thin-shelled and is eaten by centrarchid fish (e.g., redbreast sunfish; M. G. Ryon, Environmental Sciences Division, ORNL, personal communication). Assuming (1) that 25% of the average daily mass of snails exported from the pond was available as food for fish (i.e., that 50% of the mass of a live snail is due to shell material, and that only 50% of the total number of exported "snails" were alive) and (2) that *Physella* would not be transported more than several meters downstream (due to their relatively high specific gravity), one can reasonably suppose that *Physella* could be an important source of some contaminants (e.g., mercury, PCBs) to fish that lived close to the pond's outfall. However, we did not analyze contaminants in the snails or quantify the deposition patterns of the snails in EFPC to verify this supposition.

The results of the chemical analyses of samples of *Potamogeton* and filamentous algae collected from NHP-o and the noncontaminated 1504 pond are summarized in Table 3-9. Enrichment ratios were calculated for the contaminants shown in Table 3-9. These ratios, which are computed as the average concentration of a contaminant in algae or *Potamogeton* from NHP divided by the average concentration of that contaminant in the corresponding plant material from the 1504 pond, are shown in Table 3-10. Coded "blind"

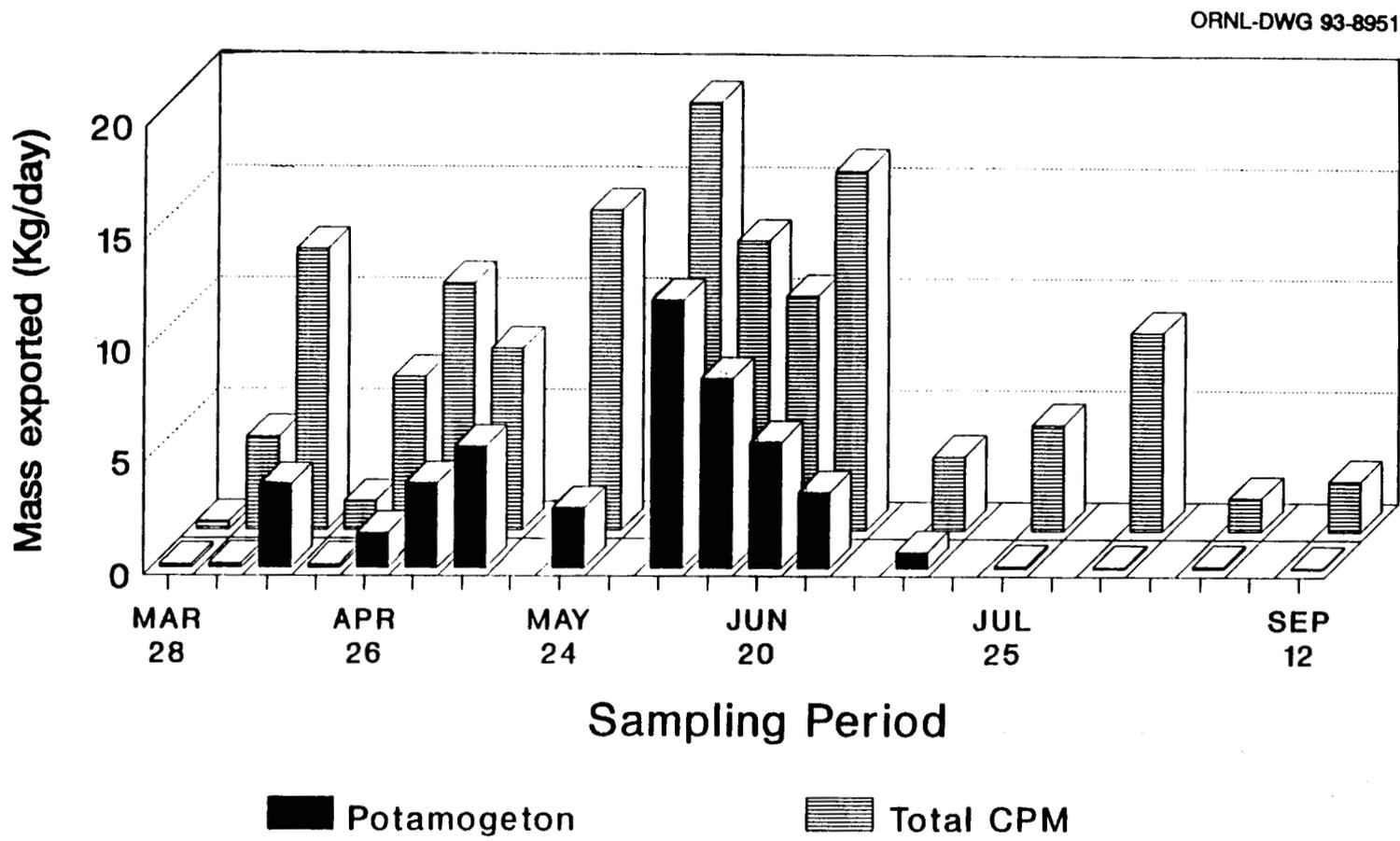


Fig. 3-5. Export rates of coarse particulate matter from New Hope Pond. Units (vertical axis) are kilograms dry weight per day.

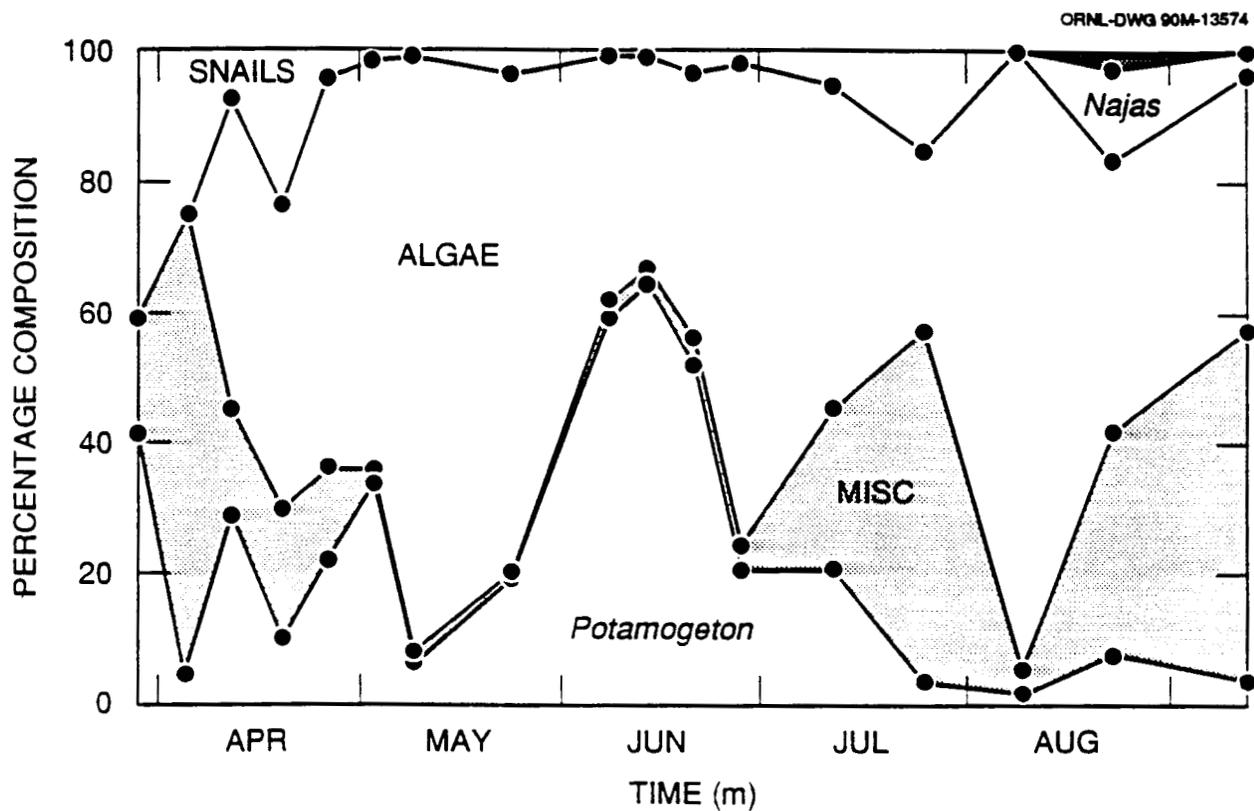


Fig. 3-6. Composition (expressed as percentage of the total) of coarse particulate matter exported from New Hope Pond.

Table 3-10. Approximate enrichment ratios^a for filamentous algae and *Potamogeton foliosus*

Contaminant	Enrichment ratio	
	Algae	<i>Potamogeton</i>
Cd	35	>17
C	>124	>12
Cr	17	6
Cu	6	13
Mn	1.3	5
Ni	13	9
U	>6.4	>3
Zn	29	4

^aThe concentration of a contaminant in plant material from New Hope Pond divided by the concentration of that contaminant in plant material from a noncontaminated pond near ORNL Building 1504.

Note: Values are calculated from data given in Table 3-9 of this document.

samples of plant material from the National Bureau of Standards (NBS) [three samples each of pine needles (SRM-1575) and citrus leaves (SRM-1572)] were included among the algae and *Potamogeton* samples that were sent to the analysis laboratory. The concentrations of metals in the standards as determined by the analysis laboratory tended to be in good general agreement with those cited by the NBS (Table 3-11). Thus, the enrichment ratios for most of the metals shown in Table 3-10 are likely to be reasonably accurate even though the concentrations of the metals in the plant samples varied substantially during the 7-month sampling period. The analysis laboratory's detection limit for uranium, however, was only 2.5 $\mu\text{g/g}$. Therefore, the true enrichment ratios of this metal, both for *Potamogeton* and filamentous algae, are probably much higher than those given in Table 3-10.

3.6 SPECIAL STUDIES: EFFECTS OF AQUATIC PLANTS FROM NEW HOPE POND ON BIOTA

3.6.1 Introduction

Laboratory experiments with snails, amphipods (*Gammarus*), and *Ceriodaphnia*, and a field experiment with snails, were used to assess the possible importance of inputs of contaminated *Potamogeton* to stream biota. Laboratory tests with snails and amphipods were used to determine whether either of these types of animals could detect differences between *P. foliosus* from NHP and *P. foliosus* from a noncontaminated pond. A 7-d laboratory test with *Ceriodaphnia* was also used to determine if leachates—prepared from *P. foliosus* collected from NHP and from a noncontaminated pond—released toxic materials as they decomposed. Finally, *P. foliosus* collected from NHP and from a noncontaminated

Table 3-11. Comparison of University of Georgia Chemical Analysis Laboratory (UGCAL) analytical data with those provided by the National Bureau of Standards (NBS) for selected metals in standard reference materials [pine needles (SRM-1575) and citrus leaves (SRM-1572)]

Metal	Pine needles		Citrus leaves	
	UGCAL	NBS 2	UGCAL	NBS
Cd	0.3 ± 0.3	<0.5 ^a	0.2 ± 0.1	0.03 ± 0.01
Co	ND	0.1 ^a	ND	ND
Cr	3.4 ± 0.5	2.6 ± 0.2	0.8 ± 0.1	0.8 ± 0.01
Cu	3.7 ± 0.8	3.0 ± 0.3	23.2 ± 18.2	16.5 ± 1.0
Mn	670 ± 1	675 ± 15	22.1 ± 2.3	23 ± 2
Ni	2.4 ± 0.4	3.5 ^a	1.0 ± 0.1	0.6 ± 0.3
U	ND	0.02 ± 0.004	ND	---
Zn	66.2 ± 2.9	---	28.5 ± 0.8	29 ± 2

^aNoncertified values.

Note: Values are means ± 1 SD for concentrations, in milligrams per gram dry weight. ND designates nondetectable levels.

pond were placed in various locations in a noncontaminated stream, and responses of native populations of the predominant snail (*Elimia clavaeformis*) to the two types of plants were used to determine whether or not the snails preferred one or the other of the two types of food. The methods used to conduct these tests, and the results obtained from them, are described in the following sections.

3.6.2 Amphipod Food Preference Test—Methods

This test was started on June 28, 1988, and used 5 replicates, each containing 20 animals. Each replicate consisted of a white plastic tray (28 × 32 cm), to which was added enough dechlorinated tap water to achieve a water depth of about 2 cm. Two small clumps of *P. foliosus*—one made of plants from NHP, the other made of plants from the noncontaminated 1504 pond—were then placed into each tray, about 15 cm apart. The pondweed clumps were about 4 to 5 cm in diameter, and each contained about 1.0 g (wet weight) of plant matter. Each of the clumps was marked with a small piece of plastic flagging to identify its source (NHP vs 1504 pond). The amphipods, which were collected from upper First Creek, were then added to the trays. Two days after the experiment was started, the number of amphipods associated with each plant type in each tray was determined. Two of the replicates were then sacrificed for analytical purposes. The remaining three replicates were allowed to continue undisturbed for five more days. Seven days after the experiment had been started, the number of amphipods associated with each plant type in each of the three remaining replicates was counted again.

3.6.3 Amphipod Food Preference Test—Results

Early in the experiment, the amphipods strongly preferred NHP *Potamogeton* over *Potamogeton* from the 1504 pond. This preference had completely reversed, however, by the end of the experiment (Table 3-12).

3.6.4 Snail Food Preference Transition Test—Methods

This laboratory test was similar to the amphipod test in that it used freshly collected contaminated and noncontaminated *P. foliosus* and free-roaming animals. It differed, however, in that the test used only 1 replicate (a fiberglass Min-o-Cool waterbath, 55 cm × 208 cm), 200 snails, and 18 clumps of plant material (9 of each type). The plant clumps were positioned randomly in the waterbath, which contained about 2 cm of dechlorinated tap water.

This experiment was started on June 16, 1988. On June 20, 20 snails selected randomly from those in the waterbath were withdrawn, marked individually with nail polish (Burris et al. 1990), and returned to the waterbath. Over the next 48 h, positions of the individually marked snails were noted on 12 occasions. This provided information about food-preference tendencies and changes in these preferences at the level of the individual animal. In each observation period, each snail was categorized as to its position (1) associated with a clump of contaminated plant material, (2) associated with a clump of noncontaminated plant material, or (3) not in association with either plant type (i.e., on the bottom of the waterbath between clumps).

3.6.5 Snail Food Preference Transition Test—Results

The number of snails making any of 11 possible transitions for the 220 transition periods is shown in Table 3-13. Collectively, the clumps of plants covered only about 5% of the area on the bottom of the waterbath, so that 95% of the area was categorized as "open." Since 40% of all transitions involved plant clumps, the snails preferred plants of either kind over open area by a factor of 8.

The large number of transitions involving the "open" category (Table 3-13) statistically weakened the test's ability to show whether or not the snails could distinguish between the contaminated and noncontaminated plants. However, the similar number of transitions made from contaminated to noncontaminated plants (8) and from noncontaminated to contaminated plants (7) suggested that the choice between contaminated and noncontaminated food was less important to the snails than was the choice between any food or no food. The number of transitions made by the snails is conservative, for it is possible that some snails moved more than once between adjacent observation periods. This possible bias, however, is likely to be small, for the time interval between observations made during the day was short (2 h). The transition frequency distribution (e.g., the number of transitions per snail) was nearly normal (Fig. 3-7), and the bias, if one was present, would have tended to skew the distribution to the right. The overall interpretation of the test, however, would not be markedly affected.

Figure 3-7 shows that although many snails tended to move frequently (e.g., most made from 4 to 7 transitions in 48 h), variability was high: one snail moved only once, and one moved 9 (or more) times. The number of snails associated with noncontaminated plant material declined steadily with time, perhaps because the animals became satiated by

Table 3-12. Number of *Gammarus* associated with clumps of *Potamogeton foliosus* collected from New Hope Pond (contaminated) and from a noncontaminated pond after 2 and 7d

Replicate	Day	Pondweed type		Neither	Missing
		Contaminated	Noncontaminated		
1	2	19	0	1	0
2	2	20	0	0	0
3	2	20	0	0	0
4	2	20 ^a	0	0	0
5	2	20	0	0	0
1	7	0	10	3	7
2	7	6 ^b	12	1	1
5	7	1 ^a	14 ^a	1 ^a	4

^aNumber includes one dead animal.

^bNumber includes three dead animals.

Note: There were 20 animals present in each replicate at the start of the test. Amphipods that were not associated with either type of plant (e.g., swimming about or in open-water areas away from the clumps) were scored as "neither." After 7 d, missing animals were presumed to have died and decomposed.

Table 3-13. Results of snail transition test

Transition type	Number of moves
C to NC	8
C to same C	34
C to new C	9
C to open	17
NC to C	7
NC to same NC	20
NC to new NC	10
NC to open	16
Open to NC	16
Open to C	23
Open to open	60
Total	220

Note: The data summarize the preference patterns of 20 snails based on 12 observation periods (= 11 transitions). C and NC refer to "islands" of contaminated and noncontaminated *Potamogeton foliosus* respectively. "Open" refers to any area located between the "islands." A transition type was scored as "new" (e.g., C to new C or NC to new NC) if a snail moved from an island of one plant type to another island of the same type), and as "same" (e.g., C to same C) if a snail associated with a particular island during one observation period was found on the same island during the next observation period.

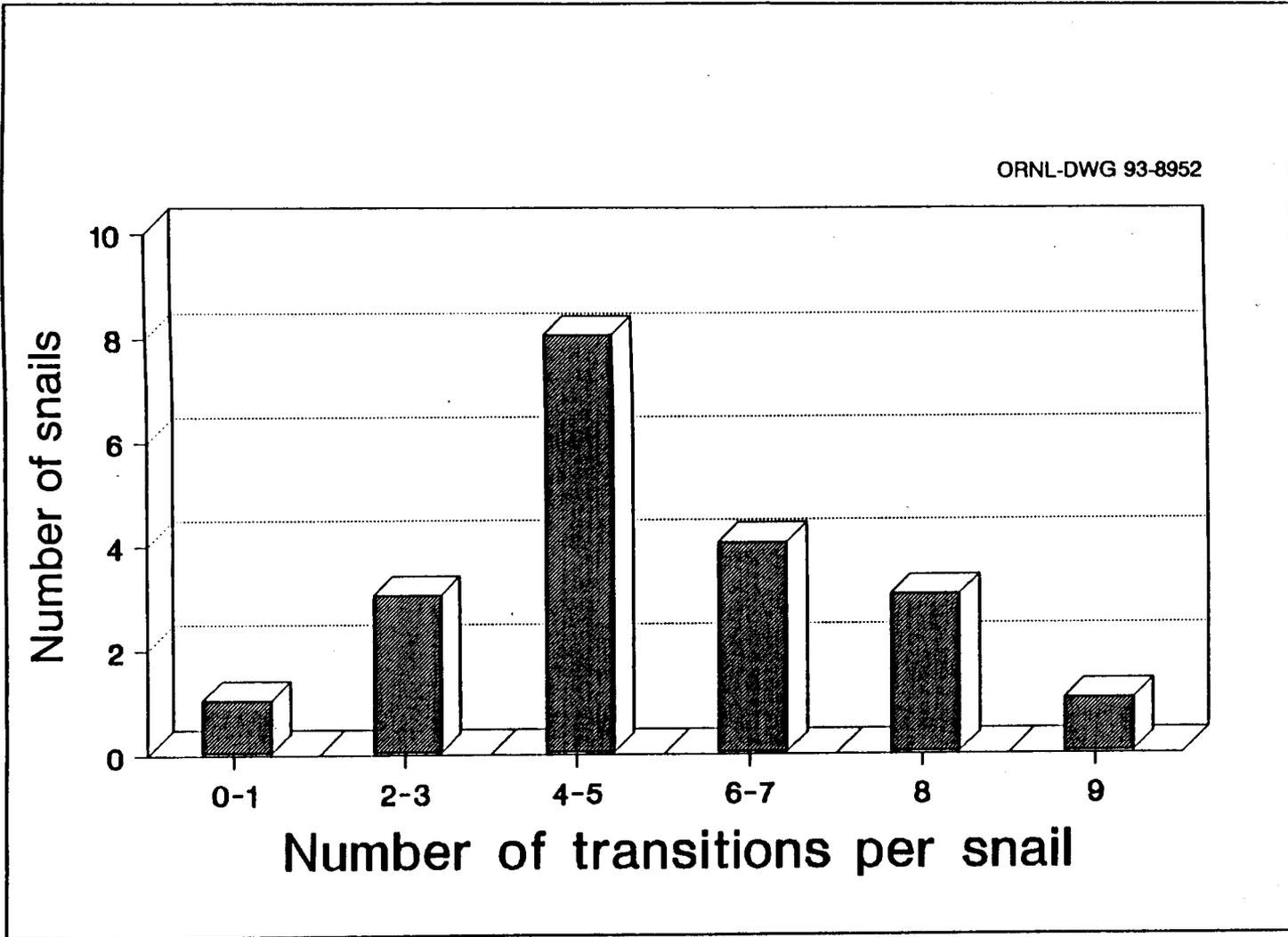


Fig. 3-7. Transition frequency distribution for snails moving among "islands" of contaminated and noncontaminated *Potamogeton* (from New Hope Pond and a pond at ORNL respectively). A few snails moved only 1 to 3 times; most moved 4 to 7 times; one moved 9 times. Eleven transitions were possible.

feeding. Over the 48-h test period, however, the number of snails associated with noncontaminated plant material equaled or exceeded that associated with contaminated plant material in 42 of the 48 cases. Thus, *Elimia* appears to be able to detect differences in food quality even within a plant species. The high mobility of the animals (e.g., Fig. 3-7) and the relatively large number of transitions involving the "open" category (Table 3-13) suggested that a more definitive test could be achieved by reducing the size of the test chamber.

3.6.6 Snail Food Preference "Beaker" Test—Methods

A snail food-preference test using smaller test chambers was set up to more precisely determine whether *Elimia* could detect differences in *Potamogeton foliosus* that had matured in different kinds of environments. This test, conducted during April 18–19, 1989, used flat-bottomed glass dishes, each 18 cm in diameter, as test chambers. About 1.5 L of dechlorinated tap water was added to each chamber, as were two small clumps of *Potamogeton* (one noncontaminated and one contaminated). Fifteen temperature-acclimated snails were placed near the center of each test chamber, about equidistant from the clumps of plant material. The number of snails associated with contaminated and noncontaminated plant material in each test chamber were counted three times during the next 48 h.

3.6.7 Snail Food Preference "Beaker" Test—Results

The results of this experiment showed that the snails strongly preferred noncontaminated plant material over contaminated plant material (Table 3-14).

Table 3-14. Results of laboratory snail preference test for contaminated vs noncontaminated *Potamogeton foliosus* in 18-cm-diam test chambers^a

Time (h)	Number of snails associated with plant type		NC:C
	Noncontaminated	Contaminated	
6.3	5.44 ± 1.71 (87)	0.75 ± 1.29 (12)	7.3
20.5	3.38 ± 1.31 (54)	0.31 ± 0.60 (5)	10.8
48.0	2.44 ± 1.26 (39)	0.75 ± 1.12 (12)	3.3

^aThe test was conducted at 14°C. Values are the mean number of snails associated with each plant type ± 1 SD. The total number of snails associated with each plant type during each observation period is given in parentheses. NC:C is the ratio of the total number of snails associated with noncontaminated and contaminated plant material in each observation period.

3.6.8 Instream Snail Food Preference Test—Methods

To verify that the apparent preference of the snails for noncontaminated plant material was not just an artifact of the test system, a food-preference test was conducted in Ish Creek, a pristine stream containing large numbers of *Elimia*. This 24-h field test was conducted during April 19–20. Small clumps of contaminated and noncontaminated *Potamogeton* were made by wrapping a small bunch (~1 g dry wt) of plant material with a twist of plastic-coated wire. Two clumps of plant material, one contaminated and one noncontaminated, were placed about 20 cm apart at each of 7 sites in the stream. At each site, the clumps were positioned near the center of the stream along an imaginary axis perpendicular to the flow of water. Twenty-four hours after positioning the clumps of plant material in the stream, we returned to the sites and counted the number of snails that were associated with each clump.

3.6.9 Instream Snail Food Preference Test—Results

The results of the instream test showed that natural populations of *Elimia* in a natural habitat strongly preferred noncontaminated *Potamogeton* over contaminated *Potamogeton* ($p < 0.05$, based on Wilcoxon’s signed rank test). There were more snails associated with the noncontaminated plants than with the contaminated plants at six of the seven sites (Table 3-15).

Table 3-15. Results of field snail preference test for contaminated vs noncontaminated *Potamogeton foliosus* in Ish Creek

Site	Number of snails associated with plant type	
	Noncontaminated	Contaminated
1	7	3
2	3	0
3	1	0
4	1	2
5	8	4
6	2	0
7	11	3
Total	33	12

Note: Values are the numbers of snails found associated with a particular type of plant 24 h after the plants had been placed in the stream.

In casual observations, it was also noted that crayfish started feeding on the plant material at some sites within 30 minutes after it had been placed in the stream. Seventy-two hours after the experiment had started, the contaminated and noncontaminated plant material at several of the sites had completely disappeared, presumably through consumption by these omnivores.

3.6.10 *Ceriodaphnia* Test of *Potamogeton* Leachates—Methods

Fresh samples of *P. foliosus* were collected from NHP and from a noncontaminated pond (1504 pond; behind ORNL Building 1504) on June 28, 1988. Leachates of the two types of plants were prepared by putting a 13.5-g mass (wet wt) of each plant type into separate 2-L flasks; each flask contained 1.6 L of diluted mineral water (1:9 v:v, Perrier water and deionized distilled water). Two replicate flasks were used for each plant type. The flasks were covered with aluminum foil to exclude light and placed in an incubator at 25°C; the contents of each flask was kept mixed (and aerated) by gently bubbling with air. After 9 d, the liquid in each flask was filtered (0.5- μ m pore-size glass fiber), and the filtrates were tested for chronic and acute toxicity to *Ceriodaphnia* neonates using standard Environmental Protection Agency test procedures (Horning and Weber 1985). Each filtrate was tested at 25% and 75% of full-strength; a set of 10 animals reared individually in diluted mineral water was used as a control in this test.

3.6.11 *Ceriodaphnia* Test of *Potamogeton* Leachates—Results

The results of the *Ceriodaphnia* test are summarized in Table 3-16. The higher concentration of the leachates made from plants from NHP were toxic. Animals in these solutions had high mortality and low fecundity. Fecundity of *Ceriodaphnia* tested in the low concentration of the leachates prepared from plants from NHP was also slightly lower (by about 15%) than that of the control, but their survival was high.

Samples of the higher concentrations of the leachates prepared from each plant type were analyzed for metals by ICP. The results of these analyses are given in Table 3-17. Only one third (10 of 30) of the samples analyzed for metals had concentrations above ICP detection limits in either leachate. None of the metals that were detected by ICP seemed to be at a concentration high enough to adversely affect *Ceriodaphnia*; of the detectable metals, only phosphorus was much higher in NHP leachate than it was in 1504 Pond leachate. Concentrations of SRP and nitrate-N in the NHP leachates were much higher in the 1504 Pond leachates. Thus, it is possible that the toxicity of the leachates prepared using plants from NHP was due to metals not tested or to one or more organic compounds released either from the plants themselves, or from microbial communities that developed in the flasks during the 9-d decomposition period.

3.7 SPECIAL STUDIES: SUMMARY AND CONCLUSIONS

Data given in Sect. 3.5 showed that (1) large amounts of plant matter produced in NHP (largely *Potamogeton*, filamentous algae, and *Najas*) were exported from the pond during the growing season; (2) the exported plant material was enriched, relative to the

Table 3-16. Results of *Ceriodaphnia* toxicity test of leachates prepared from *Potamogeton* collected from New Hope Pond (NHP) and from a noncontaminated pond near ORNL Building 1504 (1504 Pond)

Plant source	Replicate	Concentration (%)	Survival (%)	Fecundity (mean \pm 1 SD)
NHP	1	25	100	17.4 \pm 2.6
NHP	2	25	100	17.2 \pm 3.8
NHP	1	75	50	0.0 \pm ---
NHP	2	75	40	1.5 \pm 1.3
1504 Pond	1	25	70 ^a	21.3 \pm 2.5
1504 Pond	2	25	90	21.9 \pm 5.1
1504 Pond	1	75	100	23.1 \pm 4.4
1504 Pond	2	75	100	24.6 \pm 2.3
Control	---	---	100	20.1 \pm 2.6

^aIncludes one male.

Note: Survival is given as percentage; fecundity is the number of offspring (mean \pm 1 SD) produced by females that survived the entire 7-d test period.

plants from a noncontaminated pond, with PCBs and various metals; and (3) very little of the particulate matter exported from the pond made it intact (i.e., in recognizable form) for more than several kilometers once it entered EFPC. Data given in Sect. 3.6 showed that in short-term laboratory and field tests, snails, amphipods, and *Ceriodaphnia* were able to discriminate between *Potamogeton foliosus* from NHP and *P. foliosus* from a noncontaminated pond: plants from NHP were either preferred less than those from the noncontaminated pond (based on the results of food-preference tests with snails and amphipods), or yielded leachates that were toxic to *Ceriodaphnia*. In this section, an attempt is made to reconcile information given in Sects. 3.5 and 3.6 to evaluate the possible influence of NHP on biotic conditions in EFPC downstream from the pond.

"Best guess" estimates of the organic carbon inputs to the 1.4-km reach of EFPC downstream from the outfall of NHP are given in Table 3-18. In this table, the net amount of particulate organic carbon (POC) contributed to the 1.4-km reach of stream by periphytic algae in the stream was estimated from a series of ¹⁴C-uptake experiments conducted by Drs. Harry Boston and Walter Hill (Environmental Sciences Division, unpublished data). In like fashion, the amount of POC available to the stream segment due to exports from NHP (i.e., filamentous algae and *Potamogeton*) was conservatively estimated by assuming that fully 50% of the POC leaving NHP was transported intact downstream beyond EFK 22.3.

Table 3-17. Average concentrations of materials detectable in leachates (75% full-strength) prepared from *Potamogeton foliosus* collected from New Hope Pond (NHP) and from a noncontaminated pond near ORNL Building 1504 (1504 Pond)

Parameter	Leachate source		Control
	NHP	1504 Pond	
Al	0.125	0.155	<0.06
Ba	0.019	0.034	<0.08
Be	0.0013	0.0017	<0.002
Ca	19.5	26.5	17.0
Mg	1.20	1.45	0.38
Na	5.5	3.5	1.2
Si	0.56	0.60	0.53
Sr	0.058	0.074	0.057
Zn	0.010	0.012	<0.003
Hardness	49.0	62.0	38.0
Alkalinity	32.5	67.5	32.0
pH	7.64	8.02	7.80
Nitrate-N	6.01	0.06	---
SRP	1.22	0.258	0.001

Note: Values for the two leachates are means based on two samples. Concentrations for inductively coupled plasma metals in the control water are from a single analysis made on March 22, 1988. Only metals that had concentrations above detection limits in either of the two leachate types are given. Hardness and alkalinity of the solutions (expressed as milligrams CaCO₃/L) were measured by titrations; pH values are standard units; and nitrate-N and soluble reactive phosphorus (SRP) were measured colorimetrically using EPA methods 353.2 and 351.2 respectively.

Table 3-18. Sources of organic carbon to biota in a 1.4-km reach of East Fork Poplar Creek immediately downstream from the outfall of New Hope Pond (NHP)

Estimates are based on data for the period March 28 through September 12, 1988

Organic carbon source	Supply rate (mg C·m ⁻² ·d ⁻¹)
Periphyton (net primary)	166
<i>Potamogeton</i> from NHP (estimated deposition)	102
Filamentous algae from NHP (estimated deposition)	301

This analysis shows that during the growing season, the contribution of organic matter from NHP was about 2.4 times greater than that available to the stream community from periphyton growing in the stream. Additionally, the types of assumptions used to generate the values in Table 3-18 are more likely to underestimate contributions from NHP than they are to underestimate contributions from the periphyton. Thus, it is likely that for at least a 6-month period (April through September), the supply rates of particulate organic matter from NHP to biota over the 1.4-km reach of EFPC exceeded the supply rate of organic matter produced by periphyton in the stream by a factor of at least three to two.

Energy subsidies to ecosystems can be ecologically disruptive, for the flow of energy drives the cycles of materials (Odum 1989). Algae and submersed vascular aquatic plants contain little or no lignin (a microbiologically recalcitrant, important structural material in woody plants), and so are much more labile than terrestrial plants (pp. 667-706 in Wetzel 1983). Thus, particulate organic matter from NHP must be considered to be an important energy subsidy to this reach of EFPC. It is also reasonable to suppose that energy subsidies (in the form of labile organic matter) may be ecologically more disruptive in aquatic sulfate- or nitrate-enriched ecosystems than they are in more pristine aquatic habitats, because sulfate and nitrate can be used as electron acceptors by anaerobic microbes (Rich and Wetzel 1978). Thus, microbial communities in EFPC just downstream from NHP have (1) more organic matter available due to the large inputs of plants from NHP, (2) enhanced opportunities to "extract" the energy associated with this organic matter due to the presence of moderately high concentrations of nitrate and sulfate, and (3) quite probably, higher overall rates of community respiration. A higher rate of respiration (by about 50%) is likely because microbial respiration is temperature sensitive (it doubles, approximately, with each 10°C increase in temperature), and the average water temperature in upper reaches of EFPC is elevated by about 5°C relative to reference streams (e.g., Brushy Fork; M. G. Ryon, Environmental Sciences Division, personal communication; see Sect. 2.5).

"Pure" trophodynamic consequences of a "pure" energy subsidy to a mid-reach segment of White Oak Creek (WOC) were very evident following an ethylene glycol leak to the stream in September 1986. "Sewage fungi" (bacteria) proliferated, fish became stressed due to low levels of oxygen caused by the rapid consumption of oxygen as microbial respiration increased, and (somewhat later) chironomids (which can feed on bacteria) increased in abundance downstream from the leak (Smith, unpublished data). The trophodynamic consequences of sustained inputs of energy-rich organic matter, particularly when such inputs also contain toxicants that may bioaccumulate (e.g., mercury, PCBs, cadmium), remain unknown. Elevated concentrations of nutrients that commonly limit primary production in aquatic ecosystems (e.g., phosphorus, nitrogen), toxicants, and compounds (e.g., sulfate and nitrate) that can be used as alternate electron acceptors by anaerobic microbiota tend to co-occur in receiving streams. How these conditions affect processes of organic matter production and decomposition remains an important challenge in stream ecotoxicology.

The deficiencies in reproductive competency of fish in upper EFPC (Sect. 5.0) are not matched by the results obtained through the use of the EPA's *Ceriodaphnia* toxicity test (Sect. 3.3.2). This discrepancy could relate to key differences in exposure duration (e.g., 7-d for *Ceriodaphnia* vs months or years for fish living in the stream), exposure regime (e.g., episodic vs constant conditions, static-renewal vs continuously flowing), or exposure mode (e.g., uptake of contaminants by ingestion of contaminated food vs exposure to contaminants in dissolved phase). Alternatively, the discrepancy could relate

to differences in abiotic conditions that are deliberately not mimicked by the *Ceriodaphnia* test system (e.g., water temperature, or seasonal or daily fluctuations therein), or it might occur because the *Ceriodaphnia* test system does not include processes that (in EFPC) influence toxicant speciation or increase the supply rates of a toxicant to biota (e.g., the biotransformation of mercury). It may well be that discrepancies of this type ultimately can be resolved only by developing a more thorough understanding of how biotic and abiotic processes collectively influence a natural community's ability to respond to toxic insults.

3.8 INSTREAM MONITORING OF THE PERIPHYTON COMMUNITY

(H. L. Boston and W. R. Hill)

Periphyton is a complex matrix of algae and heterotrophic microbes attached to submersed surfaces that serves as a major food source for many stream invertebrates (Minshall 1978) and herbivorous fishes (Power et al. 1985). Contaminants may accumulate in the periphyton as a result of biological uptake and adsorption to organic matter (Huckabee and Blaylock 1973, Selby et al. 1985). Materials sequestered in periphyton can subsequently be transferred through the aquatic food chain. The biotic components of the periphyton may turn over on the order of days; hence, the periphyton community is especially useful for detecting short-term environmental changes (e.g., infrequent pulses of toxicants that might go undetected when considering organisms with longer life spans). We can use the periphyton to (1) evaluate biological conditions, (2) evaluate contaminant transport and food chain accumulation, (3) identify sources and frequencies of toxicity, and (4) aid in the prediction and evaluation of biotic responses to remedial action measures.

Monitoring of the periphyton communities began in March 1986, following an initial survey that suggested that the riffle areas at sites EFK 23.2, EFK 13.7, EFK 10.6 and EFK 6.3 were fairly representative of the different regions of the stream below the outfall of NHP to the downstream extent of the stream-like habitat (i.e., before the channel deepened and became more riverine). A monitoring site was also established in Brushy Fork, at BFK 7.6, to serve as a reference site not impacted by industrial activities. In October of 1986, a monitoring station at EFK 24.4 located upstream of NHP was added. During February 1987, the riffle at EFK 13.7 was destroyed by construction activities and the monitoring site was moved about 75 m upstream to a riffle in a section of the stream not shaded by riparian vegetation (EFK 13.8). At that time the sampling site at EFK 10.6 was moved downstream about 30 m to a more stable riffle area that had a similar amount of shading by riparian vegetation. Because the sampling sites were moved during October when the riparian vegetation was leafless, changes in light had little influence on the periphyton community for the October 1986 through September 1988 study period. The sampling site on Brushy Fork was located on the north side of a steep ridge and so received no direct sunlight during the winter. Because the seasonal light regime differed greatly from that for the shaded sites on EFPC, the sampling site on Brushy Fork (BFK 7.6) was moved about 100 m downstream in November 1987, to a location that received more light during the winter. These sites largely coincide with the locations of the invertebrate and fish monitoring stations (Figs. 3-8 and 2-1).

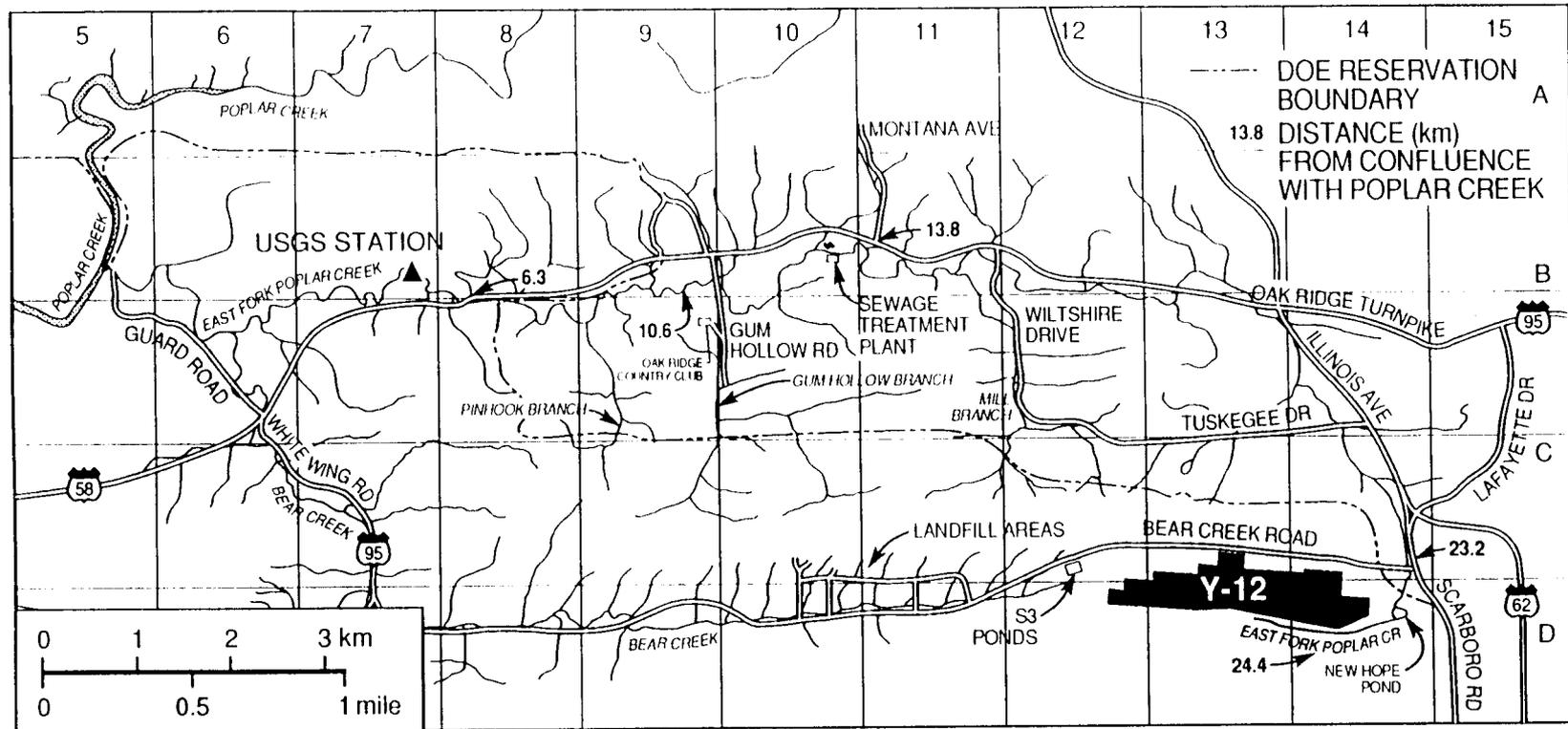


Fig. 3-8. Periphyton monitoring sites. Rocks collected from riffles (10- to 20-cm water depth) in 50- to 100-m area.

Preliminary findings have been presented in Loar (1992b). This report will consider the results of our efforts from October 1986 through September 1988. This period has been divided into two study years; October 1986 through September 1987 (Year 1), and October 1987 through September 1988 (Year 2). This division was partly for convenience, but also allowed each study period to begin at the onset of the cool weather and as leaves began to fall.

Our efforts have largely addressed the algal component of the periphyton, measuring biomass and primary production (as short-term photosynthetic rates) of algae on small rocks collected from riffles. Documentation of temporal and spatial changes in algal biomass and production provide a means of tracking sites and comparing the relationship between photosynthesis and biomass for the periphyton at various sites. To provide a basis for interpreting differences in the biomass and condition of the algal periphyton at each site we have characterized the habitat (light, water depth, canopy cover, bottom type, etc.) for 50-m areas centered on each of the monitoring sites. We have also collected discrete water quality samples in conjunction with the monthly collection of periphyton (Sect. 2.4.2). The taxonomic composition of the algal assemblages were also determined.

During the summers of 1987 and 1988 two experiments were conducted to augment the monitoring data: (1) short-term laboratory algal bioassays were conducted to evaluate the influence of water quality at each site on periphyton photosynthesis, and (2) colonization and development studies were conducted to evaluate the rate of algal colonization and growth at BFK 7.6 and several of the EFPC sites.

Adenosine triphosphate (ATP) concentrations in the periphyton have been determined by G. J. Haynes, Environmental Sciences Division (ESD) as an indicator of both algal and heterotrophic biomass. Periphyton samples were collected and analyzed to screen for metal contamination, to identify sources of contaminant entry to EFPC, stresses on the periphyton community, and the potential for food chain transfer of contaminants via the periphyton.

3.8.1 Methods

3.8.1.1 Physical characteristics of the sites

The periphyton monitoring sites (Fig. 3-8) largely coincided with fish and invertebrate sampling stations (see Fig. 2-1). The sites were characterized during September 1987 (when leaves were present on the riparian vegetation), and during March 1988 (when the riparian vegetation was leafless). On each date stream width was measured at 8–12 randomly chosen points. Stream depth and water velocity at 75% of the depth were measured at a randomly chosen point along each stream cross section. The composition of the substratum was visually estimated as percent cobble (> 10-cm upper surface diameter), gravel (> 1- and ≤ 10-cm diameter), pebbles (> 2- to ≤ 10-mm diameter), sand (0.1- to 2-mm diameter) and silt (< 0.1-mm diameter), within a 0.1 m² area centered on the point used for the determination of depth and velocity. The percentage of the area deemed colonizable by the periphyton was the sum of area covered by gravel and cobbles that was at less than 30-cm depth. The extent of the riparian canopy over each transect point was estimated using a convex mirror with a grid etched on the surface. Cover was estimated as the percentage of grid points intersected by riparian vegetation when looking into the mirror. The percentage of full sunlight reaching the water surface was measured

at each point, compared with a reading in the open, using a Li-Cor quantum meter and quantum sensor calibrated for photosynthetically active radiation (PAR) as $\mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. On August 23, 1988, 3- to 5-ozalid light meters (Friend 1961) were placed in the center of the channel in riffle areas at each site to provide a daily integrated measure of light reaching the stream.

3.8.1.2 Periphyton characterization

Three or four small rocks were collected from riffles in each study area on four dates. The rocks were taken to the laboratory where the periphyton was removed by brushing with a tooth brush. A subsample of the periphyton removed was filtered (Whatman GF-F, glass fiber filter), and then the filter was divided for determination of chlorophyll *a* (Chl *a*), ATP, dry weight (48 h at 70°C), and ash free dry matter (after dry ashing 3 h at 500°C). Methods for Chl *a* and ATP are presented in the following section. Data were expressed per unit rock upper surface area (see also following). A subsample of the periphyton was preserved in Lugol's solution for later determination of algal taxonomic composition.

3.8.1.3 Periphyton chlorophyll and carbon incorporation

To determine algal periphyton biomass and production, four small (10 to 60 cm²) relatively flat rocks were collected from shallow (< 25 cm deep) riffle areas, once a month at each site. The rocks were taken to the laboratory in water from the collection site. In the laboratory, rocks from each site were incubated for 2-h in water from the collection site containing 10 $\mu\text{Ci NaH}^{14}\text{CO}_3$. During the incubation, the water temperature was maintained within 2°C of ambient stream temperature. Approximately 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR (~16% full sun) was provided by a 1000-W metal halide lamp, and the water in the incubation chambers was circulated by submersible pumps to simulate natural conditions. After incubation the rocks were rinsed twice in distilled water to remove residual inorganic ¹⁴C. They were then placed in 30 mL of dimethylsulfoxide (DMSO) and kept in darkness for 24 h to extract soluble organic compounds and chlorophyll (Shoaf and Lium 1976, Palumbo et al. 1987). Five milliliters of extract was diluted 1:1 with 90% acetone and the Chl *a* content was determined spectrophotometrically using the equations of Jeffrey and Humphrey (1975). Corrections were made for phaeopigments (Strickland and Parsons 1972). A 500- μL aliquot of the extract was added to 10 mL of Aquasol (scintillation cocktail), and the ¹⁴C in the aliquot was determined by liquid scintillation spectrometry.

The surface area of each rock was determined by covering the upper surface with aluminum foil, determining the weight of the foil, and converting to surface area based on a known weight per unit area of foil. This procedure was repeated twice for each rock. Chlorophyll *a*, the rate of carbon incorporation, and ATP were then expressed on a surface area basis.

3.8.1.4 Periphyton ATP

ATP was determined by assaying an aliquot of the DMSO extract (Palumbo et al. 1987) using Lumac reagents and a Lumac Biocounter integrating photometer. Phosphate was added to the DMSO to about 20 mM prior to extraction to prevent loss of ATP to phosphatase activity (Palumbo et al. 1987).

3.8.1.5 Algal bioassay of water quality

To evaluate the influence of water quality at each of the study sites on the growth and photosynthesis of the algal periphyton, we performed short-term laboratory bioassays (Giddings et al. 1983) using the green alga *Haematococcus*. In these studies the photosynthetic rates of *Haematococcus* held in water from the field sites provided a relative measure of the potential influence of water quality on photosynthesis by the periphyton at the field sites. For these assays, water was collected from each of nine sites on EFPC that are used for instream toxicity testing (Loar et al. 1992b and Sect. 3.3.1 of this report) and from Brushy Fork. Bioassays were conducted on six dates (June 8, 16, 25, and July 8, 16, and 22). The water was taken to the laboratory, where an inoculum of *Haematococcus* was added to an aliquot of the water from each of the field sites and to dechlorinated tap water as a control medium. Following a 2-h preincubation, the cells in each aliquot were concentrated by centrifugation, and a 500- μ L inoculum from this concentrated solution was introduced to a fresh 4.5-mL aliquot of water from the same site in 20-mL glass scintillation vials. A solution of $\text{NaH}^{14}\text{CO}_3$ was added to each aliquot and then incubated on a gently rotating shaker table for 2-h at 25°C and about 150- μ mol PAR. At the end of the incubation period, 30 μ L of a 1N HCl solution was added to each vial to stop carbon uptake and to lower the pH to about pH 4. The solutions were then purged for 20 min with air to remove inorganic ^{14}C . Fifteen mL of Aquasol liquid scintillation cocktail was then added to each vial, and the ^{14}C content (i.e., carbon fixed by the algae) was determined by liquid scintillation counting. The rate of photosynthesis was determined based on the fraction of the added carbon that was incorporated into the cells and on the inorganic carbon content of the water from each site (determined from pH, alkalinity, conductivity, and temperature).

Data were corrected for background uptake by photoautotrophs in the water sample and nonphotosynthetic carbon incorporation. Background uptake in the water sample was determined by adding the ^{14}C solution and incubating the water sample without adding the *Haematococcus* inoculum. Nonphotosynthetic carbon uptake was determined by adding DCMU (an inhibitor of photosynthesis) to the *Haematococcus* in one of the scintillation vials from each site prior to the addition of the ^{14}C solution. For each site three replicate samples plus one background and one control for non-photosynthetic uptake were run for each date. The carbon uptake determined for the background and control vials was subtracted from each of the test vials; this was typically less than 5% of the carbon incorporation by *Haematococcus* in the test vials.

3.8.1.6 Periphyton colonization and development study

Ceramic tiles attached to bricks were placed at several sites to determine the rate of periphyton accumulation (colonization and growth). Four bricks with 24 5.3-cm² unglazed ceramic tiles were placed at EFK 24.4, EFK 23.2, EFK 13.8, EFK 6.3, and BFK 7.6, on June 22, 1988. Beginning three weeks after placement, and continuing weekly thereafter for six weeks, one tile was randomly selected at each site and placed in DMSO to extract Chl *a* to provide an estimate of algal biomass (as described for the monthly sampling program). On each date a fifth tile was selected from each site; the periphyton was brushed from the surface and preserved in Lugol's solution for the later examination of algal taxonomic composition.

3.8.1.7 Contaminant transfer

During February and May of 1987, periphyton was brushed from three rocks collected from each site, the samples were oven dried at about 65°C for 24 h and then analyzed for metals by inductively coupled plasma spectroscopy (ICP). The periphyton samples collected on February 10, were also analyzed for total mercury, via cold vapor atomic absorption. Standard reference material (NBS SRM-1645, River Sediment) was submitted along with the periphyton samples for ICP and mercury analyses as part of our QA/QC program.

3.8.2 Results and Discussion

3.8.2.1 Site characteristics

Stream width and average water depth increased as current velocity decreased with distance downstream in EFPC (Table 3-19); however, at all sites the periphyton were collected from riffles of similar depth (10 to 30 cm) and velocity (0.2 to 0.4 m/s). Silt contributes an increasing percentage to substratum texture with distance downstream (Table 3-19). At EFK 6.3, silt is an important site characteristic for the periphyton. Silt deposition may cover the periphyton community and form an unstable substratum for periphyton development that is easily removed during high discharge events. No riparian canopy (overhanging vegetation) was present at EFK 24.4 or EFK 13.8, therefore these sites receive full sunlight during midday. High banks at EFK 24.4 resulted in some shading at low sun angles and reduced the total daily PAR compared with EFK 13.8 (Table 3-19). EFK 23.2 had some overhanging riparian vegetation (about 60% canopy cover) which resulted in only about 30 to 60% of full sunlight reaching the water surface. EFK 10.6 and EFK 6.3 had many large trees shading the stream so that less than 20 % of full sun reached the stream surface during summer, and only about 50 to 60% of full sun reached the stream surface when the trees were leafless. These sites received less than 5% of total daily irradiance, when measured on a sunny day in August 1988 (Table 3-19). The reference site on Brushy Fork (the original site characterized on 9/25/87 and the new site characterized on 3/7/88) was similar to EFK 10.6 and EFK 6.3 for most physical characteristics (Table 3-19).

Table 3-19. Characteristics of stream reach sites for periphyton monitoring

Standard deviations shown in parentheses

	EFK 24.4		EFK 23.2		EFK 13.8		EFK 10.6		EFK 6.3		BFK 7.6		n
	10-16-87	3-17-88	9-21-87	3-17-88	9-28-87	3-21-88	9-28-87	3-21-88	9-28-87	4-11-88	9-25-87	3-7-88	
Width (m)	4.4 (0.2)	-	5.5 (1.1)	5.8 (1.2)	6.9 (0.9)	7.1 (1.4)	7.5 (1.4)	7.7 (1.0)	8.8 (1.3)	9.6 (1.2)	7.0 (1.3)	6.7 (1.5)	8-12
Depth (cm)	19 (6)	-	19 (9)	20 (11)	36 (23)	37 (18)	37 (18)	41 (28)	45 (23)	90 (6)	12 (5)	31 (12)	15-18
Current (cm/s)	43 (11)	-	32 (26)	33 (18)	24 (28)	30 (34)	39 (32)	43 (29)	12 (11)	20 (16)	17 (16)	25 (16)	16-18
Substratum (%)													
Silt	0	-	8	-	3	-	4	-	16	-	15	-	16-18
Sand	24	-	11	-	12	-	37	-	31	-	17	-	16-18
Pebbles	47	-	44	-	28	-	32	-	28	-	47	-	16-18
Gravel	21	-	6	-	15	-	23	-	18	-	10	-	16-18
Cobbles	6	-	31	-	42	-	4	-	7	-	9	-	16-18
Colonizable area (%)	28 (17)	-	38 (27)	-	53 (36)	-	25 (29)	-	49 (24)	-	25 (19)	-	16-18
Canopy (%)	0 (0)	-	54 (33)	(66) (19)	0 (0)	0 (0)	98 (3)	81 (14)	95 (10)	90 (5)	93 (3)	87 (5)	8
Light (%)	100 (0)	-	62 (38)	28 (24)	100 (0)	100 (0)	7 (5)	61 (21)	16 (14)	53 (30)	7 (4)	55 (22)	15-7
PAR ^a (mol·m ⁻² ·d ⁻¹)	22.3		11.6		31		1.2		1.3		1.2		3-5

^aDaily total of photosynthetically active radiation, measured on 8/23/88.

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

EFPC water was rich in the plant nutrients nitrogen and phosphorus (see Sect. 2.4.2). The Y-12 Plant was a source of soluble nitrogen (NO_3^- and NH_4^+) and SRP. Additional nutrient loading to EFK 13.8 resulted from livestock pastures adjacent to the stream. The ORWTF contributed both N and P at a discharge point above EFK 10.6. Concentrations of soluble nutrients in BF were much lower than those found in EFPC (see Sect. 2.4); however, BF was still relatively rich in nutrients compared with local streams in undeveloped areas. The concentrations of available nutrients were enough at all sites, with the possible exception of BF, so that the observed differences in nutrient concentrations should have had little influence on algal growth, biomass, or photosynthetic rates.

3.8.2.2 Periphyton characterization

Periphyton Chl *a* and ATP (per unit substratum area) that were brushed off rock surfaces tended to be higher at upstream sites than downstream sites (Table 3-20). The DMSO extraction procedure is more effective than brushing for removing ATP and chlorophyll from the rock surfaces when little periphyton is present and organisms are closely appressed to the rock surfaces. Data for Chl *a* and ATP obtained by extraction using DMSO will be used to compare the periphyton communities at the study sites. The data for Chl *a* and ATP obtained by brushing are used here for comparison with other parameters to characterize the periphyton.

Periphyton dry weight was variable at all sites, but tended to be lowest at EFK 10.6, except for the February 1988, when there was a substantial growth of blue-green cyanobacteria. The ash-free dry mass (AFDM) was typically highest for the upstream sites, EFK 24.4 and EFK 23.2, and generally behaved similarly to the other measures of biological material (Chl *a* and ATP). The fraction of dry weight attributable to organic matter (percentage organic) was high at the upstream sites and low downstream, reflecting increased siltation and increased diatom abundance. (Diatom AFDM is typically 25 to 60% Si, and silt deposition decreases the percentage of periphyton weight attributable to organic constituents.) In streams, organic matter usually accounts for 30 to 50% of periphyton weight. The high percentage of organic content of the periphyton at EFK 24.4 (60 to 81%) likely reflects a low siltation and rapid algal growth.

The autotrophic index (AFDM/Chl *a*) may provide a relative indication of the allochthonous input to the various stream reaches, where ratios greater than 100 indicate proportionally high microbial biomass to the periphyton as the result of organic loading (Weber 1973). Because organic loading is likely high at both EFK 24.4 and EFK 10.6, the low AFDM/Chl *a* at those sites may reflect low microbial biomass. At BFK 7.6 the ratio is higher, and organic loading and the contribution of heterotrophic biomass to the periphyton may be relatively high. The relative contribution of heterotrophic microbes to the periphyton can also be evaluated by the trophic index (Chl *a*/ATP), which will be discussed in Sect. 3.8.2.5 (Periphyton ATP).

3.8.2.3 Algal periphyton taxonomic composition

The algal assemblages found at the various sites during February and June 1988 are listed in Table 3-21. The algal periphyton at EFK 24.4 consisted almost exclusively of

Table 3-20. Periphyton characteristics based on samples collected by brushing the periphyton off of the surfaces of flat rocks in East Fork Poplar Creek and Brushy Fork

Site	Date	Chl <i>a</i> ($\mu\text{g}/\text{cm}^2$)	ATP ($\mu\text{g}/\text{cm}^2$)	Dry wt. (mg/cm^2)	AFDM ^a (mg/cm^2)	% Organic ^b	AFDM/Chl <i>a</i>	<i>n</i>
EFK 24.4	8/25/88	59	9.58	6.07	3.61	63	61	3
	9/24/88	24	36.6	3.78	2.28	60	95	3
	2/19/88	54	5.25	3.11	2.22	70	41	4
	6/2/88	16.6	3.40	1.86	1.50	81	90	3
EFK 23.3	8/25/88	15	2.382	4.87	3.30	68	220	
	9/24/88	30	24.024	4.00	2.21	55	74	
	2/19/88	32	3.604	3.45	1.86	55	58	
	6/12/88	13.7	4.832	2.04	1.18	58	86	
EFK 13.8	8/25/88	3.3	0.245	1.72	0.35	27	106	
	9/24/88	7.9	10.538	3.88	1.49	39	189	
	2/19/88	29.7	2.624	8.91	2.32	26	78	
	6/2/88	4.6	1.918	1.94	0.70	37	152	
EFK 10.6	8/25/88	3.6	0.092	0.80	0.33	40	92	
	9/24/88	11.5	1.735	1.92	0.50	26	43	
	2/19/88	47.2	5.729	4.63	2.45	55	52	
	6/2/88	7.1	3.053	1.85	0.67	37	94	
EFK 6.3	8/25/88	10.8	1.755	13.7	1.34	13	124	
	9/24/88	3.4	1.557	4.56	0.47	11	138	
	2/19/88	14.4	1.933	3.31	1.03	32	72	
	6/2/88	5.2	0.61	2.34	0.59	25	113	
BF	8/25/88	1.5	0.114	0.74	0.23	30	67	
	9/24/88	-	-	-	-	-	-	
	2/19/88	15.0	23.2	2.34	1.53	60	102	
	6/2/88	3.7	1.14	2.15	0.50	23	135	

^aAFDW = Ash free dry weight.

^b% Organic = percent of dry weight lost upon ignition.

Note: EFK = East Fork Poplar Creek kilometer; BF = Brushy Fork.

Table 3-21. Periphyton algal assemblages by site and date

	Date	
	June 2, 1988	Feb. 18, 1988
	Site ^a	
	EFK 24.4	
Green unicells (<5 um) A few <i>Stigeoclonium</i> filaments		Green unicells
	EFK 23.2	
Green unicells (<5 um) Filamentous cyanophyte <i>Stigeoclonium</i> filaments Diatoms (<i>Achnanthes</i> , <i>Navivula</i>)		Cyanophyte filament (<i>Oscillatoria</i>) Prostrate filament (<i>Stigeoclonium</i>) Diatoms (e.g., <i>Achnanthes</i>)
	EFK 13.8	
<i>Cladophora</i> filaments Filamentous green (<i>Stigeoclonium</i> ?) Large diatoms (e.g., <i>Navicula</i>) Cyanophyte filaments		Mostly diatoms (<i>Rhoicosphenia</i> , <i>Gomphonema</i> , <i>Diatoma</i> , <i>Navicula</i> , <i>Nitzschia</i>)
	EFK 10.6	
Green unicells Cyanophyte colonies <i>Stigeoclonium</i> Small diatoms (e.g., <i>Rhoicosphenia</i>)		Mostly diatoms (e.g., <i>Gomphonema</i> , <i>Rhoicosphenia</i>) Cyanophyte colonies Some <i>Stigeoclonium</i>
	EFK 6.3	
Large diatoms (<i>Navicula</i>)		Diatoms (<i>Navicula</i> , <i>Surirella</i> , <i>Cocconeis</i> , <i>Nitzschia</i>) Filaments (<i>Cladophora</i> , <i>Spirogyra</i> , <i>Oscillatoria</i>)
	BFK 7.6	
Prostrate green (<i>Stigeoclonium</i> ?) Diatoms (<i>Rhoicosphenia</i>) Filamentous cyanophyte		Large <i>Batrachospermum</i> Diatoms (<i>Rhoicosphenia</i> , <i>Gomphonemia</i> , <i>Surirella</i> , <i>Navicula</i> , <i>Nitzschia</i> , and <i>Melosira</i>) Some <i>Stigeoclonium</i>

^aEFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

green unicells that were patchily distributed on the rocks. At EFK 23.2 the algal assemblage was more diverse than at EFK 24.4, consisting of filamentous chlorophytes, filamentous cyanophytes, and diatoms in addition to the green unicells found at EFK 24.4. This assemblage was similar to those found at nutrient-enriched, organically enriched sites in other local systems (Boston et al., unpublished data). The algal assemblages at EFK 13.8 were dominated by short, rough, green filaments during summer and diatoms during winter. At EFK 10.6, a diverse community of green unicells, cyanophyte colonies, prostrate green filaments, and diatoms occurred during summer. During winter, diatoms and cyanophyte colonies dominated EFK 10.6. EFK 6.3 was dominated by large diatoms during both summer and winter. A cover of filaments (mostly chlorophytes) was common at EFK 6.3 following leaf-fall but before frequent winter storms began. BFK 7.6 was dominated by prostrate greens and small diatoms during summer. During winter, large clumps of *Batrachospermum* occurred along with diverse diatom assemblages.

3.8.2.4 Monthly determination of algal periphyton biomass and productivity

Periphyton Chl *a* per unit rock surface area provided a measure of algal biomass at each of the monitoring sites. At EFK 24.4 the periphyton distribution was very patchy, and it was not uncommon to find a single rock with a thick periphyton covering, while surrounding rocks were barren. To avoid excessive variance, and the analysis of many barren rocks, we chose rocks from among those with some visible periphyton cover. The data for EFK 24.4 represent average biomass and production on colonized rocks, recognizing that as few as 1 percent of the rocks might have had periphyton on a given month. These data are useful for evaluating the physiological condition of the algal periphyton at EFK 24.4 but overestimate average biomass and production and underestimate variance.

Multiple comparisons of means indicated that mean annual Chl *a* for the EFPC sites generally decreased with distance downstream (Table 3-22). Mean annual Chl *a* at BFK 7.6 was similar to that at EFK 6.3 and was low compared with other EFPC sites. The algal periphyton biomass on rocks that were colonized at EFK 24.4 reached extremely high concentrations (up to 71 $\mu\text{g}/\text{cm}^2$ during March 1988). Chlorophyll *a* at the sites with little or no riparian vegetation extending over the stream (EFK 24.4, EFK 23.2, and EFK 13.8) was greater than at the sites with substantial canopy cover (EFK 10.6, EFK 6.3, and BFK), during both years 1 and 2 (Table 3-19 and 3-22), implicating light as a major controlling factor.

Average annual periphyton primary production on rocks from riffles at the EFPC sites, based on monthly measurements of short-term carbon uptake, was similar at EFK 24.4, EFK 23.2, EFK 13.8, and EFK 6.3 (Table 3-22). Average production at EFK 10.6 was lowest among the EFPC sites, and average annual production at BFK 7.6 was lower than all of the EFPC sites. In contrast to the data for algal biomass, the average production rate was not correlated with light (e.g., mean primary production at EFK 6.3, a shaded site, was similar to that at unshaded sites). During the summer when differences in incident light for shaded and unshaded sites are greatest, there was a good correlation between light and photosynthesis, as will be discussed below.

Monthly data for periphyton biomass (as Chl *a* per unit rock surface area) for each site, and each year, are presented in Figs. 3-9a-f. Monthly data for Chl *a* at EFK 24.4 behaved differently from other sites, and was highly variable (Fig. 3-9a). As noted above,

Table 3-22. Annual data for periphyton chlorophyll *a* and primary production (expressed in micrograms per square centimeter) for years one and two

Sites with same letter designation are not significantly different ($p < 0.05$) after analysis of variance; range is for the monthly mean values

Chlorophyll <i>a</i>	EFK 24.4 ($\mu\text{g}/\text{cm}^2$)	EFK 23.2 ($\mu\text{g}/\text{cm}^2$)	EFK 13.8 ($\mu\text{g}/\text{cm}^2$)	EFK 10.6 ($\mu\text{g}/\text{cm}^2$)	EFK 6.3 ($\mu\text{g}/\text{cm}^2$)	BFK 7.6 ($\mu\text{g}/\text{cm}^2$)	<i>n</i>
Mean	23.8	19.9	17.2	10.7	8.2	8.7	48
SE 2.6	2.0	1.6	1.8	0.8	1.0		
Range	5.4-65	7.0-39	8.2-37	0.11-40	2.7-19	3.1-28	
ANOVA	A	A	A	B	C	C	
Mean	30.9	19.5	19.7	17.4	10.9	10.1	48
SE 3.3	1.7	1.5	2.2	1.2	1.3		
Range	2.9-71	6.5-30	11-32	2.9-44	0.3-29	3.6-28	
ANOVA	A B	A B	A B	C B	C D	C D	
Production							
Year 1							
Mean	52.2	49.7	56.6	27.2	45.3	26.1	48
SE 5.0	3.5	3.5	3.0	3.2	2.4		
Range	20-124	26-83	37-91	0.3-57	22-101	26-83	
ANOVA	A B D	A B D	A B	C	A D	E	
Year 2							
Mean	61.4	54.0	61.3	37.5	52.1	27.6	48
SE 5.7	3.7	6.4	3.3	4.2	3.1		
Range	17-134	11-83	27-149	11-58	0.8-105	5.3-47	
ANOVA	A B D	A B D	A B	C	A D	E	

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

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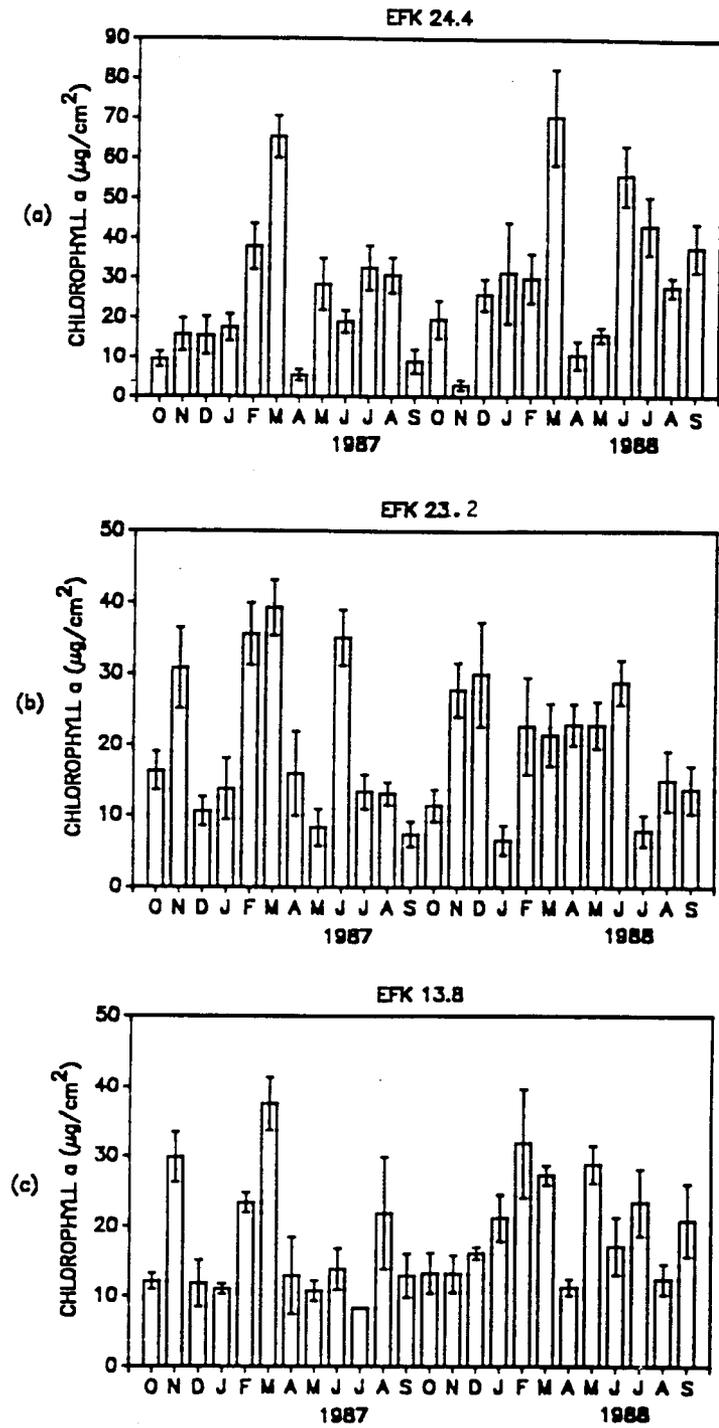


Fig. 3-9. Periphyton biomass (micrograms of chlorophyll *a* per square centimeter) on small flat rocks collected from shallow riffles. Values are the means and standard errors for four samples collected monthly from each site. EFK = East Fork Poplar Creek kilometer; BF = Brushy Fork.

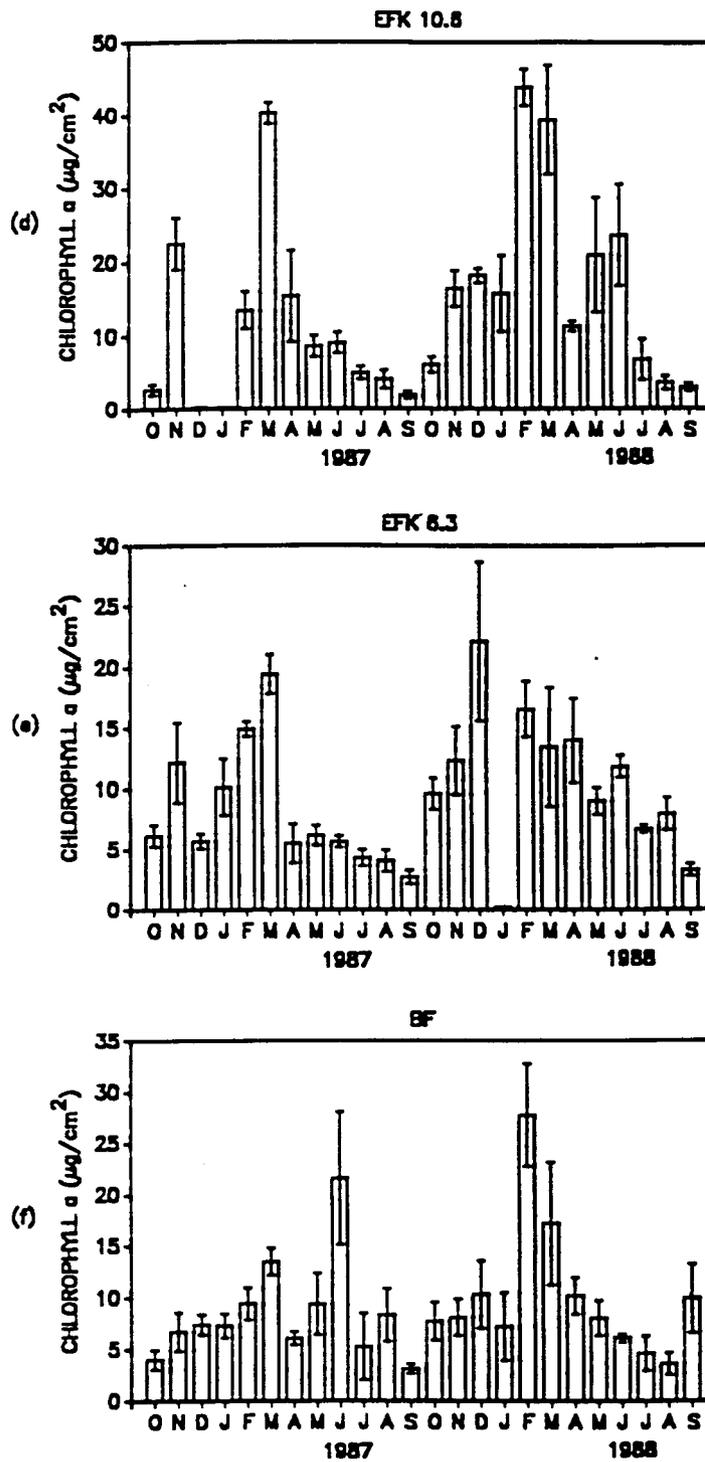


Fig. 3-9 (continued). Periphyton biomass (micrograms of chlorophyll *a* per square centimeter) on small flat rocks collected from shallow riffles. Values are the means and standard errors for four samples collected monthly from each site. EFK = East Fork Poplar Creek kilometer; BF = Brushy Fork.

rocks were not randomly collected from EFK 24.4, and these data overestimated average biomass and underestimated variability at that site. The patchy periphyton distribution and high month to month variability at EFK 24.4 are believed to result from intermittent chlorine toxicity, from the discharge of cooling water from the Y-12 Plant.

There were no distinct seasonal patterns in periphyton Chl *a* at EFK 23.2 and EFK 13.6 (Fig. 3-9b and c). Both of these sites receive fairly high light intensities year round. Sites EFK 10.6 and EFK 6.3, which are heavily shaded by riparian vegetation from April through September, tended to have the highest Chl *a* values during winter months when the periphyton received a larger fraction of full sunlight (Table 3-19 and Figs. 3-9d and e). Chl *a* tended to behave similarly month to month at these sites.

During December 1986 and January 1987, Chl *a* was greatly reduced at EFK 10.6 but was fairly normal for the season at sites upstream and further downstream. We found no evidence that natural factors (e.g., high numbers of grazers, excessive siltation, etc.) were responsible for the extremely low biomass and suspect that excess chlorine discharge from ORWTF may have been responsible. The low algal biomass at EFK 6.3 during January 1988 followed particularly intense scouring and siltation during storms the weeks prior to sampling.

With the exception of June 1987, algal biomass at BFK 7.6 tended to be higher during winter than during summer. At that site, and other sites with a riparian canopy, a greater fraction of full sunlight reached the stream during winter (Fig. 3-9f).

Production was related to algal biomass at each site; however, the relationship between biomass and production for each site was not constant seasonally and will be discussed further.

Because light does not change equally over the year for all sites (i.e., incident light varies more seasonally at sites where riparian vegetation shaded the channel), the data for algal periphyton biomass and production have been separated into the closed-canopy season (April through September), and open-canopy season (October through March), for year one and year two (Figs. 3-10 and 3-11). During summer, algal biomass was highest for the open or partly shaded sites, and primary production was related to algal biomass during both years (Figs. 3-10 and 3-11). Primary production was greater than expected at EFK 6.3, based on biomass, when compared with other sites (Figs. 3-10 and 3-11). [Note: the high biomass at EFK 24.4 also reflects the absence or low densities of invertebrate grazers (Sect. 6.1)]. During the winter, mean production and biomass were not closely related, and production was similar at all sites despite differences in biomass (Figs. 3-10 and 3-11). During the winters of both years, production was disproportionately high at EFK 13.8, EFK 6.3 and BFK 7.6, suggesting that differences in the physiological condition of the periphyton existed among the sites.

Evaluating the rate of photosynthesis per unit algal biomass provides a convenient means for comparing the rates of photosynthesis among sites where periphyton biomass differs. Chlorophyll (biomass) and the rate of carbon incorporation are positively, but not linearly, related. Self shading limits the light available for photosynthesis by the algal cells in the lower layers of the periphyton matrix, and thickening of the matrix slows rates of diffusion of inorganic carbon and nutrients to algal cells in lower layers. These limitations cause carbon incorporation to increase more slowly than does chlorophyll, as biomass accumulates and additional cells are added to the periphyton matrix. At some sites biomass accumulates to the point that photosynthetic carbon incorporation per unit bottom surface area no longer increases as additional biomass is added. In the resulting relationship, the chlorophyll-specific production (carbon uptake per unit chlorophyll per

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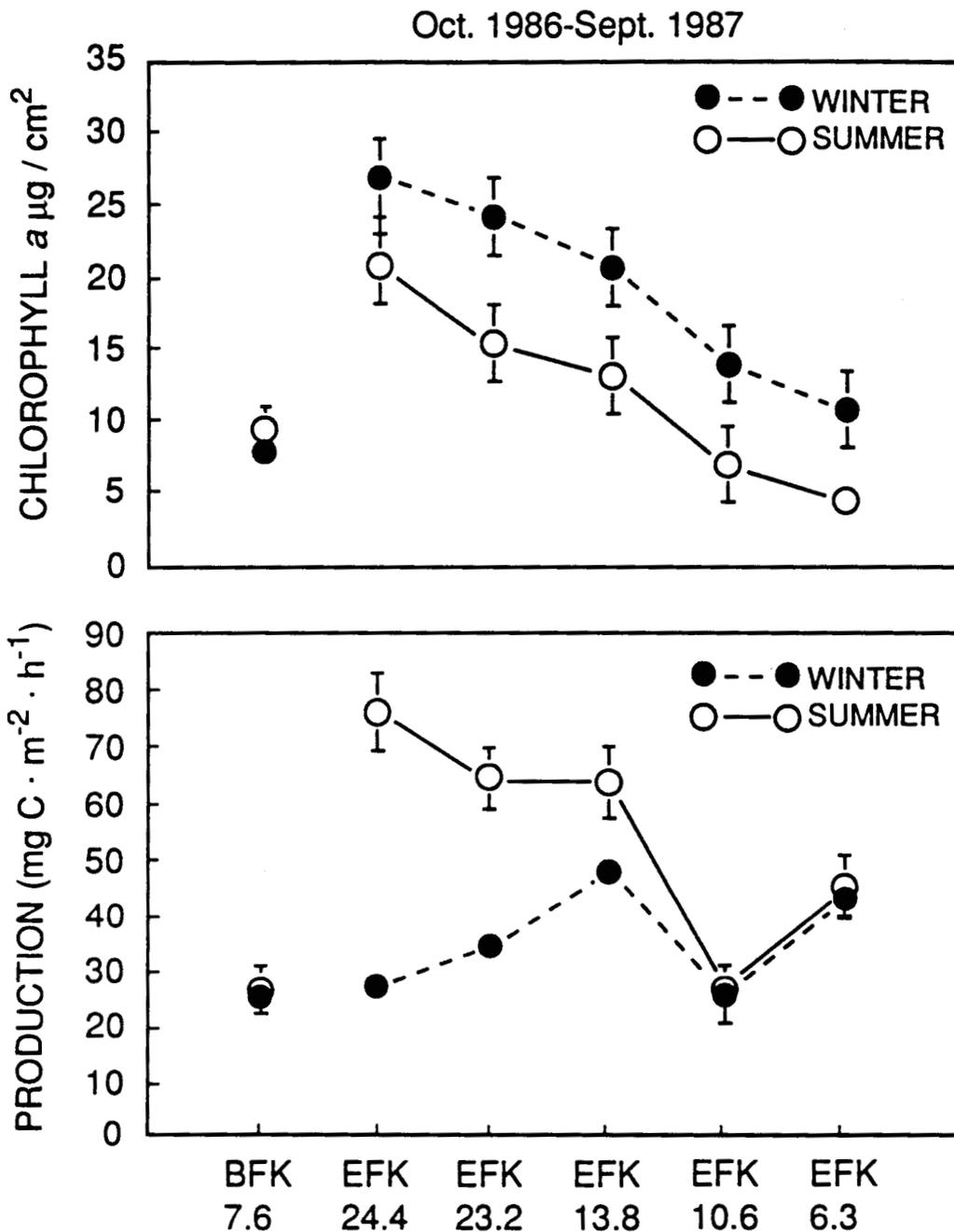


Fig. 3-10. Periphyton chlorophyll (in micrograms of chlorophyll *a* per square centimeter) and production (in milligrams of carbon per square meter per hour) on rocks collected from the study sites during winter (October through March) and summer (April through September) of year one. Values are the mean and standard error of six monthly mean values for each site. EFK = East Fork Poplar Creek kilometer; Brushy Fork kilometer.

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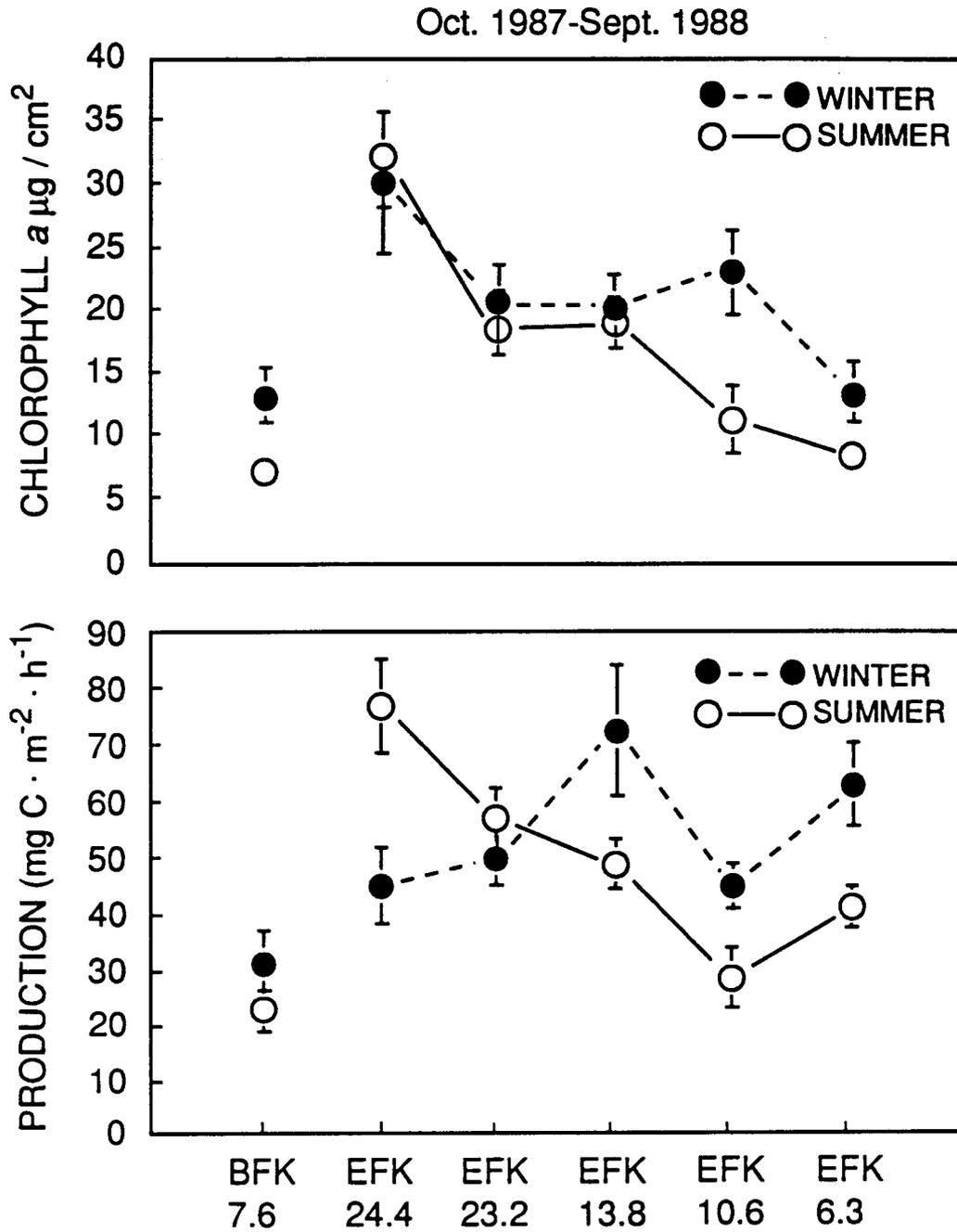


Fig. 3-11. Periphyton chlorophyll (in micrograms of chlorophyll *a* per square centimeter) and production (in milligrams of carbon per square meter per hour) on rocks collected from the study sites during winter (October through March) and summer (April through September) of year two. Values are the mean and standard error of six monthly mean values for each site. EFK = East Fork Poplar Creek kilometer; Brushy Fork kilometer.

unit time) decreases with increasing chlorophyll as soon as enough cells are present to influence the rate of resource delivery to the cells below them in the matrix.

Because the relationship between chlorophyll (biomass) and production is not linear, it is difficult to compare photosynthetic rates at sites where periphyton biomass differed substantially. To parcel out the effect that biomass has on decreasing biomass-specific production, we performed an analysis of covariance (ANCOVA) for photosynthesis at the different sites and months, using chlorophyll as the covariate. Photosynthesis and chlorophyll were log transformed to generate an approximately linear relationship between the two variables. The analysis model was: $\log \text{ photosynthesis} = \text{site} + \text{month} + \text{site} \times \text{month} + \log \text{ chlorophyll}$. All four factors were highly significant; chlorophyll effects were most important, followed by site, month, and the site-month interaction. The average annual predicted photosynthetic rates adjusted for Chl *a* (or chlorophyll-adjusted production) were highest at EFK 6.3 and EFK 13.8, lowest at EFK 10.6 and BFK 7.6, and intermediate at EFK 24.4 and EFK 23.2 for both years (Table 3-23).

Table 3-23. Analysis of covariance for periphyton primary production evaluated as a function of site and month, with chlorophyll *a* as a covariate using LS means for log-transformed data

Sites with the same letter are not different ($p < 0.05$)

Site	LS ^a mean	Year one	LS mean	Year two
EFK 24.4	3.53	A	3.52	A D
EFK 23.2	3.62	A	3.67	A B
EFK 13.8	3.82	B	3.75	B C
EFK 10.6	2.96	C	3.43	D E
EFK 6.3	3.85	B	3.88	C
BFK 7.6	3.21	D	3.29	E

^aLS = least square for log normal (ln) transformed data.

Note: $\ln \text{ Production} = \text{site} \times \text{month} \times (\text{site} \times \text{month}) + \ln \text{ Chl } a$.

Both natural and anthropogenic factors may influence the relationship between photosynthesis and biomass. The chlorophyll-adjusted production (based on the ANCOVA) provides an indication of the “condition” of the periphyton. A high chlorophyll-adjusted photosynthetic rate suggests good condition (a high biomass specific rate of growth) and reflects both inherent physiological potential and recent history. The chlorophyll-adjusted production should be greater for a site receiving high light and high nutrient concentrations compared with a site where resource availability was lower. Effects of anthropogenic stresses would additionally modify the chlorophyll-adjusted production at these sites.

Sites EFK 6.3 and EFK 13.8 had the highest average annual rates of chlorophyll-adjusted photosynthesis (Table 3-23). The periphyton at these sites differed in biomass, average primary production, and species composition. Although the water at both sites is nutrient rich, during the warm season EFK 13.8 receives full sun, while at EFK 6.3 less

than 5% of full sun reaches the water surface. The periphyton at BFK 7.6 and EFK 10.6 had the lowest average annual chlorophyll-adjusted rates of photosynthesis, although at these sites light was similar to EFK 6.3 and water nutrient concentrations at EFK 10.6 were similar to those at EFK 6.3. Therefore, based on these results and results of similar analyses of periphyton from other local streams, it appears that the ANCOVA results for the relationship between photosynthesis and biomass at the different sites were fairly independent of light, nutrients, and biomass. While these factors are surely important aspects of the "environment" for the periphyton, apparently other factors have a greater influence on the relationship between biomass and photosynthesis.

To further evaluate the relationship between biomass and photosynthesis, we plotted the monthly values for chlorophyll-adjusted photosynthesis to track the behavior of the periphyton at the various sites over time and to further compare chlorophyll-adjusted photosynthesis ("condition") among sites. In Figs. 3-12 and 3-13 the chlorophyll-adjusted production rates during the first and second years are plotted by month. In general the chlorophyll-adjusted photosynthetic rates for the periphyton from each site and the relative ranking of these were fairly constant over time. During year one the chlorophyll-adjusted photosynthetic rates for the periphyton from EFK 10.6 were low during December and January. As noted earlier, we suspect that this was the result of a chlorine discharge from ORWTF. The rates of chlorophyll-adjusted production at EFK 6.3 and BFK 7.6 both declined during January 1988 following a storm that removed much of the biomass. These sites are located downstream on EFPC and BF, respectively, and scouring during high discharge events can remove much of the active biomass and leave a community with chlorophyll-adjusted rates of photosynthesis.

There was no apparent seasonal change in chlorophyll-adjusted photosynthesis at EFK 10.6, EFK 6.3, and BFK 7.6 despite significant changes in light availability (Table 3-19) and temperature. At sites EFK 24.4 and EFK 23.2 a seasonal pattern was evident where chlorophyll-adjusted photosynthesis was generally high June through September and low October through March (Figs. 3-12 and 3-13). For periphyton from EFK 24.4, the rates of chlorophyll-adjusted photosynthesis during the summer were high compared with periphyton at other sites but were low compared with periphyton from other sites during winter.

For periphyton from EFK 24.4 we suspected that a lower-than-ambient temperature used in the carbon uptake bioassays conducted during winter could have been responsible for the relatively low chlorophyll-adjusted photosynthesis. The monthly carbon uptake measurements were conducted at the average temperature for the field sites at the time of collection, not including EFK 24.4, which was frequently warmer (up to 9°C) than the other sites due to the input of heated water from the Y-12 Plant. Therefore, while the assay temperature was always within 2°C of ambient for other sites, the assay temperature could be as much as 7°C lower than ambient, during winter (November through March), for EFK 24.4. During the remainder of the year, the chlorophyll-adjusted production at EFK 24.4 was fairly high compared with the other sites.

During February 1989, we measured biomass and photosynthesis of periphyton on about 40 rocks collected from EFK 24.4 that were incubated at ambient temperature. We found that photosynthesis was lower than expected based upon the biomass present. Those data suggested that some factor other than temperature was likely responsible for the poor "condition" of the algal periphyton at that site during winter.

The periphyton at EFK 23.2 had a similar monthly pattern of chlorophyll-adjusted production as the periphyton at EFK 24.4; however, the temperature at EFK 23.2 was not

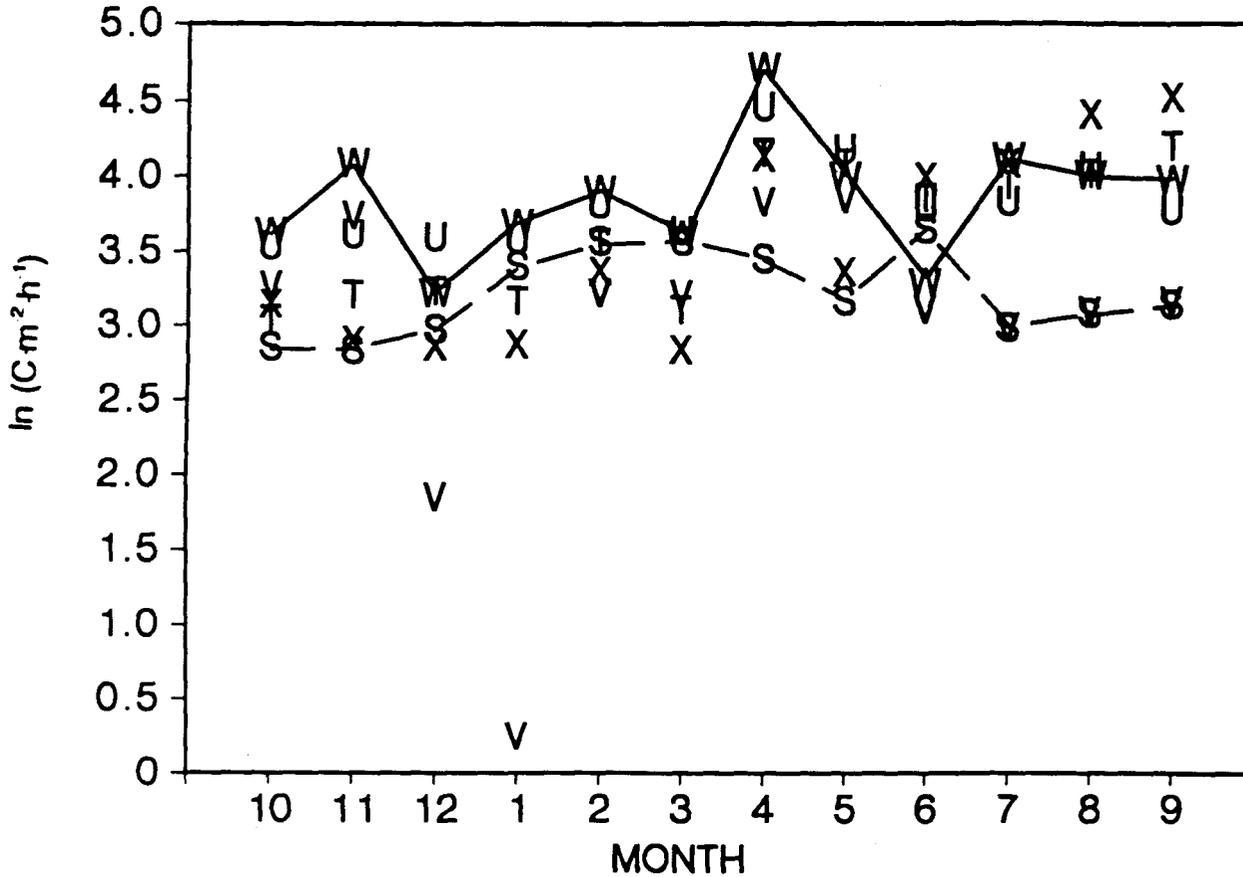


Fig. 3-12. Chlorophyll-adjusted photosynthesis [ln (carbon uptake) expressed as milligrams of carbon per square meter per hour] for year one (October 1986 through September 1987). Monthly values based on ANCOVA for log production with log chlorophyll *a* as the covariate. S = BFK 7.6, T = EFK 23.2, U = EFK 13.8, V = EFK 10.6, W = EFK 6.3, and X = EFK 24.4. The values for EFK 6.3 and BFK 7.6 are connected to track month changes. ANCOVA = analysis of covariance; BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

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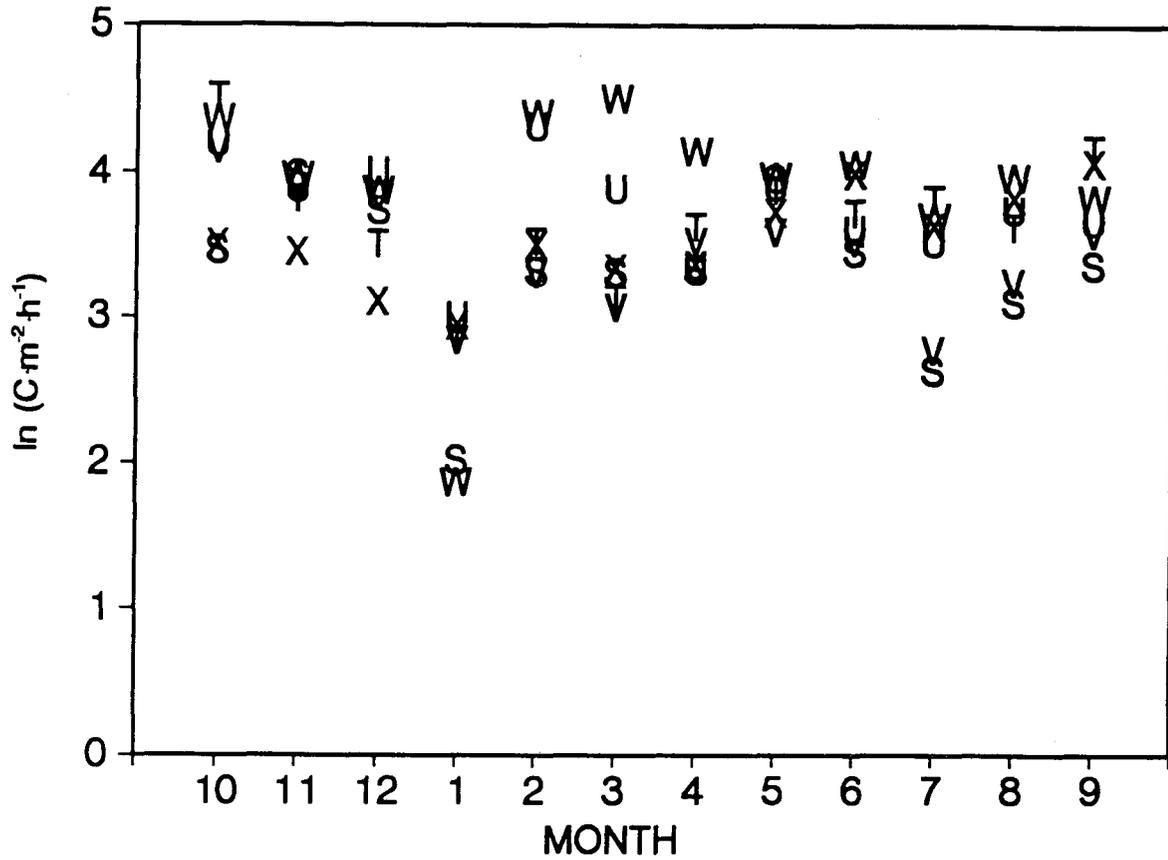


Fig. 3-13. Chlorophyll-adjusted photosynthesis [ln (carbon uptake) expressed as milligrams of carbon per square meter per hour] for year two (October 1987 through September 1988). Monthly values based on ANCOVA for log production with log chlorophyll *a* as the covariate. S = BFK 7.6, T = EFK 23.2, U = EFK 13.8, V = EFK 10.6, W = EFK 6.3, and X = EFK 24.4. ANCOVA = analysis of covariance; BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

substantially different than the temperatures during carbon uptake measurement. For periphyton from both EFK 24.4 and EFK 23.2, the chlorophyll-adjusted photosynthetic rates were lower during winter than summer. Apparently at these sites conditions for algal growth were less favorable during winter. The limited taxonomic diversity and the patchy distribution of periphyton at EFK 24.4 indicated anthropogenic stress; however, at least during the summer, the periphyton present was in fairly good physiological condition.

Although available light and nutrient concentrations in the water were low at BFK 7.6 compared with the other sites, the relatively low rates of chlorophyll-adjusted photosynthesis at BFK 7.6 more likely resulted from the influence of intense grazing pressure on the algae by snails. Periphyton communities in other reference streams (Boston et al. unpublished data) had similarly low rates of chlorophyll-adjusted photosynthesis when grazing pressure by snails kept algal biomass low. It may be that the high grazing pressure at BFK 7.6 (and some other sites) resulted in selection of an alga (*Stigeoclonium*) that has inherently low biomass specific production.

These results were largely as expected based on resource availability and evidence of toxicity; however, they do illustrate that low chlorophyll-adjusted production (poor physiological condition) may result from natural factors (e.g., grazing and limiting nutrients) as well as from toxic stress (e.g., chlorine). The generally poor condition of the algae at EFK 10.6, relative to the algae at EFK 6.3, was not expected given the similarity in environmental conditions. This reinforced earlier speculation that anthropogenic stresses may originate from ORWTF.

The ANCOVA for the photosynthesis-biomass relationship seemed useful for monitoring the periphyton communities at the sites through time, and for comparing photosynthetic rates among periphyton communities that differed in biomass. It is interesting to note that the periphyton at sites nearest the Y-12 discharge had intermediate chlorophyll-adjusted rates of photosynthesis, and on average were in better condition than periphyton from BFK 7.6 (the low nutrient reference site where grazing pressure is high). Two important qualifiers in interpreting these results are first, the analysis is comparative (i.e., the ranking of sites is relative to other sites included in the analysis), and second, both natural and anthropogenic factors influence chlorophyll-adjusted rates of photosynthesis.

3.8.2.5 Periphyton ATP

ATP is present in all living cells. The ratio of Chl *a* to ATP has been used to evaluate the physiological condition of algal periphyton (Bothwell 1985) and can provide a relative measure of microbial heterotrophic activity when significant organic enrichment is present. Periphyton ATP was measured nine times between November 1986 and September 1988 (Table 3-24). The only significant difference among the sites was for the sites with the highest and lowest average ATP, EFK 13.8 and EFK 10.6 respectively. ATP values tended to be related to data for Chl *a* (Table 3-22).

Data for ATP were also collected on four dates by brushing the periphyton from the rocks (Table 3-20). With the exception of some high ATP values for upstream sites on September 24, 1987, the data for the brushing and the direct extraction were similar. Because both the algal and heterotrophic components of the periphyton contain ATP, non-complementary changes in either component can confound interpretation of the data for ATP among sites. However, the data for ATP from September 24, 1987 (Table 3-20)

Table 3-24. Periphyton ATP content and the Chl *a*:ATP ratio (wt:wt) at points along East Fork Poplar Creek and on Brushy Fork

n = 4 for each date

Date	ATP ($\mu\text{g}/\text{cm}^2$)					
	EFK 24.4	EFK 23.2	EFK 13.8	EFK 10.6	EFK 6.3	BFK 7.6
Nov. 1986	1.07	0.74	0.82	0.31	0.82	0.56
Jan. 1987	0.32	1.66	0.88	0.01	0.30	0.58
Apr. 1987	5.06	5.35	17.8	1.78	7.06	1.06
Jul. 1987	8.93	5.04	4.24	3.80	1.97	0.92
Oct. 1987	6.65	4.83	7.92	3.13	3.97	2.95
Nov. 1987	2.92	2.32	2.68	2.20	6.10	1.82
Feb. 1988	3.76	1.33	5.48	1.48	5.04	7.86
May 1988	1.39	1.50	1.06	1.52	0.90	0.21
Sept. 1988	1.47	0.95	0.99	0.69	0.67	0.39
	Chl <i>a</i> /ATP					
Nov. 1986	17.3	56	39	82	14.7	12.1
Jan. 1987	63	97	14.9	7.6	37.9	12.7
Apr. 1987	1.1	4.3	1.4	11.4	0.7	5.9
Jul. 1987	3.7	3.9	2.1	2.5	2.4	5.0
Oct. 1987	2.8	2.4	2.1	1.9	2.6	3.33
Nov. 1987	0.9	7.5	3.5	8.3	2.1	3.06
Feb. 1988	7.5	15.4	6.8	31.6	4.9	3.80
May 1988	14	26	32	15	14	37
Sept. 1988	28	13.5	23	10	5.0	23

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

show high ATP upstream (EFK 24.4) and decreasing concentrations moving downstream. This pattern was not related to the change in algal biomass and suggests a microbial response to an organic release from Y-12. The general pattern of higher dissolved organic carbon upstream in EFPC is discussed in Sect. 2.4.

In other local streams seasonal patterns in periphyton ATP are found that are similar to algal biomass and/or the input of allochthonous organic material (Loar et al. 1992c). However, no consistent seasonal patterns were apparent for the EFPC sites or the BF reference site.

The Chl *a*:ATP ratio (wt:wt) is typically on the order of 3:1 to 10:1 in algal cultures. The ratio varies with nitrogen or phosphorus availability, becoming larger with increasing phosphorus limitation and smaller under nitrogen deficiency. Under field conditions, ratios much larger than 10:1 usually indicate the presence of detrital chlorophyll (Healey and Hendzel 1980). Ratios much lower than 3:1 suggested significant organic enrichment

and substantial microbial populations. Data for the periphyton Chl *a*:ATP ratio for the study sites varied seasonally for all sites (Table 3-24). The generally low values for the EFPC sites suggest substantial organic loading and microbial activity at all sites. Because all of the EFPC sites have high concentrations of available phosphorus, the occasionally high Chl:ATP ratio should not be a result of P deficiency. The high Chl:ATP ratio may reflect unfavorable conditions under which ATP declines more rapidly than Chl *a*, resulting in a high Chl:ATP ratio. (Compare data for ATP and Chl:ATP in Table 3-24.) Although BFK 7.6 undoubtedly receives substantial organic loading from the adjacent forest and cow pastures, the Chl:ATP ratio generally remained in a moderate range (3 to 5). The causes for the occasionally high ratios are unknown. Because ATP and the Chl:ATP ratio are influenced by a number of factors, the high degree of spatial and temporal variability encountered makes it impossible for us to draw conclusions concerning microbial activity and the condition of the algal periphyton.

3.8.2.6 Algal bioassay of water quality

The rates of carbon incorporation by the laboratory alga incubated in the water from the various sites were inconsistent among dates. ANOVA showed significant differences in the carbon uptake among sites (Table 3-25). *Haematococcus* generally showed the highest carbon uptake rates in water from EFK 10.9 and the lowest carbon uptake rates in water from EFK 20.5, EFK 18.2, and EFK 13.8. Water collected from the reference site produced intermediate responses from the alga.

There seemed to be little correlation between the photosynthetic rates of the laboratory alga in water collected from specific areas of EFPC and the chlorophyll-adjusted production or the condition of the periphyton found near those areas. [E.g., periphyton were in good condition near EFK 13.8 but *Haematococcus* had low photosynthetic rates in water collected from near that site.] Although differences in the photosynthetic rates of *Haematococcus* in water from the various sites were significant, the magnitude of the differences were not large (Table 3-25). Large differences were found in the photosynthetic rates in water collected from sites in nearby WOC (Loar et al. 1992c). However, in EFPC we were unable to identify any site specific (water chemical) factors that could account for the differences in the photosynthetic rates of *Haematococcus* in water from the different sites.

There were significant differences in the average photosynthetic rates for water from all sites among dates (Table 3-25). Although we have no explanation for the observed differences among dates, it is interesting to note that the lowest average photosynthetic rates occurred on June 8, which corresponded to the time of a fish kill below NHP. On June 8, photosynthetic rates in water collected from EFK 22.8 were the lowest among the sites. On average the photosynthetic rates in water from EFK 22.8 were good (Table 3-25). Therefore, we suspect a relation between the fish kill and the poor quality of the water for algal carbon uptake.

These results suggest that the observed differences in the condition of the periphyton at the study sites may (1) not result from differences in ambient water quality (with the exception of chlorine toxicity at EFK 24.4), (2) result from physical or biotic factors not included in these short-term carbon uptake studies, (3) result from the cumulative effects of some factor, or (4) result from intermittent stresses not encountered during these studies. Intermittent exposure to chlorine for short periods (hours or less) can have

effects that persist for weeks, and we suspect that this accounts for the composition and variability of the periphyton at EFK 24.4.

Table 3-25. Carbon uptake rates for laboratory grown alga in water from nine sites on EFPC and the Brushy Fork reference site, measured on six dates

Site number	Mean adjusted carbon uptake (mg C·m ⁻² ·h ⁻¹)	Duncan grouping
7 (EFK 10.9)	1.30	A
5 (EFK 16.1)	1.24	A B
8 (EFK 7.6)	1.16	A B C
1 (EFK 22.8)	1.13	A B C
9 (EFK 5.1)	1.11	B C
2 (EFK 21.9)	1.11	B C
10 (BFK 7.6)	1.07	B C
6 (EFK 13.8)	1.04	C
4 (EFK 18.2)	1.03	C
3 (EFK 20.5)	1.01	C

Week number	Mean adjusted carbon uptake	Duncan grouping
2	1.37	A
3	1.28	A B
6	1.20	B C
5	1.11	C D
1	1.03	D
4	0.74	E

Note: Data for average carbon uptake for each site measured on 6 dates ($n = 18$, 3 replicates on 6 dates), and data for average carbon uptake on each of 6 dates for all 10 sites ($n = 30$, 3 replicates for 10 sites) are listed based on an analysis of variance. Mean values with the same letter were not significantly different ($p < 0.05$) based on Duncan's multiple range test. (Carbon uptake data have been adjusted relative to uptake in dechlorinated tap water.) EFK = East Fork Poplar Creek kilometer; BFK = Brush Fork kilometer; EFPC = East Fork Poplar Creek.

3.8.2.7 Periphyton colonization

Ceramic tiles were first retrieved three weeks after deployment (Fig. 3-14). Substantial biomass (as Chl *a*) was present by the third week at EFK 23.2 and EFK 13.8. By the fourth week, substantial algal biomass was also present at EFK 6.3. In Fig. 3-14 the algal biomass samples are shown as (R) on rocks collected from the riffles in which the bricks were placed. The tiles placed at sites EFK 23.2 and EFK 6.3 rapidly accumulated algal biomass similar in concentration and composition to that found on rocks native to the site. Although colonization was rapid at EFK 13.8, it was 6 to 8 weeks

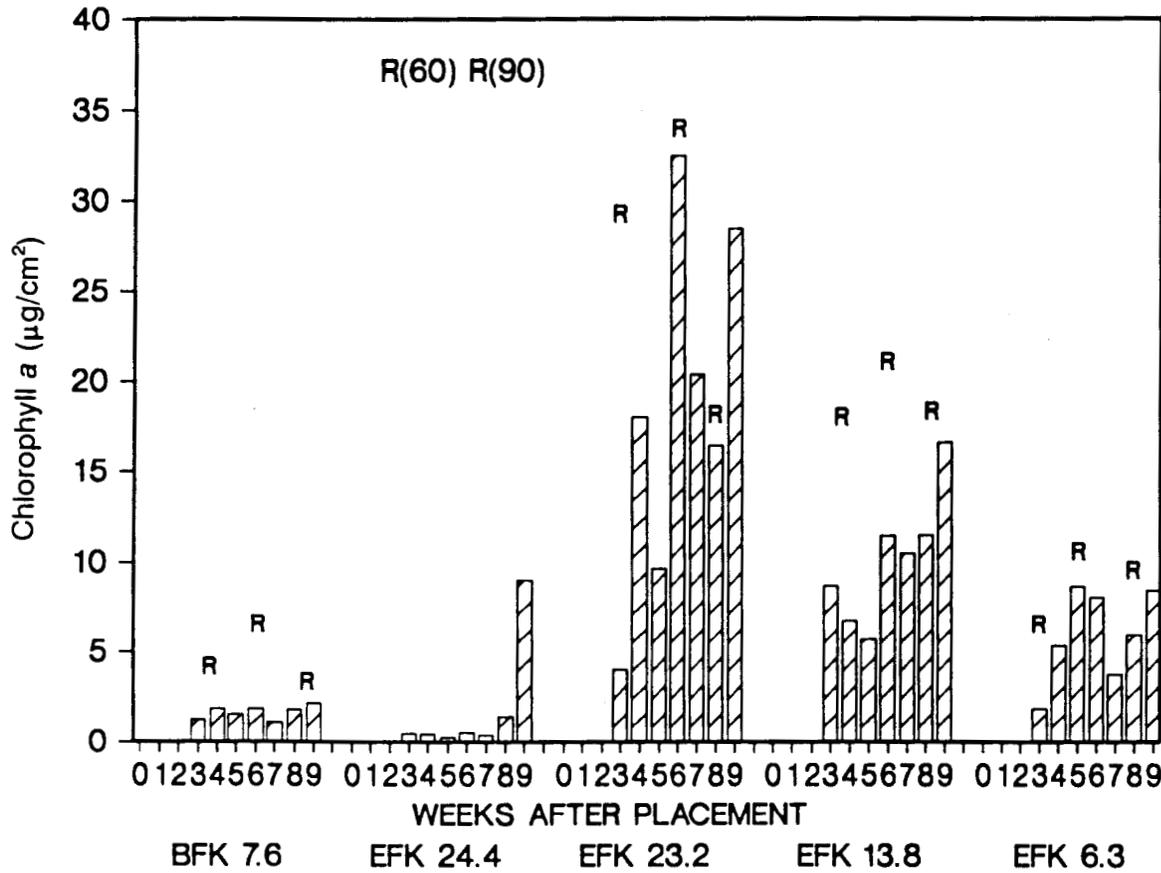


Fig. 3-14. Algal periphyton biomass (expressed as micrograms of chlorophyll *a* per square centimeter) on tiles placed at several study sites for 3 to 9 weeks. Values are the means of four replicate samples. Chlorophyll *a* on rocks adjacent to the tiles on three sampling dates is shown as (R), and is also the mean of four randomly chosen samples. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

before the biomass on the tiles appeared similar to that on rocks at the site. Thick growths of the filamentous alga *Cladophora sp.* covered the rocks at that site. *Cladophora* may be a relatively slow colonizer of bare substrata (tile).

Biomass at BFK 7.6 site did not increase greatly following week three and had not reached the densities found on the rocks adjacent to the tiles by the end of the study. Observations at BFK 7.6 and at reference sites in other systems suggest that the irregular surface of stream rocks provides protection from invertebrate grazers (mostly snails) that may efficiently remove algal biomass from tiles. At several of these reference sites the level of biomass on the tiles did eventually approach that on the surrounding rocks, which suggested that in low nutrient, low light habitats, a community apparently develops that is somehow more resistant to grazing. At sites where algal growth is more rapid (e.g., EFK 23.2) grazers seem to influence tiles and rocks equally.

Through week seven there was very little algal periphyton present on the tiles at EFK 24.4 (Fig. 3-14). By week eight, periphyton biomass had increased; however, the surrounding rocks always had much greater biomass. Similarly slow colonization was found at a site in WOC (WCK 3.9) that also receives cooling tower blowdown and experiences occasional chlorine toxicity (H. L. Boston, ORNL, unpublished data).

As discussed earlier, some rocks at EFK 24.4 and WCK 3.9 have very high algal biomass while nearby rocks are bare. We conducted experiments at WCK 3.9 during 1988 that showed that algal periphyton regrew rapidly on rocks from which most of the periphyton had been brushed off (but on which some live cells remained) while regrowth was extremely slow on rocks sterilized with chlorine. Those results suggested that conditions for algal growth at WCK 3.9 are good excepting intermittent incidents of toxicity that remove (kill) much of the population (similar to conclusions for EFK 24.4). On rocks where a few viable algal cells remain, regrowth is rapid. On rocks where no viable cells remain, recolonization is slow and so regrowth is restricted. The algal periphyton at WCK 3.9 and EFK 24.4. are dominated by similar algae: sessile, unicellular chlorophytes. This organism grows rapidly under the high-light, high-nutrient conditions at those sites but is apparently a poor colonizer of barren surfaces (e.g., new tiles or sterilized rocks). The cause for the abnormal algal community composition at sites occasionally stressed by chlorine is unknown.

3.8.2.8 Contaminant transfer

The periphyton collected from EFK 24.4 and EFK 23.2 during February and May 1987 had higher concentrations of Ag, Cd, Cu, Ni, Pb, and Zn, than did samples collected from the downstream sites or the BF reference site (Tables 3-26 and 3-27).

Concentrations of silver in the periphyton at EFK 24.4 of up to 112 ppm (dry wt) were several fold greater than usually found in streams and may reflect the input of photolab waste into the stream. Concentrations of cadmium in periphyton from all sites on EFPC were higher than typically encountered. Cadmium was particularly high at upstream sites EFK 24.4 and EFK 23.2, being 31 and 40 ppm respectively. Copper was also high throughout EFPC, especially upstream, but the concentrations were not excessive (up to 280 ppm).

Concentrations of nickel in the periphyton were also higher for the upstream sites, and the 580 ppm found at EFK 23.2 during May 1987 could be considered excessive. Lead was generally high throughout EFPC, likely reflecting urban runoff inputs.

Table 3-26. Selected elements in periphyton (parts per million dry wt) collected during February 1987

Values are the mean of three replicates

Metals	BFK 7.6	EFK 24.4	EFK 23.2	EFK 17.0	EFK 13.8	EFK 10.6	EFK 6.3
Ag	<10	112	21	<10	<9.9	<10	<10
Al	9667	3900	16000	11600	9900	8700	10100
As	<20	<20	<20	<20	<20	<20	<20
B	25	27	23	<16	19	20	22
Ba	360	170	467	157	160	393	167
Be	0.78	0.48	1.3	0.88	0.72	0.56	0.6
Ca	39000	23000	32000	27000	18000	11000	14000
Cd	1.3	31	31	6.9	4.6	4.2	2.4
Co	24	46	97	18	13	19	12
Cr	13	26	47	41	26	26	23
Cu	15	280	207	68	59	45	29
Fe	14000	12000	28000	17000	15000	12000	13000
Ga	<60	<60	<60	<60	<60	<60	<60
Li	<40	<39	44	<40	<39	<40	<40
Mg	3100	6100	8700	5600	3100	2900	2200
Mn	6700	3200	8200	2600	2000	6900	24
Mo	<8.0	16	33	<8.0	8.2	<8.0	<8.0
Na	303	953	660	237	603	1143	267
Ni	22	86	233	51	31	50	26
P	1500	13000	7700	1500	2800	7200	2500
Pb	<40	<39	63	66	<40	<40	<39
Sb	<40	<40	<40	<40	<40	<40	<40
Se	<40	<40	<40	<40	<40	<40	<40
Si	3200	1700	4200	4200	3400	3800	3600
Sn	<10	<10	<10	<10	<10	<10	<10
Sr	41	34	52	44	34	30	27
Ti	99	40	124	88	83	65	84
V	16	8.9	22	20	17	11	15
Zn	557	2500	3500	633	433	500	217
Zr	9	8.5	16	21	22	12	16

Note: BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

Concentrations of zinc in the periphyton of 2400 to 3800 ppm at the upstream sites were several times higher than typical for reference sites.

High concentrations of potentially toxic metals apparently result from Y-12 discharges and may influence periphyton taxonomic composition and/or the physiological condition at the EFK 24.4 and EFK 23.2. The potential transfer of these contaminants to grazers suggests a possible influence on invertebrate composition and numbers.

Periphyton samples collected February 10, 1987, analyzed for mercury, showed that total mercury in the periphyton was about 607 ppm at EFK 24.4 and decreased with

Table 3-27. Selected elements in periphyton (parts per million dry wt) collected during May 1987

Values are the means of three replicates

Metals	BFK 7.6	EFK 24.4	EFK 23.2	EFK 17.0	EFK 13.8	EFK 10.6	EFK 6.3
Ag	7.1	22.3	<32	<7	<5	<9	<7
Al	10100	7100	6800	9400	10800	8170	8900
As	14	<29	<64	<14	<11	<17	<13
B	14	<23	<51	<11	<8	40	<10
Ba	500	170	270	105	1433	263	102
Be	0.69	0.78	<1.3	0.72	0.74	0.62	0.58
Ca	61700	37300	66000	48700	57000	10400	11100
Cd	1	25.3	39.7	5.1	4	4.5	2
Co	21	49	131	12	19	25	11
Cr	16	22	<25	2	293	37	24
Cu	11	207	137	50	42	40	33
Fe	12300	14300	6800	14300	17700	14000	12000
Ga	<43	<85	<190	<40	<31	<52	<40
Li	<28	<58	<127	<29	34.3	<35	<29
Mg	3100	8700	6000	6700	3300	2300	2500
Mn	12700	4230	12900	1400	4570	9170	1730
Mo	<6	<12	<27	<5	12.4	7.3	9.8
Na	1490	980	780	3230	1900	1083	5970
Ni	24	126	580	35	43	74	23
P	1630	8230	7470	1600	2200	3300	3730
Pb	<28	<58	<128	54	36	36	<26
Sb	<28	<58	<128	<27	<21	35	<26
Se	<28	<58	<128	<27	<21	35	<26
Si	4400	2370	3000	2900	2370	4500	2970
Sn	<7	<14	<32	<7	<5	<9	<7
Sr	59	48	65	51	56	24	24
Ti	56	<7	<13	8	19	43	16
V	14	13	<13	14	16	12	13
Zn	437	2430	3800	360	233	410	207
Zr	7	17	<13	17	22	15	13

Note: BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

distance downstream (Table 3-28). Periphyton collected from the BF reference site had about 0.3 ppm mercury, which is fairly typical of unpolluted streams in this area. The potential effects of the mercury on the organisms comprising the periphyton depends on the fraction of the metal present as Hg^{+2} , and the potential for food chain transfer is largely dependent on the fraction that is present in organic form (e.g., methyl-mercury). The distribution of the mercury fractions in the periphyton are not known.

Table 3-28. Mercury in periphyton (parts per million dry wt) brushed from rocks collected from the study sites on February 10, 1987

Values are the means \pm 1 SD ($n = 3$)

Site ^a	Hg (ppm)
EFK 24.4	607 \pm 99
EFK 23.2	159 \pm 44
EFK 17.0	56 \pm 3.5
EFK 13.8	23 \pm 13
EFK 10.6	14 \pm 3.2
EFK 6.3	16 \pm 6.2
BFK 7.6	0.3 \pm 0.06

^aEFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

3.8.3 Summary

Our efforts to date have emphasized the documentation of the biomass and rates of primary production of the algal periphyton on rocks in riffles at five study sites in EFPC and at a reference site in Brushy Fork. The study sites were chosen to characterize major stream reaches above and below NHP, above and below ORWTF, and at the downstream extent stream-like section of EFPC. Effluent from the Y-12 Plant and ORWTF enrich the water of EFPC with plant nutrients, while the water at the reference site had only moderate concentrations of plant nutrients (Sect. 2.3). Sites EFK 10.6, EFK 6.3, and BFK 7.6 received only about 5% of incident light during much of the year due to shading by riparian vegetation. The stream silt load increased with distance downstream and may have influenced the periphyton matrix.

Light was an important factor controlling periphyton biomass, and the annual average algal biomass was high at unshaded sites (EFK 24.4, EFK 23.2, and EFK 13.8) and lower at three shaded sites (EFK 10.6, EFK 6.3 and BFK 7.6). Average primary production measured by short-term carbon incorporation was similar at EFK 24.4, EFK 23.3, EFK 13.8, and EFK 6.3, lower at EFK 10.6, and lowest at BFK 7.6.

Because the rate of carbon incorporation is not linearly related to biomass, we performed an ANCOVA and used chlorophyll-adjusted production to compare the photosynthetic rates among sites that differed in biomass. This evaluation showed that the periphyton at EFK 6.3 and EFK 13.8 had the highest rates of chlorophyll-adjusted production (i.e., were in the best "condition"); periphyton at BFK 7.6 and EFK 10.6 had the lowest rates of chlorophyll-adjusted production; and periphyton at EFK 24.4 and EFK 23.2 were intermediate. The chlorophyll-adjusted production or condition of the periphyton seemed related to both natural and anthropogenic factors.

On six dates a laboratory algal bioassay was conducted to evaluate water quality for algal growth at nine sites on EFPC and at BF. There was little difference among the sites, and that which did exist was not related to measures of periphyton condition. The

photosynthetic rates of the laboratory-grown *Haematococcus* was, on average, lowest in water collected from EFPC on June 8, 1987, which corresponded to the occurrence of a fish kill below NHP. These studies could not detect differences in water quality for algal photosynthesis among sites along EFPC that related to the behavior of the periphyton in EFPC. These results suggested that (1) intermittent factors, (2) physical or biotic factors, and/or (3) cumulative factors are responsible for the difference in algal biomass, production, and condition among the sites downstream of NHP.

Studies of algal colonization/development found rapid colonization and development at EFK 23.2, EFK 13.8, and EFK 6.3. At BFK 7.6, shade, grazing pressure, and lower nutrient levels likely combined to limit biomass accumulation. At EFK 24.4, colonization was extremely slow. We suspect that intermittent chlorine toxicity and perhaps a persistent low level of toxicity restricts the taxonomic diversity of the algae largely to one species that is a very poor colonizer of new surfaces. This alga's inability to colonize and recolonize rock surfaces may explain the patchy distribution of periphyton on the rocks at EFK 24.4. Impacts of toxic releases on the microbial component of the periphyton may contribute to the slow colonization; however, the microbial component has not been addressed sufficiently to reach conclusions.

The concentrations of ATP in the periphyton were variable at all sites and tended to be related to algal biomass. A pattern of decreasing ATP with distance downstream in EFPC, not correlated with algal biomass, was present on several dates that suggested a response to organic enrichment from Y-12 discharges. However, the pattern was not consistent. Changes in algal physiology, toxic events, and other site specific factors likely contributed to our inability to reach satisfying conclusions concerning microbial activity at the study sites.

The Chl *a*/ATP ratio was used as an indicator of the magnitude of autotrophic vs heterotrophic activity in the periphyton. This ratio was within the range of values reported in the literature; however, variability precluded any meaningful evaluation.

The periphyton at EFK 24.4 and EFK 23.2 contained high concentrations of Ag, Cd, Cu, Hg, Ni, Pb, and Zn. With the exception of mercury, other elements were found in concentrations typical of unpolluted systems at the site farthest downstream (EFK 6.3). Concentrations of these elements at the upstream sites may be sufficient to influence periphyton composition or performance and may influence other trophic levels via food chain interactions.

The following major conclusions are based on the first two years efforts:

1. Both the Y-12 Plant and ORWTF discharge nutrients that may stimulate algal growth in EFPC.
2. Algal biomass is generally high in EFPC, particularly at sites that are not shaded by riparian vegetation.
3. The algal periphyton at EFK 13.8 and EFK 6.3 had high chlorophyll-adjusted rates of primary production, suggesting that they were in good physiological condition. Algal periphyton at EFK 10.6 (below ORWTF) had low rates of chlorophyll-adjusted production.
4. Short-term bioassays of water quality for algal photosynthesis and field studies of algal colonization/development found little difference among sites downstream of NHP that correlated with the observed differences in algal biomass or production.
5. Occasional releases of toxicants from the Y-12 Plant probably result in the highly variable spatial and temporal distribution of periphyton at EFK 24.4 above NHP.

Activities at Y-12 may also have an occasional adverse impact on the algal periphyton directly below NHP; however, those effects probably do not extend far downstream.

6. Periphyton just downstream of NHP accumulate metals, and may transfer these metals to higher trophic levels.

3.8.4 Future Studies

Our efforts during next year will focus on topics that will expand our understanding of the role of the periphyton component in EFPC and allow us to better predict whole system responses to remedial actions that influence the periphyton community.

Heterotrophy on allochthonous organic matter and dissolved organics discharged by the Y-12 Plant are probably important components of energy flow in EFPC. Our first task will be to address heterotrophic activity for the study reaches in EFPC. These investigations will determine whether microbial activity is impacted in a manner similar to algal periphyton activity and will contribute to our evaluation of the importance of heterotrophy to energy flow in EFPC.

The second task will address the direct influences of the periphyton on other trophic levels by considering the periphyton in terms of food quality for grazers. Does the composition or contaminant content of the periphyton in certain reaches of EFPC influence the composition, numbers, or production of grazing organisms?

This information will contribute to the characterization of the various study reaches, allow the evaluation of the influence of Y-12 activities on the structure and dynamics of EFPC, and provide the information needed to predict the responses to proposed remedial action alternatives.

4. BIOACCUMULATION STUDIES

M. C. Black, W. Burton, J. F. McCarthy, M. J. Peterson, and G. R. Southworth

4.1 ACCUMULATION OF CONTAMINANTS BY BIOTA IN EAST FORK POPLAR CREEK (*G. R. Southworth and M. J. Peterson*)

4.1.1 Introduction

The problems of chemical contamination of water, sediments, and biota in EFPC have been examined in a number of recent reports and studies (Van Winkle et al. 1984, TVA 1985a-1985e, Loar et al. 1992b). Management of the Y-12 Plant is committed to reducing releases of substances such as mercury and PCBs to levels that will ensure that biota in EFPC are safe for human consumption. The bioaccumulation portion of BMAP (Loar et al., unpublished data) is designed to monitor those substances (mercury, PCBs) known to accumulate to undesirable levels in EFPC fish in order to evaluate the effectiveness of any remedial actions taken within, and downstream of, the Y-12 Plant. A broad suite of inorganic and organic pollutants are also monitored in EFPC biota to ensure that no other substances accumulate to unacceptable levels as new treatment processes are developed and new treated waste streams are discharged to the creek. This report presents results of contaminant monitoring conducted between December 1986 and May 1988.

4.1.2 Methods

Fish were collected for contaminant analysis from five sites in EFPC and a reference stream, Hinds Creek. Four of the EFPC sites coincided with sites used for the quantitative fish population surveys: EFK 23.4, EFK 18.2, EFK 13.8, and EFK 6.3. The fifth site was located at EFK 2.1, approximately 300 m downstream from the mouth of Bear Creek. Sites were selected to describe the longitudinal pattern of contaminant accumulation throughout the length of EFPC and to coincide with sample locations used in studies conducted in 1982 (Van Winkle et al. 1984) and 1984 (TVA 1985e) of mercury contamination in EFPC fish. Samples were occasionally taken from other reference streams—Brushy Fork, Beaver Creek, and Bull Run—to obtain more representative estimates of background levels of mercury and PCBs in rural streams similar in size to EFPC.

Fish were collected for contaminant analyses by electrofishing usually beginning at the upper boundary of the reach used for the population survey task (Sect. 6.2.2.1) and continuing upstream until a suitable number of fish were collected or a distance of approximately 800 m was covered. If adequate numbers of fish could not be obtained in this effort, electrofishing was initiated approximately 400 m downstream from the lower boundary of the population reach and proceeded upstream to that boundary, or the site was sampled again at a later date.

The initial fish collection was conducted in May 1985, and collections were made at approximately 6-month intervals thereafter. The four sets of collections presented in this report are identified by the month and year they were made: December 1986, May 1987, December 1987, and May 1988. Originally, it was planned to collect eight fish of each of

three species (redbreast sunfish, *Lepomis auritus*; bluegill, *L. macrochirus*; and carp, *Cyprinus carpio*) at each site, if possible. Redbreast sunfish were abundant at all sites; however, attempts to collect bluegill at sites in the middle reaches of EFPC were discontinued due to the scarcity of this species at those sites. Carp were likewise uncommon in the uppermost (EFK 23.4) and lowermost (EFK 2.1) reaches of EFPC; thus, collections focused on the middle reaches of the stream. An attempt was made to restrict collections of sunfish to only individuals of a size likely to be taken by sport fishermen (≥ 50 g). However, at sites where fish were not abundant, it was necessary to collect smaller fish for analysis in order to obtain an adequate sample size. In fall 1987, bluegill were collected in lower Poplar Creek and the Clinch River downstream from the mouth of Poplar Creek (eight fish per site) to investigate whether or not mercury and PCB contamination originating in the EFPC system could be detected in fish in downstream waters.

Fish collected at each site were placed on ice in a labeled ice chest and returned to the laboratory for processing. Upon return to the laboratory, fish were tagged with a unique four-digit tag wired to the lower jaw. Each fish was then weighed and measured, and scales were taken for age determination. The fish was filleted and the skin was removed from the fillet. A 1- to 2-g sample of the anterior dorsal portion of the axial muscle fillet was excised for the determination of mercury, and the remainder of the fillet was used for PCB analysis. The second fillet provided samples for duplicate analyses, archival storage, and metals/organics analysis, if needed. Samples were wrapped in heavy-duty aluminum foil, labeled, and stored at -20°C in a locked freezer until delivered to the ORNL Analytical Chemistry Division (ACD) for analysis.

Asiatic clams (*Corbicula fluminea*) were placed in EFPC to monitor for PCBs and other organic contaminants. Clams were obtained from Bull Run in Union County, Tennessee, and Beaver Creek in Knox County, Tennessee. After the clams were held for 24 h in clean-flowing water, they were put into polypropylene cages and placed at various sites in EFPC. One set of clams from the original source was frozen at this time for analysis as a control. Each cage held approximately 30–50 clams that contained 0.5–2 g (wet wt) of soft tissue each. The clams were suspended in the stream for four weeks, at which time they were removed and stored in a locked freezer before being processed for delivery to the ACD laboratory at ORNL. After freezing the clams, the shells were removed, and the frozen soft tissue was placed in a 20-ml glass vial. Composite samples weighing approximately 5 and 10 g each were taken for PCB and broad spectrum organics analyses respectively.

Mercury determinations were conducted using an approved modification of the procedure described in EPA (Environmental Protection Agency) (1979a). Samples were digested in a mixture of nitric acid, perchloric acid, and potassium dichromate, after which the mercury was reduced with stannous chloride and determined by cold vapor atomic absorption spectrophotometry. Other metals were determined by using graphite furnace atomic absorption spectrophotometry following digestion with concentrated nitric acid (EPA 1980). Organic priority pollutants were analyzed by procedures PPB 12/83 (EPA 1983) and EPA 600/4-81-055 (EPA 1980) in which the homogenized sample is extracted in methylene chloride; cleaned up using column chromatography, solvent exchange, and evaporative concentration; and analyzed by gas chromatography/mass spectrometry (GC/MS), gas chromatography with electron capture detection (GC/ECD), and high performance liquid chromatography (HPLC) with fluorimetric detection.

Cesium¹³⁷ was determined by counting the characteristic 0.662-MeV gamma emission on a gamma spectrometer equipped with a germanium-lithium (GeLi) crystal scintillation detector, using procedure EMSL-LV-0539-17 (EPA 1979b). A 5- to 10-g (wet wt) sample of freeze-dried fish was counted in a 6.8-cm-diameter plastic dish placed on top of the GeLi crystal.

Statistical evaluations of data were made using SAS procedures and software (SAS 1985a, 1985b) for ANOVA; Tukey's multiple comparison test, linear regression analysis; t-tests; and calculation of the mean, SD, standard error (SE), and CV. Tests for homogeneity of variance among various data groups were conducted using Levene's test on untransformed and log_e-transformed variables (Sokal and Rohlf 1981). Comparisons were based on untransformed data unless Levene's test indicated that transformation was needed to meet assumptions of homogeneous variances. Dunnett's test was used to compare means of various groups with controls (Zar 1984). All comparisons were conducted using $p = 0.05$.

Quality assurance was maintained using a combination of blind duplicate analyses; split sample analyses between the EPA Environmental Services Laboratory in Athens, Georgia, and ORNL; and the analysis of biological reference standards and uncontaminated fish. Recoveries of representative organics were verified by spiking uncontaminated fish or clam samples with known amounts of PCBs and analyzing them. A summary of the results of these procedures is presented in Appendix B.

4.1.3 Results and Discussion

4.1.3.1 Mercury

December 1986–May 1988 sampling

Concentrations of mercury in fish from EFPC during the last four sampling periods (December 1986 through May 1988) were elevated above those found in fish from reference streams. Redbreast sunfish (*Lepomis auritus*) averaged 0.92 $\mu\text{g/g}$ (fresh wt) total mercury, an 11-fold increase above the 0.08 $\mu\text{g/g}$ observed in fish from reference streams. Bluegill (*L. macrochirus*) and carp (*Cyprinus carpio*) contained similar concentrations, averaging 0.74 and 0.73 $\mu\text{g/g}$, respectively, in contrast to 0.08 and 0.21 $\mu\text{g/g}$ in fish from reference streams. Results of all analyses are listed in Appendix C, Tables C-1 through C-4.

A substantial proportion (30%) of the fish collected from EFPC from December 1986 through May 1988 exceeded the FDA tolerance level for mercury in fish and shellfish of 1 $\mu\text{g/g}$ (FDA 1984a). With respect to species, 41% of the redbreast sunfish, 23% of the bluegill, and 18% of the carp contained mercury at or above the FDA limit (Table 4-1). Most of the sunfish containing mercury concentrations above 1 $\mu\text{g/g}$ were collected in the reach immediately below NHP. At this site, 78% of the redbreast sunfish and 52% of the bluegill exceeded 1 $\mu\text{g/g}$. The maximum levels observed in each of the species were 3.6 $\mu\text{g/g}$ in redbreast sunfish, 2.8 $\mu\text{g/g}$ in bluegill, and 1.9 $\mu\text{g/g}$ in carp.

Mean mercury levels for sunfish were highest just below NHP and decreased steadily with distance downstream from that site (Tables 4-2 and 4-3). Redbreast sunfish were collected in adequate numbers at all sites and best illustrate this pattern. The bluegill data also follow this trend, although this species was collected in abundance only at the uppermost and lowermost sites in EFPC. Carp were rarely collected at the uppermost site

Table 4-1. Proportion of fish collected from East Fork Poplar Creek with a total mercury concentration greater than or equal to 1 $\mu\text{g/g}$ (wet wt) December 1986–May 1988

NC = no fish collected

Site	Species		
	Bluegill (<i>Lepomis macrochirus</i>)	Redbreast sunfish (<i>L. auritus</i>)	Carp (<i>Cyprinus carpio</i>)
EFK 23.4	16/31	25/32	0/1
EFK 18.2	NC	17/32	3/17
EFK 13.8	0/1	8/32	4/14
EFK 10.0	NC	NC	4/5
EFK 6.3	0/5	2/32	4/30
EFK 2.1	0/34	0/31	0/15

Note: EFK = East Fork Poplar Creek kilometer.

Table 4-2. Total mercury (measured in micrograms per gram, wet weight) in redbreast sunfish (*Lepomis auritus*) from East Fork Poplar Creek, 1986–88

Site	Season			
	December 1986	May 1987	December 1987	May 1988
EFK 23.4	1.70 \pm 0.10 (8)	1.49 \pm 0.13 (8)	1.57 \pm 0.58 (8)	1.45 \pm 0.21 (8)
EFK 18.2	0.91 \pm 0.05 (8)	1.15 \pm 0.08 (8)	1.25 \pm 0.11 (8)	0.95 \pm 0.16 (8)
EFK 13.8	0.91 \pm 0.06 (8)	0.83 \pm 0.06 (8)	1.03 \pm 0.08 (8)	0.66 \pm 0.08 (8)
EFK 6.3	0.70 \pm 0.05 (8)	0.82 \pm 0.04 (8)	0.75 \pm 0.08 (8)	0.60 \pm 0.06 (8)
EFK 2.1	0.40 \pm 0.03 (8)	0.47 \pm 0.09 (8)	0.43 \pm 0.04 (8)	0.35 \pm 0.03 (8)

Note: Values are mean \pm SE (sample size in parentheses). Background level (reference stream fish) is 0.08 \pm 0.0 $\mu\text{g/g}$ ($n = 30$). EFK = East Fork Poplar Creek kilometer.

Table 4-3. Total mercury (measured in micrograms per gram, wet weight) in bluegill (*Lepomis macrochirus*) from East Fork Poplar Creek, 1986–88

Site ^a	Season			
	December 1986	May 1987	December 1987	May 1988
EFK 23.4	1.16 ± 0.16 (7)	0.70 ± 0.11 (8)	1.76 ± 0.28 (8)	1.08 ± 0.28 (8)
EFK 18.2	NC	NC	NC	NC
EFK 13.8	0.31 (1)	NC	NC	NC
EFK 6.3	0.55 (1)	0.60 ± 0.14 (3)	0.42 (1)	NC
EFK 2.1	0.38 ± 0.08 (8)	0.38 ± 0.08 (9)	0.43 ± 0.06 (8)	0.32 ± 0.03 (8)

^aEFK = East Fork Poplar Creek kilometer.

Note: Values are mean ± SE (sample size in parentheses). Background level (reference stream fish) is 0.08 ± 0.01 µg/g (*n* = 25). NC = no fish collected.

and only sporadically at EFK 2.1. Mean concentrations of mercury in carp did not appear to follow any consistent pattern among sites (Table 4-4).

Significance of observed levels of mercury in fish

The results of mercury monitoring of EFPC fish from December 1986 to May 1988 were consistent with findings of previous studies (Van Winkle et al. 1984, TVA 1985e, Loar et al. 1992b). Fish from all sites in EFPC remain contaminated with abnormally high concentrations of mercury. The concentrations of mercury found in fish in EFPC were elevated relative to fish collected from reference streams in this study and from other local reservoirs and streams including Poplar Creek above the confluence with EFPC, Melton Hill Reservoir, and the Clinch River upstream from the mouth of Poplar Creek (Elwood 1984; Loar et al. 1981; TVA 1985e; this report, Fig. 4-1). Mercury in fish from EFPC exceeded the geometric mean concentration (0.11 µg/g) observed nationwide in the National Contaminant Biomonitoring Program (Lowe et al. 1985). Mercury concentrations in fish from lower EFPC approximated those observed in fish from reservoirs in the Tennessee River system downstream from sources of mercury contamination, such as Pickwick and Cherokee reservoirs (Elwood 1984). Mercury levels in fish from the uppermost reaches of EFPC below NHP continue to be typical of levels found previously in fish from highly contaminated sites, such as the North Fork of the Holston River (Hildebrand et al. 1980).

Table 4-4. Total mercury (measured in micrograms per gram, wet weight) in carp (*Cyprinus carpio*) from East Fork Poplar Creek, 1986-88

Site ^a	Season			
	December 1986	May 1987	December 1987	May 1988
EFK 23.4	NC	0.11 (1)	NC	NC
EFK 18.2	NC	0.31 (1)	0.83 ± 0.08 (8)	0.82 ± 0.13 (8)
EFK 13.8	NC	0.51 ± 0.09 (6)	NC	0.99 ± 0.16 (8)
EFK 10.0	1.04 ± 0.16 (5)	NC	NC	NC
EFK 6.3	0.83 ± 0.05 (8)	0.66 ± 0.13 (8)	0.82 ± 0.09 (8)	0.62 ± 0.13 (6)
EFK 2.1	NC	0.66 ± 0.02 (6)	0.56 (1)	0.53 ± 0.09 (8)

^aEFK = East Fork Poplar Creek kilometer.

Note: Values are mean ± SE (sample size in parentheses). Background level (reference stream fish) is 0.21 ± 0.03 µg/g (*n* = 9). NC = no fish collected.

In the 1986-88 collections, a substantial fraction of the sunfish collected at sites in the upper half of EFPC downstream from NHP contained mercury in excess of the 1 µg/g FDA limit (FDA 1984a). The fraction of fish exceeding 1 µg/g was higher in 1987/1988 than in 1985/1986 at all sites except EFK 2.1. Mercury contamination continues to result in excessive concentrations of mercury in fish throughout EFPC, with the levels of concern for public health occurring primarily in the reach from EFK 23.4 to EFK 13.8.

Mercury vs fish weight

Most studies have shown a positive correlation between fish weight/age and mercury levels in both contaminated and uncontaminated waters (Hildebrand et al. 1980, Elwood 1984, Van Winkle et al. 1984). This relationship complicates comparisons among sampling dates and sites. In this study, an attempt was made to minimize the effect of the mercury vs age relationship by selecting fish of similar age at each site. By restricting our collections to fish of a size likely to be kept by sport fishermen, it was hoped that most fish would be age III, IV, or V, and that no relationship between mercury content and size would be observed. ANCOVA, blocked by site and season with fish weight as the covariate, indicated no significant relationship between mercury and fish weight (or

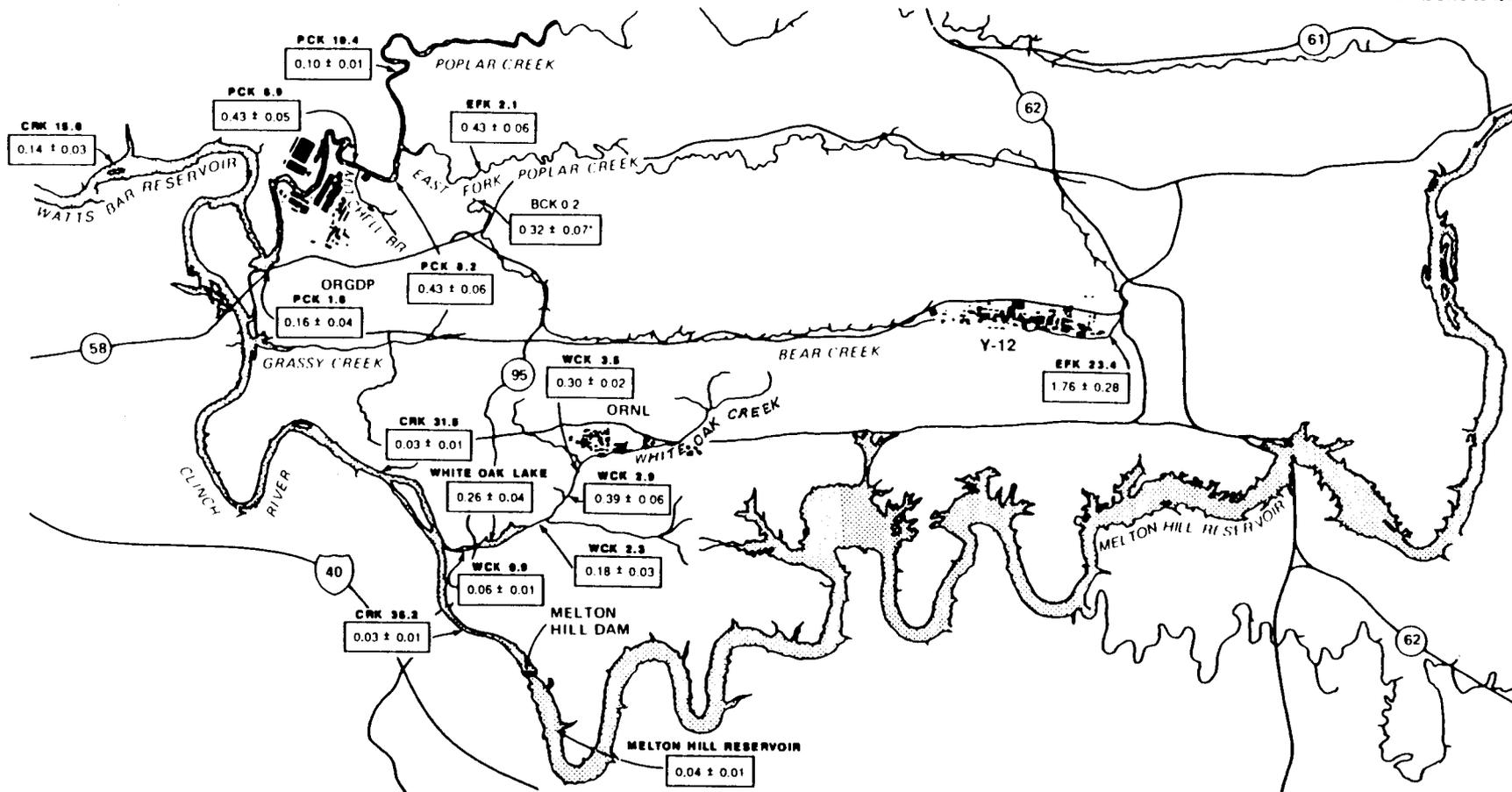


Fig. 4-1. Mercury concentrations (expressed in micrograms per gram) in bluegill (*Lepomis macrochirus*) collected in fall/winter 1987 at sites near DOE facilities in Oak Ridge. Values are mean ± SE, n = 8. Numerical designations indicate distance in kilometers above the mouth of the stream (i.e., CRK 15.0 is a site in the Clinch River 15 km upstream from its mouth).

significant mercury-season or mercury-site interactions) for any of the three species. Least-squares regression of mercury vs fish weight for each species, at each site in EFPC (seasons pooled), found none of 11 regressions to have positive slopes that were significantly different from zero; while regressions for each combination of species, site, and season found 4 of 39 evaluations to have significant positive slopes. It was concluded that no significant relationship between mercury concentration and fish weight existed in the fish collected, and no normalization procedure was required.

Differences among species

An important systematic difference in mercury concentrations among fish species in this study is the possible difference between bluegill and redbreast sunfish, since analysis of historical trends requires the comparison of these species. Comparison of mercury levels in these two species for given site-date combinations revealed significantly higher mean levels of mercury in redbreast sunfish at EFK 23.4 in December 1986 and May 1987 but not in December 1987 and May 1988. At EFK 2.1, there were no significant differences in mean mercury levels between the two species. These results parallel those reported for the May 1985–May 1986 monitoring period (Loar et al. 1992b), in which mean mercury concentrations in redbreast sunfish exceeded those in bluegill on two of three sampling dates at EFK 23.4 but were not different on any dates at EFK 2.1. The more recent data strengthen the conclusion reached previously that mercury levels in these two species are comparable in lower EFPC, but that true differences may exist in upper EFPC below NHP (EFK 23.4). While the two most recent samplings may suggest a diminishing of this relationship, it must be noted that 50% of the fish in the December 1987 redbreast collection at EFK 23.4 contained anomalously low mercury concentrations, suggesting that they were recent migrants from a far less contaminated system (probably the Clinch River).

Mercury levels in carp were similar to those in redbreast and bluegill sunfish. When mercury levels were compared between carp and sunfish species for given site-date combinations, no significant differences were noted in 8 of 12 comparisons. Mean mercury in redbreast sunfish was higher than in carp at EFK 13.8 in May 1987 and lower at EFK 2.1 in May 1988. Mercury levels in carp exceeded those in bluegill at EFK 2.1 in May 1987 and 1988. The pattern closely resembles that observed in the 1985–86 monitoring (Loar et al. 1992b).

Mercury vs distance

The downstream decrease in mercury concentrations in bluegill and redbreast sunfish that was observed in May 1985–May 1986 was also noted in the December 1986–May 1988 period. Mercury was highest in fish from EFK 23.4 and decreased steadily at sites farther downstream, with the pattern clearly repeated year after year (Fig. 4-2). The only exception to this pattern occurred in May 1985 for redbreast sunfish. Least-squares linear regression of mercury in fish vs distance downstream from EFK 23.4 were significant (slope non-zero) for bluegill and redbreast sunfish on all sampling periods since the start of BMAP (May 1985–May 1988). Similar results were not seen for carp. Regressions of mercury in carp vs distance from EFK 23.4 were generally not statistically significant (five of seven sampling periods), and the two significant regressions were contradictory, with mercury significantly decreasing with distance in May 1988 and increasing with distance

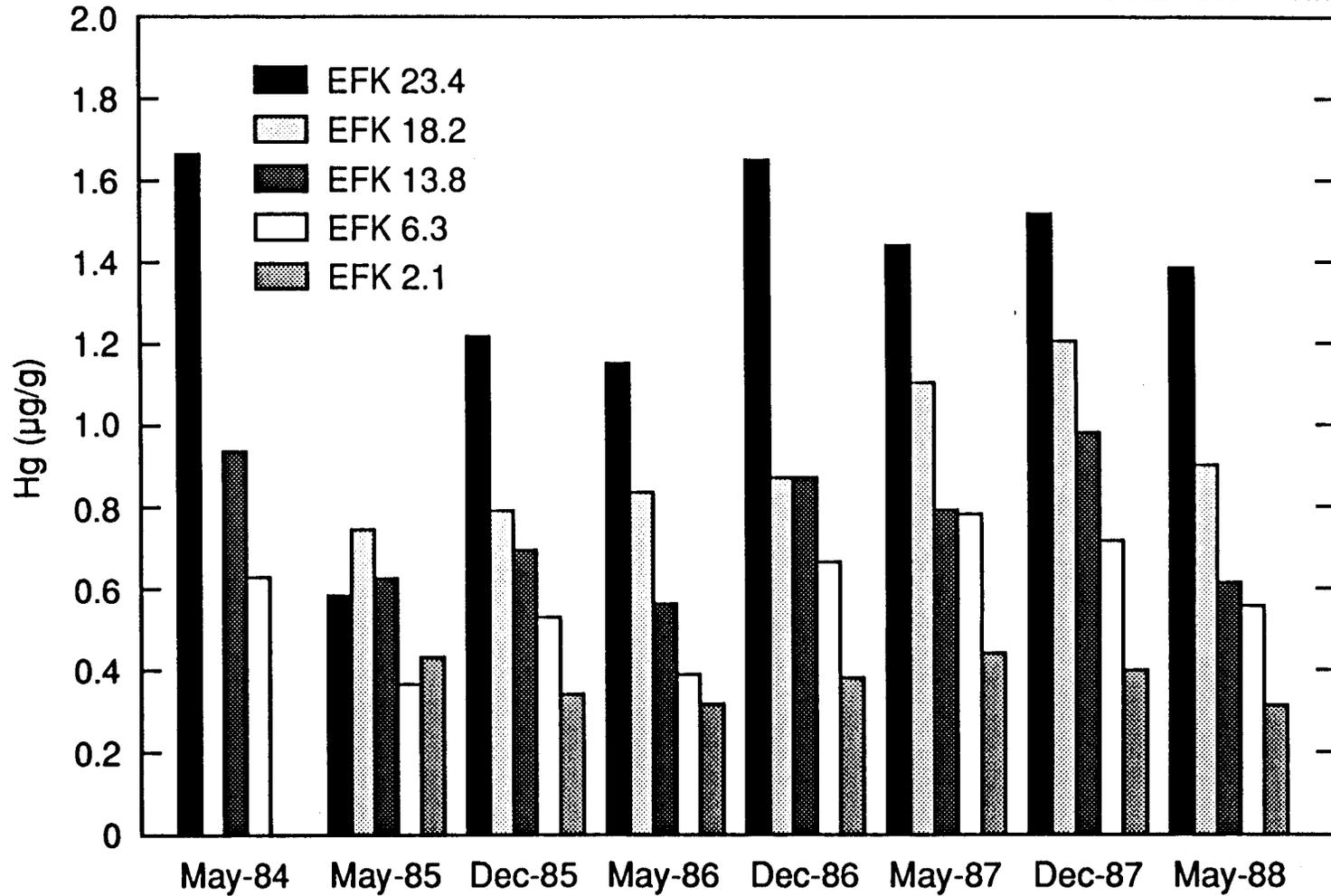


Fig. 4-2. Mean mercury concentrations (measured in micrograms per gram, wet weight, $n = 8$) in redbreast sunfish (*Lepomis auritus*) at sampling sites in EFPC (1984-88). May 1984 data are from Tennessee Valley Authority (TVA). *Instream Contaminant Study, Task 4: Fish Sampling and Analysis*, Report to U.S. Department of Energy, Oak Ridge Operations Office, Office of Natural Resources and Economic Development, Tennessee Valley Authority, Knoxville, Tenn., 1985. EFK = East Fork Poplar Creek kilometer.

in May 1987. Much of the difficulty in discerning downstream trends in the carp data may be due to the absence of data for fish from the upper reaches of the creek.

The data collected in 1987/1988 continue to support the conclusion of Van Winkle et al. (1984) and Loar et al. (1992c) that the pattern of mercury contamination in fish in EFPC is consistent with a continuing discharge of mercury from NHP and subsequent downstream dilution. As was the case in 1985/1986, there was no increase in mercury concentrations in fish in the vicinity of highly contaminated floodplain areas (upstream of site EFK 13.8) or in fish below the ORWTF, suggesting that these are not major sources of biologically available mercury to fish. The most recent data continue to support the conclusion that the levels of mercury that presently exist in EFPC fish are determined by the rate of release of biologically available mercury species from the NHP (or now, LR) discharge.

Temporal trends in mercury concentrations in fish

Mean concentrations of mercury in redbreast sunfish at each site in EFPC from May 1985 to May 1988 are depicted in Fig. 4-2. Generally, concentrations in the period reflected in this report (December 1986–May 1988) appear to be higher than those (May 1985–May 1986) noted in the first Y-12 BMAP report (Loar et al. 1992b). Linear regression of mercury concentrations in redbreast sunfish vs time indicated that mercury contamination in this species has increased a small but statistically significant amount since BMAP was started in May 1985. The slopes of mercury vs time were statistically significant for all sites on EFPC except EFK 2.1 and corresponded to average increases in mercury concentration in fish (from the product of slope of the regression and time) of 0.2 to 0.8 $\mu\text{g/g}$.

Similar results were seen for bluegill over the May 1985–May 1988 period (Fig. 4-3). A significant increase was observed at EFK 23.4, while there was not a significant change at EFK 2.1. A significant increase in mercury with time was observed in carp at EFK 18.2 but not at the other three sites. When carp data from different sites were pooled, there was not a significant change in mercury vs time.

While mercury may have increased somewhat in fish from 1985 to 1988, the data indicate that mercury concentrations in fish populations exhibit substantial temporal variability, with relatively large changes occurring between sampling periods. If data collected in 1982 (Van Winkle et al. 1984) and 1984 (TVA 1985e) are included in the regressions, the results change appreciably. No significant change in mercury was noted for redbreast sunfish between 1984 and 1988 at EFK 23.4 and EFK 13.8, the two sites where more than four specimens were collected in 1984. Evaluation of mercury in bluegill over time since 1982 yields statistically significant decreases at EFK 23.4 and EFK 2.1. Thus, it appears as though present mercury concentrations in EFPC fish are within ranges typically seen in the past and have not increased relative to the levels that resulted in posting of EFPC.

Two significant remedial actions were completed in the period of May 1985 to November 1988. Those storm and process water drains of the Y-12 Plant that contributed the most mercury to EFPC were isolated, cleaned, and relined in 1986–87. In November 1988, EFPC was rerouted around NHP and through LR. The data presented in this report do not cover the period subsequent to bypassing NHP; however, if the drain restoration project had sharply curtailed inputs of biologically available mercury to EFPC

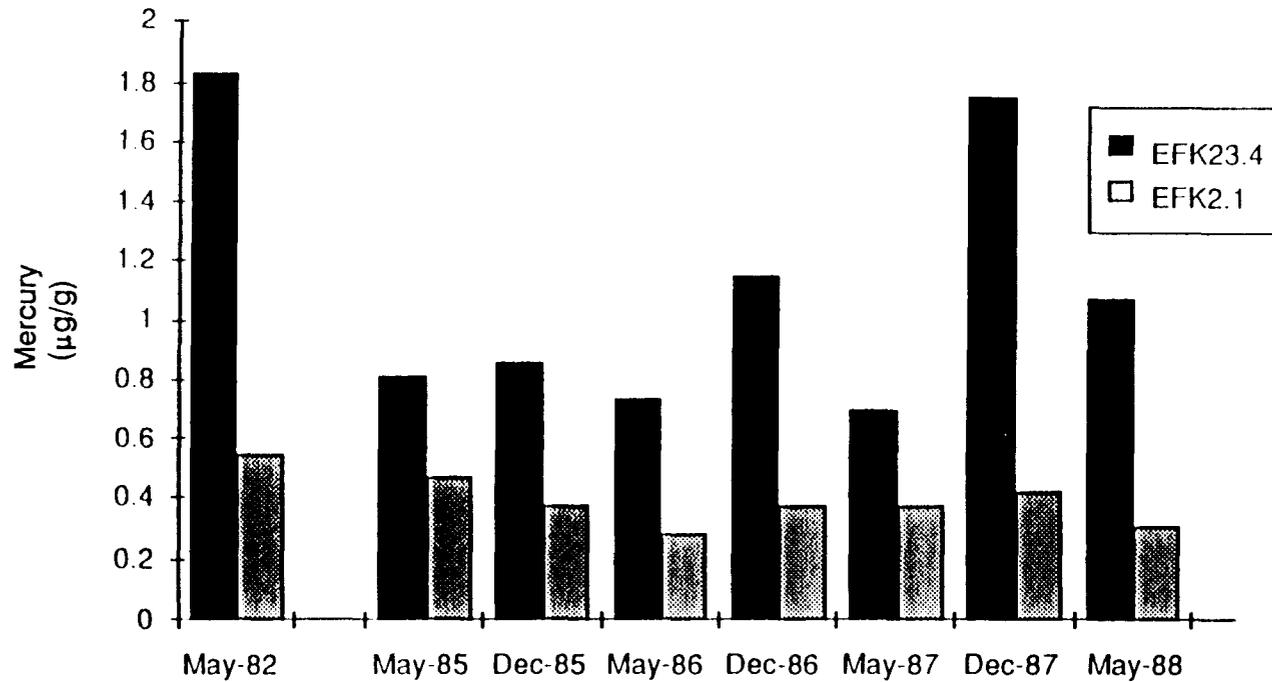


Fig. 4-3. Mean mercury concentrations (micrograms per gram, wet weight, $n = 8$) in bluegill (*Lepomis macrochirus*) at sampling sites in EFPC (1982-88). May 1982 data are from W. Van Winkle et al. *Mercury Contamination in East Fork Poplar Creek and Bear Creek*, ORNL/TM-8894, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1984. EFK = East Fork Poplar Creek kilometer.

upon completion, substantial decreases in mercury contamination in fish at EFK 23.4 would have been evident by May 1988. If the drain restoration project initiated a gradual decline in inputs of biologically available mercury, further monitoring should ultimately reveal a commensurate decrease in contamination in fish. Understanding the cause of any improvement will be complicated by the fact that NHP was bypassed before any improvement related to drain restoration could be discerned. It is likely, however, that ongoing studies by R. R. Turner of ORNL ESD on the fate and transformation of mercury in LR will provide important information for evaluating the effects of the two remedial actions.

Effects of EFPC mercury discharges in downstream receiving waters

Concentrations of mercury and PCBs were measured in bluegill in fall 1987 at sites in Poplar Creek and the Clinch River downstream from EFPC as part of a coordinated sampling for the Y-12 Plant, K-25 Site, and ORNL BMAPs. The objective of this study was to assess the relative importance of mercury and PCB releases from each plant in determining contaminant levels in fish from systems impacted by more than one of these facilities. Results of these analyses are shown in Fig. 4-1. An increase in mercury levels in fish from the Clinch River is noted downstream (CRK 15.0) from the mouth of Poplar Creek. Comparison of mean mercury concentrations in fish from the Clinch River indicated statistically significant differences (Tukey's multiple comparison test on \log_e transformed values) between CRK 15.0 and upstream sites [CRK 31.5, CRK 35.5, and Melton Hill Reservoir (MHR)]; however, the difference between CRK 15.0 and Hinds Creek (reference site) fish was not significant. The mean concentrations of mercury in fish from lower Poplar Creek did not decline proportionally to the dilution of EFPC in Poplar Creek, but rather remained at levels typical of lower EFPC at PCK 8.2 and PCK 6.9. Concentrations of mercury in fish from a site nearer the mouth of Poplar Creek (PCK 1.6) were lower. Fish from PCK 10.4 contained mercury concentrations typical of reference stream fish, indicating that neither upstream sources of mercury in Poplar Creek nor movement of fish between sites were likely explanations for the higher than expected mercury levels at PCK 8.2 and PCK 6.9. Mercury concentrations in rock bass collected in lower Bear Creek (bluegill were uncommon at that site) indicate that a source of biologically available mercury may exist in the Bear Creek watershed; however, the mercury concentrations in these fish and the small flow of Bear Creek relative to EFPC suggest that contributions from this source are unlikely to account for the level of mercury contamination in Poplar Creek fish.

Mercury concentrations in fish throughout EFPC thus appear to be determined by the rate of input of biologically available mercury at the NHP discharge (now LR) and the degree to which that discharge is diluted at downstream sites. This relationship between degree of contamination and dilution does not hold for lower Poplar Creek. While one additional mercury source to that area (Bear Creek) was identified, it does not appear to be large enough to explain the anomaly. Further dilution of Poplar Creek near its mouth and in a downstream reach of the Clinch River result in further reduction in mercury levels in fish, although the decrease is not as large as the degree of additional dilution.

While the impact of the Y-12 Plant discharge on mercury concentrations in fish in the Clinch River/Watts Bar Reservoir was evident, the mercury concentrations in fish were only slightly above background levels typical of stream fish. If mercury concentrations in bluegill at upstream sites in the Clinch River (Fig. 4-1) are used as a reference, the EFPC

discharge to the Clinch River elevates mercury concentrations in bluegill near the mouth of Poplar Creek by approximately $0.1 \mu\text{g/g}$. It appears as though mercury contamination of fish resulting from the NHP/LR discharge is a problem restricted to EFPC and lower Poplar Creek, with relatively little impact on the Clinch River.

The presence of mercury contamination in rock bass in lower Bear Creek suggests the presence of an upstream mercury source in that watershed. While mercury concentrations in Bear Creek sediments do not indicate the presence of heavy contamination, it is very unlikely that mercury in rock bass at this site came from a source related to EFPC. Rock bass are very abundant in lower Bear Creek and relatively uncommon in the nearby reaches of EFPC; thus it is most probable that the fish collected were residents of the site where they were collected rather than migrants. While the concentrations of mercury in rock bass in lower Bear Creek were not high enough to be a major human health concern, gaining further knowledge of the source of mercury contamination in this stream could be useful in understanding the mechanisms by which mercury contamination of EFPC occurs, and aid in planning effective remedial measures if needed.

4.1.3.2 Other Metals

Sunfish collected from EFK 23.4 in 1987 and 1988 contained concentrations of metals (other than mercury) that were similar to those found in fish from Hinds Creek, a reference stream (Table 4-5, Appendix C-4). Copper and selenium were slightly higher in EFPC fish than in Hinds Creek fish in January 1987. The levels of metals in EFPC fish in both 1987 and 1988 were similar to those observed in 1984 by the Tennessee Valley Authority (TVA) in fish from Melton Hill Reservoir (Table 4-5), a reference site, and from EFPC (TVA 1985c, 1986). The geometric mean concentrations of metals (As, Cd, Cu, Pb, Se, and Zn) observed in the National Contaminant Biomonitoring Program (Lowe et al. 1985) were also generally similar to levels observed in EFPC fish (Table 4-5). A comparison of the mean concentrations of metals in EFPC fish with preliminary guidance values (PGVs), which were derived by the Oak Ridge Task Force to screen for contamination that potentially threatened human health (Hoffman et al. 1984, Travis et al. 1986), indicated that only mercury approached this threshold. Detection limits for beryllium and arsenic exceeded the PGVs, which are set at levels below background as a result of the carcinogenicity of these two metals. Both metals were below detection limits in all samples. The PGV approach is very conservative and is designed to eliminate from concern any substances not exceeding the PGV (Hoffman et al. 1984).

4.1.3.3 PCBs

1986–88 Sampling

The PCB contamination detected in fish from EFPC in the 1985/1986 sampling (Loar et al. 1992b) was also observed in the 1987/1988 collections. PCB concentrations in redbreast sunfish and bluegill from EFPC were again similar, each averaging $0.46 \mu\text{g/g}$, while the mean PCB concentration in all carp collected in EFPC was $0.98 \mu\text{g/g}$. Average concentrations of PCBs in fish from the reference stream (Hinds Creek) were 0.04, 0.03,

Table 4-5. Metal concentrations (measured in micrograms per gram, wet weight) in fish from East Fork Poplar Creek below New Hope Pond (EFK 23.4) and from Hinds Creek, a reference stream

Tabular values for these two sites are mean and standard error (in parentheses)

Metal	EFK 23.4		Hinds Creek ^b	TVA ^c	USFWS ^d	PGV ^e
	Jan 1987 ^a	May 1988 ^b				
Sb	<0.3	<0.3	<0.3	---	---	5.2
As	<0.05	<0.05	<0.05	<0.03	0.16	0.0007
Be	<0.04	<0.04	<0.04	<1	---	0.004
Cd	0.015 (0.008)	0.003 (0.001)	0.007 (0.003)	0.007	0.04	1.0
Cr	<0.1	<0.1	<0.1	0.06	---	1.8
Cu	0.42 ^f (0.05)	0.27 (0.08)	0.10 ^f (0.02)	0.4	0.86	36
Pb	0.03 (0.01)	<0.02	<0.02	0.21	0.19	1.8
Li	<0.5	<0.3	<0.5	---	---	---
Ni	<1.0	<1.0	<1.0	<1.0	---	5.2
Se	0.56 ^f (0.02)	0.33 (0.05)	0.32 ^f (0.10)	0.7	0.46	12
Ag	<0.1	<0.1	<0.1	<0.3	---	0.29
Tl	<0.2	<0.2	<0.2	<1	---	0.66
U	---	0.11 (0.02)	0.08 ^g (0.01)	---	---	1.6
Zn	6.4 (0.57)	6.9 (1.69)	6.1 (0.21)	8.4	25.6	180

^a*n* = 3 bluegill (*Lepomis macrochirus*), *n* = 5 redbreast sunfish (*L. auritus*).

^b*n* = 4 bluegill.

^cTennessee Valley Authority (TVA), *Instream Contaminant Study, Task 4: Fish Sampling and Analysis*, Report to U.S. Department of Energy, Oak Ridge Operations Office, Office of Natural Resources and Economic Development, Tennessee Valley Authority, Knoxville, Tenn., 1985.

^dT. P. Lowe et al., *National Contaminant Biomonitoring Program: Concentrations of Seven Elements in Freshwater Fish, 1978-1981*, Arch. Environ. Contam. Toxicol. 14:363-388, 1985.

^ePreliminary Guidance Values (from F. O. Hoffman et al., *Preliminary Screening of Contaminants in Sediments*, ORNL TM-9370, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1984; C. C. Travis et al., *Preliminary Review of TVA Fish Sampling and Analysis Report*, Mimeograph, Report of Task Group Five to Oak Ridge Task Force, January 1986).

^fSignificantly different at *p* < 0.05.

^g*n* = 2 bluegill.

Note: EFK = East Fork Poplar Creek kilometer.

and 0.08 $\mu\text{g/g}$ for redbreast, bluegill, and carp respectively (Tables 4-6 through 4-8). Results of analyses of individual fish are listed in Appendix C, Tables C-1 through C-4. Maximum concentrations of PCBs were 3.8 $\mu\text{g/g}$ in redbreast sunfish, 2.2 $\mu\text{g/g}$ in bluegill, and 3.2 $\mu\text{g/g}$ in carp.

A small fraction of sunfish contained concentrations of PCBs in excess of the FDA limit of 2 $\mu\text{g/g}$ in fish and shellfish sold for human consumption. Overall, 3.6% of sunfish (2.5% of redbreast sunfish and 5.9% of bluegill) collected in EFPC exceeded the FDA limit. All sunfish exceeding 2 $\mu\text{g/g}$ were collected at EFK 23.4. A higher proportion (11.0%) of carp contained greater than 2 $\mu\text{g/g}$ PCBs; these were collected from all sites except EFK 2.1 and EFK 23.4 (where carp are rarely found).

The PCBs found in fish from EFPC were characterized by those isomers and congeners found in commercial mixtures Arochlor-1254 and Arochlor-1260 (referred to as PCB-1254 and PCB-1260 in this report). As was the case in the previous report (Loar et al. 1992b), roughly equal amounts of PCBs similar to the two commercial mixtures were found in sunfish at all sites in EFPC except EFK 23.4, where PCB-1254 predominated in a ratio of approximately 2:1. The ratio of PCB-1254 to PCB-1260 in carp from EFPC was roughly the reverse of this, 1:2, and was also similar to the ratio found in the 1985/1986 sampling.

Significance of observed levels

Contamination of EFPC fish by PCBs was clearly evident in the present study as it also was in the 1985/1986 collections (Loar et al. 1992b). The pattern of highest PCB concentrations in fish nearest NHP with decreasing levels at sites farther downstream continued as it did for mercury, reflecting the apparent common source and similar environmental partitioning of the two contaminants. The PCB concentrations observed were not unusual for streams flowing through industrialized areas. Fish containing PCBs were found at 94% of the stations sampled nationwide in the U.S. Fish and Wildlife Service National Pesticide Monitoring Program. The geometric mean total PCB concentration (wet weight, whole fish) in those collections was 0.53 $\mu\text{g/g}$ (Schmitt et al. 1985).

Although the concentrations of PCBs in sunfish at most sites in EFPC remained well below the 2 $\mu\text{g/g}$ FDA limit (FDA 1984b) in 1986–88, mean PCB concentrations at EFK 23.4 approached or exceeded 1 $\mu\text{g/g}$ on three of four sampling periods, and 12.5% of the sunfish collected at that site exceeded 2 $\mu\text{g/g}$ PCBs. No sunfish at this site exceeded the FDA limit in the 1985–86 collections (Loar et al. 1992b). A similar increase in the proportion of fish exceeding 2 $\mu\text{g/g}$ was not observed in carp, which are not resident in the EFK 23.4 reach and which would tend to respond more slowly to temporal changes in exposure. Thus, sunfish continue to act as indicators of PCB contamination and track fluctuations in exposure but do not accumulate concentrations approaching the FDA limit, except at EFK 23.4. Carp accumulate higher concentrations, and a small but significant proportion of fish (10–20%) inhabiting EFPC are likely to contain PCBs in excess of 2 $\mu\text{g/g}$. There is little indication of any substantial change since 1985 in the potential health risk to the public posed by eating EFPC fish.

Table 4-6. Total polychlorinated biphenyls (expressed in micrograms per gram, wet weight) in redbreast sunfish (*Lepomis auritus*) from East Fork Poplar Creek, 1986-88

Site	Season			
	December 1986	May 1987	December 1987	May 1988
EFK 23.4	1.74 ± 0.37 (8)	1.04 ± 0.40 (8)	0.61 ± 0.27 (8)	0.86 ± 0.18 (8)
EFK 18.2	0.28 ± 0.06 (8)	0.57 ± 0.11 (8)	0.76 ± 0.17 (8)	0.43 ± 0.07 (8)
EFK 13.8	0.31 ± 0.06 (8)	0.30 ± 0.07 (8)	0.62 ± 0.15 (8)	0.36 ± 0.06 (8)
EFK 6.3	0.10 ± 0.01 (8)	0.18 ± 0.04 (8)	0.18 ± 0.03 (8)	0.19 ± 0.03 (8)
EFK 2.1	0.08 ± 0.01 (8)	0.15 ± 0.04 (7)	0.14 ± 0.03 (8)	0.18 ± 0.02 (8)

Note: Values are mean ± SE (size sample in parentheses). Background level (reference stream fish) is 0.04 ± 0.01 µg/g (n = 31). EFK = East Fork Poplar Creek kilometer.

Table 4-7. Total polychlorinated biphenyls (expressed in micrograms per gram, wet weight) in bluegill (*Lepomis macrochirus*) from East Fork Poplar Creek, 1986-88

Site	Season			
	December 1986	May 1987	December 1987	May 1988
EFK 23.4	0.70 ± 0.15 (7)	0.46 ± 0.11 (8)	1.45 ± 0.21 (8)	0.66 ± 0.23 (8)
EFK 18.2	NC	NC	NC	NC
EFK 13.8	NC	NC	NC	NC
EFK 6.3	0.05 (1)	0.13 ± 0.02 (3)	0.31 (1)	NC
EFK 2.1	0.12 ± 0.03 (8)	0.17 ± 0.06 (9)	0.14 ± 0.04 (8)	0.27 ± 0.05 (8)

Note: Values are mean ± SE (size sample in parentheses). Background level (reference stream fish) is 0.03 ± 0.01 µg/g (n = 31). NC = no fish collected. EFK = East Fork Poplar Creek kilometer.

Table 4-8. Total polychlorinated biphenyls (expressed in micrograms per gram, wet weight) in carp (*Cyprinus carpio*) from East Fork Poplar Creek, 1986–88

Site	Season			
	December 1986	May 1987	December 1987	May 1988
EFK 23.4	NC	0.59 (1)	NC	NC
EFK 18.2	NC	0.97 (1)	2.03 ± 0.36 (8)	1.11 ± 0.16 (8)
EFK 13.8	NC	0.96 ± 0.18 (6)	NC	1.52 ± 0.28 (8)
EFK 10.0	0.52 ± 0.08 (5)	NC	NC	NC
EFK 6.3	1.22 ± 0.19 (8)	0.82 ± 0.24 (8)	0.62 ± 0.08 (8)	0.51 ± 0.12 (6)
EFK 2.1	NC	1.11 ± 0.21 (6)	0.17 (1)	0.28 ± 0.06 (8)

Note: Values are mean ± SE (size sample in parentheses). Background level (reference stream fish) is 0.18 ± 0.05 µg/g ($n = 9$). NC = no fish collected. EFK = East Fork Poplar Creek kilometer.

PCB vs fish weight

ANCOVA performed on data for the period December 1986–May 1988 found no significant relationship between PCB concentrations and weight in bluegill and carp (nor site-weight or season-weight interactions) but did indicate a significant relationship and season-weight interaction for redbreast sunfish. Comparisons of PCB vs fish weight for each site-season combination indicated significant positive relationships in only 5 of 20 possible comparisons for redbreast, 1 of 8 for bluegill, and 2 of 11 for carp. A significant negative relationship between PCB and weight was observed in one site-date combination for carp.

When data from all four sampling periods were combined, significant positive relationships were observed for redbreast sunfish at EFK 18.2 and carp at EFK 6.3 but not at any of the remaining nine species-site combinations. As was noted in the May 1985–May 1986 sampling (Loar et al. 1992b), it appears that a weak relationship between PCB concentration and fish weight exists in the data set for a few combinations of species, sites, and dates. Since no relationship existed with most possible combinations, and mean fish weights were generally similar among sites and dates, the data were not normalized prior to making comparisons.

Differences among species

No significant differences (T-test, with assumption of nonhomogeneous variances when appropriate) were observed in the mean total PCB concentration between bluegill and redbreast sunfish on six of eight date-site combinations where both species were abundant in December 1986–May 1988. Mean PCBs in redbreast significantly exceeded that in bluegill at EFK 23.4 in December 1986, while the opposite was observed at that site in December 1987. When fish from all four sampling periods were combined prior to evaluation, there was no significant difference between mean PCBs in bluegill and redbreast sunfish at either site.

PCB concentrations in carp significantly exceeded those in bluegill and or redbreast sunfish on eight of ten site-date combinations (T-test). When sampling periods were pooled, PCBs in carp were significantly higher at all sites in EFPC (except EFK 23.4, where carp are rarely found and no statistical analysis was carried out due to small sample size). These results agree with the findings of the May 1985–May 1986 sampling (Loar et al. 1992b), and were not unexpected since carp are longer-lived and richer in intramuscular lipids than sunfish, both of which act to increase the levels to which hydrophobic contaminants accumulate.

It does not appear that the higher concentrations in carp reflect historical accumulation from a time when exposures were much higher, an explanation for high PCB concentrations in carp that was hypothesized previously (Loar et al. 1992b). Mean PCB concentrations in carp generally were about three to five times higher than in sunfish at sites where both were collected. Neither this ratio nor mean PCB concentrations in carp have decreased substantially in recent collections, suggesting that exposure to higher PCB concentrations several years ago does not account for the difference in PCB accumulation between carp and sunfish in EFPC.

The differences in proportions of PCB-1254 and PCB-1260 in sunfish vs carp that were noted previously (Loar et al. 1992b) were also observed in these more recent data, with PCB-1260 being more predominant in carp. Since more highly chlorinated PCB congeners are less rapidly excreted (Niimi and Oliver 1983) and thus take longer to accumulate to steady state concentrations than less chlorinated congeners, the higher proportion of PCB-1260 in carp is consistent with its longer lifetime. Thus, it would appear that the biota of EFPC contain PCB concentrations commensurate with their exposure history, with short-lived sunfish averaging exposure over a shorter time period than longer-lived carp.

PCBs vs distance

The longitudinal pattern of decreasing contaminant concentrations in sunfish with increasing distance from NHP that was noted for mercury and PCBs in 1985/1986 (Loar et al. 1992b) and mercury in 1987/1988 (Sect. 4.1.3.1) was also observed for PCBs in 1987/1988 (Tables 4-6, 4-7). The relationship between PCB concentrations in redbreast sunfish and distance downstream from NHP was statistically significant ($p < 0.05$) on all sampling periods. The downstream decrease in PCBs in this species appears to be more striking in the December 1986–May 1988 data than in results reported previously (Fig. 4-4). Bluegill were collected in adequate numbers only at EFK 23.4 and EFK 2.1, and the decrease in PCB concentrations between these two sites was statistically significant on all dates except May 1988. A pattern of decreasing PCB concentrations with distance

from NHP was significant in carp in December 1987 and May 1988, but not in the two previous collections (Table 4-8). Drought conditions in 1987/1988 made it possible to collect carp from deep water that was previously inaccessible at EFK 18.2. Adding substantial numbers of fish from this site expanded the longitudinal range of sites for carp and probably accounts for the ability to discern a downstream decrease in PCB concentrations in the December 1987–May 1988 collections.

The pattern of gradual downstream decrease in PCB concentrations in sunfish in EFPC observed in all sampling periods in this study and noted in the previous annual report (Loar et al., unpublished data) indicates the presence of a continuing input of biologically available PCBs to EFPC upstream from EFK 23.4. Attempts to further localize the source by monitoring PCB uptake by caged clams provided evidence that NHP may have been the predominant source of this contamination; however, there is reason to regard this straightforward interpretation of these results with some skepticism for reasons outlined later in this section. The data again did not show an increase in PCB concentrations in redbreast sunfish below ORWTF or adjacent to the highly contaminated floodplain between EFK 18.2 and EFK 13.8, suggesting that these are not major sources of PCB contamination relative to the NHP discharge.

Temporal variation in PCB concentrations in fish

PCB concentrations in redbreast sunfish from the upper reaches of EFPC (EFK 23.4 to EFK 13.8) were generally higher in December 1986–May 1988 than in May 1985–May 1986 (Fig. 4-4). Slopes of linear regressions of PCBs vs time were positive at these three sites; however, only at EFK 18.2 was there a statistically significant increase in PCBs over the period May 1985–May 1988. Bluegill exhibited a significant increase in PCB concentrations from May 1985 to May 1988 at EFK 23.4 but not at EFK 2.1. Carp exhibited a significant decrease in PCB concentrations from May 1985 to May 1988 at EFK 6.3 and EFK 2.1 but not at the other sites. The comparability of May 1985 PCB results in carp with later results (PCB extraction methods were changed) was questioned in the previous report (Loar et al 1992b); reanalysis of May 1985 carp samples confirmed that the first set of results were anomalously high. When May 1985 data were excluded from the regression of PCBs vs time, no significant decrease was noted at EFK 2.1, but a significant decrease remained at EFK 6.3.

Mean PCB concentrations in sunfish at specific sites exhibited substantial temporal variability. Large changes in mean PCB concentrations in redbreast sunfish were commonly noted between sequential sampling periods; such changes were much greater than the corresponding variation in mean mercury concentrations (Figs. 4-1, 4-4). Since both mercury and PCBs have long turnover times in fish, the data suggest that PCB exposure of fish in EFPC is more variable or episodic in nature than mercury exposure.

Concentrations of PCBs in fish in EFPC appear to have no clear increasing or decreasing trend over the May 1985–May 1988 period. The data suggest that PCB levels may have increased somewhat since 1985, but the large amount of variation in PCB concentrations among individual fish and among sampling periods makes the significance of any apparent trend tenuous. The large amount of variation among sampling periods does indicate that PCB concentrations in sunfish in EFPC are capable of relatively rapid change and thus should provide a rapid evaluation of the effectiveness of any remedial actions that affect PCB mobilization.

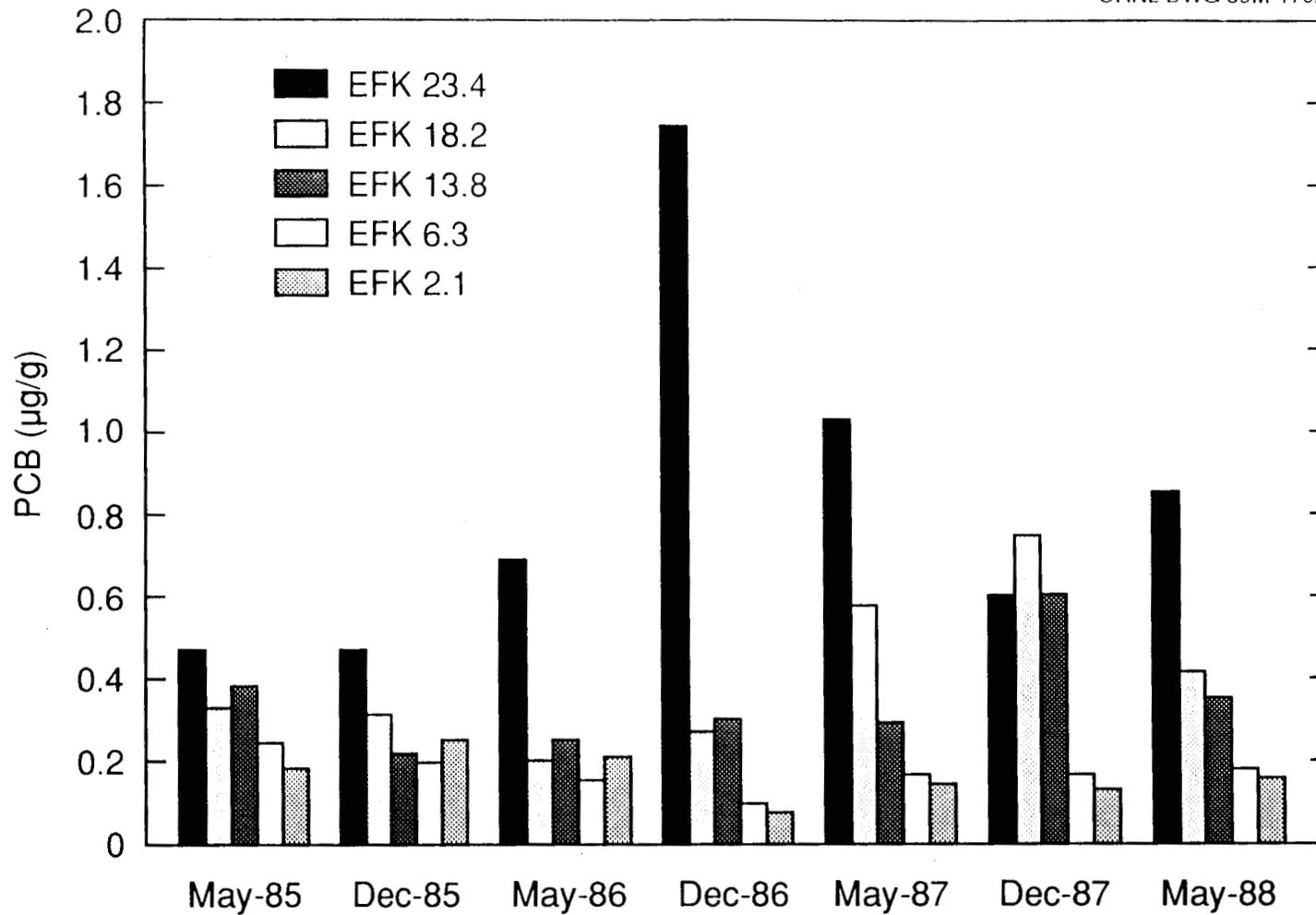


Fig. 4-4. Mean PCB concentrations (expressed as micrograms per gram, wet weight, $n = 8$) in redbreast sunfish (*Lepomis auritus*) at sampling sites in EFPC (1985-88). EFK = East Fork Poplar Creek kilometer.

The apparent increases in PCB concentrations noted in late 1986 and 1987 may have been associated with remedial actions and construction/ excavation activities within the Y-12 Plant over that period. It is possible that PCB-contaminated sediments in drains, pipes, etc. may have been disturbed in such activities, resulting in temporary increases in PCB inputs to EFPC. Completion of these activities and closure of NHP may result in future decreases in PCB concentrations in EFPC fish.

Effects of EFPC PCB discharges in downstream receiving waters

Concentrations of mercury and PCBs were measured in bluegill in fall 1987 at sites in Poplar Creek and the Clinch River downstream from EFPC as part of a coordinated sampling for the Y-12 Plant, K-25 Site, and ORNL BMAPs. The objective of this study was to assess the relative importance of mercury and PCB releases from each plant in determining contaminant levels in fish from systems impacted by more than one of these facilities. A slight increase in PCB concentrations in fish from the Clinch River is noted downstream (CRK 15.0) from the mouth of Poplar Creek (Fig. 4-5). Comparison of mean PCB concentrations in fish from the Clinch River indicated no statistically significant differences (Tukey's multiple comparison test on \log_e transformed values) between CRK 15.0 and upstream sites (CRK 31.5, CRK 35.5, and MHR); however, the difference between CRK 15.0 and Hinds Creek was significant (Dunnett's Test). As was observed for mercury, the mean concentrations of PCBs in fish from lower Poplar Creek (PCK 8.2, PCK 6.9, and PCK 1.6) did not decline proportionally to the dilution of EFPC in Poplar Creek but remained instead at levels typical or slightly higher than those of lower EFPC. Fish from PCK 10.4 contained much lower PCB concentrations, which, although statistically higher than those in Hinds Creek fish, were not significantly different from upstream sites on the Clinch River. As was the case for mercury, neither upstream sources of PCBs in Poplar Creek nor movement of fish between sites were likely explanations for the higher than expected PCB levels at PCK 8.2, PCK 6.9 and PCK 1.6. PCB concentrations in rock bass collected in lower Bear Creek (bluegill were uncommon at that site) confirmed that a source of biologically available PCBs exists in the Bear Creek watershed and the likely episodic releases of PCB-contaminated oil and sediment from the Bear Creek burial grounds could have a discernable impact on PCB concentrations in fish in Poplar Creek. Another episodic source of PCBs to lower Poplar Creek is Mitchell Branch. The slightly higher mean PCB concentration in bluegill collected downstream from this source (PCK 6.9) was not significantly different from the mean PCB concentration upstream (PCK 8.2).

Channel catfish (*Ictalurus punctatus*) were collected at PCK 6.9 in August 1988 as part of an evaluation of the importance of inputs from DOE sources in contributing to PCB contamination in Watts Bar Reservoir. Results of this study, including data on individual fish, are reported in Loar et al. (unpublished data). Concentrations of PCBs in channel catfish at PCK 6.9 can be regarded as almost the worst case example of PCB levels attainable in major food or sport fish in Watts Bar Reservoir occurring as a result of PCB inputs to the Poplar Creek system. PCBs in catfish averaged $0.71 \pm 0.37 \mu\text{g/g}$ wet wt (mean \pm SD, $n = 8$), with a range of 0.28–1.31 $\mu\text{g/g}$. PCBs averaged approximately 0.5 $\mu\text{g/g}$ at two sites in the Clinch River and one in MHR. Composite samples of

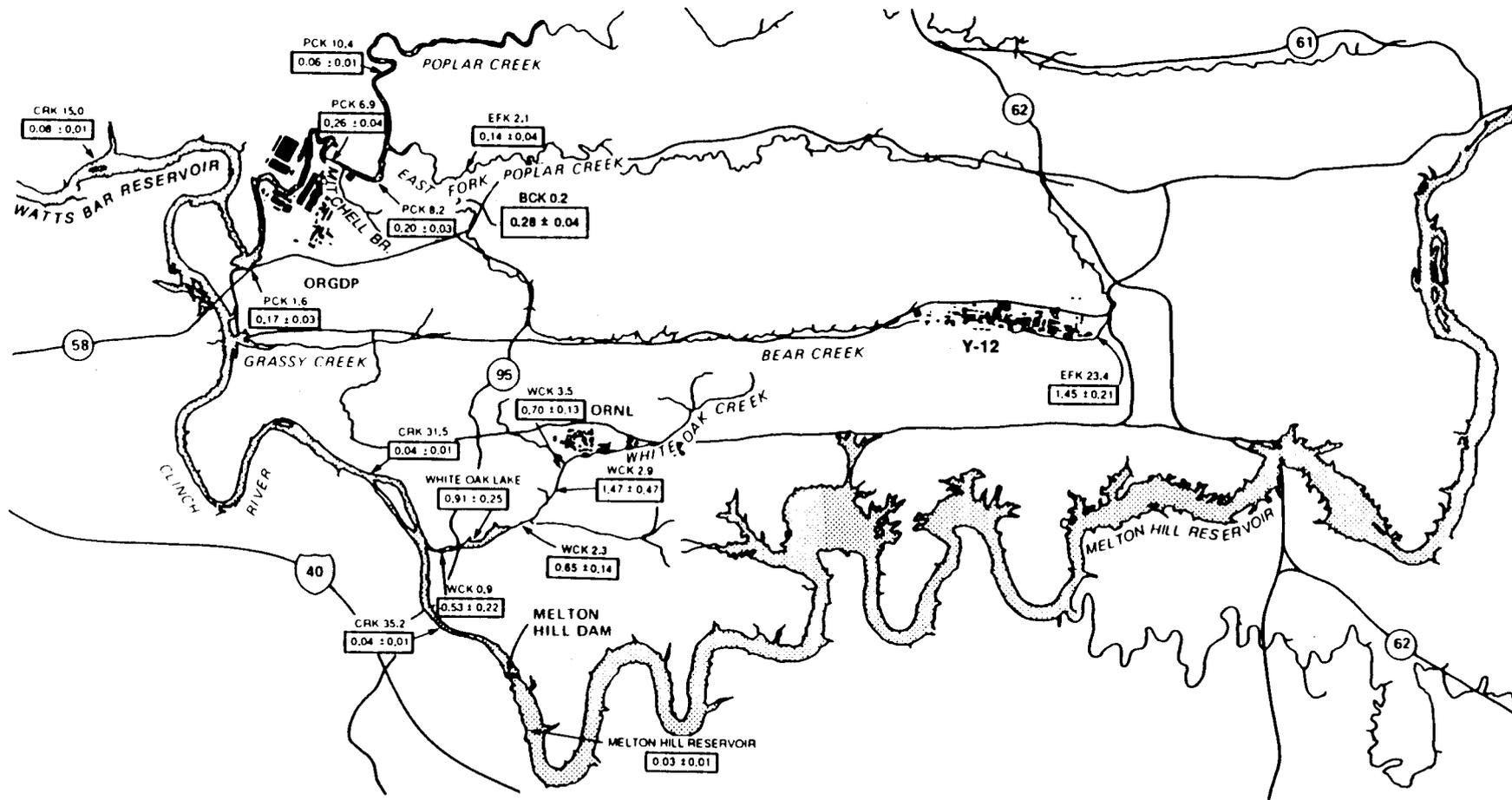


Fig. 4-5. PCB concentrations (expressed in micrograms per gram, wet weight) in bluegill (*Lepomis macrochirus*) collected in fall/winter 1987 at sites near DOE facilities in Oak Ridge. Values are mean ± SE, n = 8. Numerical designations indicate distance in kilometers above the mouth of the stream (i.e., CRK 15.0 is a site in the Clinch River 15 km upstream from its mouth).

gizzard shad, an abundant forage fish in Watts Bar Reservoir, collected at PCK 6.9 averaged $2.97 \pm 1.31 \mu\text{g/g}$, compared with averages of 0.98 ± 0.66 and 0.82 ± 0.21 at Clinch River sites.

While a headwater source clearly can account for the PCB contamination in biota in EFPC, evaluating the significance of that source in contributing to the PCB contamination in biota in waters farther downstream (Poplar Creek and the Clinch River) is not straightforward. The pattern of gradual downstream decreases in PCB concentrations in sunfish, in apparent response to dilution of a discrete source that was observed in EFPC, did not continue into lower Poplar Creek. If it is assumed that such a decrease would have continued in the absence of additional sources of PCBs, then only 25–50% of the PCB contamination in sunfish in lower Poplar Creek could be attributed to ongoing discharges at NHP. Given that lower Poplar Creek is a depositional backwater of Watts Bar Reservoir which is likely to accumulate PCB-contaminated sediments; and that it has received PCB inputs from sources in Bear Creek, EFPC, and Mitchell Branch, it is not unlikely that much of the observed contamination in biota at this site arises from mobilization of sediment-associated PCBs rather than inputs from EFPC.

PCB contamination in sunfish was barely discernible in the Clinch River a short distance below the mouth of Poplar Creek, reflecting a substantial decrease from concentrations observed upstream in lower Poplar Creek. Catfish and gizzard shad contained higher concentrations of PCBs in lower Poplar Creek (PCK 6.9) than in the Clinch River site downstream (CRK 15.0), indicating that Poplar Creek embayment, like WOC embayment (Loar et al., unpublished data) is a relative “hot spot” for PCB contamination in Watts Bar Reservoir. However, PCB concentrations in catfish and shad in the Clinch River were similar above and below the mouth of Poplar Creek, indicating that the effects of inputs from that source are attenuated considerably by dilution and dispersion.

PCBs in caged clams

Asiatic clams in polypropylene cages were suspended in EFPC at sites upstream and downstream from the NHP outfall in 1987 and 1988 in an attempt to locate the source(s) of PCB contamination in EFPC. Clams held four weeks below the NHP discharge accumulated roughly $0.5 \mu\text{g/g}$ PCBs (Table 4-9), similar to levels accumulated in 1986 (Loar et al. 1992b). As was observed previously, PCB-1254 characterized the predominant constituent PCBs in all clam samples (Loar et al. 1992b). In both 1987 and 1988, clams caged at sites above NHP experienced high mortality; survival was adequate only near the inlet to NHP. The large difference in PCB concentrations between clams immediately upstream and immediately downstream from NHP suggests that NHP was the source of much of the PCB contamination in EFPC. However, the physiological status of surviving clams in the reach of stream above NHP is of concern due to the high mortality at sites upstream from it. If these organisms were in a famished or otherwise debilitated state, accumulation of PCBs could be reduced considerably.

The downstream pattern of PCB accumulation in clams does not show the consistent decrease with distance noted in fish. No explanation for this dichotomy is obvious. Several hypothetical explanations are possible, such as response to episodic releases or poor physiological condition of clams at the uppermost site, but each remains conjecture.

Table 4-9. Polychlorinated biphenyl concentrations (expressed in micrograms per gram, wet weight) in clams (*Corbicula fluminea*) caged in East Fork Poplar Creek, April 28–May 26, 1987, and April 8–May 6, 1988

Tabular values are mean \pm 1 SD, $n = 3$ composites containing approximately ten clams each

Site ^a	1987	1988
EFK 26.7	Dead	Dead
EFK 25.1	0.18 ^b	Dead
EFK 23.7	0.14 \pm 0.10	0.04 \pm 0.01
EFK 23.4	0.57 \pm 0.20	0.32 \pm 0.04
EFK 18.2	0.50 \pm 0.06	---
EFK 13.8	0.49 \pm 0.05	0.50 \pm 0.06
EFK 6.3	---	0.33 \pm 0.02
PCK 8.2	---	0.12 \pm 0.00
BCK 4.6	---	1.01 \pm 0.22
Reference ^c	0.08 \pm 0.02	0.05 \pm 0.01

^aEFK = East Fork Poplar Creek kilometer; PCK = Poplar Creek kilometer; BCK = Bear Creek kilometer.

^bMost animals died; enough survived for only one sample.

^cReference site for clams was Beaver Creek in 1987, Bull Run in 1988.

Results of transplants of native clams (*Sphaerium fabale*) to EFK 23.4 indicated that this reach is toxic to that species, and *Corbicula* is not found upstream from EFK 6.3 despite ready access of a colonization source in Poplar Creek (Sect. 6.1.2.2, this report). Thus, a strong likelihood exists that studies of PCB accumulation in clams caged in the upper reaches of EFPC underestimate the availability of PCBs relative to sites farther downstream.

Clams were maintained in Bear Creek in 1988 in an effort to ascertain its contribution to PCB loading of lower EFPC and Poplar Creek. The high concentration of PCBs found in those clams (Table 4-9) clearly indicates that an on-going source of PCB contamination existed in Bear Creek above BCK 4.6 in spring 1988.

4.1.3.4 Other Organics

Clams (*Corbicula fluminea*) maintained in the NHP discharge for 4 weeks in May and June 1988 were analyzed for polycyclic aromatic hydrocarbons (PAHs) and other priority pollutants. Concentrations of PAHs determined by high performance liquid chromatography (HPLC)/fluorescence were markedly higher in clams after 4 weeks' residence in EFPC (Table 4-10), although the concentrations of individual compounds were not high. Mean concentrations of benzo[*a*]anthracene, benzo[*b*]fluoranthene, chrysene, and pyrene in clams from EFK 23.4 exceeded those in clams which had not been placed in EFPC by more than a factor of four. Seven other PAHs were higher in EFPC

Table 4-10. Concentrations (expressed in micrograms per gram wet weight) of polycyclic aromatic hydrocarbons in clams (*Corbicula fluminea*) from Bull Run (Union County, Tennessee) before and after being caged in East Fork Poplar Creek at EFK 23.4, May 18 to June 17, 1988

Compound	Site	
	EFK 23.4	Bull Run
Acenaphthene	0.14 ± 0.06	<0.06
Anthracene	<0.05 ^a	<0.05
Benzo[<i>a</i>]anthracene	0.037 ± 0.010	<0.001
Benzo[<i>a</i>]pyrene	0.070 ± 0.012 ^a	0.035 ± 0.005
Benzo[<i>b</i>]fluoranthene	0.35 ± 0.07 ^a	<0.06
Benzo[<i>ghi</i>]perylene	0.088 ± 0.002	0.025 ± 0.005
Benzo[<i>k</i>]fluoranthene	0.13 ± 0.10 ^a	0.04 ± 0.00
Chrysene	0.15 ± 0.01 ^a	<0.01
Dibenz[<i>a,h</i>]anthracene	0.010 ± 0.000	0.009 ± 0.000
Fluoranthene	<0.50 ^a	<0.50
Naphthalene	0.31 ± 0.10	<0.20
Phenanthrene	0.065 ± 0.013 ^a	<0.020
Pyrene	0.15 ± 0.02 ^a	0.035 ± 0.005
Indenopyrene	0.25 ± 0.03	0.12 ± 0.00

^aAlso detected (below quantification limits) in gas chromatography/electron capture detector analyses.

Note: Tabular values are mean ± SE, *n* = 4 for EFK, *n* = 2 for Bull Run. Samples are composites of approximately ten clams each. EFK = East Fork Poplar Creek kilometer.

clams. Analyses by the less sensitive GC/MS procedure indicated detectable amounts of several PAHs in clams from EFPC but not from Bull Run, but the concentrations were below the limits of quantification. No individual PAH exceeded a concentration of 1.0 µg/g in clams; the highest mean concentration was 0.35 µg/g benzo[*b*]fluoranthene.

The pattern of PAHs found in clams from EFPC is typical of that expected from the presence of petroleum or coal-derived hydrocarbon mixtures in water. Such mixtures would contain a large suite of PAHs, and the more water soluble compounds such as

phenanthrene, pyrene, fluoranthene, and benzo[*a*]anthracene would be likely to accumulate in aquatic biota.

No organic priority pollutants were detected in screening by GC/MS in bluegill collected in June 1988 (Table C-5), except for phthalates, which were also found in laboratory blanks. The pattern of PAH contamination found in clams was not found in fish. Benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene were detected in all samples (EFPC and Hinds Creek) analyzed by HPLC/fluorescence. Similar results were observed in analyses of fish from WOC (Loar et al., unpublished data). The ability of HPLC/fluorescence to detect very low concentrations of PAHs and other fluorescent compounds makes it susceptible to interpreting interferences as low levels of PAH. The two positive values for PAHs found in all samples are thus almost certainly such an analytical artifact.

4.1.3.5 Cesium-137

Mean concentrations of ¹³⁷Cs in sunfish collected from EFPC in 1986 to 1988 are listed in Table 4-11. Concentrations were similar to those observed previously in EFPC (Loar et al. 1992b). The increase in ¹³⁷Cs that was observed in May 1986 in lower EFPC was not found in this later sampling. All concentrations of ¹³⁷Cs in fish were well below the screening PGV of 100 Bq/kg used previously to assess human health concerns (Hoffman et al. 1984). It does not appear that the Y-12 Plant is a significant source of ¹³⁷Cs contamination in EFPC fish.

4.1.4 Role of New Hope Pond Sediments as a Source of Mercury in EFPC Fish

Prior to November 1988, mercury-contaminated water in upper EFPC flowed into NHP before being discharged to the downstream reaches of the creek. Approximately 50% of the mercury transported by the stream was retained in pond sediments (Elwood et al. 1987) that contained mercury in excess of 100 µg/g dry wt. While this trapping of mercury before discharge to downstream sites is desirable, microbial conversion of sediment-associated mercury to methylmercury can result in the enhanced accumulation of mercury by fish. The same mechanism would be likely to operate in the new impoundment on upper EFPC, although differences in the depth of mercury-contaminated sediments—and oxidation-reduction potential within those sediments—could conceivably result in large differences in rates of methylmercury production between the two ponds.

Because it is critical to understand the role of the reservoir of mercury in pond and creek sediments as a source of mercury contamination in fish in order to plan effective remedial strategies in EFPC, a study was carried out to assess the availability of sediment-associated mercury to fish. Blacknose dace (*Rhinichthys atralatus*) were maintained for approximately 6 months in separate 30-L aquaria containing fine surface sediment (<2 mm, wet sieved) from NHP and a site in EFPC, 5 km downstream (EFK 18.2). Mercury concentrations in the sediments were 153 and 62 µg/g dry wt, for NHP and EFPC, respectively. Dace were also maintained in a cage in EFPC at the NHP outlet and in an aquarium in which water was recycled over a bead of elemental mercury to try to mimic conditions in upper EFPC. Control fish were maintained in a tank in

Table 4-11. Cesium-137 (measured in becquerels per kilogram, wet weight) in bluegill (*Lepomis macrochirus*) and redbreast sunfish (*L. auritus*) from East Fork Poplar Creek, 1986–88

Values are mean \pm SE (sample size in parentheses, NS = not sampled)

Site ^a	Season		
	December 1986	May 1987	May 1988
EFK 23.4	9.5 \pm 1.2 (4)	15.3 \pm 3.8 (4)	<12.5 (4)
EFK 18.2	2.5 \pm 0.8 (2)	3.2 \pm 1.0 (2)	NS
EFK 13.8	NS	5.2 (1)	NS
EFK 6.3	12.5 \pm 0.7 (2)	11.2 (1)	NS
EFK 2.1	7.3 \pm 0.3 (3)	13.6 \pm 1.9 (4)	NS

^aEFK = East Fork Poplar Creek kilometer.

Bldg. 1504 with a continuous renewal of fresh dechlorinated process water. Fish were fed frozen brine shrimp twice weekly at a rate of approximately 2-3% of their weight per day. Total mercury concentrations in water, sediment, and fish were determined by the method in Sect. 4.1.2, using the eviscerated carcass with head, tail, scales, and fins removed for fish samples. Elemental mercury in water was determined by sparging the water sample with argon directly into the spectrophotometer as is done in the routine mercury analysis within 30 min of collection; however no acidification, digestion, or addition of stannous chloride (to reduce mercury to the elemental state) was carried out on the sample.

After 25 weeks of exposure to NHP and EFPC sediments, dace contained virtually the same concentration of mercury as control fish held in the laboratory for the same period of time (Fig. 4-6). Comparison of the linear regressions of mercury in dace vs time indicated that the three regressions (control, NHP, and EFPC sediment) were coincident (F-test for equality of regression lines, $p > 0.05$). The caged fish from NHP discharge accumulated approximately 0.5 $\mu\text{g/g}$ mercury over a 6-week period, while fish exposed to the "synthetic" EFPC discharge accumulated mercury at about double that rate. The rates of mercury accumulation in these two treatments differed significantly from each other and also from the control and sediment treatments.

All fish were removed from the "synthetic" EFPC discharge after 6 weeks. At that time the source of elemental mercury was removed; and the next day, six fish from the control treatment were placed in the "synthetic" treatment without changing the water. The rate of increase in mercury concentrations in these fish is depicted in Fig. 4-6 by the

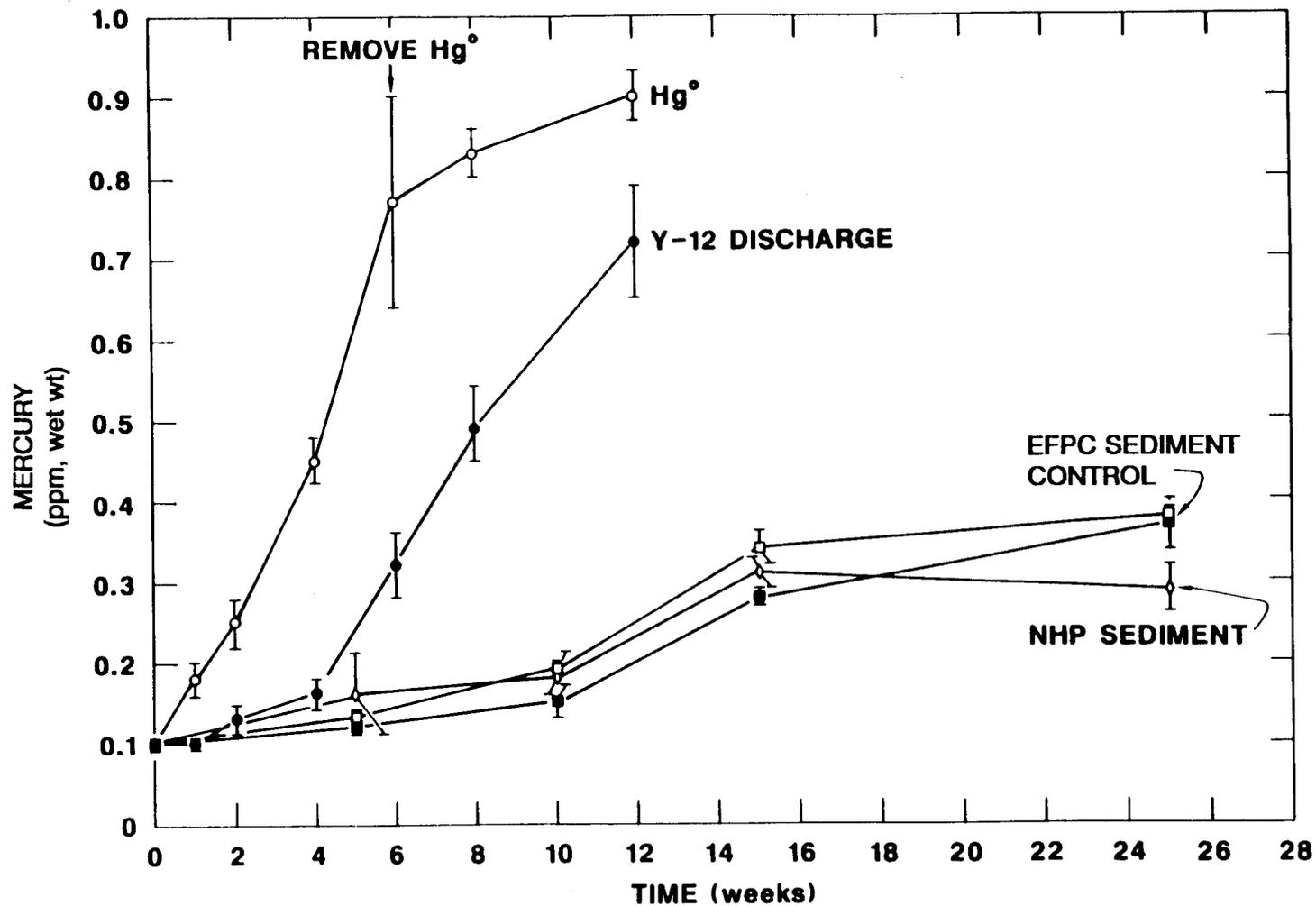


Fig. 4-6. Accumulation of mercury by blacknose dace (*Rhinichthys atralatus*). Fish were experimentally exposed to the New Hope Pond (NHP) discharge, NHP sediments, East Fork Poplar Creek (EFPC) sediments, an elemental mercury source, and dechlorinated process water (control). Values are mean \pm SE, $n = 3$. One replicate from the EFPC treatment, T = 25 weeks, was excluded as contaminated due to an anomalously high ($14 \mu\text{g/g}$) reported mercury concentration.

data points following “remove Hg°.” These two points do not depict actual mercury concentrations in those fish but rather add the difference between the mercury concentration measured in control fish at T = 6 weeks and that in fish after exposure to this treatment to the mean concentration of mercury measured in fish in the “synthetic” treatment at T = 6 weeks. The rate of mercury accumulation decreased abruptly upon removal of the elemental mercury source despite the continued presence of substantial concentrations (~5 µg/L) of total mercury in the water.

Relative to sources of mercury upstream, sediments in NHP and EFPC do not appear to be a significant source of mercury contamination for fish in EFPC. These results suggest that the closure of NHP will result in little change in mercury concentrations in fish in EFPC, unless Lake Reality functions much differently than NHP as a “reaction vessel” for the conversion of mercury inputs to more (or less) biologically available forms.

Contact between water and elemental mercury results in the rapid transfer of biologically available mercury to the water. Results of preliminary studies (not presented in this report) suggest that vapor-phase transfer of elemental mercury is a more facile means of attaining significant concentrations of biologically available mercury in water than direct contact between water and elemental mercury. The subterranean portion of EFPC upstream from the north-south pipe is an ideal environment for such transfer to occur, and it is possible that this mechanism (vapor-phase transfer to water) accounts for a significant portion of the mercury present in the water of upper EFPC.

4.1.5 Future Studies

Routine monitoring of contaminants will continue as it has previously. As colonization of LR and upstream portions of EFPC proceeds and populations become large enough to sustain collection pressure, fish sampling will be expanded into those areas. The elimination of NHP provides the opportunity to conclusively determine whether the PCB contaminated sediments in it were the primary source of PCB contamination in downstream biota, rather than ongoing discharges. This question will be addressed by continued monitoring of PCBs in sunfish and expansion of that effort to LR and upstream, measurement of PCBs in surface sediments upstream and downstream of LR, and continued methodological development and use of caged clams to monitor for PCBs. The sampling of Clinch River sunfish coordinated among the Y-12 Plant, K-25 Site, and ORNL BMAPs will be continued in order to track effects of various NPDES-permitted sources on contamination of biota in upper Watts Bar Reservoir.

4.2 CRITICAL FACTORS IN TRANSPORT, FATE, AND BIOAVAILABILITY

(J. F. McCarthy, M. C. Black, and W. Burton)

4.2.1 Introduction

The objective of this task was to increase fundamental understanding of the factors controlling bioaccumulation of contaminants by aquatic organisms and, from this understanding, develop a capability to predict the accumulation of organic contaminants, particularly PCBs, which are the predominant organic contaminants bioaccumulated in fish in EFPC. Recent research has centered on the role of environmental partitioning of the

contaminants on bioavailability, and the role of physiological factors in the organism which can affect the uptake and accumulation of contaminants. Specific research involved a series of studies that dealt with the following:

1. bioaccumulation and toxicity of PCB congeners,
2. influence of dissolved organic matter (DOM) on the bioavailability of contaminants, and
3. effects of seasonal changes and other environmental factors on uptake of contaminants by fish.

4.2.1.1 Properties of PCB congeners

Most environmental PCBs are commercial formulations, such as the Aroclors, which are mixtures of individual PCB congeners that have chlorine atoms substituted at positions between 2,2' and 6,6' on the biphenyl molecule (Fig. 4-7). The number and position of the chlorines on the biphenyl molecule influences the physicochemical properties and thus the environmental behavior of a congener. Increased numbers of chlorine substitutions increases the molecular surface area of the PCB congener thus decreasing the solubility of the congener in water (Shaw and Connel 1984). Congeners with higher numbers of chlorines are therefore more hydrophobic and have a greater affinity for sorbing to sediment, suspended particles, or DOM (Rapaport and Eisenreich 1984, Karickhoff et al. 1979, McCarthy and Jimenez 1985a). The number and position of chlorine substitution may also sterically limit the diffusion of a PCB congener through a biological membrane such as the gill or gut. For example, orthosubstitution can twist the biphenyl rings out of plane and increase the molecular cross-section of the congener, making it more difficult for the molecule to pass through a membrane (Shaw and Connell 1980). Substitution patterns also alter the metabolism of congeners. Para-substituted congeners and congeners with two adjacent unsubstituted carbon atoms are more readily metabolized than congeners with other substitution patterns (Moore et al. 1978, Matthews and Tuey 1980); however, it is not known if the increase in susceptibility to metabolic transformation affects the toxic effect of the individual congener.

Because of differences in environmental distribution, bioavailability, and biotransformation, individual congeners have the potential to accumulate to different levels in biota and may elicit different toxic effects. Therefore, the toxicity of individual PCB congeners was examined to determine whether future research should concentrate on the behavior and bioaccumulation of congeners with particular properties.

4.2.1.2 Influence of DOM on contaminant bioavailability

Hydrophobic organic contaminants, including PCB's, can sorb to natural dissolved or colloidal organic matter in natural water systems. Sorption to DOM appears to decrease the uptake and bioaccumulation of the contaminants (McCarthy and Jimenez 1985b, McCarthy et al. 1985). However, the affinity of DOM for binding the same contaminants

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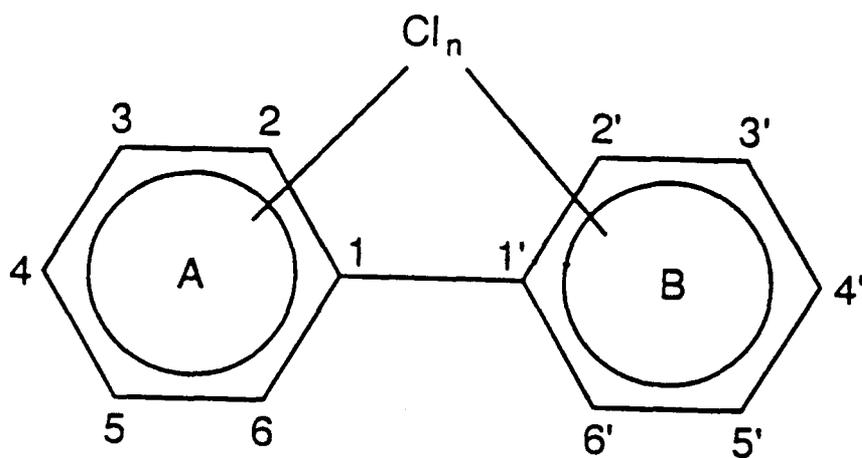


Fig. 4-7. Chemical structure of polychlorinated biphenyl. Chlorines (Cl) may be substituted at any or all of positions 2-6 and 2'-6' ($n = 1, 2, 3, \dots, 10$), yielding 209 possible configurations. The nucleus of the molecule consists of two phenyl ring structures (A and B) covalently bonded at carbons numbered 1 and 1'.

can vary widely depending on the source of natural waters (Morehead et al. 1986; Landrum et al. 1985). Our capability to predict the role of organic macromolecules on the accumulation and toxicity (Friant and Henry 1987) of hydrophobic organic contaminants in aquatic environments appears to be dependent on, but is limited by, the variability in the binding affinity between these contaminants and DOM present in natural waters.

One approach to elucidating the source of the variability in binding affinity of different sources of organic matter is to chemically fractionate DOM and determine if there are underlying similarities in binding affinities of functionally similar subcomponents of the total DOM. Nonionic macroporous sorbents such as the Amberlite XAD resins have been used both to isolate humic substances from surface and ground water (Thurman and Malcolm 1981), and to fractionate the total DOM of natural waters into subcomponents based on the hydrophobicity and charge of the molecules (Leenheer and Huffman 1979, Thurman and Malcolm 1981). Variability in the sorptive behavior of different waters (or the same water at different times of the year) might then be attributed to, and predicted from, differences in the relative abundance of key subcomponents in a particular water or in EFPC at different times of the year.

To elucidate the effect of DOM on contaminant bioaccumulation, and specifically the potential effect within EFPC, research examined

1. the role of DOM on uptake of hydrophobic contaminants in an aquatic invertebrate (*Daphnia magna*) and a fish (*Salmo gairdneri*),
2. the effect of different subcomponents of DOM on sorption of contaminants and uptake of the contaminants by *D. magna*, and
3. the range and variability of total DOM concentrations and concentrations of subcomponents of DOM in EFPC.

4.2.1.3 Effect of environmental and physiological variables on bioaccumulation

Aquatic organisms exposed to xenobiotics in the natural environment can accumulate these substances primarily by two routes, via the food chain and from direct exposure to the toxicant in water. Most hydrophobic organic contaminants, including PCBs and PAHs, are accumulated by passive diffusion across respiratory surfaces, such as fish gills. Since accumulation, metabolism, and toxicity of these compounds depends on the dose incorporated by the organism, an understanding of factors that mediate the gill uptake of these toxicants is essential.

The gill is a very efficient surface for diffusion of oxygen and hydrophobic contaminants due to its large surface area and exposure to large volumes of water. Therefore, physiological and environmental factors that alter the diffusional properties of the gill membrane or the volumes and rates of water pumped over the gill membrane can alter the uptake of oxygen. These factors may also have similar effects on the uptake of other substances that enter the gill via passive diffusion, including waterborne contaminants.

A fish's respiratory functions can vary as a result of physiological changes such as changes in activity levels, nutritional status, reproductive status, and life-stage of the organism. These physiological changes often have environmental cues including seasonal

temperature changes, changes in photoperiod, food availability, and water quality. In studies where fish respiration was manipulated by noninvasive methods (exercise and hypoxia), increases in ventilatory functions were correlated with decreased respiratory efficiency (Saunders 1962, Kerstens et al. 1979, and McKim and Goeden 1982). Gill-irritating or damaging agents (including chlorine, metals, surfactants, etc.) have caused similar changes in respiratory functions that may also result in decreased respiratory efficiency.

This task sought to quantitate the effects of environmentally induced changes in respiration (ventilation volume and ventilation rate) on the uptake of oxygen and model hydrophobic contaminants by fish gills. Our primary goal was to develop a predictive relationship between changes in oxygen uptake and/or consumption and contaminant uptake. Using this relationship, physiological data on fish respiration could be used to make extrapolations of contaminant uptake over a variety of physiological and environmental conditions.

To address these questions, research examined

1. the effect of coexposure to chlorine on the uptake of oxygen and contaminants by fish, and
2. the effect of temperature-induced changes in oxygen demand and respiration on contaminant uptake.

4.2.2 Methods and Materials

4.2.2.1 Toxicity tests with *Daphnia magna*

Acute toxicity test

Ten PCB congeners were used in this study representing a broad range of isomer groups (tri- to octa-chlorinated biphenyls) that have different physical and chemical properties. Aqueous solutions of each PCB congener were prepared using a modification of the generator column technique described by Veith and Comstock (1975). Glass beads 3 mm in diam. were coated by roto-evaporation with an excess amount of the PCB required to achieve the nominal solubility limit of the congener in the volume of water needed for the test. The coated, dry beads were placed in a stainless steel column that was plugged at each end with glass wool. Dechlorinated tap water was recirculated for 72 to 96 h through this closed system. The effectiveness of this technique in generating solutions at the aqueous solubility limit of the congener was confirmed using a radiolabeled congener (IUPAC No. 153; 2,2',4,4',5,5'-hexachlorobiphenyl). In all tests the highest nominal concentration was the aqueous solubility limit of the congener, operationally defined as being that concentration produced by the generator column technique. The concentration of IUPAC No. 153 was calculated to be 1.32 $\mu\text{g/L}$. This value agreed closely with the reported solubility of 1 $\mu\text{g/L}$ (Yalkowsky et al. 1983). Once the solution was generated, the acute toxicity test protocol followed the EPA recommendations for acute toxicity testing (EPA/600/4-85/013), except that both static and static/renewal conditions were observed concurrently. Both *D. magna* and the larvae of

the fathead minnow (FHM, *Pimphales promelas*) were tested for acute toxicity of the PCB congeners.

Chronic toxicity test

A chronic toxicity study with *D. magna* was conducted using a single PCB congener. Static-renewal test conditions were used to measure survival and reproductive success (time to first brood, number of broods per 21 d, mean number of young per brood, and total number of young per female) of the organisms. *D. magna* neonates (\leq 24-h old) were exposed to 3,3',4,4'-TCB at a concentration of 5 $\mu\text{g/L}$. The test was initiated by placing a single neonate into 100-ml glass beakers that contained 80 ml of test solution. The animals were transferred to fresh test solution and fed 1 ml of trout-chow food medium 3 times a week. Ten animals were observed for a duration of 21 days.

4.2.2.2 Measuring contaminant uptake and bioaccumulation

Fish metabolic chamber

Rainbow trout were exposed to ^{14}C -labeled benzo[*a*]pyrene (B[*a*]P) and 2,2',5,5'-tetrachlorobiphenyl (TCB) with and without DOM (Aldrich humic acid) using a fish metabolic chamber (Fig. 4-8). The fish metabolic chamber is described in detail elsewhere (Black and McCarthy 1988). DOM concentrations in the B[*a*]P and TCB exposures were 0, 0.1, 0.5, and 3 mg of carbon per liter and 0, 3.4, and 11.8 mg of carbon per liter respectively. Contaminant concentrations were measured before and after passing over the gill membranes using the fish metabolic chamber. Contaminant uptake efficiencies were calculated by dividing the concentration of B[*a*]P or TCB measured in the water that had passed over the gills (chamber B) by the concentration measured in the exposure water (chamber A). Fish respiratory functions (ventilation volume, ventilation rate, and oxygen uptake) were measured simultaneously with contaminant uptake measurements. Contaminant uptake efficiencies from exposures with DOM were compared with uptake efficiency measured with no DOM present (control exposure) for each fish and expressed as the percentage of control uptake.

In separate experiments, rainbow trout were preexposed to a PCB congener (2,2',4,4'-tetrachlorobiphenyl, 2,2',5,5'-tetrachloro-biphenyl, or 2,2',3,3',5,5'-hexachlorobiphenyl) in the fish metabolic chamber for 3 h. During this time respiratory functions and PCB uptake efficiencies were measured. Chlorine (0.04 mg/L) was added to the PCB-exposure water, and concomitant exposure to PCB and chlorine continued for 24 h. Respiratory functions and PCB uptake by the trout were monitored at 4, 8, 12, and 24 h. At the end of the 24-h chlorine exposure, the chamber was flushed with clean water and fish were allowed to recover from exposure to chlorine in clean water for 24 h. PCBs were reintroduced to the chamber at 1–4 h and 21–24 h, and respiratory and uptake measurements were monitored at these times.

To determine the effects of acute temperature change on trout respiration and contaminant uptake, trout were exposed in the metabolic chamber to ^{14}C , B[*a*]P, TCB, or naphthalene (NPH) while experiencing an acute decrease in temperature. Baseline respiration and uptake measurements were made at the acclimation temperature (18°C). The temperature of the exposure water was then gradually decreased approximately 8°C

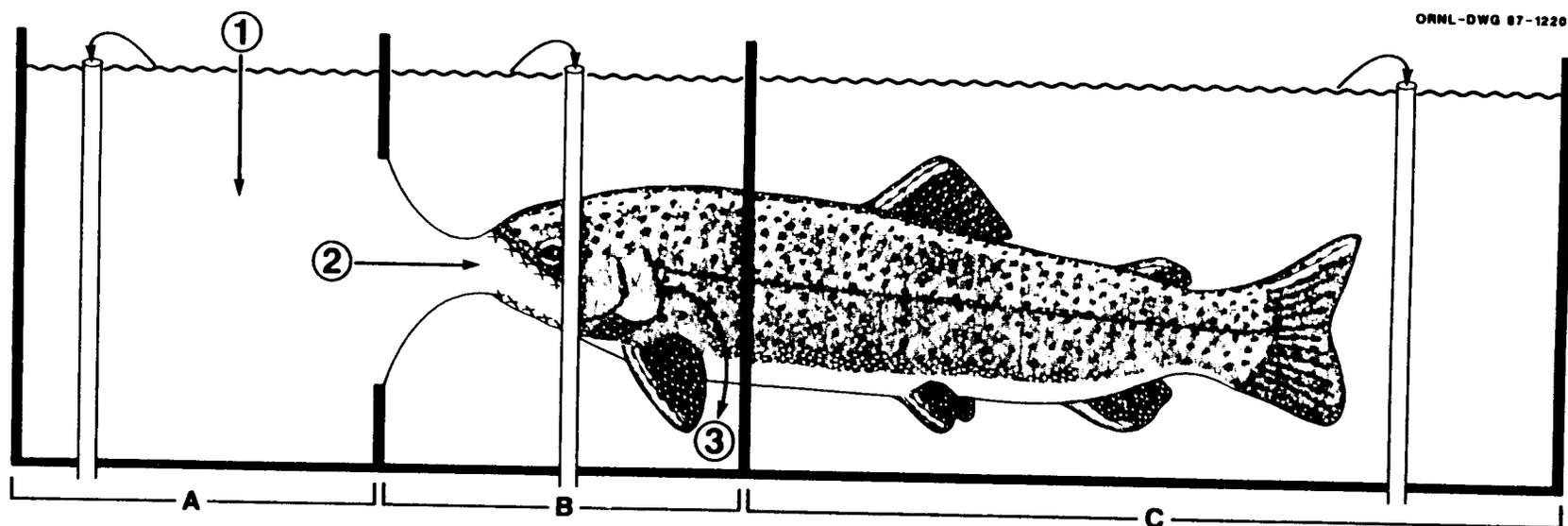


Fig. 4-8. The fish metabolic chamber. Testing procedure is as follows: (1) exposure water with O_2 and ^{14}C -labeled contaminant enters chamber A; (2) water contaminant and O_2 is pumped over the gills by the ventilatory action of the fish; and (3) water minus contaminant and O_2 extracted by the gills is expelled into chamber B.

over 3 h (0.04°C/min) and respiration and compound uptake were monitored at 15-min (B[a]P exposures) or 30-min (TCB and NPH exposures) intervals.

Time course exposure to chlorine

Rainbow trout were exposed to 0.04 mg/L chlorine (added as sodium hypochlorite) in large, flow-through tanks. Trout were removed at 0, 8, 12, and 24 h after exposure to chlorine for histopathological analysis of the gill tissue. Two groups of trout were allowed to recover from the chlorine exposures in clean water for 24 and 48 h before removal. Upon removal, trout were sacrificed and their gills were removed and preserved in 10% neutral formalin. Gill tissue samples were later dehydrated, mounted in paraffin, sectioned (4 µm), and mounted on slides. Duplicate slides from each sampling time were stained with Hematoxylin/Eosin, a general nuclear stain and Alcian Blue/Periodic Acid-Schiff's Base, a stain that detects mucopolysaccharides.

Bioaccumulation of contaminants by *D. magna*

Water samples from each fraction were filtered (Nucleopore, 0.22-µm) and adjusted to a pH of 6.5. Aqueous concentrations of B[a]P, TCB, and NPH were 1, 2, and 5 µg/L respectively; each concentration was below the compound's aqueous solubility (Mackay and Shiu 1980). Artificial, organic-free control water was prepared from Milli-Que water (DOC ≤ 0.1 mgC/l) and reagent-grade salts to a final Ca+Mg hardness of 1.0 mM, with pH adjusted to 6.5.

Before exposures, 5- to 7-d old *D. magna* without eggs in the brood chamber were held for 1 hr in clean control water. Five *D. magna* were placed in 100-ml beakers with 50 ml of a solution containing a radiolabeled contaminant and either total (unfractionated) water, a DOM fraction, or control water. Four replications were made per sample per treatment. The beakers were covered and stored in the dark at 21°C for 24 h. Animals were then removed from the water, rinsed in 50 ml distilled water, blotted dry, and weighed (each group from a beaker together) on a Cahn Model 21 electrobalance. Each group of 5 animals was added to a 10-ml ACS scintillation cocktail and counted for radioactivity. The amount of compound remaining in the water was measured in the same manner.

4.2.2.3 Measurement of contaminant binding to DOM

The binding of organic contaminants to DOM was measured using two methods: equilibrium dialysis and a Sep-Pak method. Unless otherwise noted, binding of contaminants to DOM was measured using equilibrium dialysis (Carter and Suffet 1982, McCarthy and Jimenez 1985a). Five milliliters of exposure water containing DOM were placed into a dialysis bag (Spectra/Por 6, MW cutoff of 1000 daltons) and equilibrated for 4 d in a ¹⁴C-labeled B[a]P solution. The percentage of freely dissolved B[a]P was determined from analysis of the radioactivity inside the bag (freely dissolved B[a]P + DOM-bound B[a]P) and the radioactivity outside the bag (freely dissolved B[a]P).

For the experiments on the effect of DOM on uptake of TCB by trout in the metabolic chamber, the TCB bound to DOM was separated from the freely dissolved TCB

using a C₁₈ Sep-Pack cartridge (Waters Associates). Then, 5 ml of exposure water with DOM and TCB were passed through a prerinsed (water) Sep-Pac cartridge, the eluate was collected, and the radioactivity measured by liquid scintillation counting. The percentage of freely dissolved TCB was determined by analysis of the radioactivity of the exposure water (freely dissolved + DOM-bound TCB) and the eluate (DOM-bound TCB).

4.2.2.4 Fractionation of DOM in water

Fractionation of water samples followed a modified protocol of Leenheer and Huffman (1979). Three fractions were collected: hydrophilic acid, hydrophobic acid, and hydrophobic neutral. Prior to fractionation each water sample was filtered through a 0.2- μ m glass fiber filter. For fractionation each water sample (150 ml) was acidified with concentrated H₂SO₄ to a pH < 2 and added to a purified XAD-8 column (bed vol = 5 ml) at a flow rate of 1.2 ml/min. The hydrophilic acid fraction (HL) was operationally defined as that organic material not retained by the column, while the hydrophobic acid fraction (HBA) was the organic material eluted when the column was rinsed with 0.1 N NaOH. Subsequently, the hydrophobic neutral (HBN) fraction was the organic material retained by the XAD-8 column and not eluted with base. The HL fraction was neutralized with 10 N NaOH; the HBA fraction was neutralized with HCl, and both fractions were diluted with Milli-Que water before use. The HBN fraction was extracted from the resin with 3 washes of 100 ml methanol. The methanol was pooled, diluted with 5 ml Milli-Que water, and roto-evaporated at 35°C to a final volume of 3 ml to remove the methanol. The remaining fraction (HBN) was removed and nitrogen purged for 2 h to remove any residual methanol. All fractions were analyzed for total organic carbon (TOC), and the percentage of total carbon was calculated and compared to the TOC present in the total water.

4.2.2.5 Analytical measurements

Dissolved organic carbon was measured in water samples using a Model 700 Total Carbon Analyzer (OI Corp., College Station, Texas); water samples were filtered through 0.2- μ m glass fiber filters prior to analysis. Radioactivity in water samples or in animals was measured by liquid scintillation counting using a Packard Tri-Carb 2000CA liquid scintillation counter.

4.2.3 Results and Discussion

4.2.3.1 Toxicity of PCB congeners to *D. magna*

The toxicity of most compounds is dependent on the accumulated dose to an organism. In order to study the uptake pattern for individual congeners, acute toxicity tests were conducted to determine the relative toxicity of different congeners to *D. magna* neonates and FHM (*Pimephales promelas*). Chronic toxicity tests to assess the adverse effects of sublethal concentrations of PCB congeners were also conducted.

Uptake of individual congeners increased over time as represented by 2,2',5,5'-TCB (Fig. 4-9), and the uptake curves for the congeners in this study agreed closely with the uptake of Aroclors by copepods (Wyman and O'Connor 1980). The toxicity test duration permitted the expected bioaccumulation of the congeners by *D. magna*, creating the potential for toxic response.

Forty-eight hour *D. magna* and 96-h FHM static and static-renewal toxicity tests showed that the LC50 values for both species were greater than the aqueous solubility limit for all congeners except 2,2',5-trichlorobiphenyl (TCB) (IUPAC No. 18) for FHM (Table 4-12). The FHM deaths were observed under static-renewal conditions and were possibly due to either the metabolism of the congener and the subsequent production of a toxic metabolite or to the fact that the aqueous concentration used in the test may have been greater than the aqueous solubility limit. The actual concentration of congeners in the test solutions are unavailable pending completion of analyses.

Results from the chronic toxicity test indicated that individual PCB congeners do not produce adverse effects to any aspect of the reproductive competence of *D. magna* (Fig. 4-10).

The lack of toxic effects in these tests may be explained by the dose of the congener within the organism. PCBs are believed to exhibit narcotic responses, thus acute toxicity is expected to occur when the chemical in the organism's internal body fluid is at equilibrium with that of the external (water) concentration. Because PCBs are hydrophobic compounds with high octanol-water partition coefficients and low aqueous solubilities, the congener concentration in the water and the time allowed for testing may not have been sufficient to reach equilibrium conditions. Additionally, the uptake of PCBs from the diet may be a more important route of exposure than bioconcentration from water, especially for fish (Crossland et al. 1987). Since the animals were not fed during the acute test period, this factor cannot be considered; however, chronic exposures in which *D. magna* were fed daily did not differ from acute exposures in terms of adverse effects.

The chronic results for an individual PCB congener in this study were similar to those reported by Benson and co-workers (1988). One implication, then, is that the toxic effects reported for commercial PCB mixtures may be due to other mixture components or to an interaction between different congeners.

4.2.3.2 Bioavailability of hydrophobic contaminants in water

The bioavailability of model hydrophobic contaminants by rainbow trout (*Salmo gairdneri*) and *D. magna* in the presence of DOM was investigated using selected PCB congeners and PAHs as model contaminants and naturally occurring DOM and Aldrich humic acid as DOM sources. A fish metabolic chamber was used to measure uptake of the contaminants by the gills of rainbow trout. By directly measuring gill uptake, the effect of binding to dissolved sorbents on contaminant uptake by fish can be quantified without the confounding influences of biotransformation, pharmacodynamics within the organism, and kinetic model limitations. The results of these experiments are described in detail in Black and McCarthy (1988).

In separate experiments, described in Kukkonen et al. (1988), *D. magna* were exposed to selected PAHs and PCBs in the presence of a natural water. To examine the

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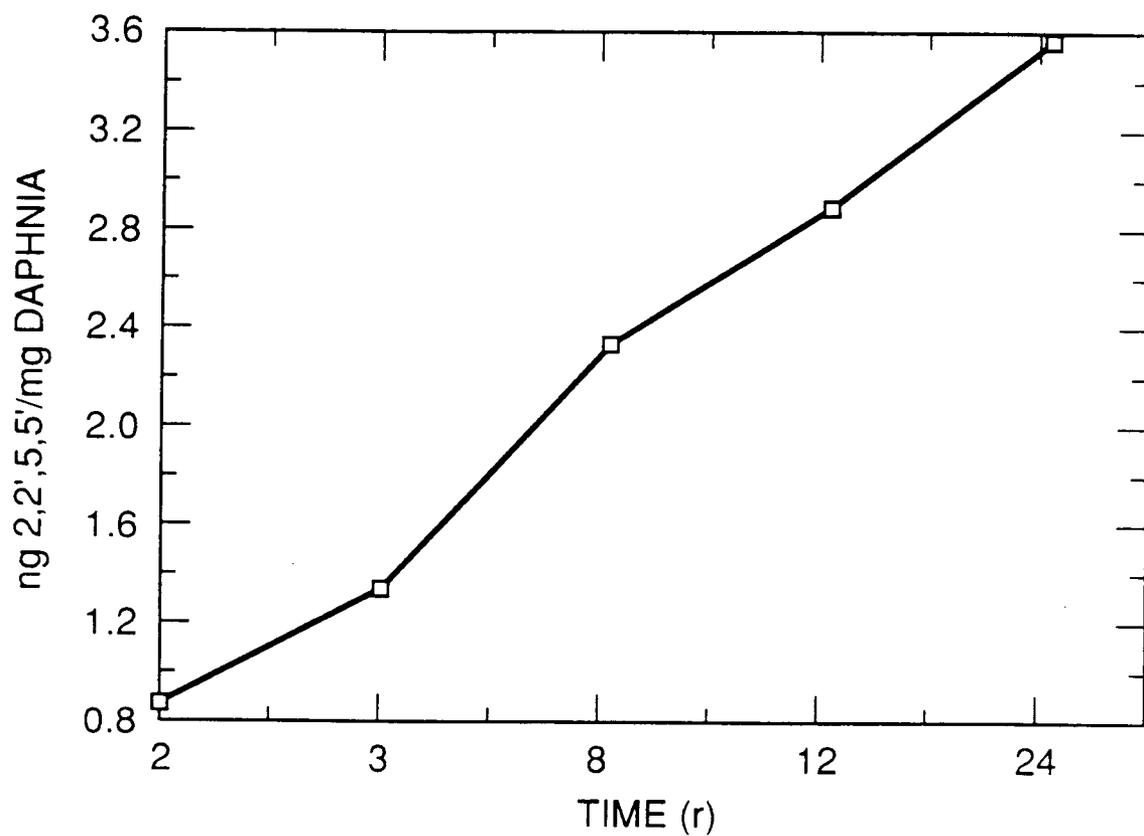


Fig. 4-9. Uptake of 2,2',5,5'-trichlorobiphenyl by *Daphnia magna* exposed in mass culture (150 animals in 3L).

Table 4-12. Acute toxicity of selected PCB congeners to *Daphnia magna* neonates and *Pimephales promelas* larvae

LC50 = the concentration which is lethal to 50% of the test organisms

Congener number	Congener name	Aqueous solubility limits (ASL) ($\mu\text{g/L}$)	LC50 (ASL)	
			<i>D. magna</i>	<i>P. promelas</i>
18	2,2',5-TCB	55	> ASL	33.8
28	2,4,4'-TCB	160	> ASL	> ASL
52	2,2',5,5'-TCB	30	> ASL	> ASL
77	3,3',4,4'-TCB	2	> ASL	> ASL
101	2,2',4,5,5'-PCB	10	> ASL	> ASL
116	2,3,4,5,6-PCB	8	> ASL	> ASL
128	2,2',3,3',4,4'-hexa-CB	0.6	> ASL	> ASL
153	2,2',4,4',5,5'-hexa-CB	1.3	> ASL	> ASL
171	2,2',3,3',4,4',6-hepta-CB	2	> ASL	> ASL
194	2,2',3,3',4,4',5,5'-octa-CB	0.2	> ASL	> ASL

Note: PCB = polychlorinated biphenyls; TCB = trichlorobiphenyl; CB = chlorinated biphenyl.

role of different fractions of the total DOM on the bioavailability of contaminants, toxicant exposures were conducted using fractionated and unfractionated DOM.

In both experimental approaches, reductions in contaminant uptake were compared with reductions in the amounts of freely dissolved contaminant measured in separate binding experiments. Comparisons were made to test the hypothesis that only freely dissolved contaminants can cross respiratory membranes and be accumulated by aquatic organisms. The relative importance of contaminant binding to each of the fractions of the natural water on contaminant bioavailability was also determined.

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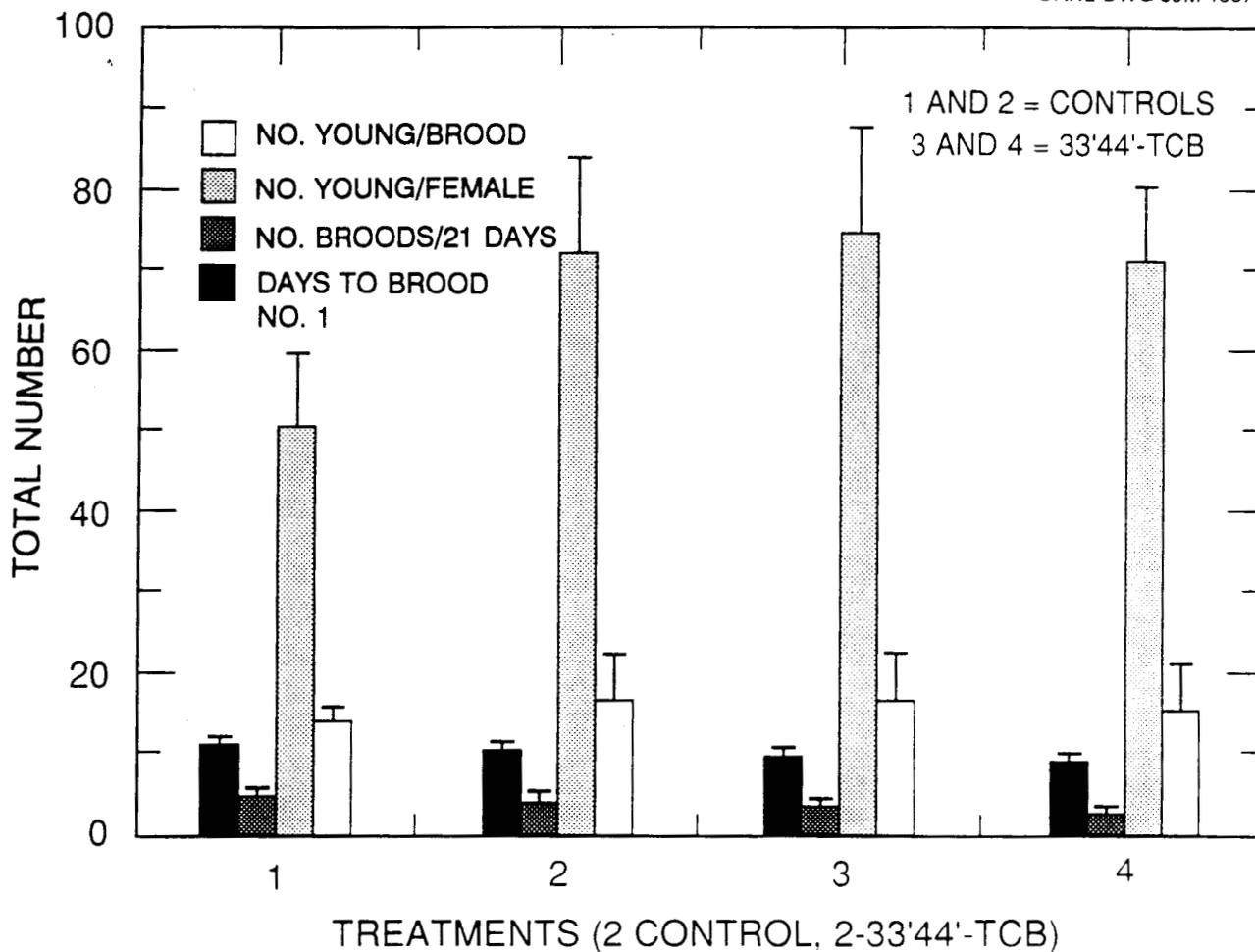


Fig. 4-10. Reproduction of *Daphnia magna* exposed to 3,3',4,4'-trichlorobiphenyl (mean of data for all animals).

Effect of DOM on uptake of contaminants by fish

The effect of DOM (Aldrich humic acid) on uptake of B[a]P and TCB by trout gills was measured using the fish metabolic chamber. These model compounds had different affinities for binding to DOM. The partition coefficients (K_p) for B[a]P and TCB were $3.6 (\pm 0.5) \times 10^6$ and $5.8 (\pm 0.4) \times 10^4$ respectively. These values compared favorably with literature values obtained using several different methodologies (McCarthy and Jimenez 1985a, Hassett and Milicic 1985).

The presence of the DOM did not alter the respiratory functions of the fish in the metabolic chamber. There were no significant changes ($p < 0.05$, Duncan's Multiple Range Test) in trout respiratory functions with DOM concentration increases in either the B[a]P or TCB exposures. Mean values for respiratory functions (\pm SE) were oxygen extraction efficiency, $57.6 (\pm 2.5)\%$; ventilation volume, $183.0 (\pm 10.1)$ mL/min; and ventilation rate, $97.4 (\pm 2.4)$ strokes/min.

DOM did reduce the apparent uptake efficiency of B[a]P and TCB by trout gills. As DOM concentrations increased, B[a]P and TCB uptake efficiencies decreased (Table 4-13). Reductions in uptake correlated with the reductions in the freely dissolved B[a]P or TCB. These observations support the hypothesis that only the freely dissolved compound can traverse the gill membrane, and thus is available for uptake by trout gills. Figure 4-11 demonstrates this relationship with measurements of the percentage of freely dissolved compound plotted against the percentage of control uptake (uptake measurements made in the presence of DOM divided by uptake in controls measured in the absence of DOM) for both hydrophobic compounds (HOC). The regression of the data points is not significantly different from the 1:1 relationship (dashed line) predicted by our hypothesis that only the freely dissolved compound is bioavailable. Thus, for all DOM concentrations, the following relationship was observed for uptake of B[a]P and TCB in the presence of DOM:

$$\text{Eff}_{(\text{with DOM})} = \text{Eff}_{(\text{no DOM})} \times f_{\text{free}} \quad (1)$$

where $\text{Eff}_{(\text{with DOM})}$ and $\text{Eff}_{(\text{no DOM})}$ are the gill uptake efficiencies in the absence and presence of DOM, respectively, and f_{free} is the fraction of PCB or PAH bound to the DOM, which can be calculated from the K_p and the concentration of DOM,

$$f_{\text{free}} = 1 / (1 + K_p \times [\text{DOM}]) \quad (2)$$

It is likely that the mechanism for the reduced bioavailability of contaminant bound to the DOM involves the inability of the DOM, including contaminant-bound DOM, to diffuse across the gill membranes. TOC concentrations measured in water collected from chambers A (before contact with gills) and B (after passing over the gills) were not significantly different ($p < 0.05$, Student's t-test), demonstrating that DOM was not taken up by the gills. The DOM molecule has a high molecular weight (ranging from <1000 to >100000 daltons [Thurman et al. 1982, Cole et al. 1984]) and contains a heterogeneous mixture of functional groups having a wide range of physicochemical properties. Diffusion of DOM and DOM-bound contaminants across the gill membrane may be hindered geometrically by the large size of the DOM molecule or chemically due to the predominately polar nature of the DOM molecule. The observation that DOM does not

Table 4-13. Physicochemical and biological uptake measurements for B[a]P and trichlorobiphenyl exposures at all dissolved organic matter (DOM) concentrations

log K_p	Physicochemical partitioning		Trout gill uptake efficiency (%)	
	DOM concn. (mg C/L)	Percentage freely dissolved ^a	Uncorrected for binding to DOM ^{a,b}	Corrected for amount bound to DOM ^c
B[a]P 6.3	0	100	52 ± 1.9 (6)	52
	0.1	66 ± 3.1 (3)	38 ± 1.0 (4)	57
	0.5	37 ± 0.5 (3)	17 ± 1.4 (6)	45
	3.0	14 ± 0.8 (3)	8 ± 1.2 (4)	56
TCB 4.8	0	100	50 ± 9.8 (6)	50
	3.4	85 ± 3.1 (6)	44 ± 8.0 (6)	51
	11.8	57 ± 2.4 (3)	26 ± 4.5 (3)	47

^aMeasured values ± 1 SE (number of observations).

$$^b\text{Uptake efficiency} = \left[\frac{([\text{HOC}]_{\text{chamber A}} - [\text{HOC}]_{\text{chamber B}})}{([\text{HOC}]_{\text{chamber A}})} \right] \times 100\%.$$

^cUptake efficiency calculated on the basis of the amount of HOC not bound to DOM = (uptake efficiency/percentage freely dissolved) × 100%.

Note: The data demonstrate the effect of contaminant binding to DOM on the percentage of freely dissolved hydrophobic compounds (HOCs) and gill uptake efficiencies. Calculation of the corrected uptake efficiency demonstrates that uptake efficiency is constant for both B[a]P and TCB if uptake calculations are based on the freely dissolved HOC concentration rather than the exposure water concentration.

diffuse across the gill membrane further supports our hypothesis that only the freely dissolved compound is bioavailable.

Predicting contaminant bioavailability in the presence of DOM. Based on this study, contaminants bound to DOM do not appear to diffuse across gill membranes. Furthermore, the fraction of freely dissolved contaminant quantitatively reflects the fraction of the contaminant that is bioavailable (Eq. 1). Therefore, the ability to determine the freely dissolved fraction of a contaminant in the natural environment would enable better predictions of contaminant uptake, bioaccumulation, and toxic effects. The fraction of freely dissolved contaminant can be calculated from the relationship between the partition coefficient (K_p) for contaminant binding to DOM and the concentration of DOM, (Eqs. 1 and 2). Since the bioavailable fraction of a contaminant in the presence of DOM is directly related to the fraction of freely dissolved contaminant (Equation 1), physicochemical determinations, such as K_p and the concentration of DOM, can be used to predict contaminant bioavailability when DOM is present. Although chemical differences exist between the commercially prepared Aldrich humic acid (used in this study) and DOM from natural sources, the mechanism of contaminant binding and the effect on bioavailability should be the same, regardless of the DOM source. However,

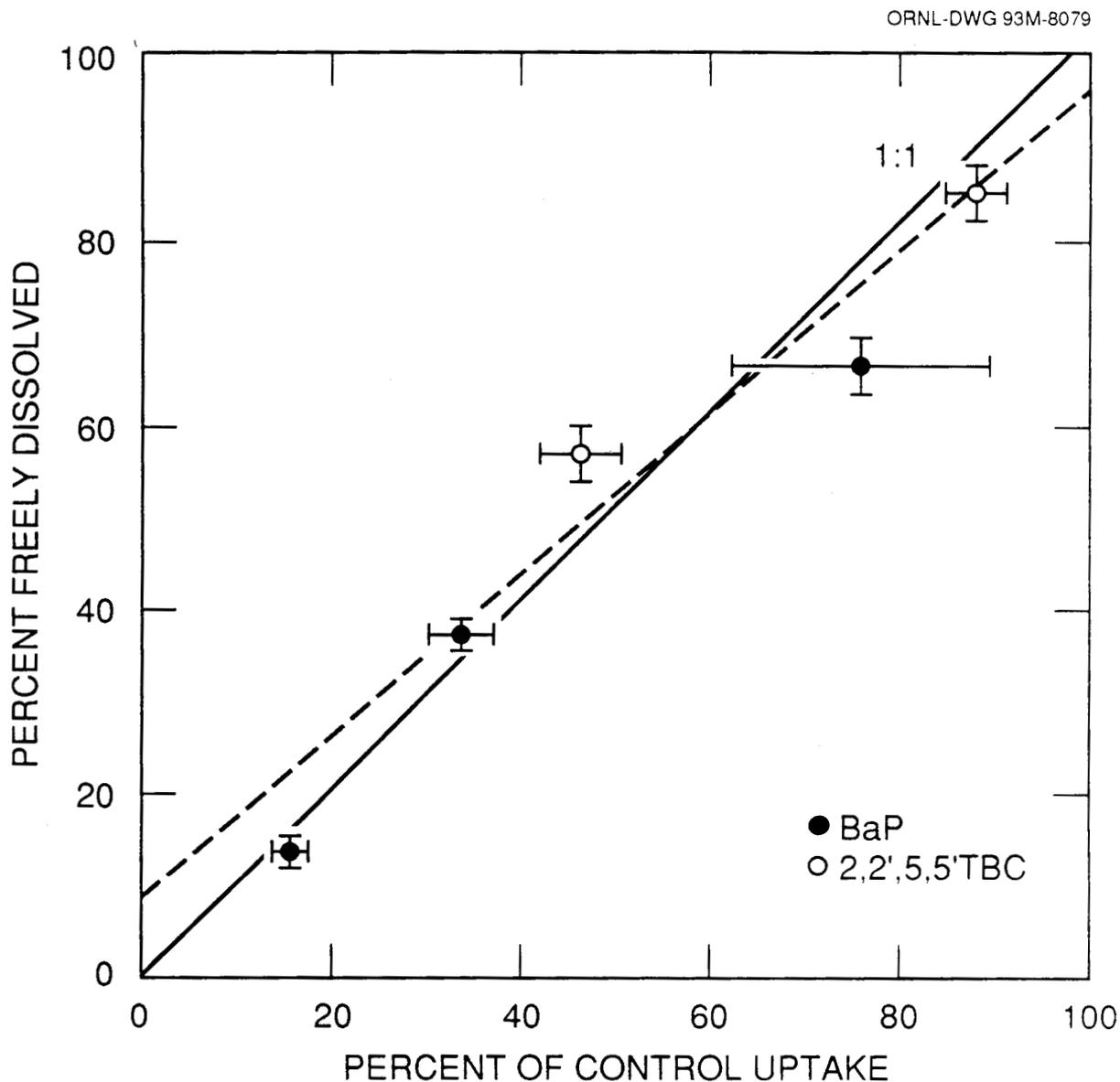


Fig. 4-11. Percentage of control uptake for benzo[*a*]pyrene (closed circles) and 2,2',5,5'-tetrachlorobiphenyl (open circles) plotted against the fraction of the total hydrophobic compound (HOC) that was freely dissolved. Each point represents mean values \pm SE for each variable. The regression equation is $y = 0.86x + 8.41$ ($r^2 = 0.91$; $n = 24$). The 95% confidence interval of the regression of the data points (dashed line) overlaps the 1:1 line (solid line) predicted by the hypothesis that only the freely dissolved HOC is available for uptake by trout gills.

another set of experiments was conducted to more rigorously test this hypothesis and to provide a more solid foundation for extrapolating physicochemical measurements of contaminant binding to predictions of contaminant bioavailability over a wide range of natural waters. These experiments are described in the following section.

Effect of DOM fractions on binding and bioavailability of contaminants

DOM from different natural sources has different chemical and structural compositions, which may vary seasonally and by location. These differences in naturally occurring DOM can affect its affinities for binding to hydrophobic contaminants (Carter and Suffet 1982, Morehead et al. 1986). The causes for the observed spatial and temporal variability of naturally occurring DOM are not well characterized, which hampers making accurate predictions of contaminant binding to naturally occurring DOM. This task investigated the roles of different fractions of DOM (hydrophobic acid, hydrophobic neutral, and hydrophilic fractions) on binding of PAHs and PCBs and on the bioavailability of these contaminants to *D. magna*. These data will be used to interpret the significance of seasonal and temporal variations of DOM in EFPC on sorption and bioavailability of contaminants in the stream.

Due to the difficulties in isolating the required volumes of the hydrophobic and hydrophilic fractions (HBAs and HLs) of the total DOM from EFPC water samples [which contain low organic carbon concentrations (<10 mg of carbon per liter)], water samples containing 50 mg of carbon per liter were selected as a source of DOM from a stream flowing through a peat deposit in Hyde County, North Carolina. This water was fractionated as previously described. The percentage of total carbon in each fraction was hydrophilic acid (HL), 21.5%, hydrophobic acid (HBA), 66.5%, and hydrophobic neutral (HBN), 12.5%.

Binding of contaminants to DOM fractions. The affinity of B[a]P, NPH, and TCB for binding to the DOM in each fraction was determined by equilibrium dialysis. The partition coefficient (K_p) of the HBA fraction for binding to B[a]P was $1.97 \times (\pm 0.23) \times 10^5$, significantly higher ($p < 0.05$) than the K_p measured for the HBN fraction [$1.18 (\pm 0.34) \times 10^5$]. The HL fraction exhibited much lower binding affinity for B[a]P [$K_p = 2.4 (\pm 0.6) \times 10^4$] than did the other two fractions (Fig. 4-12). The binding affinity of the total water appeared to reflect the sum of the binding affinities of the individual fractions, with little indication of interactive effects among the fractions (Fig. 4-12). The measured K_p for the total water agreed well with the cumulative K_p calculated from the sum of the K_p for the individual fractions [$K_{p(i)}$] and the relative contribution (f_i) of the i^{th} XAD fraction to the total DOM:

$$\text{Sum } K_p = [K_{p(\text{HL})} \times f_{\text{HL}}] + [K_{p(\text{HBA})} \times f_{\text{HBA}}] + [K_{p(\text{HBN})} \times f_{\text{HBN}}] \quad (3)$$

TCB and B[a]P appear to bind differently with respect to each DOM fraction, suggesting compound-specific binding mechanisms. The HBN fraction exhibited the greatest affinity for binding to TCB [$K_p = 1.59 (\pm 0.19) \times 10^5$]. The HBA and HL fractions exhibited significantly less affinity for binding to TCB (Fig. 4-13). The calculated sum of fraction K_p agreed with the measured K_p of the total water. This affinity of PCBs for the HBN fraction was investigated further by repeating the experiment with three other PCB congeners (2,2',4,4'-tetrachloro-biphenyl, 3,3',4,4'-tetrachlorobiphenyl, and

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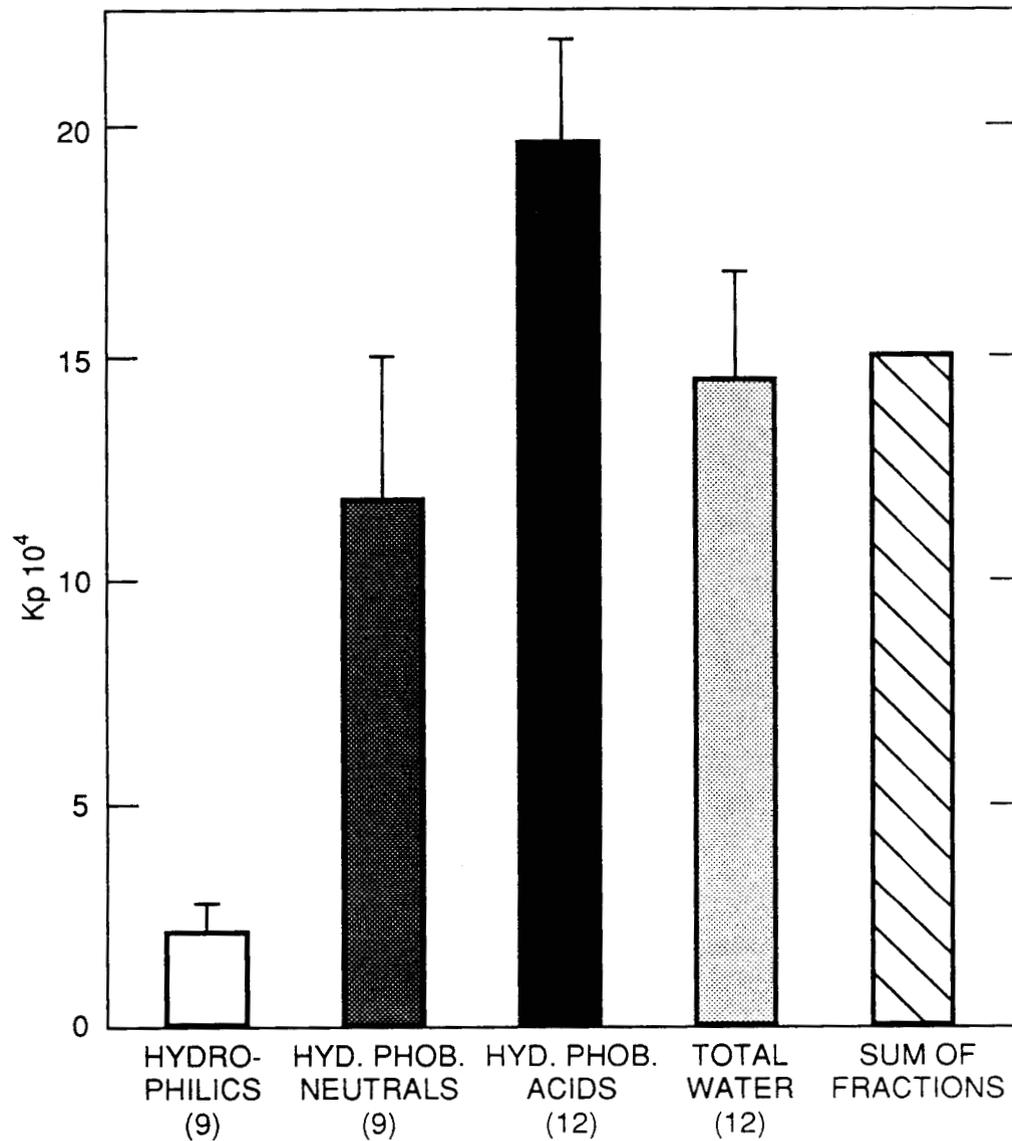


Fig. 4-12. Partition coefficients (K_p s; units of liters per kilogram of carbon) for benzo[a]pyrene in different dissolved organic carbon fractions and in the total water. Number of replicates are indicated in parentheses. Bars indicate the standard error. The sum of fractions is calculated from the K_p s of the fractions and the percentage of each fraction in the total water.

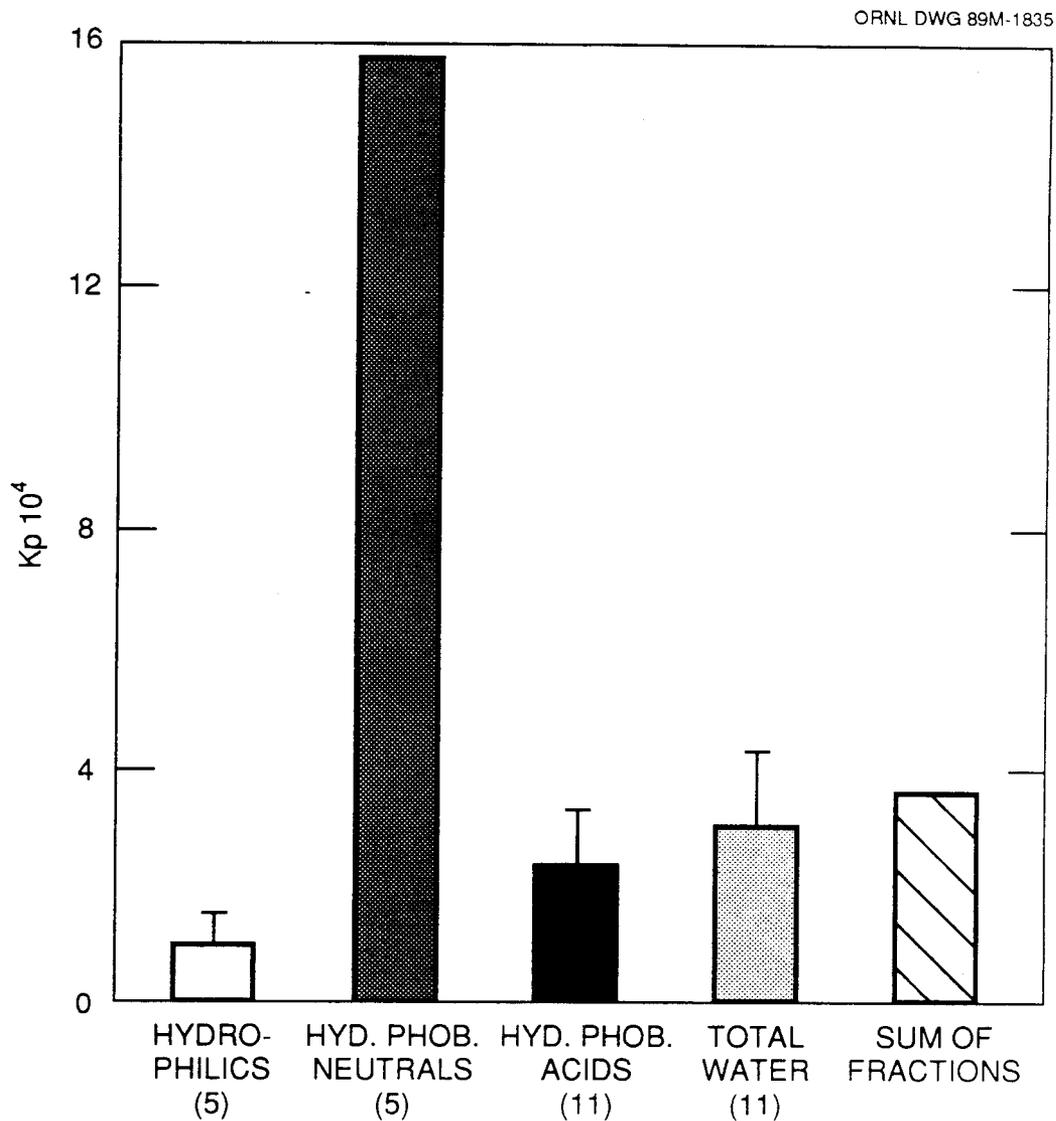


Fig. 4-13. Partition coefficients (K_p s) for 2,2',5,5'-tetrachlorobiphenyl in different fractions and in the total water. Numbers of replicates are indicated in parentheses; bars indicate the standard error. The sum of fractions is calculated from the K_p s of the fractions and the percentage of each fraction in the total water.

2,2',4,4', 5,5'-hexachlorobiphenyl). For all congeners tested, PCBs preferentially bound to the HBN fraction.

There was little binding of NPH to any of the fractions. The K_p values were low (~1000). This was the expected result based on the low hydrophobicity of NPH (McCarthy and Jimenez 1985b). NPH was included in this study as a negative control for binding and bioaccumulation experiments.

Bioavailability of contaminants to *D. magna*. Accumulation of B[a]P by *D. magna* was reduced by increasing the concentration of DOM. The magnitude of the decrease in accumulation was similar in the total water and the HBA fraction (Fig. 4-14). In both cases, DOC concentrations of 1, 2, 5, 15 and 25 mg C/L were used. The bioconcentration factor (BCF) for B[a]P in the control water was 5990 ± 670 . Accumulation was significantly lower at all DOM concentrations ($p < 0.05$). Data were fit to a logarithmic function [$BCF = (\text{control BCF}) \times e^{-a(\text{DOM})}$] to compare the effects of these DOMs (total water vs HBA fraction) on bioaccumulation of B[a]P. As in previous studies (Kukkonen et al. 1988), data fit this expression well, and there were no statistically significant differences ($p < 0.05$) between the fitted parameters for total water and HBA fraction (Fig. 4-15).

The HBN and HL fractions also reduced the bioaccumulation of B[a]P compared to the control but not to the extent of the HBA fraction (Fig. 4-14). Approximately five times more organic carbon from the HL fraction was required to reduce the BCF to the extent observed for the HBA fraction or the total water. The potency of the HBN fraction was intermediate to the two other fractions.

The observed data for bioaccumulation of B[a]P by the different DOM fractions was compared to predicted BCFs, based on the assumption that B[a]P bound to the DOM is unavailable for uptake by the organism; i.e., bioaccumulation in water containing DOM will be proportional to the fraction of the contaminant that is freely dissolved (f_{free}):

$$(\text{BCF in presence of DOM}) = (\text{control BCF}) \times (f_{\text{free}}) \quad (4)$$

The f_{free} is calculated from the measured K_p (Fig. 4-12) and DOM concentration of either the total water or the HBA fraction (Eq. 2).

In spite of large differences in the K_p of different DOM fractions, the observed reductions in bioaccumulation, expressed as a percentage of the control BCF (BCF in the presence of DOM divided by BCF in control water), agreed well with predictions based on the calculated concentration of freely dissolved B[a]P from Eq. 5 (Fig. 4-15). The resulting regression equation was as follows: percentage of freely dissolved B[a]P = $1.03 \times (\text{percentage of control BCF}) - 0.84$; where $r^2 = 0.75$, and $n = 53$. The 95% confidence interval for this regression overlapped the 1:1 line, predicted by the hypothesis that only the freely dissolved compound is available for uptake (Eq. 4). Previous research using Aldrich humic acid or natural waters demonstrated a similar relationship between reductions in the freely dissolved contaminant and accumulation in *D. magna* (McCarthy et al. 1985), *Pontoporeia hoyi* (Landrum et al. 1985), and rainbow trout (Black and McCarthy 1988). This study extends these observations to subcomponents of natural DOM and provides additional confirmation that the effects of natural DOM on bioavailability of organic pollutants can be predicted from physicochemical measurements of K_p s.

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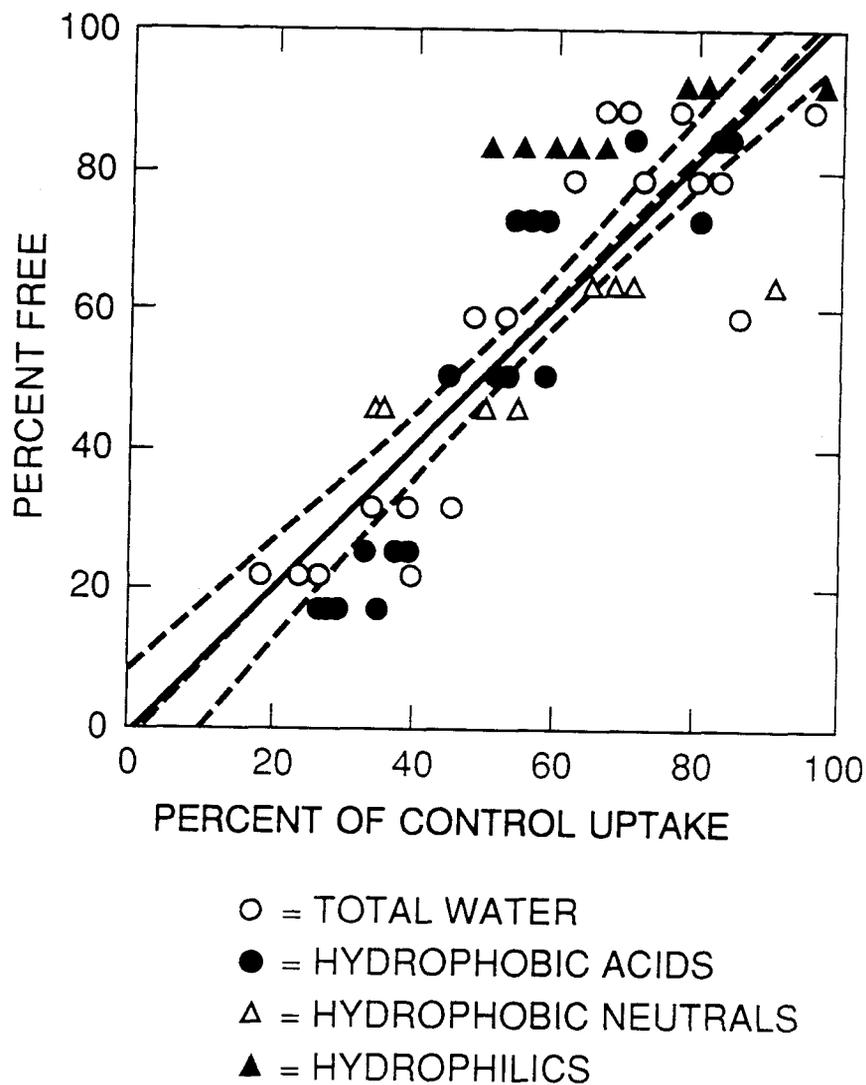


Fig. 4-14. Proportional (percentage) uptake of benzo[*a*]pyrene [bioconcentration factor (BCF) in the presence of dissolved organic carbon divided by the control BCF] plotted against the percentage of the total B[*a*]P that was freely dissolved, based on the measured K_p . This plot represents the data from individual replicate determinations of B[*a*]P uptake for the different fractions, which are indicated by different symbols on the plot. The regression equation is $y = 1.03 \times 0.84$ ($r^2 = 0.75$, $n = 52$). The 95% confidence interval (dashed curves) of the regression line (dashed line) overlaps the 1:1 line (solid line) predicted by the hypothesis that only the freely dissolved B[*a*]P is available for *D. magna*.

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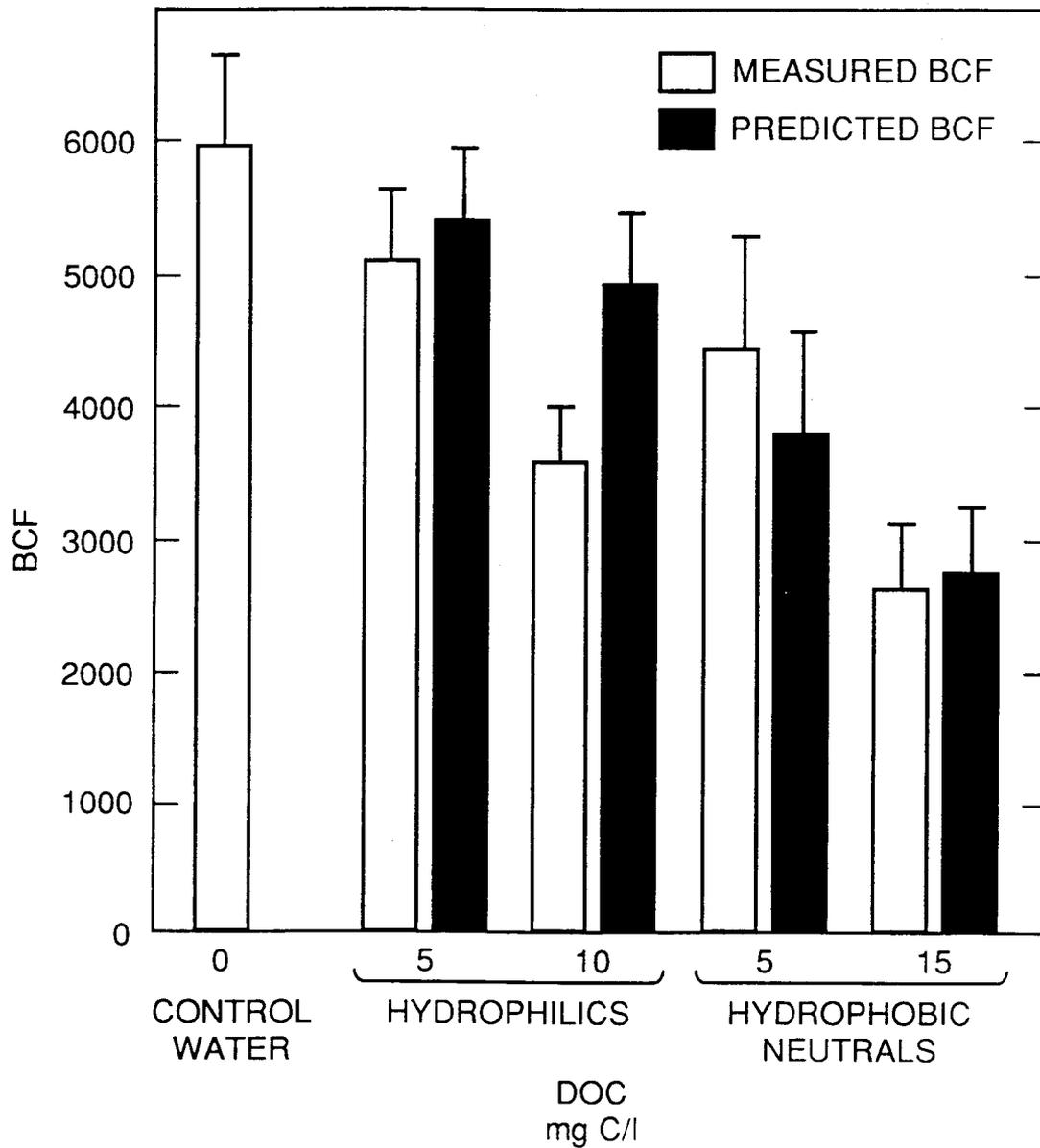


Fig 4-15. The measured and predicted bioconcentration factors (BCFs) for benzo[a]pyrene in two dissolved organic carbon concentrations of hydrophilic and hydrophobic-neutral fractions. The bars of measured BCFs are means of four replicates. The bar indicates the standard deviation.

Similar relationships between K_p of DOM fractions and bioavailability are also observed for TCB and NPH, although the data included fewer DOM concentrations than for B[a]P. The bioaccumulation of TCB (Fig. 4-16) was measured at 10 mg C/L for the HL and HBN fractions and at 5 and 10 mg C/L for the HBA fraction and the total water. The BCF of TCB in the control water was 6540. Only the HBN fraction, the higher concentrations of the HBA fraction, and the total water reduced the bioaccumulation of TCB significantly ($p < 0.05$) from the control value (Fig. 4-16). The HBN fraction was more effective in reducing the accumulation of TCB than the other carbon sources, although the effect was not significantly different from that observed for the HBA or the total water at the same DOM concentration. However, the decrease in the BCF due to this fraction was much less than anticipated on the basis of its affinity for binding TCB (Fig. 4-16). A "biological K_p " can be back-calculated based on the reduction in BCF with the HBN fraction. This value (approximately 6.4×10^4) is lower than the K_p measured using equilibrium dialysis but is still higher than those determined for the other fractions or the total water.

The bioaccumulation of NPH in *D. magna* was much less than for the other compounds. The BCF value in the control water was 36.5 ± 4.4 . There were no significant differences between BCF values for the control and DOM fractions. These results, coupled with the observed low values for K_p , confirm that NPH does not have much interaction with DOM compared to B[a]P and TCB.

In summary, the concentration of DOM determined the bioavailability of B[a]P and TCB. The observed BCF values were in good agreement with those predicted from K_p values; the reductions in freely dissolved compound were equal to the decreases in the accumulation of that compound. These observations support the hypothesis that only the freely dissolved toxicant is available for uptake by *D. magna*.

Variability in the composition of DOM in EFPC. Water samples were taken from EFPC at monthly intervals from August 1986 through September 1987. DOM samples were taken at quarterly intervals. Sample sites, as designated throughout this report, began at the outfall of NHP (EFK 23.4) and ended downstream at EFK 2.1. Initial water samples from EFK 23.4, EFK 22.9, and EFK 13.0 were taken in August 1986. The binding affinity of the DOM in these waters to TCB was measured by equilibrium dialysis. Water samples collected in October 1986 and January 1987 were fractionated to determine the relative composition of the HL, HBA, and HBN fractions.

There were no significant differences in TOC concentrations from site to site or seasonally (Fig. 4-17) with the exception of the NHP station in the January 1987 sampling, which had a much higher TOC concentration than at any other site or at any other time (11 mg of carbon per liter).

Binding studies conducted with the streamwater samples collected in August 1986 demonstrated that the affinity of the DOM for binding to TCB varied by location, even though the TOC concentration did not vary. The K_p s were 8×10^4 , 1×10^4 , and 7×10^4 for EFK 23.4, EFK 22.9, and EFK 13.0 respectively. Variation in the binding affinity of the DOM in these waters for TCB seems to be site dependent and did not follow any downstream gradient. The K_p measured at the NHP site and the ORWTF site (EFK 13.0) was higher than that found at the primarily agricultural site (EFK 22.9). Even though the total carbon present at these sites is low, the DOM present may be composed of large, complex macromolecules which have been found to augment binding to organic

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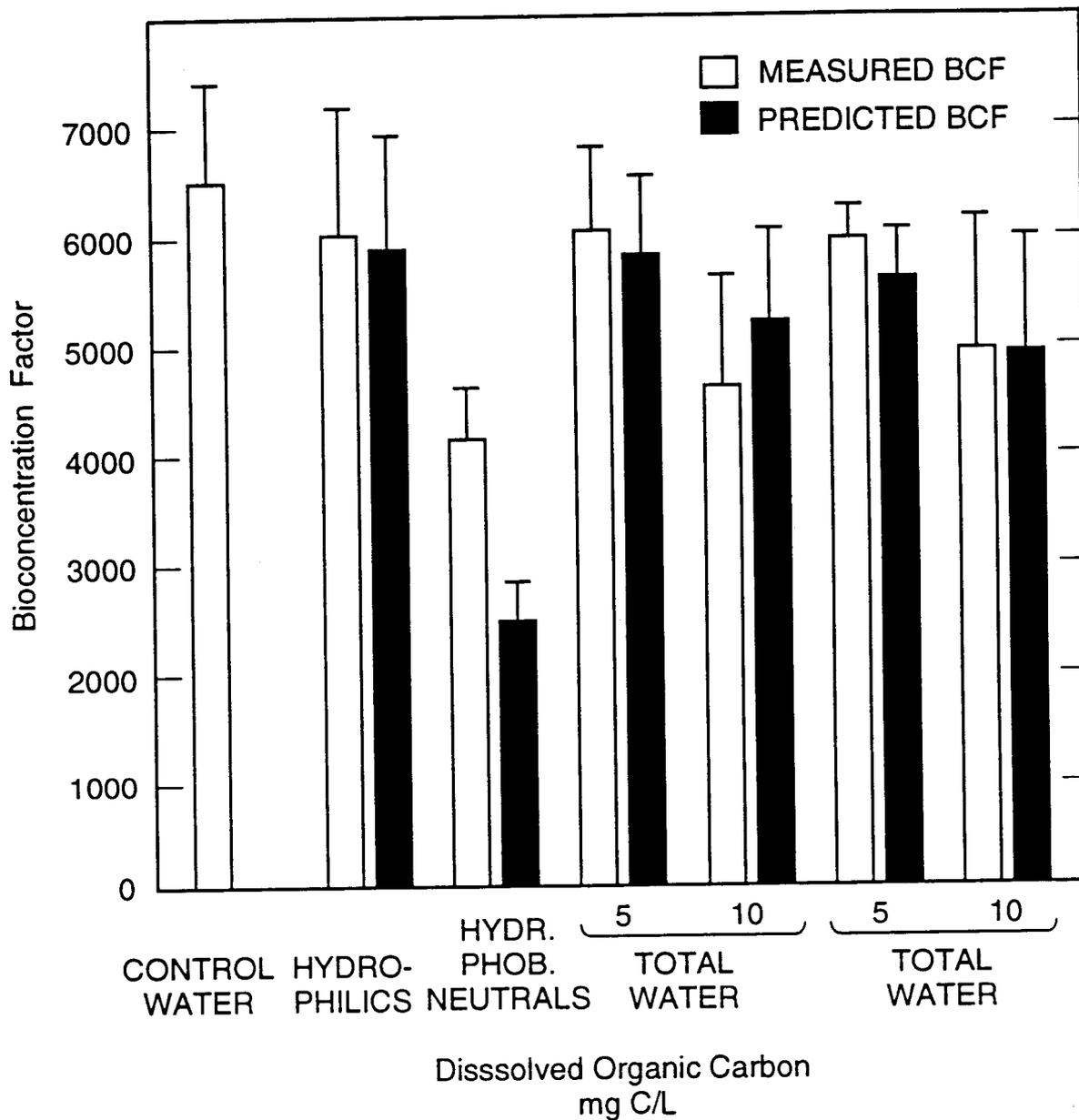


Fig. 4-16. The measured and predicted bioconcentration factors (BCFs) for 2,2',5,5'-tetrachlorobiphenyl in the different fractions and in the total water. The bars of measured BCFs are means of four replicates. The bar indicates the standard deviation.

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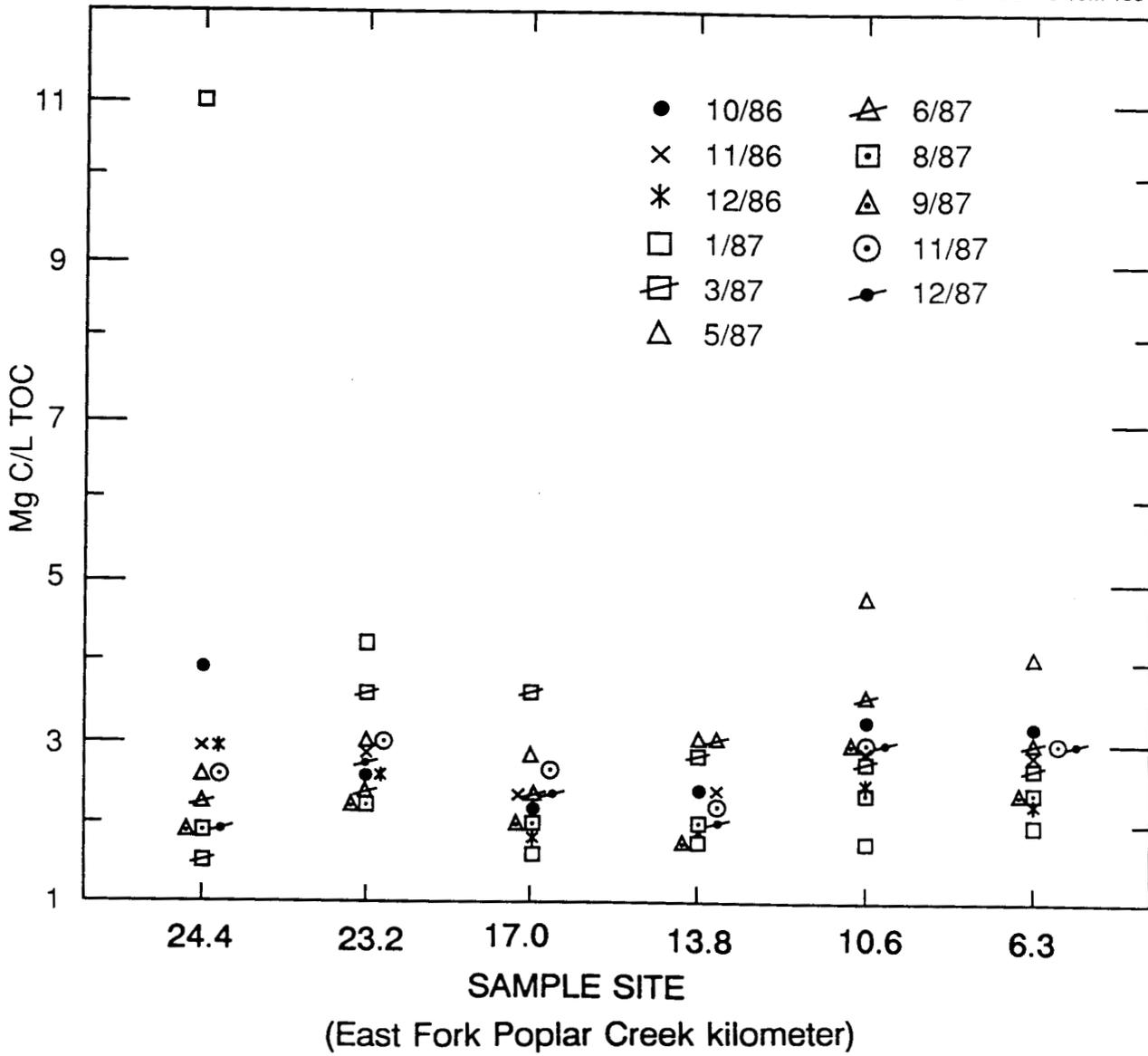


Fig. 4-17. Total organic carbon (TOC) values (measured in milligrams of carbon per liter) for each sample site in East Fork Poplar Creek. Sampling dates are listed in key.

compounds such as TCB. We therefore examined the composition of the DOM along EFPC.

The relative abundance of the different DOM fractions in EFPC water varied with season and location. In October, the HL fraction made up 90% of the total carbon present at the NHP site. Only a short distance downstream, however, the composition of the organic matter underwent a substantial change, with significant increases in the percentage of the TOC present as HBN (approximately 50% of the TOC) or HBA fractions. Since the TOC level remains relatively constant downstream from EFK 23.4 (except for one sample in January 1987), it appears unlikely that the decreased composition of HL material is due to microbial mineralization of readily metabolized low-molecular-weight organic acids or phenols discharged from NHP. Little variation in the percentage of carbon per fraction was found at the remaining downstream sites (Fig. 4-18). The January sample was similar to that of October in that 90% of the total carbon was composed of the HL fraction at the NHP site (Fig. 4-19). The HL fractions decreased from 88% to 43%, moving downstream from NHP; whereas the HBA fraction increased from 5% to 35%, moving downstream. There were generally lower amounts of the HBN fraction compared to the other two fractions (Fig. 4-19) in the January 1987 sample, and considerably less of the HBN at this sampling date than in the October 1986 sample. The reasons for the variability in the nature of the DOM in EFPC is unclear, however the implications of this variability in DOM composition on binding and bioavailability of contaminants is being investigated.

4.2.3.3 Physiological factors affecting contaminant uptake

The influence of changes in respiratory functions (e.g. ventilation volume, ventilation rate, and oxygen uptake) on contaminant uptake by fish was investigated using a fish metabolic chamber. Two experimental protocols were followed, using an invasive and a noninvasive method of altering fish respiration. Trout were exposed to PCB congeners concomitantly with chlorine, a potent gill-tissue oxidizer known to cause significant increases in fish respiration. Chlorine is also a common co-contaminant in many polluted environments and has been identified as a potential toxicant released in effluents from the Y-12 Plant (Loar et al. 1992b). In separate experiments trout were exposed to selected PAHs and PCB congeners while experiencing an acute temperature drop (approximately 8°C). In each experiment changes in trout respiratory functions were compared with changes in contaminant uptake.

Effects of chlorine on trout gills

Histological evidence of chlorine damage to gill tissue. After an 8-h exposure to chlorine, trout gill lamellae had a 3-fold increase in the number of mucous cells, a trend that continued throughout the chlorine exposure. Abnormal lesions were evident after a 12-h exposure, including hyperplasia of lamellar epithelial cells and clubbed lamellae. Lamellar fusion was seen following the 24-h sampling time. Recovery began to be evident after 48 h in clean water; abnormal lesions on the lamellae were mostly absent, yet lamellae still had elevated numbers of mucous cells. These abnormal pathologies should have two primary effects on fish respiration. Epithelial cell hyperplasia and lamellar clubbing will increase the diffusion distance for oxygen crossing the gill membrane.

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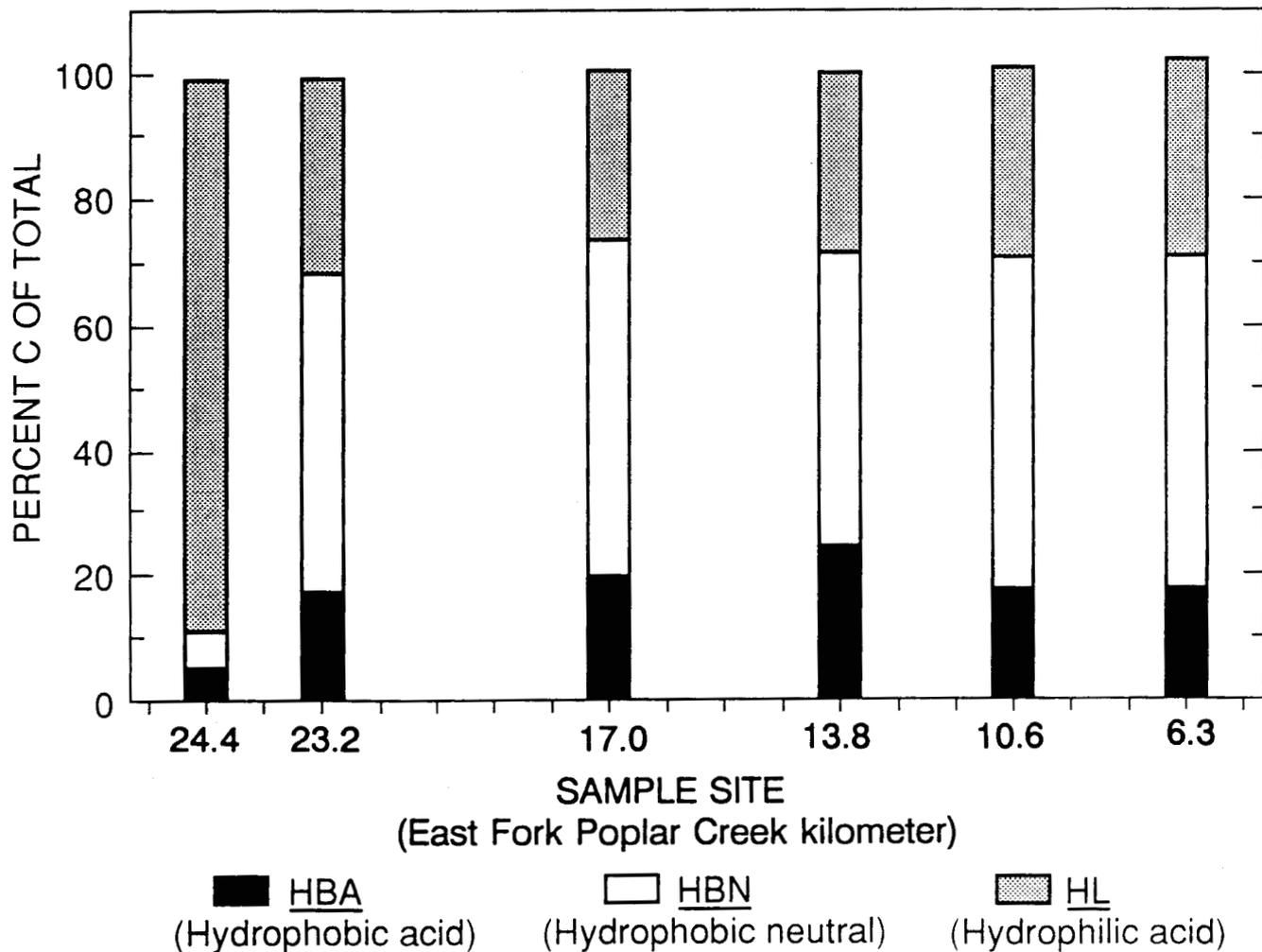


Fig. 4-18. Percentage of total organic compounds (in milligrams of carbon per liter) in each fraction (hydrophobic neutral, hydrophobic acid, and hydrophilic acid) of a water sample collected in October 1986 in East Fork Poplar Creek.

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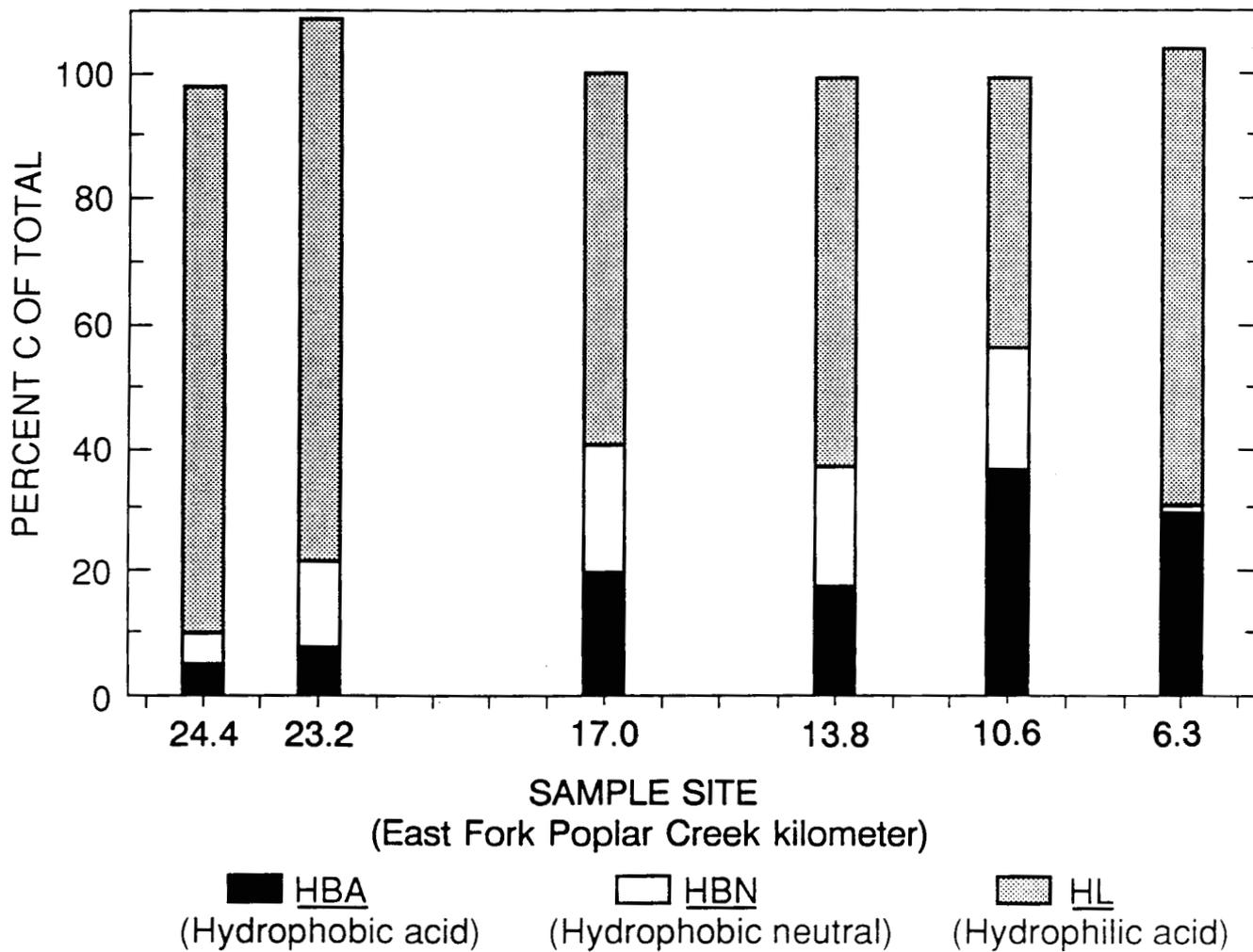


Fig. 4-19. Percentage of total organic compounds (measured in milligrams of carbon per liter) in each fraction hydrophobic neutral, hydrophobic acid, and hydrophilic acid) of a water sample collected in January 1987 in East Fork Poplar Creek.

Proliferation of mucous cells on the lamellae and lamellar fusion will decrease the functional surface area of the gill. Both of these pathologies might be expected to reduce the efficiency with which oxygen (and possibly dissolved contaminants) are taken up by trout gills.

Effects of chlorine on trout respiration and PCB uptake. Oxygen and PCB uptake efficiencies were reduced as the time of exposure to chlorine increased (Fig. 4-20). These results were expected due to the observed effects of chlorine on lamellar diffusion distances and gill functional surface area. Decreases in oxygen uptake efficiency due to chlorine-induced gill damage were paralleled by similar decreases in PCB uptake efficiency throughout the chlorine exposure period. By plotting oxygen uptake efficiencies vs PCB uptake efficiencies for all three congeners (Fig. 4-21), the relationship between the two variables was evident. For all three PCB congeners, the regressions were not significantly different ($p < 0.05$) from the 1:1 line, signifying an equivalent effect of chlorine-induced changes in gill pathology on the efficiency of oxygen and PCB uptake by the fish gill.

In order to compensate for decreased oxygen uptake efficiencies, trout exposed to chlorine had increased ventilation volumes (over 100% increase after 24-h chlorine exposure compared to preexposure values) and ventilation rates (15% increase after 24-h chlorine exposure) (Fig. 4-22). Respiratory increases resulted in the maintenance of nearly constant oxygen consumption values throughout the exposure to chlorine. Thus, the fish was able to compensate for the reductions in oxygen uptake efficiency caused by chlorine-induced changes in gill pathology. PCB consumption also remained constant, reflecting the same effect of chlorine on the uptake of both molecules. This result was expected because oxygen and PCBs both enter the gill by the same mechanism, passive diffusion across the gill membrane.

Recovery of fish respiration and oxygen and PCB uptake was monitored at 4 and 24 h after exposure to chlorine ceased. After 4-h recovery, ventilation functions were still elevated, and oxygen and PCB uptake efficiencies remained significantly lower than preexposure values. After the 24-h recovery period there was evidence of recovery. Ventilation functions and oxygen and PCB uptake efficiencies were within 15 and 12% of preexposure values respectively.

Effect of acute temperature change on fish respiration and contaminant uptake

Effect of decreased temperature on trout respiration. As the water temperature decreased, trout ventilation volumes and ventilation rates decreased 33 and 20% respectively (average of all exposures). Surprisingly, oxygen uptake efficiencies were decreased by 34 and 18% in the B[a]P and NPH exposures but were unchanged in the TCB exposures. This result was not expected because the efficiency of transfer of molecules across the gill membrane was thought to be limited by their contact time with the gill. Reductions in oxygen uptake efficiencies have been measured concurrently with increases in ventilation volumes and rates due to increased activity (Saunders 1962), hypoxia (Kerstens et al. 1979, McKim and Goeden 1982), and experimental manipulation of gill ventilation (Davis and Cameron 1971). In our experiments other physiological phenomena must be limiting oxygen uptake efficiencies in cooled trout. The effect may be due to lowered oxygen binding capacity of salmonid hemoglobins known to occur in response to decreased temperature. Low uptake efficiencies coupled with decreased

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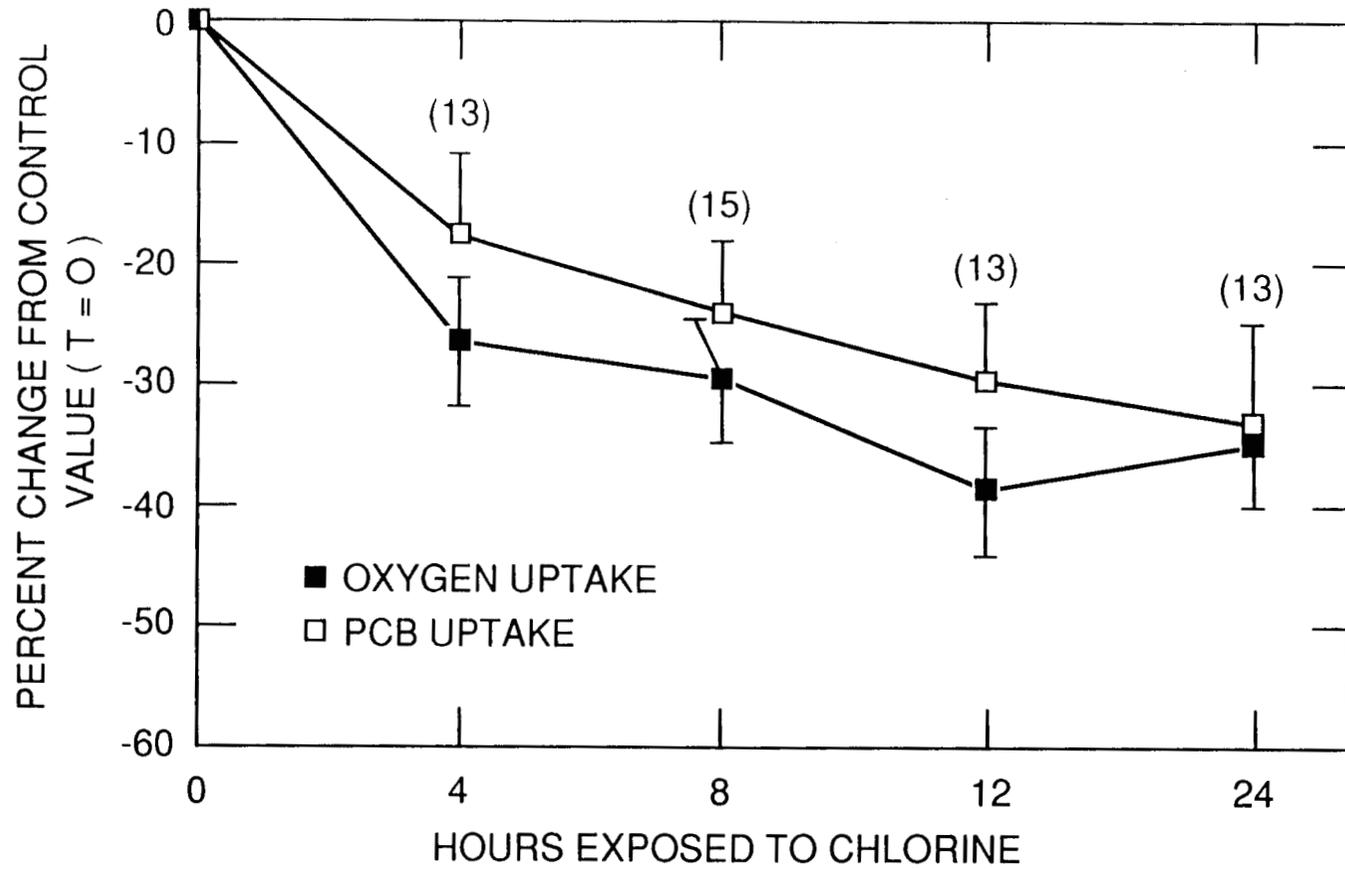


Fig. 4-20. Percentage change (± 1 SE) in O_2 uptake efficiencies (closed squares) and polychlorinated biphenyl uptake efficiencies (open squares) during the 24-h exposure to chlorine in the metabolic chamber.

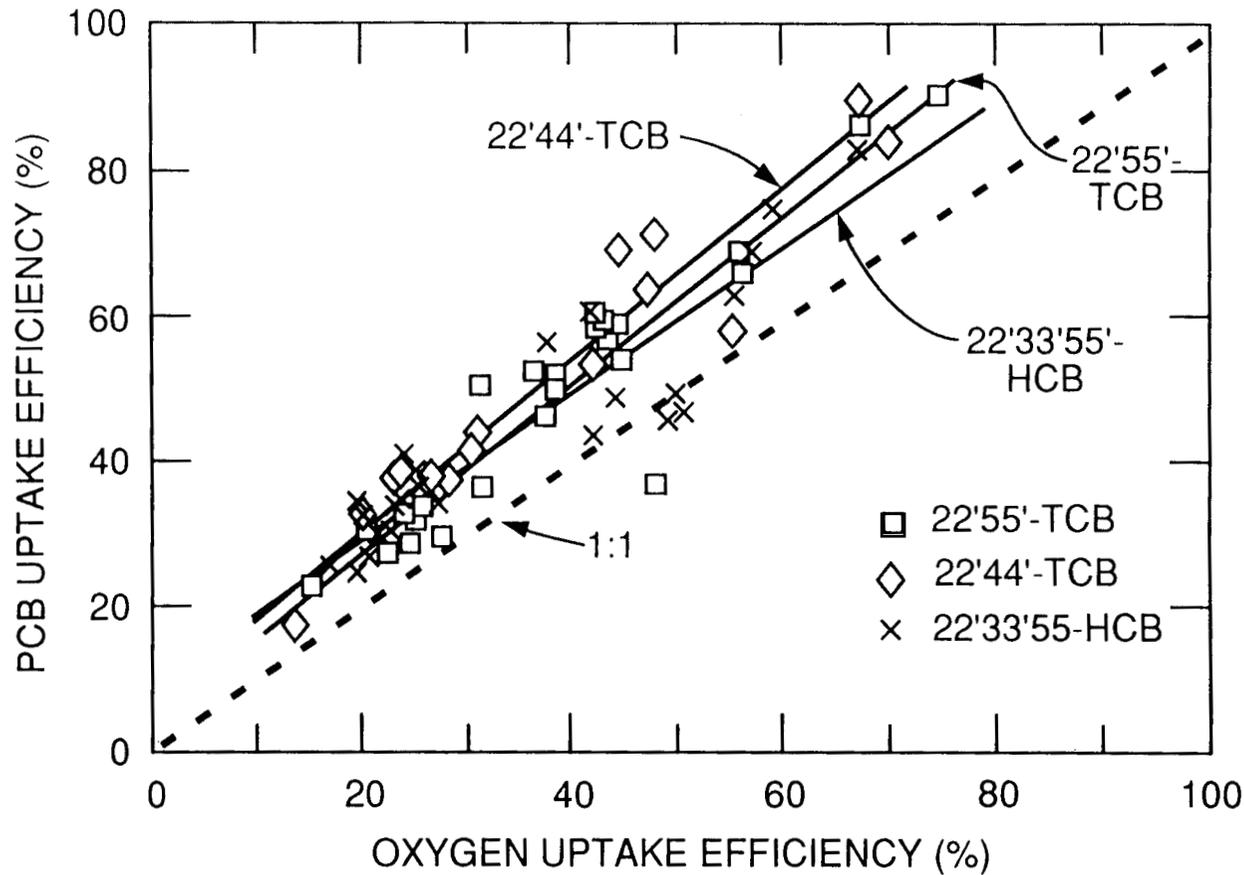


Fig. 4-21. Linear relationship between oxygen and polychlorinated biphenyl (PCB) uptake efficiencies during the metabolic chamber exposures to chlorine and PCBs for all three PCB congeners (22'44'-TCB, 22'55'-TCB, and 22'33'55'-HCB). The dashed line represents the 1:1 relationship predicted by the hypothesis that the uptake of both O_2 and PCBs are affected equally by exposure to chlorine. There were no significant differences ($p < 0.05$) in the slopes or intercepts of the regressions for the three congeners and the 1:1 line or between the three congeners.

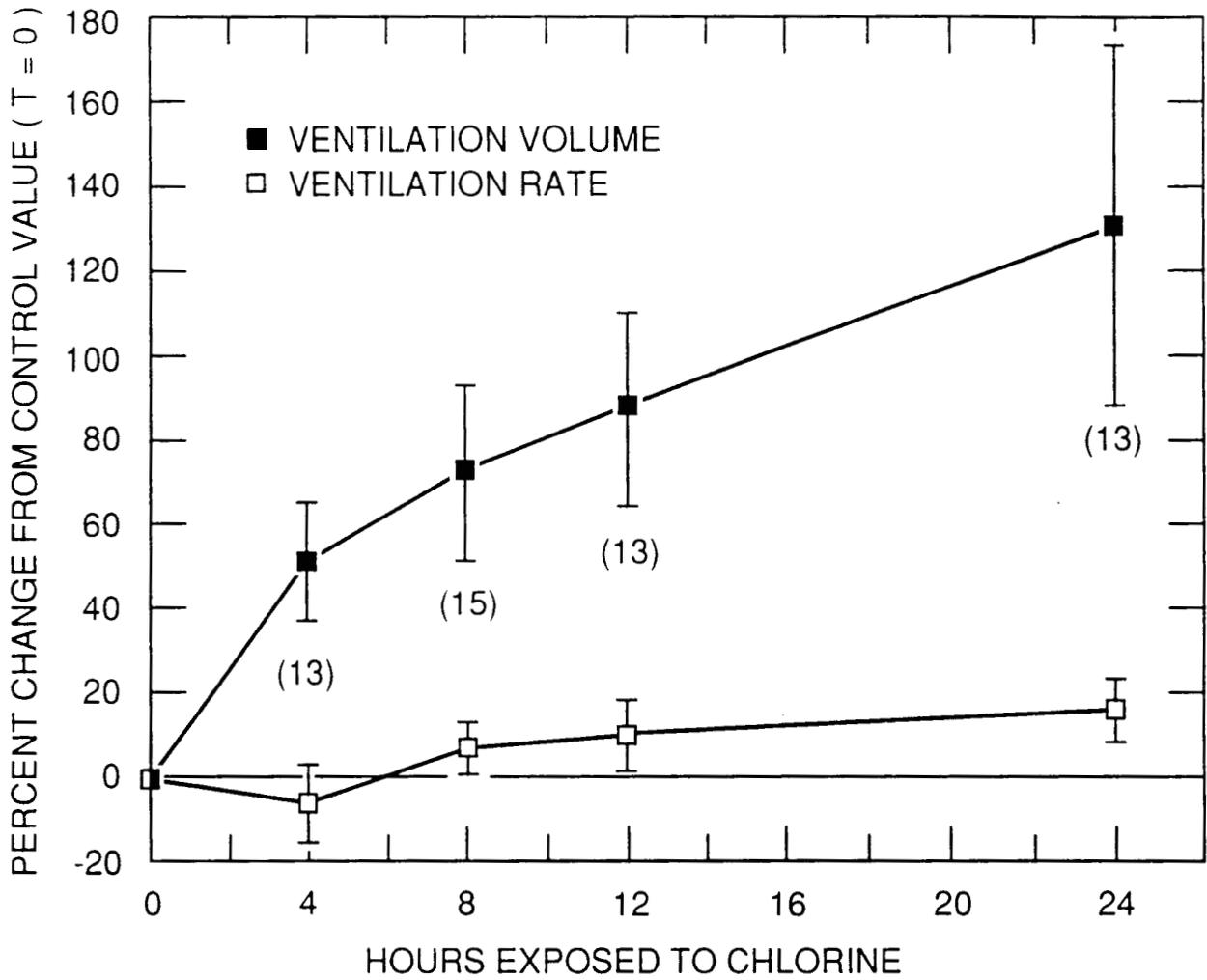


Fig. 4-22. Percentage change (\pm SE) in ventilation volume and ventilation rate compared to the control value during the 24-h exposure to chlorine and polychlorinated biphenyls in the metabolic chamber. Closed squares = ventilation volume; open squares = ventilation rate; control value (T = 0; no chlorine).

ventilatory functions resulted in decreases in the oxygen consumption of the cooled trout in all three exposures. Reductions in oxygen consumption for B[a]P, NPH, and TCB exposures were 51, 39, and 20% respectively.

Effect of decreased temperature on contaminant uptake. The temperature decrease had a similar effect on compound uptake efficiencies compared to oxygen uptake efficiencies. Uptake efficiencies for B[a]P and NPH decreased 23 and 8.5% respectively. The uptake efficiency for TCB increased slightly (7%). Significant linear relationships exist between oxygen and compound uptake efficiencies for all three compounds (Fig. 4-23), indicating that acute temperature change affected the diffusion of both molecules across the gill membrane in a similar manner. Consumption rates were also reduced for all three compounds. Percentage reductions for consumption of B[a]P, NPH, and TCB were 56, 40, and 20% respectively.

Effect of fish respiration on contaminant uptake efficiency

A consistent linear correlation was found between the efficiency of oxygen and contaminant uptake by fish gills, using both invasive (chlorine-induced gill tissue damage) and noninvasive (acute temperature change) methods to alter fish respiration (Figs. 4-21 and 4-23). This relationship was not significantly different from a 1:1 correlation between oxygen and PCB uptake efficiencies in both studies. The relationship between oxygen uptake efficiency and B[a]P and NPH uptake efficiency was also linear; however, the slope for each of these two curves was <1 (0.83 and 0.74 respectively).

Even though the relationship between oxygen and contaminant uptake was not always a 1:1 relationship, the overall trend for all compounds in both treatments was the same. Changes in oxygen uptake were quantitatively related to changes in toxicant uptake for all compounds tested. These results suggest that estimates of toxicant uptake can be made using oxygen uptake data as the basis for extrapolation. Since the two variables were linearly related over a wide range of values (for uptake efficiencies ranging from less than 20% to greater than 90%) using two dramatically different treatments (damaged gills vs temperature-induced physiological changes in metabolic demand), these experiments seem to confirm that the uptake of oxygen and hydrophobic organic contaminants occur by the same mechanism and that uptake of both molecules are affected similarly by environmental and physiological changes.

These relationships form the foundation for extrapolating contaminant dose to an organism over seasonal cycles or over the lifecycle of a population. Seasonal temperature changes and changes in metabolic demand due to growth and reproduction have well-characterized effects on oxygen demand by biota. Considerable literature exists on changes in oxygen consumption of numerous species over their life cycle or with season. The relationships documented in this research task provide a basis for using that extensive body of physiological data to predict variations in the dose of contaminant to which an organism will be exposed. It can be predicted, for example, that increases in metabolic demand due to growth, reproductive activity, or increased temperature will increase exposure of the organism to organic contaminants in the water. These relationships can be incorporated into quantitative bioenergetics models to enhance our capabilities of predicting the exposure of aquatic animals to toxicants.

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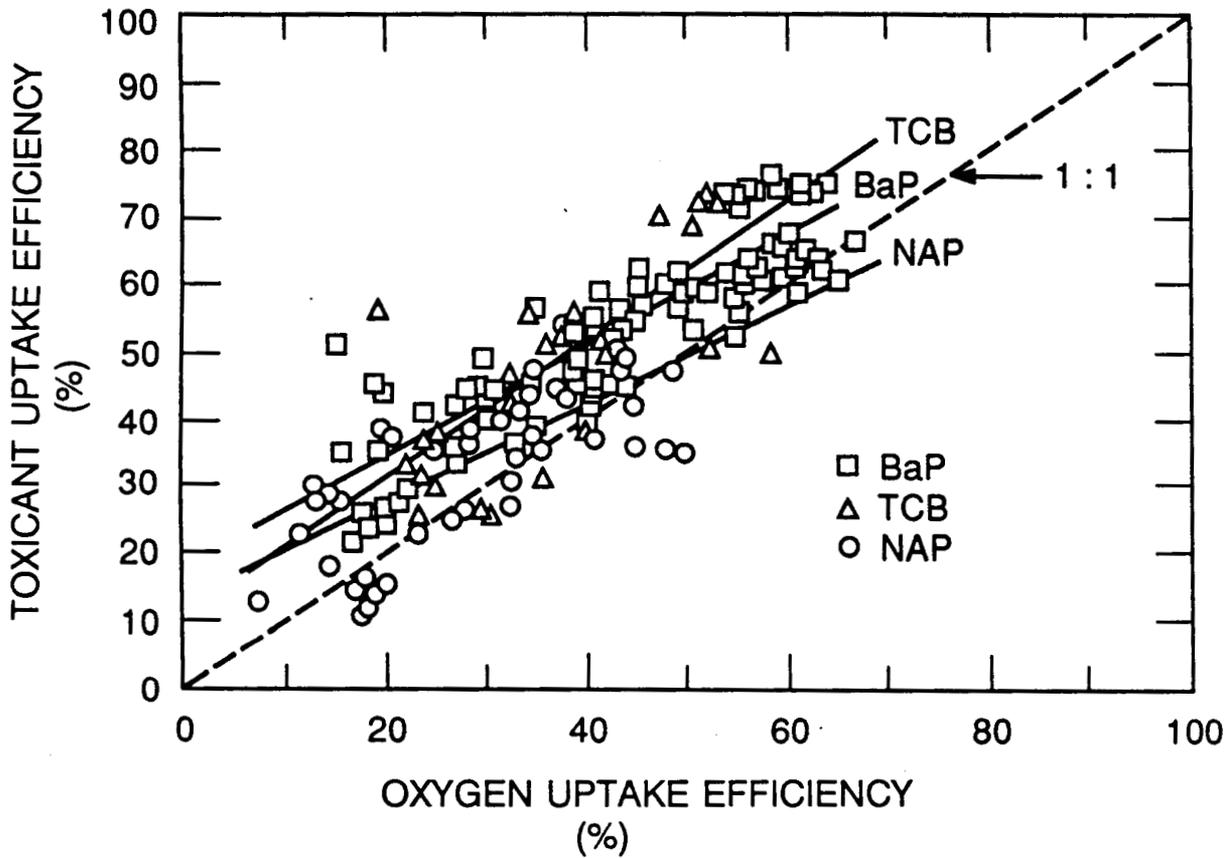


Fig. 4-23. Linear relationships between O_2 and compound uptake efficiencies for B[a]P, 2,2',5,5'-tetrachlorobiphenyl, and naphthalene during acute temperature change. The dashed line represents the 1:1 relationship between the two variables. For all three compounds the relationship between O_2 and compound uptake is linear, and for 2,2',5,5'-TCB, the regression is not significantly different from the 1:1 line ($p > 0.05$).

4.2.4 Summary and Conclusions

Based on our research, the following conclusions were reached:

4.2.4.1 Toxicity of PCB congeners

1. The uptake trends for the PCB congeners agreed with the trends for a commercial PCB mixture.
2. The LC50 values for the ten congeners were greater than the aqueous solubility limits for all but one of the congeners.
3. Sublethal concentrations of the PCB congeners used in this study did not cause adverse effects to *D. magna*.

4.2.4.2 Bioavailability of hydrophobic contaminants in water

1. Binding to DOM reduces the uptake of hydrophobic organic contaminants by fish gills. Reductions in uptake are equal to reductions in the freely dissolved compound and can be predicted from determinations of the binding coefficient, K_p , and the concentration of the DOM (Eqs. 1 and 2).
2. Binding of PAHs or PCBs to DOM or fractions of DOM reduced the bioavailability of B[a]P, NPH, and TCB to *D. magna*. The reduction in accumulation was directly related to the amount of the contaminant bound to the DOM or fraction of the DOM.
3. These data support the hypothesis that only the freely dissolved toxicant is available for uptake.
4. The concentration of TOC along EFPC did not vary substantially with season; however the partition coefficients for binding of contaminants were found to vary, depending on location. The variation in size and complexity of DOM molecules present at these sites, as reflected in the fractionation data, may account for these differences.

4.2.4.3 Physiological factors affecting contaminant uptake

1. Exposure to chlorine caused gill tissue damage resulting in changes in gill membrane diffusional properties (diffusion distance and functional surface area of the gill). Oxygen and PCB uptake efficiencies were reduced to an equivalent extent. Ventilatory functions compensated for the gill damage so as to permit oxygen consumption to remain constant, but these compensatory adjustments also maintained a constant PCB dosage throughout the chlorine exposure.

2. Manipulation of trout respiration by acute temperature changes resulted in similar changes in oxygen and compound uptake efficiencies. Data from this experiment and the chlorine exposures suggest that toxicant uptake can be estimated using oxygen uptake data.

4.2.5 Future Research

4.2.5.1 Equilibrium binding of compounds with DOM

Data presented in this report strongly suggest the presence of selective binding of hydrophobic compounds by subcomponents of naturally occurring humic materials. The removal of these compounds from the dissolved or free-water phase reduced both biological uptake and observed toxicity in the three organisms studied here. Future investigations will concentrate on further characterization of the subcomponents of organic matter responsible for binding hydrophobic contaminants and subsequent reduction in toxicity. Because partition coefficients are known to be compound specific, as well as organic matter specific, further investigation could allow development of quantitative structure-property relationships that can be used to predict partition coefficients and binding mechanisms for a variety of compounds and humic materials.

4.2.5.2 Kinetics of sorption between hydrophobic compounds and DOM

Equilibrium modeling of fate and transport processes in the environment is seldom descriptive of real world situations. For example, the rate of contaminant transport to and across fish gills could be similar (in time) to desorption or disassociation of hydrophobic compounds from humic materials. Measurement of these desorption rates will allow comparison of the rate constants for each reaction and, thus, allow determination of the rate-limiting step. Additionally, transport of hydrophobic compounds from contaminated sediment to overlying waters and biota, such as that found in the EFPC, could be controlled by the presence of humic materials. Kinetic data for each application are needed to elucidate the importance of these transport mechanisms.

4.2.5.3 Effect of physicochemical partitioning and physiological factors on contaminant accumulation in EFPC biota

Biological uptake and accumulation experiments will be conducted in parallel with the studies on steady state partitioning and the kinetics of sorption of contaminants with sediment and DOM. Routes of uptake from water, sediment, and food-chain will be considered. These experiments will also consider the role of environmental factors such as changes in respiratory function on contaminant accumulation. The thrust of these studies will be to test and confirm predictive mechanisms derived from previous work in this task.

5. BIOLOGICAL INDICATORS OF CONTAMINANT-RELATED STRESS

S. M. Adams and M. S. Greeley, Jr.

5.1 INTRODUCTION

A principal advantage of using bioindicators in a biomonitoring program is that it permits simultaneous investigation of a spectrum of biological sensitivities to stress and specificities of effects. This approach not only maximizes predictive capabilities and increases understanding of causal mechanisms responsible for any stress effects observed in organisms, it also optimizes the cost and time involved in biomonitoring programs (Adams 1990a).

The bioindicator task has been designed to address three important areas relative to the operation of the Y-12 Plant on the fish communities in EFPC: (1) the effect of Y-12 discharges on the health of fish communities, (2) the effects of past and ongoing clean-up and remedial actions on these fish communities, and (3) the causative agents or mechanisms responsible for any effects observed on the fish communities.

The initial Y-12 BMAP report (Loar et al. 1992b) presented the results of the bioindicator studies conducted from fall 1985 through spring 1986. Based on the results of these original studies, the strategy of applying bioindicators in field biomonitoring studies has been modified somewhat to not only optimize efforts and costs but to address those questions and concerns most pertinent to the operation of the Y-12 Plant. These primary concerns are (1) the effects, if any, of Y-12 Plant operations on the health of fish populations in EFPC, (2) the effects of the various clean-up and remedial action programs on fish community status, and (3) the causative agents or mechanisms responsible for any changes or effects observed on the fish communities in EFPC.

To address these issues of concern relative to operation of the Y-12 Plant, data from the bioindicator studies conducted from spring 1986 to fall 1987 will be analyzed by two principal approaches: (1) functional group analysis and (2) integrative bioindicator analysis. The functional group approach will involve analysis of each of the five major groups of biotic responses we have measured in fish (carbohydrate-protein metabolism, lipid metabolism histology, condition indices, and detoxification enzymes) to determine if there are spatial differences in fish health between areas in EFPC and if temporal differences or changes in fish health have occurred over time in various sections of EFPC. The integrative bioindicator analysis involved using all of the bioindicators together within a multivariate context to investigate holistic responses of fish to stress and to help identify the causative agents or mechanisms most responsible for observed effects on organisms. In addition to addressing spatial and temporal concerns and identifying causative mechanisms of effects, this section also includes reports on two special studies initiated in 1988; biomolecular and reproductive indicators of stress.

5.2 METHODS

5.2.1 Sampling Procedures

Sampling was conducted during spring 1986, summer 1986, spring 1987, and fall 1987 at four sites in EFPC: (1) immediately below NHP (EFK 23), (2) behind Robertsville Junior High School near Route 95 (EFK 19), (3) above ORWTF (EFK 14), and (4) near the USGS gaging station (EFK 5) (Fig. 2-1). Fish were also collected from reference sites on Brushy Fork (BF) near BFK 7.0 (Fig. 2-2).

A minimum of 12 adult redbreast sunfish were collected by electroshocking at each site during each season. Within two minutes after collection, blood samples were taken from each fish for various biochemical analyses. Blood was collected by puncturing the caudal vessels with a 20-gauge needle. Samples of approximately 0.7 mL were obtained from all fish using unheparinized 3-mL vacutainers (Becton, Dickson, & Co.). Each tube was labeled with a fish identification number and placed in a container with ice for transport to the laboratory.

5.2.2 Analytical Procedures

Total lengths and weights were recorded for fish transported from the field, and observations were made on the general condition of the fish, such as presence or absence of fin disease, body and/or mouth sores, external parasites, and general starvation status. Following sacrifice, the liver and spleen were removed from each fish for further analysis. A 100-mg section of liver for histopathological analysis was placed in a 20-mL scintillation vial with 5 mL of Bouin's fixative. A 300-mg sample of liver for detoxification enzyme analysis was placed in a small plastic bag and immediately frozen in liquid nitrogen for subsequent ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) analysis. The spleen was also removed from the body cavity, weighed to the nearest milligram, and a section was placed in Bouin's fixative for subsequent histopathological analysis. For females the ovary was removed, weighed to the nearest milligram, and placed in a separate vial with 10 mL of Bouin's fixative for future enumerations of egg numbers and size. The viscera was excised from the body cavity and the total weight recorded after all food material was removed from the stomach and intestines. Liver and visceral-somatic indices were calculated as the weights of these respective organs divided by the total body weight. Condition factor was calculated as $K = 10^5 W/L^3$, where W is body weight (expressed in grams) and L is total length (expressed in centimeters).

5.2.2.1 Lipid analysis

Following dissection and removal of the critical organs, four individuals of each sex from each site were chosen for lipid analysis. These fish were frozen at -120°C until shipment to the subcontractor. Lipid biochemical analysis was performed according to a modification of the Bligh and Dyer (1959) method and using the Iatroscan Analyzer System (Harvey and Patton 1981) for lipid class quantification. The Iatroscan system for lipid analysis combines the resolution capabilities of thin layer chromatography with the quantitative sensitivity of a flame ionization detector. Lipid analysis for each fish included

total lipids (percentage of body weight); triglycerides (percentage of total lipids); sterols, including cholesterol and/or sterol esters; phospholipids; and the two major fractions of phospholipids, phosphatidyl choline (PC) and phosphatidylethanolamine (PE).

5.2.2.2 Serum chemical analysis

Blood collected in the unheparinized tubes was allowed to clot, transferred with Pasteur pipettes to 1.5-mL conical microcentrifuge tubes labeled with the fish identification number, and centrifuged for 2 minutes in a Beckman Microfuge. The clear supernatant (serum) was drawn off with clean pipettes and transferred to labeled 1-mL conical plastic tubes. Serum glucose, serum glutamate oxaloacetate transaminase (SGOT), bilirubin, cholesterol, and triglycerides were analyzed by the ORNL Health Division and the ORNL Chemical Technology Division, using a Rotochem Ila 36-cuvette centrifugal analyzer (Burtis et al. 1973).

Glucose analysis was performed according to the method of Peterson and Young (1968); SGOT was analyzed following the procedures of the Scandinavian Committee on Enzymes (1974); cholesterol was analyzed by the method of Allain et al. (1974); triglycerides were analyzed by the procedure of Bucolo and David (1973); and bilirubin was determined by the method of Tietz (1986). All of these methods are enzymatic assays and the reagents for each assay were obtained from Smith Kline Beckman Instruments. Calibrations for all enzymatic assays are traceable to the National Bureau of Standards (NBS) reference materials. In addition, each batch of samples was run with quality control materials.

Total serum proteins were measured by the Biuret method (NCCLS 1979). The procedures for the assay are described in the Roche Diagnostic Systems (1986) information package. Assays were performed on an automated Centrifugal Fast Analyzer System (Cobas-Fara, Roche Inc.). Calibrations were performed using the Roche serum calibrator as the standard and monitor Levels 1 and 2 (American Dade, Miami, FL) as internal controls.

5.2.2.3 RNA/DNA analysis

A 50- to 100-mg section of liver was homogenized in ~1 mL of distilled water for 1 min using a teflon homogenizer while keeping the sample cold. After the homogenate was brought up to a final volume of 1.5 mL with distilled water, it was transferred to 1.5-mL microcentrifuge tubes and centrifuged for 2 min in a Beckman microfuge. The RNA content of each sample was analyzed in triplicate by adding to each of three 1.5-mL centrifuge tubes (1) 200 μ L of supernatant from the liver homogenate, (2) 1.2 mL of 95% ethanol, (3) 0.035 mL of 2 M sodium acetate, and (4) 0.015 mL of 1 M magnesium acetate. Samples were cooled for 20 min in a refrigerator before centrifuging for 2 min. After the supernatant was decanted, 1 mL of 0.3 M KOH was added to the tubes with the pellet and incubated at 37°C in a constant water bath until the pellet dissolved. Each tube then received 0.5 mL of 1.4 N perchloric acid before it was cooled for an additional 20 min in the refrigerator. The mixture was centrifuged for 2 min and the supernatant was recovered in 20-mL scintillation vials. The precipitate was then washed once with 1 mL of 0.2 N perchloric acid, centrifuged again, and the supernatant was combined with

the previous supernatant. Standards were prepared using 1100 μg of RNA and processed in exactly the same manner as the liver samples. Absorbance of the samples and standards was measured at 260 nm using a Gilford Response Spectrophotometer and a distilled water blank as a reference. Results were expressed as micrograms of RNA per milligram wet weight of liver tissue. For DNA analysis, duplicate samples were prepared by adding 3 mL of 0.2 M phosphate buffer at pH 7.0 to 100 μL of the original liver homogenate supernatant. Standards were prepared with 4.6 μg of salmon sperm DNA. The fluorescence was measured using an excitation wavelength of 360 nm and an emission wavelength of 450 nm using a Beckman LS-5 spectrofluorometer. Results were expressed as micrograms of DNA per milligram wet weight liver tissue.

5.2.2.4 Histopathological analysis

The following histopathological analyses were performed by the subcontractor at the School of Medicine, University of California, Davis: (1) percentage of liver tissue occupied by parasites, (2) percentage of liver composed of necrotic parenchyma, (3) percentage of tissue composed of macrophage aggregates, and (4) percentage of liver occupied by functional parenchyma. These analyses were performed according to the methods of Hinton and Couch (1984).

5.2.2.5 Detoxification enzymes

Microsome isolation

Fish hepatic microsomes were prepared by differential centrifugation (McKee et al. 1983) using several modifications. Fish were killed by severing their spinal cord, and the livers were immediately removed and blotted dry. Each liver sample was placed in a cooled 0.1 M phosphate buffer (0.25 mM Sucrose, 0.1 M Tris, pH 7.4). The minced tissues were homogenized in 5 volumes of buffer using 10 complete strokes of a motor driven Potter-Elvehjem glass and Teflon homogenizer. The homogenates were centrifuged at $3000 \times g^*$ for 10 min, and at $10,000 \times g$ for 20 min, using a J-21B Beckman centrifuge. The resulting supernatants were centrifuged at $105,000 \times g$ for 60 min in a Beckman L3-50 ultracentrifuge. Microsomal pellets were resuspended in 0.1 M Tris buffer (1 mM EDTA, 20% glycerol, pH 7.4) by sonication with a Braun Sonic 1510 at 50 watts for 10 to 15 seconds. All operations were performed at 0–4°C, and microsomes were frozen with liquid nitrogen and stored at -90°C or -120°C. No significant change in 7-ethoxyresorufin O-deethylase (EROD) activity was detected after 6 months of storage at this temperature. The activity of fish microsomes stored under these conditions have been reported to be stable for 1 year (Forlin and Anderson 1985).

*g = acceleration due to gravity.

Enzyme assays

The activity of EROD was measured fluorimetrically at 30°C (Burke and Mayer 1974) and is expressed as picomoles of resorufin·min⁻¹·mg⁻¹ of microsomal protein (m.p.). The final reaction buffer contained, per liter, HEPES buffer (pH 7.8), 80 mmol; magnesium acetate, 5 mmol; 7-ethoxyresorufin, 1.0 µmol; NADPH, 250 µmol; and EDTA, 100 µmol. The concentration of total protein used in the enzyme assay ranged from 0.2 to 1.0 mg·ml⁻¹, depending on the activity of the sample.

Proteins were measured by the Bio-Rad (Richmond, California) reagent method (Bradford 1976) on the Cobas Fara (Hoffman La Roche Instruments) using bovine serum albumin as a standard. Cytochrome P₄₅₀ and cytochrome b₅ content were each measured by their characteristic oxidized and reduced spectra. Cytochrome P₄₅₀ samples were oxidized with carbon monoxide and reduced with sodium dithionite (Johannesen and DePierre 1978). Cytochrome b₅ was reduced with NADH; b₅ assays were carried out prior to cyt. P₄₅₀ analysis (Stegeman et al. 1979).

NADPH-cytochrome *c* reductase was assayed spectrophotometrically by the reduction of the electron acceptor cytochrome *c* with an extinction coefficient of 21.1 cm⁻¹ mM⁻¹. The reaction mixture contained 50 mM Tris (pH 7.4), 20% glycerol, 1mM DTT, 1mM EDTA, 1.1 mg/mL horse heart cytochrome *c*, 0.175 mM NADPH and 2–10 µg of microsomal protein.

5.2.2.6 Reproductive indicators

Sampling procedures

Sampling of redbreast sunfish was conducted during late May 1988 from a reference site on BF and from four sites along EFPC: EFK 23, EFK 19, EFK 14, and EFK 5. (See Sect. 5.2.1 for complete description of sites.) These sites were sampled again during August 1988, along with an additional reference site, Hinds Creek.

A minimum of 7 adult female redbreasts (range 8–10), each 10 cm or larger in length, were collected by electroshocking from each site sampled in May; sample sizes in August increased to 10–13 females per site. Blood was taken from each fish within 5 min of collection (see Sect. 5.2.1) for later analysis of reproductive hormones. Blood samples averaged 0.7–1.0 mL per fish and were collected in unheparinized 3-mL vacutainers (Becton, Dickson, & Co.). Each tube was labeled with a fish identification number and placed in a container with ice. Fish were tagged with an identification number and maintained alive in aerated containers for transport back to the laboratory.

Analytical procedures

Total lengths and weights were recorded for each fish. Following sacrifice, ovaries were removed and weighed. Weighed pieces of tissue were then transferred to petri dishes containing a physiological saline solution for quantitative oocyte size-frequency determinations and to tubes that were subsequently stored at -120°C for future analysis of ovarian reproductive hormones. Remaining ovary portions were transferred for archival storage to 20-mL glass scintillation vials containing 5 mL Bouin's fixative. The gonadal-

somatic index (GSI) was determined for each fish as $GSI = (\text{gonad weight}/\text{total body weight}) \times 100$.

Oocyte size-frequency determinations

Oocyte size-frequency determinations were conducted by the methods of Greeley et al. (1987) and Lin et al. (1989). Weighed pieces of fresh ovary were gently teased apart in physiological saline with the aid of a dissecting microscope and watchmaker's forceps. Individual oocytes ≥ 0.3 mm in diameter were then measured to the nearest 0.1 mm with an ocular micrometer attached to the eyepiece of a dissecting microscope. Condition and approximate stage of development of the oocytes were recorded along with the presence of atretic (dead or nonviable) oocytes. Cysts of a parasite, the "white grub" (*Postodisplostomum*), occasionally found within the ovary and other organs of redbreast sunfish, were also counted in each tissue piece. Results were subsequently expressed as the number of oocytes of a certain size-class or stage (or parasitic cysts) per ovary or as the number of oocytes or cysts per gram of fish.

5.2.2.7 DNA integrity and metal-binding proteins

Measurement of DNA integrity

Alkaline unwinding is a sensitive analytical technique which has previously been used in cells in culture to detect and quantify DNA strand breaks induced by physical and chemical carcinogens (Ahnstrom and Erixon 1980; Kanter and Schwartz 1979, 1982; Daniel et al. 1985). To assess the level of strand breaks of DNA in aquatic species, existing methods were modified to allow (1) the isolation of intact, highly polymerized DNA from livers of aquatic organisms and (2) the estimation of the amount of strand breaks in the isolated DNA by allowing strand separation under carefully controlled experimental conditions.

DNA isolation is accomplished by homogenizing the intact liver of the sunfish in 1 *N* $\text{NH}_4\text{OH}/0.2\%$ Triton X-100. The DNA is further purified by differential extraction with chloroform/isoamyl alcohol/phenol (24:1:25[v:v]), and passage through a molecular sieve column (Sephadex G50). DNA strand breaks are measured in the isolated DNA by an alkaline unwinding assay (Kanter and Schwartz 1982; Shugart 1988a,b). The technique is based on the time-dependent partial alkaline unwinding of DNA followed by determination of the duplex:total DNA ratio (\bar{F} value). Since DNA unwinding takes place at single-strand breaks within the molecule, the amount of double-stranded DNA remaining after a given period of alkaline unwinding will be inversely proportional to the number of strand breaks present at the initiation of the alkaline exposure, provided renaturation is prevented. The amounts of these two types of DNA are quantified by measuring the fluorescence that results with Hoechst dye #33258 (Kanter and Schwartz 1982; Shugart 1988a, 1988b).

Rydberg (1975) established the theoretical background for estimating strand breaks in DNA by alkaline unwinding, which is summarized by the equation:

$$\ln \bar{F} = - (K/M)(t^b) \quad (5)$$

where K is a constant, t is time, M is the number average molecular weight between two breaks, and b is a constant <1 which is influenced by the conditions for alkaline unwinding.

The relative number of strand breaks (\underline{N} value) in DNA of sunfish from sampled sites can be compared to those from reference sites as follows (Kanter and Schwartz 1982, Shugart 1988b):

$$\underline{N} = (\ln \underline{F}_s / \ln \underline{F}_r) - 1 \quad (6)$$

where \underline{F}_s and \underline{F}_r are the mean \underline{F} values of DNA from the sampled sites and referenced site respectively. \underline{N} values greater than zero indicate that DNA from the sampled sites has more strand breaks than DNA from the reference site; an \underline{N} value of 5, for example, indicates five times more strand breakage. Statistical analyses were performed on the $\ln \underline{F}$ data because the observed values on this variable are independent, and hypotheses about values of \underline{N} being significantly different (positive or negative) can be translated into equivalent hypotheses about differences in the mean $\ln \underline{F}_s$ and mean $\ln \underline{F}_r$ values. Thus an \underline{N} value reported to be significantly different from zero indicates that the mean $\ln \underline{F}$ data of the sampled site was significantly different from the mean $\ln \underline{F}$ data at the reference site using appropriate statistical tests [Dunnnett's test for comparing mean response ($\ln \underline{F}$) of the reference with the mean response of the sample sites].

Metal-binding proteins

The metallothionein (MT) content was determined for sunfish obtained during the August 1988 sampling period. Sampling sites included four stations along EFPC (EFK 23, EFK 19, EFK 14, and EFK 5) as well as two streams outside the EFPC area (Hinds Creek and BF).

Metallothionein content of sunfish was measured by the Chelex-100 assay (Sloop et al., unpublished data). This assay involves (1) the preparation of a homogenate containing the soluble protein fraction of liver tissue; (2) the acid displacement and subsequent removal of protein-bound endogenous cadmium and zinc and their replacement with ^{109}Cd ; (3) the removal of large, non-MT metal-binding proteins via isoelectric precipitation; (4) the adsorption of free or weakly bound ^{109}Cd from the assay solution to Chelex-100; and (5) the quantification of MT by the scintillation counting of the fraction not adsorbed to Chelex-100.

5.2.3 Statistical Procedures

ANOVA procedures were used to test for differences in individual bioindicators among sites, between sexes, and between seasons. Interaction effects between site, sex, and seasons were also included in the ANOVA model. If the ANOVA procedure rejected a multisample (site, sex, or season) null hypothesis of equal means, then the Tukey multiple range test was used to identify significant differences among pairs of variables (e.g., sites). The Tukey test was used because it is fairly robust with respect to departures of the data from normality and homogeneity and also because it is the most widely accepted and commonly used multiple comparison test (Zar 1984).

To determine the integrated response of fish to the environmental conditions at each sampling site, all the bioindicator variables were considered jointly within a multivariate context by using canonical discriminant analysis available on the Statistical Analysis System (SAS 1985a, 1985b). This method provides a graphical representation of the positions and orientations of the various integrated site responses relative to each other. In addition, the discriminant analysis variable selection procedure (SAS 1985a, 1985b) was used to identify and prioritize the variables which contributed most to the discrimination among integrated site responses. This variable selection procedure considered all possible combinations of the observed values and, for any specified subset size, selected those variables having the best discriminating power.

Tests for homogeneity of variance of individual bioindicator response variables between sites were conducted using Levenes test (Sokal and Rohlf 1981). This is a F-distribution test which compares the ratios of the variances from two independent sample populations.

5.2.3.1 Conclusion

Differences in the mean values of the various reproductive parameters between study sites along EFPC and the primary reference site (BF) were examined by Least-Squares Analysis of Variance followed by a Duncan's Multiple Range Test. A critical p -level of 0.05 was adopted for all comparisons.

5.3 RESULTS AND DISCUSSION

5.3.1 Indicators of Contaminant Exposure

Both DNA integrity and metal binding proteins are used as indicators of contaminant exposure.

5.3.1.1 DNA integrity

A total of 176 fish were analyzed for DNA strand breaks over a period of ~15 months covering the period May 1987 through August 1988. Because intrinsic factors such as reproductive capacity, nutritional status, age, disease state, etc. can modulate to some extent the genotoxic response in fish, the selection of a suitable reference site was of paramount importance to the interpretation of the data on strand breaks in the DNA of the sampled fish from EFPC. The influence of these factors on the fish in both EFPC and the reference streams was assumed to be similar. Therefore, by calculating \bar{N} values, the contribution of these intrinsic factors to the measured DNA integrity values was essentially removed.

DNA strand breaks were measured in fish from two streams in different watersheds to determine their suitability as an appropriate reference site. There was no significant difference between the mean $\ln F$ values of fish from the Hinds Creek site at the three different sampling times ($p > 0.3$). Strand breaks in Brushy Fork fish, however, showed significant differences over the five different sampling times ($p < 0.001$). In addition, a

comparison between Hinds and Brushy Fork creeks of the mean $\ln F$ values for the June-1987 and August-1988 sampling times indicated that the two sites were significantly different.

The most appropriate reference stream for comparison of DNA integrity to EFPC fish appears to be Hinds Creek. Redbreast sunfish from Hinds Creek appeared to be under little genotoxic stress. This can be demonstrated from the minimal fluctuation in the observed mean $\ln F$ values of fish from this stream over a period of 14 months (Table 5-1). The small negative mean $\ln F$ value (average for all data = -0.11909) in the alkaline unwinding assay is indicative of highly polymerized, double-stranded DNA with few endogenous breaks (Rydberg 1975, Ahnstron and Erixon 1980).

Table 5-1. DNA integrity measured in sunfish from two reference sites

Sample date	Reference sites	
	Brushy Fork	Hinds Creek
	($\ln F^a$)	
May 1987	-0.65352 ± 0.15182	
June 1987	-1.70070 ± 1.05905	-0.21078 ± 0.07150
October 1987	-1.15272 ± 0.33850	
November 1987	-0.29060 ± 0.14055	
May 1988		-0.09710 ± 0.05170
August 1988	-0.40170 ± 0.19998	-0.10718 ± 0.13494

^a $\ln F$ values ± standard deviation.

A comparison of DNA strand breaks in sunfish collected from Hinds Creek with those in sunfish collected from EFK 23 and several other off-site streams in June 1987 is shown in Table 5-2. The DNA isolated from sunfish at all sites (excluding Bull Run Creek) had significantly more strand breaks than DNA in similar fish from the reference site. EFK 23 was also significantly different from Bull Run Creek and Beaver Creek, but not from BF. A comparison of DNA strand breaks in sunfish at EFPC sampling sites compared with Hinds Creek for the sampling periods of October 1987, May 1988, and August 1988 are shown in Table 5-3. As no fish were obtained at this reference site during the October-1987 sampling, the average $\ln F$ value for all Hinds Creek data was used (see earlier discussion concerning appropriateness of Hinds Creek as a reference site).

Concerning an assessment of the integrity of the DNA in sunfish along EFPC, two important observations can be made from the data in Table 5-3. First, for all sampling times, there was no obvious gradient of decreasing DNA strand breakage in the fish from

the headwaters of EFPC (EFK 23) to the lower reaches of EFPC (EFK 5). DNA integrity was always lower (i.e., more DNA strand breakage) at EFK 19 and EFK 14, except EFK 14 in August 1988. Second, DNA strand breakage appeared to be decreasing with time at all EFPC sampling sites except EFK 19 (Tables 5-2 and 5-3).

Table 5-2. Comparison of DNA^a integrity in sunfish at several sampling sites with sunfish at Hinds Creek

Sampling sites ^b				
Bull Run Creek	Beaver Creek	Brushy Fork	EFK 23	
(N value)				
1.98	5.45 ^c	7.10 ^c	9.74 ^c	

^aDeoxyribonucleic acid.

^bSampling date: June 1987.

^cData significantly different from reference site at $p < 0.05$. EFK = East Fork Poplar Creek kilometer.

Table 5-3. Comparison of DNA^a integrity in sunfish at EFPC sampling sites with sunfish at Hinds Creek

Sampling date	Sampling sites				
	EFK 23	EFK 19	EFK 14	EFK 4	Brushy Fork
(N value) ^b					
October 1987	7.10 ^c	11.23 ^c	11.72 ^c	9.30 ^c	8.68 ^c
May 1988	5.18 ^c	5.75 ^c	5.93 ^c	1.19 ^c	
August 1988	3.53 ^c	6.48 ^c	1.11	0.26	2.75 ^c

^aDeoxyribonucleic acid.

^bN value = relative number of stand breaks in DNA of sunfish from sampled sites (E_s) compared with those from reference sites (E_r). $N = (\ln E_s / \ln E_r) - 1$.

^cData significantly different from reference site at date of sampling, $p < 0.05$.

Note: EFK = East Fork Poplar Creek kilometer.

5.3.1.2 Summary—DNA integrity

The incidence of DNA strand breakage was measured in sunfish from several EFPC sites and reference streams between May 1987 and August 1988. Hinds Creek was shown to be a more appropriate reference site than BF for the comparison of DNA integrity. Fish at all the EFPC sampling stations initially showed levels of DNA strand breakage significantly higher than fish from the reference site. These levels appear to be decreasing with time (Table 5-3), except for those at EFK 19, where levels remained constant between May 1988 and August 1988.

At most sampling sites (EFK 19 appears to be an exception), the fish in EFPC were experiencing a decrease in genotoxic stress over the duration of this investigation. Fish at EFK 19 demonstrated a steady decline over the 14-month period and, by August 1988, fish at two EFPC sampling stations (EFK 14 and EFK 5) were not significantly different in their response compared with the reference site.

5.3.1.3 Metal binding proteins

The content of metallothionein in the livers of sunfish from Hinds Creek, BF, and EFK 5 are not statistically different from each other (Table 5-4). The levels observed are similar to those reported in sunfish maintained under laboratory conditions where exposure to heavy metals was restricted (Sloop et al. unpublished data).

Table 5-4. Metallothionein content of sunfish at East Fork Poplar Creek sampling sites and two reference sites for August 1988

Sampling site	Samples	Metallothionein ^a
Hinds Creek	17	418 ± 157
Brushy Fork	14	532 ± 322
EFK 23	12	627 ± 262 ^b
EFK 19	15	555 ± 183 ^b
EFK 14	15	963 ± 471 ^c
EFK 5	12	359 ± 182

^aNanograms of ¹⁰⁹Cd bound per gram of soluble liver protein ± 1 SD (Sloop, unpublished data).

^bUsing \bar{t} test, data significantly different ($p < 0.05$) when compared to Hinds Creek.

^cUsing \bar{t} test, data significantly different ($p < 0.01$) when compared to Hinds Creek or Brushy Fork.

Note: EFK = East Fork Poplar Creek kilometer.

Heavy metal contamination at EFK 14 is indicated by the high levels of metallothionein in the livers of sunfish. This level was significantly different from that of the fish in the two reference streams (Hinds Creek and BF).

5.3.2 Indicators of Contaminant Effects

Three major concerns are addressed in this report relative to the effects of operation of the Y-12 Plant on the biota of EFPC: (1) spatial effects, or health status of fish in various sections of EFPC compared to health of fish in a nonaffected stream; (2) temporal effects or changes in the health status of the biota in EFPC resulting from cleanup and remedial actions; and (3) evaluation of the causative agents or mechanisms responsible for any effects observed on the fish populations in EFPC.

5.3.2.1 Spatial effects

To address spatial effects or site differences in response of fish to stress, each of the bioindicators measured in this study were grouped into five functional categories representing indicators of (1) carbohydrate-protein metabolism, (2) detoxification enzymes, (3) lipid metabolism, (4) histopathology, and (5) overall fish health or condition. These responses or functional groups reflect gradients of both ecological relevance and direction of response to a stress such as a contaminant. Those variables in groups (1) and (2) respond relatively rapidly to stress but have relatively low ecological relevance whereas indicators included in groups (3) through (5) respond relatively slowly to stress and are characterized by low toxicological but high ecological relevance.

General site patterns

The relative difference in the response of each bioindicator at each of the EFPC sites compared to the reference site (BF) is shown in Figs. 5-1 to 5-5 for all seasons combined (April 1986–fall 1987). Values above the zero line in each figure indicate that the response for fish at any particular EFPC site was higher than the response for the same variable in fish from the reference site. Values below the zero line indicate lower responses in EFPC fish compared to reference fish.

Carbohydrate-protein metabolism

The indicators of carbohydrate-protein metabolism demonstrated a varied response among sites. The transaminase enzyme, SGOT, ranged from 5 to 70% lower at all the EFPC sites compared to the reference (Fig. 5-1). Changes in the transaminase enzymes are typically used to indicate tissue damage or impaired organ function (Rhodes et al. 1985, Rao and Rao 1984); however, due to the extreme variability of this parameter, it was not statistically different among any of the sites (Fig. 5-6). Serum protein is also used as an indicator of protein metabolism. Lower protein values at EFK 23 and EFK 19 compared to BF may be an indication that EFPC fish were under some type of nutritional stress, as was found for white sucker (*Catostomus commersoni*) experiencing low food

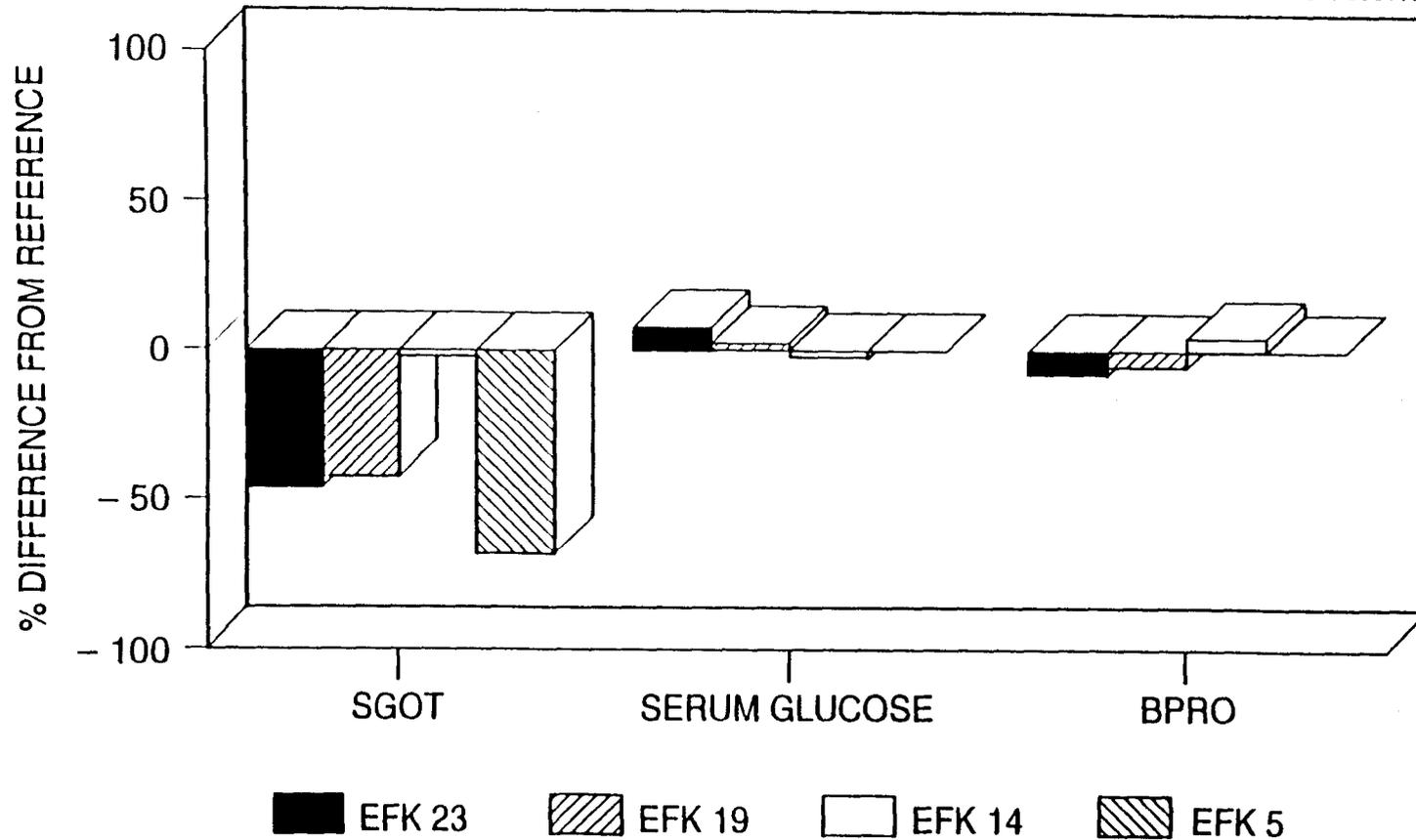


Fig. 5-1. Relative differences in the response of the carbohydrate-protein metabolism parameters at each East Fork Poplar Creek (EFPC) site compared to the reference site, Brushy Fork. Values above or below the zero line indicate that the response for fish at any particular EFPC site was higher or lower, respectively, than the same response for fish from the reference site. SGOT = serum glutamate oxaloacetate transaminase. BPRO = blood protein. EFK = East Fork Poplar Creek kilometer.

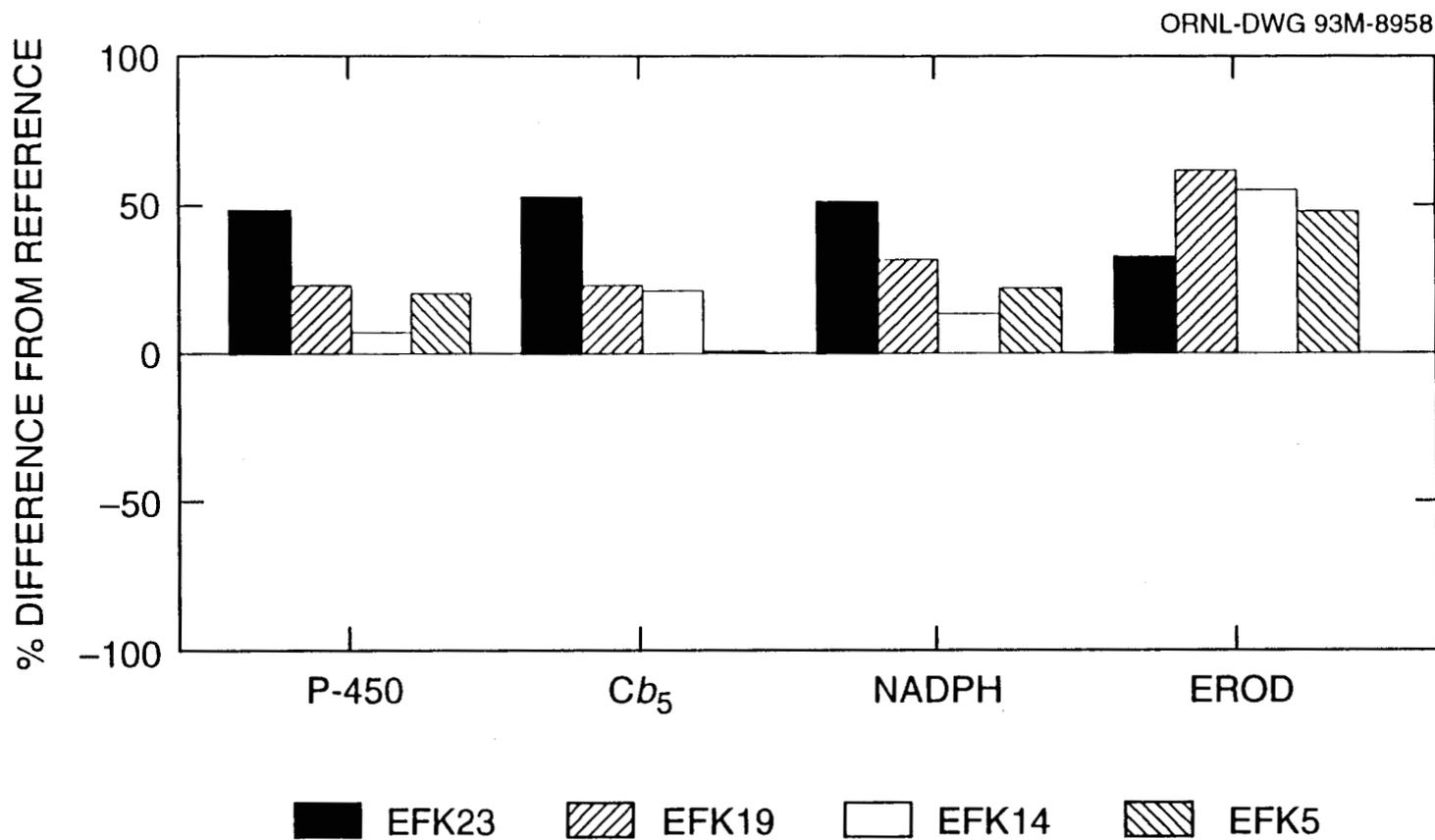


Fig. 5-2. Relative differences in the response of the detoxification enzymes at each East Fork Poplar Creek (EFPC) site compared to the reference site, Brushy Fork. Values above or below the zero line indicate that the response for fish at any particular EFPC site was higher or lower, respectively, than the same response for fish from the reference site. EFK = East Fork Poplar Creek kilometer. NADPH = Nicotinamide adenine dinucleotide phosphate, reduced form. EROD = 7-Ethoxyresorufin O-deethylase. P-450 = Cytochrome P-450. Cb_5 = Cytochrome b_5 .

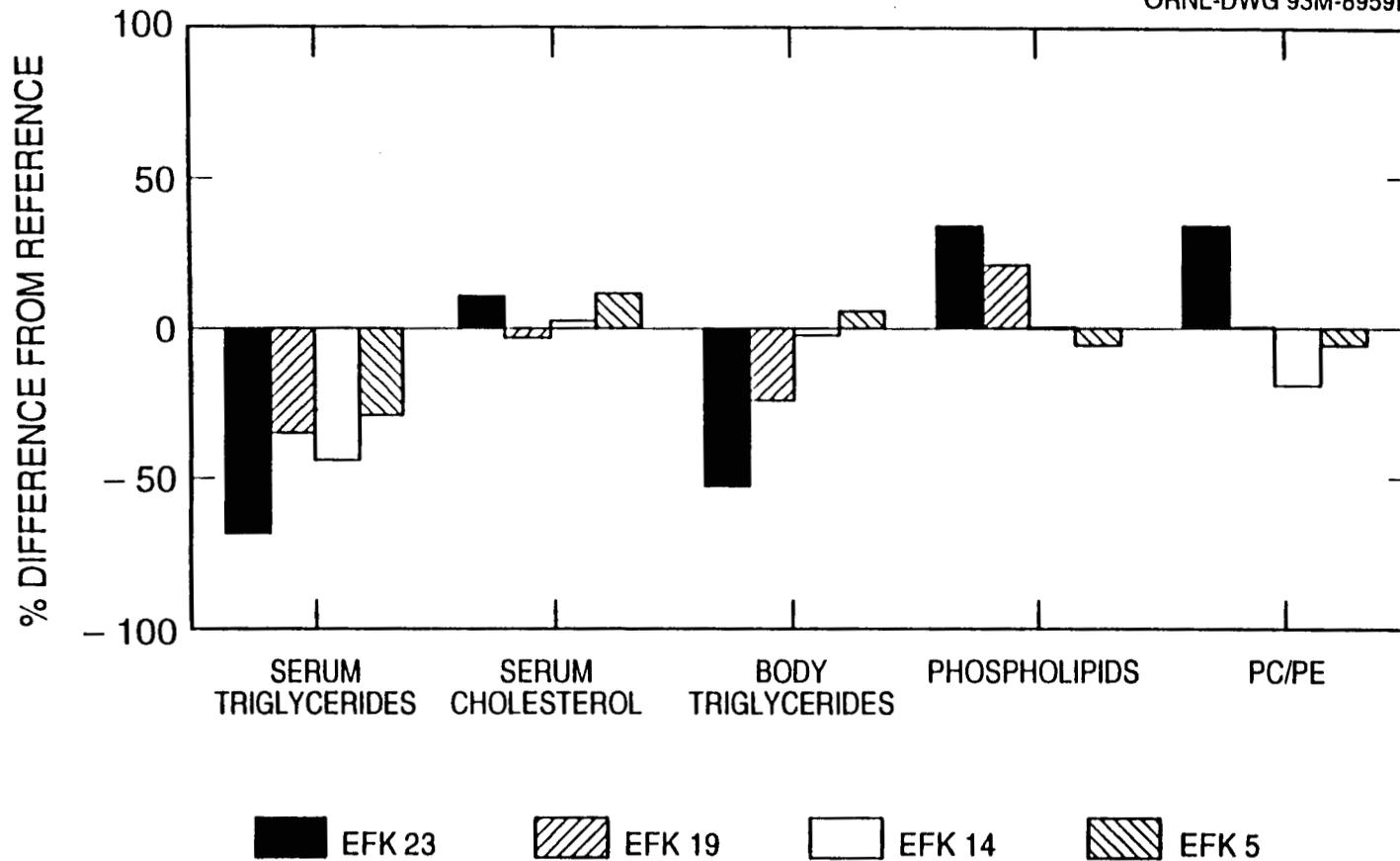


Fig. 5-3. Relative differences in the response of the lipid metabolic parameters at each East Fork Poplar Creek (EFPC) site compared to the reference site, Brushy Fork. Values above or below the zero line indicate that the response for fish at any particular EFPC site was higher or lower, respectively, than the same response for fish from the reference site. PC = phosphatidylcholine, PE = phosphatidylethanolamine. EFK = East Fork Poplar Creek kilometer.

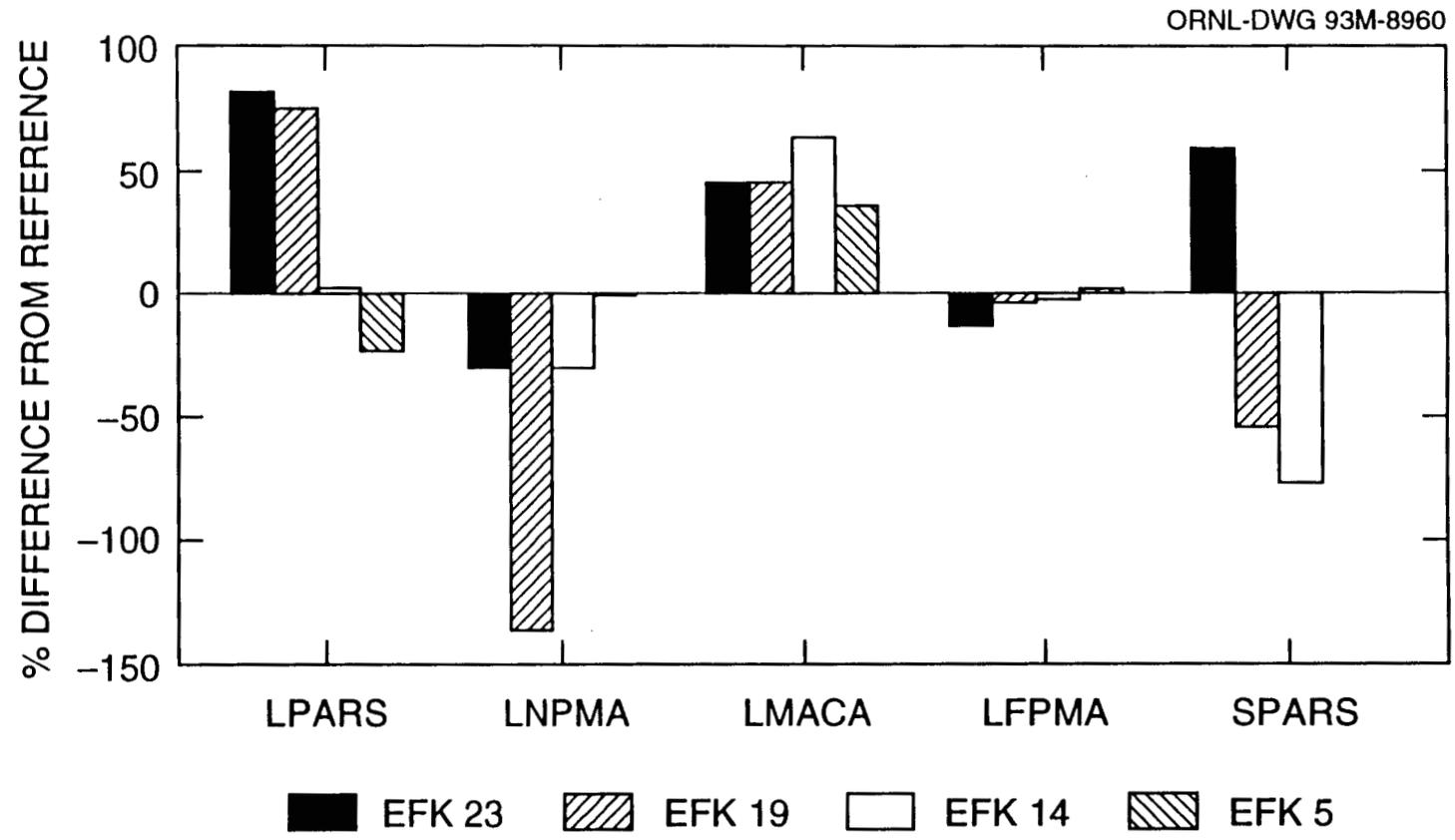


Fig. 5-4. Relative differences in the response of the histological indices at each East Fork Poplar Creek (EFPC) site compared to the reference site, Brushy Fork. Values above or below the zero line indicate that the response for fish at any particular EFPC site was higher or lower, respectively, than the same response for fish from the reference site. LPARS = liver parasites, LNPMA = necrotic liver parenchyma, LMACA = macrophage aggregates in liver, LFPMA = functional parenchyma in liver, SPARS = spleen parasites. EFK = East Fork Poplar Creek kilometer.

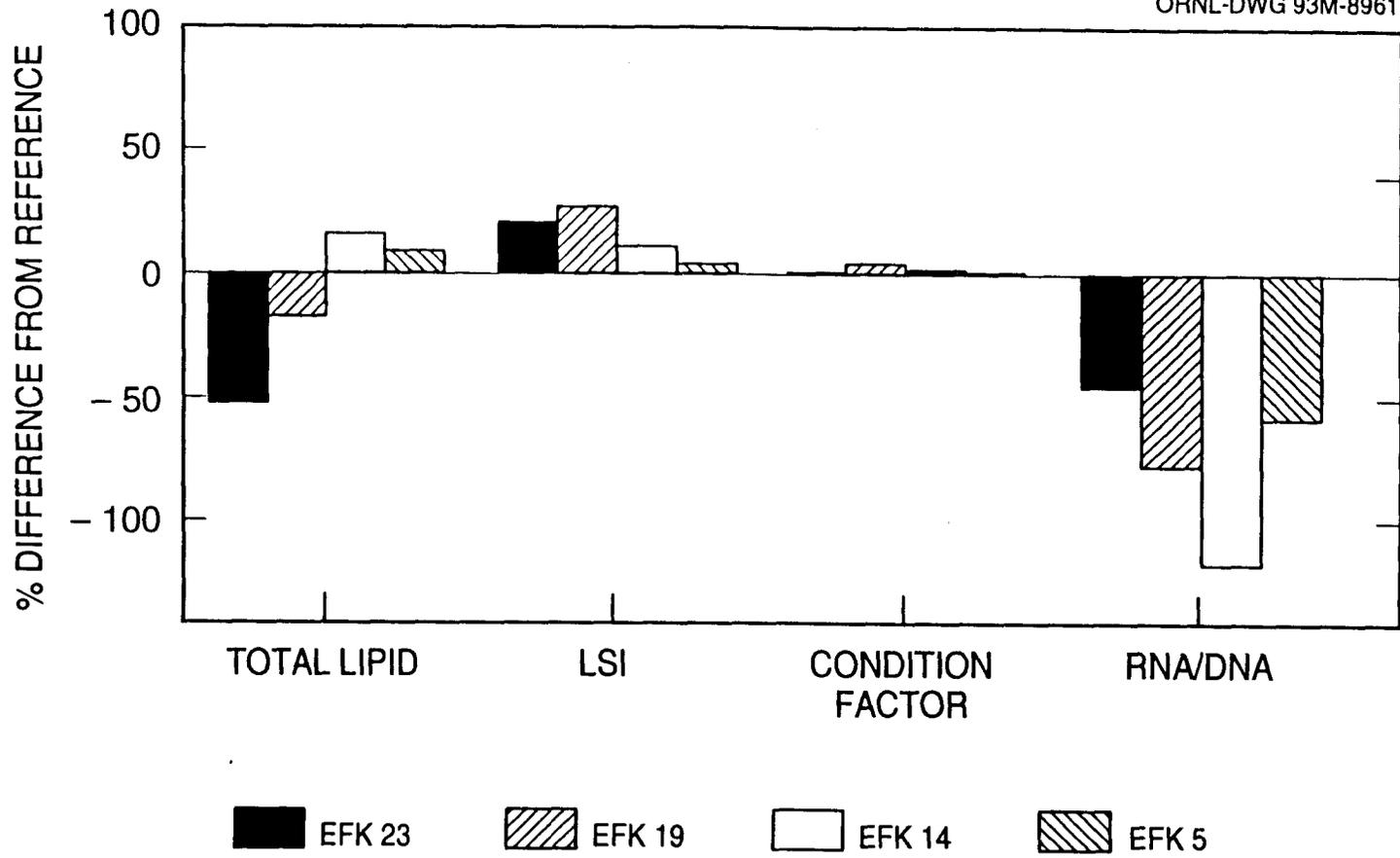


Fig. 5-5. Relative differences in the response of the condition indices at each East Fork Poplar Creek (EFPC) site compared to the reference site, Brushy Fork. Values above or below the zero line indicate that the response for fish at any particular EFPC site was higher or lower, respectively, than the same response for fish from the reference site. LSI = Liver somatic index. RNA = ribonucleic acid; DNA = deoxyribonucleic acid. EFK = East Fork Poplar Creek kilometer.

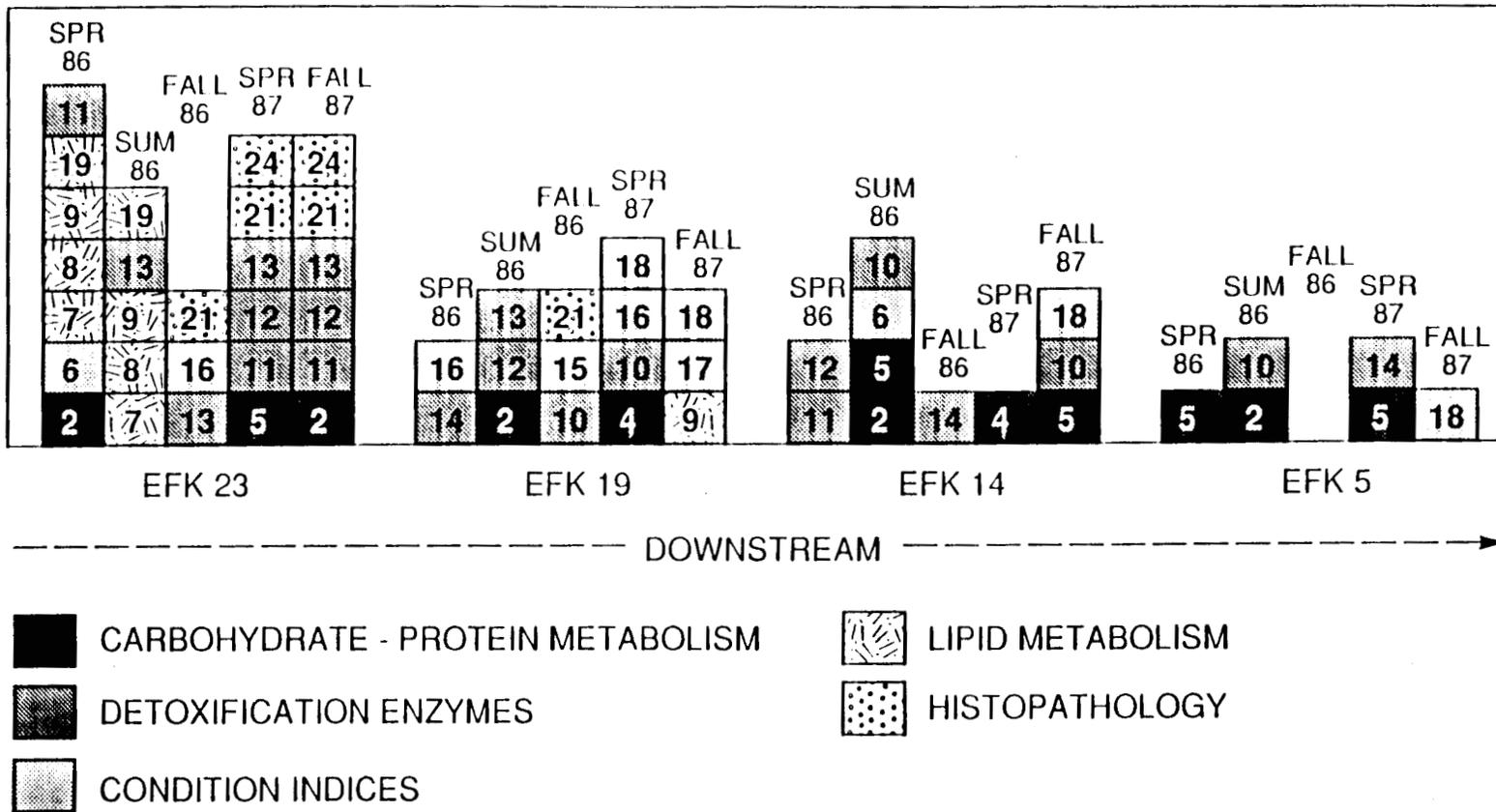


Fig. 5-6. Seasonal differences in bioindicator responses between each of the East Fork Poplar Creek (EFPC) sites and the reference area, Brushy Fork. Numbers inside each histogram represent the specific identification number given to each bioindicator, which are significantly different ($p < 0.05$) from Brushy Fork each season. Patterns enclosing each number represent functional response groups. EFK = East Fork Poplar Creek kilometer.

availability (Lockhart and Metner 1984). Even though blood glucose was slightly elevated (hyperglycemia) in fish at EFK 23, this variable is a generalized stress response in fish to a broad spectrum of environmental perturbations (Silbergeld 1974) and thus may reflect both direct (metabolic) and indirect (food-chain effects) of stress.

Detoxification enzymes

The activity or levels of liver detoxification enzymes are used to indicate exposure to various xenobiotics such as PAHs, PCBs, and pesticides (Payne and Penrose 1975). Many of these compounds commonly occur in industrial and municipal effluents and are accumulated within living tissues (Ahokas et al. 1976, Brown et al. 1986, Neff 1978).

Even though there were some differences in the magnitudes of response, detoxification enzymes were elevated in fish from all the EFPC sites as compared with the reference site (Fig. 5-2). Fish from EFK 23 had the highest levels of P_{450} , CB_5 , and NADPH and the lowest level of EROD, compared to the fish from BF. For all enzymes, excluding EROD, there appears to be a downstream gradient in activity with the highest levels immediately below NHP and the lowest levels at EFK 5. Depressed EROD activity for fish from EFK 23 could have been due to hepatotoxic damage that inhibits the ability of the liver cells to produce this enzyme (Jimenez et al. 1990). A wide array of organic compounds, including some identified in EFPC (Loar et al. 1992b) as well as metal ions, are known to cause cytotoxicity in fish. This phenomenon has been observed for pike collected from a polluted lake in Finland and sunfish exposed to organics in a laboratory situation (Jimenez et al. 1990).

Lipid metabolism

Lipid metabolic parameters can reflect both the nutritional status and level of metabolic stress in fish. The condition or status of the lipid pool is important because the vulnerability of an organism to stress depends, in part, on this condition (Shul'man 1974, Glebe and Leggett 1981). Serum triglycerides were much lower (range 30–65%) in fish from all EFPC sites than in reference fish, indicating altered or impaired lipid metabolism in the former (Fig. 5-3). Serum cholesterol is an indicator of both nutrition and steroid metabolism in fish. No consistent patterns emerged, however, for cholesterol comparisons between sites, even though levels in fish at EFK 23 and EFK 5 are about 10% higher than in reference fish.

Total body triglycerides reflect the energy available to an organism for mediating the effects of stress (Lee et al. 1983) and for use in critical physiological functions such as growth and gonadal development. These triglycerides also act as energy buffers in periods of food shortages (Adams et al. 1985). Energy available for direct physiological use was 50 and 25% lower in fish from EFK 23 and EFK 19 respectively than in BF fish (Fig. 5-3). Levels in fish from EFK 14 and EFK 5, however, were similar to BF.

Phospholipids, the nonphysiological useful energy components of lipids, were 35 and 20% higher in redbreast sunfish from EFK 23 and 19, respectively, than in BF redbreast. The ratio of the two major types of phospholipids that constitute the cell wall, PE and PC, can reflect cell wall membrane integrity. Membrane lipid structure can influence membrane fluidity, enzyme kinetics, and electrical properties (Friedman et al. 1986). The PC/PE ratio was slightly elevated in EFK 23 fish but depressed in fish from EFK 14 and EFK 5. This difference could possibly be due to the gradient in temperatures and/or

concentrations of dissolved solids between these sites, which can effect the ratio of these two cell wall components (Roche et al. 1983).

Histopathological condition

Indicators of histopathological condition in fish from EFPC showed distinct differences compared with that in the reference fish (Fig. 5-4). The percentage of the liver composed of parasites was about 75% higher in redbreast from EFK 23 and EFK 19 than in reference fish. Fish collected downstream of EFK 19 had similar or even lower percentages of parasites than BF redbreast.

An informative indicator of tissue disease or pathology is the occurrence of macrophage aggregates. These are localized centers of phagocytes (white blood cells) that invade an area that is diseased or damaged. The percentage of liver tissue composed of macrophage aggregates was consistently 40–70% higher in fish from EFPC than in BF. No downstream gradient in this pathology was indicated, however.

The percentage of liver tissue actually occupied by functional parenchyma did demonstrate, however, a downstream gradient with this condition being 15% lower in fish from EFK 23 and only 3% lower in fish from EFK 14, respectively, than BF fish. The lower amount of functional liver tissue in fish from the upper sections of EFPC may be due to cytotoxic damage to these liver cells from contaminant exposure. These lower levels of functional liver tissue imply that fish with this condition have reduced capacity to (1) produce enzymes for detoxifying contaminants, (2) store important glycogen and lipid energy reserves, (3) manufacture vitellogenin necessary for proper egg development in the female, and (4) convert and process protein and lipid compounds into physiological useful energy.

Histological analysis of the spleen indicated that the parasite load of fish from EFK 23 was 60% higher than that of reference fish. Redbreast from the lower sections of EFPC, however, had lower parasite loads than did redbreast from BF. Because the spleen is a hemopoietic (blood-producing) organ, injury due to parasitic infestations could reduce production of both red and white blood cells, possibly resulting in anemia and increased vulnerability to disease (Anderson 1990).

Condition indices

Four condition indices were measured to determine general health for fish from each site. The condition factor is a generalized indicator of overall fitness or "plumpness" and can reflect the integrated effect of both nutritional level and the metabolic costs caused by stress. Probably due to the condition factor being fairly insensitive to changes in body condition (Adams and McLean 1985), this index was very similar between fish from all sites (Fig. 5-5). One possible explanation for this, in view of the apparent lower lipid levels in fish from EFK 23 and EFK 19, is that a decline in body weight resulting from a reduction in energy reserves may be ameliorated by an increase in body water (Cunjak and Power 1986).

Total body lipid is used to indicate overall fat storage and general nutritional status of fish. Redbreast collected at EFK 23 and EFK 19 had 50 and 20% less total body lipid, respectively, than did fish from BF, even though levels at the two other EFPC sites were slightly higher than at the reference (Fig. 5-5).

The liver-somatic index (LSI) reflects both short-term nutritional status and metabolic energy demands (Heidinger and Crawford 1977, Adams and McLean 1985). In addition, the LSI was sensitive to toxicant stress and liver enlargement due to hyperplasia (increase in cell number), and hypertrophy (increase in cell size) has been reported in fish exposed to toxic compounds (Addison 1984, Heath 1987, Fletcher et al. 1982). This type of situation appeared to indeed be the case for EFPC fish where the LSI was higher at all EFPC sites than BF (Fig. 5-5). There also appeared to be a downstream gradient in the LSI which reflects a change similar to that observed for the detoxification enzymes.

The RNA to DNA ratio is used as an indicator of immediate or short-term growth in fish (Bulow 1970, Haines 1973) as well as an indicator of exposure to sublethal concentrations of toxicants (Barron and Adelman 1984). Growth is one of the ultimate indicators of fish health because it integrates all the biotic and abiotic variables acting on an organism and reflects secondary impacts of chronic stress (Waters 1977, Larken 1978). Growth, as indicated by the RNA to DNA ratio was 50–100% lower at all the EFPC sites as compared with the reference site (Fig. 5-5). There is no obvious explanation as to why growth was more depressed at EFK 14 compared with the other EFPC sites, unless it was that food availability may have been lower and/or temperatures may have been higher on a seasonal basis at this site than at other EFPC areas.

Seasonal-site comparisons

Not only can environmental variables such as water quality and temperature influence the response of an organism to stress (Cairns and van de Schalie 1980), but variables related to sampling, such as where an organism was collected (site effect) and when it was collected (seasonal effect), can influence the expression of a particular stress response as well. Given that several levels of sampling-related variables are involved in the evaluation and interpretation of the data for this study such as seasons (5), sampling sites (5), and response variables (24), it is necessary to present all this information in a synthesized form so that all the relationships between site, season, and response can be viewed together. Figure 5-6 presents such a summary of seasonal-response comparisons between sites where significant differences in bioindicators between all EFPC sites and the reference site are shown. The numbers inside each histogram represent those specific indicators (each bioindicator received a specific I.D.) at each EFPC site that are significantly different each season compared to BF. For example, in spring 1986, variable 19 (lipid metabolism) and variable 2 (carbohydrate-protein metabolism) were significantly different between fish from EFK 23 and reference fish.

The most obvious feature of the comparisons in Fig. 5-6 is that, for each season, fish from EFK 23 have the greatest number of bioindicators that are significantly different from those of BF fish. For example, during spring 1986 and 1987 and fall 1987, fish at EFK 23 had seven variables that were significantly different from fish at the reference stream during the same season. There is also an obvious downstream gradient in the number of variables significantly different for each season with the greatest number of variables occurring at EFK 23 and the smallest number of significant variables occurring at EFK 5. For example, the average number of bioindicators significantly different from the reference fish each season are 5.4 at EFK 23, 3.0 at EFK 19, 2.2 at EFK 14, and only 1.2 at EFK 5.

Another major feature of these seasonal-site comparisons is the relative importance of different functional groups in distinguishing between sites. For example,

histopathological indicators (circled variables) and enzymes (squared variables) were the two main functional groups that distinguished fish at EFK 23 from fish at BF. Histopathological parameters were not measured until fall 1986, and before this period lipid indicators were the most influential group. At EFK 19, however, condition indices and enzymes were the most important variables, while at EFK 14 and EFK 5 the variables were principally enzymes and blood parameters.

Conclusions

The seasonal-site comparisons revealed two important aspects of fish response in EFPC fish compared with the fish from the reference stream: (1) there is a gradient of response downstream of NHP with the greatest number of variables which are significantly different from BF fish occurring at EFK 23 and the least number at EFK 5, and (2) the relative importance of each functional group for distinguishing differences between EFPC and BF fish varies between sites. Enzymes are important for comparing differences between fish from each EFPC site and the reference site; while histopathological, condition index, and blood chemical parameters are also important for purposes of site comparison at EFK 23, EFK 18, EFK 14, and EFK 5.

Integrated site analysis

An informative approach for determining the overall or integrated response of fish to stress is canonical discriminant analysis. This method includes all the bioindicators together within a multivariate context and provides a graphic representation of the positions and orientations of all the various integrated site responses relative to each other.

This integrated analysis was performed on data collected from fall 1985 to fall 1987 and includes two types of data sets: (1) all data collected over this 2-year period and (2) all data collected over this period but excluding histopathological indicators and the RNA to DNA ratio. The histopathological and RNA to DNA studies were not initiated until fall 1986.

A distinct downstream gradient in integrated fish response was evident when all the bioindicators are utilized in the discriminant analysis procedure (Fig. 5-7). Fish from EFK 23 were least similar and those at EFK 5 most similar to BF fish. As indicated by the differences in the linear distances between the centers of the site means for each response, fish at EFK 19 were the most similar to those at EFK 23, while fish collected at EFK 5 were less similar to those immediately below NHP (Fig. 5-7). There was no significant difference, however, in the integrated response of fish at EFK 14 and EFK 5 due to overlapping of the 95% confidence radii of these two sites.

The variables which are most significant in discriminating among sites are also indicated in Fig. 5-7. These eight variables consist of representative indicators from each of the functional groups (see Sect. 5.3.1), including one indicator each from detoxification enzymes and protein metabolism, two from lipid metabolism, and three from the condition indices.

The integrated site responses had a similar pattern relative to each other when the histological variables and RNA to DNA ratio are excluded from the analysis (Fig. 5-8). Integrated site response also showed a downstream gradient with EFK 23 fish being least similar to BF and fish from EFK 5 being most similar to BF. Fish from EFK 23 were

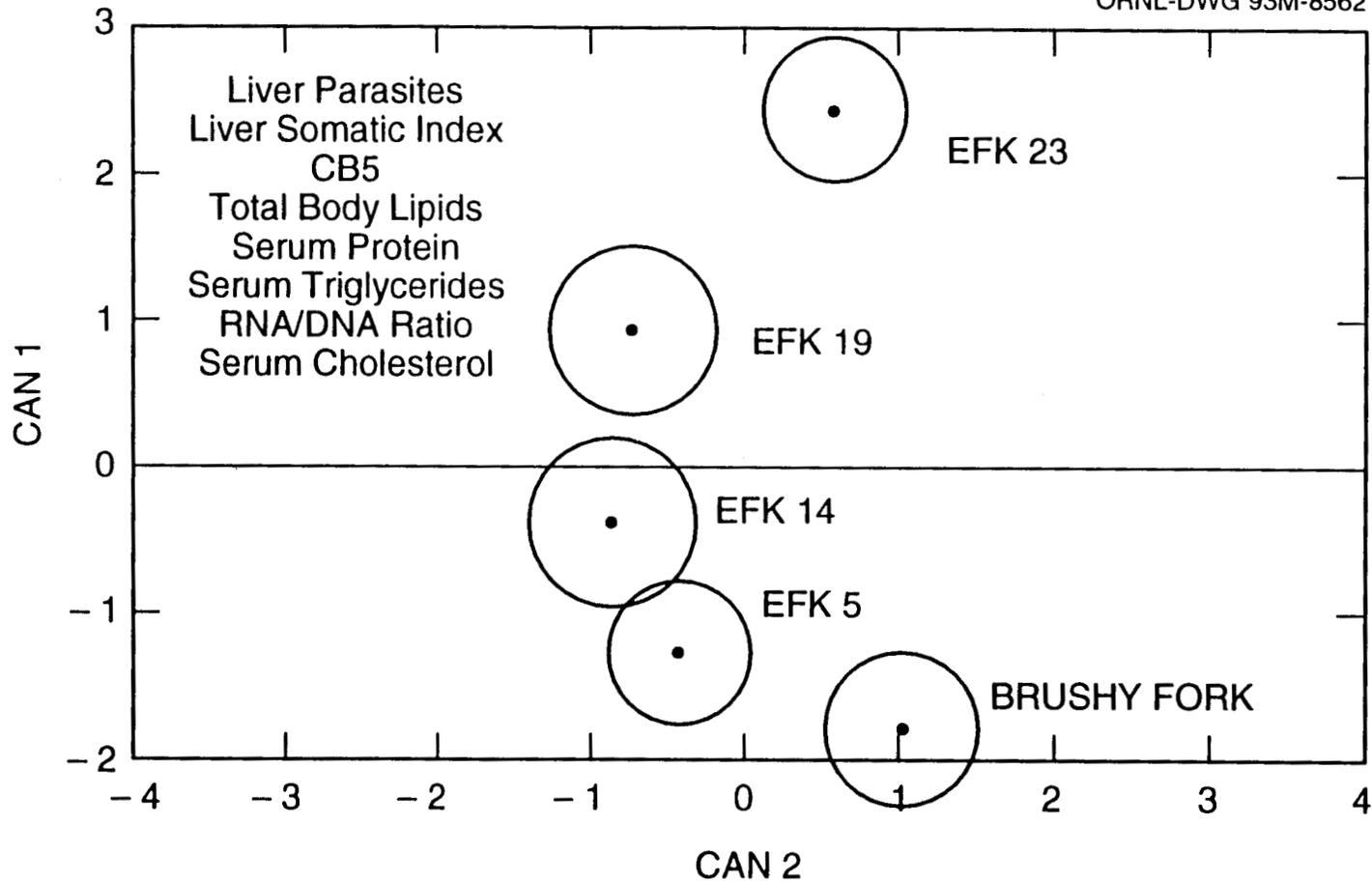


Fig. 5-7. Segregation of integrated health responses for redbreast sunfish from four sites in East Fork Poplar Creek and Brushy Fork using all the bioindicators measured from spring 1986–fall 1987. Circles represent site means and the 90% confidence radii of the site means. The most important variables for discriminating among these sites are listed in the upper left. EFK = East Fork Poplar Creek kilometer; Can = canonical discriminant analysis.

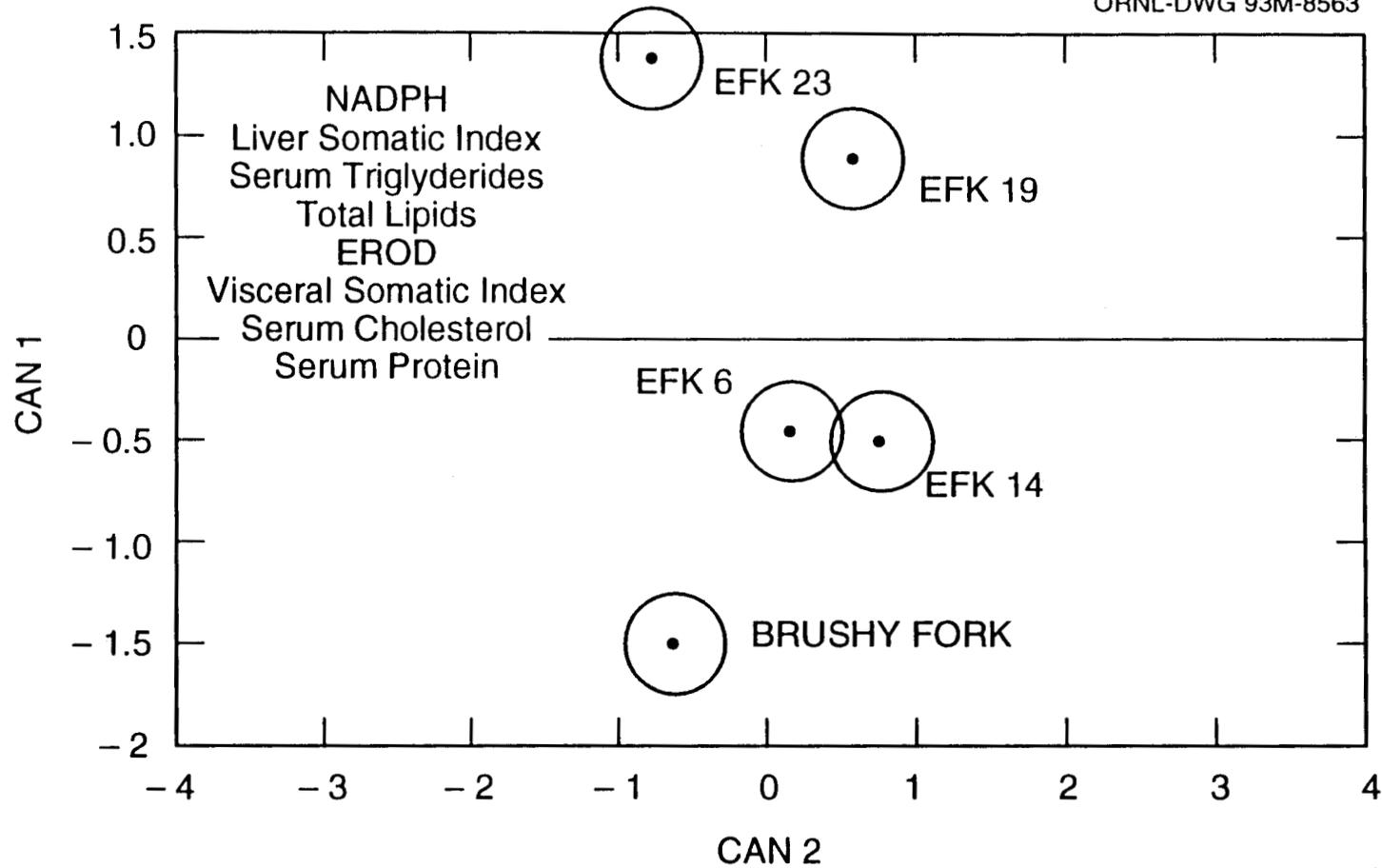


Fig. 5-8. Segregation of integrated health responses for redbreast sunfish from four sites in East Fork Poplar Creek and Brushy Fork using all bioindicators except the histological variables and the RNA/DNA ratio. Circles represent site means and the 90% confidence radii of the site means. The most important variables for discriminating among these sites are listed in the upper left. Can = canonical discriminant analysis. EFK = East Fork Poplar Creek kilometer.

more like EFK 19 redbreast than any other EFPC site. Also, EFK 14 and EFK 5 redbreast were similar to each other but significantly different from all other sites, as was also observed for the situation when all variables were included in the analysis. The important discriminating variables in this case are also listed in Fig. 5-8. Even though only five variables were common to both sets of analyses for discriminating between sites, four functional groups are represented in Fig. 5-8, including two enzymes, three condition indices, two lipid indicators, and one protein metabolism parameter.

Conclusions

Irrespective of the combinations of variables used in integrated site analysis, three major features are obvious: (1) there was a downstream gradient apparent in integrated response with fish at EFK 23 being the most dissimilar, and fish at EFK 5 being the most similar to the reference fish; (2) the integrated response of fish at the reference site was significantly different from fish at all EFPC sites; and (3) representative indicators of all functional groups were required in order to distinguish integrated responses of fish between sites.

5.3.2.2 Temporal analysis

Several types of treatment facilities have been installed at the Y-12 Plant to improve the quality of water released into EFPC. The purpose of this analysis was to determine whether any changes occurred in the health of fish at the various sites in EFPC due to implementation of these remedial actions.

Temporal changes in each indicator were determined by the significance of the slope of the regression line for all values of that indicator at each site measured between fall 1985 and fall 1987. A significant positive slope indicated that a variable increased significantly at a site over this period, a significant negative slope indicated a decrease over the period, and if a slope was not statistically different from zero, no significant change was indicated.

For redbreast sampled from BF, temporal changes in bioindicators involved primarily condition index and lipid metabolism parameters (Table 5-5). Both total body lipid and the RNA to DNA ratio increased over this period, indicating improved growth. Two of the lipid metabolism indicators, serum triglycerides and cholesterol, decreased, suggesting a switch in diet or nutritional status of redbreast sunfish in this stream. Indicators of direct contaminant exposure, the detoxification enzymes, either decreased or remained the same at this site (Table 5-5), implying that direct contaminant exposure was not a concern in this system. Any temporal changes in bioindicators for fish in this reference site should be interpreted with caution, however, since the drought during the sampling period had a large influence on stream hydrodynamics and therefore on biological conditions in the stream.

Most response parameters at EFK 23, however, either remained constant or increased over the 2-year period (Table 5-5). All indicators of protein, lipid, and carbohydrate metabolism remained the same, as did all the condition indices except the RNA to DNA ratio. Even though an increase in the RNA to DNA ratio was noted, this could have been an artifact of the short sampling period (1 year) over which this

Table 5-5. Temporal changes in bioindicator responses of redbreast sunfish from fall 1985 to fall 1987 at four sites in East Fork Poplar Creek and Brushy Fork, the reference site

Response variable	Sampling sites				
	EFK 14	EFK 5	EFK 23	EFK 19	Brushy Fork
Serum glucose	0	+	0	0	+
Serum triglycerides	0	-	-	-	-
Serum cholesterol	0	-	-	-	-
SGOT	0	0	0	-	0
Serum protein	0	0	0	0	0
Total lipid	0	-	0	-	+
Body triglyceride	0	-	0	0	0
Body cholesterol	0	+	0	0	0
EROD	0	0	-	-	-
Cytochrome P-450	+	+	0	0	0
Cytochrome <i>b</i> ₅	0	+	0	0	0
NADPH	0	0	-	-	-
Liver-somatic index	0	0	0	0	0
Visceral-somatic index	0	0	0	-	-
RNA/DNA	+	0	0	+	+
Condition factor	0	0	0	0	0

Note: A significant increase in a response over this period is indicated by a plus (+), a significant decrease by a minus (-), and no significant change by a zero (0), ($p < 0.05$). EFK = East Fork Poplar Creek kilometer; SGOT = serum glutamate oxaloacetate transaminase; EROD = 7-ethoxyresorufin *O*-deethylase; NADPH = nicotinamide adenine dinucleotide phosphate, reduced form; RNA/DNA = ribonucleic acid/deoxyribonucleic acid.

parameter was measured. All four of the detoxification enzymes either increased or remained constant, indicating that fish at this site continued to be exposed to toxicants that mobilize these enzymes.

Similar patterns of temporal bioindicator status were also observed at EFK 19 relative to EFK 23 (Table 5-5). Indicators of carbohydrate-protein metabolism increased or remained the same. All three lipid metabolism parameters decreased over this period, indicating that less energy was available to fish for critical physiological processes. A decrease in the lipid metabolic pool could have been due to an increase in metabolic stress at this site and/or decreased food availability to the population. Our extensive tagging studies conducted over three years indicated an increase in the number of sunfish

at this area (Gatz 1989); therefore, with a greater number of fish competing for food resources that are assumed to be limited, food availability per fish should decrease (Anderson 1985) with concomitant decreases in the lipid metabolic pool. All the condition indices at this site remained constant over this period, indicating little or no improvement in environmental conditions. Two of the detoxification enzymes increased and two remained constant in fish at EFK 19 (Table 5-5), demonstrating that contaminant loading to this section of the stream had not improved over this 2-year period.

The temporal response of bioindicators in redbreast was similar at EFK 14 and EFK 5; however, it was different between these two sites and the other EFPC sites (Table 5-5). Response of the enzyme parameters was the major difference between fish in the lower half of EFPC and fish from the upper half. The P_{450} and CB_5 enzymes generally increased at the upper two sites but remained the same at the lower sites. More significantly, NADPH was constant over the period in the upper half of EFPC and actually decreased in the lower half of EFPC (Table 5-5), possibly indicating decreased exposure. In addition, fish growth seemed to improve at both the lowest EFPC site and the reference stream.

Conclusions

As evidenced primarily by the temporal response in the detoxification enzymes and the RNA to DNA growth indicator, health of fish in the lower sections of EFPC seems to have improved during the sampling period (fall 1985 to fall 1987). However, apparently the health of fish in the upper sections of EFPC (EFK 23 and EFK 19) has not improved over this same period.

In streams where the primary source of stress is located at the upper end of that system (i.e., a point source discharge) and where remedial actions have been recently invoked, we would expect to see the type of temporal and spatial responses we observed in fish in EFPC. We anticipate there will be improvement in the biological integrity of the stream occurring first at the lower sections then gradually moving upstream. With the continued remedial clean-up actions at the Y-12 Plant we expect to observe increased fish health in the upper sections of EFPC in the future.

5.3.2.3 Causative mechanisms

The purpose of this section is to identify the causative agents or mechanisms responsible for the differences observed in the health status of fish at the various sites in EFPC and the reference site. Previous sections have demonstrated a downstream gradient of effects in EFPC with fish in the upper sections of the stream exhibiting higher levels of stress response than fish in the lower sections.

There are two basic mechanisms by which stress can affect an organism: (1) directly through metabolic and/or toxicological influences and (2) indirectly through the food chain (Fig. 5-9). To help identify the causative agents responsible for stress responses in EFPC fish, the importance of direct vs indirect mechanisms was evaluated. In evaluating the relative importance of these two basic mechanisms, two independent approaches were taken: (1) determining the health status of fish by a variable ranking procedure and (2) multivariate selection, based on discriminant analysis.

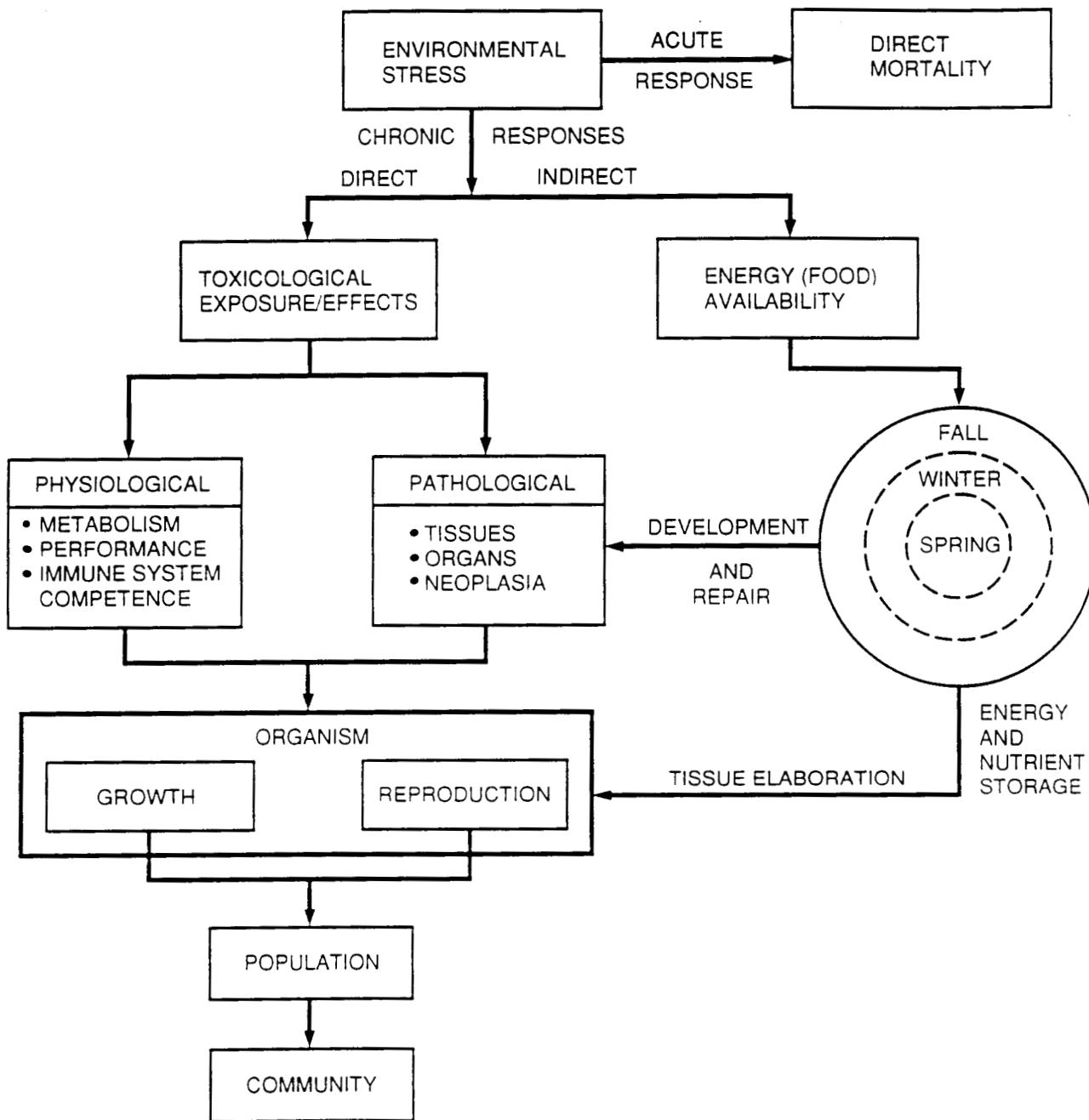


Fig. 5-9. Chronic-level responses of fish to sublethal contaminant stress. The relative importance of the direct and indirect pathways and the status of the lipid pool in influencing responses at the organism, population, and community levels are illustrated.

Health status ranking

To determine the relative health status of fish at each site, all fish were first segregated into one of four quartile ranks for each response variable. For example, if a fish from EFK 23 had a nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) value that fell within the highest 25% of all NADPH values ever measured for all fish at all sites, then that particular fish was placed in the lowest quartile for that indicator because high NADPH values are indicative of toxicant exposure or high stress. Conversely, if a fish from BF had a NADPH value within the lowest 25% of all measured values, then it was placed in the highest quartile for that parameter. For each of the bioindicator responses, fish from each site were segregated into quartiles and the total number of bioindicators for which a fish ranked in the lowest quartile were tabulated. The criterion for fish health based on this approach is the percentage of fish at each site that appeared in the lowest quartile for at least four of the indicators.

Based on this quartile ranking procedure, there appears to be a distinct downstream gradient in the quality of overall health, with the fish below NHP demonstrating the poorest health and fish from EFK 5 and BF demonstrating the highest level of health (Fig. 5-10). For example, 52% of fish from EFK 23 were in the lowest quartile for at least four variables, while only 35 and 26% of the fish from EFK 5 and BF, respectively, were in the lowest quartile for four variables.

The particular sets of variables that are responsible for the health status ranking were different between sites. It is on the basis of the differences in these sets of distinguishing variables that the mechanisms or agents ultimately responsible for the effects on fish health can be evaluated. To identify these distinguishing variables, the percentage of fish at each site that appeared in the lowest quartile for each response variable was calculated (Table 5-6). For example, 44 and 9% of the fish from EFK 23 and BF, respectively, occurred in the lowest quartile for NADPH, while 35 and 11% occurred, respectively, for triglycerides. The variables that identified fish in poor health from the upper sections of EFPC were primarily detoxification enzymes, condition indices, and indicators of lipid metabolism. Fish from the lower sections of EFPC were identified primarily by indicators of carbohydrate-protein and lipid metabolism.

Because elevated detoxification enzymes are indicators of toxicant exposure, effects observed on fish in the upper sections of EFPC were probably due directly to contaminant loading. In addition, both high levels of metallothionein and DNA damage in fish from EFK 23, EFK 19, and EFK 14 (see Sect. 5.3.1) are further evidence that fish in the upper half of EFPC experienced toxicant exposure. The concomitant effects on the various lipid parameters also observed in fish from the upper sections of EFPC (Fig. 5-11) indicate that toxicant exposure had an influence on lipid metabolism, either directly through metabolic processes or indirectly through the food chain (Fig 5-9). Such an altered metabolic disturbance can have serious implications for fish relative to energy balance especially during periods of critical energy demands such as sexual maturation, spawning, and over-winter survival (Adams et al. 1985). Relationships between detoxification enzyme levels and other response variables in fish can also be seen in Fig. 5-11. Not only was there an inverse relationship between the enzymes and lipid metabolism, there were also apparent correlative relationships between the enzymes and both the condition indices and parasite loads in the liver. All of these parameters demonstrated a downstream gradient in response, with the most severe responses typically occurring at the upper sections of EFPC and then moderating downstream.

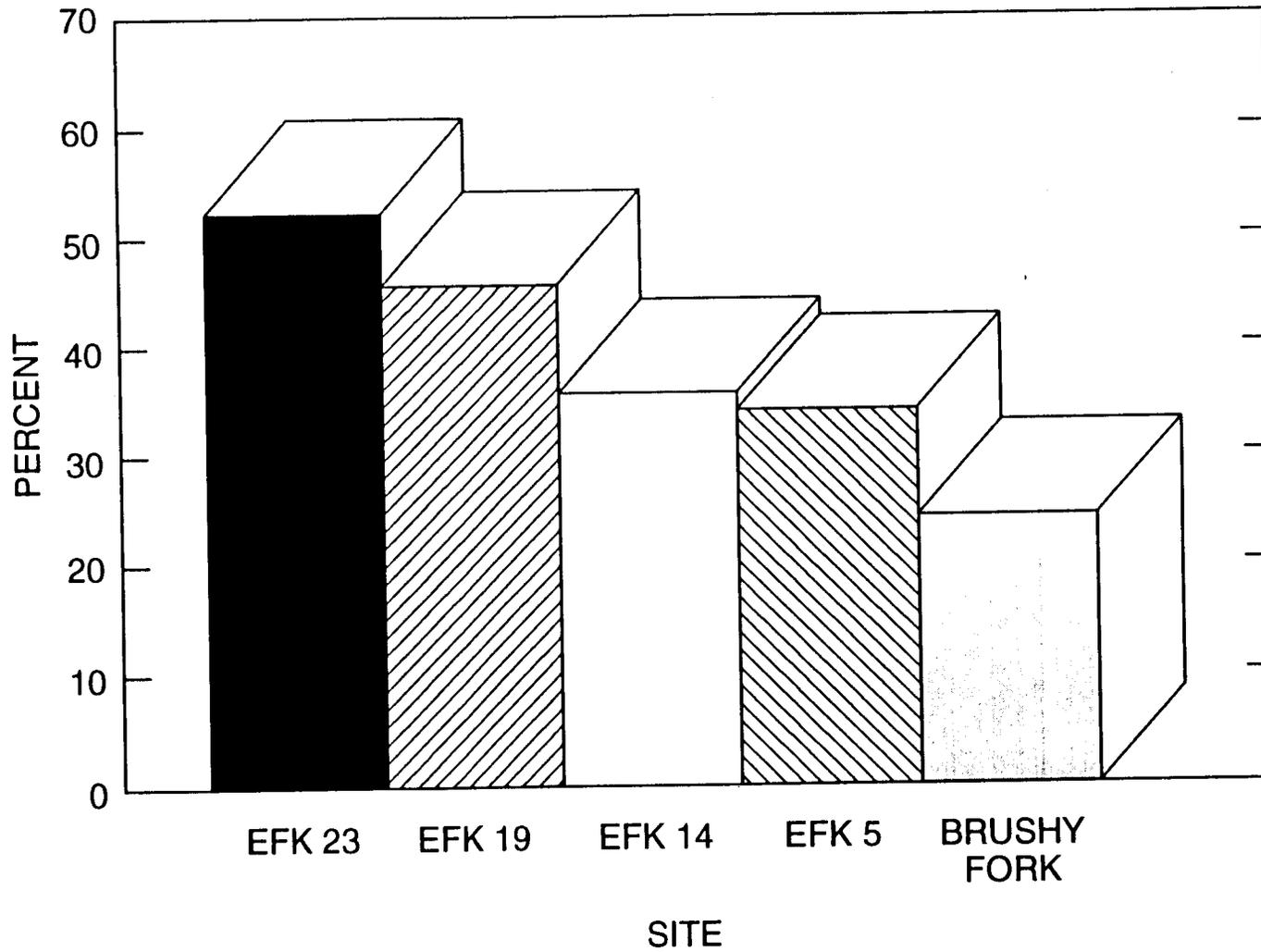


Fig. 5-10. Health status of percentage of redbreast sunfish at all East Fork Poplar Creek sites ranked in the lowest quartile for at least four bioindicators. Fish ranked in the lowest quartile for an indicator represent those individuals that are in poorest health for all fish at all sites. EFK = East Fork Poplar Creek kilometer.

Table 5-6. Percentage of redbreast sunfish at each sampling site appearing in the lowest quartile for each response variable

Response variable	Sampling sites				
	EFK 23	EFK 19	EFK 14	EFK 5	Brushy Fork
Serum glucose	19	18	25	31	23
Serum cholesterol	22	28	27	20	26
Serum protein	25	30	16	24	19
SGOT ^a	14	23	27	15	33
Total lipid	32	20	16	10	12
Body triglycerides	35	21	14	7	11
Phospholipid	3	15	29	24	19
PC/PE ^b	8	13	27	20	21
LFPMA ^c	24	10	5	2	4
EROD ^d	22	41	16	22	0
Chytochrome P-450	37	26	8	25	19
NADPH ^e	44	34	11	14	9
Condition factor	29	18	30	15	32
Visceral-somatic index	22	7	35	32	28
RNA/DNA ^f	11	3	11	15	11

^aSerum glutamate oxaloacetate transaminase.

^bPhosphatidylcholine/phosphatidylethanolamine.

^cMacrophage aggregates (functional parenchyma) in liver.

^d7-Ethoxysorufin O-deethylase.

^eNicotinamide adenine dinucleotide phosphate, reduced form.

^fRibonucleic acid/deoxyribonucleic acid.

Note: EFK = East Fork Poplar Creek kilometer.

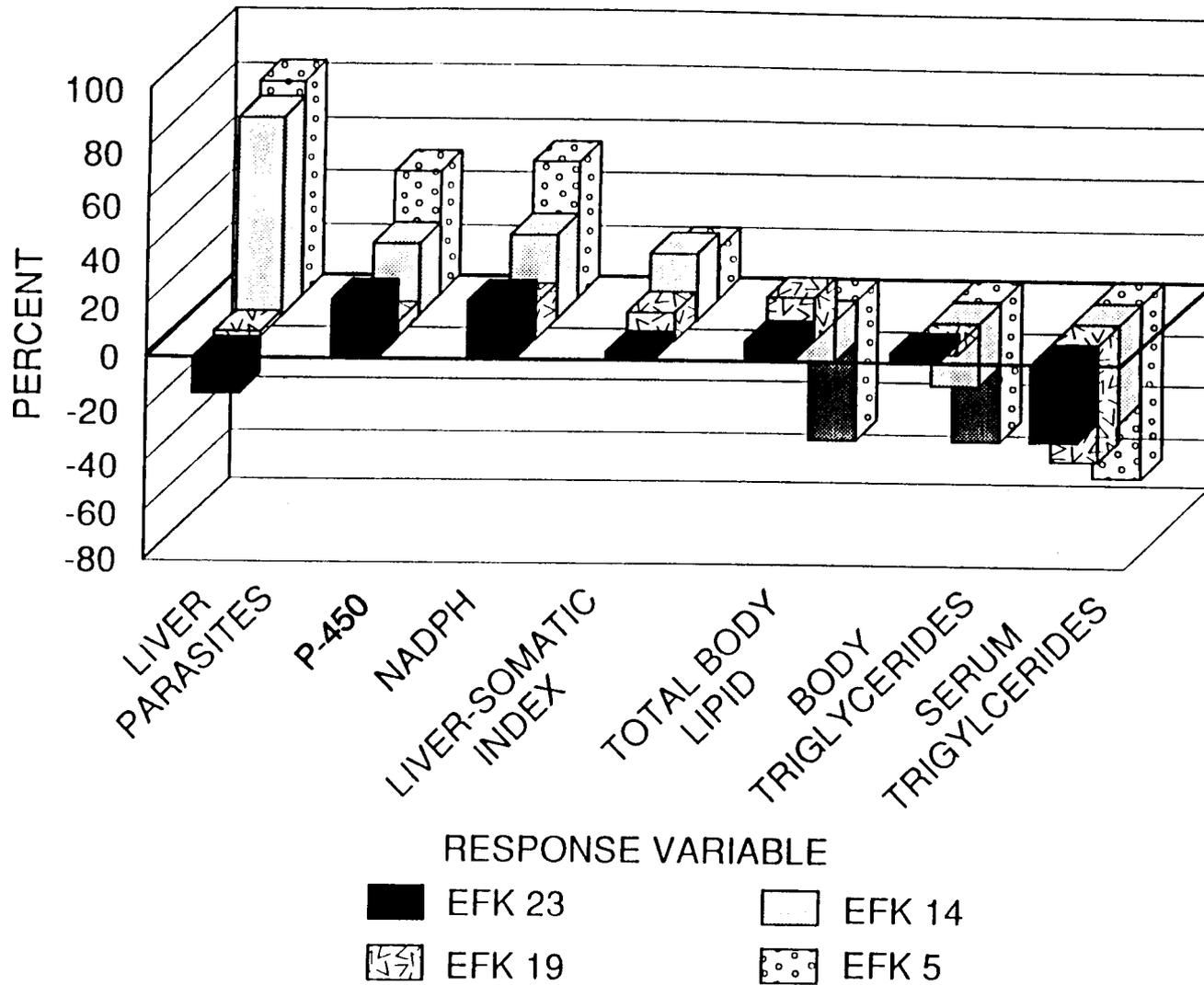


Fig. 5-11. Relative differences in the response of various bioindicators at each East Fork Poplar Creek site compared with the reference site, Brushy Fork. Values above or below the zero line indicate that the response of fish at each EFPC site was higher or lower, respectively, than the same response for fish from the reference site. EFK = East Fork Poplar Creek kilometer. NADPH = Nicotinamide adenine dinucleotide phosphate, reduced form; P-450 = Cytochrome P-450.

The relationships between toxicant exposure and other levels of effects observed in fish from EFPC are shown in Fig. 5-12. Direct contaminant exposure results in increased metabolic stress which is a negative drain on the energy and nutrient pools of organisms. Moreover, with toxicological stress, additional energy is required for repair and maintenance of damaged cells, tissues, and organs. Reduction in lipid pools due to these metabolic drains can also result in decreased calories available for growth and reproduction, where this energy is needed not only for protein (somatic) growth but also for gonadal maturation.

All of the relationships, however, between contaminant exposure, toxicological effects, energy availability, growth, and reproduction have not yet been established. In Fig. 5-12, the solid arrows represent conditions or correlative relationships that have been identified or quantified by this study. The broken arrows represent inferred conditions or relationships for which information is not yet available but for which studies have been initiated. We would expect, for example, that the indirect effects of exposure would result in altered food availability to fish, which would have an effect on the energy pool. Also, the direct toxicological effects on chemical or biological processes as they relate to the immune system and reproductive competence are not yet understood but will be investigated in future studies.

For fish from the reference stream, there was no indication of toxicological exposure as evidenced by the normal or baseline levels of detoxification enzymes and DNA damage. The indicators of health for BF fish are primarily condition indices and carbohydrate-protein metabolism responses, which reflect inadequate food availability and therefore reduced nutrition or energy intake. Drought conditions in BF during the study period were probably responsible for the reduced levels of available food, particularly in the fall. The water flow in BF is primarily from spring inputs which vary as a function of the level of the ground water table and therefore rainfall. Low stream flows result in less availability of benthic invertebrates for consumption by fish (Larimore et al. 1959) and therefore less available energy for critical physiological processes such as growth.

In EFPC, some of the observed effects (on fish) that relate to alterations in lipid metabolism, pathological condition, and overall condition could be due to or augmented by food availability. Availability in this case, however, would not be related to natural conditions such as stream flow or water level but probably to the indirect effect of toxicological exposure as manifested through the food chain (Fig. 5-12).

5.3.2.4 Summary

Two independent analyses demonstrated that the health status of fish in EFPC was due primarily to toxicological exposure; and the effects of this exposure were manifested mainly as impaired lipid metabolism, histopathological condition, and overall body condition. Evidence for toxicological exposure were the high levels of detoxification enzymes, metallothioneins, DNA damage, and liver-somatic indices observed in EFPC fish, particularly those sampled from the upper sections of the stream. Metabolic stress due to direct toxicant exposure reduces the amount and quality of energy available for maintaining immune system competence and adequate rates of growth. Further, this reduced energy availability could effect reproductive competence and reduce repair rates of damaged tissues. The effects of direct toxicant stress on chemical and biological processes in organisms as they relate to immune and reproductive competence is unknown

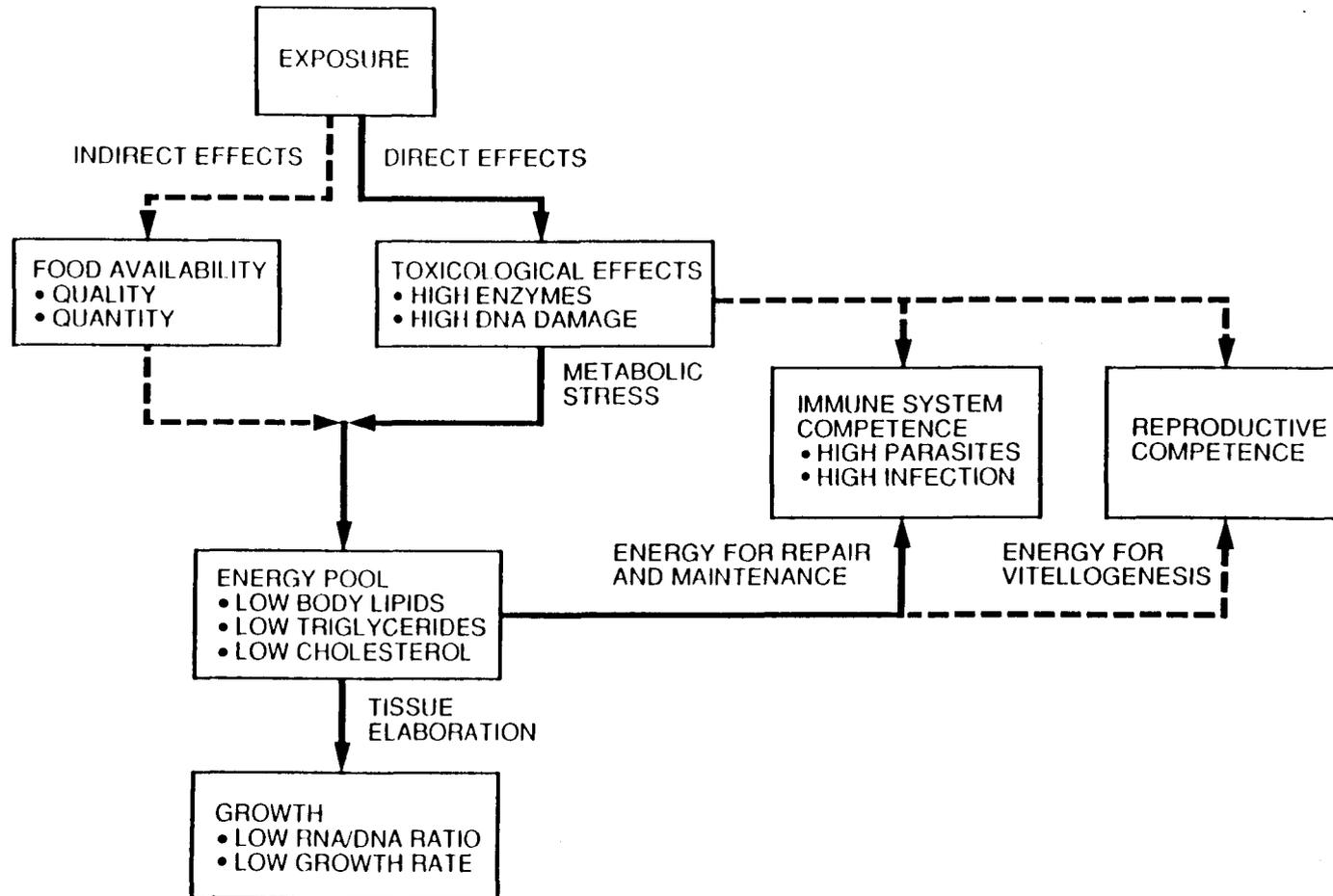


Fig. 5-12. Relationships between contaminant exposure, toxicological effects, energy availability, growth and reproduction for redbreast sunfish in East Fork Poplar Creek. Solid arrows represent conditions or correlative relationships established or quantified by this study. Broken arrows represent inferred conditions or relationships.

at this time, but future studies will address this issue. In addition, the indirect effects of exposure on food availability will also be investigated.

The health of fish in the reference stream was probably a direct result of the drought that reduced water levels in the stream, food availability for fish, and therefore the overall condition of fish, as reflected by the various condition indices and indicators of carbohydrate-protein metabolism. The health status of fish in EFPC could also be influenced by food or energy availability; but, in this case, availability would probably be related to the indirect effects of toxicants on the food chain.

Very little information exists on the effects of stress or toxicant loading on the behavior of fish. Periodic pulses of contaminants such as chlorine, mercury, and others could alter feeding behavior, reproductive behavior, or predator-prey and/or competition relationships. All of these modifications in behavior could ultimately be manifested as reduced growth and/or reproduction. Future studies will attempt to quantify the role of toxicants in influencing these important behavior-related effects.

5.3.3 Reproductive Indicators of Stress

5.3.3.1 Background

Based on such criteria as population density, biomass, and species diversity, the macroinvertebrate and fish communities in the upstream reaches of EFPC appear to have been adversely impacted by Y-12 Plant discharges near the headwaters of the stream (Loar et al. 1992b). Many of the observed population- and community-level impacts could have been due to either the direct or indirect effects of contaminants in the discharges on the reproduction of key species in the stream. However, short-term chronic toxicity tests have consistently failed to show that water taken from EFPC below NHP, the settling basin for the Y-12 Plant discharges, has any significant effect on either the reproductive competence or survival of test organisms (Loar et al. 1992b).

It is becoming increasingly apparent that short-term laboratory toxicity tests, although useful tools for testing effluent discharges, are not always sufficiently sensitive to detect the cumulative effects of low-level mixed contaminants on the biota of receiving streams such as EFPC. An alternative to such standard testing procedures would be to utilize studies involving the fish and invertebrates living in a stream. Instream organisms are more likely to be sensitive indicators of contaminant-related environmental stress, both because they are exposed to potential toxicants for considerably longer periods of time than test organisms, and because they integrate the effects of all environmental stressors acting at a contaminated site.

An evaluation of the reproductive competence of fish in EFPC, as compared with those in various reference streams, was initiated in the spring of 1988. The redbreast sunfish, *Lepomis auritus*, served as the initial subject for this preliminary study on the basis of its relative abundance and importance in the trophic ecology of EFPC. In particular, the process of oogenesis (egg development) in female redbreasts was given special attention for a number of reasons. The female egg is the primary link between the successive generations and as such is arguably the most important contributor to the reproductive success of a species. Furthermore, the number of eggs produced (fecundity) is one of the primary factors effecting the recruitment of new individuals into a population. Finally, oocyte development is a very complex process that is especially

susceptible to perturbation by xenobiotics at a wide variety of developmental stages, as evidenced by the results of recent studies (Mani and Sexena 1985, Johnson et al. 1988).

5.3.3.2 Results

Basic reproductive biology of the redbreast sunfish

The oocyte size-frequency distributions of nearly all redbreast ovaries sampled immediately before the breeding season exhibited a distinct "clutch" of synchronously-developing large oocytes interspersed within a more numerous pool of smaller oocytes (Fig. 5-13). On occasion, a less-distinct grouping of intermediate-size oocytes could also be detected, presumably representing early stages in the formation of a subsequent clutch.

The leading clutch of oocytes appears to break away from the mass of smaller oocytes in the late vitellogenesis or early maturation stage of development (Fig. 5-13). A clutch of large and synchronously developing vitellogenic oocytes is pictured (hatched area), interspersed within a more numerous pool of smaller vitellogenic and previtellogenic oocytes. In addition, a subsequent clutch (expressed as the number of oocytes per 0.1 mm increment of oocyte diameter per gram of fish) appears to be forming out of the relatively asynchronous mass of smaller oocytes. Such a pattern of oocyte development is termed "group-synchronous" development (Wallace and Selman 1981) and is relatively common among fish which spawn more than once in their lifetime (e.g., *L. auritus*). The existence of multiple clutches of developing oocytes in the same ovary further implies that the redbreast is a multiple-batch spawner (i.e., developing multiple clutches of eggs and spawning more than once per season). Although little is known of the spawning frequency of this particular species, multiple spawns are characteristic of many other centrarchids (Breder and Rosen 1966).

At the stage of ovarian development depicted in Fig. 5-13, it is possible to estimate the number of eggs a redbreast sunfish female will produce per spawn (fecundity per spawn) from the number of oocytes in the leading clutch, assuming that all oocytes in the clutch successfully complete development (Greeley et al. 1987). The fecundity of the redbreast can thus be estimated as:

$$\text{Fecundity} = \text{No. of oocytes in a developing clutch (clutch size)} \\ \text{(per spawn)}$$

$$\text{Fecundity} = \text{Mean clutch size} \times \text{total no. of spawns per year} \\ \text{(per year)}$$

Site differences in ovarian development

Spring 1988 site comparisons. In order to compare the prespawning reproductive condition of redbreast sunfish in EFPC with that in fish from a relatively noncontaminated site, female redbreasts were collected immediately prior to the beginning of the breeding season in late May 1988 from four study sites along EFPC and from a site on BF. Fish captured on the same day from the same site were typically in very similar stages of reproductive development (see Fig. 5-14 for the EFK 19 site). As illustrated by the mean oocyte size-frequency profiles for each site (Fig. 5-15), fish at most sites had very distinct

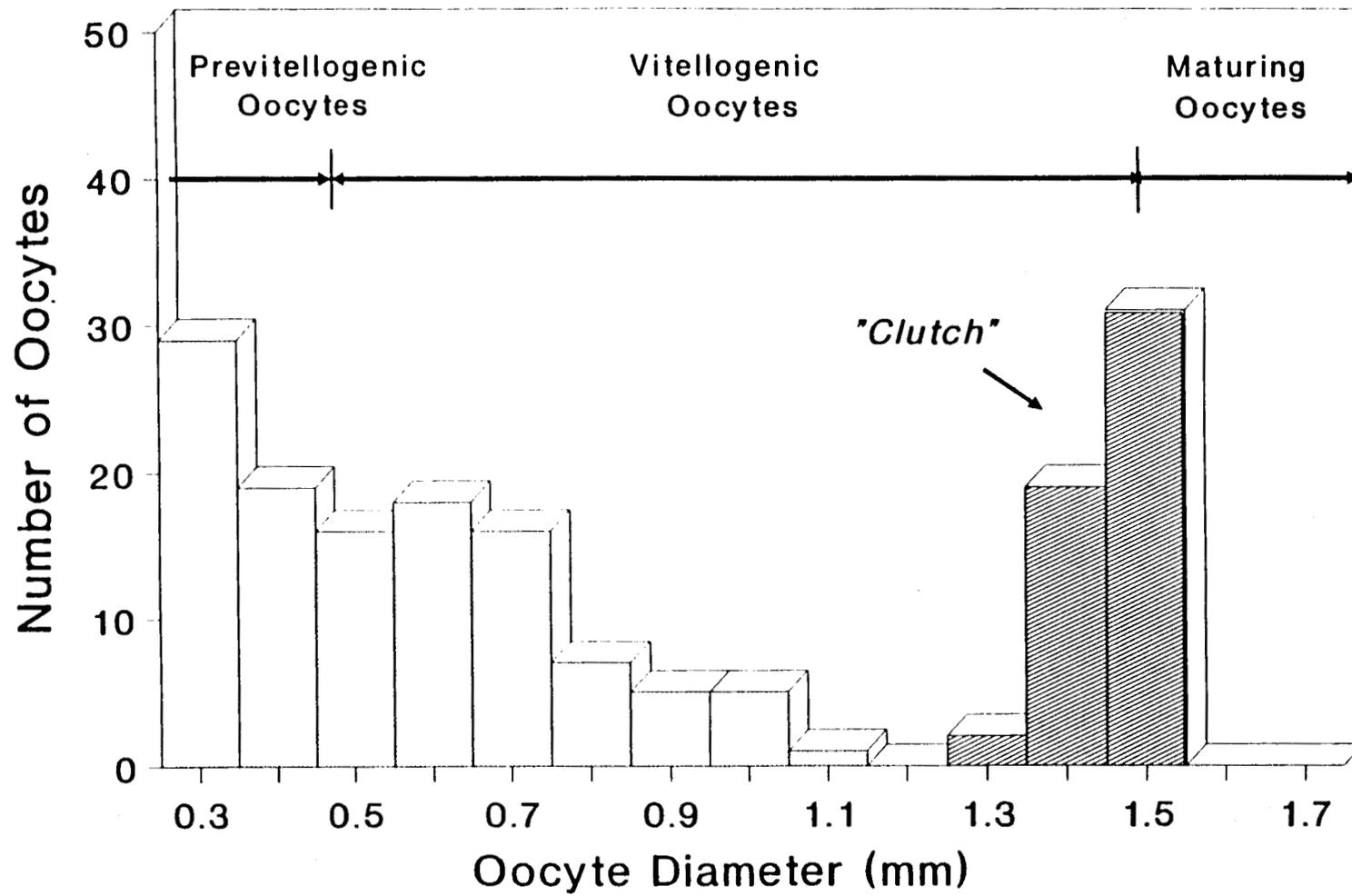


Fig. 5-13. Oocyte size-frequency profile illustrating a preliminary oocyte staging system for the redbreast sunfish, *Lepomis auritus*.

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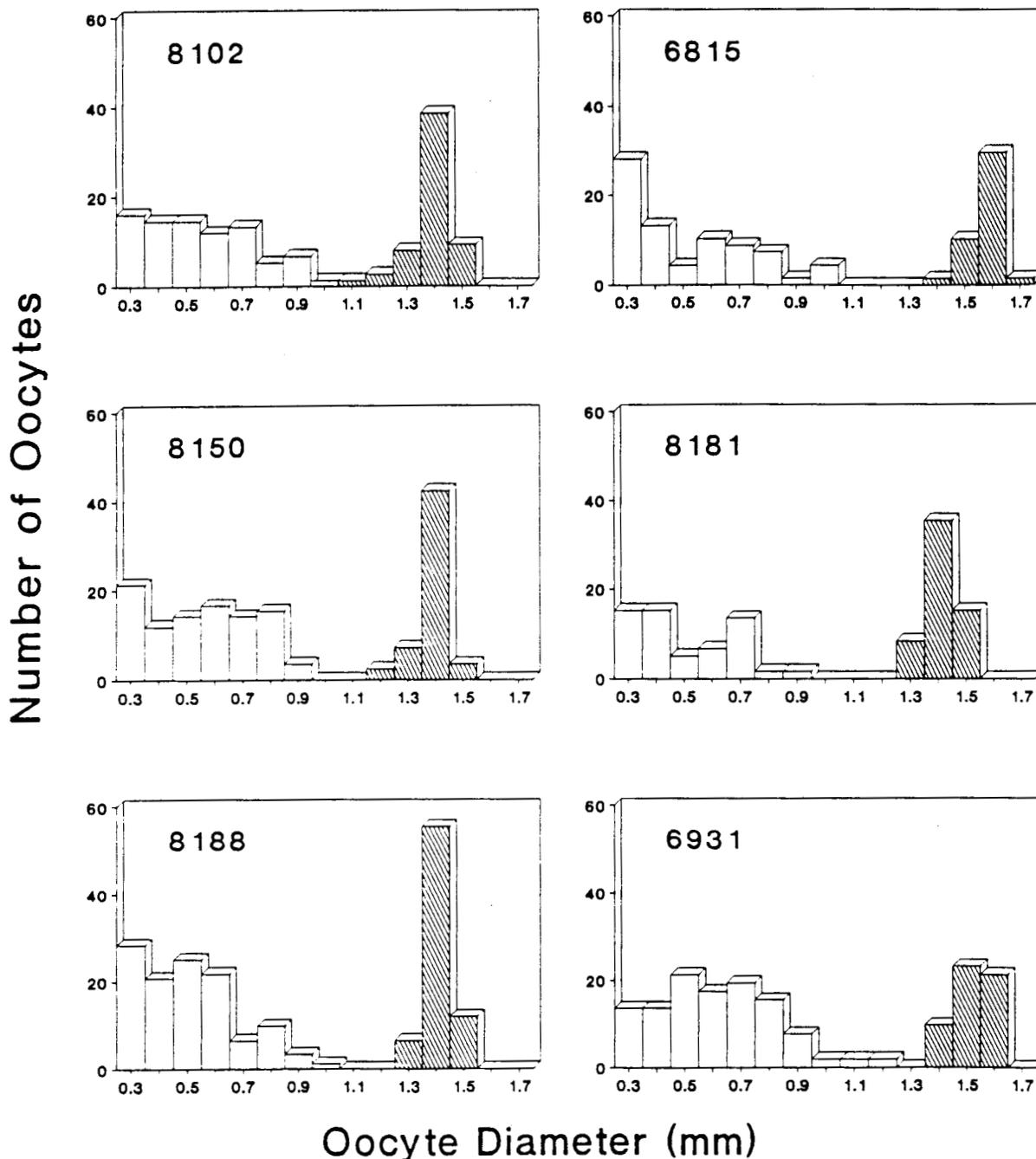


Fig. 5-14. Comparison of oocyte size-frequency profiles for six redbreast sunfish collected on May 24, 1988, from a single study site (EFK 19). The oocyte size-frequency profile illustrated in Fig. 5-13 of this document represents a seventh fish from this sample group. Measurements are expressed as the number of oocytes per 0.1 mm increment of oocyte diameter per gram of fish. Fish identification numbers are presented within each diagram. Note similarities in the developmental stage of the leading clutches (hatched bars) in each fish. EFK = East Fork Poplar Creek kilometer.

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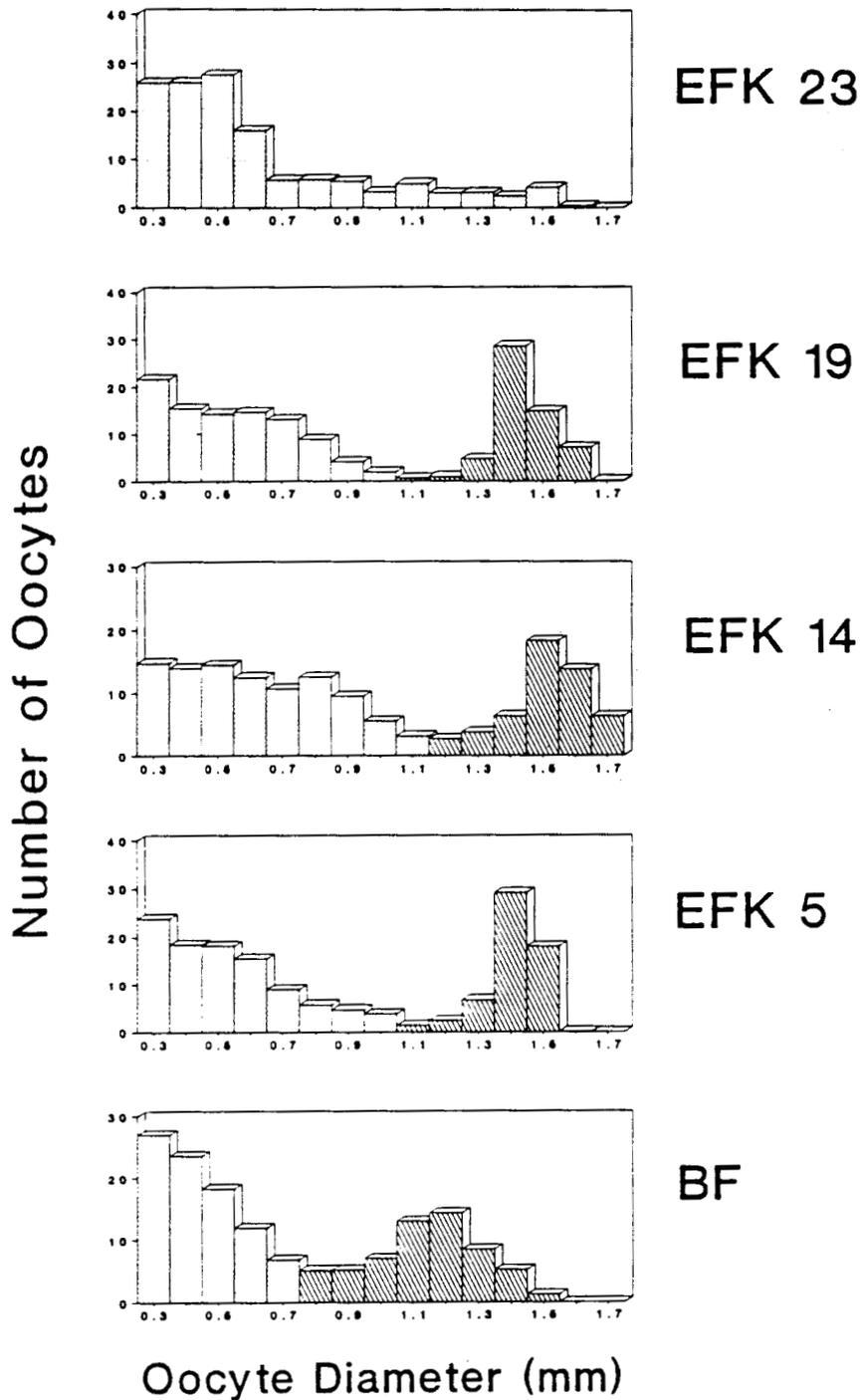


Fig. 5-15. Variation between study sites in the mean oocyte size-frequency profiles for female redbreast sunfish sampled in May 1988. Measurements are expressed as the mean number of oocytes per 0.1 mm increment of oocyte diameter per gram of fish. Note distinct clutches of synchronously developing oocytes (hatched areas) at the three downstream sites on East Fork Poplar Creek (EFK 19, EFK 14, and EFK 5) and at the reference site, Brushy Fork (BF). EFK = East Fork Poplar Creek kilometer.

and synchronous clutches of developing oocytes in either middle to late vitellogenesis (BF) or in late vitellogenesis to early maturation (EFK 19, EFK 14, and EFK 5). A slight delay was noticed in the development of oocyte clutches at the reference site (BF) compared with downstream sites along EFPC, but this can be attributed to the differing temperature regimes in the two streams.

The only study site at which a distinct clutch of developing oocytes could not be discerned was EFK 23, the location closest to the industrial outfall from the Y-12 Plant. Fish at this site were characterized by a high degree of variability in reproductive condition; although a few females had relatively well developed ovaries, others had very immature ovaries with few if any vitellogenic or maturing oocytes (Fig. 5-16). Three fish (6977, 6948, and 8358) had very immature ovaries with no obvious indications of clutch formation, while others had clutches of oocytes in various stages of development from mid-vitellogenesis through maturation.

By nearly every measure of reproductive competence applied to these samples, female redbreasts collected from EFK 23 were reproductively disadvantaged at the beginning of the breeding season in comparison with fish from other study sites. For instance, the mean number of vitellogenic oocytes was much lower at EFK 23 than at downstream sites along EFPC (Fig. 5-17). The mean size of the leading clutch was also significantly lower at EFK 23 than other study sites. Furthermore, the numbers of both atretic (nonviable) oocytes and ovarian parasites were greater at EFK 23 than other sites.

August 1988. Sampling was conducted again in early August in conjunction with the annual sampling for the main biological indicator task. Unfortunately, this sampling period coincided with the rapidly approaching end of the breeding season at several study sites. Although a few fish with relatively well developed ovaries were still found at each site sampled in August (Table 5-7), most had ovaries which appeared to be in the early stages of seasonal ovarian regression. By the time of the August samplings, it was difficult to make significant comparisons between the reproductive status of fish at the various sites sampled. Even at the primary reference site, BF, samples taken before and after the August sampling conducted at the other study sites showed marked differences in ovarian condition (Fig. 5-18). It is apparent from this example that differences between sites in August could easily be artifactual. The difference may have more to do with the sites being sampled on different days during a 2-week period of great seasonal flux in reproductive condition than with actual differences in reproductive capability between fish at different sites.

5.3.3.3 Reproductive summary

According to comparisons between sites of several criteria of reproductive competence—numbers of vitellogenic oocytes (lower immediately below NHP), egg clutch sizes (lower), numbers of damaged or dead oocytes (higher), and ovarian parasites (higher)—the reproductive potential of female *L. auritus* collected directly below NHP was compromised at the May onset of the 1988 breeding season. Some females from this site had not even begun to produce a clutch of vitellogenic oocytes by the time spawning was initiated at other sites along EFPC. By contrast, fish collected 4 km or further downstream of NHP exhibited few if any signs of reproductive dysfunction during this most critical period of the breeding season.

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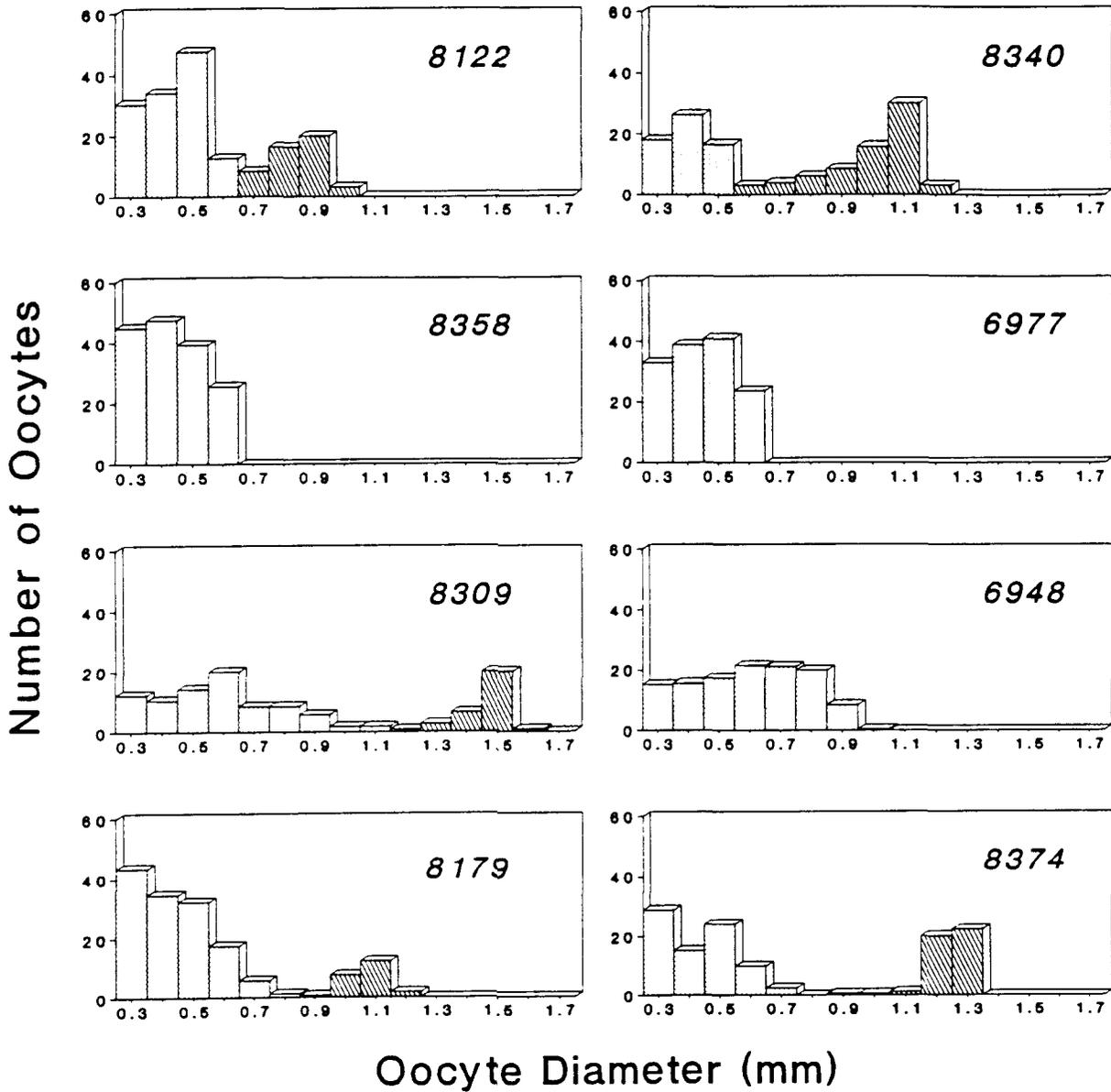


Fig. 5-16. Comparison of oocyte size-frequency profiles for eight redbreast sunfish collected on May 25, 1988, from EFK 23, the sample site nearest the outfall for Y-12 Plant discharges. Measurements are expressed as the number of oocytes per 0.1 mm increment of oocyte diameter per gram of fish. Fish identification numbers are presented within each diagram. EFK = East Fork Poplar Creek kilometer.

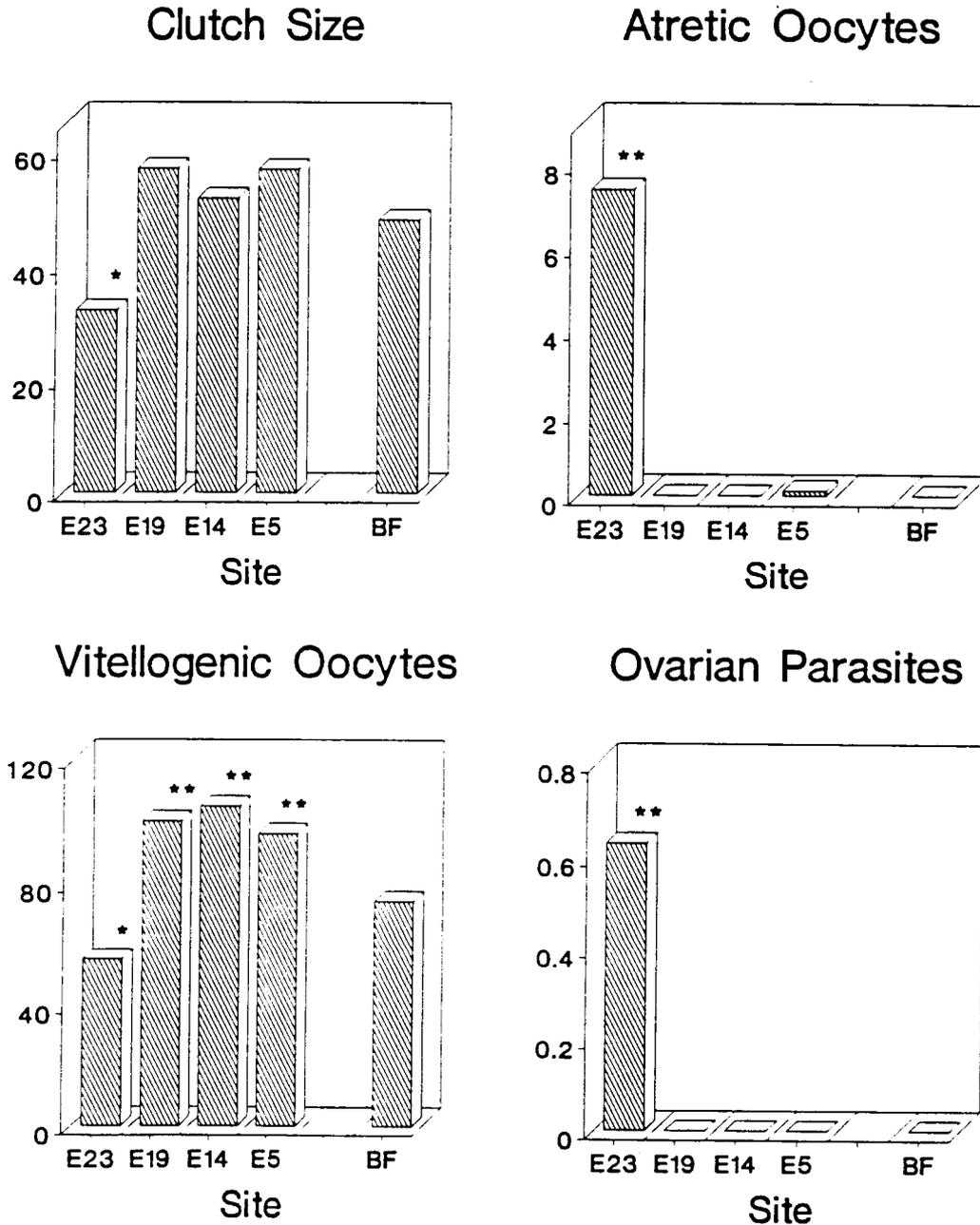


Fig. 5-17. Comparison of various reproductive indices for female redbreast sunfish collected from the study sites prior to the beginning of the breeding season in late May 1988. "Clutch size" is the mean number of oocytes in the leading clutches. "Atretic oocytes" include all dead or obviously damaged oocytes ≥ 0.3 mm in diameter. "Vitellogenic oocytes" include all yolk-containing oocytes ≥ 0.6 mm in diameter. "Ovary parasites" are cysts of the white grub (genus *Postodisplostomum*). Asterisks indicate significant ($p < 0.05$) differences from the reference site Brushy Fork (BF). * = Values less than reference values. ** = Values greater than reference values. E5 = site on East Fork Poplar Creek 5 km upstream from the confluence with Poplar Creek.

Table 5-7. Reproductive parameters for female redbreast sunfish (*Lepomis auritus*) collected in August 1988 from four sites along East Fork Poplar Creek and from two reference streams, Brushy Fork and Hinds Creek

Values are presented as means \pm SE

Site	<i>n</i>	GSI	Clutch ^a size	Atretic ^a oocytes	Ovary ^a parasites
EFK 23	13	4.9 \pm 0.8	37.1 \pm 6.4	1.47 \pm 0.94	1.57 \pm 0.45 ^b
EFK 19	12	5.2 \pm 1.2	36.9 \pm 5.4	0.33 \pm 0.27	0.41 \pm 0.22
EFK 14	12	3.6 \pm 0.6 ^b	24.9 \pm 4.8	6.35 \pm 4.71	0.09 \pm 0.09
EFK 5	13	2.2 \pm 0.4 ^b	17.3 \pm 5.2	6.04 \pm 2.53	0.16 \pm 0.13
BF	10	6.4 \pm 0.9	34.3 \pm 5.2	0.49 \pm 0.36	0.34 \pm 0.32
Hinds Creek	12	2.9 \pm 0.7 ^b	23.1 \pm 8.2	9.72 \pm 5.44	0.80 \pm 0.36

^aNumber of oocytes or parasite cysts/gram of fish.

^bValues that differ significantly ($p < 0.05$) from the primary reference site Brushy Fork (BF).

Note: EFK = East Fork Poplar Creek kilometer; BF = Brushy Fork; SE = standard error.

The results of these studies suggest that the factor(s) affecting reproduction in the upstream reaches of EFPC acted primarily by inhibiting the process of vitellogenesis or yolk formation in redbreast sunfish. There are several possible explanations for this inhibition: (1) the hormonal cues which regulate vitellogenin production in the liver could be compromised (pointing to a possible action of contaminants on the endocrine system of the fish), (2) liver cells responsible for vitellogenin production could be damaged as a result of pollutant exposure, or (3) the oocytes themselves may be damaged and unable to correctly absorb and process vitellogenin into yolk proteins. Each of these possibilities is currently being evaluated.

The results of these studies further suggest that the factors affecting reproduction of *L. auritus* at the study site closest to NHP were apparently minimized or rendered inactive (at least to *L. auritus*) by dilution, biodegradation, or water-sediment interactions within the first few kilometers of the stream. However, redbreast sunfish appear to be relatively pollution-tolerant fish, as is evident from their continuing presence at even the most heavily impacted areas of EFPC. Pollution-intolerant species of fish not currently represented in the stream might be more sensitive to presumptive reproductive hazards in EFPC and thus could potentially serve as better sentinels for such contaminants at sites downstream of NHP.

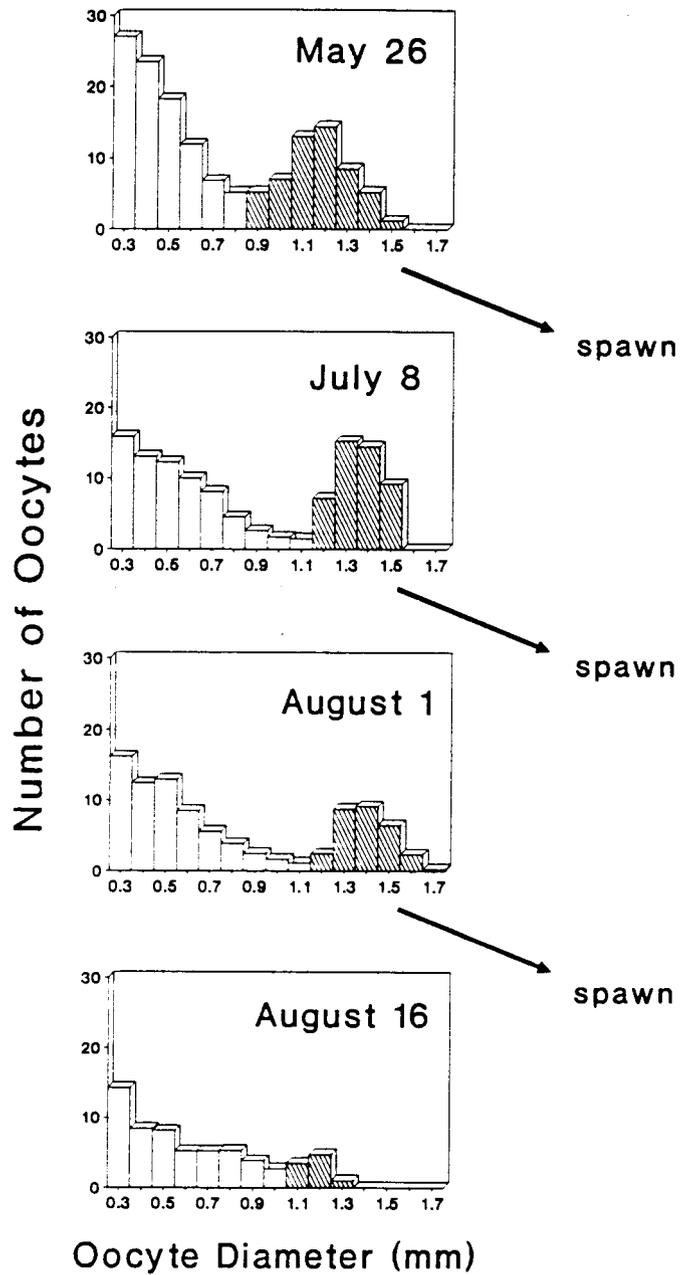


Fig. 5-18. Temporal changes in the mean oocyte size-frequency profiles for female redbreast sunfish collected from a reference site, Brushy Fork, at intervals throughout the breeding season. Data are expressed as the mean number of oocytes per 0.1 mm increment of oocyte diameter per gram of fish. Note the apparent multiple spawns, and the rapid changes in ovarian condition occurring from August 1 to August 16. Clutches of synchronously developing oocytes identified as hatched areas.

Continuing reproductive and developmental studies can provide an understanding of the deleterious effects of contaminants discharged to EFPC by the Y-12 Plant. Future directions of these studies are outlined in Sect. 5.4.

5.3.4 Summary and Synthesis

This project on biological indicators of contaminate-related stress has been designed to address three concerns relative to the effects of continuing operation of the Y-12 Plant on the biota of EFPC: (1) spatial effects, or health status of fish in various areas of EFPC (as compared with fish in a nonaffected area), (2) temporal effects or changes in the health status of fish in EFPC resulting from cleanup or remedial actions, and (3) evaluation of the causative agents or mechanisms responsible for any effects observed on the fish populations in EFPC.

The principal findings of the studies conducted from spring 1986 through fall 1987 were as follows:

1. In EFPC there was a downstream gradient in the health of fish with the lowest condition occurring in fish below NHP and the highest health in fish from EFK 5.
2. Bioindicators representative of several levels of organism functioning are necessary to evaluate the effects of chronic stress on fish.
3. The health of fish in the lower section of EFPC (EFK 18–EFK 5) improved over the study period.
4. Health of fish in the upper sections of EFPC (EFK 23–EFK 19) has not appeared to improve over this period.
5. With continued remedial actions at the Y-12 Plant, the condition of fish in the upper sections of EFPC is expected to improve in the future.
6. The condition of fish in EFPC was due primarily to toxicological exposure, particularly in the upper sections.
7. Evidence for toxicological exposure was the high level of detoxification enzymes, metallothioneins, DNA damage, and liver-somatic indices observed in EFPC fish.
8. The effects of toxicological exposure were manifested primarily as impaired lipid metabolism, histopathological condition, and overall body condition.
9. The reproductive potential of female redbreast collected directly below NHP was compromised at the onset of the 1988 breeding season, while fish collected 4 km or further downstream of NHP exhibited few, if any, indications of reproductive dysfunction.

10. Metabolic stress due to toxicant exposure reduces the amount of energy available for growth, gonad maturation, and repair of damaged tissues and also compromises the integrity of the reproductive and immune systems.
11. The health or condition of fish in the reference stream was probably affected largely by drought conditions that have occurred over the study period.
12. Lower water levels due to the drought reduce food availability for fish and therefore growth and condition.
13. In addition to direct metabolic stress, toxicants can affect fish indirectly through impairing normal behavior (such as reproduction and feeding) and alter food chain processes. Effects of stress on behavior and food chain relationships will be investigated in future studies.

5.4 FUTURE STUDIES AND NEW INITIATIVES

The annual biomonitoring studies will continue to be conducted at four sites in EFPC and a reference area. The principal bioindicators measured will be those that can provide the most cost-effective information possible for assessing and evaluating the effects of the Y-12 Plant on the biotic integrity of EFPC (see Sect. 5.3). Hinds Creek will replace BF as the primary reference site in future biomonitoring studies because, as demonstrated in this report, the condition of fish in BF has apparently been adversely affected by a drought. Fish in Hinds Creek, however, seem less affected by the drought, possibly because this is a larger stream, and critical resources such as food and habitat availability are not as limited as in Brushy Fork.

5.4.1 New Initiatives: Manipulative Caging Experiments

These experiments will be conducted during spring and summer 1989 to evaluate the stress effects on fish under semi-controlled conditions. Noncontaminated fish will be placed in large cages located in EFPC and in reference streams. The response of these fish to environmental conditions in EFPC will be determined by applying the selected suite of indicators shown in the present study in order to provide the most cost-effective information for assessing the effects of chronic stress on fish.

5.4.2 Reproductive Studies: Field Studies

Sampling of redbreast sunfish for analysis of reproductive condition is continuing at study sites along EFPC and at various reference sites. These new rounds of studies are being conducted both to expand upon the preliminary results presented in this report and to evaluate the effectiveness of remedial actions that have been carried out at the Y-12 Plant during the intervening months. In particular, the construction and operation of a new retention basin in fall 1988 and the concurrent closure of NHP are significant actions which could affect the quality of water entering EFPC downstream.

Based upon the results of these preliminary studies, several changes have been made in both the procedures and parameters to be measured during future sampling periods. The number of reference sites was increased to three: one station each on BF, Hinds Creek, and Paint Rock Creek in order to more clearly confirm the relationship between perceived reproductive impairment in *L. auritus* in EFPC and actual contaminants in the stream.

6. INSTREAM ECOLOGICAL MONITORING

A. J. Gatz, Jr., M. G. Ryon, J. G. Smith, and V. R. Tolbert

6.1 BENTHIC MACROINVERTEBRATES (*J. G. Smith and V. R. Tolbert*)

6.1.1 Introduction

During the first year of the BMAP, the benthic macroinvertebrate community of EFPC was found to be stressed to varying degrees from the headwaters to the lower reaches of the stream (Smith 1992a). The greatest evidence of stress was exhibited by the benthic community at EFK 24.4 upstream of NHP. Downstream of this site, gradual improvements were evident in the benthic community with increasing distance from the Y-12 plant. Maximum improvement was observed at the stream's mid-reach site, EFK 13.8. However, relative to a nearby reference stream, BF, impacts were still evident at all sites in EFPC.

The benthic macroinvertebrate monitoring program initiated during the first year was continued during the second year with the primary objective of further characterizing the spatial and temporal trends of this community in EFPC. Such a long-term characterization provides a better understanding of natural, temporal changes in benthic invertebrate community structure as well as an excellent baseline from which future changes can be followed. In addition to the characterization study, experimental studies were initiated during the summer of 1988 primarily to try to (1) identify the factor(s) impacting the benthic invertebrate community in upper EFPC and (2) determine the downstream extent of the Y-12 Plant's impact on the benthic community. Preliminary results from these studies are included.

6.1.2 Materials and Methods

6.1.2.1 Benthic macroinvertebrate monitoring studies

Quantitative benthic macroinvertebrate samples were taken at approximately 1-month intervals from June 1986 through May 1987, from 6 sites in EFPC and 1 site in BF (BFK 7.6) (Fig 2-1). After the September 1986 sampling period, the location from which samples had been previously collected at EFK 13.8 was moved approximately 50 m upstream due to construction-related destruction of the original site. Five randomly selected benthic macroinvertebrate samples were collected from designated riffles at each site with a Hess stream-bottom sampler (0.1-m²) fitted with a 363- μ m mesh collection net. To obtain a better estimate of total taxonomic richness, one qualitative sample was collected from riffle and nonriffle habitats (e.g., pools, leaf packs, detritus, snags, etc.) at each site in March/April 1987, with a D-frame aquatic dip net (800 \times 900- μ m mesh). Qualitative samples were washed in the field in a net (363- μ m mesh) and a white pan in order to concentrate the organisms. Both quantitative and qualitative samples were placed in pre-labeled polyurethane-coated glass jars and preserved in 80% ethanol; the ethanol was replaced with fresh ethanol within 1 week.

Supplemental information was recorded at the time of sampling. At each stream site, water temperature and specific conductance were measured with a Col-Parmer Model R-1491-20 LCD temperature-conductivity meter. Water depth, location within the riffle area (distance from permanent headstakes on the stream bank), relative current velocity (very slow, slow, moderate, or fast), and substrate type, using a modified Wentworth particle size scale (Loar et al. 1985), were all recorded for each sample.

All samples were washed in the laboratory in a standard 250- μm mesh sieve and then placed into a white tray. Organisms were removed from the debris with forceps and placed in labeled vials containing 70% ethanol. Organisms were identified to the lowest practical taxonomic level using a stereoscopic dissecting microscope. Chironomid larvae were first sorted into groups based on morphological similarities, and then one or more representatives of each group was mounted on a slide in CMC-10 mounting media and identified using a compound microscope. The remaining larvae were then identified at a magnification of 80 to 120 \times with a dissecting microscope. A blotted wet weight of all individuals within each taxon of each sample was obtained to the nearest 0.01 mg on a Mettler analytical balance.

A reference collection, composed of both ethanol-preserved and slide-mounted individuals for which the identification of each taxon has been verified, is maintained at ORNL. The remaining nonchironomid taxa and unmounted chironomids were preserved in 80% ethanol by site and collection date and maintained at ORNL.

All statistical analyses were done using standard procedures from the Statistical Analysis System (SAS 1985a, 1985b). The Shannon-Wiener index (H') was used to calculate the taxonomic diversity of benthic macroinvertebrates at each site (Pielou 1977):

$$H' = - \sum p_j \log_2 p_j \quad (7)$$

where p_j is the proportion of the benthic invertebrate community made up by species j . H' values of 3 or greater are generally found in areas of clean water, while values of 1 to 3 are found in areas of moderate pollution, and values of <1 are found in heavily polluted water (Platts et al. 1983).

Statistical comparisons were performed on transformed data [$\log_{10}(X + 1)$, where X = individual values for density, biomass, richness, etc.] (Elliott 1977). Spatial trends in density; biomass; number of taxa (taxonomic richness); number of Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT richness); and taxonomic diversity of the EFPC sites and BF were compared with a two-way analysis of variance (ANOVA), with site and date as the main effects. Significant site differences were separated with a Tukey's studentized range test (HSD); significant differences were accepted at $p < 0.05$. Between-year comparisons (i.e., Year 1, June 1985–May 1986 vs Year 2, June 1986–May 1987) were made within each site for evidence of recovery. This was accomplished with a one-way ANOVA on the various parameters (i.e., density, biomass, taxonomic richness, etc.), with year as the main effect; between-year comparisons for BF were made only with data from the months of January through May.

In order to obtain an estimate of annual community production of the invertebrates at each site, annual production rates for each taxon were determined either directly with the size-frequency method (Hynes and Coleman 1968; Hamilton 1969; Benke 1979) or indirectly by the PB method, multiplying the mean annual biomass by an estimated annual production to biomass (P/B) ratio (Benke 1984). Direct estimates of annual production were made for only a few "target" taxa which were selected primarily because they were

(1) common or abundant at some sites and/or (2) they were representative taxa of major groups of invertebrates that should occur throughout EFPC. At those sites where annual production of the target taxa was estimated directly during the first year, the resulting P/B ratios were used to estimate production during the second year using the indirect P/B ratio method. At those sites where the target taxa were not abundant enough to estimate production directly, a mean annual P/B ratio was used that was calculated from those sites where they were abundant enough to make direct estimates. Annual P/B ratios used for estimating production of the nontarget taxa were obtained from either (1) values published for specific taxa in the upper Southeast region of the United States or (2) a theoretical cohort P/B ratio of 5.0 and then corrected for the cohort production interval (CPI = the length of aquatic life during which a taxon is present and growing) (Waters 1977, Benke 1979, Waters 1979). The CPIs were obtained from either published estimates or determined from published life history information or the authors' unpublished data. The annual P/B ratios used in this report are listed in Appendix D, Table D-1. Mean values (untransformed) for P/B ratios that were weighted by the number of individuals in each taxon were compared among sites with a two-way ANOVA, with site and date as the main effects. Significant site differences were separated with a Tukey's studentized range test (HSD); significant differences were accepted at $p > 0.05$. Between-year comparisons of mean annual P/B ratios (weighted by the number of individuals in each taxon) within each site were made with a one-way ANOVA with sampling year as the main effect. Considerable heterogeneity existed in the variance of the means; however, a log transformation of the data resulted in no notable improvement. Thus, statistical analysis of these data with an ANOVA results in conservative comparisons.

6.1.2.2 Experimental studies

During the Summer of 1988, experimental studies were initiated for the EFPC BMAP with locally available invertebrates primarily to (1) try to identify the factors impacting the benthic macroinvertebrate community in upper EFPC and (2) try to determine the extent of the Y-12 Plant's influence on the benthic invertebrate community in EFPC. In these studies, two species of invertebrates were utilized for instream and/or laboratory studies including (1) the fingernail clam, *Sphaerium fabale* and (2) the hydrosychid caddisfly, *Hydropsyche depravata*.

Clam studies

Preliminary studies with *Sphaerium* keyed on evaluating the utility of this clam in an instream bioassay as an additional monitoring tool for EFPC. This test organism was selected primarily because of its abundance and local availability (approximately 2100 individuals/m²; J. G. Smith, ESD, ORNL, unpublished data) at the primary EFPC reference site, BF (BFK 7.6) and because it appears to be relatively hardy. Although there is no record of this species of clam occurring in EFPC, historical records indicate that it has been collected from lower Bear Creek, a tributary of lower EFPC (Van der Schalie and Burch 1961, unpublished data). This species of clam also occurs in Beaver Creek, which is located just east of the Oak Ridge area in Knox County, Tennessee

(J. G. Smith, Environmental Sciences Division, ORNL, Personal Observation; Heard 1977).

During 1988, two separate experiments were conducted with clams. The first experiment (EX1) was initiated in mid-July and lasted 84 days; the second experiment (EX2) was initiated in early October and lasted 88 days. In each study, growth and survival of clams were followed over the exposure period. The source of clams for both experiments was BF.

In EX1, clams were placed instream at two sites in EFPC (EFK 23.4 and EFK 13.8) and one site in BF (BFK 7.6), which served as a control (Fig. 6-1). Two size classes of clams were used: the smallest size class had shell lengths (greatest anterior to posterior length) ranging from 7.5 to 8.5 mm (small clams), and the largest size class had shell lengths ranging from 10.7 to 11.9 mm (large clams). After measuring the length of each clam to the nearest 0.01 mm with a vernier caliper, a number was placed on the shell with permanent silver ink. Twenty-five marked clams within each size class were placed into 8 × 10-in plastic photographic trays containing gravel collected from a nearby relatively undisturbed stream; windows were cut in each end to allow flow through the trays. Each tray was covered with a piece of 1-mm mesh netting; the netting was attached to the trays with strips of plastic that were held on with screws.

Two trays, one containing "small" clams and one containing "large" clams, were placed instream in a riffle at each study site. Trays were secured to the stream bottom by attaching wire from opposite corners to rebar which had been driven into the streambed. At approximately 3-week intervals clams were retrieved, measured, placed back into their respective trays, and returned to the water. Mortality was noted each time the clams were retrieved.

In EX2, clams were placed instream at all sites used in EX1 (i.e., EFK 23.4, EFK 13.8, and BFK 7.6), as well as two additional control sites: Hinds Creek at kilometer 20.6 (HCK 20.6), located in northeast Anderson County, Tennessee, and Bull Run Creek at kilometer 20.0 (BRK 20.0), located in south central Union County, Tennessee (Fig. 6-1). One tray, containing gravel and 25 individually marked and measured clams ranging in size from 7.25 to 8.30 mm, was placed in a riffle at each site and secured to the streambed as in Ex. 1. The clams were retrieved at approximately 3-week intervals, measured, placed back into their respective trays, and returned to the water. Mortality was noted each time the clams were retrieved.

Caddisfly studies

During the first year of the BMAP, several major groups of invertebrates were found to be either absent or rare in upper EFPC (Smith 1992a). Particularly notable was the rare occurrence of invertebrates in upper EFPC which obtain their food by filtering suspended particles from the water column (i.e., filter feeders). Filter-feeding organisms, such as hydropsychid caddisflies and black flies (Diptera: Simuliidae), frequently achieve their greatest densities downstream of surface release impoundments (e.g., Mackay and Waters 1986, Wotton 1982). Thus, filter-feeders should potentially be prominent members of the benthic invertebrate community in the upper EFPC just downstream of the outfall of NHP. As a preliminary step in determining the reason(s) for the absence of these invertebrates at this site, a series of studies were conducted with a locally available and abundant filter-feeding caddisfly, *Hydropsyche depravata*. The primary objectives of the studies with this caddisfly were to try to determine (1) if this filter-feeding invertebrate

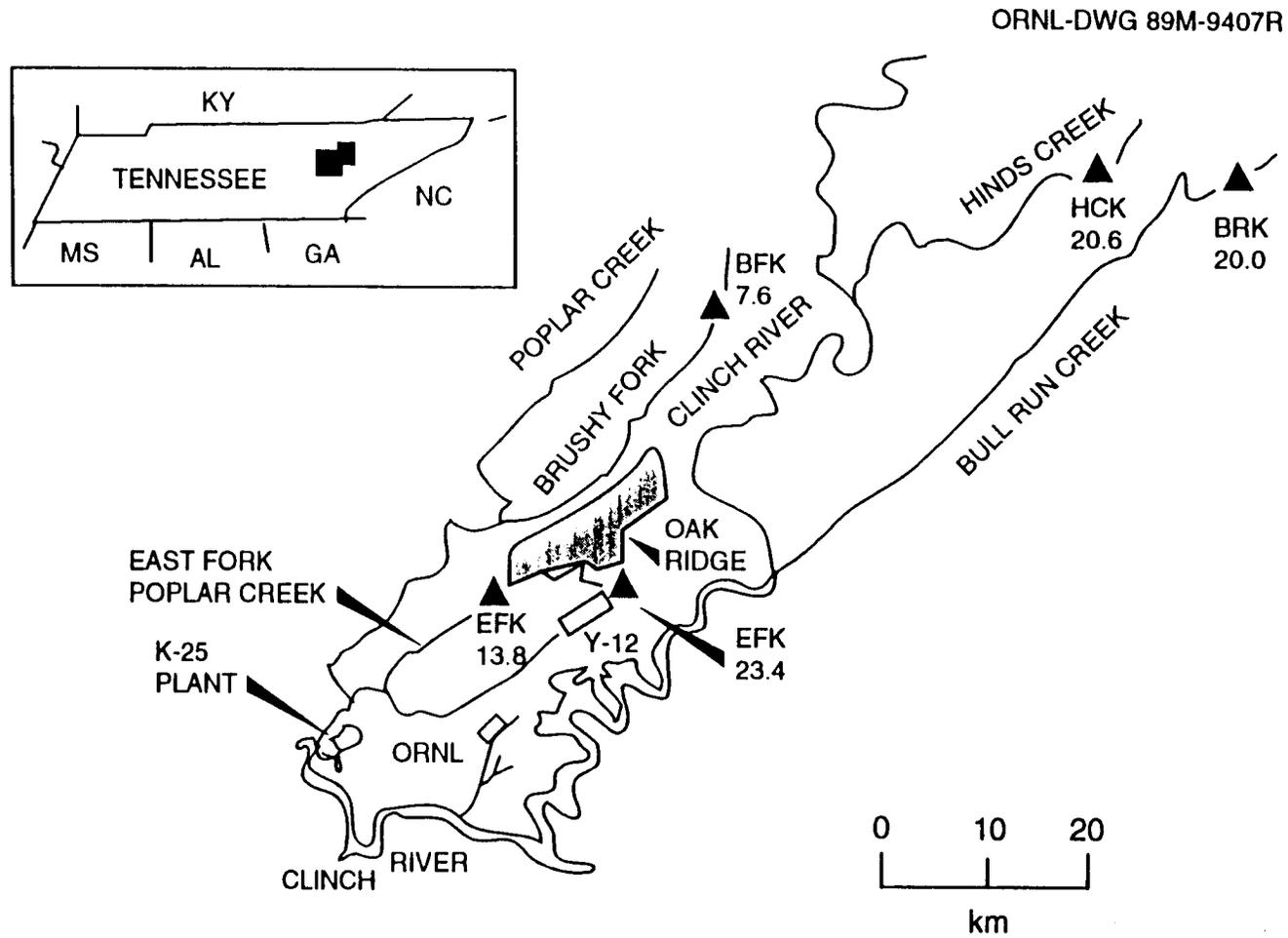


Fig. 6-1. Sites used for in situ clam (*Sphaerium fabale*) studies. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; BRK = Bull Run Creek kilometer; HCK = Hinds Creek kilometer.

could grow and survive in upper EFPC, (2) what factors may be responsible for preventing survival and growth, and (3) the extent of the Y-12 Plant's influence on its distribution in EFPC. This species was chosen as a test organism because it is (1) abundant at EFK 13.8 but scarce in upper EFPC (Smith 1992a), (2) relatively abundant downstream of weirs in WOC near ORNL (Loar et al. 1992b), and (3) moderately tolerant to organic pollutants (Schuster and Etnier 1978). The source of all caddisflies used in these studies was EFK 14.0.

Initially the distribution and relative abundance of *H. depravata* in upper EFPC was determined semiquantitatively at five sites including EFK 23.1, EFK 21.9, EFK 21.4, EFK 21.3, and EFK 18.6. One sample was collected from a riffle at each site by kicking the substrate vigorously for one minute and allowing the suspended organisms and material to drift downstream into an aquatic dip net (800- \times 900- μ m mesh). The samples were placed into glass jars and returned to the laboratory where the caddisflies were removed from the debris and counted.

To determine whether *H. depravata* could grow in upper EFPC, larvae obtained from EFK 14.0 were placed in artificial stream channels located alongside EFPC at EFK 23.1 just downstream of Bear Creek Road. As a control, larvae were also placed into streamside channels at EFK 14.0. Channels were placed at each site in triplicate. Each channel was made from plastic gutter and filled with natural substrate consisting of a mixture of sand, gravel, and small cobbles. Water was diverted into the artificial channels through a 12-m-long, 10-cm-diam capture pipe placed upstream of the channels. The water was carried into a head tank (a 120-L plastic garbage pail) which emptied into each channel via pieces of 2.5-cm-diam tygon tubing. Approximately 90 caddisflies were placed into each channel. At about 2-week intervals, 10 individuals were removed from each channel, weighed, and their head capsule widths measured with an ocular micrometer mounted in a dissecting microscope.

Because differences in water temperature among the sites in EFPC could mask other aspects of water quality that may have affected the distribution of *H. depravata*, a laboratory experiment was designed to determine the effects of ambient water quality on growth and emergence of individuals. In this experiment, small, round, recirculating, plexiglass chambers (Mackay 1981) were placed in a constant temperature water bath of 22°C. Each chamber contained a substrate mixture of coarse sand, small "pea" gravel, and small ceramic cylinders. Current in the chambers was achieved by circulating a fine stream of air bubbles under a spirally-ascending ceiling in the channel of each chamber. Four replicate chambers were used for each of two treatments including exposure of caddisfly larvae to water collected from EFK 23.1 and EFK 14.0, and three replicate chambers were used to express larvae to dechlorinated tap water. Into each chamber were placed 15 larvae (third and fourth instar), obtained from EFK 14.0. Prior to placement into the tanks, each individual was weighed to the nearest 0.01 mg with a Mettler electronic balance. Each day, a slurry of TetraMin fish food was placed into each tank that was equal to 1% of the total larval weight. Water in each tank was replaced with fresh water obtained from its respective source every 2 days. At 2-week intervals, each individual was weighed. Comparisons of instantaneous growth rates (IGR) were made among treatment tanks with a one-way ANOVA.

$$\text{IGR} = \frac{(\log W_F - \log W_I)}{\text{time}}, \quad (8)$$

where W_F = final weight, and W_I = initial weight.

Another laboratory experiment was conducted to evaluate the effects of various temperatures on survival of *H. depravata*. Three thermal regimes were tested in triplicate using flow-through channels receiving dechlorinated tap water. Into each channel were placed 30 larvae (third and fourth instar), which were fed a slurry of enough TetraMin Staple fish food twice daily to fill the capture nets of all the channels. One group of larvae was held at the collection temperature, 22°C, for the duration of the experiment. The second group of larvae was initially exposed to a water temperature of 22°C; after 24 h the temperature was lowered 1°C daily to a low of 12°C. The third group of larvae was initially exposed to a water temperature of 22°C for 24 h before increasing the temperature 1°C daily until death.

In order to determine whether food quality might have been a factor in limiting the occurrence of *H. depravata* in upper EFPC, a study of quality of potentially available food was conducted. In this study, ATP, which is found in living materials, was used as an indicator of food quality. Grab samples of water (approximately 2 L each) were collected every 2 weeks from seven sites in EFPC, including EFK 23.1, EFK 21.9, EFK 21.4, EFK 21.3, EFK 18.6, EFK 16.4, and EFK 13.8. Equal volumes of water were filtered from each site, and the filtrate was analyzed for ATP following the procedure described in Sect. 3.8.1.

6.1.3 Results

6.1.3.1 Benthic macroinvertebrate monitoring studies

Taxonomic composition

A total of 113 distinguishable benthic macroinvertebrate taxa were collected in quantitative samples from EFPC from June 1986 through May 1987 (Table E-1, Appendix E). A majority of the taxa collected were insects which had 93 representative taxa. The remaining 20 taxa comprised several major groups including crustaceans [Amphipoda (sideswimmers), Isopoda (aquatic sow bugs), and Decapoda (crayfish)], Hirudinea (leeches), Oligochaeta (aquatic worms), Nematoda (roundworms), Turbellaria (flatworms), Hydracarina (water mites), Gastropoda (snails), and Bivalvia (mussels). A majority of the insects collected were from the order Diptera (true flies), which was represented by 55 distinguishable taxa. Most of the dipterans were from the family Chironomidae (true midges, 49 taxa). In addition to dipterans, seven additional orders of insects were represented, including Coleoptera (beetles), Ephemeroptera (mayflies), Hymenoptera (wasps and bees), Megaloptera (hellgrammites and fishflies), Odonata (damselflies and dragonflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). An additional 14 taxa were collected in qualitative samples which represented 7 major groups including Ephemeroptera, Odonata, Trichoptera, Coleoptera, Diptera, and Bivalvia (Table E-1).

In Brushy Fork, a total of 103 distinguishable taxa were collected in quantitative samples during the second year (Table E-1). As in EFPC, a majority of the taxa were insects (86 taxa) of which most were dipterans (47 taxa) including 37 chironomid taxa. In addition to dipterans, seven additional orders of insects were collected including Coleoptera, Ephemeroptera, Hymenoptera, Megaloptera, Odonata, Plecoptera, and Trichoptera. The non-insect taxa collected included Nematoda, Oligochaeta, Turbellaria,

Amphipoda, Decapoda, Isopoda, Hydracarina, Gastropoda, and Bivalvia. An additional 13 taxa representing 5 major taxonomic groups were collected in a qualitative sample including Turbellaria, Hirudinea, Odonata, Trichoptera, and Diptera (Table E-1).

Density and biomass*

Mean density and biomass of the benthic macroinvertebrates at each site in EFPC and Brushy Fork from June 1986 through May 1987 are presented in Table 6-1. For comparison, density and biomass from June 1985 through May 1986 are also represented in Table 6-1. Density of invertebrates increased with increasing distance from the Y-12 Plant, with the highest density observed at EFK 13.8; density values at EFK 10.6 and EFK 6.3 were similar to those found at EFK 23.4 and EFK 18.2. Density (including and excluding crayfish and mollusks) was significantly higher at EFK 13.8 and significantly lower at EFK 24.4, compared with the other four EFPC sites and BFK 7.6 (Appendix F, Table F-1). With the exclusion of a crayfish and some mollusks, densities at EFK 23.4, EFK 18.2, EFK 10.6, and EFK 6.3 were statistically indistinguishable from one another and from BFK 7.6. However, with mollusks and crayfish included in the analysis, densities at EFK 23.4 and EFK 18.2 were significantly less than at BFK 7.6. Although the annual means at BFK 7.6 and EFK 10.6 were similar (Table 6-1), the results of the ANOVA/Tukey test indicated that the density (including all taxa) at BFK 7.6 was significantly greater. This was due to the large increase in the density at EFK 10.6 during the last three sampling periods (Fig. 6-2). The mean densities during the first nine sampling periods were 230.6 and 85.6 organisms per 0.1 m² for BFK 7.6 and EFK 10.6 respectively. During the last three sampling periods, monthly densities at EFK 10.6 increased from 229.8 organisms per 0.1 m² in March to 1241.4 organisms per 0.1 m² in May, thus increasing the annual mean density substantially. Therefore, the ANOVA/Tukey test accurately detected the difference between densities at these two sites.

Strong spatial trends were not evident for biomass in EFPC; although, as for density, the highest mean biomass occurred at EFK 13.8 and the lowest at EFK 24.4 (Tables 6-1 and F-1). The mollusks and/or crayfish strongly influenced the biomass at EFK 23.4, EFK 18.2, EFK 13.8, and BFK 7.6. Biomass at EFK 13.8 was significantly greater than at any other EFPC site both with and without mollusks and crayfish. These groups also influenced the differences observed in biomass between the EFPC sites and BFK 7.6. When all taxa were included, biomass at BFK 7.6 was significantly greater than at any EFPC site; but when mollusks and crayfish were excluded, biomass at EFK 13.8 was significantly greater than at BFK 7.6. Biomass at BFK 7.6, EFK 6.3, and EFK 23.4 did not differ significantly when mollusks and crayfish were excluded from the analysis.

Monthly changes in both density and biomass (exclusive of Decapoda and Mollusca) were evident at all EFPC sites and BFK 7.6. Most sites exhibited at least one distinct peak in density, although monthly changes at EFK 24.4 and EFK 23.4 were not as

*Comparisons in benthic macroinvertebrate density and biomass have been made between the communities at different sites both with and without Mollusca (snails and mussels) and Decapoda (crayfish), because these taxa are typically very heavy but numerically unimportant and can thus suppress the importance of the weight changes of the other organisms. Therefore, unless otherwise noted, trends presented in both spatial and temporal patterns in density include both Decapoda and Mollusca, while trends in biomass exclude these two groups.

Table 6-1. Mean annual density and biomass of benthic macroinvertebrates in East Fork Poplar Creek and Brushy Fork, Year 1 (June 1985–May 1986) and Year 2 (June 1986–May 1987)

Site ^a	Density ^b (n/0.1 m ²)	Density ^c (n/0.1 m ²)	Biomass ^b (wet wt/0.1 m ²)	Biomass ^c (wet wt/0.1 m ²)
EFK 24.4				
Year 1	93.0**** (21.8)	93.0**** (12.9)	86.2 (14.6)	86.2 (15.8)
Year 2	39.7 (10.3)	39.7 (10.3)	66.0 (12.4)	65.6 (12.4)
EFK 23.4				
Year 1	231.1**** (44.3)	230.1**** (24.5)	435.1*** (251.4)	170.2 (29.9)
Year 2	106.5 (19.5)	104.4 (19.2)	1,063.1 (261.1)	244.7 (39.9)
EFK 18.2				
Year 1	254.5 (91.6)	254.0 (42.5)	476.6 (275.9)	139.5 (17.8)
Year 2	206.9 (68.2)	206.3 (68.2)	261.5 (101.2)	125.4 (25.6)
EFK 13.8				
Year 1	251.1**** (32.7)	234.0**** (20.7)	2,489.9*** (505.0)	1,365.1**** (200.1)
Year 2	595.0 (74.9)	578.4 (75.3)	4,062.5 (503.9)	3,321.67 (543.22)
EFK 10.6				
Year 1	131.8*** (40.6)	129.9*** (22.7)	186.5* (75.5)	178.1* (52.9)
Year 2	254.9 (108.6)	254.3 (108.7)	194.5 (68.6)	187.4 (67.8)
EFK 6.3				
Year 1	105.7 (22.1)	102.5 (13.5)	384.7 (114.5)	286.9 (46.9)
Year 2	155.9 (54.5)	154.8 (54.5)	403.33 (127.4)	365.3 (125.8)
BFK 7.6				
Year 1	286.6 ^d (73.5)	265.1**** ^d (39.2)	6,461.2**** ^d (1,491.8)	583.2**** ^d (87.6)
Year 2	253.7 (28.7)	210.22 (21.7)	17,861.2 (2,269.8)	210.2 (37.0)
	274.9 ^d (49.7)	151.0 ^d (38.4)	21,709.9 ^d (3,219.7)	255.0 ^d (75.3)

^aEFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

^bIncludes all taxa.

^cExcludes decapoda and mollusca.

^dMean for January–May only ($n = 5$); ANOVA based on January–May only.

Note: Mean values were calculated from mean monthly values; thus $n = 12$ for the annual means. Values in parentheses are \pm one standard error of the mean. An asterisk (*) indicates significant between-year differences based on an ANOVA where: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$.

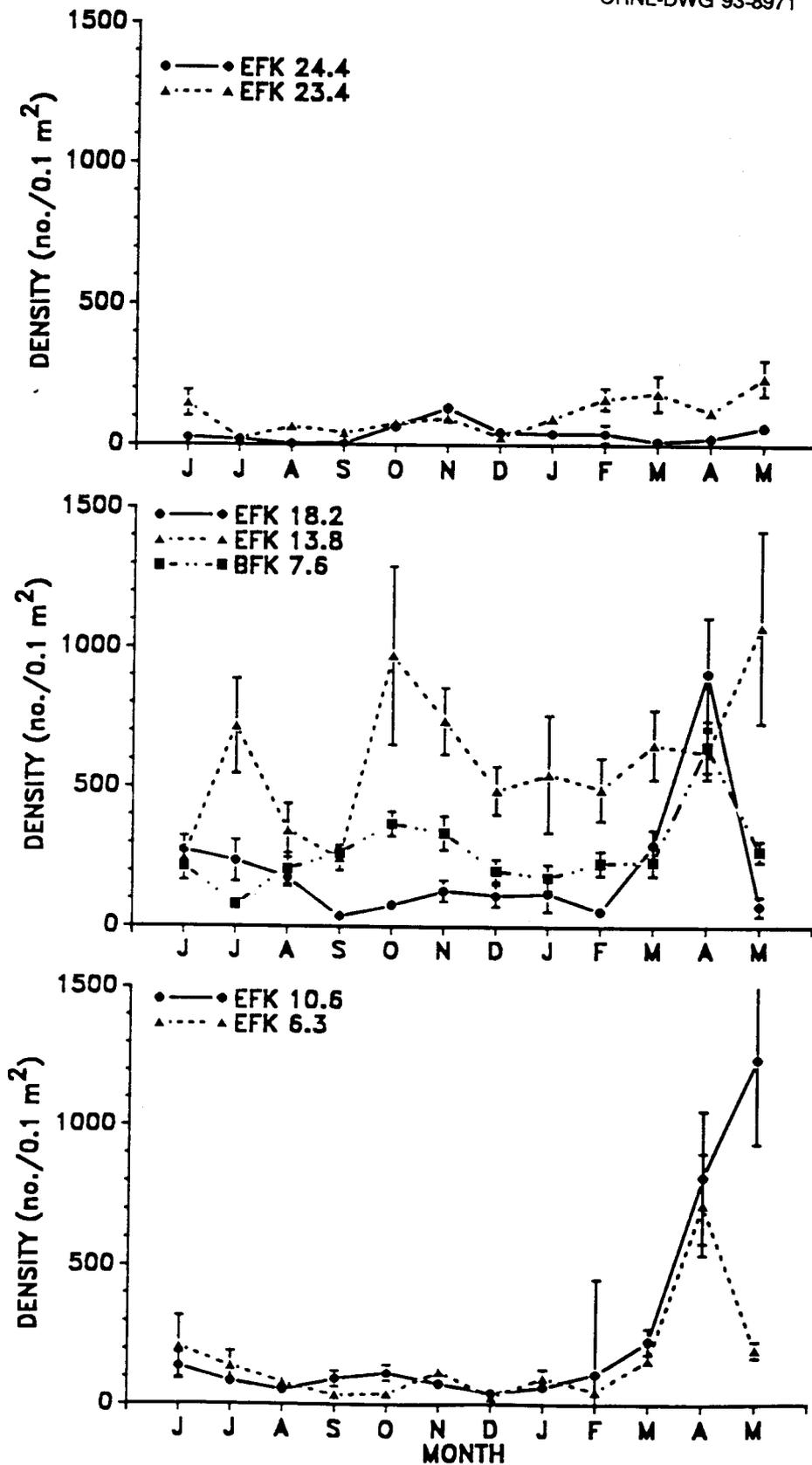


Fig. 6-2. Monthly trends in density of benthic macroinvertebrates in East Fork Poplar Creek and Brushy Fork, June 1986–May 1987. Vertical bars are ± 1 SE. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

dramatic as those at the other sites (Fig. 6-2). The largest peaks in density generally occurred during the spring months (March through May), although EFK 13.8 exhibited additional notable peaks in the summer and fall.

Monthly changes in biomass were generally greater than those for density (Fig. 6-3). The largest peaks in biomass typically occurred during the spring and early summer. Biomass at EFK 13.8 increased dramatically during the fall and remained high through the winter and spring months.

Between-year comparisons of density within each site in EFPC showed that there were no significant changes at EFK 18.2 or EFK 6.3, either with or without mollusks and crayfish (Table 6-1). Significant reductions in density during the second year were observed at EFK 24.4 and EFK 23.4, while at EFK 13.8 and EFK 10.6 density increased significantly. At BFK 7.6 there was no significant difference between years when all taxa were included; but when mollusks and crayfish were excluded, the density during the first year was significantly greater than during the second year.

Comparisons in biomass (exclusive of mollusks and crayfish) showed that there were no significant differences between years one and two at EFK 24.4, EFK 23.4, EFK 18.2, and EFK 6.3; while at EFK 13.8 and EFK 10.6, biomass was significantly greater in the second year, and at BFK 7.6 biomass was significantly greater during the first year (Table 6-1). The inclusion of mollusks and crayfish in between-year comparisons of biomass at the EFPC sites affected interpretation only at EFK 23.4 where biomass was significantly greater during the second year than first year; this difference was largely due to the periodic collection of a few crayfish. Mollusks and crayfish strongly influenced the analysis of biomass at Brushy Fork however, with their inclusion showing a trend opposite to that shown when these taxa were excluded (Table 6-1).

Dominant taxa*

Many of the between-site differences in density and biomass of the benthic macroinvertebrates were generally due to a few major taxonomic groups including the Chironomidae (midges), Coleoptera (beetles), Oligochaeta (aquatic worms), and Trichoptera (caddisflies).

Numerically, chironomids were the most dominant taxon at all sites in EFPC except EFK 13.8; this group accounted for 27.9% at EFK 13.8 and more than 61% at the other five sites in EFPC (Fig 6-4). Their relative abundance was greatest at the three sites upstream of EFK 13.8. The relative abundance of chironomids at BFK 7.6 was considerably less, where they accounted for only 10.8% of the total community. Because of their small size, the relative biomass of chironomids at all EFPC sites and BFK 7.6 was considerably less than their relative abundance (Fig. 6-5). Their greatest contribution to the biomass (exclusive of the mollusks and crayfish) in EFPC was at EFK 18.2 (49.7%), and their smallest contribution was at EFK 13.8 (2.3%); chironomids made up only 2.7% of the biomass at BFK 7.6.

The relative abundance of oligochaetes in EFPC varied considerably (Fig. 6-4). Relative densities of oligochaetes were highest at EFK 24.4 (23.9%), EFK 18.2 (18.8%), and EFK 10.6 (14.9%). Relative densities of this group at the other three EFPC sites and BFK 7.6 were similar, ranging from 1.3% to 3.9%. The relative biomass (excluding

*For the benthic macroinvertebrate section of this report, the term dominant is used synonymously with numerical dominance. Taxa were considered numerically dominant if they were collected at 50% or more of the study sites and comprised 10% or more of the average density at two or more sites.

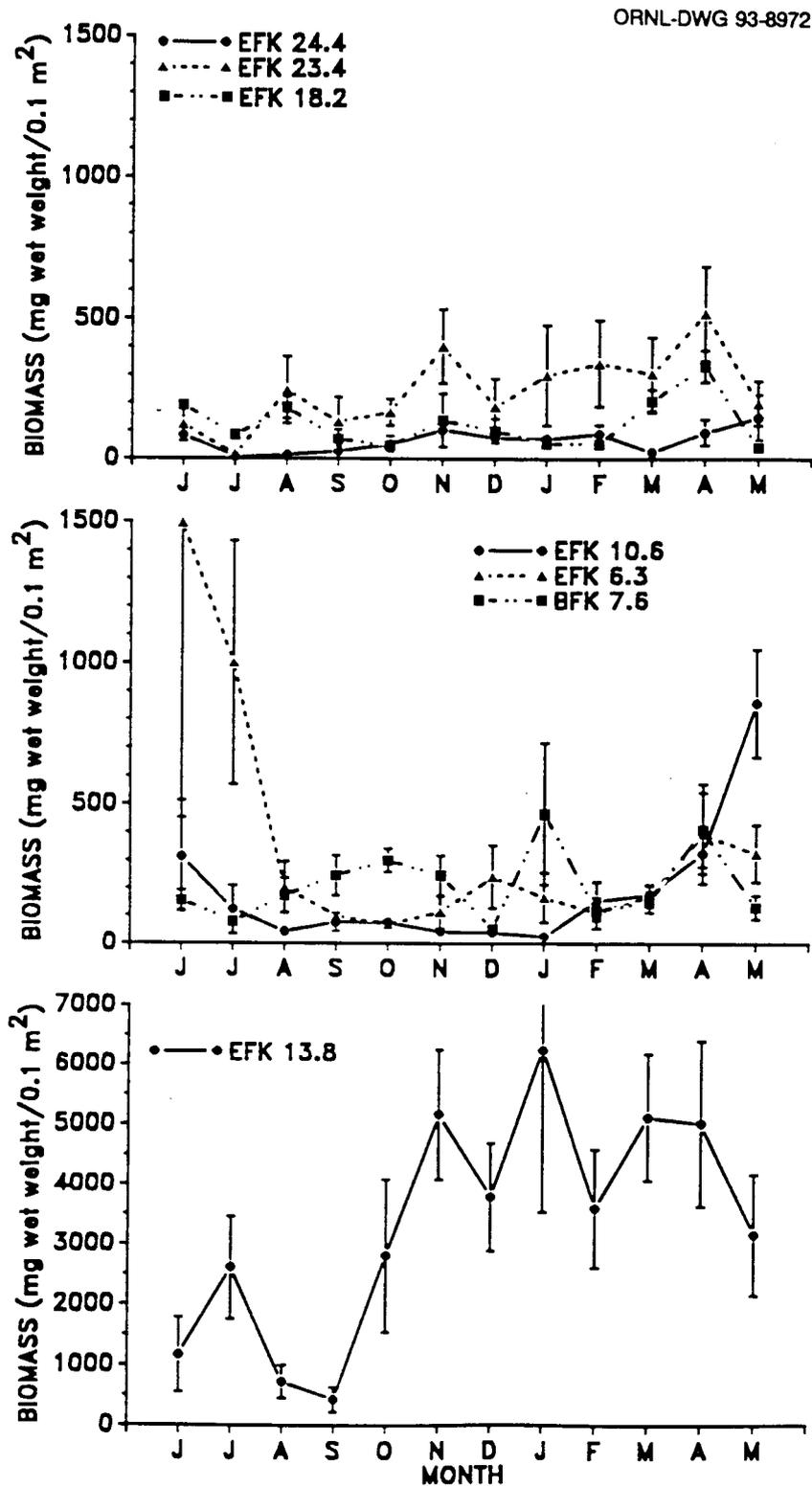


Fig. 6-3. Monthly trends in biomass (excluding Decapoda and Mollusca) of benthic macroinvertebrates in East Fork Poplar Creek and Brushy Fork, June 1986–May 1987. Vertical bars are ± 1 SE. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

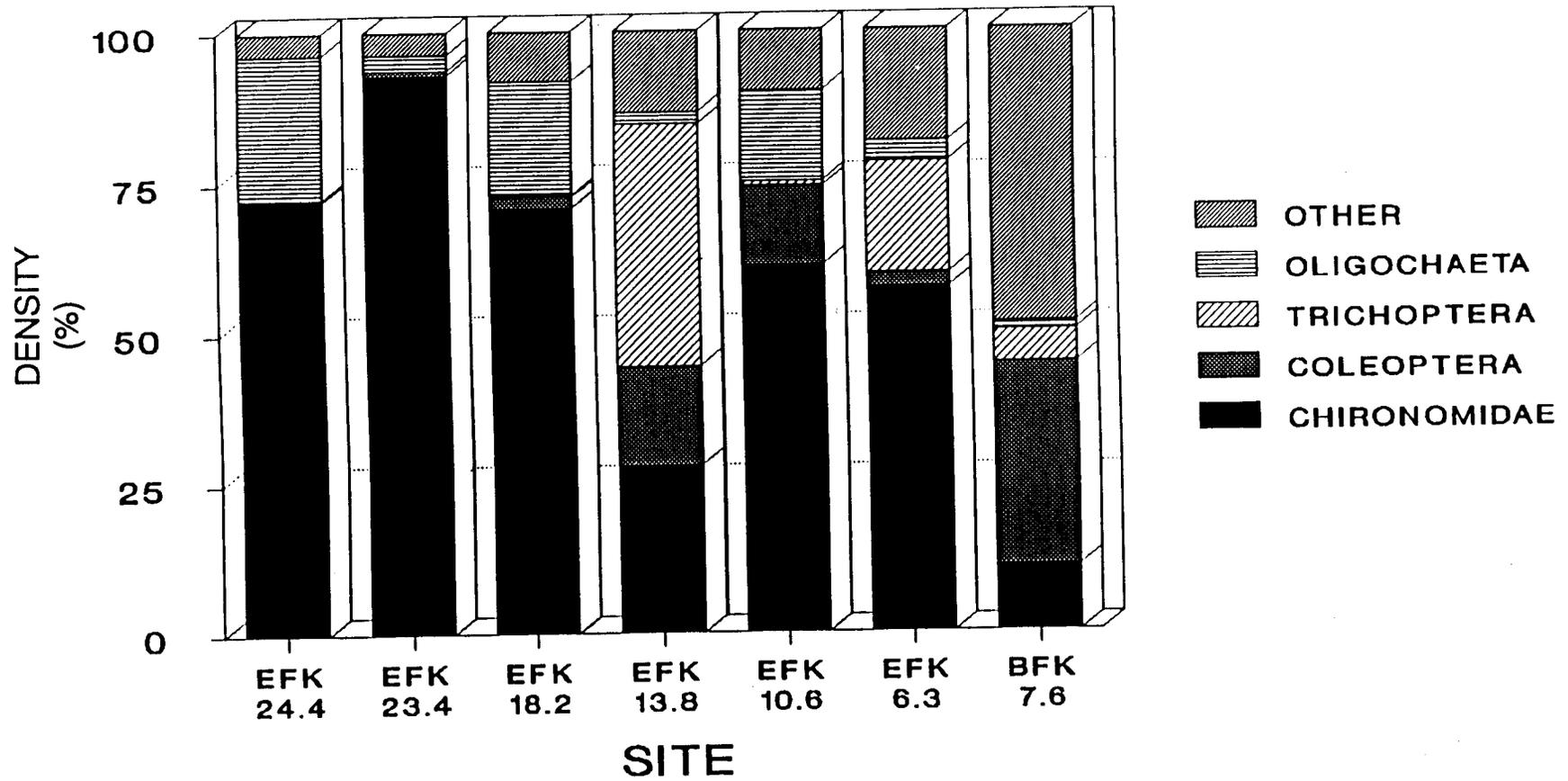


Fig. 6-4. Relative abundance (percentage of annual mean density) of benthic macroinvertebrates in East Fork Poplar Creek and Brushy Fork, June 1986-May 1987. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

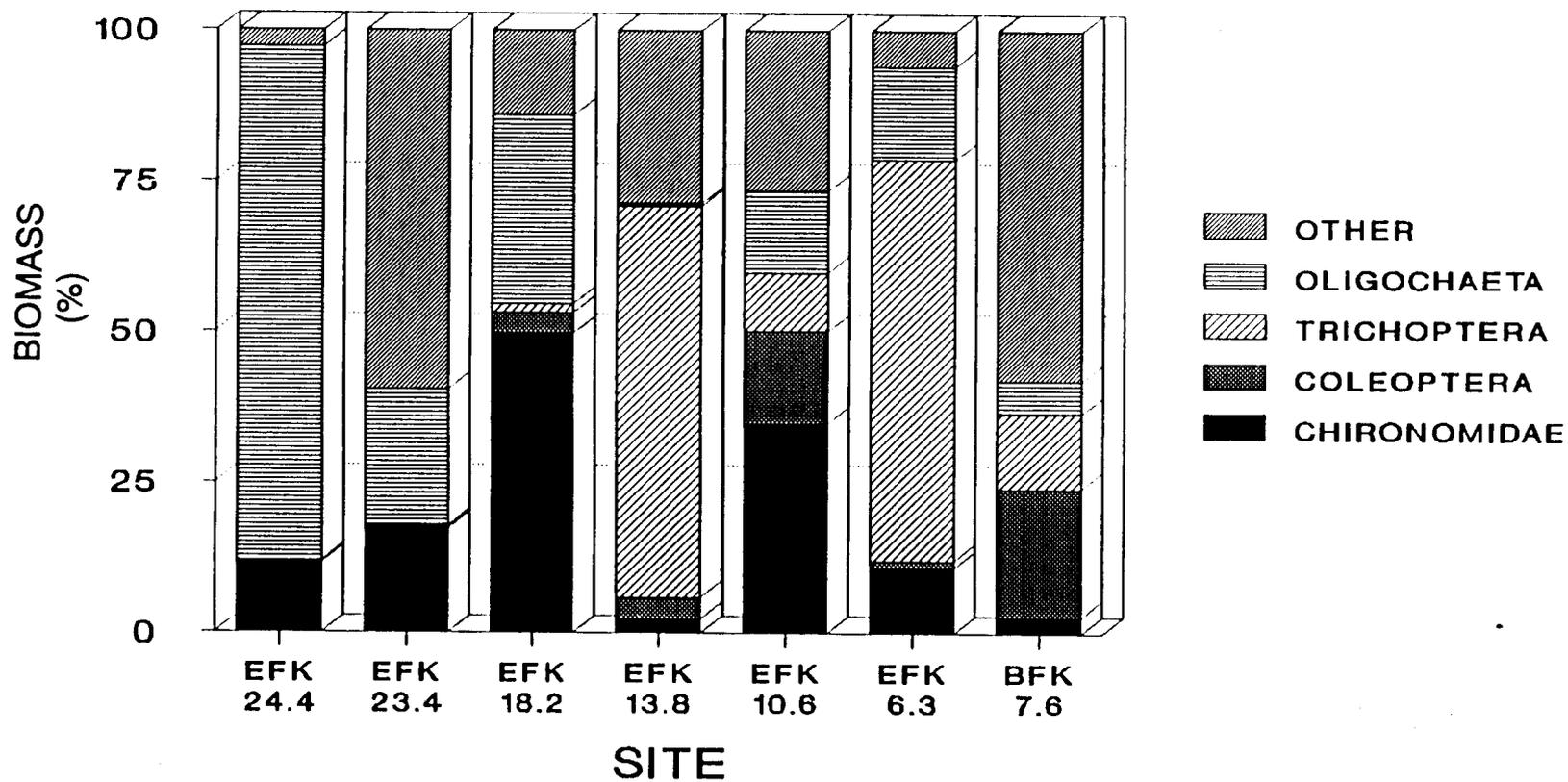


Fig. 6-5. Relative biomass (percentage of annual mean biomass) of benthic macroinvertebrates (excluding Decapoda and Mollusca) in East Fork Poplar Creek and Brushy Fork, June 1986-May 1987. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

mollusks and crayfish) of oligochaetes was highest at the three upstream-most sites (> 22%) and lowest (0.5%) at EFK 13.8 (Fig. 6-5). The relative biomass of oligochaetes at BFK 7.6 (5.5%) was somewhat less than at EFK 10.6 and EFK 6.3.

The relative densities of coleopterans in EFPC exceeded 3.0% at only EFK 13.8 and EFK 10.6, where they comprised 16.5% and 13.0%, respectively, of the total density (Fig. 6-4). At BFK 7.6, the relative abundance of this group was 33.8%. The relative biomass (excluding mollusks and crayfish) of the coleopterans followed a similar pattern, although their relative biomass at EFK 13.8 was lower largely because of the relatively high numbers of the much heavier trichopterans (Fig. 6-5). The relative biomass of the beetles ranged from 0.1% at EFK 24.4 to 15.3% at EFK 10.6; relative biomass of the beetles at BFK 7.6 was 21.3%.

Caddisflies were rarely collected at those sites above EFK 13.8 and were also collected infrequently at EFK 10.6, where their relative abundances did not exceed 0.9%; thus, where their relative abundance was low, their relative biomass was also low. At EFK 13.8 and EFK 6.3, however, caddisflies comprised 40.3% and 22.0% of the community respectively. Their contribution to the total biomass (excluding mollusks and crayfish) at these two sites was even greater, contributing 65.0% and 66.6% to the total biomass at EFK 13.8 and EFK 6.3 respectively. Additionally, relative densities and biomass of this group were about five to seven times greater at these two sites than at BFK 7.6.

Several other taxa contributed considerably to total density and biomass (excluding mollusks and crayfish) at some sites (Figs. 6-4 and 6-5). Mayflies (Ephemeroptera) were relatively abundant at BFK 7.6 and EFK 13.8, where they comprised 10.6% and 9.2%, respectively, of the total community density. However, they contributed little to the biomass at EFK 13.8, while at BFK 7.6 they made up 21.9% of the total biomass.

Dipterans (true flies excluding Chironomidae) were relatively abundant at EFK 10.6, where they made up 8.9% of the total density. Their major contribution at some other sites, however, was to biomass (excluding mollusks and crayfish). At BFK 7.6, EFK 10.6, and EFK 23.4 their contribution to the total biomass was 29.3%, 17.5%, and 54.8% respectively. This was largely due, however, to the collection of a few large-bodied individuals (i.e., Tabanidae at BFK 7.6 and Tipulidae at EFK 10.6 and EFK 23.4).

Mollusks, including snails (Gastropoda) and mussels (Bivalvia), were relatively abundant at BFK 7.6 (34.0%), but were rarely collected from any EFPC site except EFK 13.8, where they comprised only about 2.7% of the total density. Their contribution to the biomass at BFK 7.6 was substantial, where, when included in analyses of biomass, they comprised approximately 97% of the total biomass.

Three other taxa which contributed substantially to the biomass at some of the sites included Megaloptera (hellgrammites), Odonata (dragonflies and/or damselflies), and Decapoda (crayfish). Megalopterans accounted for 25.8% of the biomass (excluding mollusks and crayfish) at EFK 13.8, and odonates accounted for 7.8% of the biomass (excluding mollusks and crayfish) at EFK 18.2. When considered in the analyses of biomass of all taxa, crayfish accounted for 16.8%, 51.4%, and 74.6% of the biomass at EFK 13.8, EFK 18.2, and EFK 23.4 respectively. However, collection of these relatively large bodied organisms at these sites was infrequent.

Community structure

Richness. Mean taxonomic richness (number of taxa per sample) of benthic invertebrates exhibited a distinct longitudinal gradient in EFPC (Tables 6-2 and F-2). Richness was lowest at EFK 24.4 where the number of taxa per sample was significantly lower than at all other sites. Richness then increased significantly with distance from the Y-12 Plant reaching a maximum at EFK 13.8. Finally, relative to EFK 13.8, richness decreased significantly at EFK 10.6 and EFK 6.3 but was similar to richness at EFK 18.2. Richness at the reference site, BFK 7.6, was significantly greater than at all EFPC sites.

Temporal trends in taxonomic richness in EFPC and BF are exhibited in Fig. 6-6. Richness was highly variable over time, but general seasonal trends were evident at most sites. Minima typically occurred during the summer or early fall, and maxima typically occurred during the winter and/or spring months.

The mean number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa collected per sample (EPT richness) followed a trend similar to that of total community richness but was more pronounced (Tables 6-2 and F-4). EPT richness was significantly greater at EFK 13.8 than all other sites in EFPC and significantly greater at BFK 7.6 than all EFPC sites. EPT richness was lowest at EFK 24.4 but was not statistically distinguishable from EFK 23.4. EPT richness at EFK 18.2 and EFK 10.6 was similar but significantly less than at EFK 6.3.

Within-site comparisons of community and EPT richness values between years one and two of the BMAP (June 1985 through May 1986 vs June 1986 through May 1987) showed that, in general, values during the second year tended to be higher (Table 6-2). The difference however, was significant for only EFK 23.4, EFK 13.8, and EFK 10.6 for total richness and only at EFK 13.8 for EPT richness. Neither total nor EPT richness differed significantly between years at BFK 7.6.

Taxonomic diversity. Spatial trends in taxonomic diversity were similar to those exhibited by community and EPT richness but were not as distinct (Tables 6-2 and F-2). Diversity increased with distance from the Y-12 Plant, reaching a maximum at EFK 13.8; however, diversity of the community at EFK 13.8 was not statistically distinguishable from diversity at EFK 10.6 or EFK 18.2, sites at which both EPT and total richness were clearly depressed relative to EFK 13.8. However, diversity did show, as richness parameters did, that maximum impact on the benthic community occurs at the two sites closest to the Y-12 Plant.

Temporal variability in taxonomic diversity of benthic invertebrates was considerable at all sites (Fig. 6-7). Most EFPC sites exhibited decreases in diversity during the winter months before showing some increase during the spring months, although EFK 13.8 changed little over the winter and spring months. After exhibiting a peak during September, diversity declined at BFK 7.6 in October, fluctuated considerably during the winter, and then increased throughout the spring.

Secondary production

Target taxa. Estimates of annual production for the target taxa in EFPC and BF are presented in Table 6-3. Estimates for the target taxa in BF are only for the second year of the BMAP since sampling of this stream was not initiated until January 1986. Three taxa were collected in sufficient numbers at some sites in EFPC to obtain direct estimates

Table 6-2. Total taxonomic richness, total EPT^a richness, taxonomic richness, mean EPT richness, and taxonomic diversity (H') of benthic macroinvertebrates in East Fork Poplar Creek and Brushy Fork

Year 1 (June 1985–May 1986) and Year 2 (June 1986–May 1987)

Site	Total richness ^b	EPT richness	Taxonomic richness ^c	EPT richness ^d	Diversity
EFK 24.4					
Year 1	16	2	2.9 (0.13)	0.03 (0.02)	0.92 (0.06)
Year 2	21	3	3.1 (0.30)	0.1 (0.03)	0.97 (0.09)
EFK 23.4					
Year 1	28	4	5.1** (0.23)	0.2 (0.06)	0.77**** (0.06)
Year 2	34	4	6.0 (0.28)	0.15 (0.07)	1.21 (0.13)
EFK 18.2					
Year 1	42	3	8.7 (0.40)	0.5 (0.14)	1.83 (0.07)
Year 2	48	6	9.7 (0.28)	0.7 (0.17)	2.0 (0.18)
EFK 13.8					
Year 1	60	9	11.6**** (0.43)	2.1**** (0.20)	1.94** (0.08)
Year 2	75	16	16.3 (0.56)	2.9 (0.21)	2.22 (0.05)
EFK 10.6					
Year 1	51	5	8.8** (0.39)	0.3 (0.10)	2.02 (0.07)
Year 2	56	6	10.0 (0.36)	0.5 (0.17)	2.17 (0.11)
EFK 6.3					
Year 1	58	8	8.1 (0.37)	1.5 (0.15)	1.73 (0.10)
Year 2	62	7	9.2 (0.89)	1.8 (0.18)	1.70 (0.20)

Table 6-2 (continued)

Site	Total richness ^b	EPT richness	Taxonomic richness ^c	EPT richness ^d	Diversity
BFK 7.6					
Year 1	68 ^e	19 ^e	24.7 ^e (0.86)	6.7 ^b (0.75)	3.27 ^{b,**} (0.11)
Year 2	108	29	21.4 (1.55)	4.9 (0.44)	2.74 (0.11)
	74 ^e	24 ^e	23.2 ^e (2.50)	5.7 ^b (0.72)	2.79 ^b (0.19)

^aTotal number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa collected in quantitative samples.

^bTotal number of taxa collected in quantitative samples.

^cMean number of taxa collected per sample.

^dMean number of EPT taxa collected per sample.

^eTotal or mean for January–May only ($n = 5$); ANOVA based on January–May only.

Note: Mean values were calculated from mean monthly values, thus $n = 12$ for the annual means. Values in parentheses are ± 1 SE of the mean. An asterisk (*) indicates significant between-year differences based on an ANOVA where: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

of production, including *Baetis*, *Hydropsyche*, and *Stenelmis*. These same taxa were not abundant enough in BF to obtain direct estimates of production; therefore, three other trophically and taxonomically similar taxa, *Cheumatopsyche*, *Ephemerella*, and *Optioservus*, were selected. Species identifications were possible for only *Hydropsyche depravata*; for this species, it was possible to associate larval material with adult material. From the results obtained on population dynamics of *Baetis* in EFPC and on *Cheumatopsyche* and *Ephemerella* in BF, it appeared that these taxa were only represented by one species, although an additional species of *Baetis* may have rarely occurred at EFK 13.8. It was not clear, however, whether the two beetles, *Optioservus* and *Stenelmis*, were represented by one or more species.

Direct estimates of production for *Baetis* were possible for EFK 13.8 and EFK 18.2 only. The highest estimate of production for *Baetis* was at EFK 13.8 where in Years 1 and 2 production was 1.44 and 2.71 g wet wt·m⁻²·year⁻¹ respectively (Table 6-3). Production of *Baetis* at EFK 18.2 was considerably less, where estimates were 0.71 and 0.07 g wet wt·m⁻²·year⁻¹ during years one and two respectively. Relative to these two sites, production of *Baetis* at the other four sites was very low; production at these four sites during both years ranged from 0.0 at EFK 24.4 to 0.05 g wet wt/m² at EFK 10.6 during the first year (Table 6-3).

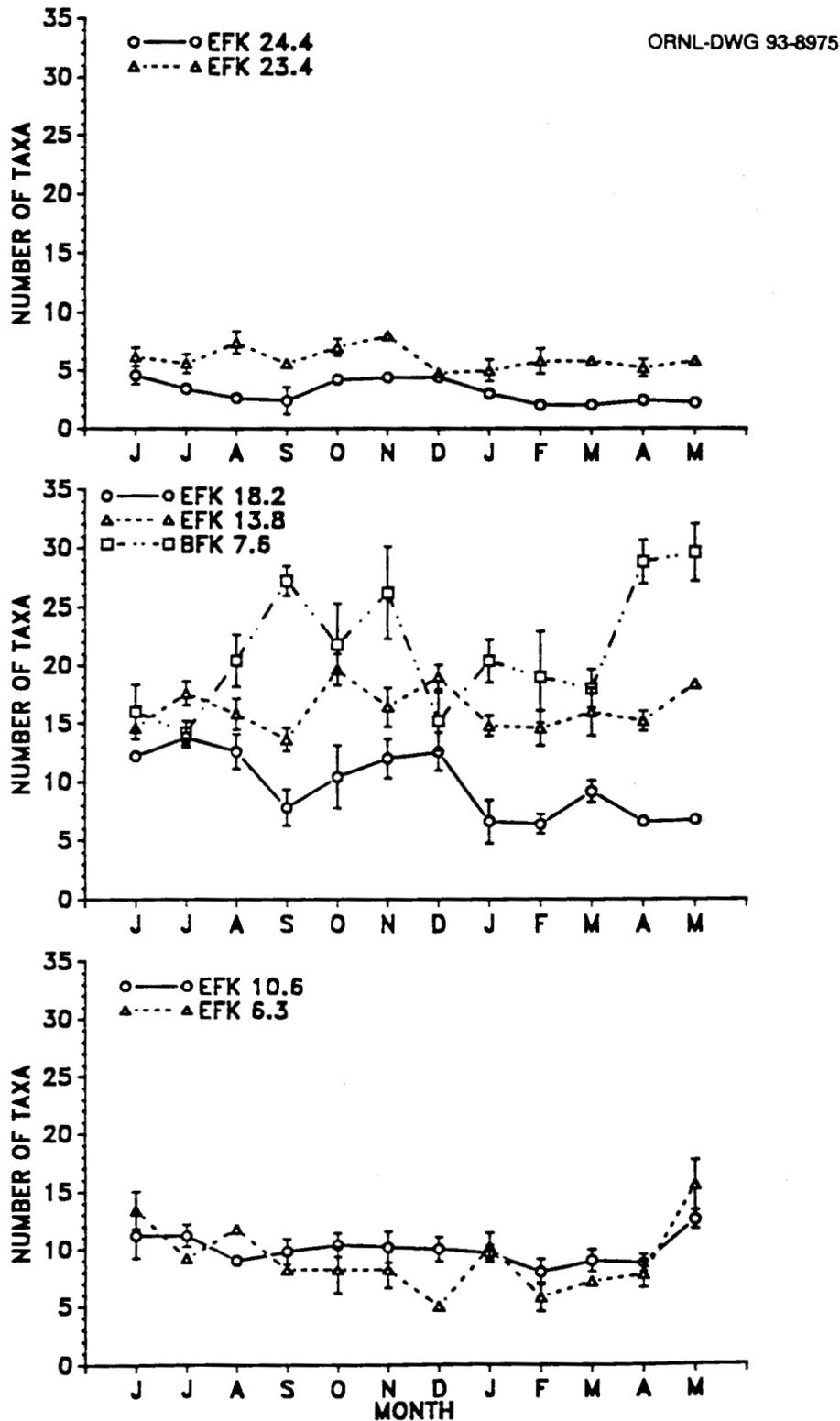


Fig. 6-6. Monthly trends in total taxonomic richness (mean number of benthic macroinvertebrate taxa per sample) in East Fork Poplar Creek and Brushy Fork, June 1986–May 1987. Vertical bars are ± 1 SE. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

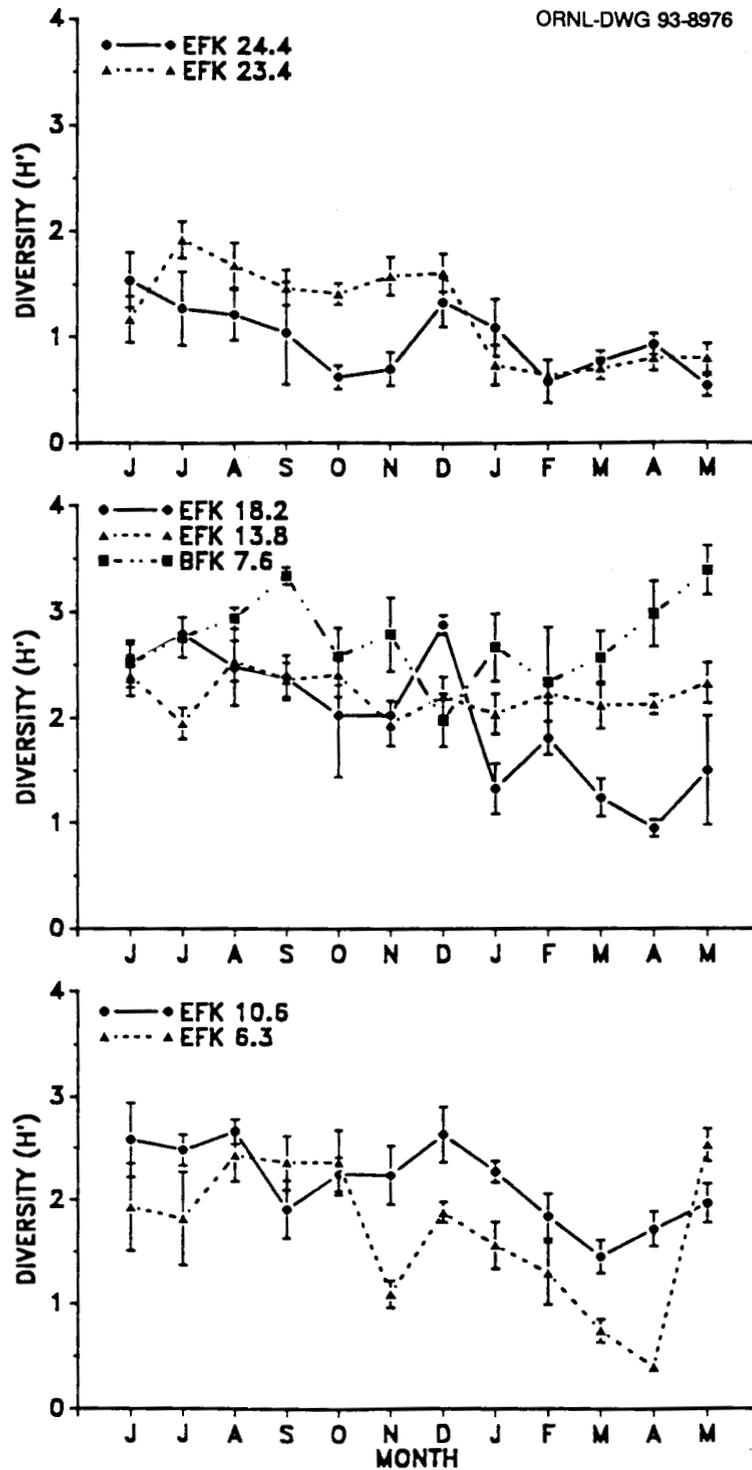


Fig. 6-7. Monthly trends in taxonomic diversity of benthic macroinvertebrates in East Fork Poplar Creek and Brushy Fork, June 1986–May 1987. Vertical bars are \pm SE. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table 6-3. Annual means for density (number per square meter) and biomass (milligrams wet weight per square meter), and annual production (in grams of wt weight per square meter per year) and P/B ratios for target taxa in East Fork Poplar Creek and Brushy Fork during Year 1 (June 1985–May 1986) and Year 2 (June 1986–May 1987)

Values in parenthesis below density and biomass are ± 1 SE

Site/Taxon	Density		Biomass		Production		P/B
	YR1	YR2	YR1	YR2	YR1	YR2	
EFK 24.4							
<i>Baetis</i> ^a	0 (0)	<1 (0.2)	0.0 (0.0)	0.5 (0.3)	0.0	0.005	10.5
<i>Hydropsyche</i> ^b	<1 (0.4)	<1 (0.2)	0.2 (0.2)	0.1 (1.1)	0.02	0.1	11.0
<i>Stenelmis</i> ^c	0 (0)	1 (0.5)	0.0 (0.0)	0.7 (0.4)	0.0	0.001	1.8
EFK 23.4							
<i>Baetis</i> ^a	<1 (0.2)	1 (0.8)	0.4 (0.2)	0.2 (0.2)	0.004	0.002	10.5
<i>Hydropsyche</i> ^b	5 (4.3)	1 (0.4)	6.8 (5.9)	4.5 (3.0)	0.07	0.05	11.0
<i>Stenelmis</i> ^c	8 (1.5)	8 (2.5)	13.1 (2.5)	8.0 (1.9)	0.02	0.01	1.8
EFK 18.2							
<i>Baetis</i> ^d	58 (38.0)	8 (5.2)	65.4 (40.9)	5.8 (4.6)	0.71	0.07	11.3
<i>Hydropsyche</i> ^b	1 (0.7)	2 (0.5)	2.8 (1.9)	1.9 (0.7)	0.03	0.02	11.0
<i>Stenelmis</i> ^c	13.7 (3.2)	32.3 (4.6)	16.2 (3.9)	38.3 (5.7)	0.03	0.07	1.8

Table 6-3 (continued)

Site/Taxon	Density		Biomass		Production		P/B
	YR1	YR2	YR1	YR2	YR1	YR2	
EFK 13.8							
<i>Baetis</i> ^d	202 (100.2)	475 (195.6)	219.6 (103.4)	279.9 (99.9)	1.44	2.71	9.7
<i>Hydropsyche</i> ^d	1120 (235.2)	2390 (406.6)	11582.0 (2817.9)	21601.9 (3666.2)	117.34	257.06	11.9
<i>Stenelmis</i> ^d	80 (16.8)	927 (174.6)	122.7 (25.3)	1038.0 (180.4)	0.19	2.18	2.1
EFK 10.6							
<i>Baetis</i> ^a	5 (5.3)	1 (1.0)	4.7 (4.7)	0.7 (0.6)	0.05	0.01	10.5
<i>Hydropsyche</i> ^b	6 (2.4)	21 (11.9)	44.9 (24.9)	182.9 (99.7)	0.49	2.01	11.0
<i>Stenelmis</i> ^d	37 (16.8)	32 (128.0)	45.5 (25.3)	244.0 (100.0)	0.06	0.37	1.5
EFK 6.3							
<i>Baetis</i> ^a	<1 (0.5)	1 (0.8)	0.5 (0.5)	1.5 (1.0)	0.01	0.02	10.5
<i>Hydropsyche</i> ^d	306 (116.4)	327 (147.6)	1839.2 (519.3)	2412.3 (1166.8)	22.19	24.36	10.1
<i>Stenelmis</i> ^c	17 (5.5)	36 (20.1)	18.6 (6.6)	38.6 (17.4)	0.03	0.07	1.8

Table 6-3 (continued)

Site/Taxon	Density		Biomass		Production		P/B
	YR1	YR2	YR1	YR2	YR1	YR2	
BFK 7.6							
<i>Cheumatopsyche</i>	-	107 (43.2)	-	110.0 (37.1)	-	0.42	4.4
<i>Ephemerella</i>	-	69 (37.6)	-	200.3 (120.8)	-	2.52	18.2
<i>Optioservus</i>	-	812 (124.8)	-	490.0 (87.6)	-	1.00	2.34

^aProduction of *Baetis* for both years determined by P/B method (mean annual biomass times the ratio of estimated annual production to biomass) using an average of the values obtained during Year 1 for EFK 13.8 and EFK 18.2.

^bProduction of *Hydropsyche* for both years determined by P/B method using an average of the values obtained during Year 1 for EFK 6.3 and EFK 13.8.

^cProduction of *Stenelmis* for both years determined by P/B method using an average of the values obtained during Year 1 for EFK 10.6 and EFK 13.8.

^dProduction estimated with the size-frequency method for Year 1, and the P/B value obtained during Year 1 was used to estimate production in Year 2.

Note: Year 1 = June 1985 through May 1986; Year 2 = June 1986 through May 1987. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; SE = standard error.

Direct estimates of *Hydropsyche* production were possible for EFK 6.3 and EFK 13.8 only (Table 6-3). Production of *Hydropsyche* was highest at EFK 13.8 where estimates were 117.34 and 257.06 g wet wt·m⁻²·year⁻¹ for the first and second years respectively. Production of *Hydropsyche* at EFK 6.3 was high relative to the other target taxa but considerably lower than for this species at EFK 13.8; production of this species at EFK 6.3 during the first and second years was 22.19 and 24.36 g wet wt·m⁻²·year⁻¹ respectively. This species, however, contributed very little to the total production at the other sites in EFPC, where estimates were generally much less than 1 g wet wt·m⁻²·year⁻¹. Direct estimates of production for *Stenelmis* were possible for EFK 10.6 and EFK 13.8 only (Table 6-3). As with *Baetis* and *Hydropsyche*, production of *Stenelmis* was considerably greater at EFK 13.8 than at any other site in EFPC, with estimates of 0.19 and 2.18 g wet wt·m⁻²·year⁻¹ during years 1 and 2 respectively. Additionally, as with the other two target taxa, production more than doubled from Year 1 to Year 2 at EFK 13.8. At EFK 10.6, production was 0.06 and 0.37 g wet wt·m⁻²·year⁻¹ during years 1 and 2 respectively. Production of this beetle at the remaining four sites did not exceed 0.07 g wet wt·m⁻²·year⁻¹ and was generally much less.

Production of the target taxa at BFK 7.6 was generally comparable to production of the target taxa in EFPC except for *Hydropsyche* (Table 6-3). Production estimates for the target taxa at BFK 7.6 were 0.42, 2.52, and 1.0 g wet wt·m⁻²·year⁻¹ for *Cheumatopsyche*, *Ephemerella*, and *Optioservus* respectively.

Community production. Estimates of secondary production of the benthic macroinvertebrate communities in EFPC and BF during the first and second years are presented in Table 6-4. As for the target taxa, greatest production of the benthic community in EFPC occurred at EFK 13.8, where production (152.7 and 321.0 g wet wt·m⁻²·year⁻¹ in years 1 and 2 respectively) was at least four times greater than at any other site during both years. The lowest estimate of production of the benthic community was at EFK 24.4 (10.5 and 7.5 g wet wt·m⁻²·year⁻¹ in years 1 and 2 respectively) where estimates were at least two times lower than at all other sites in EFPC. In general, total production of the benthos at the remaining four sites was relatively similar, ranging from 20.6 to 41.2 g wet wt·m⁻²·year⁻¹. Except for EFK 13.8, production of the benthic community changed very little between the first and second years, with slight increases occurring at EFK 23.4, EFK 10.6, and EFK 6.3 and slight decreases occurring at EFK 24.4 and EFK 18.2. At EFK 13.8, production almost doubled during the second year. Production of the benthos at BFK 7.6 during the second year was considerably higher than at any site in EFPC (Table 6-4). However, when mollusks were excluded, total production resulted in estimates at BFK 7.6 that were relatively similar to or slightly less than at most sites in EFPC.

In general, longitudinal trends in the distribution of production among the major taxonomic groups were similar between years one and two (Figs. 6-8 and 6-9). The relative contribution of chironomids was considerable at most sites in EFPC in both years. Generally their contribution was greatest at the three sites nearest the Y-12 Plant and least at EFK 13.8. Considerable changes were observed, however, in their relative contribution between Years 1 and 2 at EFK 10.6 and EFK 23.4. At EFK 23.4, the reduction in the relative contribution of the chironomids in the second year was largely due the collection of more crayfish which, although not very abundant, periodically contributed considerably to the biomass. At EFK 10.6, the increase in the relative production of the chironomids during the second year was largely due to the reduction of

Table 6-4. Total community, EPT (Ephemeroptera, Plecoptera, and Trichoptera), and Mollusca production, and mean community production to biomass ratios (P/B) (± 1 SE) of benthic macroinvertebrates in East Fork Poplar Creek and Brushy Fork

Site	Production (g wet wt·m ⁻² ·year ⁻¹)			P/B ratio
	Total	EPT	Mollusca	
EFK 24.4				
Year 1	10.5	<0.1	0.0	18.0 \pm 0.88
Year 2	7.5	<0.1	<0.1	17.3 \pm 1.04
EFK 23.4				
Year 1	25.3	0.15	0.6	22.3 \pm 0.36****
Year 2	35.0	0.1	<0.1	20.8 \pm 0.61
EFK 18.2				
Year 1	24.3	0.7	<0.1	19.0 \pm 0.73***
Year 2	20.6	0.3	0.1	17.2 \pm 0.76
EFK 13.8				
Year 1	152.7	119.7	6.6	14.9 \pm 0.84****
Year 2	321.0	261.1	4.8	12.5 \pm 0.80
EFK 10.6				
Year 1	24.1	0.6	0.1	18.2 \pm 0.48****
Year 2	24.5	2.1	0.1	15.7 \pm 1.19
EFK 6.3				
Year 1	36.8	22.9	0.6	16.4 \pm 1.18
Year 2	41.2	24.8	0.2	17.1 \pm 1.50
BFK 7.6 ^a				
Year 2	579.8	5.6	563.8	6.3 \pm 0.60

^aSamples were collected from this site only five months during the first year; therefore, secondary production estimates were not made.

Note: Data are for Year 1 (June 1985 through May 1986) and Year 2 (June 1986 through May 1987). An asterisk (*) indicates significant between-year differences based on an ANOVA where * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

dipterans of the family Simuliidae, whose production decreased from 15.9 g wet wgt/m² in the first year to 4.2 g wet wt·m⁻²·year⁻¹ in the second year. The relative contribution of chironomids to total production at BFK 7.6 (exclusive of mollusks) during the second year was similar to their contribution at EFK 13.8.

The combined contribution of EPT taxa was considerable at EFK 6.3 and EFK 13.8, where they accounted for more than 60% of the total production in both years (Table 6-4 and Figs. 6-8 and 6-9). These taxa also contributed considerably to the total production

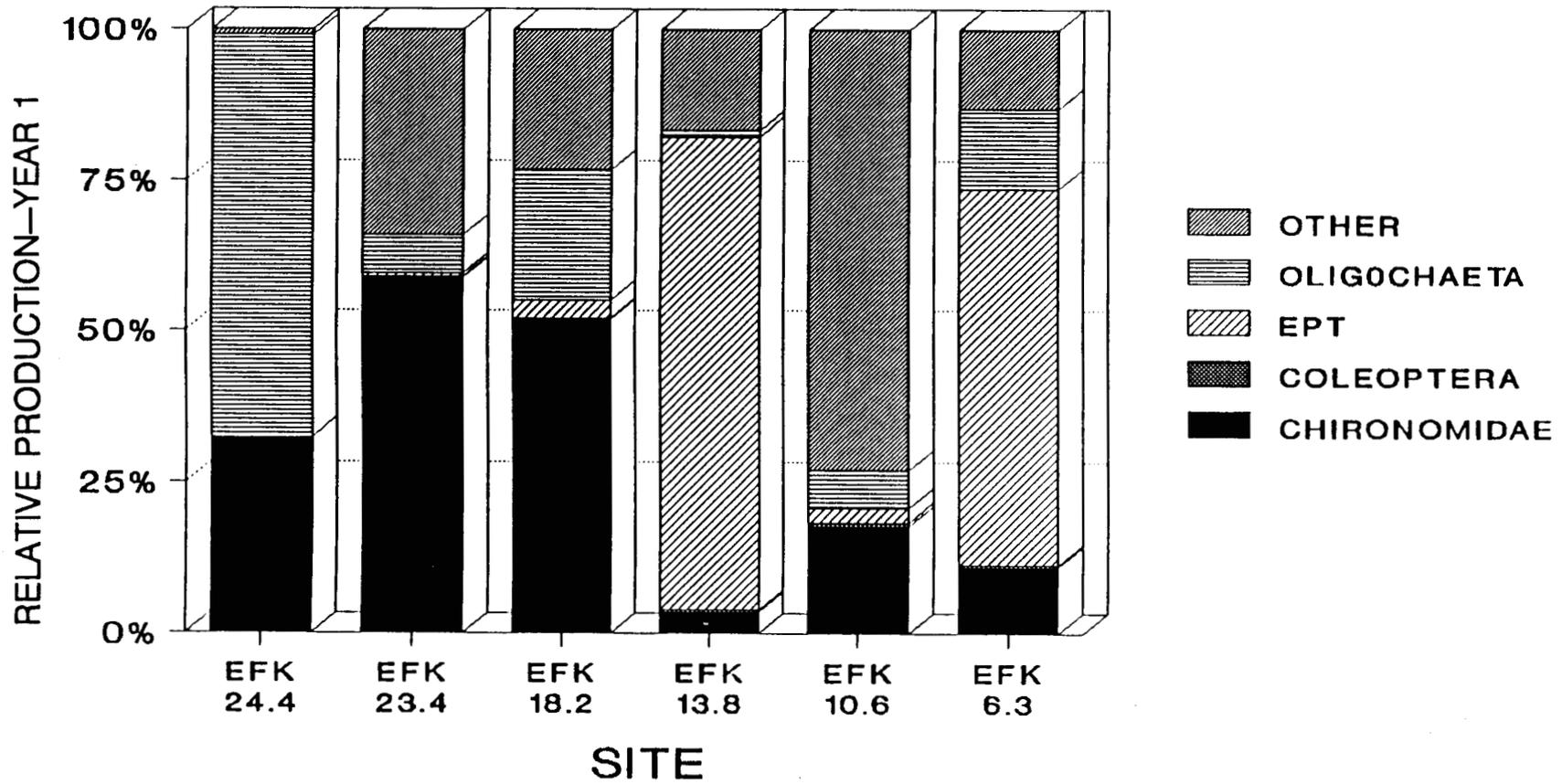


Fig. 6-8. Relative production (percentage of total annual production excluding Decapoda and Mollusca) of major groups of benthic macroinvertebrates in East Fork Poplar Creek, June 1985–May 1986. EPT = Ephemeroptera, Plecoptera, and Trichoptera. EFK = East Fork Poplar Creek kilometer.

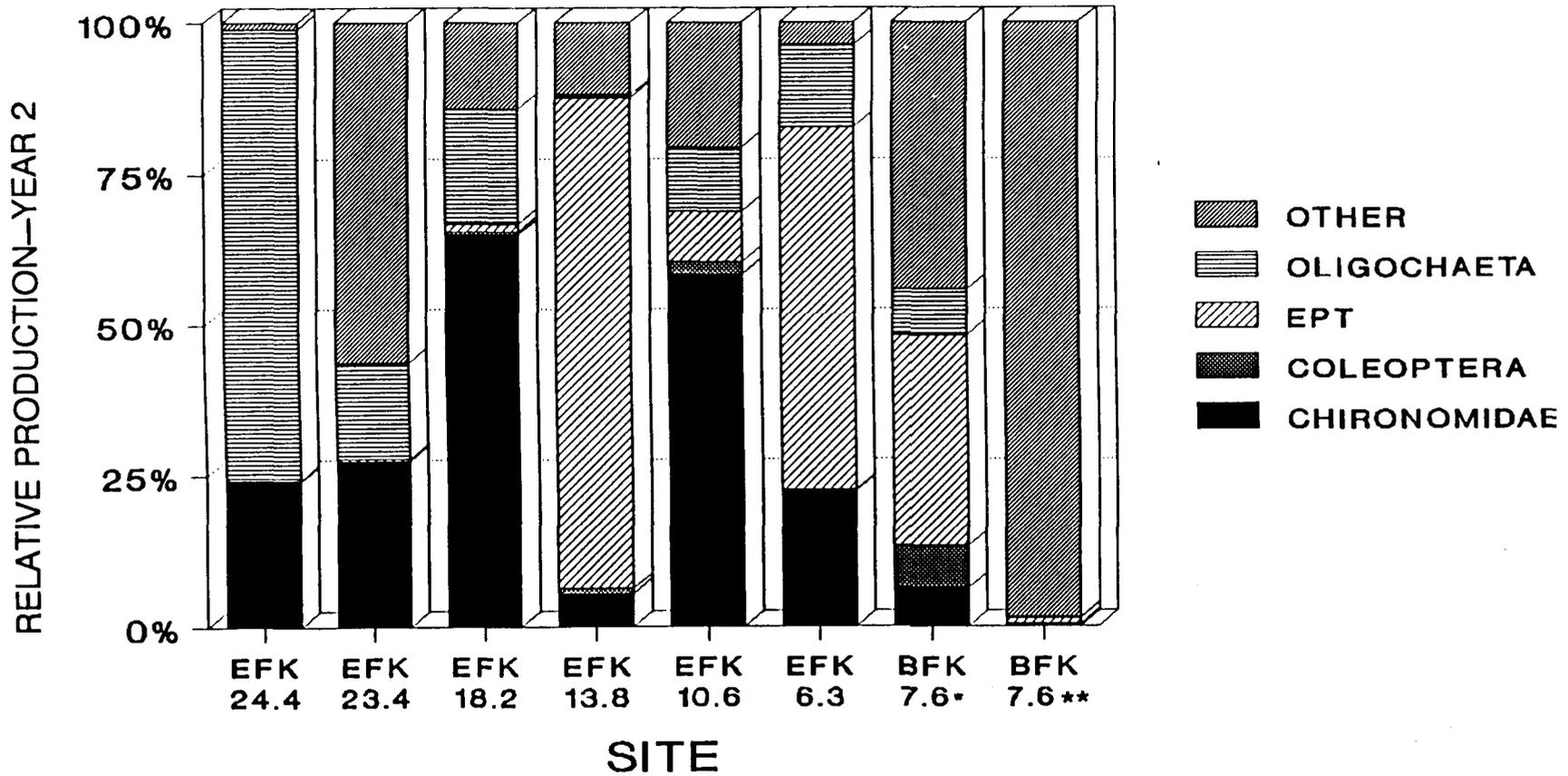


Fig. 6-9. Relative production (percentage of total annual production excluding Mollusca) of major groups of benthic macroinvertebrates in East Fork Poplar Creek and Brushy Fork, June 1986–May 1987. EPT = Ephemeroptera, Plecoptera, and Trichoptera. *Excludes Mollusca; **Includes Mollusca. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

(excluding mollusks) at BFK 7.6 (Fig. 6-9). Most of the production within these three groups was attributable to either the ephemeropterans (EFK 18.2 and BFK 7.6) or trichopterans (all EFPC sites except EFK 18.2 during the first year) (Figs. 6-10 and 6-11). Production by plecopterans was negligible at all EFPC sites and BFK 7.6.

By far the greatest contributors to the total production at BFK 7.6 were the mollusks (Table 6-4). Mollusks, however, contributed little to total production at any site in EFPC. Major contributors to total production in those taxa grouped in the "Other" category in Figs. 6-8 and 6-9 were Decapoda, Oligochaeta, and Diptera (excluding Chironomidae). Oligochaetes were particularly important at EFK 24.4, where they accounted for more than 66% of the total production in both years.

Mean annual P/B ratios showed relatively consistent longitudinal trends in both years in EFPC (Table 6-4 and F-3). Mean annual P/B ratios were significantly less at EFK 13.8 than at all other EFPC sites during both years, and at EFK 23.4 mean P/B ratios were significantly greater than at all other sites during both years. With the exception of EFK 6.3 during the first year, mean P/B ratios at the remaining four sites in EFPC were similar during both years. During the second year, mean annual P/B ratios were significantly less at BFK 7.6 than at all EFPC sites.

Several EFPC sites exhibited significant changes in mean P/B ratios from the first to second year (Table 6-4). P/B ratios decreased significantly during the second year at EFK 23.4, EFK 18.2, EFK 13.8, and EFK 10.6; while mean P/B ratios did not change significantly between years at the other two EFPC sites.

6.1.3.2 Experimental studies

Clam studies

In the first experiment with clams, considerable differences were seen between sites in the survival of the clams (Fig. 6-12). Survival of clams was greatest at BFK 7.6, where, after 81 d, 87% of the small clams and 100% of the large clams survived. Percent survival of the large clams declined considerably over time at EFK 13.8 and EFK 23.4, where their respective survival rates were only 41% and 8% after 81 d. At EFK 23.4 no small clams were alive after 81 d; while at EFK 13.8, survival of small clams was 100% after 38 d, but the tray containing these clams was lost soon after the thirty-eighth day during high flows.

Considerable differences were observed in growth of clams between size groups and sites (Fig. 6-13). Growth of clams in both size classes was greatest at BFK 7.6; although, prior to being lost, the small clams at EFK 13.8 appeared to have grown at a rate similar to those at BFK 7.6. Little or no growth had been exhibited by large clams at either EFK 23.4 or EFK 13.8 after 81 d. Likewise, little or no growth was exhibited by small clams at EFK 23.4 during the study. The greatest amount of growth was exhibited by the small clams at BFK 7.6, where after 81 d the average length increase of small clams was 1.29 mm (SD \pm 0.23). Relative to the small clams, growth of the large clams appeared to be considerably slower at BFK 7.6, where after 81 d the average length increase was only 0.27 mm (SD \pm 0.22).

The survival rate of clams at EFK 23.4 during the second experiment was similar to the survival rate observed during the first experiment (Fig. 6-14). The number of clams surviving at this site declined considerably over time, with only a 4% survival rate after 88 d. At EFK 13.8 however, survival after 88 d was 94%, which was similar to the 100% survival of clams at the 3 reference sites.

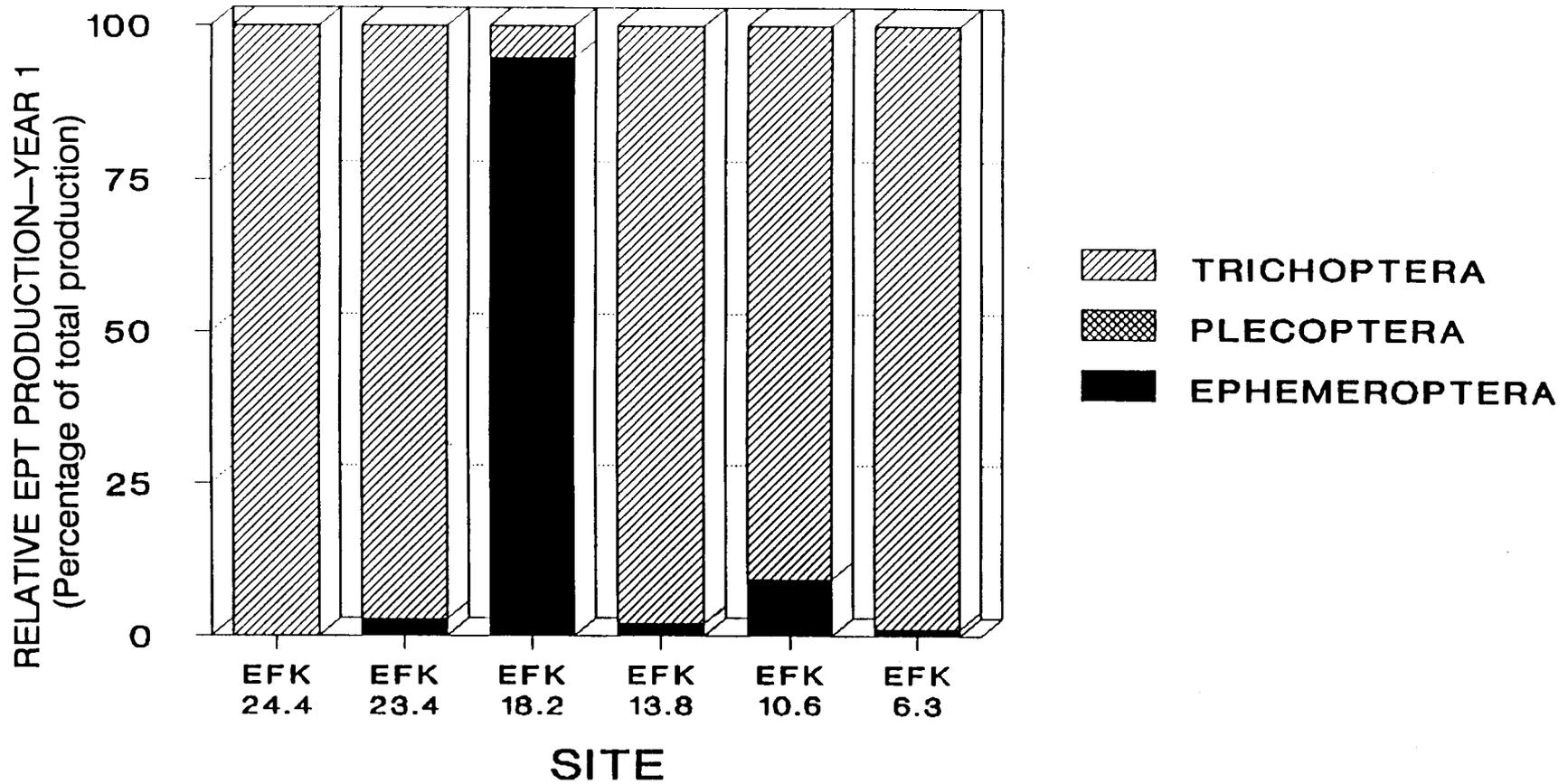


Fig. 6-10. Relative contribution of Ephemeroptera, Plecoptera, and Trichoptera to the total production of these three taxa in East Fork Poplar Creek, June 1985–May 1986. EFK = East Fork Poplar Creek kilometer.

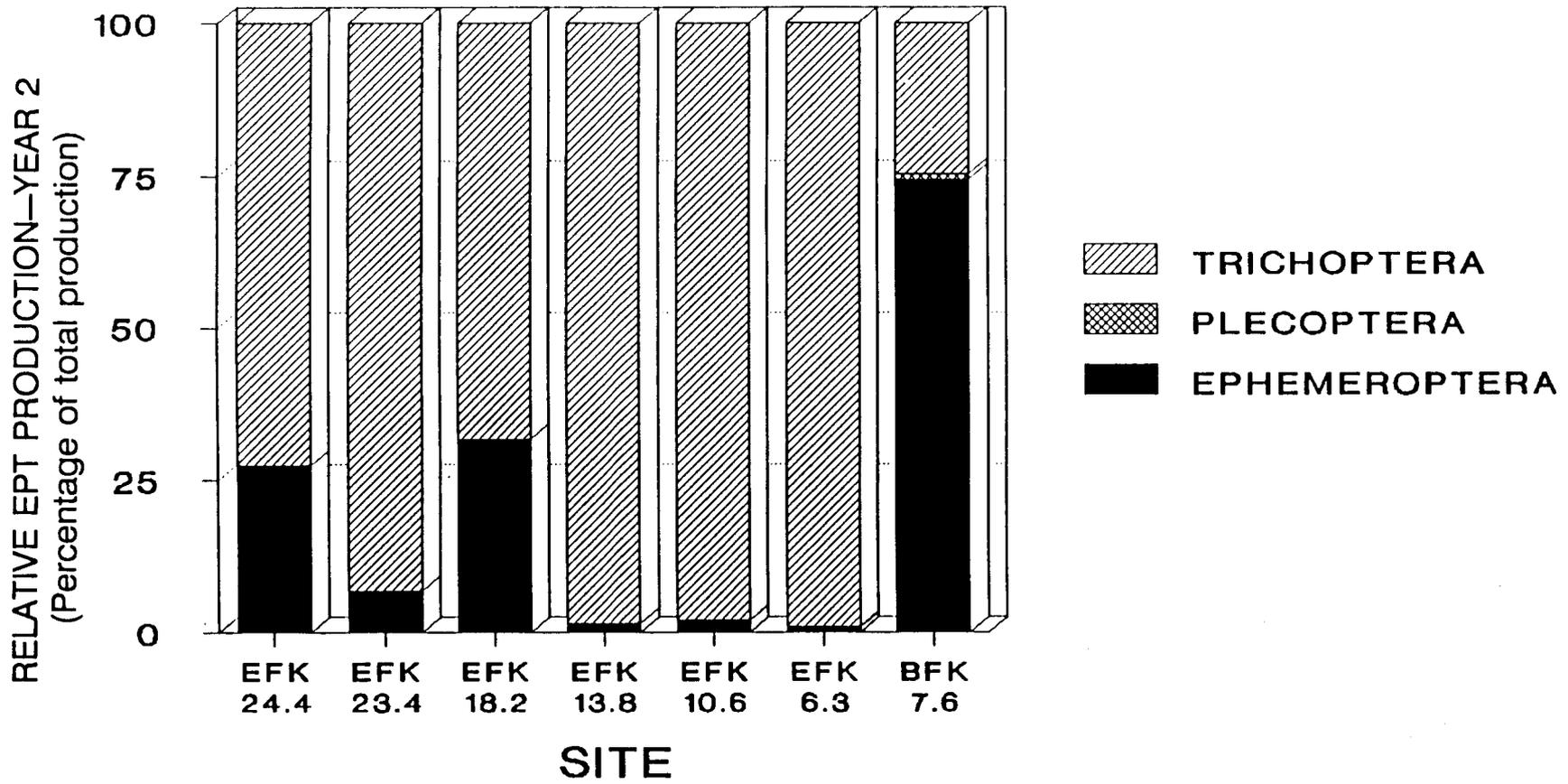


Fig. 6-11. Relative contribution of Ephemeroptera, Plecoptera, and Trichoptera to the total production of these three taxa in East Fork Poplar Creek, June 1986–May 1987. EFK = East Fork Poplar Creek kilometer. BFK = Brushy Fork kilometer.

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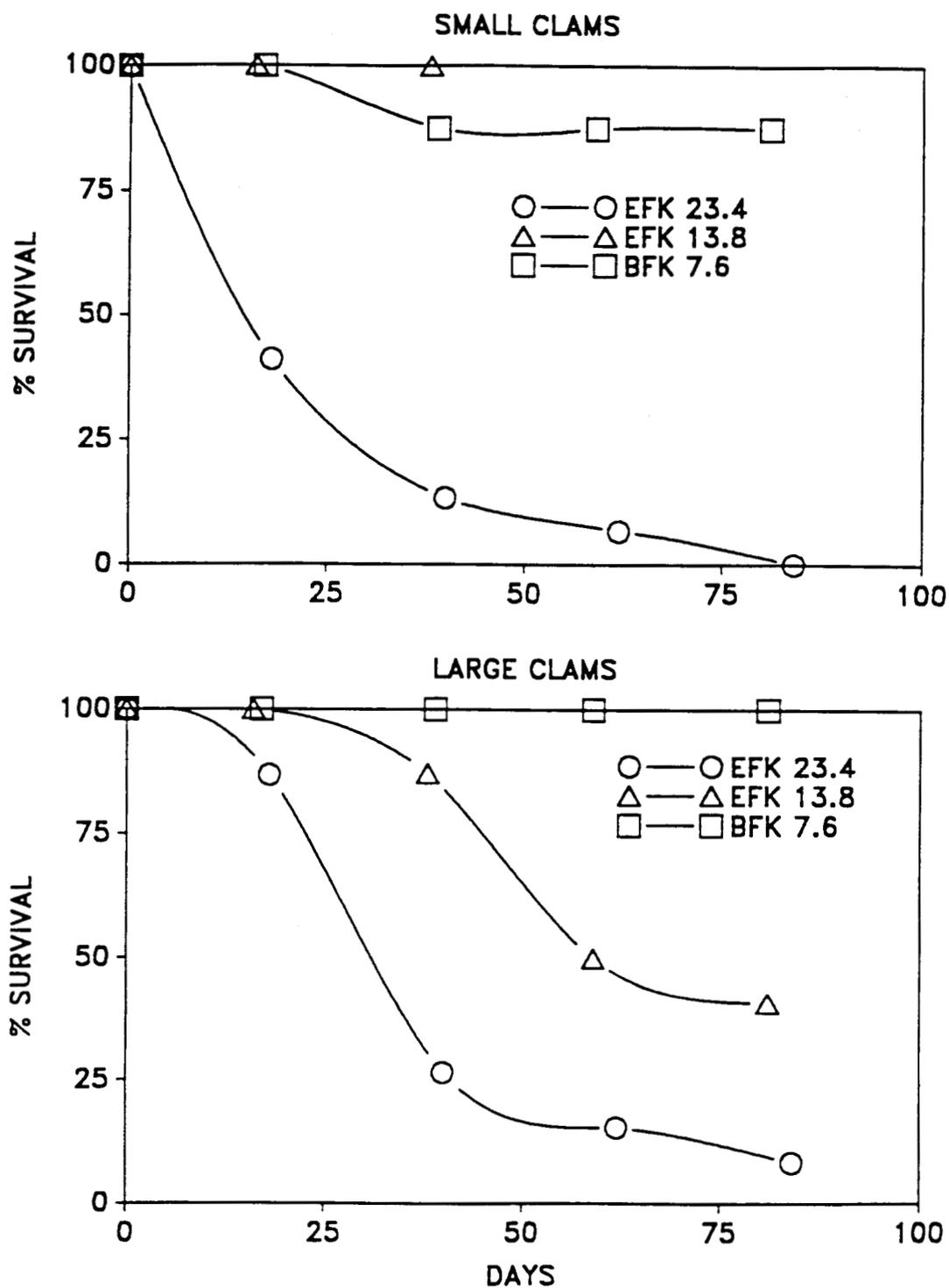


Fig. 6-12. Percentage of surviving small and large clams (*Sphaerium fabale*) placed in situ in East Fork Poplar Creek and Brushy Fork during Experiment 1, July–October 1988. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

ORNL-DWG 93-8982

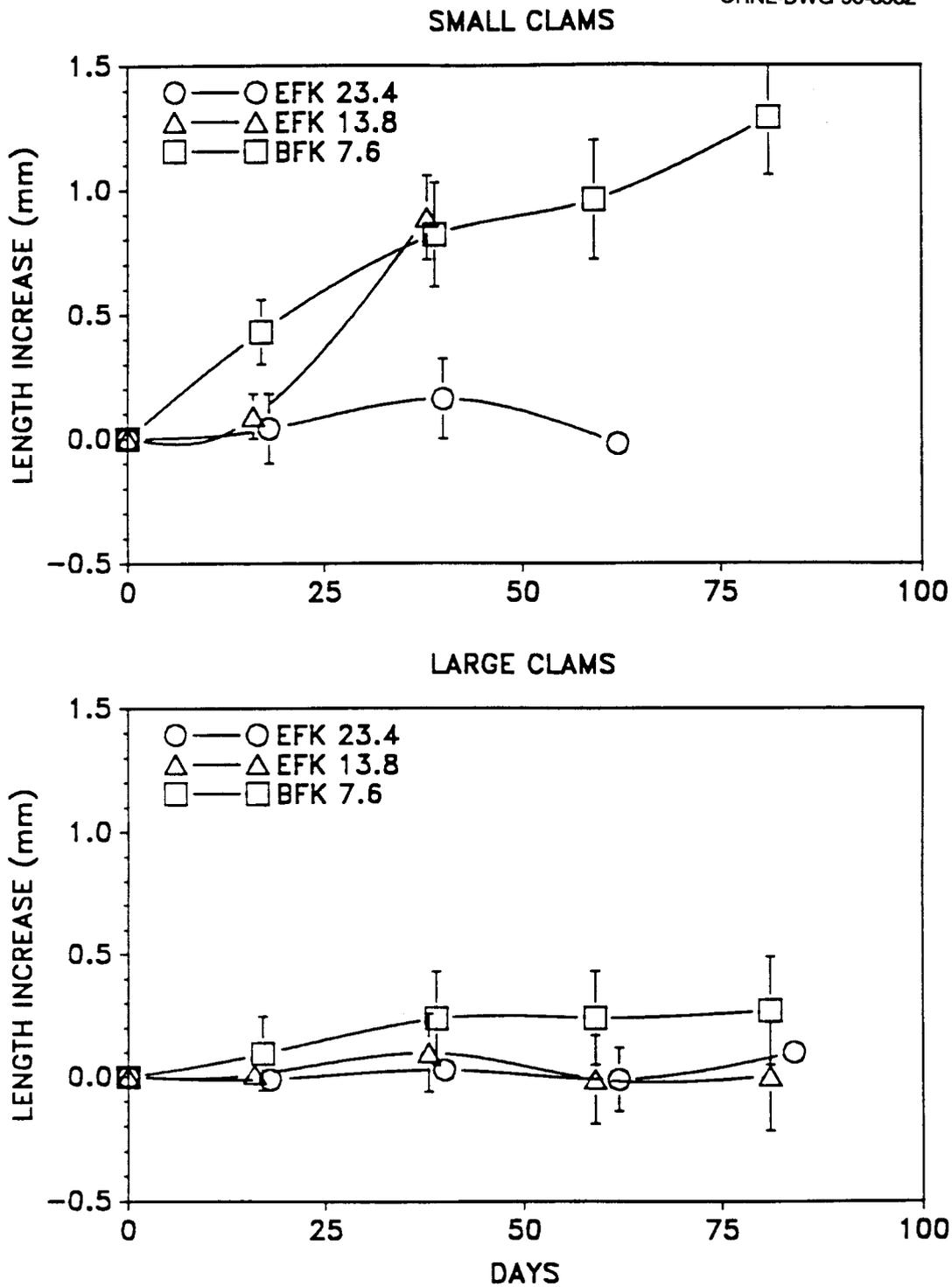


Fig. 6-13. Growth of small and large clams (*Sphaerium fabale*) placed in situ in East Fork Poplar Creek and Brushy Fork during Experiment 1, July–October 1988. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

ORNL-DWG 89-9198

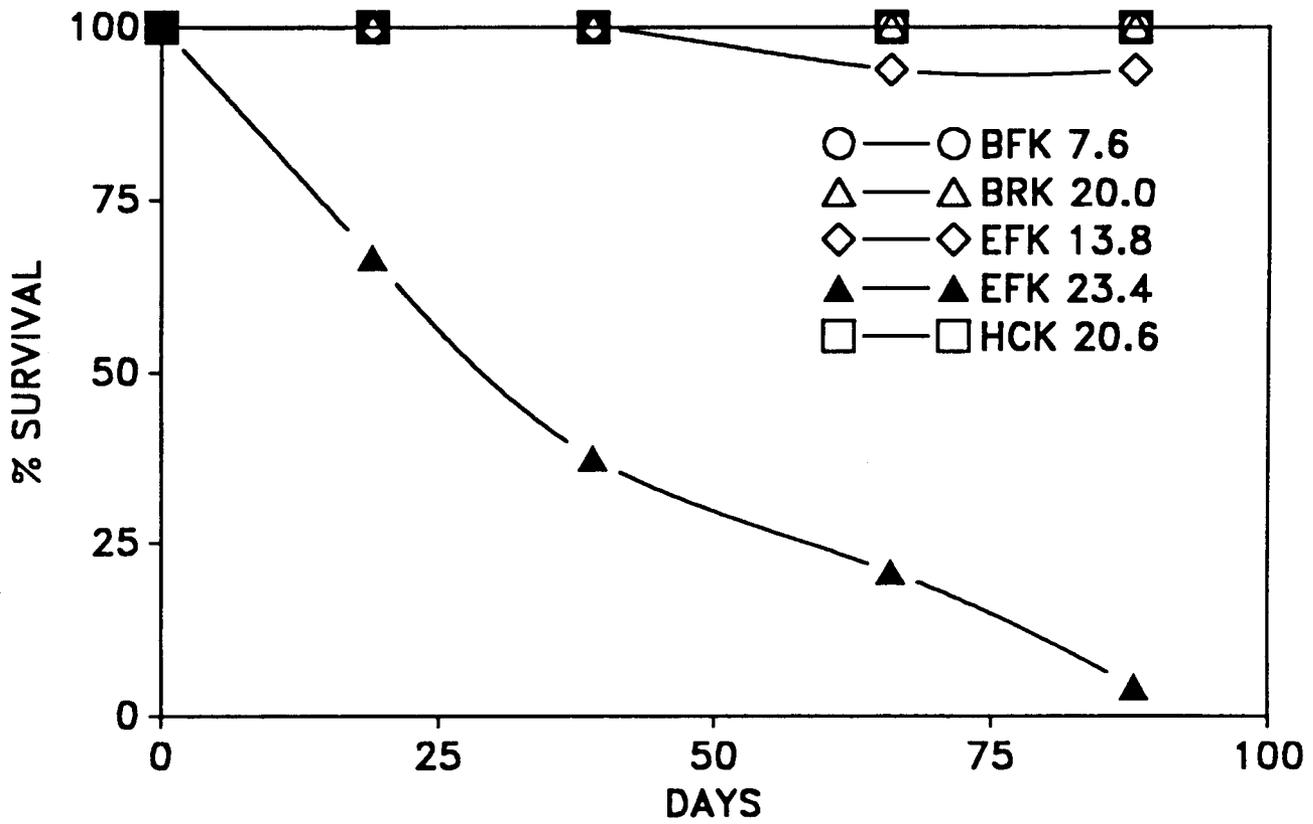


Fig. 6-14. Percentage of surviving clams (*Sphaerium fabale*) placed in situ in East Fork Poplar Creek and control streams during Experiment 2, October-December 1988. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; BRK = Bull Run Creek kilometer; HCK = Hinds Creek kilometer.

There was little difference in growth between the clams at EFK 13.8 and the three reference sites, where length increases at these sites averaged between 0.2 and 0.3 mm over the 88-d exposure period (Fig. 6-15). Clams at EFK 23.4 however, exhibited little or no growth over the same time period.

Caddisfly studies

Results from the semiquantitative survey of *H. deprivata* in upper EFPC suggested that the number of caddisflies increased with increasing distance from the Y-12 Plant (Fig. 6-16). The total number of caddisflies collected per sample was 4, 4, 32, 44, and 298 at EFK 23.1, EFK 21.9, EFK 21.4, EFK 21.3, and EFK 18.6 respectively.

The results of the streamside artificial channel comparisons of growth at EFK 14.0 and EFK 23.1 were inconclusive. The channels at EFK 23.1 received considerable amounts of silt during stormflow events to the extent that most of the experimental population was lost due to drift from the channels or mortality approximately 6 weeks after initiating the experiment. The experimental populations at EFK 14.0, however, continued to grow and emerge throughout the summer.

Figures 6-17 and 6-18 illustrate the results of the laboratory experiment on the effect of ambient water quality from EFK 23.1 on growth and emergence of *H. deprivata*. Under conditions of constant temperatures and equal quantities of food, no significant differences were found in growth of individuals maintained in either dechlorinated tap water or water from EFK 23.1 or EFK 14.0. Similarly, there appeared to be no differences between treatments in the emergence of adults (Fig. 6-18).

In the temperature tolerance experiment, no mortality was observed in larvae maintained at 22°C or 12°C. In contrast, in the channels containing the larvae exposed to increasing temperatures, all larvae were found dead at 31°C.

Results of the ATP analysis demonstrated that the amount of living material available in the water column that could potentially be consumed by filter feeding organisms was relatively similar at EFK 23.1 and EFK 13.8, where concentrations averaged 1562 and 1301 ng/L respectively (Fig. 6-19). Mean concentrations of ATP at the remaining 5 sites were about one-half to one-third of those found at EFK 23.1 and EFK 14.0, ranging from 531 to 726 ng/L.

6.1.4 Discussion

The ultimate goals of the benthic invertebrate monitoring program are to provide (1) part of the information needed to show that the effluent limitations established at the Y-12 Plant protect the classified uses of EFPC (see Sect. 1. Introduction) and (2) documentation of the ecological effects resulting from implementation of the water pollution control program at the Y-12 Plant. As a part of achieving these goals, the "health" of, and changes occurring in, the benthic community of EFPC are being assessed relative to a single, relatively undisturbed reference site on BF (BFK 7.6). Results of other benthic invertebrate studies on relatively undisturbed reference streams both on and off of the ORR have shown that considerable spatial and temporal differences can exist in the composition and structure of benthic invertebrate communities both between and within streams (Smith 1992b and 1992c). Thus, use of a single reference site could

ORNL-DWG 89-9199

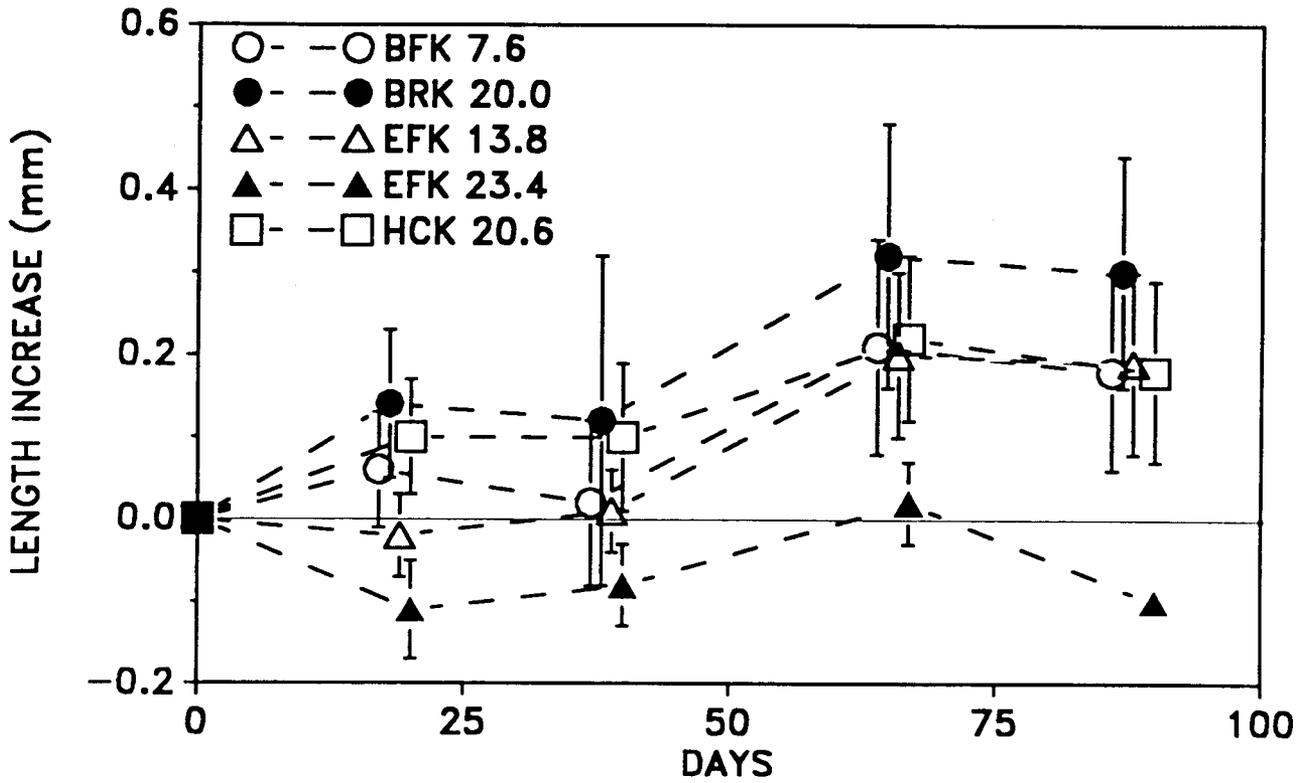


Fig. 6-15. Growth of clams (*Sphaerium fabale*) placed in situ in East Fork Poplar Creek and control streams during Experiment 2, October–December 1988. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; BRK = Bull Run Creek kilometer; HCK = Hinds Creek kilometer.

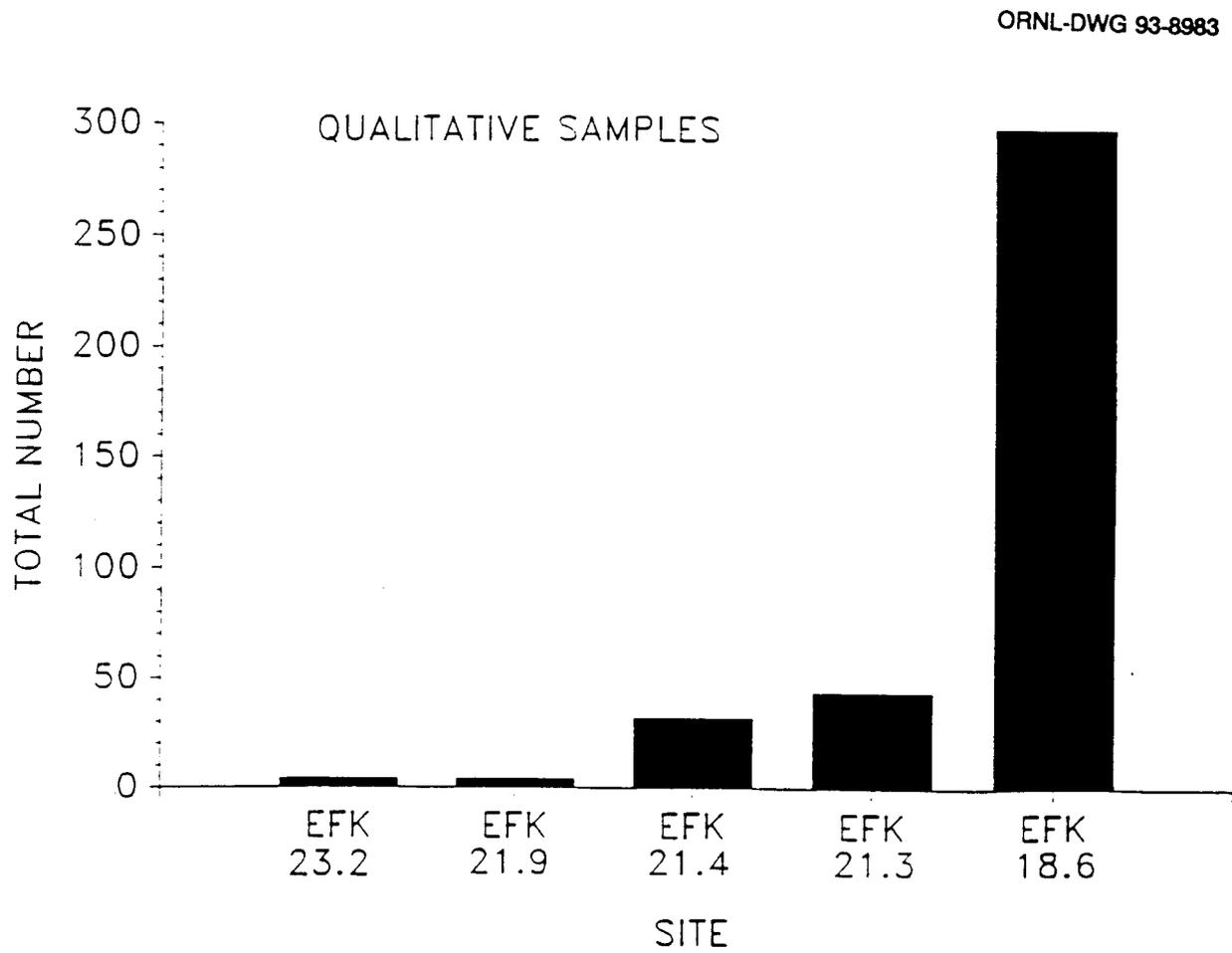


Fig. 6-16. Total numbers of the caddisfly, *Hydropsyche depravata*, collected in qualitative samples from East Fork Poplar Creek, October 1988. EFK = East Fork Poplar Creek kilometer.

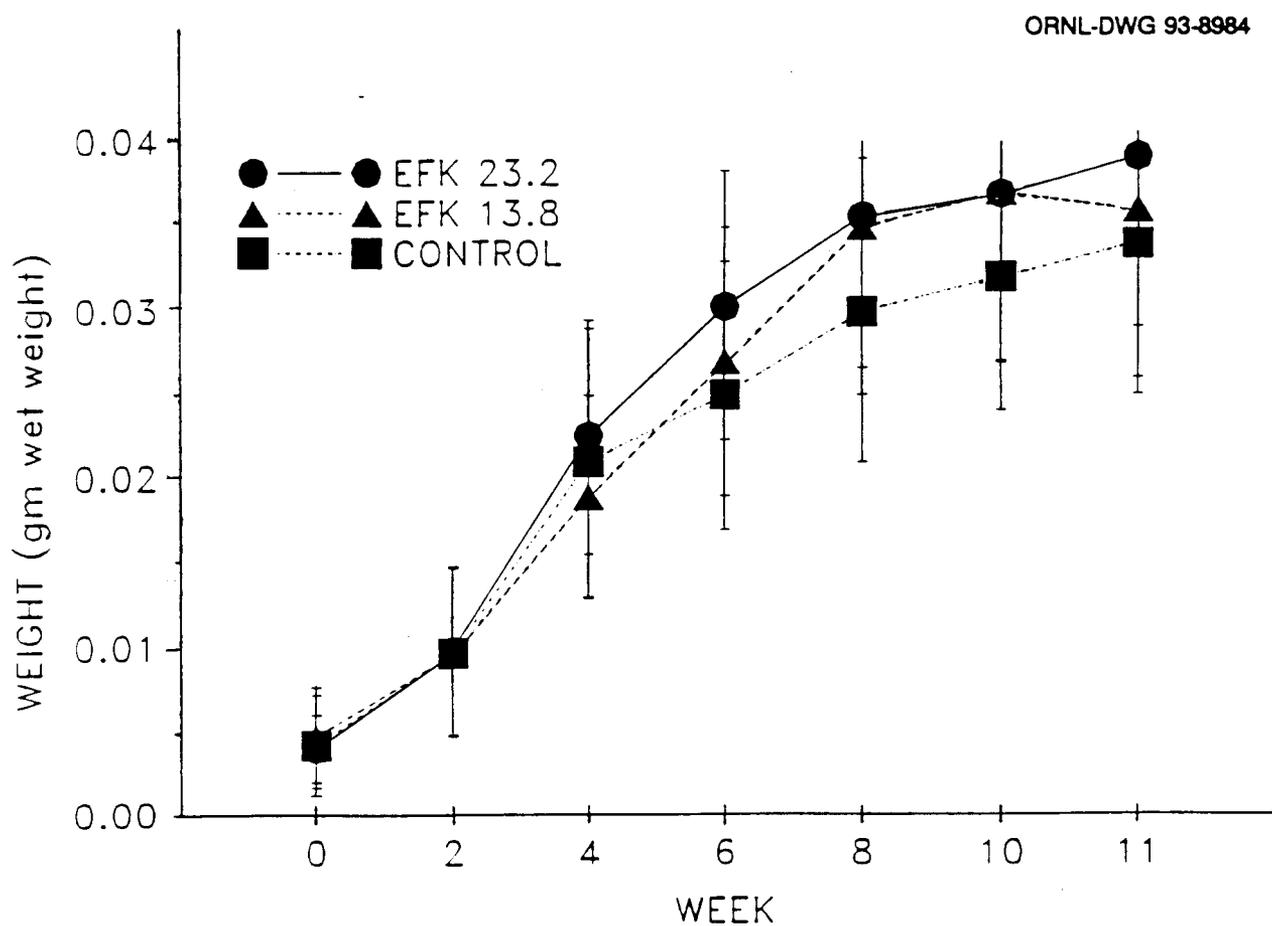


Fig. 6-17. Mean weight increase of caddisflies (*Hydropsyche depravata*) exposed to water from East Fork Poplar Creek in artificial circular stream channels in the laboratory, September-December 1988. Vertical bars are ± 1 SE. EFK = East Fork Poplar Creek kilometer.

ORNL-DWG 93-8985

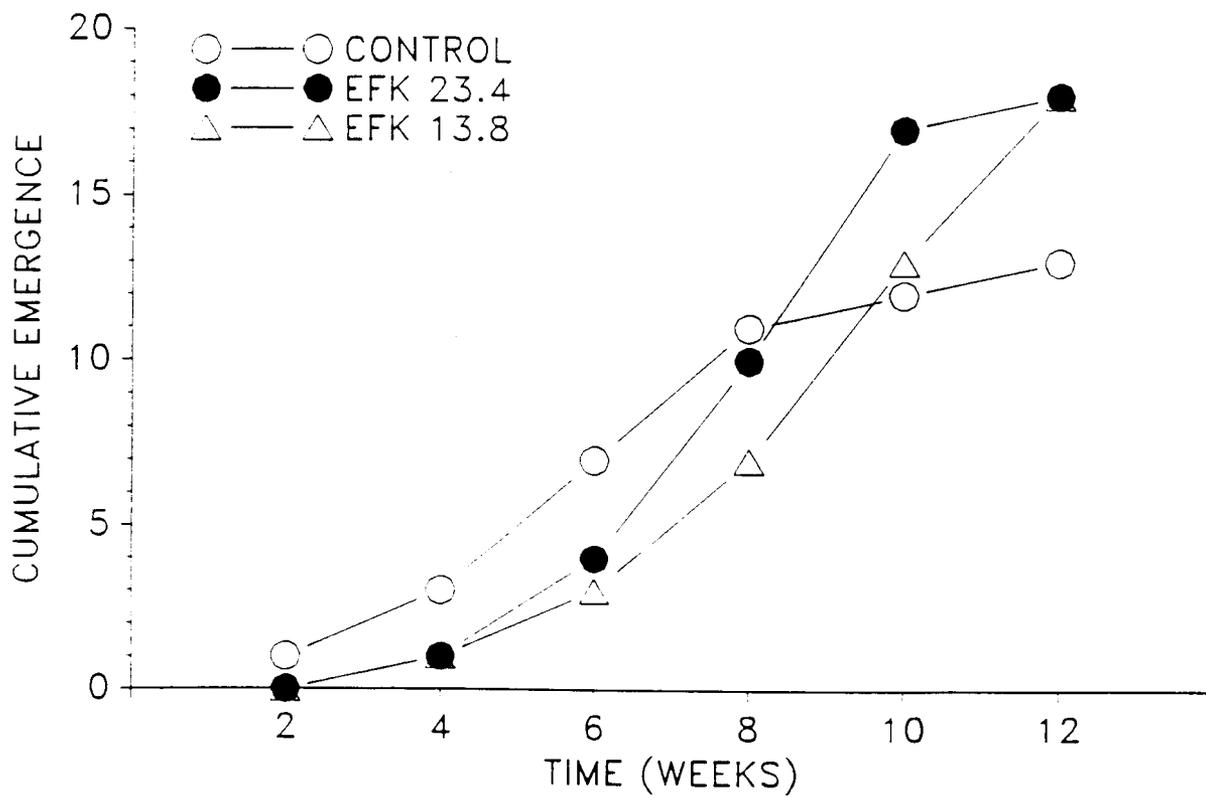


Fig. 6-18. Cumulative emergence of *Hydropsyche depravata* exposed to water from East Fork Poplar Creek in artificial circular stream channels in the laboratory, September–December 1988. EFK = East Fork Poplar Creek kilometer.

ORNL-DWG 93-8986

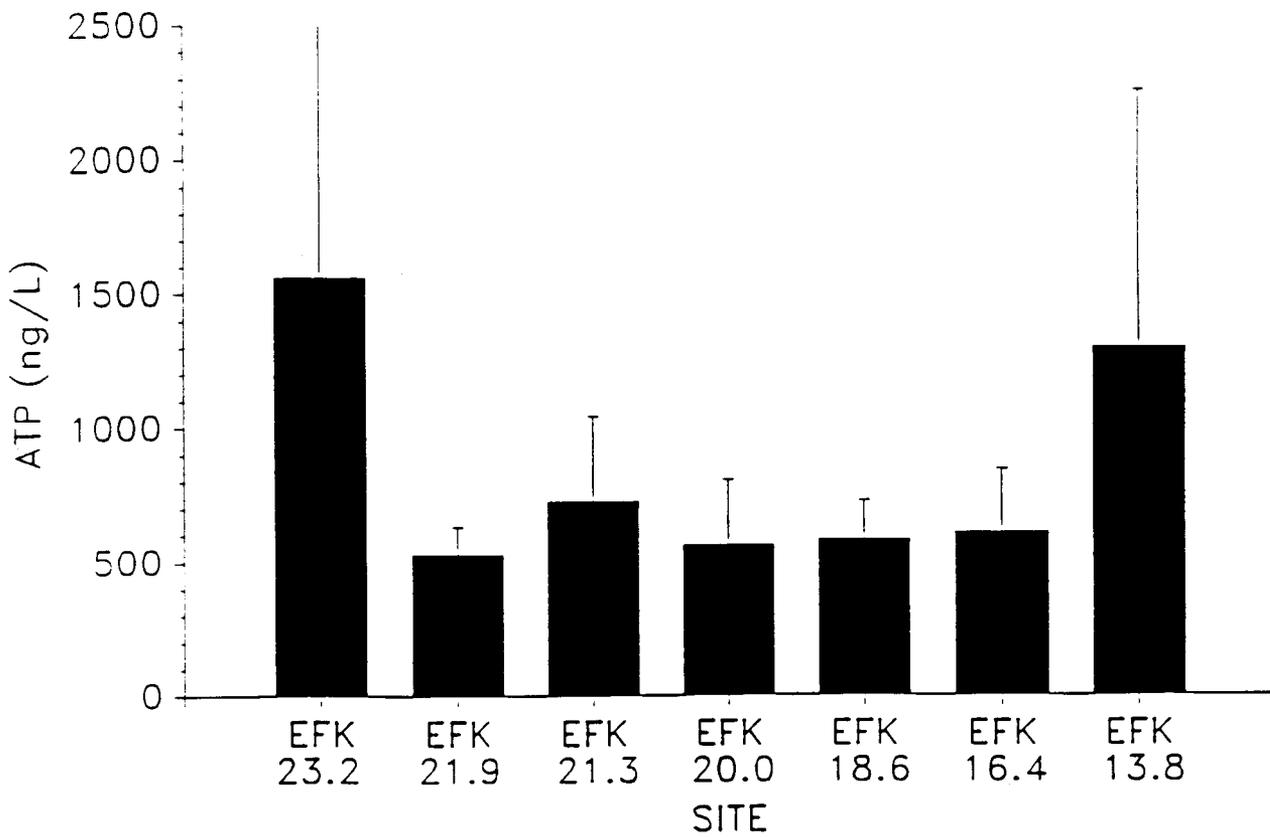


Fig. 6-19. Mean adenosine triphosphate (ATP) concentration from water samples collected from East Fork Poplar Creek, October–November 1988. Vertical bars are ± 1 SE. EFK = East Fork Poplar Creek kilometer.

potentially lead to erroneous conclusions. However, fairly consistent patterns in some community structure parameters of these reference sites have been observed that provide a good baseline for comparing changes in both reference and nonreference sites, reducing the possibility of making erroneous conclusions. Results obtained to date for these other reference sites, particularly for mean community richness and EPT richness, show that BFK 7.6 is relatively similar to other reference sites. For example, EPT richness at five reference sites on the WOC watershed exceeded 4.8 taxa per sample, while EPT richness at the nonreference sites was less than 2.5 taxa per sample (Smith, unpublished data). In the current study, EPT richness at BFK 7.6 was almost 5.0 taxa per sample while in EFPC, EPT richness was <3.0 taxa per sample at all sites. Thus these similarities among reference sites indicate that BFK 7.6 appears to be a suitable reference site for comparison with EFPC.

Results obtained during the second year of the EFPC BMAP continue to show, as was found in the first year (Smith 1992a), that the benthic community is under varying degrees of stress. Richness, diversity, density, biomass, and production were lower at EFK 24.4 than at all other sites, showing that this site remains the most impacted. With increasing distance from the Y-12 Plant, the benthic community exhibited signs of increasing improvement with the most improvement being exhibited at EFK 13.8. At the two sites downstream of EFK 13.8, the benthic community exhibited signs of additional stress, most likely resulting from the effluent discharge of ORWTF. In light of recent stream theory (e.g., Vannote et al. 1980), one could possibly argue that this spatial trend in EFPC (i.e., increasing values for each parameter with increasing distance from the headwaters that peak in the middle reaches of a stream before declining) was natural and not disturbance related. However, the magnitude of differences in some of the parameters between the most and least disturbed sites, and between all EFPC sites and BFK 7.6, provide strong evidence that this trend was stress induced. For example, data from undisturbed reference streams in WOC watershed (Smith, unpublished data) show that taxonomic and EPT richness typically exceed 20 taxa per sample and 5 EPT taxa per sample, respectively, values which are very similar to those for BFK 7.6 but considerably greater than for any site in EFPC. Thus, even if substantial spatial differences would be expected under natural conditions, the data from relatively undisturbed streams in the Oak Ridge area suggest that reductions in parameters such as taxonomic and EPT richness, of the magnitude seen in EFPC, were much greater than would be expected in either small or large undisturbed streams.

The benthic community at BFK 7.6 exhibited significant changes in biomass, density, and diversity between the first and second year (January through May only), which demonstrates how changes occur naturally between years in minimally disturbed streams. Comparisons between years in EFPC showed that little or no change had occurred in the invertebrate community at EFK 24.4, EFK 18.2, or EFK 6.3. For example, at EFK 24.4 there were no significant differences between the first and second year in biomass, richness, or diversity, although density decreased during the second year, and at EFK 18.2 and EFK 6.3 there were no differences between years in any of these parameters. Similarly, there was little change between years in production at these sites.

At EFK 23.4 the results were not as clear. Density at this site was greater during the first than the second year; while biomass (all taxa), community richness, diversity, and production were all greater during the second year, and EPT richness and biomass (excluding Decapoda and Mollusca) did not differ between years. It is likely that these between-year differences represent natural annual variability and that no changes have

occurred that can be attributable to remedial actions. This hypothesis is strengthened by the fact that there was little change between years in the relative abundance of the dominant taxa, particularly the chironomids, which comprised >90% of the total community density in both years.

The communities at EFK 10.6 and EFK 13.8 exhibited significant changes from the first year to the second year, where—with the exception of EPT richness, diversity, and production at EFK 10.6—data on density, biomass, and total richness suggested that improvements may have occurred at both sites during the second year. A major factor which may have been responsible for the between-year differences at EFK 13.8 was the change in the location from which samples were collected (i.e., approximately 50 m upstream of the original riffle) as a result of the destruction of the original riffle by construction activity in early October 1986. Natural annual variability in combination with between-site differences in physical factors, such as substrate (e.g., size of substrate, amount of silt), flow characteristics (i.e., current velocity) and biological factors (e.g., amount of food availability), most likely account for much of the observed differences in the community at these two riffles at EFK 13.8. Similarly, there was little change between years at EFK 10.6, where chironomids also dominated in both years. The major difference between years at this site was the reduction in the relative abundance of Simuliidae (blackflies). This change, however, was most likely the result of natural annual variability.

The accuracy of secondary production estimates for benthic invertebrates depends on a number of factors, including the procedures and equipment used in collecting samples, the efficiency of processing samples, and the method(s) of calculation (Waters 1979, Benke 1984). Direct calculation methods are the most accurate (Benke 1984) but require considerably more effort and are therefore less feasible in studies such as BMAP. The use of production to biomass ratios (P/B) are less accurate, but their accuracy can be enhanced when corrected for cohort production intervals (CPI) (Waters 1979). Although estimates of community production from combined direct and indirect methods are not as accurate as using direct methods alone, they still provide a better estimate of the food potentially available to higher trophic levels (e.g., fishes) than do estimates of biomass. Such estimates also provide another means of comparing the "health" of benthic communities between sites and between years at a given site.

At those sites in EFPC where the target taxa were most abundant, production estimates were generally within the range of values found for the target taxa at BF and of published values for related taxa (e.g., Behmer and Hawkins 1986, Kruger and Waters 1983, Neves 1979). Although secondary production of *Hydropsyche depravata* was high at EFK 13.8 and EFK 6.3 relative to the production of the other target taxa at these and the other sites, values similar to these have been reported in other studies for related species of caddisflies (e.g., Flössner 1976, Benke 1984, Mackay and Waters 1986). High densities or production values are often associated with moderately enriched conditions such as those sometimes found below the outfalls of ponds and surface release reservoirs or those associated with organic pollution (e.g., Mackay and Waters 1986, Schuster and Etnier 1978); thus, the relatively high production and density of *H. depravata* at EFK 13.8 and EFK 6.3 suggested the existence of moderately enriched conditions. This also provides additional evidence of some improvement from upstream perturbations. The low abundance and production of this species and other filter-feeding taxa just downstream of NHP (EFK 23.4) was surprising since ponds typically provide an abundant supply of food for filter-feeders living downstream of their outfalls. This suggests that conditions in upper EFPC may have been chronically toxic or periodically acutely toxic.

For relatively undisturbed streams on ORR, data show that estimates of community production (excluding mollusks) generally ranged from ~10 to 50 g wet wt·m⁻²·year⁻¹ (Table 6-5); either with or without mollusks, these values generally fell within or near the lower to middle range of published values (Table 6-6). Production at moderately impacted sites on ORR appeared to be similar to or higher than production at reference sites, while production estimates at the most highly impacted sites fell below the lower end of the normal range (i.e., < 10 g wet wt·m⁻²·year⁻¹). Thus, except for the most highly impacted sites where production was notably reduced, production at the moderately to relatively unimpacted sites was similar. Production estimates at all sites in EFPC except EFK 13.8 and EFK 24.4 fell within the normal range for ORR streams. The high values obtained at EFK 13.8 provide further evidence of improvement relative to those sites further upstream and were probably due to the absence of or considerable reduction in chemical and physical perturbations in combination with an abundant food supply resulting from the breakdown of organic matter. The low values obtained at EFK 24.4 provide further evidence of considerable impact occurring in the upper reaches of EFPC. Thus, differences in production between moderately impacted sites and reference sites may not be evident from total community estimates, but differences *are* apparent in the relative contribution of the major taxa to the total production. In general, the greater the impact, the greater the contribution of chironomids; although oligochaetes may also be major contributors.

The magnitude of production is determined by the biomass and rate of biomass turnover, or annual production to biomass ratio (P/B), and the annual P/B ratio is in turn determined primarily by the length of aquatic life, or the CPI (Benke 1984). As was observed, chironomids were major contributors to community production at the upstream-most sites in EFPC. These small organisms typically produce several generations each year which results in short CPIs and, thus, high annual P/B ratios. Thus, the higher mean annual P/B ratios at the sites exhibiting the greatest impact reflected the dominance of chironomids and/or other organisms with short CPIs (e.g., Simuliidae at EFK 10.6 during the first year). In contrast, the lower P/B ratios at EFK 13.8, EFK 6.3, and particularly BFK 7.6 reflect the dominance of heavier organisms with longer CPIs (e.g., Trichoptera and snails). Organisms which produce several generations per year and have short CPIs would be expected to dominate in areas frequently disturbed, while longer-lived species would be expected to become more dominant in minimally disturbed or undisturbed areas (Resh et al. 1988). For example, in the event of a major or frequent disturbance, such as the release of a toxicant, longer-lived taxa may be partially or completely eliminated. Unless a resistant (egg) or insusceptible (adult) stage exists at the time of the disturbance, longer-lived taxa may not be able to attempt to recolonize for several months. In contrast, shorter-lived species, which produce several generations a year, would most likely have resistant and/or insusceptible stages occurring at most times and would therefore be able to recolonize fairly rapidly.

Preliminary studies were conducted with clams, keyed on assessing their utility as indicators of water quality conditions in EFPC. As with the data on benthic invertebrate community structure, results on growth and mortality of clams placed in situ in EFPC suggest that upper EFPC cannot yet support invertebrates which are as sensitive as, or more sensitive than the species of clam used in this study. However, because this study was designed to evaluate the utility of this technique as an additional monitoring tool and

Table 6-5. Production estimates and mean annual production to biomass (P/B) ratios for benthic macroinvertebrate communities in streams on the Department of Energy's Oak Ridge Reservation

Site	Production (g wet wt·m ⁻² ·year ⁻¹)		P/B
	Total	Mollusca	
First Creek^a			
FCK 0.1	21.6	0.3	15.0
FCK 0.8 ^b	87.9	67.8	6.3
Fifth Creek^a			
FFK 0.2	7.1	0.1	12.2
FFK 1.0 ^b	50.1	0.3	9.9
Melton Branch^a			
MEK 0.6	19.2	0.5	16.1
MEK 1.2	16.4	<0.1	14.6
MEK 2.1 ^b	12.9	0.1	10.3
Mitchell Branch^{c,d}			
MIK 0.45	8.0	<0.1	12.9
MIK 0.54	4.5	0.0	14.9
MIK 0.71	0.3	<0.1	11.5
MIK 0.78	19.8	<0.1	14.2
MIK 0.86	22.8	0.0	11.7
MIK 1.43 ^b	10.9	<0.1	12.4
Northwest Tributary^a			
NTK 0.2	188.7	163.5	11.5
NTK 1.0 ^b	24.3	<0.1	9.4
White Oak Creek^a			
WCK 2.3	51.4	0.3	15.1
WCK 2.9	35.3	0.6	17.0
WCK 3.4	34.9	0.2	22.4
WCK 3.9	20.5	0.1	20.3
WCK 5.1	15.8	<0.1	19.4
WCK 6.8 ^b	96.8	73.2	8.4

^aStreams in White Oak Creek watershed located near Oak Ridge National Laboratory. FCK = First Creek kilometer; FFK = Fifth Creek kilometer; MEK = Melton Branch kilometer; MIK = Mitchell Branch kilometer; NTK = Northwest Tributary kilometer; WCK = White Oak Creek kilometer.

^bReference site.

^cStream located at the Oak Ridge K-25 Site.

^dMollusca production values and P/B ratios for Mitchell Branch sites are unpublished data of J. G. Smith, Environmental Sciences Division, Oak Ridge National Laboratory.

Source: J. G. Smith, unpublished data.

Table 6-6. Published estimates of benthic invertebrate community production

Includes total community estimates from riffle habitat only

Stream	Production (g wet wt·m ⁻² ·year ⁻¹)	Reference ^a
Bear Brook	28.1	Fisher and Likens 1973
Caribou River	32.7	Kruger and Waters 1983
Blackhoof River	43.4	Kruger and Waters 1983
Ball Creek ^b	47.3	Huryn and Wallace 1987
Hinau Stream ^c S. Branch	50.4	Hopkins 1976
Rold Kilde ^b	62.3	Iversen 1988
North Branch Creek	132.4	Kruger and Waters 1983
Horokiwi Stream ^c Bush	147.8	Hopkins 1976
Bisballe Bæk	150.0	Mortensen and Simonsen 1983
Hinau Stream ^c N. Branch	195.2	Hopkins 1976
Horokiwi Stream ^c Gyton	338.7	Hopkins 1976

^aComplete references are listed in Sect. 7.

^bEstimates were converted from dry mass to wet mass assuming that dry mass is 17% of the wet mass.
Source: T. F. Waters, *Secondary Production in Inland Waters*, Adv. Ecol. Res. 19:91-164 (1977).

^cEstimates were converted from ash free dry mass to wet mass assuming that dry mass is 17% of the wet mass and ash free dry mass is 90% of the dry mass (Waters 1977).

not to test a specific hypothesis, statistical analyses were not possible. Thus, the accuracy of the results obtained in the clam studies are not known. Future studies will incorporate the changes necessary for statistical analyses.

Preliminary attempts to determine the ability of the caddisfly *Hydropsyche depravata* to grow and survive in streamside channels in upper EFPC were unsuccessful due to various mechanical problems with the artificial stream channels. However, several other studies with (or associated with) this species of caddisfly did provide some information on its distribution and some potential factors related to its distribution. The results of the

collection of semiquantitative samples suggested that the relative abundance of this caddisfly increased gradually with increasing distance from the Y-12 Plant, with relatively high numbers occurring within approximately 5 km (EFK 18.6) of the outfall of NHP. The occurrence of large numbers of this caddisfly at EFK 18.6 contrasts with the findings from EFK 18.2, where this caddisfly rarely occurs. Two factors may potentially account for this discrepancy. First, only quantitative samples collected through May 1987 have been processed, while the qualitative samples were collected during the fall of 1988. Thus it is possible that some recovery occurred during this time period. The second factor relates to the possibility of greater habitat stability (i.e., stable attachment sites on the substratum) resulting from the small amount and infrequent occurrence of rain during the summer and fall of 1988. The substratum in the vicinity of EFK 18.2 and EFK 18.6 consists of a relatively fine mixture of small gravel, sand, and silt, which is subject to considerable disturbance during storm events. At EFK 13.8, where densities of this caddisfly remain relatively high throughout the year, the substrate consists largely of a mixture of gravel, rubble, and small boulders, which provides a more stable and thus a more suitable habitat for this caddisfly. The combination of stable flows from the Y-12 Plant discharges and stable habitat conditions resulting from the lack of rain may have provided suitable conditions for more successful colonization in this portion of EFPC during this time period.

Several factors which may be responsible for the absence of *H. depravata* in upper EFPC were examined, including temperature, water quality, and food availability. Similar concentrations of ATP (an indicator of food quality) at EFK 23.1 and EFK 13.8 suggests that food is probably not a factor limiting the occurrence *H. depravata* in upper EFPC. However, further studies on the food of this caddisfly would be needed to confirm these results, such as (1) seasonal concentrations of ATP, (2) site comparisons in the size distribution of potential food particles, (3) the differences in the food quality of the various size fractions, and (4) the effects of available food from each site on growth.

Laboratory studies on the effects of water from EFPC failed to demonstrate that water from EFK 23.1 affects growth or survival of caddisflies. However, water samples were collected and replaced only every 2 d. Thus, it would be possible to miss periodic releases of toxicants, which may be a major perturbation to the benthic community in upper EFPC.

Temperature tolerance experiments with *H. depravata* showed that this species may not be able to tolerate temperatures as high as 31°C. However, it is not known how long the larvae remained at this temperature before they died. Although temperatures as high as 31°C have been recorded at EFK 23.4 (Loar 1992b), it is not known if the temperature was elevated long enough to be lethal. Thus it is not yet clear whether elevated temperatures may be lethal to *H. depravata* in upper EFPC.

The cause or causes of impact to the benthic invertebrate community in upper EFPC remain unknown. Several possible causes of impact were suggested in the first report including siltation, elevated temperatures, altered flow regime, nutrient enrichment, and/or the presence of toxicants (e.g., chlorine, metals, organics) (Smith 1992a). However, the impact to the benthic community is most likely caused by a combination of several factors rather than any one thing. The moderately high densities of invertebrates in upper EFPC, particularly the chironomids, are indicative of enriched conditions, but the paucity of species also suggests the influence of additional factors. The dominance of organisms in upper EFPC that are capable of producing several generations per year suggests the possibility of the periodic release of a toxicant or the existence of sublethal concentrations

of toxicants. Unexposed adults and/or resistant eggs are most likely present throughout much of the year, which would allow rapid recolonization following a toxic excursion. Added to the possibility of periodic releases of toxicants or the occurrence of sublethal concentrations of toxicants is the occurrence of elevated temperatures. It has been estimated that, at a given concentration of a toxicant, survival of an organism is generally reduced by half with a temperature increase of 10°C (Hynes 1960). Results from studies on the distribution of *H. depravata* suggest the possible influence of siltation. Further studies are needed to elucidate the possible causal factors.

6.1.5 Summary and Conclusions

Results obtained during the second year of the BMAP show that the benthic macroinvertebrate community in upper EFPC continues to be impacted by effluents from the Y-12 Plant; however, the extent of the plant's influence is unknown as yet. Monitoring data through May 1987 showed no evidence of recovery in the benthic community in the upper reaches of EFPC, and results from instream clam studies during the summer and fall of 1988 suggested continued impact through this time period. Although its causes are as yet unidentified, the impact was most likely the result of a combination of several factors such as elevated temperatures, sublethal concentrations of a toxicant, periodic release of toxicants, siltation, and nutrient enrichment.

6.1.6 Future Studies

Beginning in the third year, sampling frequency for quantitative benthic invertebrate samples was reduced from monthly to quarterly. Qualitative samples will no longer be collected unless results from quantitative samples indicate a need for data supplementation. Instream studies with clams will continue, but procedures will be modified so that results can be analyzed statistically. As conditions improve in upper EFPC, more sensitive indicators of potentially sublethal toxic conditions will be incorporated into the clam studies (e.g., effects on reproduction). Laboratory and field studies to isolate the factors impacting the invertebrate community in upper EFPC will continue with caddisflies or other suitable taxa (e.g., temperature tolerance tests and instream studies on growth and mortality). Future analyses of the monitoring data will be streamlined and will focus on the more sensitive parameters (e.g., density, taxonomic richness, and EPT richness), while less sensitive parameters will be dropped (e.g., taxonomic diversity). Analyses will also be keyed more to longitudinal and between-year trends than to seasonal trends unless important seasonal changes are observed.

6.2 FISHES (*M. G. Ryon*)

6.2.1 Introduction

Fish population and community studies can be used to assess the ecological effects of water quality and/or habitat degradation. These studies offer several advantages over other indicators of environmental quality (see review by Karr et al. 1986) and are

especially relevant to assessment of the biotic integrity of EFPC. Fish communities, for example, include several trophic levels, and species that comprise the potential sport fishery in EFPC [e.g., bluegill (*Lepomis macrochirus*), redbreast sunfish (*L. auritus*), and largemouth bass (*Micropterus salmoides*)] are at or near the end of food chains. Consequently, they integrate the direct effects of water quality and habitat degradation on primary producers (periphyton) and consumers (benthic invertebrates) that are utilized for food. Because of these trophic interrelationships, the well-being of fish populations has often been used as an index of water quality (e.g., Weber 1973, Greeson et al. 1977, Karr et al. 1986). Moreover, statements about the condition of the fish community are better understood by the general public (Karr 1981).

The initial objectives of the instream fish monitoring task (Subtask 4b of the BMAP, as described in Loar et al. 1989) were (1) to characterize spatial and temporal patterns in the distribution and abundance of fishes in EFPC and (2) to document any effects on fish community structure and function resulting from implementation of the Water Pollution Control Program at the Y-12 Plant (Sect. 1).

6.2.2 Methods

6.2.2.1 Field sampling procedures

Quantitative sampling of the fish populations at six sites in EFPC and a reference stream, BF (Fig. 2-3), was conducted by electrofishing during four periods: October through December 1986, March 1987, October 1987, and March 1988. The resulting data were used to estimate population size (numbers and biomass per unit area), determine age structure and growth rates, and calculate Index of Biotic Integrity (IBI) values. Sampling reaches ranged from 100 to 128 m in mean length at all sites except EFK 24.4 where a much longer section (227 m) was needed to adequately determine that fish were not present. The reach sampled at EFK 23.4 was reduced in October 1987 from 116 m to 92 m as a result of extremely high fish densities. Fish sampling sites either overlapped or were within 100 m of the sites included in the instream benthic invertebrate monitoring task (Sect. 6.1), except for EFK 10.0, where benthos were sampled at EFK 10.9. Lengths of the sampling reaches were similar to those used in 1985 and 1986 except where the density of fish allowed a reduction in length (Table 2-8).

Qualitative sampling of EFPC watershed was done as part of the sampling efforts of other tasks and as part of a survey of fish on ORR (Ryon and Loar 1992). Areas sampled included stretches of EFPC between population sites, all tributaries, NHP, and downstream areas of EFPC near the confluence with Poplar Creek.

All stream sampling was conducted using one or two Smith-Root Model 15A backpack electrofishers, depending on stream size. Each unit includes a self-contained, gasoline-powered generator capable of delivering up to 1200 V of pulsed direct current. A pulse frequency of 90 to 120 Hz was used, and the output voltage was adjusted to the optimal value (generally 400 V or less) based on the specific conductance of the water. The circular (ring) electrode at the end of the fiberglass anode pole was fitted with a nylon net (0.64-cm mesh) so that the electrofisher operator could also collect stunned fish.

After a 0.64-cm-mesh seine was stretched across the upper and lower boundaries of the reach to restrict fish movement, a 2- to 7-person sampling team made three consecutive passes through the study reach in an upstream direction. If fish numbers

captured during the first pass were extremely low or zero, then only one pass was made. Depending upon the turbidity of the water, the consecutive passes could not always be made immediately. Rather, fish were processed after each pass to allow sufficient time for the water to clear before another pass was initiated. Stunned fish were collected and stored by pass in wire mesh cages (0.64-cm diam) or buckets with small holes during further sampling.

Fish were anesthetized with MS-222 (tricaine methanesulfonate), identified, measured to the nearest 0.1 cm (total length), and weighed using Pesola spring scales to the nearest 0.1 g (for fish <100 g) or gram (for fish >100 g). At sites with high fish densities, individuals were recorded by 1-cm size classes and species. If 25 individuals of a species-size class were measured and weighed, additional members of that size class were only measured. Length-weight regressions (SAS 1985b) were later used to estimate missing weight data. Other data recorded (if possible to determine) included sex, reproductive state, disposition (i.e., dead or kept for laboratory identification and reference collection), and presence of any abnormalities (e.g., external parasites, skeletal deformities).

After processing fish from all passes, the fish were allowed to fully recover from the anesthetic and returned to the stream. Any additional mortality occurring as a result of processing was noted at that time. In addition, conductivity, pH, water temperature, turbidity, dissolved oxygen, cloud cover, shocking time, and length, width, and depth of sample reach were recorded at each sampling site.

NHP was sampled using a Smith-Root Type IV, boat-mounted electrofisher with pulsed DC current and one boom. Since the purpose of the qualitative sampling was to detect additional species of fish not located in quantitative sampling, the time and effort expended varied, but at least 30 minutes of shocking time was performed for each sampling period. Captured specimens were identified in the boat, and only species new to the EFPC watershed or difficult to identify were preserved for laboratory identification and placement in the reference collection.

6.2.2.2 Population size analysis

Species population estimates were calculated using the method of Carle and Strub (1978). Biomass was estimated by multiplying the population estimate by the mean weight per individual. To calculate density and biomass per unit area, total numbers and biomass were divided by the surface area (expressed in square meters) of the study reach. For each sampling date, surface area was estimated by multiplying the length of the reach by the mean width based on measurements taken at 5-m intervals.

6.2.2.3 Growth and condition factor analysis

Condition factors (K) were calculated for individual fish by site and species using the formula:

$$K = 100 (\text{weight}/\text{length}^3) ,$$

with weight in grams and total length in centimeters (Hile 1936). Fish without measured weights were not used in calculations of condition factors. Comparisons of condition

factors between sites and between sampling periods were made using an ANOVA procedure (PROC GLM) on untransformed data (SAS 1985b). If the GLM procedure indicated significant differences in condition factors between groups, the Tukey test was performed to identify those groups that were significantly different.

Analysis of growth was done using PROC GLM and PROC MEANS procedures (SAS 1985a,b) with length, weight, and age data for redbreast and bluegill sunfish. Growth was analyzed by calculating the true growth; this is the instantaneous rate of increase in weight for the most recent year of growth in EFPC and the reference stream, BF. This analysis involves the following steps: (1) determine age from scales (see Sect. 6.2.2.4), and measure to successive annuli; (2) establish relation of scale size to fish size; (3) backcalculate the length at the start and close of the last complete year contained on the scale at each age for each fish; (4) calculate the functional slope (b) of the length-weight regression for each fish population; (5) take the natural logarithm of lengths determined in (3) for each fish and subtract (equals instantaneous rate of increase in length for each fish); and (6) average the instantaneous rates of increase for each age-group and multiply by b, which gives the instantaneous rate of increase in weight at each age (Ricker 1975). The formula for backcalculating length (Carlander 1981) was:

$$BCL = a + [(TL - a)/RL]AL,$$

where BCL = backcalculated length

a = intercept of body-scale regression,

TL = total length of fish at capture,

RL = scale measurement to edge of scale, and

AL = scale measurement to each annulus.

For these analyses, a common value of 20 mm was used for the constant a, as determined and recommended by Carlander (1982). The formula for the length-weight regression (Ricker 1975) was:

$$\log \text{ weight} = \log a + b(\log \text{ length})$$

and was determined using PROC GLM (SAS 1985b) to provide the slope value, b. Because calculation of the true growth rate restricts the growth evaluation to the most recent year of growth, problems with mortality biases are avoided (Ricker 1975, Gutreuter 1987). Statistical comparisons of true growth were made using the Tukey test.

6.2.2.4 Age determination

Scales were taken for age determination from target species [redbreast sunfish, bluegill, and rock bass, (*Ambloplites rupestris*)] collected during the fall 1986 and 1987 population surveys. Because of the low densities of these species at the population sites in EFPC, additional sampling above and/or below the site was conducted to get a larger sample size. Scale data for collected during the same periods from the same species in a reference stream (BF; see Sect. 2.3) were used for comparison.

Scales were taken from an area above the lateral line and slightly anterior to the insertion of the dorsal fin. Impressions of the scales were made using a Wildco scale press

and acetate slides. Enlarged images of the scales were projected onto a screen using an Bruning 4020 microfiche reader with a 16-mm lens. Where possible, at least 10 scales from each fish were mounted and compared. For actual measurements of annuli, the best representative scale was used. Scales identified as regenerated (latinucleate) and those that were damaged or highly irregular in shape were not read. In some cases, no age data were obtained because all scales were unsuitable. In this analysis, ages were determined by one person; questionable scales were reviewed by a second observer and used for age analysis only if a consensus could be reached.

The following data were recorded for each scale examined: number of annuli, total length of scale radius (distance from focus to anterior margin), and length of radius to various annuli. The annulus was determined by examining:

1. the intersection of the outermost margin of closely spaced (i.e., slow-growth) circuli with the innermost margin of widely spaced (i.e., rapid-growth) circuli;
2. the occurrence of cutting over of circuli at the lateral edges of the anterior field;
3. the increase in radii width or formation of holes in the radii; and
4. the termination or origin of radii.

As part of the age analysis, length-frequencies of the target species were constructed from all population surveys. Plots of number of individuals vs total length using 2-cm intervals were made. For the fall population surveys, the length-frequency plots were compared to the size range of fish of known age at that site, as determined by the scale analyses.

6.2.2.5 IBI analysis

The fish population data at each site were analyzed using the IBI, a fish community assessment which includes measures of species richness and composition, trophic composition, and fish abundance and condition (Karr 1981, Karr et al. 1986). As suggested by Karr and others (Karr et al. 1986, Ohio EPA 1988), modifications were made to the basic IBI metrics to reflect differences in the Clinch River System in the Oak Ridge area which includes EFPC (Table G-3). In addition to the IBI modified for the Clinch River in the Oak Ridge area, a modified IBI (Table G-4) developed by the Ohio EPA (1987; 1988) for use in headwater streams (drainage area less than 52 km²) was applied to the EFPC data. A detailed discussion of the IBI and the modifications made for the Clinch River version and the Ohio EPA headwater version are given in Appendix G.

6.2.3 Results

6.2.3.1 Species richness and composition

Quantitative surveys of EFPC and BF during the 4 sampling periods located a total of 30 and 23 species respectively (Table 6-7). The lowermost site on EFPC, EFK 6.3, had

Table 6-7. Fish species composition in East Fork Poplar Creek and the reference site, Brushy Fork, for the period November 1986–March 1988

Numerals indicate number of sampling periods ($n = 4$) that a given species was collected at a site;
X = species taken only during qualitative samples

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Petromyzontidae						
Chestnut lamprey (<i>Ichthyomyzon castaneus</i>)					1	
American brook lamprey (<i>Lampetra appendix</i>)						4
Clupeidae						
Gizzard shad (<i>Dorosoma cepedianum</i>)	1	1	1	1	2	x
Cyprinidae						
Bigeye chub (<i>Notropis amblops</i>)						4
Bluntnose minnow (<i>Pimephales notatus</i>)		3	2	4	4	
Blacknose dace (<i>Rhinichthys atratulus</i>)	4	4	4	4	1	4
Carp (<i>Cyprinus carpio</i>)	x	x	x		2	x
Creek chub (<i>Semotilus atromaculatus</i>)	2	4	4	3	x	2
Emerald shiner (<i>Notropis atherinoides</i>)		1		1	4	3
Golden shiner (<i>Notemigonus chrysoleucas</i>)				1		
Rosefin shiner (<i>Lythurus ardens</i>)		1	1			2
Spotfin shiner (<i>Cyprinella spilopteras</i>)						2
Striped shiner (<i>Luxilus chysoccephalus</i>)	4	4	4	4	4	4
Stoneroller (<i>Campostoma anomalum</i>)	4	4	4	4	4	4
Catostomidae						
Black redbhorse (<i>Moxostoma duquesnei</i>)					1	4
Golden redbhorse (<i>Moxostoma erythrurum</i>)			1		2	1
Northern hogsucker (<i>Hypentelium nigricans</i>)				4	3	4

Table 6-7 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Smallmouth buffalo (<i>Ictiobus bubalus</i>)					2	
Spotted sucker (<i>Minytrema melanops</i>)	1	1	1	1	4	3
White sucker (<i>Catostomus commersoni</i>)	4	3	2	2	1	4
Ictaluridae						
Yellow bullhead (<i>Ameiurus natalis</i>)	2	1	1	3	2	
Cyprinodontidae						
Black spotted topminnow (<i>Fundulus alivaceus</i>)						1
Poeciliidae						
Western mosquitofish (<i>Gambusia affinis</i>)	4	4	4	3	1	
Atherinidae						
Brook silverside (<i>Labidesthes sicculus</i>)			1		4	
Percichthyidae						
Striped bass (<i>Morone saxatilis</i>)					x	
Centrarchidae						
Bluegill (<i>Lepomis macrochirus</i>)	3	2		3	4	1
Green sunfish (<i>Lepomis cyanellus</i>)	x					
Longear sunfish (<i>Lepomis megalotis</i>)					x	
Redbreast sunfish (<i>Lepomis auritus</i>)	4	4	4	4	4	4
Redear sunfish (<i>Lepomis microlophus</i>)		x	x		x	
Warmouth (<i>Lepomis gulosus</i>)		1	2	3	3	
Rock bass (<i>Ambloplites rupestris</i>)		1	3	4	1	4
Largemouth bass (<i>Micropterus salmoides</i>)	2	1			1	x

Table 6-7 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Smallmouth bass (<i>Micropterus dolomieu</i>)					x	x
Spotted bass (<i>Micropterus punctulatus</i>)						x
Percidae						
Blueside darter (<i>Etheostoma jessiae</i>)						4
Greenside darter (<i>Etheostoma blenniodes</i>)						3
Logperch (<i>Percina caprodes</i>)					4	1
Redline darter (<i>Etheostoma rufilineatum</i>)						1
Stripetail darter (<i>Etheostoma kennicotti</i>)				2		
Snubnose darter (<i>Etheostoma simoterum</i>) ^a			1	4	2	4
Yellow perch (<i>Perca flavescens</i>)		x	x		1	
Sauger (<i>Stizostedion canadense</i>)					x ^b	
Sciaenidae						
Freshwater drum (<i>Aplodinotus grunniens</i>)					2	x
Cottidae						
Banded sculpin (<i>Cottus carolinae</i>)			4	4	4	4
Total species	14	20	21	20	33	30
Total species (without qualitative)	12	17	18	20	27	24

^aMay also include specimens of *Etheostoma duryi*, as these species cannot easily be separated in field.

^bSpecimen was taken downstream of EFK 6.3 during sampling for bioaccumulation task.

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

27 species including species moving up from reservoirs (e.g., smallmouth buffalo, *Ictiobus bubalus*) and downstream from small tributaries (e.g., blacknose dace, *Rhinichthys atratulus*). Intermediate sites on EFPC (EFK 10.0 to EFK 24.3) had 12 to 20 species, while the uppermost site, EFK 24.4, did not have any fish. NHP was surveyed during all four sampling periods, and only during the spring 1987 sampling period were any fish collected. Four large creek chub (*Semotilus atromaculatus*) and two hybrid sunfish (introduced for other experiments) were found on March 5, 1987.

Qualitative surveys of EFPC and BF added two to three species at most sites, with surveys at EFK 6.3 and BFK 7.6 revealing an additional six species. Several of these species were found during quantitative surveys of other EFPC site(s). Species found only during qualitative sampling were striped bass (*Morone saxatilis*), longear sunfish (*Lepomis megalotis*), green sunfish (*Lepomis cyanellus*), reardear sunfish (*Lepomis microlophus*), smallmouth bass (*Micropterus dolomieu*), spotted bass (*Micropterus punctulatus*), and sauger (*Stizostedion canadense*).

In general, the species richness increased as stream size increased (Table 6-7), with the reference stream BF having the highest mean richness (Table 6-8). This pattern indicates the influence of larger areas with more diverse habitat, greater exposure to species sources, and potentially better water quality at lower EFPC sites. In quantitative surveys of EFPC, 11 species were found that were not found in BF, and an additional 7 species were found in BF but not in EFPC. In general, species found only in EFPC were associated with reservoirs or were considered more tolerant to habitat and water quality degradation. Fish found only in BF were less tolerant stream species.

The downstream increase in species richness for EFPC during 1986–88 was similar to that seen in 1985–86 (Loar 1992b), except that EFK 6.3 had a higher total richness than BFK 7.6 in some 1986–88 samplings. Mean species richness increased at all EFPC sites between the two sampling phases, with an increase of between two to eight species. EFK 6.3 showed the most dramatic increase with eight additional species, including white sucker (*Catostomus commersoni*), smallmouth buffalo, golden redhorse (*Moxostoma erythrurum*), yellow perch (*Perca flavescens*), chestnut lamprey (*Ichthyomyzon castaneus*), and brook silverside (*Labidesthes sicculus*). EFK 18.2 increased in mean species richness from six to ten, adding the new species rosefin (*Lythurus ardens*) and emerald (*Notropis atherinoides*) shiners, spotted sucker (*Minytrema melanops*), and rock bass. The two species with the greatest increase in distribution in EFPC were the spotted sucker and the yellow bullhead (*Ameiurus natalis*). Interestingly, the mean species richness for BFK 7.6 decreased by two species between the earlier sampling phase of 1985–86 and the phase of November 1986 to March 1988.

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Table 6-8. Total fish density (expressed in individuals per 10 m²), total biomass (expressed in grams per 10 m²), and species richness for November 1986 to March 1988 in East Fork Poplar Creek, and the reference stream, Brushy Fork

Sampling periods	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
November-December 1986							
Density	0	45.94	9.42	3.86	3.75	2.26	2.22
Biomass	0	221.8	59.8	22.7	38.2	61.8	44.6
Richness	0	12	13	10	13	19	17
March 1987							
Density	0	29.62	21.82	5.18	5.32	1.55	6.64
Biomass	0	139.4	50.7	82.5	68.8	295.7 ^a	135.8
Richness	0	7	11	12	12	17	16
October 1987							
Density	0	32.45	14.01	5.47	5.41	2.40	9.42
Biomass	0	327.7	25.1	31.5	41.5	13.8	88.1
Richness	0	9	8	10	16	14	23
March 1988							
Density	0	33.61	14.13	7.20	12.47	2.58	9.91
Biomass	0	222.1	10.4	44.9	132.3	689.9 ^a	74.5
Richness	0	7	8	13	18	19	17
Mean for 1986-1988							
Density	0	35.41	14.85	5.43	6.74	2.20	7.05
Biomass	0	227.8	36.5	43.4	70.2	265.3 ^b	85.8
Richness	0	9	10	11	15	17	18
Mean for 1985-1986 ^c							
Density	0	3.40	6.80	1.80	4.00	1.00	6.60
Biomass	0	70.4	9.20	106.9 ^d	45.5	50.2 ^e	155.7
Richness	0	6	6	9	12	9	20

^aCarp and smallmouth buffalo were a large part of the biomass at this sample date.

^bBiomass excluding carp and smallmouth buffalo was only 50.3 g/10 m².

^cData taken from J. M. Loar et al., 1992. *First Report on the Y-12 Plant Biological Monitoring and Abatement Program*, Y/TS-886, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

^dBiomass excluding carp was only 47.0 g/10 m².

^eBiomass excluding carp was only 29.8 g/10 m².

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

species with the greatest increase in distribution in EFPC were the spotted sucker and the yellow bullhead (*Ameiurus natalis*). Interestingly, the mean species richness for BFK 7.6 decreased by two species between the earlier sampling phase of 1985–86 and the phase of November 1986 to March 1988.

6.2.3.2 Density and biomass

The population surveys of EFPC conducted during the four sampling periods from November 1986 to March 1988 provided data to determine species biomass and density values for each period. The total biomass and densities at each site for each sampling period are given in Table 6-8. Individual species values are given in Appendix H, Tables H-1–H-4.

Densities in EFPC decrease in inverse proportion to stream size for each sampling period. The only deviation from this pattern is a density increase from EFK 13.8 to EFK 10.0. Densities at BFK 7.6 are generally higher than all EFPC sites except EFK 23.4 and EFK 18.2. This pattern differed somewhat from that found in the 1985–86 sampling (Loar 1992b). In 1985–86, peaks in density occurred at EFK 18.2 and EFK 10.0, with the highest values at EFK 18.2. Also, BFK 7.6 showed densities that were the highest or next highest in comparison with EFPC sites. In 1986–88, the highest densities were at EFK 23.4, and BFK 7.6 had only the third highest densities. The greatest shift in the pattern of densities between the two sampling phases was the increased density at EFK 23.4 during 1986–88.

A seasonal pattern in densities was not apparent at any site. The fall samples did not demonstrate a higher density, which would normally be expected based on recruitment of young-of-the-year fish. This again varied from the pattern shown in 1985–86. The lower sites on EFPC (EFK 13.8 to EFK 6.3) and BFK 7.6 did show a trend toward increasing densities over the four sampling periods. Two situations that had the potential to affect density values occurred during the 1986–88 sampling. In the EFK 23.4 area, large fish kills occurred in November 1986 and July 1987 (see Sect. 6.2.4). Both kills involved mainly stonerollers (*Camptostoma anomalum*), which constituted ~98% of the kill. However, the routine population surveys following the kills did not indicate any significant decline in stoneroller populations, either in total numbers or percentage of the overall fish populations (Ryon et al., unpublished data). The other situation occurred in EFK 13.8 area and involved a manhole on the City of Oak Ridge sewer line located adjacent to EFPC. Beginning in March 1987, raw sewage was frequently observed overflowing from the manhole and entering the sampling reach during and after periods of precipitation. A ditch from the manhole to EFPC contained sewage fungus, indicating a potential chronic problem. However, the population survey data did not suggest the occurrence of a significant impact. Density and species richness were not entirely synchronous with downstream trends, but the number of intolerant species [e.g., banded sculpin (*Cottus carolinae*) and snubnose darter (*Etheostoma simoterum*)] had increased since spring 1987.

In comparison with the first year, there was a substantial increase in density difference during 1986–88. Mean densities at all EFPC sites were greater by a range of 2- to 10-fold. Some of this increase could be attributed to the addition of new species at sites in EFPC, but the increase of individual species densities was also an important factor (Appendix H). The trend for the upstream sites on EFPC demonstrated the greatest increases, with the 10-fold increase occurring at EFK 23.4.

Individual species density showed an increasing trend for the striped shiner (*Luxilus chrysocephalus*), stoneroller, bluntnose minnow (*Pimephales notatus*), and banded sculpin from November 1986 to March 1988. The species that occurred at the highest density at most sites in EFPC was the stoneroller. This was followed by the striped shiner, bluntnose minnow, and western mosquitofish (*Gambusia affinis*). In BF, the dominant species by density were the banded sculpin and snubnose darter. This validates the apparent trend for EFPC to contain less sensitive species, because three of the four most dominant species are considered tolerant species in the IBI (see Appendix G). The biomass data did not follow a pattern related to stream distance in the EFPC sampling for 1986–88. The greatest mean biomass for the sampling periods occurred at EFK 23.4 (Table 6-8), with EFK 6.3 having a high biomass when smallmouth buffalo or carp (*Cyprinus carpio*) were captured in the sample. The site with the lowest biomass varied, but EFK 18.2 had the lowest mean biomass. Biomass at BFK 7.6 was generally close to the middle of the range for the EFPC sites, never greater or lower than any EFPC site.

The lack of a pattern for biomass in 1986–88 differed from earlier sampling in 1985–86. During that period, high biomass values were correlated with low densities and BF had a higher mean biomass than all EFPC sites. Another change that was apparent from the first sampling phase was a general increase in biomass values at most sites. Mean biomass increased from 1.5 to 5.3 times at all EFPC sites except EFK 13.8.

In the 1986–88 sampling, the mean biomass at EFK 13.8 and BFK 7.6 was about half that of the earlier sampling. The decline in biomass in BF can be traced to major reductions in the biomass of redhorse, other sucker species, rock bass, and redbreast sunfish. These reductions may be related to drought; EFPC sites would not be affected due to flow augmentation and larger species would tend to remain further downstream in BF during drought conditions. The primary effect of the drought is thought to be smaller pool areas without a decrease in water quality, because other sensitive species (e.g., darters) that occur in riffles showed increased biomass. The lower biomass at EFK 13.8 reflected decreased biomass from redbreast sunfish and the absence of carp during the 1986–88 sampling. When compared to the 1985–86 mean biomass (excluding carp), the biomass values at EFK 13.8 are very similar between the sampling phases (Table 6-8).

The stoneroller and the redbreast sunfish most frequently contributed the dominant share of the biomass at EFPC sites. Six other species were also dominant at EFPC sites, including species with large size and few numbers (e.g., smallmouth buffalo at EFK 6.3) and species with small size and large numbers (e.g., western mosquitofish at EFK 18.2). In BF, the dominant biomass species during all sampling periods was the black redhorse (*Moxostoma duquesnei*), an intolerant species rarely seen in EFPC.

The species that contributed the most biomass during the 1986–88 period were quite different from those in the 1985–86 sampling. In the earlier sampling phase, redbreast sunfish was the species contributing the most or next highest biomass in 15 of 40 possible sample date-site combinations. The next most frequent species were the striped shiner (6) and bluegill sunfish (4). By the 1986–88 sampling phase, stonerollers were the species contributing the most or next highest biomass in 12 of 40 possible sample date-site combinations. The next most frequent species were the striped shiner (9) and redbreast sunfish (7). In addition to the frequency shifts in species biomass, the mean biomass of these primary contributors also changed. The mean biomass of redbreast sunfish when it was a dominant contributor changed from 15.8 g/10 m² in 1985–86 to 11.0 g/10 m² in 1986–88. In contrast, the mean biomass of striped shiners changed from 8.2 g/10 m² to

33.0 g/10 m². This shift in species biomass dominance was especially evident at EFK 23.4, which went from a site dominated by redbreast and bluegill sunfish to a site dominated by stonerollers and striped shiners. The cause of this shift was not readily apparent but may have been related to a change in food availability as a result of a general enrichment of EFPC, especially below NHP.

6.2.3.3 Age, growth, and condition

Growth and condition of the EFPC watershed fish were analyzed by calculating condition factors for all species and by estimating growth rates for redbreast sunfish and bluegill. Although data were analyzed for growth of rock bass, these data are not presented because of low numbers of specimens at some sites, and the lack of rock bass at most sites made comparisons of little value. The age structure was determined by examining scales and length-frequencies for each sample period. Although data were analyzed for age structure of rock bass and bluegill, these data are also not presented because of low sample numbers.

Analysis of the growth of redbreast sunfish for 1986 indicated a stronger initial growth (age class 2+) at EFK 23.4 (Fig. 6-20 and Table H-5). The trend continued for that age class in the next year of growth, age class 3+ in 1987 (Fig. 6-21 and Table H-6). Statistical comparisons confirmed that growth of 2+ redbreast at EFK 23.4 in 1986 was significantly greater than at EFK 10.0, EFK 13.8, and BFK 7.6 (Table H-7). However, the 3+ redbreast growth in 1987 was not statistically significantly different. The other sites and age classes did not demonstrate any consistent significant differences in growth. The growth analyses of 1986 and 1987 bluegill (Tables H-8 and H-9 and Figs. 6-22 and 6-23) indicated similar growth patterns at all sites considering the sample size. A consistent pattern of statistically significant differences in growth was not seen in 1986 or 1987 (Table H-10).

Condition factors were calculated for the fish in EFPC and the reference stream, BFK 7.6, and compared for differences between sites and between sampling periods. Comparisons between sampling periods for each site and species showed that the condition factors for the March 1987 and 1988 periods were significantly greater than for the November–December 1986 or October 1987 periods. Of 24 comparisons where a significant difference was indicated, the spring period was the highest in 16 comparisons and the fall period was highest only once. This trend was also seen in 1985–86 (Loar 1992b) and indicates the expected preparation for spawning. Neither spring period was consistently higher than the other.

Comparisons between sites within a sampling period did not in general show any pattern of significant differences (Appendix H, Tables H-11 and H-12). Sites that have been identified as impacted by the biomass, density, and/or species richness data (EFK 23.4 and EFK 18.2) do not show significantly reduced condition factors for any species. In fact, individuals in these areas often have high condition factors, suggesting that they are transient individuals strong enough to traverse or remain in less than optimal areas (e.g., the creek chubs taken in NHP).

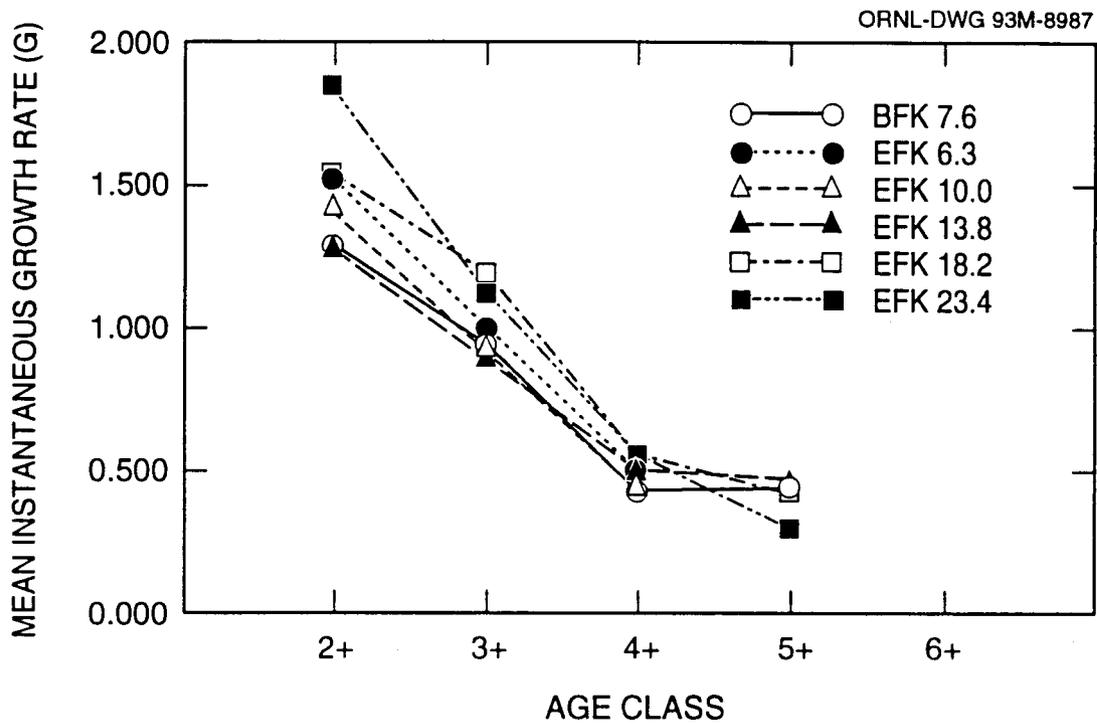


Fig. 6-20. True rate of growth in weight (in grams) of redbreast sunfish in East Fork Poplar Creek and a reference stream, Brushy Fork, during 1986. BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

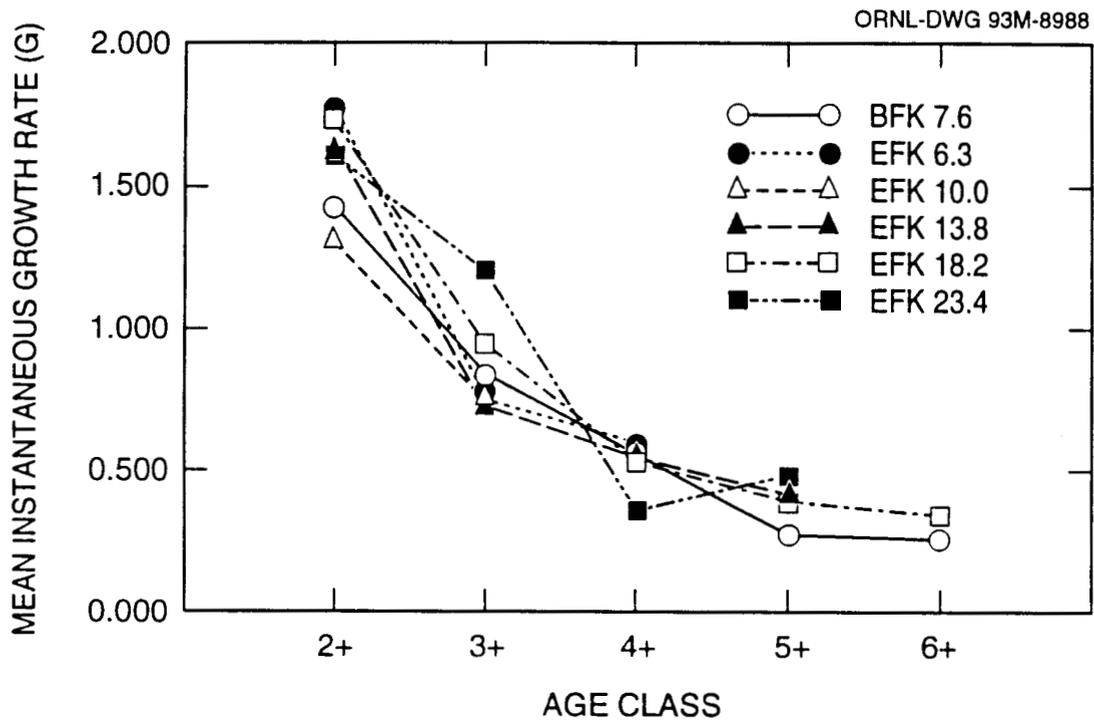


Fig. 6-21. True rate of growth in weight (in grams) of redbreast sunfish in East Fork Poplar Creek and a reference stream, Brushy Fork, during 1987. BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

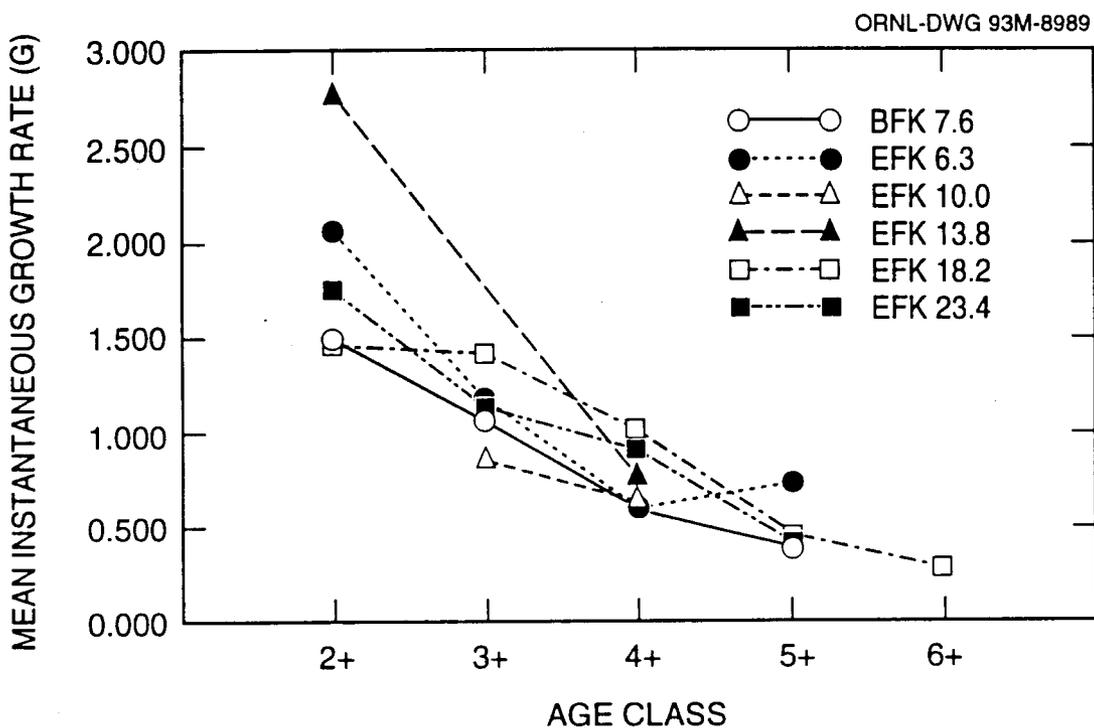


Fig. 6-22. True rate of growth in weight (in grams) of bluegill in East Fork Poplar Creek and a reference stream, Brushy Fork, during 1986. BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

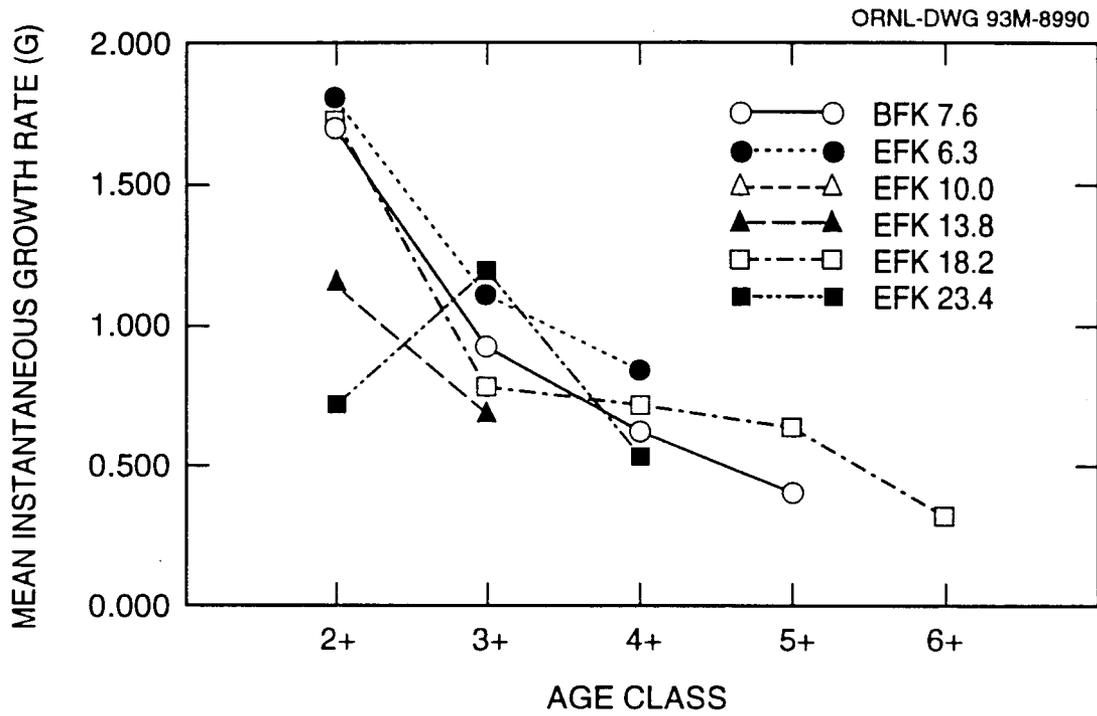


Fig. 6-23. True rate of growth in weight (in grams) of bluegill in East Fork Poplar Creek and a reference stream, Brushy Fork, during 1987. BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

The age structure of redbreast sunfish at EFPC sites indicated some differences between sites and between the four sampling periods from fall 1986 to spring 1988 (Figs. 6-24, 6-25 and 6-26). The total number of redbreast taken in the population surveys was plotted by total length in 2-cm size classes. These plots were compared with the age-class determinations made from the scale analysis data used in the growth interpretation. At EFK 13.8, EFK 10.0, and EFK 6.3, the age structure indicated similar-sized 0+ age class (mostly 2–4 cm in length) and the presence of individuals in most size classes over all four sampling periods (Figs. 6-25 and 6-26). At EFK 23.4, the presence of individuals at all sizes in all sampling periods was evident (Fig. 6-24). However, the 0+ age class was skewed upward toward 4–6 cm in length. The size difference between the sites may be an indication of early spawning at EFK 23.4 or may suggest that no spawning occurs within the population reach, and the 0+ age class present at the site represents migrations from downstream. The age structure at EFK 18.2 fits the expected patterns but shows a steady decline in numbers in each age class over the four sampling periods (Fig. 6-24). This general decline suggests a causative factor that is affecting all classes equally and not just the most recent reproductive effort. The low population numbers at BF make comparisons of any patterns in EFPC vs the reference site very speculative.

6.2.3.4 IBI analysis

An IBI modified for the Clinch River area (including EFPC) was applied to the population data collected from June 1985 to March 1988. This includes data first presented in Loar (1992b). The IBI modification (Ohio EPA 1987; 1988) for headwater systems (watershed areas < 52 km²) was also applied to the data for comparison.

The IBI values for EFPC (Table 6-9) indicate that the fish communities were in very poor to poor standing. The reference community at BF was in better shape with a fair rating. These ratings reflect the decline in the species composition of the Clinch River fauna from historical records. As might be expected, there was a trend for higher values downstream from the Y-12 Plant (Fig. 6-27). EFK 23.4 and EFK 18.2 consistently rated as very poor communities with a gradual change downstream to EFK 6.3, which was consistently rated as merely a poor community. However, as indicated by the rating of only fair for the reference site on BF, the decline in EFPC was part of a general historical trend and was not solely attributable to discharges from the Y-12 Plant.

Changes in the IBI values from 1985 to 1988 showed a slight improvement in the fish communities (Fig. 6-27). IBI values for EFK 23.4 and EFK 18.2 improved from ratings of very poor to bordering on poor, primarily due to a significant increase in densities. At EFK 13.8 and EFK 6.3 the ratings improved from very poor to poor with a steady, gradual increase in the numerical rating at each site. EFK 10.0 showed much greater fluctuation; ratings of very poor to almost fair and then back to poor occurred within one sampling period. The changes in ratings at the lower sites were a result of improvements in numbers of darter species, intolerant species, and benthic insectivores.

This general but slight improvement in the total IBI score masked some greater improvements in individual metrics. The number of darter species, the occurrence of intolerant species, and the number of lithophilic spawners all increased at EFK 6.3 and EFK 13.8 from 1985 to 1988. The total number of species also increased at EFK 6.3

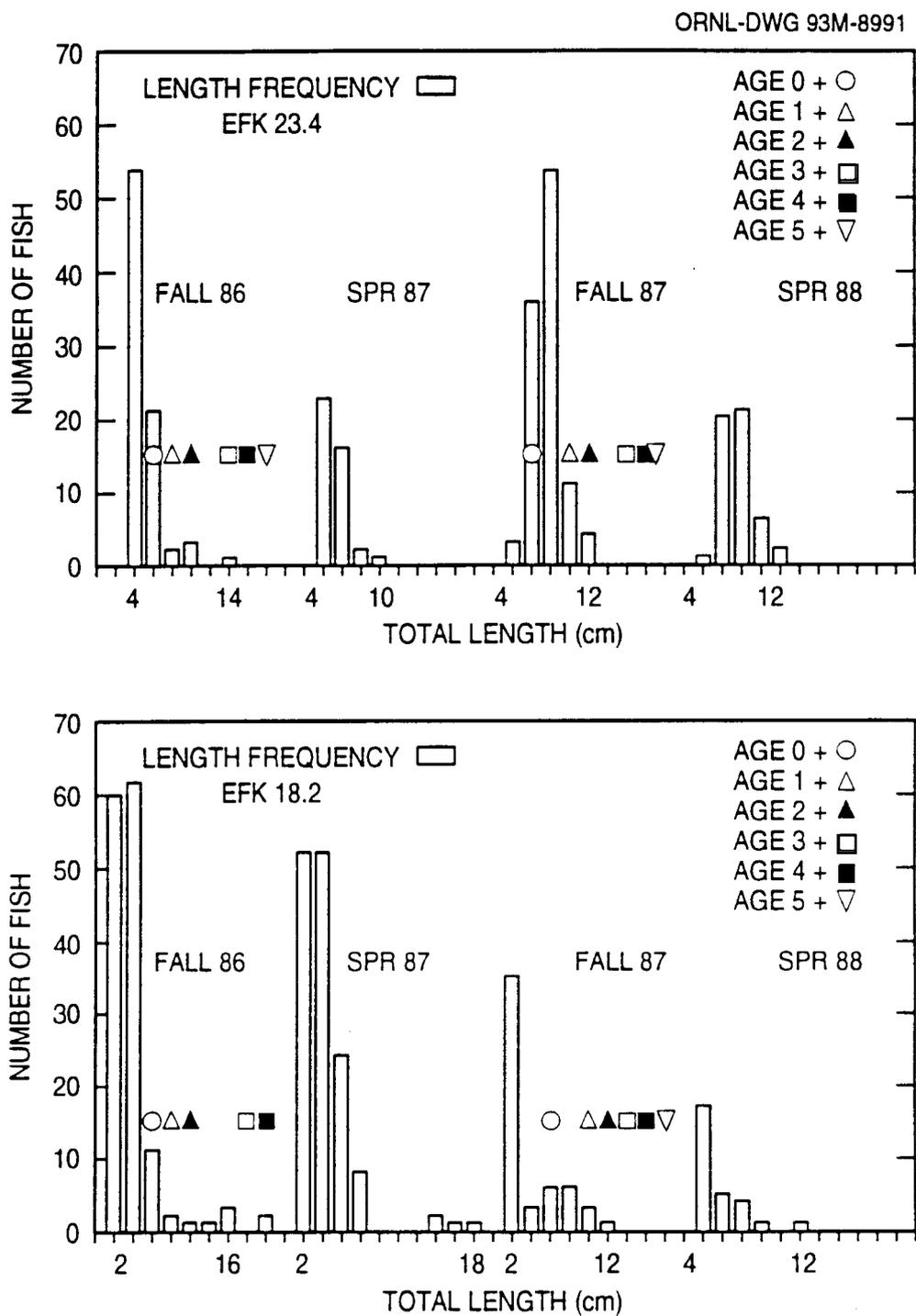


Fig. 6-24. Length frequency, total length in 2-cm size classes, of redbreast sunfish taken in population surveys of East Fork Poplar Creek at EFK 23.4 and EFK 18.2, for October 1986, March 1987, October 1987, and March 1988. Mean size of age classes for the fall samples were determined from scale analysis data. EFK = East Fork Poplar Creek kilometer.

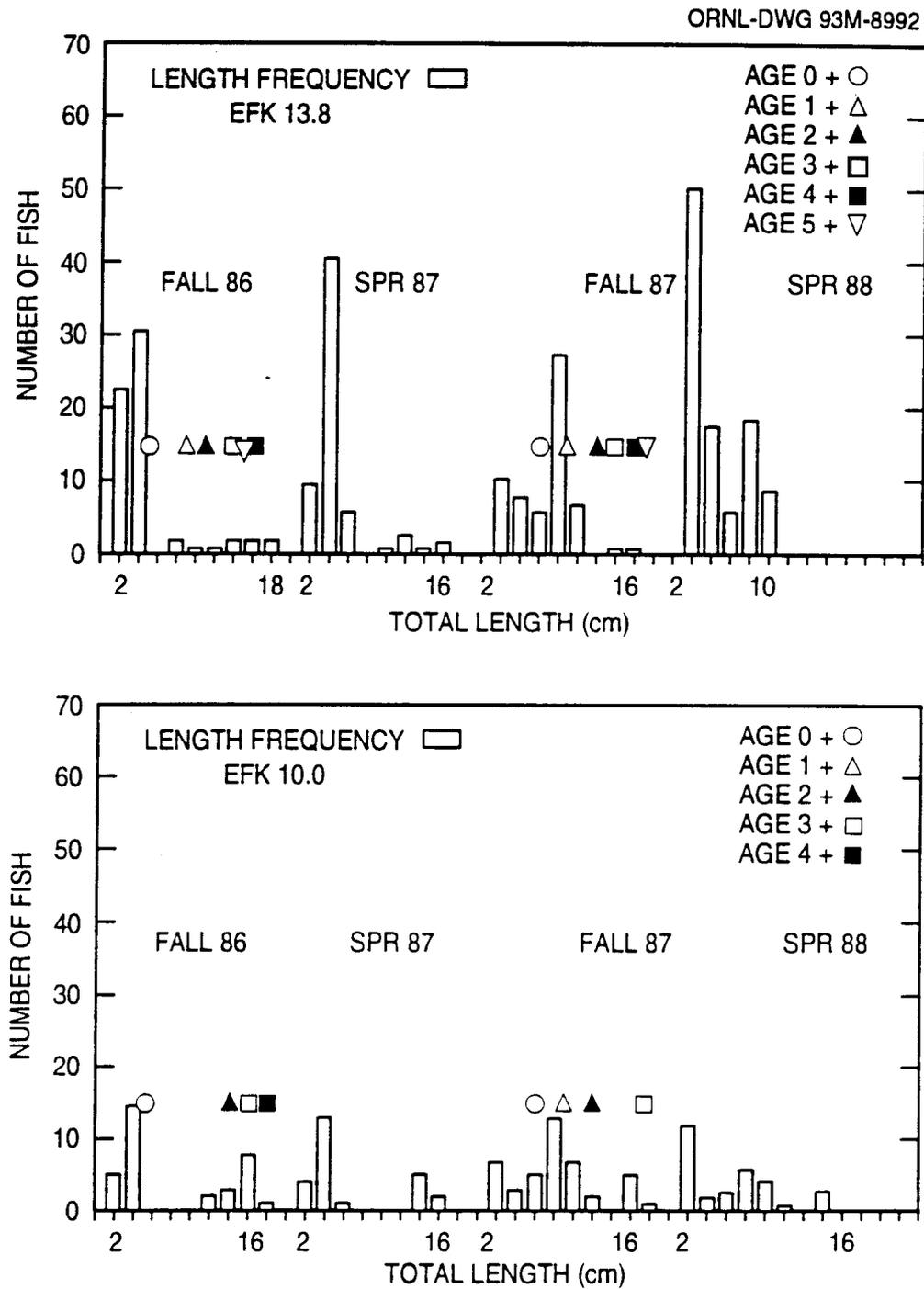


Fig. 6-25. Length frequency, total length in 2-cm size classes, of redbreast sunfish taken in population surveys of East Fork Poplar Creek at EFK 13.8 and EFK 10.0, for October 1986, March 1987, October 1987, and March 1988. Mean size of age classes for the fall samples were determined from scale analysis data. EFK = East Fork Poplar Creek kilometer.

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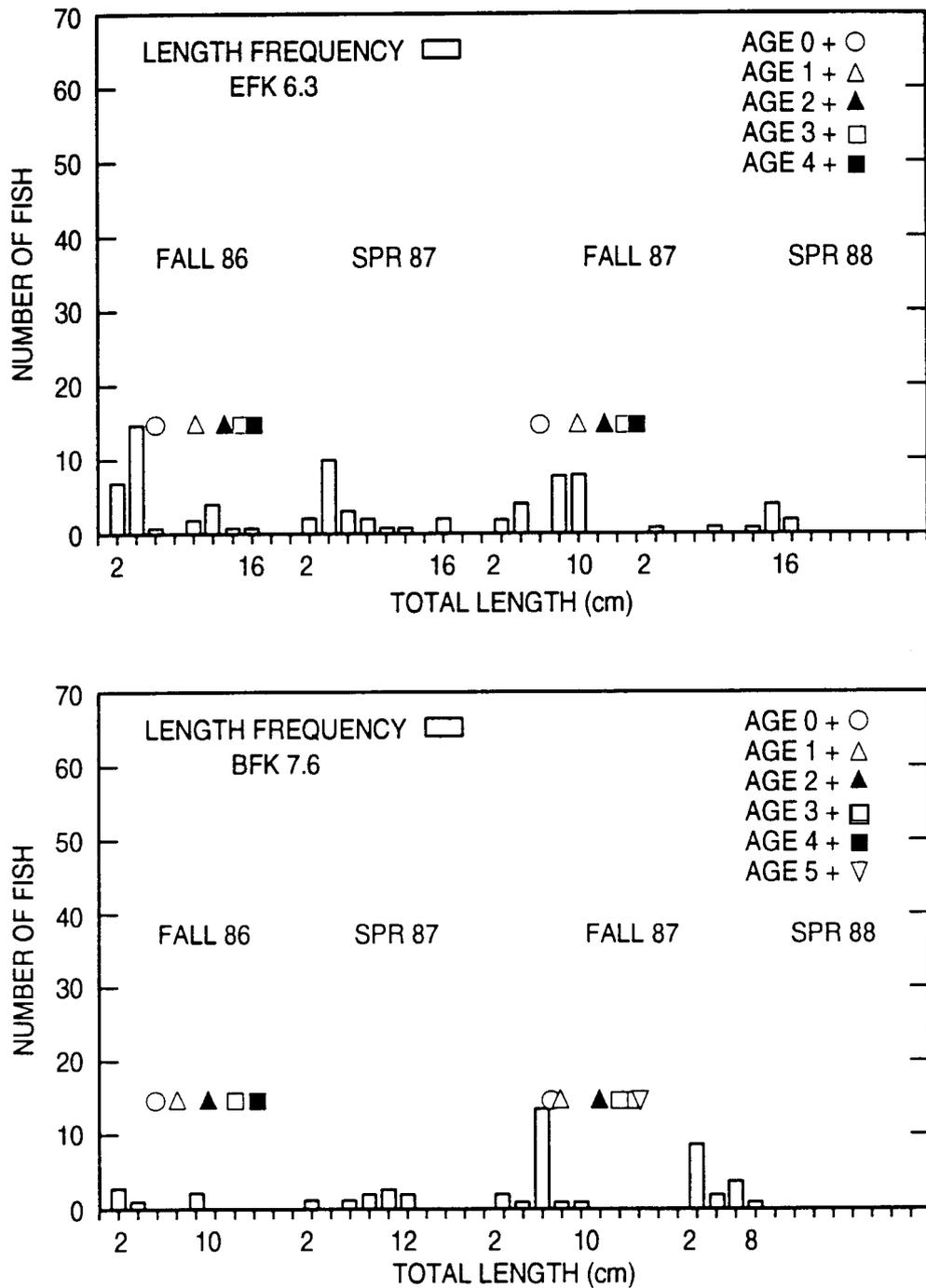


Fig. 6-26. Length frequency, total length in 2-cm size classes, of redbreast sunfish taken in population surveys of East Fork Poplar Creek at EFK 6.3 and a reference stream, Brushy Fork at BFK 7.6, for October 1986, March 1987, October 1987, and March 1988. Mean size of age classes for the fall samples were determined from scale analysis data. EFK = East Fork Poplar Creek kilometer, BFK = Brushy Fork kilometer.

Table 6-9. Index of Biotic Integrity (IBI) Ratings^a of East Fork Poplar Creek and Brushy Fork for sampling periods from June 1985 to March 1988 using methodologies developed for the Clinch River area and for headwater^b streams

Sampling period	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Spring 1985						
CR IBI ^c	20 VP	26 VP-P	22 VP	30 P	24 VP-P	NS ^d
HW IBI ^c	38 P-F	32 P	36 P-F	40 F	32 P	NS
Fall 1985						
CR IBI	22 VP	20 VP	22 VP	26 VP-P	28 P	42 F
HW IBI	32 P	30 P	34 P	38 P-F	40 F	52 G
Spring 1986						
CR IBI	20 VP	20 VP	26 VP-P	36 P-F	28 P	36 F
HW IBI	42 F	30 P	38 P-F	50 G	40 F	52 G
Summer 1986						
CR IBI	22 VP	22 VP	26 VP-P	28 P	26 VP-P	36 P-F
HW IBI	34 P	36 P-F	38 P-F	38 P-F	40 F	54 G-E
Fall 1986						
CR IBI	28 P	26 VP-P	22 VP	24 VP-P	28 P	40 F
HW IBI	48 G	38 P-F	36 P-F	44 F	46 F-G	52 G
Spring 1987						
CR IBI	26 VP-P	26 VP-P	28 P	28 P	30 P	40 F
HW IBI	44 F	38 P-F	40 F	40 F	38 P-F	52 G
Fall 1987						
CR IBI	26 VP-P	22 VP	24 VP-P	28 P	32 P	42 F
HW IBI	44 F	38 P-F	38 P-F	44 F	40 F	54 G-E

Table 6-9 (continued)

Sampling period	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Spring 1988						
CR IBI	26 VP-P	24 VP-P	28 P	28 P	32 P	42 F
HW IBI	44 F	36 P-F	42 F	50 G	36 P-F	52 G
Mean Values						
CR IBI	23.8 VP-P	23.3 VP-P	24.8 VP-P	28.5 P	28.5 P	39.7 F
HW IBI	40.8 F	34.8 P-F	37.8 P-F	36.9 P-F	39.0 P-F	52.6 G-E

^aNumerical values are on a scale from 12 to 60 with characterization as follows, very poor 12–22 (VP), poor 28–34 (P), fair 40–44 (F), good 48–52 (G), and excellent 58–60 (E).

^bBased on guidelines given in Ohio EPA (Environmental Protection Agency), *Biological Criteria for the Protection of Aquatic Life: Volume III, Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Microinvertebrate Communities*, Ohio Environmental Protection Agency, Division of Water Quality Monitoring and Assessment, Columbus, Ohio, 1987; and *Biological Criteria for the Protection of Aquatic Life: Volume II, Users' Manual for Biological Field Assessment of Ohio Surface Streams*, Ohio Environmental Protection Agency, Division of Water Quality Monitoring and Assessment, Columbus, Ohio, 1988.

^cCR = Clinch River; HW = headwater; EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

^dNS = not sampled.

and EFK 10.0, suggesting a gradual improvement in EFPC starting at the lower sampling sites. Upstream improvements that were incorporated into the IBI were limited to increases in density.

The headwater IBI values for EFPC gave higher ratings for most sites. The general evaluation rated EFPC fish communities as poor to fair with occasional good ratings (Table 6-9). The reference site also rated higher, from good to excellent. The significant difference between the two IBI methods in the patterns for EFPC was the high rating (poor-fair to good) for EFK 23.4. This indication of quality at the upper site was a result of the scaling of the headwater IBI to watershed area. EFK 23.4 has a very small watershed, resulting in low ranges for metric levels. However, EFPC is not a typical stream due to the significant flow augmentation from the Y-12 Plant. The added flow allowed much larger fish communities at the sites than would be normal for their watershed areas, especially at EFK 23.4. The added flow was compensated for in the Clinch River IBI by taking a combination of discharge and watershed area for scaling the metric values. Since the headwater IBI was based on reference values obtained by the Ohio EPA (1987; 1988), a similar compensation was not possible. The two IBI methods also differ due to the fact of greater variation in site ratings in the headwater IBI. This IBI was more sensitive to small changes in fish community structure and reveals smaller

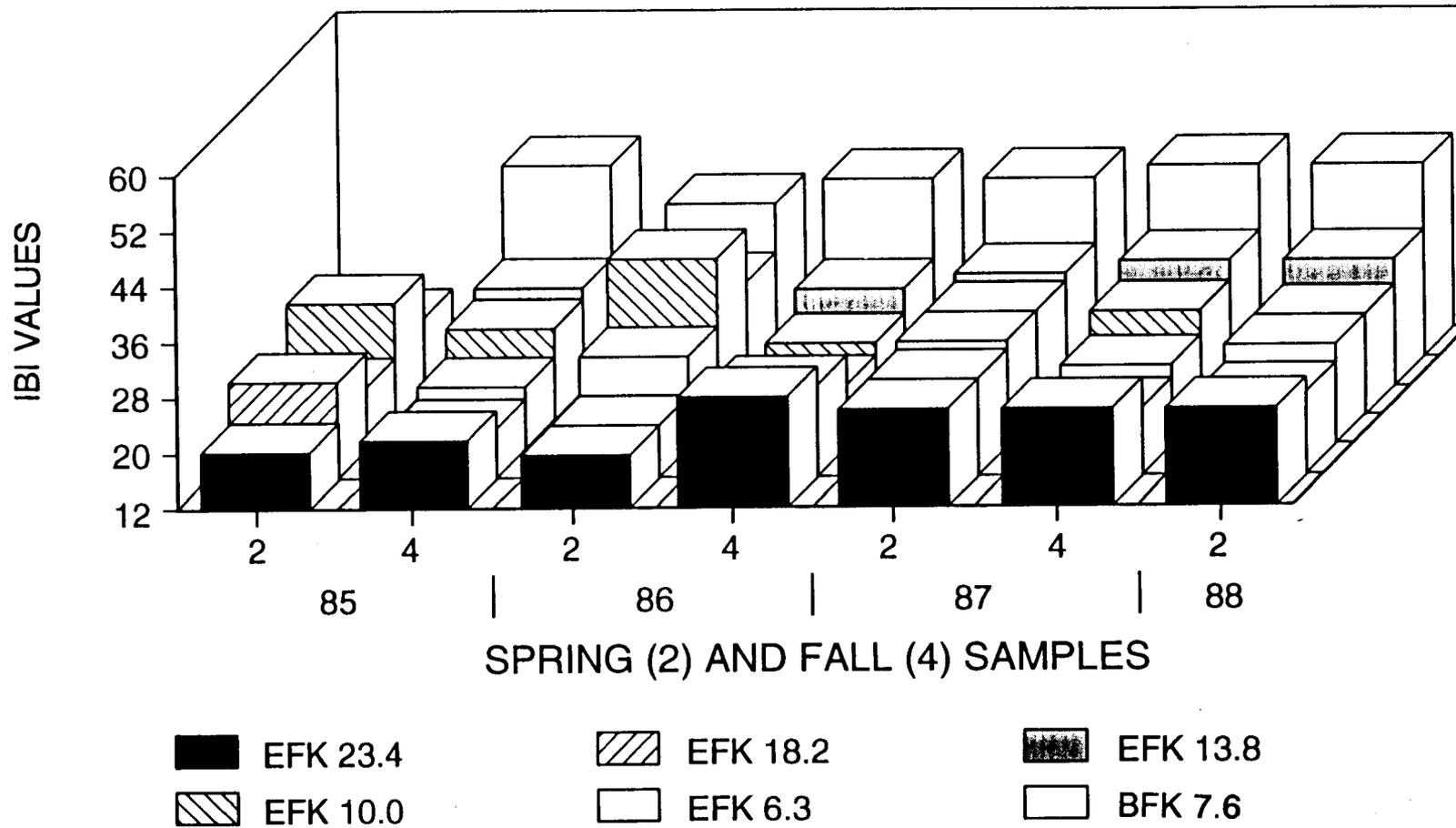


Fig. 6-27. Index of Biotic Integrity (IBI) values for fish communities at sites in East Fork Poplar Creek and a reference stream, Brushy Fork, for the sampling period of 1985 to 1988. IBI values range from a minimum of 12 to a maximum of 60.

changes in the overall rating rather than masking them as the Clinch River IBI does. Thus variation from poor-fair to good ratings occurred more frequently in the headwater IBI analysis (e.g., at EFK 13.8, Table 6-9).

6.2.4 Fish Kills

During the 1986–88 period, two reportable incidents of fish kills occurred in the EFPC watershed below the outfall of NHP. They were similar in nature and caused by several factors.

The first incident was observed on November 21, 1986, and proceeded through December 15, 1986. The kill was limited to a 0.5 km stretch immediately below NHP outfall and was restricted primarily to stonerollers. A total of 1148 dead or dying fish were found (all but two were stonerollers), most with symptoms of hemorrhaging from gills, anus, and/or fins. Ninety percent of the fish died within the first two days; and, throughout the fish kill period, live fish were observed in the area. Examination of the affected fish indicated the cause of death to be bacterial hemorrhagic septicemia (BHS), a stress-mediated disease, caused by the organism, *Aeromonas hydrophila*. Based on water quality data, patterns of collecting activities in the area, and Y-12 Plant operations, possible stressors included electrofishing activity, elevated levels of chlorine and mercury (associated with sewer cleaning at the Y-12 Plant), and/or changes in environmental conditions in NHP that promoted growth of the bacteria.

A second fish kill occurred in July 1987, again limited to the area below NHP and involving mostly stonerollers. A total of 747 dead fish (97.6% stonerollers) were collected, with 96% collected during the first two weeks. The symptoms were the same as in the November 1986 fish kill and BHS was again the cause of death. Examination of possible causes again suggested elevated mercury and/or chlorine levels, electrofishing, or cumulative stress.

The possible effects of the fish kills on the population site below NHP (EFK 23.4) are discussed in the text, and a complete, detailed account of the events and investigations following the episodes are given in Ryon et al. 1990.

6.2.5 Conclusions

This report represents the third and fourth years of studies designed to characterize the fish populations of EFPC; and, although some conclusions are still preliminary in nature, much has been established regarding patterns in EFPC. Although the data gathered in 1986–88 support some trends first observed in 1985–86 (Loar 1992b), there were also many significant differences.

One general factor that influenced the 1986–88 data was the drought conditions present during the sampling period. Rainfall for 1986 and 1987 was substantially lower than in past seasons (see Sect. 2). This manifested itself in smaller sample volumes and compressed populations at some sites. Effects of the drought were more evident in BF than in EFPC; for example, there was a decrease in mean species richness and biomass in BF between the 1985–86 sampling and the 1986–88 sampling. The flow augmentation to EFPC from the Y-12 Plant moderated much of the impact of the drought on EFPC.

The data collected during 1986–88 indicate that EFPC was in poor, stressed condition, although some recovery was suggested. Species richness increased in EFPC, and at the lower sites was comparable to the reference site, BF. However, in comparison to historical data, the richness was still depressed. Throughout the 1986–88 period, and particularly since the first sampling phase of 1985–86, the mean density and biomass values of EFPC increased steadily. The density values in particular were much improved, even in comparison with BF. Growth and condition factor analyses did not demonstrate any significant trends between EFPC and BF. Beyond these data, the general health of EFPC was not as positive. The Clinch River IBI analysis indicated that EFPC was composed of very poor to poor communities, while BF rated significantly better with a consistent index of fair. Individual IBI metrics reinforced this conclusion; for example, EFPC was dominated by tolerant species while BF was dominated by more sensitive species. Improvement in the IBI ratings has been slow although steady since the initial surveys in 1985.

In more specific terms, improvement and recovery were indicated at some EFPC sites although offset by a noticeable decline or no change at other sites. At EFK 24.4 no change was detected during the 1986–88 sampling. The total absence of fish may have been due in part to elevated temperatures (see Sect. 2), chlorine discharges from the Y-12 Plant (Loar 1992b), or isolation from lower EFPC by NHP. The effects in this section of EFPC should be more easily evaluated in future monitoring following the closure of NHP in late 1988. A new East Fork Basin (Lake Reality) was opened as NHP was closed, and the levels of the inlet and outlet culverts should allow colonization by strong swimmers from lower EFPC. The presence of fish in NHP on March 5, 1987, prior to closing, suggests that some fish may be able to survive the water conditions in the EFK 24.4 area.

Data for EFK 23.4 during 1986–88 reflected significant changes from that gathered in the 1985–86 sampling period, but the effects of discharges from the Y-12 Plant were still apparent. The mean species richness improved slightly from the level indicated in the 1985–86 sampling, but continuation of the trend through individual sampling dates in 1986–88 was not apparent. The richness levels were still far below the expected level for a stream with the amount of water that is available at EFK 23.4. The most significant change was a tremendous increase in mean density and biomass values. The levels measured at EFK 23.4 were among the highest seen in area sampling (Ryon, unpublished data) and were particularly impressive considering the stresses applied to the site. The major components of the increased biomass and density were stonerollers and striped shiners. These species dominated the 1986–88 samples and represented a complete shift from the redbreast and bluegill sunfish that dominated the 1985–86 sampling. The shift in dominance was particularly impressive because the stoneroller population was twice stricken by large losses during fish kills in November 1986 and July 1987. After both kills, neither the overall ratio of stonerollers to the community nor the absolute density of stonerollers decreased. Evaluation of EFK 23.4 by the IBI reemphasized the degraded conditions. Ratings of very poor improved only slightly to very poor bordering on poor, despite the large increases in density. The lack of intolerant species and darter species and the presence of large numbers of tolerant species indicated that the community was occupied only by species strong enough to exist under stressful conditions. The source of the stress may have been elevated temperatures (see Sect. 2), altered habitat, or some chronic effluent effects. The tremendous increase in biomass and densities of a herbivore

(the stoneroller) and a generalist feeder (the striped shiner) suggest that a significant enrichment was occurring at the site.

At EFK 18.2 the situation appeared to be declining or at best remained in very poor condition without improvement. In comparison with data from the 1985–86 sampling, the mean species richness, density, and biomass in the 1986–88 sampling increased. The increase may actually reflect the depressed mean values of 1985–86, which resulted from a fish kill rather than a substantial improvement of the conditions at EFK 18.2. During the 1986–88 sampling, there was a steady decline in the species richness and biomass over the four sampling periods. The biomass values were the lowest of any on EFPC. The density values did not show a trend. The degraded conditions at EFK 18.2 were also reflected by the IBI analysis, which rated the community as very poor bordering on poor, nor did this rating improve during the 1985–88 sampling. Individual metrics suggest that the conditions must have declined for less tolerant species, as none were taken at the site since the fall of 1986. Moreover, the site was dominated by mosquitofish and striped shiner (two tolerant species), which accounted for more than 80% of the population density. The decline was further reflected in the age structure data for redbreast sunfish, which demonstrated decreasing numbers in all size classes over the four sampling periods. The general decline at EFK 18.2 can be traced in part to the steady siltation occurring in the sampling reach. Data collected on depths during the 1986–88 period showed an approximate 50% reduction of the mean water depth in the last two samples. Also, personal observations of the topography of the site over the period have noted extensions of a large, shifting, gravel and sediment bar within the site, with sediment deposits filling in deeper holes along the banks. These effects are related to the position of the site within the city of Oak Ridge and are not directly related to Y-12 Plant effluent.

The next downstream site, EFK 13.8, demonstrated a steady and significant pattern of improvement. The mean species richness and density have improved from both the early 1985–86 sampling and during the four sampling periods in 1986–88. The biomass at the site declined slightly from what was measured in the 1985–86 period, but increased, in general, during the later sampling. The IBI also reflects improvement, with the rating going from very poor to poor (with values increasing for metrics) on number of darters, number of intolerant species, and percentage lithophilic spawners. Data on the age structure of redbreast sunfish demonstrated successful reproduction and good representation of all age classes. The improvement at this site indicated that recovery of the lower portion of EFPC was occurring and should become even more evident with further monitoring.

The improvement noted at EFK 13.8 continued downstream at EFK 10.0. The mean species richness, density, and biomass have all increased from that shown in the 1985–86 sampling and increased throughout the 1986–88 sample period. Although the IBI ratings did not change from an integrity class of poor, some individual metrics such as number of darters and intolerant species did increase. Also, the age structure of redbreast sunfish indicated successful reproduction and strong representation in all age classes.

The most downstream site on EFPC, EFK 6.3, demonstrated improvement in conditions from those recorded in the early 1985–86 sampling. In comparison with the early sampling, EFK 6.3 had increased mean species richness, showing the greatest improvement of any site in EFPC. The mean density and biomass also improved from the early sampling phase, although the trend leveled off in the 1986–88 period. Analysis by IBI showed an improvement from very poor to poor with a constant increase of the

overall numerical rating. The metrics for number of darters, number of intolerant species, and percentage of lithophilic spawners all increased in the later sampling phase.

6.2.6 Future Studies

For the next sampling year, a variety of research plans for assessing impacts on the fish populations of EFPC will be implemented. In the coming years, more detailed experimental approaches will be added to determine areas of specific impact.

As a first step, the regular, quantitative monitoring of the density, biomass, and richness of the populations at the established sites will be continued on a spring-fall sampling regime. Included in this sampling will be scale sampling during the fall period. Qualitative sampling will be limited to stream areas not previously covered in sampling.

In addition to the normal sampling, some evaluative procedures will be scheduled. To further assess the role of habitat differences in explaining community differences, each site will be analyzed for canopy, flow, and pool/riffle ratios during winter-flow conditions. A method for evaluating solar heat input (Platts et al. 1987) to the stream may be employed as well as an evaluation of degree days. Temperature tolerances of fish species in EFPC will be examined through a literature review. These efforts should provide insight into the influence of temperature on community structure in EFPC. Further development of the IBI will continue in order to improve analytical capabilities. Productivity estimates will be made using a modification (Railsback et al. 1989) of the procedures of Garman and Waters (1983).

As part of the growth evaluation, a validation program for the scale analysis of age will be developed. This will involve analyzing otoliths and scales from sunfish taken for the bioaccumulation (Sect. 4) and biological indices (Sect. 5) programs and determining the degree of correspondence.

6.3 FISH MOVEMENT AND GROWTH IN EFPC, JULY 1985–JULY 1987

(A. J. Gatz, Jr.)

6.3.1 Introduction

Knowledge of the movement and growth of sunfishes in EFPC is necessary to interpret the data from a variety of other analyses used in monitoring the creek. For example, the accumulation of contaminants by EFPC fishes is being studied by site and becomes a meaningful measure of exposure at a given stream location only if the fishes are relatively immobile. Similarly, without knowledge of the amount of movement the fishes show seasonally, it is difficult to interpret fluctuations in fish abundance on a quarterly or semiannual basis. Moreover, the results of the various biological indicator analyses using redbreast sunfish can be placed into context only after seasonal growth patterns are quantified.

Although sunfish are generally considered to be sedentary, no work has been done on redbreast sunfish (*Lepomis auritus*), the most common sunfish species in EFPC. Information on movement and growth at a series of sites on EFPC is necessary to accomplish these needs. For example, comparisons of growth rates of sunfish at different EFPC sites in conjunction with the ongoing studies of food availability will permit

assessment of whether or not effluents from the Y-12 Plant (or elsewhere) are having an adverse sublethal effect (i.e., impairment of growth) on the fish populations. Further comparisons of growth rates between fish in EFPC and those in Brushy Fork will permit assessment of whether or not growth is generally depressed in EFPC.

6.3.2 Methods

Sampling was conducted in regions that included four of the biological monitoring sites. Moving downstream, these sampling regions were (1) EFK 22.7, the 950 m immediately below the outfall from NHP; (2) EFK 17.9, the 300 m downstream and the 700 m upstream from the Oak Ridge Turnpike crossing of EFPC near the Rocky Top convenience store at stoplight 13 (in spring 1987 this site was expanded an additional 1250 m upstream); (3) EFK 13.4, the 850 m upstream from the ORWTF outfall; and (4) EFK 4.7, the 600 m downstream and 1000 m upstream from the USGS gaging station. The sites were chosen based on their accessibility and suitability for sampling. In addition, two control reaches in BF (BFK 7.6) (800 m and 850 m) were studied. Sampling started in July 1985 at the four sites in EFPC and continued quarterly (except for winter 1987). Sampling started in October 1985 in BF and continued on the same schedule as at EFPC.

Movement and growth was studied by recapturing tagged native centrarchids (sunfishes and basses) and carp. Fishes were collected with backpack electrofishers, weighed and measured, marked by sewing individually numbered and color-coded Floy fingerling tags through the epaxial musculature at the anterior base of the dorsal fin, and then released within 25 m of the location of their capture. Numbered stakes every 100 m and flagging every 50 m indicated the stream kilometers so that precise stream locations were recorded.

6.3.3 Results

6.3.3.1 Sampling effectiveness

Total numbers and proportion of population tagged

Nearly 6000 fishes were tagged and released. Table 6-10 shows the numbers of fishes tagged and released at the various sampling sites on the different sampling occasions. The higher numbers of fishes tagged early in the study reflect repeated samplings of the same site in a single season rather than higher population densities. A single sampling per site each season became the norm starting January 1986; although EFK 17.9 and EFK 13.4 were sampled twice in October 1986, and EFK 13.4 was sampled twice in July 1987. The numbers of fishes tagged at most sites were fairly constant throughout 1986 and 1987 except that lower numbers of sunfishes were tagged in the region below NHP (EFK 22.7) during the 1987 samplings.

The same sites were sampled twice to obtain estimates of the percentages of the fish populations captured and tagged using the electrofishers (Table 6-11). The average percentage of the estimated population captured in one day of sampling was only 20.7% and repeating the procedure a second day still only yielded an average of 33.5% of the estimated population marked. Obviously large numbers of sunfishes avoided capture and we did not expect extremely high (e.g., >50%) percentages of marked fishes to be

Table 6-10. Number of fishes tagged and released at the sampling sites in the first 2 years of the study

Site	Date							Total
	Jul 85	Oct 85	Jan 86	Apr 86	Oct 86	Apr 87	Jul 87	
EFK 22.7	771	463	179	191	191	40	62	1897
EFK 17.9	147	0	52	60	83	197 ^a	114 ^a	673
EFK 13.4	347	184	56	87	179	36	166	1055
EFK 4.7	282	281	104	94	69	104	69	1003
BFK 7.6	0	261	90	131	198	126	152	958
Total	1547	1189	481	583	720	503	563	5586

^aSite expanded to include another 1150 meters upstream of original site. Total length = 2250 m.
 EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table 6-11. Estimated percentages and associated numbers of sunfishes tagged during sampling on three occasions

Site, date	M	n	m	N _e	Sample %	Total %
EFK 13.4 Fall 1986	104	111	36	321	32.4	55.8
EFK 13.4 Summer 1987	125	74	13	712	17.6	26.1
EFK 17.9 Fall 1986	54	33	4	446	12.1	18.6

Note: M = number of sunfishes tagged on first day of sampling; n = total number of sunfishes obtained on the second day of sampling; m = number of sunfishes captured/marked on first day of sampling that were also captured on the second day of sampling; N_e = estimated population size for the entire site; Sample % = estimated percentage of sunfishes at the site that were captured on day one (= M/N_e); and Total % = the estimated percentage of sunfishes at the site that were captured during both samplings (= {M + [n-m]}/N_e).
 EFK = East Fork Poplar Creek kilometer.

recaptured in later samples. In fact, the percentages of tagged sunfishes recaptured (see next section) was about as high as could reasonably be expected given our sampling efficiency.

Recaptures

Over 650 recaptures of tagged sunfishes were made in the two years of this study. The following results represent an analysis of those recaptures. This analysis indicated both seasonal and year-to-year variations in movement and growth. Recaptures of carp are reported in a following subsection.

The percentage of recaptures was highest in the samples taken 3 to 6 months after the sunfishes were marked (Table 6-12). This result, and the existence of numerous sunfishes collected during the first year of the study that had obviously lost a tag, indicated that tag loss was a severe impediment to gathering long term data on individual fish. There were no recaptures that covered the entire 24 months of the study and only 2 recaptures over a 21-month interval. However, because the initial recapture rates increased in the second year of the study, these numbers might be expected to increase with further study.

Table 6-12. Percentages of sunfishes tagged on a given date that were recaptured on the subsequent date indicated

Date of recapture	Date of tagging					
	Summer 85	Fall 85	Winter 86	Spring 86	Fall 86	Spring 87
Fall 1985	3.5%	--	--	--	--	--
Winter 1986	1.1%	5.9%	--	--	--	--
Spring 1986	0.9%	3.6%	7.3%	--	--	--
Summer 1986	0.3%	2.2%	0.4%	13.7%	--	--
Fall 1986	0.1%	0.5%	1.0%	6.0%	--	--
Spring 1987	0.1%	0.1%	1.0%	3.0%	12.5%	--
Summer 1987	0	0.1%	0	1.2%	6.0%	14.7%

Survivorship/tag retention by site

In most cases the numbers of marked sunfishes recaptured represented the same proportion of the total number marked at all sites in both EFPC and BF. For example, Table 6-13 shows the numbers of recaptures made during the summer 1987 sampling period compared with captures made during the two most recent marking periods. This result implies equal survivorship and tag retention at all sites, both in EFPC and in BF. The sole exception to this pattern involves sunfishes originally tagged in spring 1986 and recaptured one year later in spring 1987. For these fishes, the number of recaptures in BF was significantly higher than in EFPC (Table 6-14). Lumping all EFPC sites to get more statistically acceptable expected values still gives a significant X^2 result: $X^2 = 18.080$, $df = 1$, and $p < 0.001$.

Table 6-13. Comparison of the number of recaptures made in summer 1987 with the number expected if recapture rates were equal at all sites

A	Site	Observed	Expected
	EFK 22.7	4	5.9
	EFK 17.9	35	29.0
	EFK 13.4	2	5.3
	EFK 4.7	10	15.3
	BFK 7.6	23	18.5

B	Site	Observed	Expected
	EFK 22.7	5	11.4
	EFK 17.9	4	5.0
	EFK 13.4	16	10.5
	EFK 4.7	3	4.1
	BFK 7.6	15	11.8

Note: Part A shows data for sunfishes marked in spring 1987. For these fishes, a hypothesis of equal rates of recapture at all sites cannot be rejected: $X^2 = 6.839$, D.F. = 4, $p > 0.10$. Part B shows data for sunfishes marked in fall 1986. For these fishes, also, a hypothesis of equal rates of recapture at all sites cannot be rejected: $X^2 = 7.581$, D.F. = 4, $p > 0.10$. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; D.F. = degrees of freedom.

Table 6-14. Comparison of the numbers of sunfishes marked in spring 1986 and recaptured one year later in spring 1987 with the numbers expected if recapture rates were equal at all sites

Site	Observed	Expected
EFK 22.7	2	5.9
EFK 17.9	2	2.5
EFK 13.4	0	2.7
EFK 4.7	2	2.9
BFK 7.6	12	4.0

Note: For these fishes, a hypothesis of equal rates of recapture at all sites is rejected: $X^2 = 21.657$, D.F. = 4, $p < 0.001$. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; D.F. = degrees of freedom.

6.3.3.2 Movement

Species similarity

There were no significant ($p < 0.05$) differences in patterns of movement seen among any of the species of sunfishes, hence data for all sunfishes (except the few largemouth and spotted bass collected) were pooled for the analysis presented here. The species involved, listed in decreasing order of abundance, were redbreast sunfish, bluegill sunfish, rockbass, hybrids, and warmouth.

Lack of movement

The sedentary nature of sunfishes was verified by the initial results: 58% of all recaptures over time intervals of 3 months or longer were sunfishes that had moved <100 m (Table 6-15). These fishes were recaptured either within the same 50-m reach as that in which they had been marked 3 or more months earlier or in one of the two 50-m reaches immediately adjacent to that in which they had been marked and released. A further 19% of recaptures over similar time intervals were of sunfishes that had moved between 100 and 200 m, hence 77% of all sunfishes recaptured were within 200 m of the site of their initial capture 3 or more months earlier.

Among site differences

Although overall most sunfishes move little, significantly different patterns of movement occurred among the various sites. Three sites showed exceedingly low amounts of movement—EFK 4.7, EFK 13.4, and the combined sites in BF. These sites were not significantly different from one another when all data were combined (Table 6-16). For these sites, 73% of all recaptures were made <100 m from the point of first capture and 92% within 200 m. Only 1.6% of the sunfishes moved ≥ 1000 m.

Table 6-15. Patterns of movement by site for sunfishes in East Fork Poplar Creek

Site	Distance (m)				Total
	0-50	100-200	250-950	≥ 1000	
EFK 22.7	108 (151.3)	47 (49.7)	71 (38.5)	33 (19.5)	259
EFK 17.9	43 (45.6)	19 (15.0)	5 (11.6)	11 (5.9)	78
EFK 13.4	90 (76.6)	30 (25.1)	8 (19.5)	3 (9.8)	131
EFK 4.7	80 (63.1)	20 (20.7)	7 (16.1)	1 (8.1)	108
BFK 7.6	60 (44.4)	9 (14.6)	6 (11.3)	1 (5.7)	108
Totals	381	125	97	49	652

Note: Expected values for null hypothesis of no difference between sites appear in parentheses below the observed values. The null hypothesis must be rejected: $X^2 = 103.45$, D.F. = 12, $p < 0.001$. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; D.F. = degrees of freedom.

Table 6-16. Similarity of patterns of movement by sunfishes at EFK 13.4, EFK 4.7, and Brushy Fork

Site	Distance (m)				Total
	0-50	100-200	250-950	≥ 1000	
EFK 13.4	30 (95.7)	30 (24.5)	8 (8.7)	3 (2.1)	131
EFK 4.7	80 (78.9)	20 (20.2)	7 (7.2)	1 (1.7)	108
BFK 7.6	60 (55.5)	9 (14.2)	6 (5.1)	1 (1.2)	108
Totals	230	59	21	5	315

Note: Expected values for null hypothesis of no difference between sites appear in parentheses below the observed values. The null hypothesis is not rejected: $X^2 = 4.84$, D.F. = 6, $p > 0.50$. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; D.F. = degrees of freedom.

In distinct contrast, movements ≥ 1000 m were an order of magnitude more common at both EFK 17.9 (14.1% of all recaptures; see Table 6-17) and EFK 22.7 (12.7% of all recaptures). Intermediately long movements (250–950 m) were common at EFK 22.7 (27.4%) but not at EFK 17.9 (6.4%); the data for these two sites show significant differences from each other (Table 6-17) as well as from the two sites further downstream and BF (Table 6-15).

Table 6-17. Patterns of movement by sunfishes at EFK 17.9 and EFK 22.7

Site	Distance (m)				Total
	0–50	100–200	250–950	≥ 1000	
EFK 22.7	108 (116.1)	47 (50.7)	71 (58.4)	33 (33.8)	259
EFK 17.9	43 (34.9)	19 (15.3)	5 (17.6)	11 (10.2)	78
Totals	151	66	76	44	337

Note: Expected values for null hypothesis of no difference between sites appear in parentheses below the observed values. The null hypothesis must be rejected: $X^2 = 15.40$, D.F. = 3, $p < 0.005$. EFK = East Fork Poplar Creek kilometer; D.F. = degrees of freedom.

Seasonal patterns

Sufficient data exist to analyze for seasonal patterns of movement at only three sites, EFK 22.7, EFK 13.4 and EFK 4.7. No significant differences in movement with season were seen at either of the two downstream sites in EFPC. These two sites did not differ from each other in any patterns of movement analyzed; combined data for these sites are shown in Table 6-18.

A distinct seasonal pattern of movement existed from summer 1985 to summer 1986 at EFK 22.7 (Table 6-19). The sunfishes were sedentary through the summer and fall and on into winter. Then they showed increased movement from winter to spring and high movement from spring to summer. This same sampling schedule was not followed in 1986–87, so verification for a second year was not possible.

Evidence for spawning movements

Spawning movement was likely to have been a major contributing factor to the unique patterns of movement seen at EFK 22.7 and EFK 17.9. Supportive evidence is of several types: (1) nest sites were obvious in portions of EFK 17.9 but not in EFK 22.7, (2) the time of the peak in long-distance movements coincided with the time of reproduction, and (3) several sunfishes, originally tagged at EFK 22.7, were recaptured

Table 6-18. Patterns of movement of sunfishes by season at the EFK 13.4 and EFK 4.7

Season	Distance (m)			Total
	0-50	100-450	≥ 500	
Summer-fall	24 (30.3)	15 (10.2)	3 (1.5)	42
Fall-winter	18 (15.9)	4 (5.4)	0 (0.8)	22
Winter-spring	12 (10.8)	3 (3.7)	0 (0.5)	15
Spring-summer	29 (26.0)	6 (8.8)	1 (1.3)	36
Totals	83	28	4	115

Note: The expected values for the null hypothesis of no differences by season appear in parentheses below the observed values. The null hypothesis cannot be rejected: $X^2 = 8.60$, D.F. = 6, $p > 0.10$ (D.F. = degrees of freedom). EFK = East Fork Poplar Creek kilometer.

Table 6-19. Patterns of movement of sunfishes by season below New Hope Pond (EFK 22.7)

Season	Distance (m)			Total
	0-50	100-450	≥ 500	
Summer-fall	13 (11.9)	11 (10.6)	0 (1.4)	24
Fall-winter	24 (18.9)	14 (16.9)	0 (2.3)	38
Winter-spring	8 (14.9)	20 (13.3)	2 (1.8)	30
Spring-summer	21 (20.3)	14 (18.2)	6 (2.5)	41
Totals	66	59	8	133

Note: The expected values for the null hypothesis of no differences by season appear in parentheses below the observed values. The null hypothesis is rejected: $X^2 = 18.35$, D.F. = 6, $p < 0.01$ (D.F. = degrees of freedom). EFK = East Fork Poplar Creek kilometer.

first at EFK 17.9 in spring and then recaptured once again at the New Hope site the next summer.

Differences between years

The same patterns of movement were not seen in both years over the same season at all sites. For example, large numbers of long distance movements (interpreted herein as spawning movements) occurred at EFK 22.7 between fall 1986 and spring 1987 but not between fall 1985 and spring 1986 (Table 6-20). Similarly, fall to spring movements differed between years at EFK 17.4 (Table 6-21), and spring to summer movements differed between years at EFK 17.9 (Table 6-22). No such between-year differences were identified at EFK 4.7, and no further between-year differences were identified for any other seasons at any of the sites.

Carp

There were 5 carp recaptured over time intervals of 3 months or more and none moved > 200 m between captures. Of those recaptured, four were from EFK 4.7. One individual moved 50 m downstream from summer to winter; one moved 150 m upstream from fall to spring; and the other two moved, respectively, 100 and 200 m upstream from one summer to the next. The other recapture of a carp was from EFK 13.4; it moved 200 m upstream from spring to summer. From these limited returns, carp appeared to be sedentary in EFPC.

Table 6-20. Between year differences in patterns of movement of sunfishes at EFK 22.7

Year	Distance (m)				Total
	0-50	100-200	250-950	≥ 1000	
Fall 1985- spring 1986	12 (8.2)	3 (4.1)	11 (8.2)	0 (5.6)	26
Fall 1986- spring 1987	4 (7.8)	5 (3.9)	5 (7.8)	11 (5.4)	25
Totals	16	8	16	11	51

Note: Expected values for null hypothesis of no difference between years appear in parentheses below the observed values. The null hypothesis must be rejected: $X^2 = 17.74$, D.F. = 3, $p > 0.001$ (D.F. = degrees of freedom). EFK = East Fork Poplar Creek kilometer.

Table 6-21. Between year differences in patterns of movement of sunfishes at EFK 13.4

Year	Distance (m)		Total
	0-50	≥ 100	
Fall 1985- spring 1986	10 (7.4)	0 (2.6)	10
Fall 1986- spring 1987	15 (17.6)	9 (6.4)	24
Totals	25	9	34

Note: Expected values for null hypothesis of no difference between years appear in parentheses below the observed values. The null hypothesis must be rejected: $X^2 = 5.10$, D.F. = 1, $p > 0.025$ (D.F. = degrees of freedom). EFK = East Fork Poplar Creek kilometer.

Table 6-22. Between year differences in patterns of movement of sunfishes at EFK 17.9

Year	Distance (m)			Total
	0-50	100-450	≥ 500	
Spring 1986- summer 1986	6 (9.3)	5 (5.7)	8 (4.0)	19
Spring 1987- summer 1987	22 (18.7)	12 (11.3)	4 (8.0)	38
Totals	28	17	12	57

Note: Expected values for null hypothesis of no difference between years appear in parentheses below the observed values. The null hypothesis is rejected: $X^2 = 7.90$, D.F. = 2, $p < 0.025$ (D.F. = degrees of freedom). EFK = East Fork Poplar Creek kilometer.

6.3.3.3 Growth

Heterogeneity

The predominant feature shown by most of the growth data was their heterogeneity. Some fish thrived during a given time interval at a given site while other fish lost weight. For example, Table 6-23 shows the high degree of variation in weight gain by sunfishes 12.1 to 15-cm standard length at EFK 22.7 between spring and summer 1986. Although similarly widely ranging weight changes did not always occur (e.g., 12 sunfishes in this

Table 6-23. Weight changes (in grams) and descriptive statistics for the 14 sunfishes, 12.1 to 15 cm standard length, marked in spring 1986 at EFK 22.7 and recaptured in summer 1986

Weight changes					Mean	SD	SE
≤ 0 g	1–10 g	10–20 g	20–30 g	> 30 g			
-8	3	12	27	45	15.1	17.8	4.8
-6	3	18	30	51			
	4	19					
	5						
	9						

Note: EFK = East Fork Poplar Creek kilometer.

same size class at the same site all lost from 0 to 9 g from winter to spring 1986) the results shown in Table 6-23 were not atypical.

Seasonal Patterns

Despite the heterogeneity, statistically significant differences in amount of weight gained per 3-month interval can be identified for most size classes at most sites (Table 6-24). At EFK 4.7 all three size classes averaged negative growth from fall to winter, low but positive growth from summer to fall, somewhat higher positive growth from winter to spring, and the best growth from spring to summer. Many, but not all, of these differences were statistically significant. At EFK 13.4, very few significant differences with season were identified, but maximum average growth occurred from spring to summer for all but the largest sunfishes, as was the case at EFK 4.7. In contrast, growth at EFK 22.7 averaged the highest between summer and fall for two of the size classes, although never significantly higher than from spring to summer. Minimal growth at EFK 22.7 occurred from winter to spring, a time of good growth at EFK 4.7. The difference may relate to the high level of movement by sunfishes from EFK 22.7 during this period (Table 6-17).

Differences between years

In the 2 years of the study, amounts of growth that could be called statistically significant varied with the seasons being compared. No differences occurred between the 2 years in spring to summer growth (Table 6-25), but differences did occur in fall to spring (Table 6-26) and fall to summer growth (Table 6-27).

Among-site differences

Mean growth increments varied among sites for some size classes in some seasons. For example, in both winter to spring growth (Table 6-28) and spring to summer growth

Table 6-24. Mean weight changes in sunfishes (expressed in grams) over 3-month intervals from July 1985 to July 1987 at three sites in East Fork Poplar Creek

Solid lines connect means not significantly different from each other

Site	Size class	Mean weight change and season ^a			
		F-W	S-F	W-Sp	Sp-S
EFK 4.7	≤ 12 cm	<u>-1.6</u>	<u>3.4</u>	6	<u>18.5</u>
	12.1–15 cm	<u>-12.3</u>	<u>3.7</u>	<u>13.2</u>	<u>31.2</u>
	< 15 cm	<u>-19.0</u>	<u>13.5</u>	<u>14.0</u>	no data
EFK 13.4	≤ 12 cm	<u>-4.5</u>	<u>1.2</u>	<u>2.3</u>	<u>7.2</u>
	12.1–15 cm	<u>-5.0</u>	<u>-2.0</u>	<u>5.0</u>	<u>21.5</u>
	> 15 cm	<u>-9.7</u>	<u>-2.5</u>	<u>1.2</u>	<u>4.0</u>
EFK 22.7	≤ 12 cm	<u>-2.0</u>	<u>-1.8</u>	<u>9.0</u>	<u>19.3</u>
	12.1–15 cm	<u>-3.9</u>	<u>-0.9</u>	<u>14.5</u>	<u>17.8</u>
	> 15 cm	<u>-7.4</u>	<u>-6.0</u>	<u>2.9</u>	<u>22.0</u>

^aF = fall, S = summer, Sp = spring, and W = winter.

Note: EFK = East Fork Poplar Creek kilometer.

(Table 6-29), EFK 4.7 seemed to be a favorable site for all size classes. In contrast, other sites were good for some size classes and poor for others, even in the same season. For example, spring to summer at EFK 22.7 was good for sunfishes > 15 cm and poor for those ≤ 12 cm (Table 6-29). Fall-1986 to spring-1987 growth was the same at all sites for sunfishes ≤ 15 cm, but major differences among sites occurred among the largest sunfishes (> 15 cm; Table 6-30). These differences may have also been related to spawning movements. They also showed that the same site was not necessarily the best in all seasons.

Table 6-25. Mean growth increments (expressed in grams) from spring to summer in 1986 and 1987

Size class	EFK 4.7		EFK 13.4		EFK 12.9		EFK 22.2		BFK 7.6	
	1986	1987	1986	1987	1986	1987	1986	1987	1986	1987
≤ 12 cm	18.5	17.0	7.2	6.2	9.0	12.6	5.0	^a	12.8	11.9
12.1–15 cm	31.3	12.5	12.5	^a	17.8	22.4	15.5	23.5	19.0	19.3
> 15 cm	^a	11.8	1.2	^a	2.9	-9.1	13.5	-0.2	^a	7.3

^aSample size of one or zero, so no statistical tests possible.

Note: The null hypothesis of no difference in mean growth increment between years cannot be rejected for any size group at any site: all t-test data < 2, and all *p*'s > 0.10. EFK = East Fork Poplar Creek kilometer. BFK = Brushy Fork kilometer.

Table 6-26. Mean growth increments (in grams) from fall to spring 1985–1986 compared with 1986–1987 for three East Fork Poplar Creek sites

Size class	EFK 4.7			EFK 13.4			EFK 22.7		
	85/86	86/87	<i>p</i>	85/86	86/87	<i>p</i>	85/86	86/87	<i>p</i>
≤ 12 cm	^a	5.7	^a	1.2	7.7	<.001	-1.9	5.2	<.001
12.1–15 cm	-0.8	9.3	>.10	-1.7	17.8	>.001	-1.2	10.4	>.05
> 15 cm	-16.0	22.6	<.01	^a	36.1	^a	-3.2	7.9	>.20

^aSample size of one or zero, so no statistical tests possible.

Note: The null hypothesis of no difference in mean growth increment between years is rejected for at least one size class at each of the three sites. EFK = East Fork Poplar Creek kilometer.

Discussion

How much of the pattern of movement in the upper end of EFPC was due to the after effects of the fish kill on July 23, 1985, (see Appendix G in Loar et al. 1992b) is unknown. Although it might be that some of the increased movement seen was due to fishes colonizing the area decimated by the fish kill, there are some data contrary to this interpretation. For example, data for sunfishes marked in spring 1987 and recaptured in summer 1987 continued to show the same pattern that was seen shortly after the fish kill even though the recolonization of the decimated reach had long since been accomplished.

Table 6-27. Mean growth increments (in grams) from fall to summer 1985–1986 compared with 1986–1987 for all East Fork Poplar Creek sites combined

Size class	Fall 1985 – summer 1986	Fall 1986 – summer 1987	T-test data	<i>p</i>
≤ 12 cm	5.5	15.7	3.43	<.01
12.1–15 cm	8.3	21.0	2.32	<.05
>15 cm	0.8	28.9	2.36	>.05

Note: The null hypothesis of no difference in mean growth increment between years is rejected for two of the three size classes.

Table 6-28. Growth increments from winter 1985 to spring 1986 at the various sites

Size class	Growth increments and sites		
	EFK 13.4	EFK 22.7	EFK 4.7
≤ 12 cm	<u>-4.5</u>	<u>-2.0</u>	<u>6</u>
12.1–15 cm	<u>-5.0</u>	<u>-3.9</u>	<u>13.2</u>
> 15 cm	<u>-9.7</u>	<u>-7.4</u>	<u>14.0</u>

Note: Solid underlines connect growth increments not significantly different from each other. EFK = East Fork Poplar Creek kilometer.

It seems more likely that the high degree of movement was associated to a large degree with spawning movements (see following) although other long distance movements also occurred.

Only 11.2% of the sunfishes recaptured had moved more than 500 m and 77% had moved 200 m or less, so it appeared that samples taken at a given site for the study of bioaccumulation would have largely reflected the local conditions. Differences in movement patterns between sites bear further study, however, especially because the highest movement was seen at EFK 22.7, closest to Y-12 discharges. The elevated amount of movement at this site may have been related to any of several features of the site: (1) discharges from Y-12 could induce movements, (2) the headwater nature of the habitat at the site may lead to higher frequencies of movements, or (3) an absence of spawning habitat may induce higher movement in breeding season. Another possibility relates to the massive fish kill (July 23, 1985) that left several kilometers of stream immediately downstream from the NHP site (EFK 22.7) essentially fishless. The absence

Table 6-29. Growth increments from spring to summer at the various sites, 1986 and 1987 data combined

Size class	Growth increments and sites				
	EFK 22.7	EFK 13.4	EFK 17.9	BFK 7.6	EFK 4.7
≤ 12 cm	<u>5.0</u>	<u>6.8</u>	<u>11.8</u>	<u>12.2</u>	<u>17.8</u>
12.1-15 cm	<u>16.2</u>	<u>17.8</u>	<u>19.2</u>	<u>20.3</u>	<u>21.9</u>
> 15 cm	<u>-5.3</u>	<u>0.2</u>	<u>7.6</u>	<u>9.5</u>	<u>11.8</u>

Note: Solid underlines connect growth increments not significantly different from each other. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table 6-30. Growth increments from fall 1986 to spring 1987 at the various sites

Size class	Growth increments and sites				
	EFK 22.7	EFK 4.7	BFK 7.6	EFK 13.4	EFK 17.9
≤ 12 cm	<u>5.2</u>	<u>5.7</u>	<u>6.4</u>	<u>7.7</u>	<u>10.3</u>
12.1-15 cm	<u>9.3</u>	<u>10.4</u>	<u>15.5</u>	<u>15.5</u>	<u>17.8</u>
> 15 cm	<u>7.9</u>	<u>22.6</u>	<u>24.0</u>	<u>36.1</u>	<u>43.0</u>

Note: Solid underlines connect growth increments not significantly different from each other. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

of competitors may well have increased the amount of movement shown by the nearby fishes, especially those below NHP.

Tag loss was a problem in the first year of the study. Collections in July 1986 contained roughly equal numbers of sunfishes that had lost their tags and that had retained their tags. The proportion of obvious tag losses was much lower in the second year of the study; for example, only 3% of the sunfishes tagged in spring 1987 had obviously lost tags. On a more positive note, the proportion of fishes tagged that were recaptured from the first to second year of the study rose dramatically (Table 6-12). The recapture rate, however, dropped rapidly after the first 3-month interval following initial marking (Table 6-12). Further examination of this trend is necessary.

It is difficult to know how much of the difference in recapture rate over time was due to improvements in tag-attachment technique and how much was due to differences in water temperature and water quality during the course of the study. The large external

lesions noted on many fishes at EFK 22.7 in July 1985 were not seen in the second year of the study. If this reflects improved water quality, one might expect lower infection rate and better tag retention during the course of the study than was seen. Further support for the idea of improved water quality in the headwaters of EFPC comes from the fact that the recapture rates at EFK 22.7 were the lowest for all sites between July and October 1985 and the highest for the three subsequent sampling intervals. Evidence (Tables 6-13 and 6-14) suggests tag retention and survivorship was about the same at all sites in EFPC and BF over most time intervals.

Larger samples were needed to meet the objectives of the growth study. The extreme heterogeneity in most samples made it difficult to show statistically significant differences by site, season, or size class. Still, the current information was sufficient to make some general statements about the growth cycle for the sunfishes. Integration of these results with the results of scale analyses should enhance our understanding of the growth patterns of these fishes.

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Appendix A

**MEAN MONTHLY TEMPERATURES IN EAST FORK POPLAR
CREEK AND BRUSHY FORK, JULY 1986-JULY 1988**

Table A-1. Mean ± 1 SD monthly water temperatures (in degrees Celsius) in East Fork Poplar Creek and Brushy Fork, July 1985–July 1988

Absolute minimum and maximum values are given in parentheses and the number of days in the temperature record is also given

1985	BFK 7.6	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3
Jul	ND	ND	25.3±2.1 (22.0–30.4) 20	ND	23.4±1.3 (21.0–26.0) 21	23.0±1.0 (21.2–25.2) 20	22.7±0.9 (21.0–24.6) 19
Aug	ND	ND	24.1±2.1 (30.8–30.0) 31	ND	22.1±1.6 (19.4–25.4) 31	22.0±1.3 (20.0–24.8) 31	21.6±1.4 (19.4–25.0) 31
Sep	ND	ND	22.8±2.4 (18.2–28.8) 29	ND	19.9±2.3 (15.4–24.0) 29	20.3±1.8 (16.4–23.4) 30	19.3±1.9 (15.2–22.6) 29
Oct	16.8±1.2 (13.8–19.0) 17	ND	22.0±1.9 (17.2–25.6) 30	19.7±1.9 (14.4–23.0) 31	18.3±2.0 (13.2–21.2) 31	18.9±1.5 (15.0–21.2) 34	17.7±1.7 (13.2–20.4) 31
Nov	13.6±2.0 (8.0–17.2) 30	ND	19.7±1.8 (16.0–22.6) 18	16.9±2.0 (12.0–20.8) 30	15.4±2.1 (10.2–18.8) 30	16.5±1.6 (12.6–19.2) 30	15.1±1.9 (10.4–18.0) 30
Dec	6.7±2.9 (2.6–14.8) 31	ND	ND	10.5±2.7 (5.0–16.8) 29	8.6±2.8 (4.2–15.6) 24	10.7±2.2 (6.6–16.0) 21	9.0±2.6 (4.6–15.2) 23
1986							
Jan	5.1±1.0 (3.2–7.0) 31	ND	12.1±1.9 (7.0–16.0) 19	9.0±2.0 (5.0–13.2) 19	6.8±2.1 (1.0–11.4) 19	8.3±1.8 (3.2–11.8) 19	7.7±1.8 (3.0–11.4) 19
Feb	ND	ND	13.8±1.7 (9.2–17.8) 28	11.2±2.1 (5.8–15.4) 28	9.7±2.2 (4.2–13.8) 28	10.7±1.9 (6.6–13.8) 28	10.2±1.9 (5.6–13.8) 28

Table A-1 (continued)

1986	BFK 7.6	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3
Mar	11.7±2.2 (6.8-16.2) 13	ND	15.7±2.4 (10.2-22.6) 30	13.2±2.6 (7.2-19.8) 30	12.1±2.8 (6.0-17.6) 30	12.6±2.3 (8.0-17.8) 30	12.4±2.6 (7.2-17.6) 30
Apr	14.3±2.2 (9.6-20.0) 30	ND	19.3±3.0 (13.6-28.0) 30	16.9±2.7 (11.0-24.0) 30	16.2±2.5 (10.8-24.0) 30	16.4±2.0 (10.8-23.0) 30	16.4±2.1 (12.0-21.4) 30
May	16.9±1.8 (11.2-20.8) 29	ND	22.0±2.7 (15.2-22.2) 30	20.1±2.0 (13.6-24.0) 29	19.7±2.1 (13.0-23.8) 30	19.4±1.7 (14.0-22.4) 30	19.2±1.7 (14.2-22.2) 30
Jun	19.7±1.2 (17.0-22.6) 30	ND	24.5±2.3 (20.2-29.0) 30	23.2±1.5 (19.6-26.2) 30	23.2±1.2 (19.6-25.8) 30	22.4±1.1 (19.6-24.6) 30	22.1±0.9 (19.8-24.0) 30
Jul	21.6±1.3 (18.0-24.4) 31	ND	25.7±2.1 (21.0-31.0) 31	24.8±1.4 (20.8-28.0) 31	25.0±1.4 (21.0-28.0) 25	24.3±1.1 (20.8-27.0) 31	23.9±1.0 (20.8-25.6) 31
Aug	19.6±1.6 (15.1-22.9) 20	ND	24.9±2.0 (19.5-28.9) 12	23.0±1.8 (17.8-26.0) 19	ND	23.1±1.4 (19.2-25.1) 19	22.7±1.5 (19.0-24.0) 19
Sep	18.2±1.3 (15.2-22.0) 30	ND	24.0±1.8 (20.0-28.2) 30	21.4±1.2 (18.2-24.6) 30	ND	21.8±1.0 (19.1-23.9) 30	21.1±0.9 (19.4-22.9) 30
Oct	15.1±2.9 (10.5-22.0) 31	ND	21.4±2.2 (13.8-24.0) 30	18.0±2.4 (13.8-24.0) 30	ND	ND	17.6±2.8 (14.2-23.0) 31
Nov	12.6±2.4 (7.0-17.7) 30	ND	18.6±2.2 (13.1-24.0) 30	14.9±2.4 (8.9-20.3) 30	15.6±2.7 (7.7-19.2) 12	15.5±2.1 (10.7-20.0) 30	14.2±2.2 (9.6-19.0) 30
Dec	9.0±1.9 (0.9-12.0) 30	ND	14.0±2.3 (11.1-17.7) 30	11.2±1.2 (8.5-13.8) 21	ND	11.4±1.7 (9.1-14.5) 31	10.4±1.6 (7.1-13.2) 13

Table A-1 (continued)

1987	BFK 7.6	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3
Jan	7.1±1.2 (4.1-9.9) 30	ND	12.4±1.6 (8.3-15.9) 30	9.6±1.6 (6.1-13.0) 29	8.6±1.7 (4.7-12.2) 26	9.6±1.3 (6.8-12.8) 30	8.5±1.4 (5.0-11.5) 14
Feb	8.1±1.0 (4.7-10.9) 28	ND	12.7±1.5 (9.3-17.2) 28	10.2±1.3 (6.1-14.4) 28	9.5±1.2 (5.5-13.0) 28	10.2±1.0 (6.5-12.8) 28	9.2±1.0 (5.6-11.5) 28
Mar	11.3±1.5 (8.2-15.5) 29	ND	15.6±2.4 (11.5-22.5) 30	ND	12.5±2.1 (8.6-17.1) 30	13.0±1.7 (9.7-17.2) 30	11.7±1.5 (8.1-15.8) 29
Apr	14.8±1.5 (11.1-18.1) 21	18.1±7.0 (6.5-43.5) ^a 21	18.6±2.8 (13.0-25.9) 21	6.9±2.3 (11.8-22.6) 21	16.5±1.9 (12.3-20.3) 21	16.1±1.6 (12.3-19.5) 21	15.9±1.7 (11.7-19.3) 21
May	18.2±1.7 (13.7-21.7) 31	21.2±2.3 (14.8-26.5) 31	22.7±12.9 (16.0-28.8) 31	21.3±2.0 (15.6-25.1) 31	21.2±1.9 (16.2-24.7) 31	20.2±1.7 (16.0-23.8) 31	19.9±1.8 (8.8-22.7) 31
Jun	19.8±1.1 (16.8-22.8) 30	23.1±1.6 (19.6-28.4) 30	24.2±2.1 (19.3-29.1) 30	23.0±1.3 (18.9-25.3) 30	23.0±1.0 (19.6-25.3) 30	22.3±0.9 (19.3-24.2) 30	21.9±0.9 (19.1-23.5) 30
Jul	21.0±1.1 (18.2-23.3) 29	25.8±4.9 (17.5-44.2) ^a 29	25.9±2.1 (22.8-29.4) 11	24.1±1.4 (20.6-29.5) 29	24.0±1.1 (21.3-30.1) 29	23.2±0.8 (21.5-30.1) 29	23.0±0.9 (20.6-29.6) 29
Aug	21.2±1.3 (17.6-24.6) 31	25.0±2.2 (21.8-34.4) 31	25.6±2.1 (20.4-30.4) 31	24.5±1.4 (20.0-27.3) 31	24.4±1.3 (20.2-26.9) 31	24.0±0.7 (21.9-25.2) 31	23.5±0.9 (20.5-25.1) 31
Sep	18.4±1.7 (14.3-21.8) 30	23.4±1.3 (19.0-26.9) 30	23.1±2.1 (18.5-28.6) 30	21.3±1.6 (17.3-24.4) 30	20.8±1.7 (16.8-24.1) 30	21.3±1.0 (19.1-22.9) 30	20.4±1.5 (17.0-22.9) 30
Oct	12.1±1.6 (8.4-16.6) 31	21.0±1.0 (18.8-23.8) 31	18.7±1.9 (14.5-23.3) 31	15.5±1.8 (11.1-19.9) 31	14.3±1.8 (10.3-19.4) 31	16.4±1.3 (14.6-20.2) 31	14.3±1.6 (11.0-19.5) 31

Table A-1 (continued)

1987	BFK 7.6	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3
Nov	10.0±2.7 (3.7-14.8) 30	20.4±1.3 (16.3-24.1) 30	17.2±2.4 (11.8-22.9) 30	13.3±2.9 (7.1-19.1) 30	12.5±2.9 (5.7-17.2) 30	14.1±1.4 (11.3-16.4) 30	12.5±2.5 (6.7-16.8) 30
Dec	7.5±2.0 (2.7-12.0) 31	18.1±1.1 (12.3-20.4) 31	14.2±1.8 (9.5-18.3) 31	10.9±2.0 (6.0-15.3) 31	9.6±2.2 (4.4-14.7) 31	11.9±0.9 (10.1-14.7) 31	10.0±1.9 (5.5-14.0) 31
1988							
Jan	4.2±3.1 (0-11.1) 24	15.3±1.4 (9.2-18.6) 26	ND	8.1±2.0 (3.7-12.5) 25	6.6±2.4 (1.7-11.9) 25	9.3±1.5 (7.1-11.8) 25	6.9±2.3 (2.7-11.2) 25
Feb	7.4±2.1 (2.9-13.0) 29	15.5±1.1 (10.7-18.4) 29	12.8±2.1 (8.0-18.3) 29	10.0±2.3 (5.1-15.7) 29	8.9±2.4 (3.7-14.8) 29	9.6±1.9 (5.6-14.5) 29	8.9±2.1 (4.9-13.9) 29
Mar	11.1±2.3 (6.6-16.8) 31	16.5±1.3 (10.8-21.0) 31	13.9±5.1 (1.6-32.3) ^a 31	13.3±2.5 (7.7-19.6) 31	12.4±2.4 (6.8-18.0) 31	12.5±2.1 (8.1-17.4) 31	11.3±4.9 (5.0-29.9) ^a 31
Apr	14.2±1.8 (10.3-18.8) 31	19.0±1.5 (15.6-23.4) 30	18.3±2.9 (7.4-25.4) 30	16.4±2.5 (10.8-22.5) 30	15.7±2.2 (10.9-21.1) 30	15.7±1.8 (11.8-20.2) 30	15.3±5.9 (5.3-38.8) ^a 30
May	16.5±1.7 (12.6-21.0) 31	22.2±1.6 (18.6-26.0) 31	22.0±2.8 (16.4-28.2) 31	19.8±2.2 (14.6-24.8) 31	19.0±1.9 (14.4-23.6) 31	18.5±1.7 (14.1-22.4) 31	18.0±2.3 (8.6-34.6) ^a 31
Jun	19.3±2.1 (13.9-24.1) 30	22.3±1.4 (19.5-26.6) 30	23.0±2.3 (18.6-28.7) 30	22.1±2.1 (16.5-26.3) 30	22.0±2.0 (16.9-26.2) 30	21.5±1.8 (16.8-25.0) 30	21.1±1.8 (16.6-24.5) 30
Jul	21.1±1.7 (15.9-24.8) 25	22.8±2.0 (18.6-26.7) 25	23.8±2.8 (18.1-37.3) ^a 25	23.2±2.0 (17.0-27.4) 25	23.3±1.8 (18.2-27.1) 24	22.8±1.4 (18.5-25.5) 25	22.7±1.5 (18.5-25.5) 25

^aExtreme values in temperature data may be due to equipment error. A review of the raw data, however, reveals normal trends around the peak temperature. The data, therefore, are unchanged.

Note: Data were obtained (1) at 2-h intervals for July 1985 through March 1987, using a Ryan-Peabody thermograph (Model J-90), (2) at 20-min intervals for April through June 1987, using a Ryan Tempmentor digital thermograph, and (3) at 1-h intervals for July 1987-July 1988, using a Ryan Tempmentor digital thermograph. ND = no data available. BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

Appendix B

**RESULTS OF QUALITY ASSURANCE/QUALITY CONTROL
ANALYSES OF MERCURY, POLYCHLORINATED
BIPHENYLS, AND ORGANICS
IN FISH SAMPLES**

Appendix B

RESULTS OF QUALITY ASSURANCE/QUALITY CONTROL ANALYSES OF MERCURY, POLYCHLORINATED BIPHENYLS, AND ORGANICS IN FISH SAMPLES

B.1 MERCURY

Sixty-one pairs of blind duplicate samples of fish muscle tissue were analyzed for mercury and showed a relatively low degree of variation; the mean coefficient of variation (C.V.) between sample pairs was 9%, with a mean standard deviation (SD) of $0.06 \mu\text{g/g}$. The mean absolute difference between duplicate samples was $0.08 \mu\text{g/g}$. The multiple analyses of mercury in EPA reference fish ($n = 45$) agreed well with the expected value, averaging $2.51 \pm 0.09 \mu\text{g/g}$ (mean \pm SD) vs an expected value of $2.52 \mu\text{g/g}$; the average recovery was $100 \pm 4\%$. Split duplicate fish ($n = 20$) samples analyzed for mercury by the ORNL Analytical Chemistry Division (ACD) and the U.S. EPA Environmental Services Laboratory in Athens, Georgia, differed little. Fish analyzed by ORNL averaged $0.87 \mu\text{g/g}$ mercury, while those analyzed by the EPA lab averaged $0.83 \mu\text{g/g}$. The mean difference between individual samples analyzed by EPA and ORNL was $0.04 \mu\text{g/g}$ and was not significantly different from zero ($p > 0.05$); but the mean coefficient of variation (18%) and standard deviation ($0.14 \mu\text{g/g}$) were larger than values observed for duplicate analyses within the ORNL lab. Mercury levels in sunfish from the uncontaminated reference site (Hinds Creek) were typical of background levels in stream fish, averaging $0.08 \pm 0.03 \mu\text{g/g}$ ($n = 45$).

B.2 OTHER METALS

Four pairs of blind duplicate samples were analyzed for metals, and relatively low variation was observed for those metals exceeding detection limits. Mean coefficients of variation for Cd, Cu, Se, and Zn were 42, 41, 11, and 26% respectively; mean standard deviations were 0.014, 0.08, 0.04, and $1.8 \mu\text{g/g}$ respectively. The relatively high coefficients of variation are due in large part to the very low concentrations of metals in these samples. Results of analyses of reference tissues indicated good recoveries and quantitation of the metals (Table B-1).

B.3 PCBs

The results of PCB analyses of 52 pairs of blind duplicate fish samples were somewhat more variable than results of mercury analyses, as is generally the case. The mean absolute difference and standard deviation between duplicates was $0.25 \pm 0.18 \mu\text{g/g}$, with a mean coefficient of variation of 28%. The variabilities in the measurement of PCB-1254 and PCB-1260 were similar, with mean absolute differences between duplicates of 0.11 and $0.18 \mu\text{g/g}$ respectively; standard deviations were 0.08 and $0.13 \mu\text{g/g}$ and the mean coefficients of variation were 29% and 34% respectively. Samples of

Table B-1. Analyses of reference tissues for metals (units are milligrams per gram dry weight)

Metal	Measured	Expected	Sample ^a	Mean % Recovery \pm	SD Range
As	2.46 2.51 2.46	2.43	Fish	102 \pm 1	101-103
Cd	0.15 0.15 0.16	0.16	Fish	96 \pm 4	94-100
Cu	2.22 2.40 2.02	2.21	Fish	100 \pm 9	91-109
Cr	0.96 0.98 0.50	0.58	Fish	93 \pm 6	86-97
Hg	2.51 2.52 2.57 2.61	2.52	Fish	101 \pm 2	100-104
Ni	0.54 0.56 0.53	0.54	Fish	101 \pm 3	98-104
Pb	0.24 0.25 0.29	0.26	Fish	100 \pm 10	92-112
Se	1.25 1.21	1.1	Bovine liver	112 \pm 3	110-114
Zn	47.4 43.5 41.1	43.6	Fish	101 \pm 7	94-109

^aSample types were secured as follows: freeze-dried fish tissue (EPA trace metals in fish) from U.S. EPA, Cincinnati, Oh; and bovine liver was freeze-dried bovine liver (National Bureau of Standards #1577).

uncontaminated fish and clams were spiked at 1 $\mu\text{g/g}$ each of PCB-1254 and PCB-1260 and analyzed along with fish or clam samples. Mean recoveries averaged 92 \pm 18% for total PCBs, and 87 \pm 19% and 97 \pm 15% ($n = 39$) for PCB-1254 and PCB-1260 respectively. Samples of PCB-contaminated carp and channel catfish were homogenized and split for analysis by the ORNL/ACD laboratory and the EPA Environmental Services

Laboratory, Athens, Georgia. Mean levels of total PCBs and PCB-1260 did differ significantly in the nineteen samples analyzed by the two laboratories ($p < 0.05$) primarily because the results for PCB-1254 obtained by the EPA lab were significantly higher than those from the ORNL lab in the December 1986 samples. Results (EPA vs ORNL) averaged 1.85 ± 1.38 vs 1.26 ± 0.87 , 0.82 ± 0.83 vs 0.41 ± 0.38 , and 1.02 ± 0.64 vs 0.85 ± 0.62 $\mu\text{g/g}$ for total PCBs, PCB-1254, and PCB-1260 respectively. If the December 1986 samples were excluded from the comparison, the results of EPA vs ORNL PCB analyses did not differ significantly. The mean difference between sample pairs was 0.12 $\mu\text{g/g}$ (1.46 vs 1.34 $\mu\text{g/g}$). PCB-1254 and PCB-1260 also did not differ, with mean differences of 0.18 (0.62 vs 0.44 $\mu\text{g/g}$) and 0.07 (0.83 vs 0.90 $\mu\text{g/g}$). The variability between duplicate samples analyzed by ORNL and EPA (May 1987–May 1988) was similar to the variability between duplicates analyzed at ORNL, with a mean standard deviation between duplicates of 0.33 , 0.23 , and 0.15 $\mu\text{g/g}$ and a mean coefficient of variation of 25% , 51% , and 21% for total PCBs, PCB-1254, and PCB-1260 respectively. Samples of sunfish collected from a reference site (Hinds Creek) were used as analytical controls; these exhibited very low levels of total PCBs, averaging 0.03 ± 0.02 $\mu\text{g/g}$ ($n = 46$).

B.4 ORGANICS SCREENING ANALYSES

Uncontaminated fish and clam samples were spiked with a mixture of six priority pollutants (PCB-1254, PCB-1260, di-*N*-butylphthalate, 2-ethylhexylphthalate, pyrene, and benzo[*a*]pyrene) and analyzed to ensure that these contaminants would be recovered and quantified in the extraction, clean-up, and gas chromatographic analysis. In the GC/MS analysis, the recovery of di-*N*-butylphthalate and 2-ethylhexylphthalate averaged $57 \pm 3\%$ and $56 \pm 7\%$ respectively, and that of pyrene and benzo[*a*]pyrene averaged $63 \pm 6\%$ and $70 \pm 7\%$ respectively ($n = 4$ for each substance). Recoveries of PAHs were better in the HPLC procedure, averaging $105 \pm 11\%$ for pyrene and $80 \pm 12\%$ for benzo[*a*]pyrene.

Appendix C

CONTAMINANT CONCENTRATIONS IN BIOTA FROM EAST
FORK POPLAR CREEK AND REFERENCE SITES,
DECEMBER 1986–MAY 1988

Table C-1. Mercury, polychlorinated biphenyls (PCBs), and ¹³⁷Cs (in micrograms per gram wet weight) in fish from East Fork Poplar Creek and reference sites, December 1986–January 1987

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 23.4	12/17/86	REDBRE	M	6937	82.3	16.8	1.80	1.11	0.87	0.24	.
EFK 23.4	12/17/86	REDBRE	M	6938	111.3	19.2	1.70	0.68	0.35	0.33	9.4
EFK 23.4	12/17/86	REDBRE	F	6947	65.2	15.2	1.60	1.63	1.30	0.33	.
EFK 23.4	12/17/86	REDBRE	M	6962	83.7	16.6	1.40	1.19	0.93	0.26	10.2
EFK 23.4	12/17/86	REDBRE	F	6974	62.8	15.1	1.80	3.79	2.90	0.89	.
EFK 23.4	12/17/86	REDBRE	M	6975	140.6	18.9	1.40	1.31	0.80	0.51	.
EFK 23.4	12/17/86	REDBRE	F	6989	68.7	15.6	1.60	1.32	0.57	0.75	.
EFK 23.4	12/17/86	REDBRE	F	6996	63.3	15.4	2.30	2.88	1.90	0.98	.
EFK 18.2	12/15/86	REDBRE	F	6911	51.6	14.1	0.91	0.24	0.14	0.10	.
EFK 18.2	12/15/86	REDBRE	M	6917	24.1	11.0	0.73	0.64	0.46	0.18	.
EFK 18.2	12/15/86	REDBRE	M	6945	124.8	19.2	0.88	0.28	0.20	0.08	.
EFK 18.2	12/15/86	REDBRE	M	6965	89.9	17.1	0.92	0.30	0.17	0.13	.
EFK 18.2	12/15/86	REDBRE	F	6999	78.2	16.3	1.00	0.15	0.13	0.02	.
EFK 18.2	12/15/86	REDBRE	M	7574	153.9	19.4	0.86	0.25	0.16	0.09	<2.2
EFK 18.2	12/15/86	REDBRE	M	7578	34.5	12.5	0.79	0.19	0.11	0.08	.
EFK 18.2	12/15/86	REDBRE	F	7596	88.2	17.2	1.20	0.16	0.10	0.06	4.2
EFK 13.8	12/17/86	REDBRE	F	6901	44.4	13.2	0.97	0.15	0.06	0.09	.
EFK 13.8	12/17/86	REDBRE	M	6924	51.2	14.0	0.82	0.60	0.50	0.10	.
EFK 13.8	12/17/86	REDBRE	M	6929	97.2	17.6	0.63	0.28	0.12	0.16	.
EFK 13.8	12/15/86	REDBRE	F	6942	53.0	14.3	0.85	0.15	0.10	0.05	.
EFK 13.8	12/17/86	REDBRE	M	6944	112.5	18.2	0.92	0.21	0.09	0.12	5.2
EFK 13.8	12/17/86	REDBRE	M	6952	121.1	18.9	1.20	0.31	0.11	0.20	.
EFK 13.8	12/17/86	REDBRE	F	6955	42.0	13.1	1.10	0.22	0.10	0.12	.
EFK 13.8	12/17/86	REDBRE	M	6976	66.6	15.4	0.78	0.59	0.20	0.39	.
EFK 6.3	1/07/87	REDBRE	F	6910	51.8	15.5	0.86	0.12	0.02	0.10	.
EFK 6.3	1/07/87	REDBRE	F	6918	64.1	15.8	0.79	0.08	0.03	0.05	.
EFK 6.3	1/07/87	REDBRE	M	6921	83.9	17.4	0.63	0.17	0.06	0.11	.
EFK 6.3	1/07/87	REDBRE	M	6949	93.5	17.8	0.61	0.08	0.03	0.05	.
EFK 6.3	1/07/87	REDBRE	F	6958	44.8	14.3	0.71	0.08	0.04	0.04	.
EFK 6.3	1/07/87	REDBRE	M	6967	104.8	18.0	0.52	0.08	0.03	0.05	11.2
EFK 6.3	1/07/87	REDBRE	M	6971	90.1	17.8	0.61	0.10	0.02	0.08	.
EFK 6.3	1/07/87	REDBRE	F	6998	48.4	14.1	0.87	0.08	0.03	0.05	.
EFK 2.1	1/08/87	REDBRE	M	6909	80.6	17.0	0.40	0.12	0.04	0.08	.
EFK 2.1	1/08/87	REDBRE	M	6931	23.3	11.6	0.37	0.11	0.06	0.05	.
EFK 2.1	1/08/87	REDBRE	F	6932	56.8	14.5	0.46	0.07	0.04	0.03	.
EFK 2.1	1/08/87	REDBRE	M	6941	130.0	19.0	0.44	0.07	0.03	0.04	15.4
EFK 2.1	1/08/87	REDBRE	M	6943	54.7	14.1	0.39	0.06	0.03	0.03	.
EFK 2.1	1/08/87	REDBRE	F	6966	61.5	15.4	0.49	0.06	0.03	0.03	.

Table C-1 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 2.1	1/08/87	REDBRE	M	6977	95.3	17.8	0.41	0.07	0.03	0.04	10.2
EFK 2.1	1/08/87	REDBRE	M	6994	72.4	16.2	0.25	0.05	0.03	0.02	.
HINDSCR	1/08/86	REDBRE	M	8012	40.1	13.6	0.06	0.02	0.01	<0.01	.
EFK 23.4	12/17/86	BLUGIL	M	6956	70.8	15.6	0.78	0.42	0.20	0.22	.
EFK 23.4	12/17/86	BLUGIL	M	6959	75.0	15.8	0.96	0.47	0.34	0.13	.
EFK 23.4	12/17/86	BLUGIL	F	6920	60.5	15.8	1.00	0.18	0.05	0.13	.
EFK 23.4	12/17/86	BLUGIL	F	6928	53.3	14.5	1.20	0.65	0.51	0.14	26
EFK 23.4	12/17/86	BLUGIL	F	6940	40.0	13.2	1.30	1.31	0.50	0.81	.
EFK 23.4	12/17/86	BLUGIL	M	6986	34.4	12.8	0.87	0.70	0.62	0.17	.
EFK 23.4	12/17/86	BLUGIL	M	0100	78.7	16.0	2.00	1.11	0.88	0.23	15.4
EFK 13.8	12/17/86	BLUGIL	M	6964	33.2	12.2	0.31
EFK 6.3	01/07/87	BLUGIL	M	6987	136.0	18.0	0.55	0.05	0.03	0.02	.
EFK 2.1	01/08/87	BLUGIL	F	6915	33.3	12.1	0.32	0.15	0.05	0.10	.
EFK 2.1	01/08/87	BLUGIL	M	6925	69.3	15.7	0.43	0.16	0.08	0.08	.
EFK 2.1	01/08/87	BLUGIL	M	6926	101.6	17.4	0.23	0.04	0.02	0.02	.
EFK 2.1	01/08/87	BLUGIL	M	6934	142.0	18.7	0.82	0.19	0.09	0.10	18
EFK 2.1	01/08/87	BLUGIL	M	6953	90.0	17.6	0.41	0.23	0.03	0.20	.
EFK 2.1	01/08/87	BLUGIL	M	6957	59.2	14.8	0.37	0.09	0.04	0.05	.
EFK 2.1	01/08/87	BLUGIL	M	6961	99.2	17.2	0.38	0.10	0.06	0.04	10.6
EFK 2.1	01/08/87	BLUGIL	M	6988	23.7	11.5	0.04	0.03	0.02	0.01	.
HINDSCR	1/08/87	BLUGIL	M	6900	82.3	16.4	0.05	0.02	0.01	<0.01	.
HINDSCR	1/08/87	BLUGIL	M	6907	49.4	14.4	0.05	0.02	0.01	0.01	.
HINDSCR	1/08/87	BLUGIL	F	6916	56.5	14.9	0.06	0.03	0.02	0.01	.
HINDSCR	1/08/87	BLUGIL	M	6919	65.2	14.8	0.09	0.03	0.02	<0.01	.
HINDSCR	1/08/87	BLUGIL	M	6930	83.5	16.9	0.05	0.02	<0.01	<0.01	.
HINDSCR	1/08/87	BLUGIL	F	6935	49.5	14.1	0.09	0.03	0.02	0.01	.
HINDSCR	1/08/87	BLUGIL	M	6970	108.1	17.9	0.06	0.02	0.01	<0.01	.
HINDSCR	1/08/87	BLUGIL	.	6980	45.5	14.0	.	0.03	0.02	<0.01	.
HINDSCR	1/08/87	BLUGIL	M	6995	85.2	16.5	0.05	0.03	0.02	<0.01	.
EFK 10.0	01/13/87	COCARP	M	6991	2170	54.5	1.00	0.39	0.11	0.28	.
EFK 10.0	01/13/87	COCARP	M	6982	1612	54.0	1.60	0.39	0.03	0.36	.
EFK 10.0	01/13/87	COCARP	M	6912	2094	53.6	1.00	0.70	0.11	0.59	.
EFK 10.0	01/13/87	COCARP	M	6979	2444	57.5	1.00	0.74	0.24	0.50	.
EFK 10.0	01/13/87	COCARP	F	6990	3352	60.0	0.61	0.38	0.10	0.28	.

Table C-1 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 6.3	01/07/87	COCARP	F	6993	2046	56.5	0.75	0.67	0.11	0.56	.
EFK 6.3	01/07/87	COCARP	M	6908	1460	48.0	0.56	1.06	0.44	0.62	.
EFK 6.3	01/07/87	COCARP	F	6936	2436	56.0	0.78	2.08	0.98	1.10	.
EFK 6.3	01/07/87	COCARP	F	6954	1856	54.5	0.97	0.72	0.03	0.69	.
EFK 6.3	01/07/87	COCARP	F	6960	2264	54.4	0.77	0.64	0.12	0.52	.
EFK 6.3	01/07/87	COCARP	M	6913	3100	60.0	0.92	1.56	0.87	0.69	.
EFK 6.3	01/07/87	COCARP	F	6903	1626	51.0	0.97	1.74	0.95	0.79	.
EFK 6.3	01/07/87	COCARP	M	6923	3160	63.7	0.91	1.29	0.19	1.10	.
HINDSCR	01/08/87	COCARP	F	6914	4370	69.7	0.31	0.03	0.01	0.02	.
HINDSCR	05/13/86	COCARP	F	8038A	.	.	.	0.07	0.02	0.05	.
HINDSCR	05/13/86	COCARP	F	8087A	.	.	.	0.09	<0.01	0.08	.
HINDSCR	05/13/86	COCARP	F	8035A	.	.	.	0.02	0.01	0.01	.
HINDSCR	05/13/86	COCARP	F	8095A	.	.	.	0.07	<0.01	0.06	.

^aEFK = East Fork Poplar Creek kilometer; HINDSCR = Hinds Creek.

^bSpecies: REDBRE = redbreast sunfish (*Lepomis auritus*); BLUGIL = bluegill (*Lepomis macrochirus*); COCARP = carp (*Cyprinus carpio*).

^cM = male; F = female.

^dTotal mercury in fish axial muscle, in micrograms per gram wet weight.

^eTotal PCBs (sum of PCB-1254 and PCB-1260) in fish axial muscle, in micrograms per gram wet weight.

^fPCB-1254 (Arochlor-1254) in fish axial muscle, in micrograms per gram wet weight.

^gPCB-1260 (Arochlor-1260) in fish axial muscle; in micrograms per gram wet weight.

^hCesium-137 in fish axial muscle; in micrograms per gram wet weight.

Table C-2. Mercury, polychlorinated biphenyls (PCBs), and ¹³⁷Cs in fish (in micrograms per gram wet weight) from East Fork Poplar Creek and reference sites, May 1987

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 23.4	05/22/87	REDBRE	M	7466	162.7	20.4	1.65	3.70	1.90	1.80	7.6
EFK 23.4	05/22/87	REDBRE	M	7671	39.1	12.9	1.15	0.33	0.15	0.18	.
EFK 23.4	05/22/87	REDBRE	M	7672	72.3	15.4	1.25	0.77	0.42	0.35	.
EFK 23.4	05/22/87	REDBRE	M	7673	118.6	17.8	1.32	0.92	0.38	0.54	10.6
EFK 23.4	05/22/87	REDBRE	F	7675	48.8	13.9	1.25	0.41	0.25	0.16	.
EFK 23.4	05/22/87	REDBRE	M	7684	56.1	15.7	1.35	0.43	0.09	0.34	.
EFK 23.4	05/22/87	REDBRE	M	9115	44.1	13.7	2.25	1.42	0.26	1.16	.
EFK 23.4	05/22/87	REDBRE	F	9170	54.3	15.7	1.68	0.36	0.19	0.17	.
EFK 18.2	05/27/87	REDBRE	F	7139	52.3	13.0	1.22	0.83	0.31	0.52	.
EFK 18.2	05/27/87	REDBRE	M	7237	128.4	17.7	1.16	0.70	0.21	0.49	.
EFK 18.2	05/27/87	REDBRE	M	7429	87.6	15.5	1.40	0.24	0.11	0.13	.
EFK 18.2	05/27/87	REDBRE	F	9119	78.8	15.3	1.17	0.20	0.08	0.12	.
EFK 18.2	05/27/87	REDBRE	M	9139	194.3	18.9	0.80	0.53	0.12	0.41	1.7
EFK 18.2	05/27/87	REDBRE	M	9155	134.8	17.8	1.39	0.46	0.14	0.32	.
EFK 18.2	05/27/87	REDBRE	F	9196	117.7	16.6	1.19	0.40	0.07	0.33	.
EFK 18.2	05/27/87	REDBRE	M	9197	109.3	16.0	0.88	1.16	0.37	0.79	<3.2
EFK 13.8	05/27/87	REDBRE	M	7658	134.4	17.6	0.90	0.65	0.26	0.39	.
EFK 13.8	05/27/87	REDBRE	M	9074	157.9	18.1	0.80	0.30	0.08	0.22	.
EFK 13.8	05/27/87	REDBRE	F	9116	98.2	15.8	0.96	0.09	0.04	0.05	.
EFK 13.8	05/27/87	REDBRE	F	9133	74.6	15.0	0.80	0.18	0.08	0.10	.
EFK 13.8	05/27/87	REDBRE	M	9135	143.2	18.6	0.97	0.22	0.10	0.12	.
EFK 13.8	05/27/87	REDBRE	M	9136	143.6	18.6	0.82	0.46	0.21	0.25	.
EFK 13.8	05/27/87	REDBRE	M	9171	160.6	19.5	0.46	0.11	0.04	0.07	.
EFK 13.8	05/27/87	REDBRE	F	9172	66.6	15.4	0.96	0.38	0.13	0.25	.
EFK 6.3	05/28/87	REDBRE	F	7193	52.2	13.5	0.75	0.19	0.07	0.12	.
EFK 6.3	05/26/87	REDBRE	F	7473	58.0	13.6	0.63	0.10	0.05	0.05	.
EFK 6.3	05/28/87	REDBRE	F	7477	77.6	15.5	0.83	0.21	0.10	0.11	.
EFK 6.3	05/28/87	REDBRE	F	7499	82.6	15.0	0.93	0.21	0.12	0.09	13.2
EFK 6.3	05/28/87	REDBRE	M	9117	70.2	14.5	0.94	0.42	0.19	0.23	.
EFK 6.3	05/28/87	REDBRE	F	9134	70.6	14.0	0.87	0.05	0.02	0.03	.
EFK 6.3	05/28/87	REDBRE	F	9138	75.6	14.9	0.93	0.10	0.06	0.04	.
EFK 6.3	05/26/87	REDBRE	M	9198	115.9	18.9	0.67	0.13	0.03	0.10	11.8
EFK 2.1	05/26/87	REDBRE	M	7467	72.7	15.5	0.79	0.09	0.05	0.04	.
EFK 2.1	05/26/87	REDBRE	M	7478	88.0	16.6	0.35	0.27	0.17	0.10	.
EFK 2.1	05/26/87	REDBRE	M	9118	40.1	12.7	0.62	0.08	0.05	0.03	.
EFK 2.1	06/01/87	REDBRE	F	9159	105.5	16.8	0.71	0.11	0.07	0.04	.
EFK 2.1	05/26/87	REDBRE	M	9175	92.3	17.0	0.32	0.04	0.02	0.02	7.8
EFK 2.1	06/01/87	REDBRE	M	9176	37.7	12.3	0.11	0.24	0.13	0.11	.
EFK 2.1	06/01/87	REDBRE	M	9179	129.1	18.7	0.41	0.24	0.11	0.13	7.2

Table C-2 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
HINDSCR	06/05/87	REDBRE	F	0072	53.5	14.6	0.08	0.03	0.03	<0.01	.
HINDSCR	06/05/87	REDBRE	F	9156	50.9	14.2	.	0.13	0.05	0.08	.
HINDSCR	06/15/87	REDBRE	F	6009	59.0	14.9	0.12	0.03	0.02	0.01	.
HINDSCR	06/15/87	REDBRE	F	6010	76.1	16.9	0.15	0.02	0.01	0.01	.
HINDSCR	06/15/87	REDBRE	M	6014	64.2	15.0	0.04	0.11	0.11	<0.01	.
HINDSCR	06/15/87	REDBRE	M	6017	132.0	18.8	0.11	0.04	0.04	<0.01	.
BULLRUN	06/16/87	REDBRE	F	6023	46.0	13.7	0.11	0.02	0.02	<0.01	.
BULLRUN	06/16/87	REDBRE	M	6034	160.9	18.7	0.08	0.01	<0.01	0.01	.
BULLRUN	06/16/87	REDBRE	F	6037	40.4	12.4	0.06	0.03	0.03	<0.01	.
BULLRUN	06/16/87	REDBRE	M	6038	46.3	13.9	0.05	0.02	0.02	<0.01	.
BRSHYFK	06/19/87	REDBRE	M	6064	84.0	15.0	0.02	0.16	0.16	<0.01	.
BRSHYFK	06/19/87	REDBRE	F	6065	46.0	13.0	0.04	0.07	0.06	0.01	.
BRSHYFK	06/19/87	REDBRE	F	6069	53.9	14.0	0.03	0.29	0.29	<0.01	.
BRSHYFK	06/19/87	REDBRE	M	6079	115.2	18.2	0.03	0.24	0.24	<0.01	.
BEAVRCR	06/23/87	REDBRE	M	6080	69.8	16.1	0.04	0.04	0.03	0.01	.
BEAVRCR	06/23/87	REDBRE	F	6084	62.2	15.4	0.11	0.04	0.03	0.01	.
BEAVRCR	06/23/87	REDBRE	M	6091	53.0	14.3	0.06	0.06	0.05	0.01	.
BEAVRCR	06/23/87	REDBRE	F	6095	41.3	15.5	0.08	0.05	0.04	0.01	.
EFK 23.4	06/18/87	REDBRE	M	6040	63.5	15.4	1.40	0.81	0.21	0.60	.
EFK 23.4	06/18/87	REDBRE	M	6043	62.4	15.0	1.10	0.86	0.29	0.57	.
EFK 23.4	06/18/87	REDBRE	M	6044	51.6	14.7	2.00	1.20	0.37	0.83	.
EFK 23.4	06/18/87	REDBRE	M	6048	100.0	17.0	0.66	0.36	0.14	0.22	.
EFK 23.4	08/06/87	REDBRE	M	6099	123.0	20.0	0.76
EFK 23.4	08/06/87	REDBRE	M	7195	88.6	17.2	1.20
EFK 23.4	08/06/87	REDBRE	M	7427	44.6	12.9	1.70
EFK 23.4	08/06/87	REDBRE	M	7468	161.5	19.1	1.90
EFK 23.4	08/06/87	REDBRE	M	7536	41.7	12.9	1.10
EFK 23.4	08/06/87	REDBRE	M	9048	109.5	17.2	1.50
EFK 23.4	08/06/87	REDBRE	F	9050	36.3	12.5	1.80
EFK 23.4	08/06/87	REDBRE	M	9051	71.0	15.7	1.60
EFK 23.4	08/06/87	REDBRE	M	9058	79.9	16.0	1.30
HINDSCR	5/13/86	REDBRE	M	80521	158.1	19.1	0.06
HINDSCR	5/13/86	REDBRE	M	80522	158.1	19.1	0.07
HINDSCR	5/13/86	BLUGIL	F	80671	47.2	13.0	0.08
HINDSCR	5/13/86	BLUGIL	F	80672	47.2	13.0	0.07
HINDSCR	5/13/86	REDBRE	F	80741	51.0	13.3	0.10
HINDSCR	5/13/86	REDBRE	F	80742	51.0	13.3	0.10

Table C-2 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 23.4	05/22/87	BLUGIL	M	9114	137.2	19.2	0.19	0.19	0.14	0.05	7.2
EFK 23.4	05/22/87	BLUGIL	F	7314	59.6	15.2	1.04	0.62	0.48	0.14	.
EFK 23.4	05/22/87	BLUGIL	M	7426	49.0	14.8	0.63	0.62	0.15	0.47	.
EFK 23.4	05/22/87	BLUGIL	M	7447	29.6	12.1	0.62	0.73	0.53	0.20	.
EFK 23.4	05/22/87	BLUGIL	M	7465	63.2	14.7	0.60	0.15	0.07	0.08	.
EFK 23.4	05/22/87	BLUGIL	M	9173	21.6	11.2	1.01	0.28	0.18	0.10	.
EFK 23.4	05/22/87	BLUGIL	M	9174	69.7	15.3	1.00	0.92	0.58	0.34	12.4
EFK 23.4	05/22/87	BLUGIL	F	7475	59.7	14.8	0.49	0.17	0.10	0.07	.
EFK 6.3	05/28/87	BLUGIL	M	7063	133.6	17.6	0.79	0.10	0.03	0.07	.
EFK 6.3	05/28/87	BLUGIL	M	7060	98.4	16.0	0.68	0.14	0.09	0.05	7.4
EFK 6.3	05/28/87	BLUGIL	.	7072	42.8	12.6	0.32	0.15	0.06	0.09	.
EFK 2.1	05/26/87	BLUGIL	M	7428	79.2	16.0	0.33	0.07	0.03	0.04	.
EFK 2.1	06/01/87	BLUGIL	M	7639	131.3	17.5	0.32	0.13	0.09	0.04	6.8
EFK 2.1	06/01/87	BLUGIL	M	7676	66.6	14.8	0.30	0.24	0.08	0.16	.
EFK 2.1	06/01/87	BLUGIL	M	7678	80.0	15.6	0.25	0.02	0.02	<0.01	.
EFK 2.1	06/01/87	BLUGIL	M	7679	110.2	18.4	0.90	0.58	0.03	0.55	.
EFK 2.1	05/26/87	BLUGIL	M	9137	47.4	12.6	0.32	0.10	0.05	0.05	.
EFK 2.1	05/26/87	BLUGIL	M	9154	67.6	15.7	0.06	0.06	0.03	0.03	.
EFK 2.1	06/01/87	BLUGIL	F	9158	93.5	17.2	0.62	0.24	0.12	0.12	.
EFK 2.1	05/26/87	BLUGIL	F	9199	36.2	12.5	0.32	0.08	0.03	0.05	.
EFK 23.4	08/06/87	BLUGIL	M	7125	30.6	12.2	0.90
EFK 23.4	08/06/87	BLUGIL	M	9049	57.1	14.8	0.60
EFK 23.4	08/06/87	BLUGIL	F	9052	70.4	15.5	0.87
EFK 23.4	08/06/87	BLUGIL	M	9053	54.4	15.1	0.99
EFK 23.4	08/06/87	BLUGIL	M	9054	57.9	14.3	0.41
EFK 23.4	08/06/87	BLUGIL	.	9055	41.4	13.4	1.30
EFK 23.4	08/06/87	BLUGIL	M	0062	51.0	14.2	1.10
HINDSCR	06/05/87	BLUGIL	M	8362	52.6	13.8	.	0.03	0.02	0.01	.
HINDSCR	06/05/87	BLUGIL	M	0078	66.2	15.0	.	0.01	0.01	<0.01	.
HINDSCR	06/05/87	BLUGIL	F	0013	43.6	13.6	.	0.01	0.01	<0.01	.
HINDSCR	06/05/87	BLUGIL	M	9178	62.8	14.7	.	0.02	0.01	0.01	.
HINDSCR	06/05/87	BLUGIL	F	7637	70.8	15.3	.	0.03	0.03	<0.01	.
HINDSCR	06/05/87	BLUGIL	M	0041	49.3	13.7	0.06	0.04	0.03	0.01	.
EFK 23.4	05/22/87	COCARP	F	1271	3240	59.7	0.11	0.59	0.31	0.28	.
EFK 18.2	05/27/87	COCARP	M	1276	2870	59.5	0.31	0.97	0.20	0.77	.
EFK 13.8	05/27/87	COCARP	M	1277	1750	50.7	0.73	1.42	0.12	1.30	.
EFK 13.8	05/27/87	COCARP	M	1278	2470	57.4	0.74	1.17	0.21	0.96	.
EFK 13.8	05/27/87	COCARP	M	1279	2040	55.0	0.58	0.99	0.06	0.93	.

Table C-2 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 13.8	05/27/87	COCARP	M	1280	2070	54.5	0.23	1.31	0.43	0.88	.
EFK 13.8	05/27/87	COCARP	M	1281	2840	60.4	0.53	0.44	0.18	0.26	.
EFK 13.8	05/27/87	COCARP	F	1282	2450	55.2	0.23	0.42	0.16	0.26	.
EFK 6.3	05/26/87	COCARP	M	1272	1750	50.5	0.14	0.60	<0.01	0.60	.
EFK 6.3	05/26/87	COCARP	F	1273	4160	65.0	0.10	2.28	0.08	2.20	.
EFK 6.3	05/28/87	COCARP	F	1283	3610	60.0	0.91	0.64	0.16	0.48	.
EFK 6.3	05/28/87	COCARP	F	1284	1960	51.2	0.88	0.31	0.05	0.26	.
EFK 6.3	05/28/87	COCARP	M	1285	1770	51.0	0.78	0.64	0.07	0.57	.
EFK 6.3	05/28/87	COCARP	F	1286	1070	42.8	0.45	0.31	0.06	0.25	.
EFK 6.3	05/28/87	COCARP	F	1287	1830	50.5	1.11	0.45	0.07	0.38	.
EFK 6.3	05/28/87	COCARP	F	1288	3840	62.2	0.87	1.34	0.39	0.95	.
EFK 2.1	05/26/87	COCARP	M	1274	1700	51.2	0.55	1.10	<0.01	1.10	.
EFK 2.1	05/26/87	COCARP	M	1275	2130	52.5	0.63	0.55	0.03	0.52	.
EFK 2.1	06/01/87	COCARP	F	1290	1573	47.0	0.69	0.97	0.20	0.77	.
EFK 2.1	06/01/87	COCARP	F	1291	1845	52.1	0.71	0.64	<0.01	0.64	.
EFK 2.1	06/01/87	COCARP	M	1292	1354	48.0	0.67	1.58	0.18	1.40	.
EFK 2.1	06/01/87	COCARP	M	1293	1461	47.5	0.69	1.82	0.22	1.60	.
HINDSCR	01/08/87	COCARP	F	1300	4370	69.7	0.30	0.07	0.03	0.04	.
HINDSCR	01/08/87	COCARP	F	1301	4370	69.7	0.27	0.04	0.01	0.03	.
HINDSCR	01/08/87	COCARP	F	1302	4370	69.7	.	0.04	0.01	0.03	.
HINDSCR	01/08/87	COCARP	F	1303	4370	69.7	.	0.05	0.01	0.04	.

^aEFK = East Fork Poplar Creek kilometer; HINDSCR = Hinds Creek; BULLRUN = Bull Run Creek; BRSHYFK = Brushy Fork; BEAVRCR = Beaver Creek.

^bSpecies: REDBRE = redbreast sunfish (*Lepomis auritus*); BLUGIL = bluegill (*Lepomis macrochirus*); COCARP = carp (*Cyprinus carpio*).

^cM = male; F = female.

^dTotal mercury in fish axial muscle, in micrograms per gram wet weight.

^eTotal PCBs (sum of PCB-1254 and PCB-1260) in fish axial muscle, in micrograms per gram wet weight.

^fPCB-1254 (Arochlor-1254) in fish axial muscle, in micrograms per gram wet weight.

^gPCB-1260 (Arochlor-1260) in fish axial muscle; in micrograms per gram wet weight.

^hCesium-137.

Table C-3. Mercury, polychlorinated biphenyls (PCBs), and ¹³⁷Cs in fish (measured in micrograms per gram wet weight) from East Fork Poplar Creek and reference sites, December 1987–January 1988

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 23.4	12/16/87	REDBRE	M	7062	75.3	15.8	0.07	0.11	0.10	0.01	.
EFK 23.4	12/16/87	REDBRE	F	7063	74.4	15.8	3.59	0.75	0.59	0.16	.
EFK 23.4	12/16/87	REDBRE	M	7065	59.8	15.2	0.05	0.07	0.06	0.01	.
EFK 23.4	12/16/87	REDBRE	F	7066	56.3	14.5	0.04	0.05	0.05	<0.01	.
EFK 23.4	12/16/87	REDBRE	M	7067	47.1	13.7	2.98	0.64	0.52	0.12	.
EFK 23.4	12/16/87	REDBRE	F	7068	49.4	14.7	0.12	0.13	0.11	0.02	.
EFK 23.4	12/23/87	REDBRE	M	7133	60.8	14.7	2.50	2.29	2.00	0.29	.
EFK 23.4	12/23/87	REDBRE	M	7134	99.6	17.0	3.20	0.84	0.63	0.21	.
EFK 18.2	12/16/87	HYBRID	.	7079	162.8	19.7	1.34	0.71	0.33	0.38	.
EFK 18.2	12/16/87	REDBRE	F	7076	80.6	17.2	1.45	0.31	0.12	0.19	.
EFK 18.2	12/16/87	REDBRE	M	7080	79.5	16.7	1.06	0.40	0.20	0.20	.
EFK 18.2	12/16/87	REDBRE	F	7081	37.4	12.9	1.63	0.34	0.11	0.23	.
EFK 18.2	01/04/88	REDBRE	F	7171	54.7	14.5	0.70	0.33	0.11	0.22	.
EFK 18.2	01/04/88	REDBRE	M	7172	130.0	20.4	1.34	1.20	<0.01	1.20	.
EFK 18.2	01/04/88	REDBRE	M	7173	133.4	20.1	1.15	0.79	0.27	0.52	.
EFK 18.2	01/04/88	REDBRE	M	7174	144.4	21.0	1.55	1.38	0.28	1.10	.
EFK 18.2	01/04/88	REDBRE	F	7175	130.0	20.8	1.11	1.32	0.02	1.30	.
EFK 13.8	12/16/87	REDBRE	M	7077	122.8	18.6	1.30	1.50	0.30	1.20	.
EFK 13.8	12/16/87	REDBRE	F	7078	48.8	13.9	0.99	0.60	0.22	0.38	.
EFK 13.8	01/14/88	REDBRE	F	7181	50.4	15.1	1.25	0.27	0.03	0.24	.
EFK 13.8	01/21/88	REDBRE	M	7182	94.4	17.7	1.18	0.72	<0.01	0.72	.
EFK 13.8	01/21/88	REDBRE	M	7183	123.2	19.7	1.04	0.93	<0.01	0.93	.
EFK 13.8	01/21/88	REDBRE	F	7184	75.2	15.8	1.06	0.35	0.07	0.28	.
EFK 13.8	01/21/88	REDBRE	F	7185	34.3	12.0	0.78	0.23	0.10	0.13	.
EFK 13.8	01/21/88	REDBRE	F	7186	36.9	13.5	0.66	0.34	0.13	0.21	.
EFK 6.3	12/17/87	REDBRE	M	7100	127.7	18.6	0.73	0.27	0.13	0.14	.
EFK 6.3	12/17/87	REDBRE	M	7101	101.3	17.6	0.65	0.36	0.14	0.22	.
EFK 6.3	12/17/87	REDBRE	F	7102	54.1	15.0	1.02	0.21	0.07	0.14	.
EFK 6.3	12/17/87	REDBRE	F	7103	50.1	13.6	0.46	0.21	0.11	0.10	.
EFK 6.3	12/17/87	REDBRE	F	7104	50.3	15.2	1.04	0.09	0.05	0.04	.
EFK 6.3	12/17/87	REDBRE	F	7105	52.6	14.9	0.91	0.13	0.05	0.08	.
EFK 6.3	12/17/87	REDBRE	F	7106	40.3	12.7	0.59	0.11	0.06	0.05	.
EFK 6.3	12/17/87	REDBRE	M	7107	62.5	14.6	0.61	0.09	0.05	0.04	.
EFK 2.1	12/17/87	REDBRE	M	7083	139.5	20.0	0.36	0.33	0.17	0.16	.
EFK 2.1	12/17/87	REDBRE	M	7084	107.4	18.0	0.33	0.21	0.11	0.10	.
EFK 2.1	12/17/87	REDBRE	F	7085	92.0	16.9	0.67	0.16	0.10	0.06	.
EFK 2.1	12/17/87	REDBRE	F	7086	53.1	15.0	0.52	0.07	0.04	0.03	.
EFK 2.1	12/17/87	REDBRE	M	7087	118.4	18.5	0.32	0.16	0.12	0.04	.
EFK 2.1	12/17/87	REDBRE	F	7088	39.9	12.6	0.47	0.15	0.09	0.06	.
EFK 2.1	12/17/87	REDBRE	F	7089	35.8	12.6	0.50	0.10	0.05	0.05	.

Table C-3 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 2.1	12/17/87	REDBRE	F	7090	31.6	12.4	0.50	0.11	0.05	0.06	.
EFK 2.1	12/22/87	REDBRE	M	7116	110.3	18.0	0.44	0.09	0.04	0.05	.
EFK 2.1	12/22/87	REDBRE	M	7117	93.8	17.3	0.30	0.04	0.02	0.02	.
EFK 2.1	12/22/87	REDBRE	M	7118	55.2	14.5	0.47	0.09	0.03	0.06	.
BEARCR	12/22/87	REDBRE	M	7128	89.0	18.3	0.19	2.6	1.5	1.1	.
BEARCR	12/22/87	REDBRE	F	7129	59.2	16.0	0.24	0.08	0.05	0.03	.
BEARCR	12/22/87	REDBRE	F	7130	48.3	14.2	0.42	0.35	0.18	0.17	.
BEARCR	12/22/87	REDBRE	M	7131	30.8	12.5	0.09	0.23	0.16	0.07	.
HINDSCR	12/30/87	REDBRE	M	7138	61.3	15.6	0.05	0.01	0.01	<0.01	.
HINDSCR	12/30/87	REDBRE	M	7139	77.8	17.3	0.06	0.02	0.01	0.01	.
HINDSCR	12/30/87	REDBRE	M	7140	68.6	16.7	0.05	0.02	0.01	0.01	.
HINDSCR	12/30/87	REDBRE	F	7141	48.9	15.1	0.11	0.02	<0.01	0.01	.
HINDSCR	12/30/87	REDBRE	F	7142	45.9	14.8	0.12	0.02	0.02	<0.01	.
HINDSCR	12/30/87	REDBRE	M	7143	38.2	13.4	0.04	0.02	0.01	0.01	.
HINDSCR	12/30/87	REDBRE	F	7144	36.4	13.0	0.10	0.01	0.01	<0.01	.
HINDSCR	12/30/87	REDBRE	F	7145	27.2	13.1	0.16	0.02	0.02	<0.01	.
EFK 23.4	12/16/87	BLUGIL	F	7071	115.1	18.0	2.80	0.80	0.64	0.16	.
EFK 23.4	12/16/87	BLUGIL	M	7072	95.4	17.0	0.88	0.88	0.76	0.12	.
EFK 23.4	12/16/87	BLUGIL	M	7073	83.0	16.3	0.98	1.01	0.82	0.19	.
EFK 23.4	12/16/87	BLUGIL	F	7074	65.0	15.3	1.94	2.24	1.60	0.64	.
EFK 23.4	12/16/87	BLUGIL	F	7075	49.2	14.4	0.90	1.32	1.10	0.22	.
EFK 23.4	12/23/87	BLUGIL	F	7135	46.4	14.7	1.67	1.87	1.00	0.87	.
EFK 23.4	12/23/87	BLUGIL	M	7136	66.4	16.6	2.50	2.20	1.20	1.00	.
EFK 23.4	12/23/87	BLUGIL	F	7137	65.8	16.3	2.43	1.26	0.36	0.90	.
EFK 6.3	12/17/87	BLUGIL	F	7108	40.4	13.2	0.42	0.31	0.12	0.19	.
EFK 2.1	12/17/87	BLUGIL	.	7091	72.9	16.0	0.62	0.41	0.08	0.33	.
EFK 2.1	12/17/87	BLUGIL	M	7092	71.6	15.3	0.41	0.06	0.03	0.03	.
EFK 2.1	12/17/87	BLUGIL	M	7093	36.4	12.6	0.57	0.24	0.06	0.18	.
EFK 2.1	12/17/87	BLUGIL	F	7094	41.9	13.0	0.56	0.16	0.06	0.10	.
EFK 2.1	12/22/87	BLUGIL	M	7110	135.2	19.0	0.44	0.17	0.07	0.10	.
EFK 2.1	12/22/87	BLUGIL	M	7111	53.4	15.2	0.05	0.03	0.01	0.02	.
EFK 2.1	12/22/87	BLUGIL	M	7112	88.3	16.7	0.35	0.12	0.06	0.06	.
EFK 2.1	12/22/87	BLUGIL	F	7114	57.0	14.7	0.50	0.10	0.04	0.06	.
EFK 2.1	12/22/87	BLUGIL	M	7115	58.1	15.7	0.48	0.10	0.04	0.06	.

Table C-3 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
HINDSCR	12/30/87	BLUGIL	F	7146	54.6	15.3	0.08	0.02	0.02	<0.01	.
HINDSCR	12/30/87	BLUGIL	F	7147	57.5	15.1	0.08	0.01	0.01	<0.01	.
HINDSCR	12/30/87	BLUGIL	F	7148	74.8	16.4	0.11	0.01	0.01	<0.01	.
HINDSCR	12/30/87	BLUGIL	.	7149	58.4	15.0	0.09	0.03	0.03	<0.01	.
HINDSCR	12/30/87	BLUGIL	M	7150	42.5	13.4	0.10	0.03	0.03	<0.01	.
HINDSCR	12/30/87	BLUGIL	F	7151	51.7	14.9	0.15	0.02	0.02	<0.01	.
HINDSCR	12/30/87	BLUGIL	F	7152	41.5	14.2	0.08	0.02	0.02	<0.01	.
HINDSCR	12/30/87	BLUGIL	F	7153	38.2	13.4	0.10	0.02	0.01	0.01	.
BEARCR	12/22/87	ROCKBA	F	7120	74.7	16.7	0.23	0.37	0.22	0.15	.
BEARCR	12/22/87	ROCKBA	M	7121	116.7	19.1	0.24	0.28	0.17	0.11	.
BEARCR	12/22/87	ROCKBA	F	7122	87.5	17.3	0.57	0.28	0.13	0.15	.
BEARCR	12/22/87	ROCKBA	F	7123	73.8	16.4	0.13	0.42	0.22	0.20	.
BEARCR	12/22/87	ROCKBA	F	7124	113.4	19.5	0.71	0.09	0.05	0.04	.
BEARCR	12/22/87	ROCKBA	F	7125	89.9	17.9	0.26	0.27	0.11	0.16	.
BEARCR	12/22/87	ROCKBA	M	7126	98.0	18.0	0.14	0.13	0.06	0.07	.
BEARCR	12/22/87	ROCKBA	M	7127	118.6	19.8	0.26	0.40	0.25	0.15	.
EFK 18.2	01/04/88	COCARP	M	7163	2035	53.0	0.72	1.87	0.67	1.20	.
EFK 18.2	01/04/88	COCARP	F	7164	1965	51.8	0.93	2.40	0.50	1.90	.
EFK 18.2	01/04/88	COCARP	M	7165	2920	59.5	0.59	3.20	1.00	2.20	.
EFK 18.2	01/04/88	COCARP	M	7166	4050	66.7	0.99	2.80	1.20	1.60	.
EFK 18.2	01/04/88	COCARP	M	7167	2890	62.0	0.87	2.52	0.52	2.00	.
EFK 18.2	01/04/88	COCARP	M	7168	4165	65.5	1.13	0.03	0.01	0.02	.
EFK 18.2	01/04/88	COCARP	M	7169	2525	56.0	0.95	2.33	0.73	1.60	.
EFK 18.2	01/04/88	COCARP	F	7170	1000	39.4	0.49	1.06	0.50	0.56	.
EFK 6.3	12/17/87	COCARP	M	7098	1650	52.5	0.81	0.75	0.03	0.72	.
EFK 6.3	12/17/87	COCARP	M	7099	1855	55.5	0.85	0.58	<0.01	0.58	.
EFK 6.3	12/17/87	COCARP	M	7097	1615	56.0	0.55	0.36	0.05	0.31	.
EFK 6.3	01/13/88	COCARP	F	7176	4005	64.5	0.92	0.92	0.20	0.72	.
EFK 6.3	01/13/88	COCARP	F	7177	3035	64.0	1.17	0.53	0.06	0.47	.
EFK 6.3	01/13/88	COCARP	F	7178	3005	60.5	1.12	0.95	0.08	0.87	.
EFK 6.3	01/13/88	COCARP	M	7179	535	32.8	0.47	0.35	0.14	0.21	.
EFK 6.3	01/13/88	COCARP	M	7180	1400	45.1	0.68	0.49	0.10	0.39	.
EFK 2.1	12/22/87	COCARP	M	7119	223	25.1	0.56	0.17	0.04	0.13	.

Table C-3 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
HINDSCR	12/30/87	COCARP	M	7155	1880	54.5	0.24	0.20	0.02	0.18	.
HINDSCR	12/30/87	COCARP	M	7156	1430	49.2	0.31	0.03	<0.01	0.03	.
HINDSCR	12/30/87	COCARP	F	7157	1550	48.6	0.17	0.46	<0.01	0.46	.
HINDSCR	12/30/87	COCARP	M	7158	740	40.1	0.09	0.11	<0.01	0.11	.
HINDSCR	12/30/87	COCARP	M	7159	2500	59.5	0.22	0.14	0.04	0.10	.
HINDSCR	12/30/87	COCARP	F	7160	3550	66.1	0.27	0.12	0.03	0.09	.
HINDSCR	12/30/87	COCARP	M	7161	810	39.5	0.13	0.44	<0.01	0.44	.
HINDSCR	12/30/87	COCARP	M	7162	1470	48.3	0.17	0.09	0.03	0.06	.

^aEFK = East Fork Poplar Creek kilometer; HINDSCR = Hinds Creek; BEARCR = Bear Creek.

^bSpecies: REDBRE = redbreast sunfish (*Lepomis auritus*); BLUGIL = bluegill (*Lepomis macrochirus*); COCARP = carp (*Cyprinus carpio*); ROCKBA = rock bass (*Ambloplites rupestris*).

^cM = male; F = female.

^dTotal mercury in fish axial muscle, in micrograms per gram wet weight.

^eTotal PCBs (sum of PCB-1254 and PCB-1260) in fish axial muscle, in micrograms per gram wet weight.

^fPCB-1254 (Arochlor-1254) in fish axial muscle, in micrograms per gram wet weight.

^gPCB-1260 (Arochlor-1260) in fish axial muscle; in micrograms per gram wet weight.

^hCesium-137.

Table C-4. Mercury, polychlorinated biphenyls (PCBs), and ¹³⁷Cs in fish (expressed in micrograms per gram wet weight) from East Fork Poplar Creek and reference sites, May 1988

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 23.4	05/18/88	REDBRE	M	7951	90.0	16.6	0.95	1.49	0.96	0.53	.
EFK 23.4	05/18/88	REDBRE	M	7950	72.0	15.2	1.72	0.37	0.12	0.25	.
EFK 23.4	05/18/88	REDBRE	M	7953	150.0	20.2	1.72	0.16	0.10	0.06	.
EFK 23.4	05/18/88	REDBRE	F	7958	52.0	13.8	1.77	0.76	0.54	0.22	.
EFK 23.4	05/18/88	REDBRE	M	7957	58.0	14.1	1.25	0.64	0.06	0.58	.
EFK 23.4	05/25/88	REDBRE	M	7618	50.8	13.2	0.75	1.12	0.39	0.73	.
EFK 23.4	06/01/88	REDBRE	M	7690	65.5	15.6	2.49	1.60	1.0	0.60	.
EFK 23.4	06/01/88	REDBRE	M	7691	68.8	15.1	0.94	0.75	0.44	0.31	.
EFK 18.2	05/19/88	REDBRE	F	7978	90.0	16.5	2.01	0.18	0.04	0.14	.
EFK 18.2	05/19/88	REDBRE	M	7994	95.8	15.6	1.08	0.48	0.06	0.42	.
EFK 18.2	05/19/88	REDBRE	M	7995	79.9	14.8	0.66	0.34	0.15	0.19	.
EFK 18.2	05/19/88	REDBRE	M	7996	98.1	16.6	0.95	0.30	0.10	0.20	.
EFK 18.2	05/19/88	REDBRE	M	7983	55.0	14.2	0.66	0.28	0.10	0.18	.
EFK 18.2	05/19/88	REDBRE	M	7997	62.9	14.4	0.53	0.47	0.17	0.30	.
EFK 18.2	05/19/88	REDBRE	M	7982	60.0	14.4	0.81	0.74	0.21	0.53	.
EFK 18.2	05/19/88	REDBRE	M	7980	72.0	14.9	0.93	0.64	0.25	0.39	.
EFK 13.8	05/23/88	REDBRE	M	7601	98.0	16.1	0.38	0.09	0.04	0.05	.
EFK 13.8	05/23/88	REDBRE	M	7602	95.0	16.2	0.42	0.30	0.18	0.12	.
EFK 13.8	05/23/88	REDBRE	M	7603	82.0	15.7	0.39	0.18	0.07	0.11	.
EFK 13.8	05/23/88	REDBRE	M	7604	50.5	13.0	0.76	0.49	0.12	0.37	.
EFK 13.8	05/23/88	REDBRE	M	7606	63.5	14.2	0.70	0.58	0.22	0.36	.
EFK 13.8	05/23/88	REDBRE	M	7608	169.1	20.3	0.71	0.29	0.12	0.17	.
EFK 13.8	05/23/88	REDBRE	F	6925	78.2	16.4	0.83	0.50	0.16	0.34	.
EFK 13.8	05/23/88	REDBRE	F	8137	85.6	16.5	1.05	0.46	0.13	0.33	.
EFK 6.3	05/31/88	REDBRE	F	7666	.	16.8	0.83	0.20	0.13	0.07	.
EFK 6.3	05/31/88	REDBRE	M	7630	126.9	17.8	0.63	0.24	0.05	0.19	.
EFK 6.3	05/31/88	REDBRE	M	7631	153.2	19.4	0.83	0.15	0.04	0.11	.
EFK 6.3	05/31/88	REDBRE	M	7632	85.3	15.6	0.42	0.08	0.06	0.02	.
EFK 6.3	05/31/88	REDBRE	M	7633	76.6	15.0	0.47	0.14	0.06	0.08	.
EFK 6.3	05/31/88	REDBRE	M	7669	73.6	14.5	0.63	0.34	0.14	0.20	.
EFK 6.3	05/31/88	REDBRE	M	7667	68.1	14.9	0.49	0.14	0.05	0.09	.
EFK 6.3	05/31/88	REDBRE	M	7635	.	.	0.49	0.19	0.09	0.10	.
EFK 2.1	05/27/88	REDBRE	M	7621	84.5	15.6	0.35	0.16	0.08	0.08	.
EFK 2.1	05/27/88	REDBRE	M	8164	116.0	17.4	0.50	0.07	0.03	0.04	.
EFK 2.1	05/27/88	REDBRE	M	7622	62.8	14.6	0.33	0.12	0.06	0.06	.
EFK 2.1	05/27/88	REDBRE	F	8144	76.6	15.3	0.34	0.17	0.08	0.09	.
EFK 2.1	05/27/88	REDBRE	F	8183	52.3	13.5	0.41	0.28	0.11	0.17	.
EFK 2.1	05/27/88	REDBRE	M	7629	96.5	16.5	0.32	0.18	0.09	0.09	.
EFK 2.1	06/01/88	REDBRE	M	7698	80.7	15.4	0.30	0.25	0.13	0.12	.
EFK 2.1	06/01/88	REDBRE	M	7699	68.4	14.9	0.24	0.21	0.09	0.12	.

Table C-4 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (µg/g)	ΣPCB ^e (µg/g)	1254 ^f (µg/g)	1260 ^g (µg/g)	¹³⁷ Cs ^h (µg/g)
HINDSCR	06/02/88	REDBRE	M	7683	76.3	16.7	0.09	0.04	0.03	0.01	.
HINDSCR	06/02/88	REDBRE	M	7684	71.6	15.7	0.05	0.04	0.03	0.01	.
HINDSCR	06/02/88	REDBRE	F	7685	63.2	15.5	0.12	0.04	0.03	0.01	.
HINDSCR	06/02/88	REDBRE	M	7686	64.5	14.7	0.04	0.03	0.03	<0.01	.
HINDSCR	06/02/88	REDBRE	M	7687	63.5	14.8	0.03	0.06	0.05	0.01	.
HINDSCR	06/02/88	REDBRE	F	7689	77.9	15.5	0.10	0.02	0.02	<0.01	.
HINDSCR	06/02/88	REDBRE	M	7693	62.2	14.7	0.05	0.03	0.03	<0.01	.
HINDSCR	06/02/88	REDBRE	F	7697	60.2	14.3	0.10	0.03	0.03	<0.01	.
EFK 23.4	05/18/88	BLUGIL	F	7970	132.0	19.9	0.67	1.96	1.50	0.46	.
EFK 23.4	05/18/88	BLUGIL	M	7955	96.0	16.7	1.07	0.46	0.18	0.28	.
EFK 23.4	05/18/88	BLUGIL	F	7956	94.0	16.9	1.33	0.27	0.07	0.20	.
EFK 23.4	05/18/88	BLUGIL	M	7971	80.0	16.8	0.54	0.18	0.09	0.09	.
EFK 23.4	05/18/88	BLUGIL	F	7967	74.0	16.5	2.28	0.67	0.36	0.31	.
EFK 23.4	05/18/88	BLUGIL	M	7954	82.0	15.6	2.14	1.33	0.79	0.54	.
EFK 23.4	05/18/88	BLUGIL	M	7962	60.0	15.4	0.26	0.22	0.05	0.17	.
EFK 23.4	05/18/88	BLUGIL	M	7959	78.0	15.4	0.34	0.21	0.09	0.12	.
EFK 2.1	05/27/88	BLUGIL	M	7624	53.3	13.5	0.39	0.32	0.13	0.19	.
EFK 2.1	06/01/88	BLUGIL	M	7676	84.1	15.7	0.34	0.32	0.10	0.22	.
EFK 2.1	05/27/88	BLUGIL	M	7677	128.6	18.9	0.19	0.24	0.23	0.01	.
EFK 2.1	05/27/88	BLUGIL	M	7678	112.6	17.5	0.39	0.11	0.04	0.07	.
EFK 2.1	05/27/88	BLUGIL	M	7679	78.2	15.0	0.31	0.43	0.23	0.20	.
EFK 2.1	05/27/88	BLUGIL	M	7680	102.4	17.0	0.41	0.06	0.02	0.04	.
EFK 2.1	05/27/88	BLUGIL	M	7681	98.3	16.4	0.19	0.30	0.22	0.08	.
EFK 2.1	05/27/88	BLUGIL	M	7682	80.1	15.4	0.32	0.38	0.11	0.27	.
HINDSCR	06/02/88	BLUGIL	M	7200	49.6	14.1	0.07	0.02	0.02	<0.01	.
HINDSCR	06/02/88	BLUGIL	M	7201	86.8	16.9	0.07	0.07	0.07	<0.01	.
HINDSCR	06/02/88	BLUGIL	M	7202	84.2	16.6	0.08	0.04	0.03	0.01	.
HINDSCR	06/02/88	BLUGIL	M	7688	94.5	17.0	0.07	0.03	0.02	0.01	.
HINDSCR	06/02/88	BLUGIL	M	7692	85.4	16.2	0.08	0.03	0.02	0.01	.
HINDSCR	06/02/88	BLUGIL	M	7694	81.6	16.5	0.07	0.05	0.05	<0.01	.
HINDSCR	06/02/88	BLUGIL	M	7695	68.9	15.8	0.07	0.06	0.04	0.02	.
HINDSCR	06/02/88	BLUGIL	M	7696	77.1	15.4	0.06	0.02	0.02	<0.01	.
EFK 18.2	05/19/88	COCARP	M	7986	2798	59.4	0.52	1.53	0.70	0.83	.
EFK 18.2	05/19/88	COCARP	M	7987	3172	60.1	0.64	0.71	0.13	0.58	.
EFK 18.2	05/19/88	COCARP	F	7988	2102	55.6	0.91	0.90	0.08	0.82	.
EFK 18.2	05/19/88	COCARP	M	7989	2809	59.2	0.79	1.45	0.15	1.30	.
EFK 18.2	05/19/88	COCARP	M	7990	1958	53.8	0.35	0.62	0.13	0.49	.
EFK 18.2	05/19/88	COCARP	F	7991	2169	52.8	0.65	0.88	0.25	0.63	.
EFK 18.2	05/19/88	COCARP	M	7992	3192	65.9	1.34	1.85	0.25	1.60	.
EFK 18.2	05/19/88	COCARP	M	7993	2100	55.6	1.33	0.94	0.10	0.84	.

Table C-4 (continued)

Site ^a	Date	Spp. ^b	Sex ^c	Tag #	Wt. (g)	Lgth. (cm)	Hg ^d (μg/g)	ΣPCB ^e (μg/g)	1254 ^f (μg/g)	1260 ^g (μg/g)	¹³⁷ Cs ^h (μg/g)
EFK 13.8	05/23/88	COCARP	M	7609	2921	58.2	0.44	1.03	0.56	0.47	.
EFK 13.8	05/23/88	COCARP	M	7610	2131	54.1	0.87	0.39	0.17	0.22	.
EFK 13.8	05/23/88	COCARP	M	7611	1935	48.8	0.67	2.32	0.52	1.80	.
EFK 13.8	05/23/88	COCARP	M	7612	2702	57.4	1.04	1.70	0.50	1.20	.
EFK 13.8	05/23/88	COCARP	M	7613	2290	52.5	1.25	2.82	0.32	2.50	.
EFK 13.8	05/23/88	COCARP	F	7614	3315	60.2	0.65	1.15	0.19	0.96	.
EFK 13.8	05/23/88	COCARP	M	7615	2343	54.2	1.86	1.04	0.23	0.81	.
EFK 13.8	05/23/88	COCARP	M	7616	1568	.	1.13	1.68	0.18	1.50	.
EFK 6.3	05/31/88	COCARP	M	7660	2324	55.8	0.52	0.47	0.21	0.26	.
EFK 6.3	05/31/88	COCARP	M	7661	3015	65.6	0.41	0.87	0.11	0.76	.
EFK 6.3	05/31/88	COCARP	M	7662	2031	52.9	1.25	0.86	0.21	0.65	.
EFK 6.3	05/31/88	COCARP	F	7663	1862	53.0	0.47	0.16	0.04	0.12	.
EFK 6.3	05/31/88	COCARP	M	7664	1156	47.0	0.60	0.32	0.03	0.29	.
EFK 6.3	05/31/88	COCARP	M	7665	652	35.6	0.46	0.37	0.16	0.21	.
EFK 2.1	05/27/88	COCARP	M	7626	482	30.4	0.45	0.29	0.13	0.16	.
EFK 2.1	05/27/88	COCARP	M	7627	391	28.7	0.51	0.20	0.08	0.12	.
EFK 2.1	05/27/88	COCARP	F	7628	1638	47.7	0.59	0.41	0.16	0.25	.
EFK 2.1	06/01/88	COCARP	M	7671	2946	61.8	0.75	0.35	0.05	0.30	.
EFK 2.1	06/01/88	COCARP	F	7672	2004	55.7	0.91	0.59	0.15	0.44	.
EFK 2.1	06/01/88	COCARP	M	7673	1835	33.2	0.10	0.18	0.10	0.08	.
EFK 2.1	06/01/88	COCARP	M	7674	1503	50.2	0.68	0.12	0.04	0.08	.
EFK 2.1	06/01/88	COCARP	F	7675	1493	48.5	0.23	0.10	0.04	0.06	.
RE-ANALYSIS OF JAN 1988 CARP											
HINDSCR	12/30/87	COCARP	M	7155	1880	54.5	0.24	0.21	0.03	0.18	.
HINDSCR	12/30/87	COCARP	M	7156	1430	49.2	0.31	0.05	0.01	0.04	.
HINDSCR	12/30/87	COCARP	F	7157	1550	48.6	0.16	0.39	<0.01	0.39	.
HINDSCR	12/30/87	COCARP	M	7158	740	40.1	0.09	0.11	<0.01	0.11	.
HINDSCR	12/30/87	COCARP	M	7159	2500	59.5	0.20	0.12	0.03	0.09	.
HINDSCR	12/30/87	COCARP	F	7160	3550	66.1	0.24	0.07	<0.01	0.07	.
HINDSCR	12/30/87	COCARP	M	7161	810	39.5	0.14	0.46	<0.01	0.46	.
HINDSCR	12/30/87	COCARP	M	7162	1470	48.3	0.17	0.12	0.06	0.06	.

^aEFK = East Fork Poplar Creek kilometer; HINDSCR = Hinds Creek.

^bSpecies: REDBRE = redbreast sunfish (*Lepomis auritus*); BLUGIL = bluegill (*Lepomis macrochirus*); COCARP = carp (*Cyprinus carpio*).

^cM = male; F = female.

^dTotal mercury in fish axial muscle, in micrograms per gram wet weight.

^eTotal PCBs (sum of PCB-1254 and PCB-1260) in fish axial muscle, in micrograms per gram wet weight.

^fPCB-1254 (Arochlor-1254) in fish axial muscle, in micrograms per gram wet weight.

^gPCB-1260 (Arochlor-1260) in fish axial muscle; in micrograms per gram wet weight.

^hCesium-137.

Table C-5. Metals (other than mercury) in sunfish (measured in micrograms per gram wet weight) from East Fork Poplar Creek and Hinds Creek, a reference site

Site ^a	Spp ^b	Tag	Date	Sex ^c	Wgt	Lgth	Cd	Cr	Cu	Pb	Se	Zn
EFK 23.4	BLUGIL	6983	1/27/87	M	126.7	18.0	0.013	<0.1	0.39	<0.02	0.55	4.4
EFK 23.4	BLUGIL	0102	1/27/87	M	105.0	18.3	0.005	<0.1	0.38	<0.02	0.49	8.5
EFK 23.4	BLUGIL	0056	1/27/87	M	88.8	17.1	0.009	<0.1	0.33	<0.02	0.65	7.4
EFK 23.4	REDBRE	0047	1/27/87	M	110.0	18.2	0.070	<0.1	0.70	0.06	0.51	6.3
EFK 23.4	REDBRE	0029	1/27/87	M	81.6	16.4	0.009	<0.1	0.50	0.05	0.59	5.6
EFK 23.4	REDBRE	0049	1/27/87	M	91.0	16.2	0.003	<0.1	0.28	0.02	0.59	5.6
EFK 23.4	REDBRE	0082	1/27/87	M	107.6	17.2	0.004	<0.1	0.37	<0.02	0.45	5.0
EFK 23.4	REDBRE	0110	1/27/87	M	81.6	17.3	0.003	<0.1	0.44	<0.02	0.63	8.7
EFK 23.4	BLUGIL	7970	5/18/88	F	132	19.9	0.002	<0.1	0.30	<0.02	0.35	4.0
EFK 23.4	BLUGIL	7956	5/18/88	F	961	6.7	0.002	<0.1	0.06	0.02	0.40	7.7
EFK 23.4	BLUGIL	7954	5/18/88	M	821	5.6	0.006	<0.1	0.45	<0.02	0.17	11.3
EFK 23.4	BLUGIL	7971	5/18/88	F	801	6.8	0.002	<0.1	0.28	<0.02	0.38	4.5
HINDS CR	BLUGIL	6930	1/21/87	M	83.5	16.9	0.004	0.17	0.10	<0.02	0.50	5.8
HINDS CR	BLUGIL	6995	1/21/87	M	85.2	16.5	0.013	0.16	0.17	<0.02	0.46	6.5
HINDS CR	BLUGIL	7202	6/02/88	F	84.2	16.6	<0.002	<0.1	0.06	<0.02	0.14	6.3
HINDS CR	BLUGIL	7694	6/02/88	M	81.6	16.5	0.010	<0.1	0.07	<0.02	0.16	5.6

OTHER METALS BELOW DETECTION LIMIT IN ALL SAMPLES

Metal	Ag	As	Be	Li	Ni	Sb	Tl
Detection limit ($\mu\text{g/g}$ wet wt)	<0.1	<0.05	<0.05	<0.5	<1	<0.3	<0.2

^aEFK = East Fork Poplar Creek kilometer; HINDSCR = Hinds Creek.

^bBLUGIL = Bluegill (*Lepomis macrochirus*); REDBRE = redbreast sunfish (*Lepomis auritus*).

^cM = male, F - female.

Table C-6. Organic contaminants in sunfish (expressed in micrograms per gram wet weight) from East Fork Poplar Creek and Hinds Creek, a reference site

Analyses by GC/MS and HPLC

Site ^a	Species	Tag	Date	Sex	Wgt	Lgth	Compounds detected ($\mu\text{g/g}$)
EFK 23.4	BLUGIL	6983	1/27/87	M	126.7	18.0	PCB-1254—3.9 PCB-1260—0.7
EFK 23.4	BLUGIL	0102	1/27/87	M	105.0	18.3	PCB-1254—0.8
EFK 23.4	BLUGIL	6914	1/27/87	M	71.1	16.5	NONE
EFK 23.4	REDBRE	0038	1/27/87	M	67.7	15.6	PCB-1254—0.9
EFK 23.4	REDBRE	0029	1/27/87	M	81.6	16.4	PCB-1254—3.2
EFK 23.4	REDBRE	0117	1/27/87	M	67.0	15.3	PCB-1254—3.1
EFK 23.4	REDBRE	0082	1/27/87	M	107.6	17.2	PCB-1254—1.4 PCB-1260—0.1
EFK 23.4	REDBRE	0032	1/27/87	M	65.5	15.9	PCB-1254—3.2 PCB-1260—0.3
EFK 23.4	BLUGIL	7203	6/28/88	M	68.6	16.2	Benzo[<i>g,h,i</i>]perylene ^b —0.04 Indenopyrene ^b —0.30
EFK 23.4	BLUGIL	7204	6/28/88	M	56.6	14.9	Benzo[<i>g,h,i</i>]perylene ^b —0.05 Indenopyrene ^b —0.31
EFK 23.4	BLUGIL	7205	6/28/88	M	72.0	16.3	Benzo[<i>g,h,i</i>]perylene ^b —0.06 Indenopyrene ^b —0.35
EFK 23.4	BLUGIL	7206	6/28/88	M	61.6	15.5	Benzo[<i>g,h,i</i>]perylene ^b —0.06 Indenopyrene ^b —0.40
HINDSCR	BLUGIL	7207	6/29/88	M	68.6	16.2	Benzo[<i>g,h,i</i>]perylene ^b —0.03 Indenopyrene ^b —0.20

^aEFK = East Fork Poplar Creek kilometer; HINDSCR = Hinds Creek.

^bThe presence of similar concentrations of benzo[*g,h,i*]perylene and indenopyrene in all samples (Reference site and EFK 23.4), plus the absence of other PAH characteristic of hydrocarbon contamination (pyrene, phenanthrene, benzo[*a*]anthracene, chrysene, etc.) indicates that these results are probably artifacts and do not indicate PAH contamination.

Note: BLUGIL = Bluegill (*Lepomis macrochirus*); REDBRE = redbreast sunfish (*Lepomis auritus*).

Table C-7. Polychlorinated biphenyls (PCBs) (expressed in micrograms per gram wet weight) in composite samples of clams caged in East Fork Poplar Creek for 4 weeks

Site	Exposure period	Sample I.D.	PCB-1254 ($\mu\text{g/g}$)	PCB-1260 ($\mu\text{g/g}$)	Total PCB ($\mu\text{g/g}$)
EFK 26.7	4/28-5/26/87	A		NO SAMPLE (CLAMS DEAD)	
		B		NO SAMPLE (CLAMS DEAD)	
		C		NO SAMPLE (CLAMS DEAD)	
EFK 25.1	4/28-5/26/87	A	0.18	0.01	0.09
		B		NO SAMPLE (CLAMS DEAD)	
		C		NO SAMPLE (CLAMS DEAD)	
EFK 23.7	4/28-5/26/87	A	0.25	0.01	0.26
		B	0.11	<0.01	0.11
		C	0.06	<0.01	0.06
EFK 23.4	4/28-5/26/87	A	0.75	0.02	0.77
		B	0.36	0.02	0.38
		C	0.54	0.02	0.56
EFK 18.2	4/28-5/26/87	A	0.34	0.10	0.44
		B	0.41	0.11	0.52
		C	0.47	0.08	0.55
EFK 13.8	4/28-5/26/87	A	0.35	0.08	0.43
		B	0.41	0.11	0.52
		C	0.40	0.12	0.52
BEAVERCR	4/28/88	A	0.05	0.01	0.06
		B	0.10	<0.01	0.10
		C	0.09	<0.01	0.09
EFK 26.7	4/8-5/6/88	A		NO SAMPLE (CLAMS DEAD)	
		B		NO SAMPLE (CLAMS DEAD)	
		C		NO SAMPLE (CLAMS DEAD)	
EFK 25.1	4/8-5/6/88	A		NO SAMPLE (CLAMS DEAD)	
		B		NO SAMPLE (CLAMS DEAD)	
		C		NO SAMPLE (CLAMS DEAD)	
EFK 24.0	4/8-5/6/88	A	0.02	0.01	0.03
		B	0.03	0.01	0.04
		C	0.03	0.01	0.04
EFK 23.4	4/8-5/6/88	A	0.32	0.01	0.33
		B	0.27	0.01	0.28
		C	0.34	0.01	0.35
EFK 13.8	4/8-5/6/88	A	0.35	0.12	0.47
		B	0.36	0.09	0.45
		C	0.44	0.13	0.57
EFK 6.3	4/8-5/6/88	A	0.23	0.08	0.31
		B	0.27	0.08	0.35
		C	0.24	0.08	0.32
BEARCR	4/8-5/6/88	A	0.70	0.10	0.80
		B	0.91	0.10	1.01
		C	1.10	0.13	1.13
BULLRUN	4/8-5/6/88	A	0.04	0.01	0.05
		B	0.04	0.01	0.05
		C	0.04	0.02	0.06

^aEFK = East Fork Poplar Creek kilometer; BEAVERCR = Beaver Creek; BEARCR = Bear Creek; BULLRUN = Bull Run.

Table C-8. Concentrations of organic chemicals (in micrograms per gram wet weight) detected in clams caged for 4 weeks at East Fork Poplar Creek kilometer, May 18, 1988–June 17, 1988

Each sample is a composite of approximately ten clams

Compound ^a	Sample ^b					
	EFPC-1	EFPC-2	EFPC-3	EFPC-4	Bull Run-1	Bull Run-2
Acenaphthene	0.07	0.12	0.07	0.31	<0.06	<0.06
Anthracene	<0.05	<0.05	<0.05	<0.05 (0.18)	<0.05	<0.05
Benzo[a]anthracene	0.027	0.030	0.040	0.050	<0.001	<0.001
Benzo[a]pyrene	0.08	0.05	0.05 (0.16)	0.10	0.03	0.04
Benzo[b]fluoranthene	0.25	0.25	0.37 (0.14)	0.54	<0.06	<0.06
Chrysene	0.10	0.15	0.15 (0.12)	0.18 (0.16)	<0.01	<0.01
Dibenz[a,h]anthracene	0.020	<0.006	<0.006	<0.006	<0.006	0.012
Naphthalene	<0.2	0.24	0.37	0.41	<0.2	<0.2
Phenanthrene	0.04	0.05	0.07 (0.12)	0.10	<0.02	<0.02
Pyrene	0.12	0.14	0.15 (0.12)	0.20 (0.16)	0.03	0.04
Indenopyrene	0.31	0.23	0.17	0.29	<0.10	<0.10
Fluoranthene	<0.5 (0.16)	<0.5	<0.5 (0.40)	<0.5 (0.42)	<0.5	<0.5
Benzo[g,h,i]perylene	0.08	0.09	0.09	0.09	0.03	<0.02
Benzo[k]fluoranthene	0.044	0.120	0.270	0.090 (0.08)	<0.04	0.04
Di-N-octylphthalate	(0.14)		(0.23)	(0.25)		

^aResults in parentheses are estimated concentrations from gas chromatography/mass spectrometry (GC/MS) analyses in which compounds were detected but were below limits of quantification. Other results are from high performance liquid chromatography (HPLC)/fluorescence.

^bClams removed from Bull Run on May 18, 1988, frozen, and analyzed along with those exposed to East Fork Poplar Creek (EFPC) water.

Table C-9. Detection limits of organic compounds (in milligrams per gram wet weight)

Compound	Detection limit ^a (mg/g)
Capillary Column GC/MS	
Phenol	<2.0
Bis(2-Chloroethylether)	<2.0
2-Chlorophenol	<2.0
1,3-Dichlorobenzene	<2.0
1,4-Dichlorobenzene	<2.0
Benzyl alcohol	<2.0
1,2-Dichlorobenzene	<2.0
2-Methylphenol	<2.0
Bis(2-Chlorodisopropyl)ether	<2.0
4-Methylphenol	<2.0
N-Nitroso-di-n-propylamine	<2.0
Hexachloroethane	<2.0
Nitrobenzene	<2.0
Isophorone	<2.0
2-Nitrophenol	<2.0
2,4-Dimethylphenol	<2.0
Benzoic acid	<10.0
Bis(2-Chloroethoxy)methane	<2.0
2,4-Dichlorophenol	<2.0
1,2,4-Trichlorobenzene	<2.0
Naphthalene	<2.0
4-Chloroaniline	<2.0
Hexachlorobutradiene	<2.0
4-Chloro-3-methylphenol	<2.0
2-Methylnaphthalene	<2.0
Hexachlorocyclapentadiene	<2.0
2,4,6-Trichlorophenol	<2.0
2,4,5-Trichlorophenol	<10.0
2-Chloronaphthalene	<2.0
2-Nitroaniline	<10.0
Dimethylphthalate	<2.0
Acenaphthalene	<2.0
3-Nitroaniline	<10.0
Acenaphthene	<2.0
2,4-Dinitrophenol	<10.0
Nitrophenol	<10.0
Dibenzofuran	<2.0
2,4-Dinitrotoluene	<2.0
2,6-Dinitrotoluene	<2.0
Diethylphthalate	<2.0
4-Chlorophenyl-phenylether	<2.0
Fluorene	<2.0
4-Nitroaniline	<10.0

Table C-9 (continued)

Compound	Detection limit ^a (mg/g)
4,6-Dinitro-2-methylphenol	<10.0
N-Nitrosodiphenylamine	<2.0
4-Bromophenyl-phenylether	<2.0
Hexachlorobenzene	<2.0
Pentachlorophenol	<10.0
Phenanthrene	<2.0
Anthracene	<2.0
Di-N-butylphthalate	<2.0
Fluoranthene	<2.0
Pyrene	<2.0
Butylbenzylphthalate	<2.0
3,3-Dichlorobenzidene	<10.0
Benz[<i>a</i>]anthracene	<2.0
Bis(2-ethylhexyl)phthalate	<2.0
Chrysene	<2.0
Di-N-octylphthalate	<2.0
Benzo[<i>b</i>]fluoranthene	<2.0
Benzo[<i>k</i>]fluoranthene	<2.0
Benzo[<i>a</i>]pyrene	<2.0
Indeno[1,2,3- <i>cd</i>]pyrene	<2.0
Dibenz[<i>a,h</i>]anthracene	<2.0
Benzo[<i>g,i</i>]perylene	<2.0
Capillary Column GC/ECD	
Alpha-bhc	<0.02
Beta-bhc	<0.04
Delta-bhc	<0.04
Gamma-bhc	<0.02
Heptachlor	<0.04
Aldrin	<0.04
Heptachlor epoxide	<0.04
Endosulfan I	<0.04
Dieldrin	<0.04
4,4'-dde	<0.04
Endrin	<0.2
Endosulfan II	<0.08
4,4'-DDD	<0.2
Endosulfan sulfate	<0.2
4,4'-DDT	<0.08
Endrin ketone	<0.4
Methoxychlor	<0.2
Alpha chlordane	<0.04
Gamma chlordane	<0.04
Toxaphene	<2

Table C-9 (continued)

Compound	Detection limit ^a (mg/g)
HPLC with Fluorescence Detection	
Naphthalene	<0.2
Acenaphthene	<0.06
Phenanthrene	<0.02
Anthracene	<0.05
Fluoranthene	<0.5
Pyrene	<0.01
Benz[<i>a</i>]anthracene	<0.001
Benzo[<i>b</i>]fluoranthene	<0.06
Benzo[<i>k</i>]fluoranthene	<0.04
Benzo[<i>a</i>]pyrene	<0.02
Dibenz[<i>a,h</i>]anthracene	<0.006
Benzo[<i>g,h,i</i>]perylene	<0.02
Indeno[1,2,3- <i>cd</i>]pyrene	<0.1

^aOptimum detection limit in 10-g sample. Sample weights varied between 5 and 10 g; detection limits were higher in some samples.

Appendix D

**ANNUAL P/B RATIOS USED TO CALCULATE SECONDARY
PRODUCTION**

Table D-1. Annual production/biomass ratios (P/B) and cohort production intervals (CPI) used for annual production estimates for benthic macroinvertebrates from East Fork Poplar Creek, June 1985 through May 1987 and Brushy Fork, June 1986 through May 1987

Unless otherwise noted, values given for the higher taxa were also used for their respective lower taxa

Taxon	P/B ^a	CPI ^b
Turbellaria	6.0	10.0
Nematoda	10.0	6.0
Oligochaeta	10.0	6.0
Isopoda	5.0	12.0
Amphipoda	5.0	12.0
Decapoda	1.5	36.0
Hydracarina	10.0	6.0
Insecta		
Collembola	10.0	6.0
Ephemeroptera	5.0	12.0
Baetidae	10.0	6.0
<i>Baetis</i> ^c	10.5	3.0
<i>Pseudocloeon</i>	7.5	8.0
Caenidae	10.0	6.0
Ephemerellidae ^d	6.0	10.0
Ephemeridae	5.0	12.0
Heptageniidae	5.0	12.0
Leptophlebiidae	6.5	9.0
Oligoneuriidae	10.0	6.0
Siphonuridae	10.0	6.0
Tricorythidae	30.0	2.0
Odonata		
Anisoptera	2.5	24.0
Zygoptera	5.0	12.0
Plecoptera	5.0	12.0
Capniidae	10.0	6.0
Chloroperlidae	5.0	12.0
Leuctridae	6.7	9.0
Nemouridae	10.0	6.0
Perlidae	5.0	12.0
<i>Phasganophora</i>	2.5	24.0
Perlodidae	5.0	12.0
Taeniopterygidae	10.0	6.0
Hemiptera		
Veliidae	40.0	1.5

Table D-1 (continued)

Taxon	P/B ^a	CPI ^b
Megaloptera	2.5	24.0
<i>Sialis</i>	5.0	12.0
Trichoptera	5.0	12.0
Glossosomatidae		
<i>Agapetus</i>	5.0	12.0
<i>Glossosoma</i>	10.0	6.0
Hydropsychidae		
<i>Cheumatopsyche</i> ^d	10.0	6.0
<i>Diplectrona modesta</i>	5.0	12.0
<i>Hydropsyche</i> ^e	11.0	4.7
<i>Hydropsyche</i> ^f	6.5	9.0
Hydroptilidae	7.5	8.0
Leptoceridae		
<i>Ceraclea</i>	6.0	10.0
Limnephilidae	5.0	12.0
<i>Goera</i>	6.0	10.0
<i>Neophylax</i>	6.0	10.0
Philopotamidae	10.0	6.0
Polycentropodidae	5.0	12.0
Psychomyiidae	5.0	12.0
Rhyacophilidae	5.0	12.0
Coleoptera		
Dryopidae	2.5	24.0
Dytiscidae	10.0	6.0
Elmidae	2.5	24.0
<i>Optioservus</i> ^d	2.5	24.0
<i>Stenelmis</i> ^g	1.8	24.0
Gyrinidae	5.0	12.0
Hydrophilidae	21.0	2.8
Psephenidae	2.5	24.0
Hymenoptera	5.0	12.0
Diptera	5.0	12.0
Ceratopogonidae	10.0	6.0
Chironomidae		
Chironominae		
Chironomini	15.0	4.0
Tanytarsini	26.0	2.3
Diamesinae	10.0	6.0
Orthocladinae	24.0	3.0
Tanypodinae	17.1	3.5
Empididae	5.0	12.0
Psychodidae	5.0	12.0
Simuliidae	15.0	4.0

Table D-1 (continued)

Taxon	P/B ^a	CPI ^b
Tabanidae	6.7	9.0
Tipulidae	5.0	12.0
Gastropoda	3.3	18.0
Ancylidae	8.6	7.0
Bithyniidae	5.0	12.0
Bivalvia		
Corbiculidae	2.5	24.0
Sphaeriidae	3.3	18.0
Unionidae	2.5	24.0

^aValues derived from a theoretical annual P/B of 5.0 and corrected for CPI and are expressed in months (T. F. Waters. "Secondary Production in Inland Waters," Adv. Ecol. Res. **10**:91-164, 1977; T. F. Waters. "Influence of Benthos Life History Upon the Estimation of Secondary Production," J. Fish. Res. Bd. Can. **36**:1425-1430, 1979).

^bValues are derived from published life history information and expressed in months.

^cProduction for *Baetis* was derived empirically during the first year (June 1985 through May 1986) at EFK 13.8 and EFK 18.2. During the second year (June 1986 through May 1987), production estimates at EFK 13.8 and EFK 18.2 were obtained from their empirically derived P/B ratios of 9.7 and 11.3, respectively, from the first year, and at all other sites a P/B ratio of 10.5 was used.

^dProduction at BFK 7.6 was derived empirically.

^eProduction for *Hydropsyche* was derived empirically during the first year (June 1985 through May 1986) at EFK 6.3 and EFK 13.8. During the second year (June 1986 through May 1987) production estimates at EFK 6.3 and EFK 13.8 were obtained from their empirically derived P/B ratios of 10.1 and 11.9, respectively, from the first year, and at all other EFPC sites a P/B of 11.0 was used.

^fValue for Brushy Fork derived from a theoretical annual P/B of 5.0 and corrected for CPI.

^gProduction for *Stenelmis* was derived empirically during the first year (June 1985 through May 1986) at EFK 10.6 and EFK 13.8. During the second year (June 1986 through May 1987), production estimates at EFK 10.6 and EFK 13.8 were obtained from their empirically derived P/B ratios of 1.5 and 2.1, respectively, from the first year, and at all other sites a P/B of 1.8 was used.

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Appendix E

**CHECKLIST OF BENTHIC MACROINVERTEBRATES IN EAST
FORK POPLAR CREEK AND BRUSHY FORK
JUNE 1986-MAY 1987**

Table E-1. Checklist of benthic macroinvertebrate taxa collected from East Fork Poplar Creek and Brushy Fork, June 1986 through May 1987

Taxon	Site ^a						
	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.6	EFK 6.3	BFK 7.6
Turbellaria							
Tricladida	-	-	X ^b	X	X	X	X
Planariidae	-	-	-	X	-	X	X
<i>Dugesia</i>	-	-	-	-	-	-	Q
Nematoda							
	X	X	X	X	X	X	X
Oligochaeta							
Tubificidae	X	X	X	X	X	X	X
	-	X	-	-	-	X	-
Hirudinea							
Glossiphoniidae	-	-	X	-	-	-	-
<i>Placobdella</i> <i>parasitica</i>	-	X	Q	-	-	-	-
	-	-	-	-	-	-	Q
Crustacea							
Isopoda							
Asellidae							
<i>Asellus</i>	-	X	X	X	X	X	-
<i>Lirceus</i>	-	X	X	X	-	X	X
Amphipoda							
Gammaridae							
<i>Crangonyx</i>	-	X	X	-	X	X	-
Decapoda							
Cambaridae							
<i>Cambarus</i>	-	X	X	X	X	-	X
Hydracarina	-	-	-	-	-	-	X
Parasitengona	-	-	-	X	-	-	-
Insecta							
Ephemeroptera							
Baetidae							
<i>Baetis</i>	X	X	X	X	X	X	X
<i>Cloeon</i>	-	Q	Q	Q	-	-	-
<i>Pseudocloeon</i>	-	-	-	X	-	-	X
Baetiscidae							
<i>Baetisca</i>	-	-	-	-	-	-	X
Caenidae							
<i>Caenis</i>	-	-	X	X	-	-	X
Ephemerellidae							
<i>Ephemerella</i>	-	-	-	-	-	-	X
<i>Eurylophella</i>	-	-	Q	Q	Q	Q	Q

Table E-1 (continued)

Taxon	Site ^a						
	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.6	EFK 6.3	BFK 7.6
Ephemeridae	-	-	-	X	-	-	-
<i>Hexagenia</i>	-	-	X	Q	-	Q	X
Heptageniidae							
<i>Stenacron</i>	-	-	-	X	-	Q	X
<i>Stenonema</i>	-	X	X	X	X	X	X
Leptophlebiidae	-	-	-	-	-	Q	-
Oligoneuriidae							
<i>Isonychia</i>	-	X	-	Q	-	-	X
Siphonuridae							
<i>Ameletus</i>	-	-	-	-	-	-	Q
<i>Siphonurus</i>	-	-	-	-	-	-	X
Tricorythidae							
<i>Tricorythodes</i>	-	-	-	X	X	-	-
Odonata							
Anisoptera	-	X	-	-	-	-	-
Aeshnidae	-	-	-	-	X	X	-
<i>Basiaeschna</i>							
<i>janata</i>	-	-	-	-	-	Q	-
<i>Boyeria</i>	-	-	-	-	-	-	X
<i>Boyeria vinosa</i>	-	Q	Q	X	Q	X	-
Gomphidae	-	-	X	-	-	Q	Q
<i>Dromogomphus</i>	-	-	-	-	Q	-	-
<i>Dromogomphus</i>							
<i>spinosus</i>	-	-	-	-	-	Q	-
<i>Gomphus</i>	-	-	Q	-	-	-	-
<i>Hagenius</i>							
<i>brevistalis</i>	-	-	-	-	-	-	Q
<i>Ophiogomphus</i>							
<i>mainensis</i>	-	-	-	X	-	-	X
<i>Progomphus</i>	-	-	X	-	-	-	-
<i>Progomphus</i>							
<i>obscurus</i>	-	-	X	-	-	-	-
<i>Stylogomphus</i>							
<i>albistylus</i>	-	-	-	X	-	X	X
Libellulidae							
<i>Erythemis</i>							
<i>simplicolli</i>	-	X	-	-	-	-	-
<i>Plathemis</i> <i>hydia</i>	-	Q	-	Q	-	-	-
Macromiidae							
<i>Macromia</i>	-	Q	Q	Q	X	Q	-

Table E-1 (continued)

Taxon	Site ^a						
	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.6	EFK 6.3	BFK 7.6
Odonata (cont.)							
Zygoptera							
Calopterygidae							
<i>Calopteryx</i>	-	-	Q	Q	Q	-	Q
<i>Calopteryx maculata</i>	-	-	-	-	-	Q	-
Coenagrionidae	-	-	-	X	-	-	-
<i>Argia</i>	X	Q	Q	X	Q	Q	Q
<i>Enallagma</i>	-	X	Q	Q	-	Q	Q
<i>Ischnura</i>	-	-	-	-	-	-	X
Plecoptera							
Capniidae	-	-	-	X	X	-	X
Chloroperlidae							
<i>Sweltsa</i>	-	-	-	X	-	-	-
Leuctridae							
<i>Leuctra</i>	-	-	-	-	-	-	X
Nemouridae							
<i>Amphinemura</i>	-	-	-	X	-	X	X
Perlidae	-	-	-	X	-	-	X
<i>Phasganophora</i>	-	-	-	X	-	-	-
Perlodidae	-	-	-	-	-	-	X
<i>Yugus</i>	-	-	-	-	-	-	Q
Taeniopterygidae							
<i>Taeniopteryx</i>	-	-	-	-	-	X	X
Megaloptera							
Corydalidae							
<i>Corydalis</i>	-	-	-	X	-	-	-
<i>Corydalis cornutus</i>	-	X	-	X	X	-	-
<i>Nigronia serricornis</i>	X	-	-	X	X	X	X
Sialidae							
<i>Sialis</i>	-	-	-	-	-	X	X
Trichoptera							
Glossosomatidae							
<i>Agapetus</i>	-	-	-	-	-	-	X
<i>Glossosoma</i>	-	-	-	-	-	-	X
Hydropsychidae							
<i>Cheumatopsyche</i>	-	-	X	X	X	X	X
<i>Diplectrona modesta</i>	-	-	-	-	-	-	X
<i>Hydropsyche</i>	X	X	X	X	X	X	X

Table E-1 (continued)

Taxon	Site ^a						
	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.6	EFK 6.3	BFK 7.6
Trichoptera (cont.)							
Hydroptilidae							
<i>Hydroptila</i>	-	-	-	X	-	X	-
<i>Leucotrichia</i>	-	-	-	X	-	-	-
<i>Ochrotrichia</i>	-	-	-	-	-	-	X
Leptoceridae							
<i>Ceraclea</i>	-	-	-	-	-	-	X
<i>Oecetis</i>	-	-	-	-	-	-	Q
<i>Triaenodes</i>	-	-	-	-	-	-	Q
Limnephilidae							
<i>Goera</i>	-	-	-	-	-	-	X
<i>Neophylax</i>	-	-	-	-	-	-	X
<i>Pycnopsyche</i>	-	-	-	-	-	-	X
Philopotamidae							
<i>Chimarra</i>	-	-	-	-	-	-	X
Polycentropodidae							
<i>Cynellus</i>	-	-	-	-	-	-	Q
<i>Phylocentropus</i>	-	-	-	-	-	-	Q
<i>Polycentropus</i>	-	-	-	-	-	-	X
Psychomyiidae							
<i>Lype diversa</i>	-	-	-	Q	Q	-	X
<i>Psychomyia</i>	-	-	-	-	-	-	X
Rhyacophilidae							
<i>Rhyacophila</i>	-	-	-	-	-	-	Q
Hymenoptera							
	-	-	-	-	-	X	X
Coleoptera							
Dryopidae							
<i>Helichus</i>	-	-	-	-	-	-	X
Dytiscidae							
	-	-	Q	-	-	-	Q
Elmidae							
<i>Dubiraphia</i>	-	X	X	X	X	X	-
<i>Optioservus</i>	X	X	X	X	X	X	X
<i>Stenelmis</i>	X	X	X	X	X	X	X
Halipilidae							
<i>Peltodytes</i>	-	Q	Q	Q	-	-	-
Hydrophilidae							
<i>Berosus</i>	-	-	-	X	-	-	-
Psephenidae							
<i>Psephenus herricki</i>	-	-	-	X	X	X	X

Table E-1 (continued)

Taxon	Site ^a						
	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.6	EFK 6.3	BFK 7.6
Diptera							
Ceratopogonidae	-	Q	X	Q	X	X	X
Chironomidae							
Tanypodinae	X	X	X	-	-	X	X
<i>Ablabesmyia</i>	-	X	X	Q	X	X	X
<i>Labrundinia</i>	-	X	-	-	-	-	-
<i>Larsia</i>	-	-	X	X	-	X	X
<i>Larsia?</i>	-	Q	-	X	-	-	-
<i>Natarsia</i>	X	Q	X	X	X	X	X
<i>Nilotanypus</i>	-	X	-	-	X	X	-
<i>Nilotanypus?</i>	-	-	-	-	-	-	X
<i>Procladius</i>	X	X	X	-	-	-	-
<i>Thienemannimyia</i> gp ^c	X	X	X	X	X	X	X
<i>Thienemannimyia</i> gp [?]	-	-	X	-	-	-	-
<i>Zavrelimyia</i>	-	X	-	-	-	-	-
Diamesinae							
<i>Diamesa</i>		X		X			X
<i>Potthastia</i>					X	X	
Orthoclaadiinae							
<i>Brillia</i>	-	X	X	X	X	X	X
<i>Cardiocladius</i>	X	X	-	X	X	X	Q
<i>Cardiocladius?</i>	-	-	-	X	-	X	-
<i>Corynoneura</i>	-	-	-	-	X	X	X
<i>Cricotopus/</i>							
<i>Orthocladus</i> ^d	X	X	X	X	X	X	X
<i>Cricotopus?</i>	-	-	-	X	-	-	-
<i>Eukiefferiella</i>	-	-	-	X	-	-	X
<i>Eukiefferiella?</i>	-	-	-	-	-	-	X
<i>Hydrobaenus</i>	-	-	-	X	X	Q	-
<i>Nanocladius</i>	-	-	X	-	-	Q	X
<i>Parakiefferiella</i>	-	-	-	-	X	X	-
<i>Parakiefferiella?</i>	-	-	-	-	X	X	-
<i>Parametriocnemus</i>	-	-	-	X	-	X	X
<i>Rheocricotopus</i>	X	X	X	X	X	X	X
<i>Rheocricotopus?</i>	-	-	-	X	X	-	-
<i>Synorthocladus</i>	-	-	-	-	-	-	X
<i>Thienemanniella</i>	-	-	X	X	X	X	X
<i>Tvetenia</i>	-	-	-	-	-	-	X
Chironominae							
Chironomini							
<i>Chironomus</i>	-	-	X	X	X	X	-
<i>Chironomus</i>	-	-	X	X	X	X	X
<i>Chironomus?</i>	-	-	-	-	X	-	-

Table E-1 (continued)

Taxon	Site ^a						
	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.6	EFK 6.3	BFK 7.6
Chironomidae (cont.)							
<i>Cryptochironomus</i>	-	-	X	X	X	X	X
<i>Demicryptochironomus</i>	-	-	-	-	-	-	X
<i>Dicrotendipes</i>	-	-	X	X	X	X	X
<i>Dicrotendipes?</i>	-	-	-	X	-	-	-
<i>Endochironomus</i>	-	-	-	-	X	X	-
<i>Microtendipes</i>	-	-	-	-	-	-	X
<i>Paralauterborniella</i>	-	-	-	-	-	-	X
<i>Paratendipes</i>	-	-	-	-	-	X	X
<i>Paratendipes?</i>	-	-	-	-	-	-	X
<i>Phaenopsectra</i>	-	-	X	X	X	X	X
<i>Phaenopsectra?</i>	-	-	-	-	-	X	X
<i>Polypedilum</i>	X	-	X	X	X	X	X
<i>Polypedilum?</i>	-	-	X	-	X	-	-
<i>Stenochironomus</i>	-	X	-	X	X	X	-
<i>Stictoichironomus</i>	-	-	X	-	-	-	X
<i>Tribelos</i>	-	-	-	-	-	-	X
Tanytarsini							
<i>Cladotanytarsus</i>	-	-	X	-	-	-	X
<i>Micropsectra</i>	-	-	-	-	-	X	-
<i>Micropsectra?</i>	-	-	-	X	-	-	-
<i>Paratanytarsus</i>	-	-	X	X	-	X	Q
<i>Paratanytarsus?</i>	-	-	-	-	-	X	X
<i>Rheotanytarsus</i>	-	-	X	X	X	X	X
<i>Rheotanytarsus?</i>	-	-	-	X	-	X	-
<i>Stempellinella</i>	-	-	-	-	-	-	X
<i>Tanytarsus</i>	X	X	X	X	X	X	X
<i>Tanytarsus?</i>	-	-	X	-	-	X	-
Empididae							
<i>Hemerodromia</i>	X	X	X	X	X	X	X
Psychodidae							
<i>Pericoma</i>	-	-	-	-	-	-	X
Simuliidae							
<i>Simulium</i>	-	-	X	X	X	X	X
Tabanidae							
<i>Tabanus</i>	-	Q	X	X	X	-	X
Tipulidae							
<i>Antocha</i>	-	-	-	X	-	X	X
<i>Hexatoma</i>	-	-	-	-	-	-	X
<i>Tipula</i>	X	X	-	X	Q	X	X
Mollusca							
Gastropoda							
Ancyliidae							
<i>Ferrissia</i>	-	-	X	X	X	X	X

Table E-1 (continued)

Taxon	Site ^a						
	EFK 24.4	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.6	EFK 6.3	BFK 7.6
Bithyniidae	-	-	-	-	-	X	-
Lymnaeidae	-	X	X	-	X	X	-
<i>Fossaria</i>	-	X	-	X	-	-	-
Physidae							
<i>Physella</i>	-	X	X	X	X	Q	X
Planorbidae							
<i>Gyraulus</i>	-	-	-	X	X	X	-
Pleuroceridae							
<i>Elimia</i>	-	-	-	-	-	-	X
<i>Pleurocera</i>	-	-	-	-	-	-	X
Bivalvia							
Corbiculidae							
<i>Corbicula</i>							
<i>fluminea</i>	-	-	-	-	-	Q	X
Sphaeriidae							
<i>Pisidium</i>	-	Q	-	X	-	-	X
<i>Sphaerium</i>	-	-	-	X	-	-	X
<i>Sphaerium</i>							
<i>striatinum</i>	-	-	-	-	-	-	X
Unionidae	-	-	-	-	-	-	X
<i>Villosa</i>	-	-	-	-	-	-	X

^aBFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer.

^bAn X indicates that taxon was collected at least once; a blank indicates that a lower level of classification (e.g., family, genus, species) was possible at one or more sites; a hyphen indicates that the taxon was not collected or that the taxon was identified to a lower level at one or more sites; and a Q indicates that the taxon was collected in qualitative samples only.

^cLowercase gp denotes group.

^dBecause of the difficulty in reliably distinguishing the species groups within the genera *Cricotopus* and *Orthocladius*, they have been lumped into the *Cricotopus/Orthocladius* group for all data analyses except in the discussion of taxonomic composition.

Appendix F

**STATISTICAL ANALYSES OF BENTHIC
MACROINVERTEBRATE DATA**

Table F-1. Comparisons of mean benthic macroinvertebrate density and biomass both with and without Decapoda and Mollusca in East Fork Poplar Creek and Brushy Fork, June 1986 through May 1987

Site						
Density - All Taxa						
<u>EFK 13.8</u>	<u>BFK 7.6</u>	<u>EFK 10.6</u>	<u>EFK 18.2</u>	<u>EFK 6.3</u>	<u>EFK 23.4</u>	<u>EFK 24.4</u>
Density - Excluding Decapoda and Mollusca						
<u>EFK 13.8</u>	<u>BFK 7.6</u>	<u>EFK 10.6</u>	<u>EFK 18.2</u>	<u>EFK 6.3</u>	<u>EFK 23.4</u>	<u>EFK 24.4</u>
Biomass - All Taxa						
<u>BFK 7.6</u>	<u>EFK 13.8</u>	<u>EFK 23.4</u>	<u>EFK 6.3</u>	<u>EFK 18.2</u>	<u>EFK 10.6</u>	<u>EFK 24.4</u>
Biomass - Excluding Decapoda and Mollusca						
<u>EFK 13.8</u>	<u>BFK 7.6</u>	<u>EFK 6.3</u>	<u>EFK 23.4</u>	<u>EFK 10.6</u>	<u>EFK 18.2</u>	<u>EFK 24.4</u>

Note: Sites not connected by the same line are significantly different ($p < 0.05$) based on Tukey's studentized range (HSD) test. Sites are arranged in order of highest to lowest values from left to right. Differences are based on 12 sampling periods. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table F-2. Comparisons of benthic macroinvertebrate taxonomic richness, EPT richness^a, and taxonomic diversity in East Fork Poplar Creek and Brushy Fork, June 1986 through May 1987

Parameter per site ^b						
Richness						
<u>BFK 7.6</u>	<u>EFK 13.8</u>	<u>EFK 10.6</u>	<u>EFK 18.2</u>	<u>EFK 6.3</u>	<u>EFK 23.4</u>	<u>EFK 24.4</u>
EPT Richness						
<u>BFK 7.6</u>	<u>EFK 13.8</u>	<u>EFK 6.3</u>	<u>EFK 18.2</u>	<u>EFK 10.6</u>	<u>EFK 23.4</u>	<u>EFK 24.4</u>
Diversity						
<u>BFK 7.6</u>	<u>EFK 13.8</u>	<u>EFK 10.6</u>	<u>EFK 18.2</u>	<u>EFK 6.3</u>	<u>EFK 23.4</u>	<u>EFK 24.4</u>

^aRichness of Ephemeroptera, Plecoptera, and Trichoptera only.

^bEFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Note: Sites not connected by the same line are significantly different ($p < 0.05$) based on Tukey's studentized range (HSD) test. Sites are arranged in order of highest to lowest values from left to right.

Table F-3. Comparisons of mean annual production to biomass (P/B) ratios of the benthic macroinvertebrate communities in East Fork Poplar Creek and Brushy Fork during Year 1 (June 1985 through May 1986) and Year 2 (June 1986 through May 1987)

Site per year ^a						
Year 1						
<u>EFK 23.4</u>	<u>EFK 18.2</u>	<u>EFK 10.6</u>	<u>EFK 24.4</u>	<u>EFK 6.3</u>	<u>EFK 13.8</u>	
Year 2						
<u>EFK 23.4</u>	<u>EFK 24.4</u>	<u>EFK 6.3</u>	<u>EFK 18.2</u>	<u>EFK 10.6</u>	<u>EFK 13.8</u>	<u>EFK 7.6</u>

^aEFK = East Fork Poplar Creek kilometer.

Note: Comparisons are based on 60 samples. Sites not connected by the same line are significantly different ($p < 0.05$) based on Tukey's studentized range test (HSD). Sites are arranged in order of highest to lowest values from left to right.

Appendix G

METHODOLOGY FOR INDEX OF BIOTIC INTEGRITY

Appendix G

METHODOLOGY FOR INDEX OF BIOTIC INTEGRITY

The fish population data at each site were analyzed using the Index of Biotic Integrity (IBI). The IBI evaluates “the ability to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region” (Karr and Dudley 1981). The IBI includes measures of species richness and composition, trophic composition, and fish abundance and condition using 12 metrics (Table G-1) originally based on studies in the Midwest (Karr 1981, Karr et al. 1986). Application of the IBI results in a numerical value between 12 and 60 that can be translated into a comparative descriptive evaluation of the fish community. Because of geographic differences in species distribution, many of the 12 metrics have been modified in order to use the IBI in different regions of the U.S. (Fausch et al. 1984, Miller et al. 1988). Also, more widespread use of the IBI has led to suggestions for replacement metrics that may better address the intent of the original metrics in a particular region (Ohio EPA 1988). These modifications have not diminished the value of the evaluation (Miller et al. 1988).

As suggested by Karr and others (Karr et al. 1986, Ohio EPA 1988), modifications were made to the basic IBI metrics to reflect differences in the Clinch River System in the Oak Ridge area, which includes EFPC. Using historical surveys of the area (Fitz 1968, Etnier 1978), regional distribution information (Lee et al. 1980, Ryon and Loar 1988, M. G. Ryon ESD/ORNL, unpublished data), and unpublished species accounts (Etnier 1987), a baseline set of species was selected that would be present under best conditions in the EFPC area (Table G-2). These species were rated for tolerance to stream impacts, evaluated for spawning pattern, and grouped by trophic level using Saylor (personal communication, 1988), Ohio EPA (1987), Etnier (1987), Karr et al. (1986), Becker (1983), Trautman (1981), and Smith (1979). Additional surveys were made in the Hinds Creek watershed, a comparably sized system located east of the EFPC system in a similar hydrologic and geologic valley and part of the Clinch River system. These surveys were performed using the same techniques as the fish population surveys to evaluate the relationship of abundance and species composition to watershed area (M. G. Ryon ESD/ORNL, unpublished data). The resulting modified IBI is shown in Table G-3.

Several major changes were made in the metrics of the IBI as designed by Karr for implementation in the EFPC evaluations. Metrics 5, 6, 7, 8, 10, and 11 were modified for the Clinch River area. Additionally, there were modifications resulting from the geographic species changes.

The use of the intolerant species (metric 5) was modified to reflect a range of species sensitivity from very intolerant to moderately intolerant to slightly intolerant species. A similar approach was used by Ohio EPA (1987, 1988). The range allowed a more flexible interpretation of this metric and in a situation with limited species, such as EFPC, gave greater sensitivity to the analysis. The number of species in the three categories (very, moderately, and slightly) were multiplied by 1.25, 1.0, or 0.8 to get the values for metric 5 in Table G-3.

Metric 6, proportion of green sunfish, was changed to the proportion of tolerant species because the green sunfish was not a common species in the Clinch River system. The proportion of tolerant species has been used in other regions to deal with this

Table G-1. Index of biotic integrity metrics used to assess stream fish communities in the Midwest

Category	Metric	Scoring criteria		
		5	3	1
Species richness and composition	1. Total number of fish species	Expectations for metrics 1-5 vary with stream size and region		
	2. Number and identity of darter species			
	3. Number and identity of sunfish species			
	4. Number and identity of sucker species			
	5. Number and identity of intolerant species			
	6. Proportion of individuals as green sunfish	<5%	5-20%	>20%
Trophic composition	7. Proportion of individuals as omnivores	<20%	20-45%	>45%
	8. Proportion of individuals as benthic insectivorous cyprinids	>45%	45-20%	<20%
	9. Proportion of individuals as top carnivores	>5%	5-1%	<1%
Fish abundance	10. Number of individuals in sample	Expectations vary with stream size and other factors		
	11. Proportion of individuals as hybrids	0%	>0-1%	>1%
	12. Proportion of individuals with disease, tumors, fin damage, and skeletal anomalies	0-2%	>2-5%	>5%

Source: J. R. Karr et al., "Assessing Biological Integrity in Running Waters: A Method and its Rationale," *Illinois Natural History Survey, Special Publication 5*, 1986.

Table G-2. List of species found in streams in the Clinch River drainage near Oak Ridge, Tennessee, with information on trophic group, tolerance rankings, and reproductive guilds

Species	Trophic group ^a	Tolerance ranking ^b				Breed group ^c
		TOL	VIN	MIN	SIN	
<i>Ichthyomyzon castaneus</i>						
<i>Lampetra appendix</i>					X	
<i>Dorosoma cepedianum</i>	GEN	X				
<i>Campostoma anomalum</i>						
<i>Cyprinus carpio</i>	GEN	X				
<i>Notemigonus crysoleucas</i>	GEN	X				
<i>Lythrurus ardens</i>						X
<i>Notropis amblops</i>	BIN				X	X
<i>N. atherinoides</i>						X
<i>Luxilus chrysocephalus</i>	GEN	X				X
<i>Cyprinella galactura</i>				X		
<i>C. spiloptera</i>		X				
<i>N. telescopus</i>			X			
<i>N. volucellus</i>	GEN			X		
<i>Pimephales notatus</i>						
<i>Rhinichthys atratulus</i>	GEN					X
<i>Semotilus atromaculatus</i>	GEN	X				
<i>Catostomus commersoni</i>	GEN	X				X
<i>Hypentelium nigricans</i>	BIN			X		X
<i>Ictiobus bubalus</i>	GEN					
<i>Minytrema melanops</i>	GEN			X		X
<i>Moxostoma duquesnei</i>	BIN				X	X
<i>M. erythrurum</i>	BIN				X	X
<i>Ameiurus melas</i>	GEN	X				
<i>A. natalis</i>	GEN	X				
<i>Ictalurus punctatus</i>	GEN					
<i>Fundulus notatus</i>		X				
<i>F. olivaceus</i>						
<i>Gambusia affinis</i>		X				
<i>Labidesthes sicculus</i>						
<i>Ambloplites rupestris</i>	PIS				X	
<i>Lepomis auritus</i>						
<i>L. cyanellus</i>		X				
<i>L. gulosus</i>	GEN					

Table G-2 (continued)

Species	Trophic group ^a	Tolerance ranking ^b				Breed group ^c
		TOL	VIN	MIN	SIN	
<i>L. macrochirus</i>	GEN					
<i>L. megalotis</i>	GEN				X	
<i>Micropterus dolomieu</i>	PIS					
<i>M. punctulatus</i>	PIS					
<i>M. salmoides</i>	PIS					
<i>Etheostoma blenniodes</i>	BIN				X	X
<i>E. duryi</i>	BIN				X	
<i>E. jessiae</i>	BIN			X		X
<i>E. kenricotti</i>	BIN				X	
<i>E. rufilineatum</i>	BIN			X		X
<i>E. zonale</i>	BIN		X			X
<i>Perca flavescens</i>						
<i>Percina caprodes</i>	BIN				X	X
<i>P. evides</i>	BIN		X			X
<i>P. sciera</i>	BIN		X			X
<i>E. simoterum</i>	BIN				X	
<i>Aplodinotus grunniens</i>	BIN					
<i>Cottus carolinae</i>	BIN			X		

^aTrophic group information is given for use in metrics 7, 8, and 9. BIN = benthic insectivore; GEN = generalist feeder; PIS = piscivore.

^bAn evaluation of tolerance ranking is assigned to intolerant or tolerant species; species of intermediate tolerance are not indicated. TOL = tolerant; VIN = very intolerant; MIN = moderately intolerant; SIN = slightly intolerant.

^cBreeding group information is given for metric 11, lithophilic spawner.

problem (Miller et al. 1988) and was even suggested as a replacement metric in the original IBI (Karr et al. 1986). The original intent of the metric, to indicate degradation by following changes in abundance of an extremely tolerant species, was still satisfied by using changes in the proportion of all tolerant species.

The proportion of omnivore species, metric 7, was replaced by the proportion of generalist feeders. Because omnivores do not occur in all sizes of streams and the number of omnivore species is limited in EFPC, use of this metric could be misleading. The concept of a generalist feeder—or a species that readily switches between benthic invertebrates, terrestrial insects, periphyton, and zooplankton, depending on the abundance of the food item—has been used as a substitute for omnivores by Leonard and

Table G-3. Index of biotic integrity metrics used to assess fish communities in streams near Oak Ridge, Tennessee, in the Clinch River system

Category	Metric	Scoring criteria		
		5	3	1
Species richness and composition	1. Total number of fish species ^a	>30	30–15	<15
	2. Number and identity of darter species	>6	6–4	<4
	3. Number and identity of sunfish species	>4	4–2	1–0
	4. Number and identity of sucker species	>4	4–2	1–0
	5. Number and identity of intolerant species ^b	>13	13–6	<6
	6. Proportion of individuals as tolerant species	<5%	5–20%	>20%
Trophic composition	7. Proportion of individuals as generalist feeders	<20%	20–45%	>45%
	8. Proportion of individuals as benthic insectivores	>45%	45–20%	<20%
	9. Proportion of individuals as piscivores	>5%	5–1%	<1%
Fish abundance	10. Density, individuals/m ²			
	EFK 23.4	>1.8	1.8–0.8	<0.8
	EFK 18.2	>1.4	1.4–0.6	<0.6
	EFK 13.8	>1.4	1.4–0.5	<0.5
	EFK 10.0	>1.3	1.3–0.4	<0.4
	EFK 6.3	>1.2	1.2–0.2	<0.2
	BFK 7.6	>1.5	1.5–0.6	<0.6
	11. Proportion of individuals as lithophilic spawners ^c	>36%	36–18%	<18%
	12. Proportion of individuals with disease skin tumors, fin damage, skeletal anomalies, or external parasites	0–2%	>2–5%	>5%

^aNumber of native species, excluding recent introductions or stocked species.

^bIntolerant species ranked as very intolerant, moderately intolerant, or slightly intolerant with a correction factor of 1.25, 1.0, or 0.8, respectively, applied to the number in each category to achieve the numbers used in the criteria rankings.

^cPercentages Source: Ohio Environmental Protection Agency (EPA), *Biological Criteria for the Protection of Aquatic Life: Volume III, Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Microinvertebrate Communities*, Ohio Environmental Protection Agency, Division of Water Quality Monitoring and Assessment, Columbus, Ohio, 1987; *Biological Criteria for the Protection of Aquatic Life: Volume II, Users Manual for Biological Field Assessment of Ohio Surface Streams*, Ohio Environmental Protection Agency, Division of Water Quality Monitoring and Assessment, Columbus, Ohio, 1988.

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Orth (1986). The original intent of the metric was to reflect disturbance of the food base by an increase in omnivory. An increase in the proportion of species that can switch between widely varying food items should reflect similar disturbances.

The proportion of insectivorous cyprinids, metric 8, was another metric frequently modified for other regions (Miller et al. 1988) and for the Clinch River area was changed to focus on benthic insectivores. This approach was similar to that used by Leonard and Orth (1986) but not as broad (all insectivores) as that used by Ohio EPA (1988). The goal of this metric was to identify impacts on benthic invertebrates, by monitoring the abundance of insectivores. Because species that feed in midwater and surface areas of streams may not reflect changes in benthic invertebrate abundance, this metric was limited to insectivores that could be classified as benthic feeding specialists. Adjustment of the metric to include all insectivores was considered to be too broad for the Clinch River area.

Metric 10, number of individuals in a sample (Table G-3) was modified to reflect the area sampled. Thus, the density of fish in a sample was used for the Clinch River area. This approach made site comparisons more standard and was especially useful for the BMAP studies, because the population sampling at each site was restricted to sampling sites of known length. This approach may limit species richness but provides good population estimate and density values. The supplemental sampling in the Hinds Creek watershed to provide abundance data was therefore designed to simulate the BMAP sampling. As part of the modification of this metric, the densities were scaled to a combination of the watershed area and a measured discharge. The Y-12 Plant uses the Clinch River as a source for cooling and process water, and this additional water is discharged at the EFPC headwaters. Thus the upper sites in EFPC have a much larger flow than indicated by their watershed area. Multiplying the watershed area and the discharge provided a method that compensated for the significant augmentation of flows that occurs in EFPC.

The proportion of hybrid individuals, metric 11, was designed to reflect disturbances on reproductive isolation and success (Karr et al. 1986). However, hybrids can be difficult to identify in the field and often occur at sites of high biotic integrity (Ohio EPA 1988). This metric was replaced by examining the proportion of individuals in the sample that are simple lithophilic spawning species, as suggested by Ohio EPA (1988). Since lithophilic spawners release their eggs in gravel without parental care, they are affected by increases in siltation and pollutants (Berkman and Rabeni 1987) and the proportion reflects the reproductive concerns contained in Karr's original metric.

The modifications in the IBI for the Clinch River area reflected changes appropriate for use in East Tennessee and were similar to species lists used for other areas of East Tennessee by TVA (Saylor, personal communication, 1988) and changes implemented by other agencies (Ohio EPA 1987, 1988). The IBI was at a preliminary stage for this report and the specific calculations may vary as additional reference sites are sampled (e.g., for abundance metrics) or specific metrics are further refined. However, the descriptive classifications (very poor, poor, fair, good, or excellent) for each site should not vary significantly even with further refinements.

In addition to the IBI modified for the Clinch River in the Oak Ridge area, a modified IBI developed by the Ohio EPA (1987, 1988) for use in headwater streams (drainage area less than $\sim 32 \text{ km}^2$) was applied to the EFPC data. In this system, Karr's original metrics dealing with suckers, sunfish, and top carnivores were replaced with metrics dealing with headwater species, minnows, and pioneering species (Table G-4). Other metrics for darters, insectivores, and lithophilic spawners were slightly modified to

Table G-4. Index of biotic integrity metrics used to assess headwater stream fish communities near Oak Ridge, Tennessee, in the Clinch River system

Metric	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
1. Total species ^a						
5	>6	>12	>14	>14	>16	>14
3	3-6	6-12	7-14	7-14	8-16	7-14
1	<3	<6	<7	<7	<8	<7
2. Number of darter per sculpin						
5	>1	>3	>3	>4	>4	>3
3	1	2-3	2-3	2-4	2-4	2-3
1	0	<2	<2	<2	<2	<2
3. Number of headwater species						
5	>3					
3	3-2					
1	<2					
4. Number of minnow species						
5	>3	>5	>6	>6	>7	>6
3	2-3	3-5	3-6	3-6	3-7	3-6
1	<2	<3	<3	<3	<3	<3
5. Number of sensitive species ^b						
5	>1	>4	>5	>5	>6	>5
3	1	2-4	2-5	3-5	3-6	2-5
1	0	<2	<2	<3	<3	<2
6. Proportion of tolerants ^c						
5	<34%					
3	34-57%					
1	>57%					
7. Proportion of omnivores ^c						
5	<8%	<13%	<14%	<15%	<15%	<14%
3	8-14%	13-25%	14-28%	15-28%	15-30%	14-28%
1	>14%	>25%	>28%	>28%	>30%	>28%

Table G-4 (continued)

Metric	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
8. Proportion of insectivores ^c						
5	>20%	>37%	>42%	>44%	>46%	>42%
3	10-20%	18-37%	21-42%	25-46%	24-46%	21-42%
1	<10%	<18%	<21%	<22%	<24%	<21%
9. Proportion of pioneering species ^c						
5	<30%					
3	30-55%					
1	>55%					
10. Number of individuals in sample ^d						
5	>3.0					
3	1.0-3.0					
1	<1.0					
11. Number of lithophilic spawners						
5	>2	>5	>6	>6	>7	>6
3	1-2	2-5	3-6	3-6	3-7	3-6
1	<1	<2	<3	<3	<3	<3
12. Proportion of individuals with disease						
5	0-2%					
3	>2-5%					
1	>5%					

^aNumber of native species, excluding recent introductions or stocked species.

^bIntolerant species ranked as very intolerant or moderately intolerant.

^cSource (for percentages): Ohio Environmental Protection Agency (EPA), *Biological Criteria for the Protection of Aquatic Life: Volume III, Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Microinvertebrate Communities*, Ohio Environmental Protection Agency, Division of Water Quality Monitoring and Assessment, Columbus, Ohio, 1987; *Biological Criteria for the Protection of Aquatic Life: Volume II, Users Manual for Biological Field Assessment of Ohio Surface Streams*, Ohio Environmental Protection Agency, Division of Water Quality Monitoring and Assessment, Columbus, Ohio, 1988.

^dDensity, number of fish per square meter.

EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

deal with the changes inherent in smaller stream systems. This headwater IBI was modified to fit EFPC data, primarily by adjusting some parameters for drainage areas, and used for comparisons with the Clinch River IBI. Some reservations as to the results of the headwater IBI evaluation were necessary, because some of the values for the metrics were based on data for 300 reference sites in Ohio and as such may not be totally applicable to EFPC.

Appendix H

**DENSITY, BIOMASS, GROWTH, AND CONDITION FACTOR
DATA FOR FISH POPULATION SURVEYS OF EAST FORK
POPLAR CREEK, OCTOBER 1986–MARCH 1988**

Table H-1. Fish densities and biomass (in parentheses) for October–December 1986 in East Fork Poplar Creek and the reference site, Brushy Fork

Density expressed as number of fish per 10 m²; biomass in grams per 10 m²

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Petromyzontidae						
American brook lamprey						0.03 (0.2)
Clupeidae						
Gizzard shad	0.04 (2.8)	0.36 (37.8)		0.04 (5.1)	0.26 (18.8)	
Cyprinidae						
Bigeye chub						0.02 (<0.1)
Bluntnose minnow		0.04 (<0.1)		0.17 (0.2)	0.11 (0.3)	
Blacknose dace	3.06 (4.0)	0.98 (0.9)	0.11 (0.2)	0.09 (0.1)	0.03 (<0.1)	0.03 (<0.1)
Creek chub	0.77 (18.9)	0.12 (0.1)	0.05 (0.1)			
Emerald shiner					0.05 (0.2)	0.09 (0.2)
Spotfin shiner						0.02 (<0.1)
Striped shiner	18.10 (30.8)	1.47 (3.8)	0.89 (4.0)	0.59 (3.6)	0.30 (1.7)	0.10 (1.1)

Table H-1 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Stoneroller	19.30 (88.9)	0.05 (0.1)	1.22 (5.4)	1.53 (5.7)	0.48 (2.1)	0.53 (2.5)
Catostomidae Black redhorse						0.06 (20.0)
Northern hogsucker				0.05 (7.3)	0.02 (0.1)	0.06 (4.6)
Spotted sucker	0.13 (8.4)	0.04 (3.3)			0.03 (1.7)	
White sucker	2.29 (22.7)	0.03 (0.1)	0.05 (0.8)		0.02 (0.3)	0.06 (7.3)
Ictaluridae Yellow bullhead	0.04 ^a		0.02 (0.3)	0.04 (4.0)	0.05 (25.0)	
Poeciliidae Western mosquitofish	0.11 (<0.1)	4.83 (1.5)	0.52 (0.2)	0.37 (0.2)		
Atherinidae Brook silverside					0.02 (<0.1)	
Centrarchidae Bluegill	0.39 (18.6)	0.04 (3.0)		0.07 (0.1)	0.30 (1.2)	
Redbreast sunfish	1.59 (7.5)	1.42 (6.2)	0.88 (7.8)	0.41 (9.7)	0.38 (4.7)	0.07 (0.5)
Warmouth			0.05 (3.0)		0.02 (<0.1)	

Table H-1 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Rock bass		0.02 (0.9)		0.09 (1.5)		0.08 (3.3)
Largemouth bass	0.06 (15.5)	0.02 (2.1)			0.04 (0.4)	
Percidae						
Blueside darter						0.02 (<0.1)
Greenside darter						0.02 (<0.1)
Logperch					0.02 (0.3)	
Redline darter						0.02 (<0.1)
Snubnose darter				0.19 (0.2)	0.04 (<0.1)	0.24 (0.2)
Sciaenidae						
Freshwater drum					0.03 (4.8)	
Cottidae						
Banded sculpin			0.05 (0.3)	0.11 (0.6)	0.06 (0.2)	0.77 (4.6)
Total density	45.94 ^b	9.42	3.86 ^c	3.75	2.26	2.22
Total biomass	221.8	59.8	22.7	38.2	61.8	44.6

^aBiomass not recorded in the field.

^bIncludes values for hybrid sunfish of 0.06 fish/10 m² and 3.8 g/10 m².

^cIncludes values for hybrid sunfish of 0.02 fish/10 m² and 0.7 g/10 m².

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table H-2. Fish densities (expressed as number of fish per 10 m²) and biomass (expressed in grams per 10 m², in parentheses) for March 1987 in East Fork Poplar Creek and the reference site, Brushy Fork

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	EFK 6.3	BFK 7.6
Petromyzontidae							
American brook lamprey							0.03 (0.3)
Clupeidae							
Gizzard shad			0.13 (12.4)			0.04 (3.5)	
Cyprinidae							
Bigeye chub							0.09 (0.1)
Bluntnose minnow		0.02 (0.1)	0.03 (0.1)	0.35 (1.2)	0.38 (0.9)		
Blacknose dace	2.69 (4.3)	0.03 (<0.1)	0.14 (0.5)	0.07 (0.1)			0.05 (0.1)
Carp						0.03 (65.2)	
Creek chub	0.29 (4.3)	0.04 (0.1)	0.04 (0.1)	0.04 (0.1)			
Emerald shiner						0.09 (0.4)	0.03 (<0.1)
Rosefin shiner		0.02 (0.1)					

Table H-2 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	EFK 6.3	BFK 7.6
Striped shiner	8.52 (28.1)	2.18 (5.9)	0.92 (16.6)	0.28 (2.3)	0.04 (0.3)	0.04 (0.3)	0.39 (5.2)
Stoneroller	16.07 (83.6)	0.12 (1.6)	2.57 (32.4)	3.65 (23.0)	0.26 (1.7)	0.26 (1.7)	0.98 (8.3)
Catostomidae							
Black redhorse					0.04 (35.2)	0.04 (35.2)	0.13 (39.6)
Golden redhorse					0.02 (5.6)	0.02 (5.6)	0.04 (13.2)
Northern hogsucker				0.07 (33.0)			0.10 (5.0)
Smallmouth buffalo					0.05 (167.8)	0.05 (167.8)	
Spotted sucker			0.03 (1.2)		0.09 (5.8)	0.09 (5.8)	0.03 (9.1)
White sucker	1.17 (15.8)	0.05 (1.0)	0.03 (6.6)				0.04 (11.6)
Poeciliidae							
Mosquitofish	0.10 (<0.1)	15.46 (7.7)	0.03 (<0.1)				
Atherinidae							
Brook silverside					0.03 (<0.1)	0.03 (<0.1)	
Centrarchidae							
Bluegill		0.05 (2.9)			0.06 (2.0)	0.06 (2.0)	

Table H-2 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Redbreast sunfish	0.78 (3.3)	3.81 (26.7)	1.09 (9.9)	0.35 (5.3)	0.28 (4.0)	0.10 (18.8)
Warmouth		0.02 (3.4)		0.02 (1.2)		
Rock bass			0.08 (1.9)	0.07 (0.9)	0.02 (0.2)	0.07 (5.9)
Percidae						
Blueside darter						0.07 (0.1)
Logperch					0.03 (0.5)	
Stripetail darter				0.02 (<0.1)		
Snubnose darter				0.04 (<0.1)		1.48 (1.0)
Yellow perch					0.02 (2.6)	
Cottidae						
Banded sculpin			0.09 (0.7)	0.36 (1.7)	0.07 (17.4)	3.01
Total density	29.62	21.82 ^a	5.18	5.32	1.55	6.64
Total biomass	139.4	50.7	82.5	68.8	295.7	135.8

^aTotals include values for hybrid sunfish of 0.02 fish per 10 m² and 1.3 g/10 m².
 Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table H-3. Fish densities (expressed as number of fish per 10 m²) and biomass (expressed in grams per 10 m², in parentheses) for October 1987 in East Fork Poplar Creek and the reference site, Brushy Fork

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Petromyzontidae						
American brook lamprey						0.10 (1.2)
Cyprinidae						
Bigeye chub						1.04 (0.8)
Bluntnose minnow		0.05 (0.1)	0.19 (0.2)	0.31 (0.3)	0.53 (0.5)	
Blacknose dace	0.62 (2.1)	0.17 (0.5)	0.18 (0.6)	0.11 (0.2)		0.20 (0.2)
Creek chub		0.02 (0.2)	0.03 (<0.1)	0.05 (0.1)		0.08 (0.1)
Emerald shiner		0.02 (<0.1)			0.14 (0.6)	0.43 (0.1)
Rosefin shiner						0.05 (<0.1)
Spotfin shiner						0.11 (0.2)
Striped shiner	12.47 (97.3)	6.32 (17.7)	1.68 (5.5)	1.11 (6.9)	0.29 (1.6)	1.80 (15.1)
Stoneroller	9.79 (107.7)	1.08 (3.1)	1.86 (9.3)	1.46 (3.4)	0.50 (1.5)	0.33 (0.9)

Table H-3 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Catostomidae						
Black redhorse						0.07 (16.9)
Northern hogsucker				0.07 (9.6)	0.02 (1.1)	0.25 (6.9)
Spotted sucker					0.02 (1.3)	0.06 (10.3)
White sucker	1.57 (51.4)			0.03 (0.1)		0.13 (16.2)
Ictaluridae						
Yellow bullhead	0.05 (0.8)			0.03 (0.5)		
Cyprinodontidae						
Black spotted topminnow						0.02 (<0.1)
Poeciliidae						
Western mosquitofish	4.11 (1.2)	5.80 (1.2)	0.57 (0.1)	1.15 (0.3)	0.17 (<0.1)	0.02 (<0.1)
Atherinidae						
Brook silverside					0.14 (0.3)	
Centrarchidae						
Bluegill	0.77 (26.5)			0.04 (3.3)	0.10 (1.4)	0.03 (1.2)
Redbreast sunfish	2.87 (31.3)	0.55 (2.4)	0.86 (9.3)	0.59 (12.7)	0.29 (4.5)	0.30 (1.7)

Table H-3 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Warmouth			0.04 (3.2)	0.04 (0.8)	0.04 (0.5)	
Rock bass			0.03 (3.1)	0.05 (2.7)		0.21 (8.8)
Largemouth bass	0.12 (2.8)					
Percidae						
Blueside darter						0.30 (0.2)
Greenside darter						0.05 (0.4)
Logperch					0.02 (0.3)	0.04 (0.3)
Stripetail darter				0.03 (<0.1)		
Snubnose darter				0.19 (0.2)	0.08 (0.1)	2.41 (1.0)
Cottidae						
Banded sculpin			0.03 (0.1)	0.15 (0.5)	0.06 (0.1)	1.69 (5.6)
Total density	32.45 ^a	14.01	5.47	5.41	2.40	9.42
Total biomass	327.7	25.1	31.5	41.5	13.8	88.1

^aTotals include values for hybrid sunfish of 0.08 fish per 10 m² and 6.7 g/10 m².
 Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table H-4. Fish densities (expressed as number of fish per 10 m²) and biomass (expressed in grams per 10 m², in parentheses) for March 1988 in East Fork Poplar Creek and the reference site, Brushy Fork

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	EFK 13.8	BFK 7.6
Petromyzontidae							
Chestnut lamprey					0.03 (0.3)		
American brook lamprey							0.19 (2.1)
Cyprinidae							
Bigeye chub							0.55 (0.6)
Bluntnose minnow			0.26 (0.7)	1.16 (2.2)	1.21 (1.8)		
Blacknose dace	0.82 (3.1)	0.05 (0.2)	0.18 (0.9)	0.11 (0.2)			0.12 (0.1)
Carp					0.02 (47.7)		
Creek chub		0.03 (0.1)	0.03 (0.1)	0.07 (0.2)			0.06 (0.1)
Emerald shiner				0.02 (0.2)	0.04 (0.2)		
Golden shiner				0.02 (0.2)			
Rosefin shiner			0.04 (0.1)				0.81 (0.3)
Striped shiner	17.62 (84.6)	0.66 (3.4)	3.01 (16.6)	2.31 (8.3)	0.30 (1.5)		1.77 (9.9)

Table H-4 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	BFK 7.6
Stoneroller	13.22 (97.8)	0.04 (1.0)	1.51 (14.7)	6.83 (77.8)	0.21 (1.2)	0.73 (8.9)
Catostomidae						
Black redhorse						0.05 (18.0)
Golden redhorse			0.04 (0.3)		0.02 (16.4)	
Northern hogsucker				0.12 (11.2)	0.04 (1.6)	0.02 (0.1)
Smallmouth buffalo					0.15 (579.5)	
Spotted sucker				0.02 (0.7)	0.08 (20.3)	0.04 (4.4)
White sucker	0.47 (12.8)	0.03 (0.9)		0.02 (0.9)		0.09 (12.5)
Ictaluridae						
Yellow bullhead		0.03 (0.1)		0.04 (6.6)	0.06 (3.7)	
Poeciliidae						
Western mosquitofish	0.04 (0.1)	13.02 (3.9)	0.14 (0.1)	0.37 (0.2)		
Atherinidae						
Brook silverside			0.04 (0.1)		0.03 (0.1)	

Table H-4 (continued)

Species	EFK 23.4	EFK 18.2	EFK 13.8	EFK 10.0	EFK 6.3	EFK 6.3	BFK 7.6
Centrarchidae							
Bluegill	0.21 (4.9)			0.02 (6.7)	0.04 (2.8)		
Redbreast sunfish	1.14 (12.6)	0.27 (0.9)	1.71 (8.7)	0.38 (6.7)	0.12 (2.4)		0.22 (1.2)
Warmouth				0.05 (3.4)	0.03 (1.8)		
Rock bass			0.12 (2.1)	0.07 (3.0)			0.16 (4.9)
Percidae							
Blueside darter							0.51 (0.6)
Greenside darter							0.02 (<0.1)
Logperch					0.04 (0.6)		
Snubnose darter			0.03 (0.1)	0.14 (0.2)	0.05 (0.1)		2.53 (2.0)
Sciaenidae							
Freshwater drum					0.02 (7.5)		
Cottidae							
Banded sculpin			0.09 (0.6)	0.72 (3.7)	0.11 (0.6)		2.04 (8.8)
Total density	33.61 ^a	14.13	7.20	12.47	2.58		9.91
Total biomass	222.1	10.4	44.9	132.3	689.9		74.5

^aTotals include 0.09 fish per 10 m² and 6.3 g/10 m² for hybrid sunfish.

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table H-5. Data for calculation of true growth rates^a of redbreast sunfish in East Fork Poplar Creek compared with the reference stream, Brushy Fork, in the fall of 1986

Site	Year class ^b	<i>n</i>	Increment growth ^c	SD	Length-Wt regress ^d	Age-class growth ^e
BFK 7.6	2	51	0.425	0.081	3.02	1.284
	3	18	0.313	0.123	3.02	0.945
	4	4	0.144	0.056	3.02	0.435
	5	3	0.148	0.039	3.02	0.447
EFK 6.3	2	12	0.498	0.143	3.06	1.524
	3	26	0.330	0.102	3.06	1.010
	4	3	0.159	0.020	3.06	0.487
EFK 10.0	2	13	0.477	0.063	3.00	1.431
	3	36	0.311	0.096	3.00	0.933
	4	7	0.146	0.039	3.00	0.438
EFK 13.8	2	15	0.446	0.134	2.88	1.284
	3	47	0.313	0.103	2.88	0.901
	4	14	0.179	0.049	2.88	0.516
	5	1	0.164	-	2.88	0.472
EFK 18.2	2	11	0.508	0.180	3.04	1.544
	3	9	0.395	0.155	3.04	1.201
	4	16	0.185	0.063	3.04	0.562
	5	5	0.142	0.035	3.04	0.432
EFK 23.4	2	27	0.614	0.110	3.02	1.854
	3	39	0.373	0.105	3.02	1.126
	4	30	0.185	0.080	3.02	0.559
	5	5	0.099	0.043	3.02	0.299

^aTrue growth rates as defined by W. E. Ricker, *Computation and Interpretation of Biological Statistics of Fish Populations*, Bul. 191, Chapter 9, pp. 203-233, "Growth in Length and Weight," Department of the Environment, Fisheries and Marine Service, Ottawa, 1975.

^bRepresents the last annulus of growth, for example a year class of 2 represents fish which have completed 2 years of growth.

^cThe mean of the difference of natural logarithms of initial and final length for the last complete year of growth; this is the instantaneous rate of increase in length.

^dThe slope of the regression between length and weight based on all fish at each site, as calculated by the PROC GLM procedure of SAS Institute, Inc., *SAS User's Guide: Statistics, Version 5 Edition*, SAS Institute, Inc., Cary, North Carolina, 1985.

^eThe product of the slope and instantaneous growth rate which equals the true growth rate for the last year of growth for that age class.

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; SD = standard deviation.

Table H-6. Data for calculation of true growth rates^a of redbreast sunfish in East Fork Poplar Creek compared with a reference stream, Brushy Fork, in 1987

Site	Year class ^b	<i>n</i>	Increment growth ^c	SD	Length-Wt regress ^d	Age-class growth ^e
BFK 7.6	2	16	0.470	0.086	3.03	1.424
	3	32	0.274	0.079	3.03	0.830
	4	7	0.180	0.048	3.03	0.545
	5	3	0.088	0.041	3.03	0.267
	6	1	0.083	-	3.03	0.251
EFK 6.3	2	32	0.589	0.127	3.01	1.773
	3	7	0.245	0.091	3.01	0.737
	4	1	0.193	-	3.01	0.589
EFK 10.0	2	2	0.447	0.007	2.95	1.312
	3	6	0.265	0.030	2.95	0.782
EFK 13.8	2	19	0.557	0.103	2.92	1.636
	3	2	0.246	0.106	2.92	0.718
	4	12	0.183	0.046	2.92	0.534
	5	2	0.141	0.036	2.92	0.412
EFK 18.2	2	43	0.564	0.117	3.06	1.726
	3	11	0.306	0.096	3.06	0.936
	4	15	0.171	0.066	3.06	0.523
	5	18	0.123	0.076	3.06	0.377
	6	2	0.109	0.043	3.06	0.334
EFK 23.4	2	57	0.501	0.083	3.19	1.598
	3	2	0.378	0.318	3.19	1.206
	4	1	0.109	-	3.19	0.348
	5	1	0.147	-	3.19	0.469

^aTrue growth rates as defined by W. E. Ricker, *Computation and Interpretation of Biological Statistics of Fish Populations*, Bul. 191, Chapter 9, pp. 203-233, "Growth in Length and Weight," Department of the Environment, Fisheries and Marine Service, Ottawa, 1975.

^bRepresents the last annulus of growth, for example a year class of 2 represents fish which have completed 2 years of growth.

^cThe mean of the difference of natural logarithms of initial and final length for the last complete year of growth; this is the instantaneous rate of increase in length.

^dThe slope of the regression between length and weight based on all fish at each site, as calculated by the PROC GLM procedure of SAS Institute, Inc., *SAS User's Guide: Statistics, Version 5 Edition*, SAS Institute, Inc., Cary, North Carolina, 1985.

^eThe product of the slope and instantaneous growth rate which equals the true growth rate for the last year of growth for that age class.

Note: EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer; SD = standard deviation.

Table H-7. Comparison between sampling sites on East Fork Poplar Creek and Brushy Fork of the mean true growth (g) of redbreast sunfish collected during 1986 and 1987

Age class	Sites					
	1986					
Age 2+	EFK 23.4 <i>n</i> =27 1.85	EFK 18.2 <i>n</i> =11 1.54	EFK 6.3 <i>n</i> =12 1.52	EFK 10.0 <i>n</i> =13 1.43	EFK 13.8 <i>n</i> =15 1.29	BFK 7.6 <i>n</i> =51 1.28
Age 3+	EFK 18.2 <i>n</i> =9 1.20	EFK 23.4 <i>n</i> =39 1.13	EFK 6.3 <i>n</i> =26 1.01	BFK 7.6 <i>n</i> =18 0.94	EFK 10.0 <i>n</i> =36 0.93	EFK 13.8 <i>n</i> =47 0.90
Age 4+	EFK 18.2 <i>n</i> =16 0.56	EFK 23.4 <i>n</i> =30 0.56	EFK 13.8 <i>n</i> =42 0.51	EFK 6.3 <i>n</i> =3 0.49	EFK 10.0 <i>n</i> =7 0.44	BFK 7.6 <i>n</i> =4 0.44
Age 5+	EFK 13.8 <i>n</i> =1 0.47	BFK 7.6 <i>n</i> =3 0.46	EFK 18.2 <i>n</i> =5 0.43	EFK 23.4 <i>n</i> =5 0.30		
	1987					
Age 2+	EFK 6.3 <i>n</i> =32 1.77	EFK 18.2 <i>n</i> =43 1.73	EFK 13.8 <i>n</i> =19 1.63	EFK 23.4 <i>n</i> =57 1.60	BFK 7.6 <i>n</i> =16 1.43	EFK 10.0 <i>n</i> =2 1.32
Age 3+	EFK 23.4 <i>n</i> =2 1.20	EFK 18.2 <i>n</i> =11 0.94	BFK 7.6 <i>n</i> =32 0.83	EFK 10.0 <i>n</i> =6 0.78	EFK 6.3 <i>n</i> =7 0.74	EFK 13.8 <i>n</i> =2 0.72

Table H-7 (continued)

Age class	Sites				
Age 4+	EFK 6.3	BFK 7.6	EFK 13.8	EFK 18.2	EFK 23.4
	<i>n</i> =1	<i>n</i> =7	<i>n</i> =12	<i>n</i> =15	<i>n</i> =1
	0.58	0.55	0.53	0.52	0.35
Age 5+	EFK 23.4	EFK 13.8	EFK 18.2	BFK 7.6	
	<i>n</i> =1	<i>n</i> =2	<i>n</i> =18	<i>n</i> =3	
	0.47	0.41	0.38	0.27	

Note: *n* = number of fish measured and weighed. Values connected by the same line are not significantly different ($p > 0.05$) based on Tukey's studentized range test (HSD). EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table H-8. Data for calculation of true growth rates^a of bluegill in East Fork Poplar Creek compared with the reference stream, Brushy Fork in the fall of 1986

Site	Year class ^b	<i>n</i>	Increment growth ^c	SD	Length-Wt regress ^d	Age-class growth ^e
BFK 7.6	2	14	0.474	0.136	3.15	1.493
	3	21	0.343	0.116	3.15	1.080
	4	13	0.193	0.132	3.15	0.608
	5	9	0.123	0.038	3.15	0.387
EFK 6.3	2	4	0.603	0.103	3.41	2.056
	3	9	0.346	0.113	3.41	1.180
	4	2	0.179	0.010	3.41	0.610
	5	1	0.217	-	3.41	0.740
EFK 10.0	3	1	0.271	-	3.17	0.859
	4	2	0.205	0.091	3.17	0.650
EFK 13.8	2	1	0.812	-	3.42	2.777
	3	0	-	-	-	-
	4	1	0.225	-	3.42	0.770
EFK 18.2	2	1	0.458	-	3.21	1.470
	3	4	0.444	0.071	3.21	1.425
	4	2	0.319	0.027	3.21	1.024
	5	2	0.140	0.024	3.21	0.449
	6	1	0.090	-	3.21	0.289
EFK 23.4	2	11	0.537	0.151	3.27	1.756
	3	17	0.343	0.130	3.27	1.122
	4	13	0.281	0.210	3.27	0.919
	5	7	0.128	0.029	3.27	0.419

^aTrue growth rates as defined by W. E. Ricker, *Computation and Interpretation of Biological Statistics of Fish Populations*, Bul. 191, Chapter 9, pp. 203-233, "Growth in Length and Weight," Department of the Environment, Fisheries and Marine Service, Ottawa, 1975.

^bRepresents the last annulus of growth; for example, a year class of 2 represents fish which have completed 2 years of growth.

^cThe mean of the difference of natural logarithms of initial and final length for the last complete year of growth; this is the instantaneous rate of increase in length.

^dThe slope of the regression between length and weight based on all fish at each site, as calculated by the PROC GLM procedure of SAS Institute, Inc., *SAS User's Guide: Statistics, Version 5 Edition*, SAS Institute, Inc., Cary, North Carolina, 1985.

^eThe product of the slope and instantaneous growth rate which equals the true growth rate for the last year of growth for that age class.

Note: BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer. *n* = number of fish included in sample.

Table H-9. Data for calculation of true growth rates^a of bluegill in East Fork Poplar Creek (EFK) compared with the reference stream, Brushy Fork (BFK 7.6) in 1987

Site	Year class ^b	<i>n</i>	Increment growth ^c	SD	Length-Wt regress ^d	Age-class growth ^e
BFK 7.6	2	52	0.514	0.097	3.32	1.706
	3	24	0.279	0.083	3.32	0.926
	4	8	0.188	0.075	3.32	0.624
	5	5	0.121	0.034	3.32	0.402
EFK 6.3	2	17	0.550	0.155	3.30	1.815
	3	11	0.337	0.067	3.30	1.112
	4	2	0.257	0.085	3.30	0.848
EFK 10.0	2	2	0.547	0.0003	3.30	1.805
EFK 13.8	2	3	0.356	0.093	3.25	1.157
	3	2	0.213	0.119	3.25	0.692
EFK 18.2	2	16	0.529	0.124	3.26	1.725
	3	10	0.240	0.096	3.26	0.782
	4	4	0.222	0.031	3.26	0.724
	5	2	0.196	0.058	3.26	0.639
	6	1	0.100	-	3.26	0.326
EFK 23.4	2	11	0.223	1.083	3.27	0.729
	3	8	0.365	0.082	3.27	1.194
	4	3	0.164	0.013	3.27	0.536

^aTrue growth rates as defined by W. E. Ricker, *Computation and Interpretation of Biological Statistics of Fish Populations*, Bul. 191, Chapter 9, pp. 203–233, "Growth in Length and Weight," Department of the Environment, Fisheries and Marine Service, Ottawa, 1975.

^bRepresents the last annulus of growth; for example, a year class of 2 represents fish which have completed 2 years of growth.

^cThe mean of the difference of natural logarithms of initial and final length for the last complete year of growth; this is the instantaneous rate of increase in length.

^dThe slope of the regression between length and weight based on all fish at each site, as calculated by the PROC GLM procedure of SAS Institute, Inc., *SAS User's Guide: Statistics, Version 5 Edition*, SAS Institute, Inc., Cary, North Carolina, 1985.

^eThe product of the slope and instantaneous growth rate which equals the true growth rate for the last year of growth for that age class.

Note: BFK = Brushy Fork kilometer; EFK = East Fork Poplar Creek kilometer. *n* = number of fish included in sample.

Table H-10. Comparison between sampling sites on East Fork Poplar Creek and Brushy Fork of the mean true growth (g) of bluegill collected during 1986 and 1987

Age class	Sites					
	1986					
Age 2+	EFK 13.8 <i>n</i> =1 2.78	EFK 6.3 <i>n</i> =4 2.06	EFK 23.4 <i>n</i> =11 1.76	EFK 7.6 <i>n</i> =14 1.49	EFK 18.2 <i>n</i> =1 1.47	
Age 3+	EFK 18.2 <i>n</i> =4 1.42	EFK 6.3 <i>n</i> =9 1.18	EFK 12.4 <i>n</i> =17 1.12	EFK 7.6 <i>n</i> =21 1.08	EFK 10.0 <i>n</i> =1 0.86	
Age 4+	EFK 18.2 <i>n</i> =2 1.02	EFK 23.4 <i>n</i> =13 0.92	EFK 13.8 <i>n</i> =1 0.77	EFK 10.0 <i>n</i> =2 0.65	EFK 6.3 <i>n</i> =2 0.61	BFK 7.6 <i>n</i> =13 0.61
Age 5+	EFK 6.3 <i>n</i> =1 0.74	BFK 18.2 <i>n</i> =2 0.45	EFK 23.4 <i>n</i> =7 0.42	EFK 7.6 <i>n</i> =9 0.39		
	1987					
Age 2+	EFK 6.3 <i>n</i> =17 1.82	EFK 10.0 <i>n</i> =2 1.81	EFK 18.2 <i>n</i> =16 1.73	EFK 7.6 <i>n</i> =52 1.71	EFK 13.8 <i>n</i> =3 1.16	BFK 23.4 <i>n</i> =11 0.73
Age 3+	EFK 23.4 <i>n</i> =8 1.19	EFK 6.3 <i>n</i> =11 1.11	BFK 7.6 <i>n</i> =24 0.93	EFK 18.2 <i>n</i> =10 0.78	EFK 13.8 <i>n</i> =2 0.69	

Table H-10 (continued)

Age class	Sites			
Age 4+	EFK 6.3	EFK 18.2	BFK 7.6	EFK 23.4
	<i>n</i> =2	<i>n</i> =4	<i>n</i> =8	<i>n</i> =3
	0.85	0.72	0.62	0.54
Age 5+	EFK 18.2	BFK 7.6		
	<i>n</i> =2	<i>n</i> =5		

Note: *n* = number of fish measured and weighed. Values connected by the same line are not significantly different ($p > 0.05$) based on Tukey's studentized range test (HSD). EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table H-11. Comparison between sampling sites on East Fork Poplar Creek and Brushy Fork of mean condition factors (k) of fish species collected in the four sampling periods between October 1986 and March 1988

Species	Sites					
	October-November 1986					
Bluegill	EFK 18.2 <i>n</i> =4 1.75	EFK 23.4 <i>n</i> =19 1.63	EFK 10.0 <i>n</i> =4 1.48	EFK 6.3 <i>n</i> =28 1.46		
Redbreast sunfish	EFK 13.8 <i>n</i> =64 1.73	EFK 23.4 <i>n</i> =57 1.70	EFK 10.0 <i>n</i> =30 1.68	EFK 6.3 <i>n</i> =31 1.67	BFK 7.6 <i>n</i> =6 1.66	EFK 18.2 <i>n</i> =110 1.62
Rock bass	EFK 18.2 <i>n</i> =1 1.85	BFK 7.6 <i>n</i> =7 1.79	EFK 10.0 <i>n</i> =6 1.67			
	March 1987					
Bluegill	EFK 18.2 <i>n</i> =6 1.80	EFK 6.3 <i>n</i> =5 1.57				

Table H-11 (continued)

Species	Sites					
Redbreast sunfish	EFK 6.3 <i>n</i> =21 3.07	BFK 7.6 <i>n</i> =9 2.12	EFK 13.8 <i>n</i> =65 1.88	EFK 18.2 <i>n</i> =122 1.87	EFK 10.0 <i>n</i> =23 1.83	EFK 23.4 <i>n</i> =42 1.59
Rock bass	EFK 13.8 <i>n</i> =5 1.89	BFK 7.6 <i>n</i> =6 1.86	EFK 10.0 <i>n</i> =5 1.79	EFK 6.3 <i>n</i> =1 1.64		
October 1987						
Bluegill	BFK 7.6 <i>n</i> =2 1.71	EFK 10.0 <i>n</i> =2 1.58	EFK 23.4 <i>n</i> =30 1.57	EFK 6.3 <i>n</i> =7 1.56		
Redbreast sunfish	EFK 10.0 <i>n</i> =43 1.74	EFK 13.8 <i>n</i> =62 1.70	EFK 6.3 <i>n</i> =23 1.65	EFK 23.4 <i>n</i> =97 1.65	BFK 7.6 <i>n</i> =19 1.56	EFK 18.2 <i>n</i> =53 1.41

Table H-11 (continued)

Species	Sites					
Rock bass	BFK 7.6 <i>n</i> =20 1.74	EFK 13.8 <i>n</i> =1 1.70	EFK 10.0 <i>n</i> =3 1.52			
March 1988						
Bluegill	EFK 10.0 <i>n</i> =1 2.68	EFK 6.3 <i>n</i> =3 1.71	EFK 23.4 <i>n</i> =9 1.54			
Redbreast sunfish	EFK 18.2 <i>n</i> =28 1.89	EFK 10.0 <i>n</i> =31 1.81	BFK 7.6 <i>n</i> =17 1.69	EFK 6.3 <i>n</i> =8 1.68	EFK 23.4 <i>n</i> =49 1.67	EFK 13.8 <i>n</i> =95 1.66
Rock bass	EFK 13.8 <i>n</i> =8 1.88	BFK 7.6 <i>n</i> =10 1.82	EFK 10.0 <i>n</i> =5 1.57			

Note: *n* = number of fish measured and weighed. Values connected by the same line are not significantly different ($p > 0.05$) based on Tukey's studentized range (HSD) test. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

Table H-12. Comparison between sampling sites on East Fork Poplar Creek, New Hope Pond, and Brushy Fork of mean condition factors (k) of fish species collected in the four sampling periods between October 1986 and March 1988

Species	Sites					
	October–November 1986					
Gizzard shad	EFK 18.2 <i>n</i> =42 0.96	EFK 10.0 <i>n</i> =2 0.89	EFK 23.4 <i>n</i> =1 0.75	EFK 6.3 <i>n</i> =19 0.65		
Northern hogsucker	EFK 6.3 <i>n</i> =1 1.10	BFK 7.6 <i>n</i> =5 1.07	EFK 10.0 <i>n</i> =3 0.93			
Stoneroller	EFK 6.3 <i>n</i> =31 1.02	EFK 13.8 <i>n</i> =75 1.02	EFK 23.4 <i>n</i> =169 1.01	EFK 10.0 <i>n</i> =75 0.98	BFK 7.6 <i>n</i> =47 0.96	EFK 18.2 <i>n</i> =5 0.90
White sucker	EFK 13.8 <i>n</i> =3 1.11	EFK 6.3 <i>n</i> =1 0.98	BFK 7.6 <i>n</i> =5 0.97	EFK 23.4 <i>n</i> =104 0.96	EFK 18.2 <i>n</i> =3 0.84	

Table H-12 (continued)

Species	Sites					
March 1987						
Creek chub	NHP <i>n</i> =4 1.27	EFK 23.4 <i>n</i> =15 1.08	EFK 13.8 <i>n</i> =2 1.06	EFK 10.0 <i>n</i> =2 0.91	EFK 18.2 <i>n</i> =4 0.83	
Stoneroller	EFK 18.2 <i>n</i> =8 1.43	BFK 7.6 <i>n</i> =54 1.31	EFK 13.8 <i>n</i> =136 1.26	EFK 6.3 <i>n</i> =26 1.08	EFK 10.0 <i>n</i> =85 1.08	EFK 23.4 <i>n</i> =160 0.97
October 1987						
Bluntnose minnow	EFK 18.2 <i>n</i> =4 0.96	EFK 10.0 <i>n</i> =22 0.84	EFK 6.3 <i>n</i> =26 0.80	EFK 13.8 <i>n</i> =11 0.73		
Blacknose dace	EFK 23.4 <i>n</i> =23 1.09	EFK 18.2 <i>n</i> =16 1.07	EFK 13.8 <i>n</i> =12 1.06	EFK 10.0 <i>n</i> =7 1.05	BFK 7.6 <i>n</i> =19 0.88	
Striped shiner	EFK 23.4 <i>n</i> =204 0.97	EFK 18.2 <i>n</i> =165 0.90	EFK 13.8 <i>n</i> =81 0.86	EFK 10.0 <i>n</i> =77 0.86	BFK 7.6 <i>n</i> =149 0.85	EFK 6.3 <i>n</i> =21 0.80

Table H-12 (continued)

Species	Sites				
	March 1988				
Bluntnose minnow	EFK 10.0 <i>n</i> =70 1.00	EFK 13.8 <i>n</i> =19 0.97	EFK 6.3 <i>n</i> =101 0.91		
Blacknose dace	EFK 18.2 <i>n</i> =4 1.63	EFK 13.8 <i>n</i> =13 1.26	EFK 23.4 <i>n</i> =30 1.15	EFK 10.0 <i>n</i> =8 1.13	BFK 7.6 <i>n</i> =10 1.03
Banded sculpin	EFK 13.8 <i>n</i> =6 1.58	EFK 6.3 <i>n</i> =9 1.48	BFK 7.6 <i>n</i> =110 1.34	EFK 10.0 <i>n</i> =49 1.31	
Snubnose darter	EFK 13.8 <i>n</i> =1 1.64	EFK 10.0 <i>n</i> =11 1.40	EFK 6.3 <i>n</i> =4 1.17	BFK 7.6 <i>n</i> =72 1.16	

Note: *n* = number of fish measured and weighed. Values connected by the same line are not significantly different ($p > 0.05$) based on Tukey's studentized range (HSD) test. EFK = East Fork Poplar Creek kilometer; BFK = Brushy Fork kilometer.

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