ELF Communications System Ecological Monitoring Program: Aquatic Ecosystem Studies – Final Report

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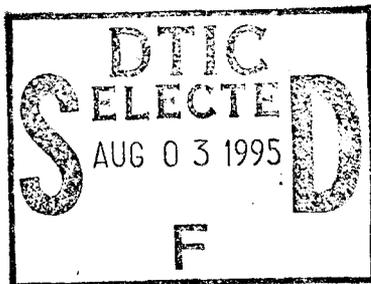
Prepared for:

Submarine Communications Project Office
Space and Naval Warfare Systems Command
Washington, D.C. 20363-5100

Submitted by:

IIT Research Institute
10 West 35th Street
Chicago, Illinois 60616-3799

19950802 003

REPORT DOCUMENTATION PAGE

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE November 1994	3. REPORT TYPE AND DATES COVERED Final Report, 1982-1994

4. TITLE AND SUBTITLE

ELF Communications System Ecological Monitoring Program:
Aquatic Ecosystem Studies—Final Report

5. FUNDING NUMBERS

C: N00039-81-C-0357
N00039-84-C-0070
N00039-88-C-0065
N00039-93-C-0001

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IIT Research Institute
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8. PERFORMING ORGANIZATION REPORT NUMBER

D06212-5

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Submarine Communications Project Office
Space and Naval Warfare Systems Command
Washington, D.C. 20363-5100

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Unclassified/Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The U.S. Navy has completed a program that monitored biota and ecological relationships for possible effects from electromagnetic (EM) fields produced by its Extremely Low Frequency (ELF) Communications System. This report documents the results and conclusions of aquatic studies conducted near its transmitting antenna in Michigan.

From 1982 through 1993 researchers from the Michigan State University (MSU) monitored aquatic flora and fauna on matched reaches of the Ford River. A treatment site was located immediately adjacent to the antenna, whereas a control site was situated at a distance downstream. Functional and structural components of the periphyton, insect, and fish communities were monitored. The research team also measured ambient factors such as temperature, discharge, and water quality indicators. Data were analyzed using a variety of statistical tests; however, BACI techniques were emphasized.

Results indicated a relative increase in algal biomass at the treatment site after the antenna became fully operational, but no changes in any other parameter or organism. MSU concludes that algal biomass was affected by ELF EM exposure. Since neither the other ecological characteristics of the periphyton nor the insect and fish communities showed any effects, MSU infers little EM impact to riverine habitats.

(ABSTRACT PREPARED BY IIT RESEARCH INSTITUTE)

14. SUBJECT TERMS

bioelectromagnetic effects
ELF Communications System
ELF Ecological Monitoring Program
environmental studies
extremely low frequency fields
long-term ecological monitoring

15. NUMBER OF PAGES 166

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unclassified

FOREWORD

This report by researchers from Michigan State University (MSU) summarizes the results and conclusions of their study of aquatic biota. In this effort, MSU monitored periphyton, insects, and fish exposed to electromagnetic fields produced by the U.S. Navy's ELF Communications System in Michigan. The Space and Naval Warfare Systems Command (SPAWAR) funded this MSU study through contracts N00039-81-C-0357, N00039-84-C-0070, N00039-88-C-0065, and N00039-93-C-0001 to IIT Research Institute (IITRI). IITRI, a not-for-profit organization, provided engineering support to MSU and managed their study through subcontract agreements.

MSU initiated their studies in late 1982. Their early efforts focused on selecting study sites, validating assumptions made in proposals, and characterizing critical study aspects. As these tasks were accomplished in 1983 and 1984, MSU then emphasized accumulating a data base through 1993. The MSU research team and IITRI evaluated each study variable for continued funding before contract renewals in 1984, 1988, and 1993. As a result, several originally proposed study elements were either expanded or discontinued in subsequent periods of performance.

Since its inception, scientific peers have reviewed the technical quality of this study on an annual basis. In similar fashion, a draft of this report has been reviewed by peers with experience in phycology, aquatic entomology, ichthyology, statistics, and electromagnetics. MSU authors have considered, and addressed, peer critiques before submitting a revised manuscript to IITRI. Except for added prefatory and title pages, MSU's manuscript is here issued by IITRI on behalf of SPAWAR without further changes or editing by IITRI or SPAWAR.

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Availability Codes		
Dist	Avail and / or Special	
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Respectfully submitted,
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IITRI D06212-5

I.   COVER PAGE

A.   SUBCONTRACTOR: MICHIGAN STATE UNIVERSITY
                                EAST LANSING, MICHIGAN 48824

B.   SUBCONTRACT NUMBER: DO6205-93-C-007

C.   TITLE OF REPORT: ELF COMMUNICATIONS SYSTEM
                                 ECOLOGICAL MONITORING PROGRAM
                                 FINAL REPORT FOR AQUATIC
                                 ECOSYSTEMS- TASKS 5.8, 5.9,
                                 5.10

D.   REPORTING YEAR: 07/10/82 - 09/30/94

E.   REPORT IDENTIFICATION NUMBER: AE-150

II. FRONTISPAGE

A. Subcontractor: Michigan State University
                       East Lansing, Michigan 48824

B. Subcontract Number: D06205-93-C-007

C. Title of Report: ELF Communications System Ecological
                     Monitoring Program Final Report for
                     Aquatic Ecosystems - Tasks 5.8, 5.9, 5.10

D. Reporting Period: 7/10/82 - 9/30/94

E. Report Identification Number: AE-150

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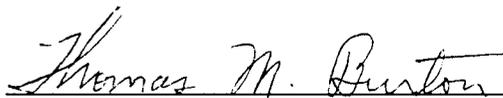
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iii

III. TABLE OF CONTENTS

IV.   GLOSSARY AND ACRONYMS

AFDW-biomass - ash-free dry weight of organic matter that
accumulates on rock or other substrate surfaces on the stream
bottom. This organic matter is produced by algae, bacteria,
and fungi and/or by the flocculation and settling of
suspended organic matter from the water column.

Alkalinity - a chemical measure of the amount of anions in
the water determined by titration with dilute acid; a rough
measure of the acid neutralizing capacity of the water
derived primarily from the carbonate and bicarbonate ions in
it.

ANCOVA - analysis of covariance; a statistical analysis in
which treatment means are compared by standardizing for
differences in a common covariant (a parameter that varies
with parameter in question).

ANOVA - analysis of variance; a statistical procedure for
comparing whether treatment means are esentially the same or
not; it is essentially an arithmetic process for partitioning
a total sum of squares into components associated with
recognized sources of variation.

BACI - Before and After, Control and Impact analysis-
statistical analysis which compares differences between
control and impact sites, both before and after antenna
operation by comparing differences in the variance for each
site before and after the operation of the antenna (see
Stewart-Oaten et al 1986 for details - reference section of
element 2).

Backcalculated length - length of fish at previous age
estimated from body-scale relationship between distance
between annuli on scales or otoliths and fish length at
capture.

Benthos (Benthic) - organisms that live on or in the river
bottom in or on substrates such as sand, gravel, and organic
detritus.

Biomass - the weight of a fish stock, or of some defined
portion of it.

Body-scale relationship - method of backcalculation where
length is determined from the distance between annuli.

Biovolume - a crude estimate of biomass of algal cells where volume is calculated from the shape and size of individual cells using geometric formulae. Individual cell volumes are then multiplied by algal species counts and summed to get total biovolume.

Catch rates - the catch of fish, in numbers or in weight per defined unit of fishing effort.

C.C. - correlation coefficient (r); a measure of the degree to which variables vary together or a measure of the intensity of association.

C-F - collector-filter-feeding aquatic invertebrates; invertebrates that feed by collecting particles of detritus or algae from the water by use of nets or other collecting devices.

C-G - collector-gatherer aquatic invertebrates; invertebrates that feed by collecting detrital particles from substrates in the river.

Chi-square test - statistical test for goodness of fit for observed and expected frequencies.

Chlorophyll a - the primary photosynthetic pigment of most plants; in this study, it is extracted using acetone and used as a crude measure of plant productivity or standing crop.

Conductivity - a measure of the ionic strength of the water.

CPUE - the catch of fish, in numbers or in weight per defined unit of fishing effort.

C.V. - coefficient of variation; a quantity of use to the experimenter in evaluating results from different experiments involving the same character but possibly conducted by different persons.

Degree days - daily accumulation of degrees ($^\circ$ C) above a pre-set threshold value (in our study the threshold was 2° C).

DeLury method - removal method of population estimation. Population is estimated from the relation of fishing success to cumulative fishing effort. Assumes fish catchability does not change throughout all sampling passes, and the population is significantly reduced with each pass. Three removals were used in this study.

Diatoms - a group of algae that often dominate unpolluted rivers (very few other kinds of algae are present in the Ford River most of the time); they are characterized by having the cells encased in two silaceous covers known as valves.

Discharge (Q) - the amount of water passing a particular point on a river over a given time period, usually expressed in cubic meters per second; it is calculated from measurements of width, depth, and velocity by taking at least 20 verticals of depth, mean velocity, and the width between the verticals across a stream or from depth measurements based on depth (stage)-discharge relationships determined empirically for the river segment being studied.

DO - Dissolved Oxygen; the amount of oxygen dissolved in water.

Electrofishing - method used in fisheries to collect/capture fish. Electric current is applied to the water which temporarily incapacitates the fish so that they can be collected.

Electrofishing efficiency - percent of the total population of fish taken by electrofishing crew.

ELF - Extremely Low Frequency electromagnetic radiation; it is derived primarily from local electric power lines or from the ELF antenna that will be used by the Navy to communicate with submarines at sea.

EPROM - Erasable Progammable Read Only Memory chip; the type of chip used to temporarily store data in the Omnidata data pods used in our ambient monitoring program; these data are transferred by use of an EPROM reader into an Apple computer and summarized.

FCD - Ford Control Downstream - site on Ford River presently used as the control site (see Fig. VII.1).

FCD-N - Ford Control Downstream New - a periphyton monitoring site located 130 m downstream of FCD.

FCU - Ford Control Upstream

FEN - Ford Experimental New - a fyke net site 400m upstream of FEX used to monitor fish movement past the antenna.

FEX - Ford Experimental - site on Ford River presently used as the primary experimental or test site; it is located where

the N-S leg of the ELF antenna crosses the Ford River (see Fig. VIII.1).

FEX-Line - Ford Experimental Line - an insect studies site located 35 m downstream of FEX (about 5 m downstream of the point where the antenna crosses the river).

FEX-N - Ford Experimental New - a periphyton monitoring site located 40 m downstream of FEX (about 10 m downstream of the point where the antenna crosses the river).

FFG - Functional Feeding Groups - aquatic insects species are categorized into feeding groups according to their predominant feeding mode (See Merritt and Cummins, 1984 - reference after element 4).

FS1 - Ford Site One - one of the original study sites. Not used presently.

Freidman's test - non-parametric test comparing distributions; the null hypothesis being that the populations within a block are identical against the alternative that at least one treatment comes from populations which have a different location in one direction.

Fyke net - portable passive gear used at FCD and FEX. Nets are set in tandem, one capturing upstream migrants the other capturing downstream migrants. Nets block entire width of stream and are used in areas with unstable substrate.

Grazer - as used in this study; an invertebrate herbivore that feeds on algae on rocks and other substrates on the stream bottom.

Gross Primary Production (GPP) - the total amount of energy fixed by green plants in the process of photosynthesis in a given time period; it is equal to plant respiration plus net primary production.

Growth - incremental increase in mean length and weight. Backcalculation of lengths and body-scale relationship were used to monitor growth in this study.

H' - taxon diversity (after Shannon-Weiner). An information theory index which weights the number of taxa and the apportionment of numbers of individuals among the taxa.

Handling (Tagging) Mortality - mortality caused by weighing, measuring, tagging, etc. Calculated from recaptured fish

found dead in the gear in this study.   Probably
underestimated.

Hardness - a rough chemical measure of the amount of cations
in the water determined by titration.

Holobiotic - an organism that spends its entire life in one
environmental medium; e.g., an aquatic beetle, Optioservus
sp., whose larval and adult stages are aquatic.

J' - taxon evenness (after Shannon-Weiner). An index which
evaluates the apportionment of numbers of individuals within
each taxon.

-k/day - processing coefficient. An exponential decay model
describing the rate biological material (in our case, leaves)
decays per day, $\log_e$ (% remaining/100)/ days.

Kruskal-Wallis test - non-parametric statistical test
comparing distributions; the null hypothesis being that the
populations sampled are continuous and identical, except
possibly for location.

Lee's Phenomenon - commonly seen in backcalculated length
estimates. In the larger fish, backcalculated lengths at
early ages are less than the true average size at that age.
Usually due to differential growth or mortality. Reverse
Lee's Phenomenon can occur also, especially in non-exploited
populations or where predator-prey relationships do not exist
or are poorly defined.

Lincoln index - an estimate of population size based on the
proportion of marked organisms that are captured in a later
sampling effort (see Southwood, 1978 - see references after
element 2).

Mann-Whitney U test - non-parametric statistical test of two
samples which gives rise to a t-test or ANOVA. Null
hypothesis is that two samples come from populations having
the same distribution.

Mark-recapture studies - a method for determining population
size or movement of organisms based on recapture of marked
individuals.

MDW/IND - mean dry weight (mg.) per individual.

N - Nitrogen when used as follows (otherwise refers to the
number of samples taken):

ammonium-N: ammonium-Nitrogen
nitrate-N: nitrate-Nitrogen
nitrite-N: nitrite-Nitrogen
inorganic-N: inorganic-Nitrogen; the sum of the
three N species above.
organic-N: organic-Nitrogen; total Kjeldahl
nitrogen minus ammonium nitrogen.

Naiads - the immature (nymph) stages of insects that undergo
incomplete metamorphosis; e.g. dragonfly naiads.

Net Primary Production (NPP) - the amount of energy or carbon
that is fixed by the process of photosynthesis that is not
used in self maintenance (respiration) by the plant; it
supports herbivore or detritivore food chains.

Numerical dominance - the ratio between numbers of
individuals from one taxon and the total numbers of
individuals found in a sample. The percentage gives the
numerical dominance of that taxon.

P - predators; animals that ingest other animals.

PAR - Photosynthetically Active solar Radiation = solar
radiation that most plants are able to use in photosynthesis;
similar to visual range for humans.

PCA - Principal Components Analysis; a statistical procedure
used to ordinate data in relation to environmental variables.

Percent recapture - the ratio between numbers of marked
animals recaptured and the total number of animals marked.

Periphyton - algae, bacteria and fungi attached to the
substrate, rocks, twigs or any other debris in the stream.
Our studies emphasize periphytic algae attached to bottom
substrates.

Phaeophytin a - the breakdown product of chlorophyll a; the
ratio of chlorophyll a to phaeophytin a is sometimes used as
a very crude estimate of the health of algal populations.

Predators - animals that ingest other animals.

Relative weight (Wr) - weight at length values calculated
from fish being studied. Used in comparative analysis of
condition against weight at length values calculated from
populations in the literature.

RIA - Randonmized Intervention Analysis; statistical analysis which compares mean differences between sites before and after antenna impact; a non-parametric equivalent of BACI in which the test statistic is compared to a random distribution of the data set.

S - shredder invertebrates; those that feed on large leaf fragments by shredding holes in this leaf material.

S - taxon richness. The number of taxa in a sample.

Shannon-Wiener diversity - diversity index which uses number of species and abundance within species to compute a values which is comparable between sites and years (see H' above).

Shredder - see S (first definition) above.

Standard weight (Ws) - mean weight at length values calculated from a number of populations from the literature. Wr values are measured against these values to comparatively determine the condition of fish being studied.

TB - total biomass; total weight of all organisms in the taxa being discussed.

TM - Two Mile Creek; a weir site.

T-test - statistical test of the difference between two means to analyze variance.

Turbidity - a measure of the light blocking particles suspended in the water.

Univoltine - one generation per year; used to describe aquatic insect life cycles.

VI Tag - Visible implant tag. A tag implanted in the clear tissue posterior to the eye of a fish, so that the code on the tag is visible.

Weir - semi-permanent traps used to capture fish. Made of hardware cloth held in place with rerod. Applied at beginning of study season and extracted at the end of the season. Weirs have removable weir boxes which, when in place, deter fish movement. When boxes are removed, weir is negotiable by all fish.

Yearling fish - fish that are one + years old but are not yet sexually mature.

V.   SUMMARY

Potential effects of extremely low frequency (ELF) electromagnetic radiation on an aquatic ecosystem were studied from 1983 to 1993.   These studies were conducted at two sites on the Ford River, a fourth order brown water, trout stream in Dickinson County, Michigan.   The north-south leg of the U.S. Navy submarine communication antenna crossed the Ford River between Channing and Ralph, Michigan (Figure VI.1). The antenna or experimental site was either directly under or within a few hundred meters of the antenna, while the primary control site was about 8 km downstream (Figure VI.1).   The control site received 4.9 to 6.5 times less exposure to ground electric fields and from 300 to 334 times less exposure to magnetic flux when the antenna was operational at 76 Hz than did the antenna site (Tables VI.1, VI.2).   Three of the most important groups of organisms in the Ford River, benthic algae, benthic larval insects and fish, were studied intensively.   Effects of ELF exposure on benthic algal and larval insects were monitored at the control (FCD) and antenna (FEX) sites, while the emphasis of the fish studies was on movement of fish under the antenna before and after antenna operation began. Antenna testing at 4-6 amps took place at intermittent intervals from May to October, 1986; at 15 amps from April to November, 1987; and at 75 and 150 amps for most working days in 1988 and 1989 respectively.   Pre-operational data were collected from June 1983 to May 1986; transitional data were collected during antenna testing from 1986 to 1989; and full operation data were collected from 1989 to 1993.   Results by work element are reported below.

Task One: Ambient Monitoring

The ambient monitoring program had two primary objectives: (1) to provide the background data on physical and chemical parameters needed to correlate observations on biological community dynamics with environmental parameters; and (2) to determine whether or not observed changes in community structure were related to physical and chemical changes in the Ford River rather than ELF electromagnetic radiation. The parameters monitored were water and air temperature, photosynthetically active radiation, stream discharge, and several measurements of water quality. Water quality measurements included; pH, dissolved oxygen, alkalinity, hardness, turbidity, specific conductance, total and soluble reactive phosphorus, inorganic nitrogen (ammonium-N+nitrite-N+nitrate-N), organic nitrogen, chloride, and silicate-Si. Land use changes in the watershed were also monitored through ground observations and GIS mapping based on 1979 and 1986 photographs.

1

The ambient monitoring program demonstrated that there were either no statistically significant differences in physical and chemical parameters monitored at the control and antenna sites or that differences were quite small and were probably not biologically significant. Most of the differences that did occur were slight increases in a downstream direction with the upstream, antenna site having slightly lower values than did the downstream, control site.

Task Two: Benthic Algal (Periphyton) Responses to ELF Electromagnetic Exposure

The objective of the benthic algal studies was to determine if ELF electromagnetic radiation had caused changes in the structure or the function of the algal communities in the Ford River. Community structure parameters monitored were: relative abundance of dominant diatom species, species diversity, and species evenness. Community function parameters monitored for potential ELF effects included: diatom cell density, biovolume, chlorophyll *a* standing crop, chlorophyll *a* daily accumulation rate, organic matter standing crop, organic matter daily accumulation rate, algal primary production and bioflim respiration.

No significant differences were found for any of the community structure variables (diversity, evenness, relative abundance of dominant diatoms) when the between site differences in before and after periods were compared using Before and After Control and Impact (BACI). Thus, 76 Hz ELF electromagnetic radiation apparently has not changed the basic makeup of the diatom community in the Ford River.

Significant differences were found when the between site differences in before and after periods were compared using BACI tests for chlorophyll *a* standing crop, chlorophyll *a* daily accumulation rate, organic matter standing crop and organic matter daily accumulation rate (Table V.1). These four community function variables all increased in magnitude at the antenna site relative to the control site from before to after periods. The largest and most consistent increases were in chlorophyll *a* standing crop and chlorophyll *a* daily accumulation rates. Mean chlorophyll *a* standing crop and daily accumulation rate data represent two separate measures of chlorophyll, since they were collected from separate slides at separate time periods. Both measures of chlorophyll *a* increased at the antenna site relative to the control site from pre to post operational periods during the period from mid-June to September when the diatom community was dominated by *Cocconeis placentula*. The increase in chlorophyll *a* at the antenna site also occurred during the 1986-1988 period of low amperage testing. It did not increase further from the testing period to the fully operational period indicating a low threshold for response

2

Table V.1   Summary of BACI Statistics for Biological Parameters at
             Control (FCD) and Experimental (FEX) sites. The comparisons
             were made using months in which the Ford River was free
             of ice cover (April - October). Before period = 1984-1985,
             After period = 1989-1993.

Parameter	N	BACI P values	Power[†] (.50)	Power (.20)
Organic Matter Standing Crop	48	0.021*	99	42
Organic Matter Daily Accumulation	46	0.000**	93	34
Chlorophyll a Standing Crop[§]	50	<0.05*	99	43
Chlorophyll a Daily Accumulation	46	0.000**	40	12
Density	50	0.094	31	12
Cell Volume	50	0.609	99	99
Biovolume	50	0.133	95	37
Diversity	50	0.681	99	99
Evenness	50	0.365	99	99
Achnanthes minutissima relative abundance	47	0.605	99	92
Cocconeis placentula relative abundance	46	0.108	99	76

-The following were log (x+1) prior to BACI analysis: chlorophyll a
standing crop, cell volume, biovolume; The following were arcsin $\sqrt{x}$
transformed: *Achnanthes minutissima* relative abundance, *Cocconeis
placentula* relative abundance.
-[†] power to detect a difference equal to 50% or 20% respectively of
before period grand mean.
- * $P<0.05$, ** $P<0.01$
-[§] t statistic corrected for positive serial correlation.

3

and lack of a linear dose response if ELF exposure caused the change. No significant changes in diatom cell density, cell volume, diatom biovolume or in algal production and biofilm respiration were detected in the means between the control and antenna sites between the before and after operational periods.

BACI statistics cannot be used to establish cause and effect. Rather, they indicate that a change has taken place in differences between the two sites between the before and after operation periods. Alternative explanations for this change then need to be explored before changes related to ELF exposure are suggested as the cause. Alternative explanations for the changes in function that were examined were related to differential changes or differential responses to physical or chemical changes that might have occurred at the control and antenna sites. No convincing alternative explanation for the changes in function were found. The antenna and control sites were well matched with few significant differences in physical and chemical parameters occurring between them. Changes in water temperature or changes in nutrients between sites were examined in detail, since these were the parameters that explained most variance in the data from the control site in stepwise regression models. Water temperature was slightly different between sites, but these discrepancies were not large enough to explain the between site differences found for the four community function variables, especially the large change in chlorophyll *a* at the antenna site. Nutrient concentrations were not significantly different between the sites for the pre or post operational periods when data collected from all years were compared, except for inorganic-N. Inorganic-N was greater at the control site than at the antenna site in 1985 during the before operational period. This difference led to a difference in the means between the control and antenna sites between the before and after periods (higher inorganic-N at the control site during the before period, no difference between the sites for the after period) according to BACI analyses. Experiments with nutrient additions in 1986 suggested that diatoms in the river only responded to nutrients when both N and P were increased simultaneously. No such increase in both parameters occurred. Thus, neither water temperature nor nutrient differences offer convincing alternative explanations for the observed increases in chlorophyll. The higher chlorophyll *a* levels during the antenna testing and post operational periods were attributable to 76 Hz ELF electromagnetic radiation or to some differential response to an environmental variable that was not monitored. The most parsimonious explanation was that the change was related to ELF antenna operation with magnetic flux exposure being the most likely cause.

4

Task Three: Aquatic Insect Responses to ELF Electromagnetic Exposure

Three levels of biotic organization for aquatic insects were examined to determine their responses to physical factors as well as to extremely low intensity electromagnetic fields; Community structure of insects living on natural benthic substrata and artificial benthic leafpacks, including diversity, equitibility, richness, numerical dominance, (2) community function for insects on substrata and leafpacks, including functional feeding guild dynamics, changes in total insect mass, and leaf processing dynamics, and (3) population dynamics, including growth rate changes for common taxa on substrates and leafpacks, and changes in movements of a major insect predator.

Changes in biotic factors were related to physical factors, including mean water temperature, cumulative degree days, mean discharge and siltation. The sites included a control site, denoted as FCD (Ford Control Downstream), and two experimental sites. FEX (Ford Experimental) was original to the monitoring program. In July, 1990 a second experimental site, denoted as FEX.LINE, was chosen underneath the ELF above ground antenna line where it crosses the Ford River.

Substrates were collected monthly, from April through November. Coefficient of determination values each month showed that the summer (June through August) had the lowest CV values, both for biotic and physical variables. Collections of leafpack insects were made over a three month fall period, with seven samples being taken and processed after 7, 14, 21, 28, 54, and 84 days' incubation at each of the sites. Movement studies of the dragonfly predator, Ophiogomphus colubrinus, took place during the summer.

Two-way Analysis of Variance tests were performed on structural and functional community parameter estimates for insects on substrates and leafpacks. Multiple linear regression tests and ANCOVA tests were used to determine which physical factors were most correlated with the biotic parameter estimates. Cumulative multiple linear regression tests were performed for one biotic variable (i.e., total insect mass in substrates). Each year of data was added for each test to determine whether there was any inflection point for the coefficient of multiple determination values at the experimental site relative to the control site, or for the partial standard regression coefficient values for discharge, temperature, or for cumulative ELF ground field exposures. Periphyton density estimates were regressed with total insect mass values each season.

Before and After, Control and Impact analysis (B.A.C.I.) was used to assess seasonal before versus after ELF activation differences for structural and functional

community parameter estimates for insects on natural substrates. Before impact data spanned April, 1984 through May, 1986. After impact data spanned June 1989 through August 1993. For data of a given parameter that did not pass Tukey's test for additivity, the Random Intervention Analysis technique (R.I.A.) was used. ANCOVA tests were performed on growth rate data for the common species of collector-gatherer mayflies (i.e., Paraleptophlebia mollis, Ephemerella invaria and Ephemerella subvaria) and one species of predator stonefly, Isoperla transmarina. Leaf processing rates (-k/day) were determined for Tag Alder (Alnus rugosa) leaves. Student t-tests were used to determine whether there were signficant site differences with respect to leaf processing rates over the years.

Population estimates, percent recapture success, and distances travelled were computed for movement studies of the dragonfly naiad, Ophiogomphus colubrinus from 1985 through 1989. Student t-tests were used to determine whether there were significant differences in distances travelled by marked animals at FEX versus those marked at FCD after 24 or 48 hr. Multiple linear regression tests were used to determine the importance of discharge and cumulative ELF ground electrical field exposures in explaining the variation in mean distances the animals moved at each site.

Over the 11 year monitoring period, weather patterns showed distinctive patterns, ranging from cool, wet springs, summers, and falls to very hot springs, summers, and falls. These patterns, by chance alone, were positively related to increasing intensity and duration of ELF fields until full activation of ELF power in the fall of 1989. Had the Ford River not experienced cooler and wetter seasons after 1989, effects of physical factors on aquatic insects would have been difficult to separate from potential effects of ELF fields. The long term dataset has been invaluable. Actual mean discharge values over the years showed that the springs of 1984, 1985, and 1991 through 1993 were relatively high. Spring discharges were relatively low during the transition years of 1987 through 1989. Temperatures were high during low discharges and lower during high stream discharges. Deviations in discharge and water temperatures were lower in the summer than in the spring and fall.

It was anticipated that the most direct affect on the rheophilic insect community would be from the ELF ground field exposure. First operation began in 1986 at low amperage (4 - 6A). In 1987, testing amperage was increased to 15A. From November 1987 to May 1989, the system operated on most weekdays at 75A. In mid-May of 1989 power was increased to 150A. Continuous operation of the ELF antenna at 150A was initiated on 7 October, 1989. Any potential effects that ELF ground field exposures may have on

structural or community parameters for insects in substrates would have been most easily detected during the summer months from June through August because coefficient of variation values were at their lowest for the biotic as well as for the physical variables studied.

Natural physical factors accounted for more of the variation in the biotic variables for insects in substrata than did the ELF fields. Discharge was the most important physical factor in explaining variation in the biotic variables. Periods of high discharge were negatively related to taxon richness, total insect mass, and to a lesser extent, taxon evenness at both FEX and FCD. Conversely, high discharge was positively related to numerical dominance and to biomass dominance of chironomids. Because the study spanned over 10 years, data were gathered over a wide range of weather conditions, including an extremely dry and hot year (1988), some very cool and wet years (1984, 1992, 1993), and some years with moderate rainfall and temperature (1989, 1990). Had the system been monitored from 1986 through 1989, ELF fields may have been invoked as having an impact on the structural and functional community parameters. During those years, spring and summer weather became warmer and falls and winters became more mild. This trend was matched by increasing amperage and duration of ELF fields. Although B.A.C.I. and R.I.A. tests showed significant before versus after differences in five of 27 cases, only one parameter appeared to be affected by ELF ground electric fields. The total insect mass in the summer was the only parameter estimated that may have been affected by the ELF operation. (The four remaining variables showed high variability around their mean values over the 10 years.) Serial multiple linear regression tests showed that discharge accounted for more of the variation in the summer insect mass at the test site than cumulative ELF ground electric field exposures or cumulative degree days. Analysis of the data indicated no ELF effects on the aquatic insect community (Table V2).

One periphyton parameter estimate, periphyton density, was positively related to total insect mass for insects on substrata in the spring and fall. In the spring, as water temperatures rose, periphyton density increased along with total insect mass. In the fall, as water temperatures decreased, the two biotic estimates were significantly correlated with one another. There was no relationship in the summer. Each estimate was significantly correlated with discharge and water temperatures in the spring and fall, but only insect mass was significantly correlated with discharge and water temperature in the summer.

Leaf processing rates did not differ between sites over the years. The insects colonizing leafpacks showed no detectable differences before or after the operation of the

7

Table V2. Summary of Statistics for Insects in Substrates
2-Way ANOVA, Multilinear Regressions: 1984 - 1993
B.A.C.I. and R.I.A., Before: April 1984-May, 1986; After:
June 1989-Aug. 1993

Parameter, Season	2-Way ANOVA: Signif.Factors	Mult.Reg.: When R^2>.30	BACI Tests	RIA Tests
H', Spring	Friedman: n.s.	Not Appropriate	N/A	n.s.
Summer	Site, Year, Interaction	FEX: Discharge, CDD	n.s.	
Fall	Site, Year	FEX: Discharge	n.s.	
J', Spring	Friedman: n.s.	Not Appropriate	N/A	n.s.
Summer	Site, Year, Interaction	FEX: Discharge, CDD	n.s.	
Fall	Site, Year	R^2 <.30 for Discharge & CDD	n.s.	
S', Spring	Site, Year, Interaction	FEX: Discharge	n.s.	
Summer	Site, Year	FEX: Discharge FCD: Discharge	n.s.	
Fall	Site, Year, Interaction	R^2 < .30 for Discharge & CDD	n.s.	
# INDIVID., Spring	Site, Yr, Interaction	FEX: Discharge FCD: Discharge	n.s.	
Summer	Site, Yr, Interaction	FCD: Discharge	N/A	***
Fall	Site, Yr, Interaction	FEX: Discharge, CDD	N/A	***
CHIRO.# DOM.Spg	Site, Yr, Interaction	R^2 < .30 for Discharge & CDD	**	
Summer	Friedman: Site	R^2 < .30 for Discharge & CDD	n.s.	
Fall	Site, Year	FEX: Year, CDD	N/A	***
INSECT MASS				
Spring	Year, Interaction	FEX:Discharge	n.s.	
Summer	Site, Yr, Interaction	FEX:Discharge FCD:Discharge,CDD, ELF	N/A	***
Fall	Site, Yr, Interaction	R^2<.30 for Disch.& CDD	n.s.	
CHIRO.MASS DOM.				
Spring	Year	R^2 <.30 for Discharge & CDD	n.s.	
Summer	Site, Year	R^2 <.30 for Discharge & CDD	n.s.	
Fall	Friedman: Site	R^2 <.30 for Discharge & CDD	n.s.	
COLL.GATH., Spg	Site, Year	R^2 <.30 for Discharge & CDD	n.s.	
Summer	Site, Year	R^2 <.30 for Discharge & CDD	n.s.	
Fall	Site, Year	R^2 <.30 for Discharge & CDD	n.s.	
PRED/PREY, Spg	Year	R^2 <.30 for Discharge & CDD	n.s.	
Summer	Site, Year, Interaction	R^2 <.30 for Discharge & CDD	n.s.	
Fall	Site, Year	R^2 <.30 for Discharge & CDD	n.s.	

CDD=Cumulative Degree Days   *** = p < 0.001
COLL.GATH.=Collector-Gatherer   n.s. = not significant
       Mass Dominance
PRED/PREY=Predator/Prey Ratio

ELF antenna. Multiple linear regression analysis indicated that cumulative degree days accounted for more of the variability than did stream discharge for the estimated parameters of diversity, equitibility, richness, numbers of individuals, total insect mass adjusted for leaf mass, and chironomid numerical dominance for insects on leafpacks (Table V3).

Growth rates of three species of insects on leafpacks were not shown to be affected by ELF fields. Although there was variability in growth rates over the years, changes in mean dry weight values per individual were similar between the sites (Table V4).

Movements of the dragonfly naiad, Ophiogomphus colubrinus, were not shown to be affected by ELF fields. The direction and distances travelled of marked animals were shown to be related to discharge events in the river.

Table V3: Summary of Statistics for Insects on Fresh
Leafpacks After Four Week's Incubation
2-Way ANOVA, Multilinear Regressions: 1984 - 1992

Parameter	2-Way ANOVA: Significant Factors	Multilinear Regression (When $R^2 >$ .30
Diversity	Site, Year, Interaction	FEX&FCD: Cum.Deg.Days
Evenness	Years	FEX&FCD: Cum.Deg.Days
Richness	Years, Interaction	N/A
No. Individuals	Years	FCD: Cum.Deg.Days
Chiro. No. Dominance	Years	FCD: Cum.Deg.Days
Total Insect Mass	Years	FEX&FCD: Cum.Deg.Days

Chiro. No. Dominance = Chironomid Numerical Dominance

Table V4. ANCOVAS for Testing Differences Between Sites for
Changes in MDW/IND values for Three Insect Taxa on
Leafpacks, 1984 - 1992

A. FEX vs. FCD

Year	E. subvaria	E. invaria	I. transmarina
1984	none	none	none
1985	none	none	none
1986	FEX<FCD	none	none
1987	none	none	none
1988	none	none	too few data
1989	FEX>FCD	none	none
1990	FEX<FCD	none	none
1991	none	none	none
1992	FEX<FCD	none	too few data

B. FEX vs. FEX.LINE

Year	E. subvaria	E. invaria	I. transmarina
1990	FEX<FexLine	none	none
1991	FEX>FexLine	FEX>FexLine	FEX>FexLine
1992	none	none	too few data

Task Four (Elements 7 and 8): Fish Community Composition, Abundance, and Movement

The operation of the ELF antenna, like any human modification of a stream ecosystem has the potential to effect changes at several levels of organization. The intent of this task was to determine if the ELF antenna operations were associated with any changes in the physiology and behavior of individual fish, in the attributes of populations of common fish species and in the composition of the entire fish assemblage in the Ford River.

The original study design was established to allow for two types of comparisons of these attributes. One of these was a comparison between a control site where fish were not exposed to ELF effects and a treatment site where fish were exposed to ELF effects. If ELF operations affected fish behavior or physiology, fish populations or the fish community, the effects should be expressed as differences in measurement variables between the control and treatment sites. A control site on the Ford River was selected that would minimize between-stream differences that were not ELF-related, such as temperature or stream flow differences among watersheds. The second comparison was between time periods, each with a measurable difference in ELF operations (pre-operational, transitional and operational periods).

Fish were collected systematically by use of directional fyke (trap) nets at the control (FCD) and treatment (FEX) sites from May through August for eleven years, 1983-1993. These sampling devices allowed for the capture of fish that were moving past the sampling location in an upstream or downstream direction. All captured fish were identified to species, measured for total length, weighed and marked with a fin clip distinctive to each site. Fish were then released beyond the sampling nets in the direction of their movement that was intercepted by the nets. The use of directional nets and site-specific fin clips allowed for the collection of vital data on individual fish, population attributes and community composition, as well as the movement behavior of individual fish.

Of the many individual, population and community attributes that were measured, few exhibited a significant effect from ELF operations either in comparisons between sites or comparisons among operation periods. The only significant differences that may be attributable to ELF operations were a decline in the Shannon-Weaver diversity index throughout the study period, and an increase in brook trout growth rates in the operational period. For both phenomena, the results at the control site (FCD) were similar to those at the treatment site (FEX).

The failure to find differences between FCD and FEX in the measurement variables may be attributable to several factors. First, electromagnetic fields such as those produced by the ELF Antenna may have no direct impact on fish growth, survival or reproduction. Second, the control site was not an effective control to the ELF operation treatment. Hundreds of individual fish moved between the FCD and FEX sites throughout the study, suggesting that the fish community in the Ford River is mixed over distances greater than the distances between the sampling sites. Some of the fish that were collected at FCD probably had been exposed to any potential ELF effects for at least part of their life, and some of the fish that were collected at FEX probably had been exposed for a period as brief as a few hours. At best, the mean period and intensity of exposure to ELF effects was lower at FCD than at FEX, however the sampling protocol was not adequate to estimate the differences in exposure times. Furthermore, no site was sampled where there was no exposure to potential ELF effects.

Another complication in comparing data between FCD and FEX sites is that for population and community variables, only one replicate was obtained for each year. This prevented accurate assessment of measurement error, which further eroded the strength of statistical methods in measuring differences attributable to site or period effects. This is reflected in the Minimum Detectable Difference estimates obtained for the BACI one-way ANOVA for species diversity and for individual species biomass data (Table V5). The BACI tests were based on $\log(x+1)$ transformations of annual values, which results in a Minimum

Detectable Difference estimate for transformed values, and it is not possible to convert this back to parameter units (e.g., g/net-day for biomass). For the Shannon-Weaver diversity index, the Minimum Detectable Difference of transformed vales between periods was 0.21, and the greatest difference between periods was 0.04, between pre-operational and operational periods. For the biomass data, burbot are representative: the Minimum Detectable Difference of transformed values was 0.89, but the greatest difference between periods was 0.32, between pre-operational and transitional periods. This summary shows that only large scale changes in species composition or in population biomass could have been detected between study periods.

The most telling data on the effects of ELF operations on fish in the Ford River is the number of observations of fish movements that involved crossing the ELF corridor. If ELF operations inhibited fish movements, the effect was not measurable with the data collected in this study. Rather, these data show that fish moved through the ELF corridor, whether the antenna was operating or not. However, when stream flow was unusually low, the fish tended to move between any sampling points less frequently, regardless of whether the move involved traversing the ELF Antenna corridor.

Furthermore, the fish community data did not exhibit any large scale changes in composition at either study site over three years of transitional operation and five years of full operation of the antenna. For example, none of the common species disappeared during the study, and no rare species became common during the study. Only burbot showed a measurable decline in biomass, and this began two years before the transitional period began. Given the dynamic nature of stream fish communities, the lack of large scale changes in community composition suggests that any ELF impacts on the fish assemblage were subtle at most. Although the study design does not allow conclusive interpretation that ELF operation has no impacts on the fish community of the Ford River, a more complete study design is likely to find no more than a subtle impact or a more long-term impact of ELF operations.

Table V5. Summary of representative data and analyses of fish community and population parameters. BACI ANOVA tested for differences between sites across three time periods (pre-operational, transitional and operational). Power analyses could not be performed on Analysis of Covariance tests.

Parameter	Pre-operational means ± S.E.		Transitional means ± S.E.		Operational means ± S.E.		Statistical Test Used[1]	p value	Min. Det. Diff.[2]
	Control	Antenna	Control	Antenna	Control	Antenna			
SPECIES DIVERSITY	2.04 ±0.06	2.11 ±0.07	1.84 ±0.17	1.79 ±0.17	1.35 ±0.08	1.26 ±0.10	BACI ANOVA	0.66	0.21
Mean Diff.[1] Trans. Val.	0.01		-0.01		-0.03				
BIOMASS (g/net-day)									
Brook Trout	348.40 ±25.65	322.51 ±89.23	179.29 ±51.38	208.91 ±128.94	205.46 ±36.36	162.80 ±22.43	BACI ANOVA	1.00	0.69
Mean Diff. Trans. Val.	0.08		0.08		0.07				
White Sucker	222.18 ±168.37	182.08 ±86.26	158.35 ±52.86	141.98 ±68.44	245.94 ±53.02	159.14 ±39.98	BACI ANOVA	0.63	0.99
Mean Diff. Trans. Val.	0.00		0.07		0.22				
Burbot	64.92 ±34.87	105.46 ±45.47	41.72 ±12.82	75.26 ±30.53	24.51 ±3.70	20.58 ±2.96	BACI ANOVA	0.27	0.89
Mean Diff. Trans. Val.	-0.24		-0.22		0.08				

Table V5, Continued. Summary of representative data and analyses of fish community and population parameters. BACI ANOVA tested for differences between sites across three time periods (pre-operational, transitional and operational). Power analyses could not be performed on Analysis of Covariance tests.

Parameter	Pre-operational means ± S.E.		Transitional means ± S.E.		Operational means ± S.E.		Statistical Test Used[1]	p value	Min. Det. Diff.[2]
	Control	Antenna	Control	Antenna	Control	Antenna			
Creek Chub	121.34 ±58.70	96.90 ±43.56	103.35 ±6.60	63.97 ±12.54	97.79 ±27.15	43.71 ±10.68	BACI ANOVA	0.09	0.49
Mean Diff. Trans. Val.	0.08		0.22		0.37				
Common Shiner	85.96 ±43.47	44.50 ±20.51	68.90 ±2.84	30.54 ±8.06	134.86 ±8.69	59.84 ±6.36	BACI ANOVA	0.44	0.36
Mean Diff. Trans. Val.	0.27		0.39		0.36				

[1] For BACI ANOVA of species diversity and biomass, data were log(x+1) transformed, and power analysis of the ANOVA was based on these transformed data. Therefore, the mean and standard error values are given for the actual measurements, and the mean difference between transformed data are given for each period. The minimum detectable difference value should be applied to the mean differences between transformed data, and cannot be converted to correspond with the measurement values.

[2] Minimum detectable difference estimates are based on values of alpha = 0.05 and beta = 0.20.

15

VI. PROJECT RATIONALE AND APPROACH

Our research plan was directed at determining the effects of extremely low frequency (ELF) electromagnetic fields and gradients produced by the ELF Communications Systems on aquatic plant and animal life. Three major groups of organisms (benthic algae (periphyton), larval insects and fish) were selected for intensive study. Sensitive life history events and community processes critical to the basic structure and function of stream ecosystems were monitored. These included: species composition of the benthic algal, insect, and fish communities; density, diversity, evenness and relative abundance data for algae, insects and fish; community primary production and respiration and chlorophyll *a* and organic matter biomass and accumulation rate changes for the biofilm community on rocks in streams; insect and fish migration patterns; organic matter processing by macroinvertebrates; and dynamics of fish population growth, reproduction, and survival. Since many of these processes and events were mutually dependent on one another and possible interactions were complex, a holistic approach with a multi-disciplinary effort was imperative. The design incorporated studies of ecosystem properties with studies of behavior and biology of individual species so that any effects of ELF could be quantified at the population, community and ecosystem levels.

We selected stream ecosystems as representative aquatic ecosystems rather than lakes or marshes because; (1) upstream-downstream paired plots on the same system provided less variability than between lake comparisons would have provided; (2) migratory behavior was more likely to be important in stream organisms; and (3) our expertise and interests were oriented toward stream ecology.

The effects of ELF on stream ecosystems were tested using a before-after, contol-impact, paired plot design on sections of the Ford River, a fourth order, brown water trout stream, in Dickinson County, Michigan. Plots were selected to afford one area of study away from the antenna corridor for use as a control site (FCD), and another area near or directly under the antenna cable for use as the experimental antenna site (FEX) (Figure VI.1). These plots were intensively studied and sampled for a 3 year period of time before operation of the antenna began (1983-1986); for a three year period when low amperage testing of the antenna was in progress (1986-1988); and for a period when the antenna was being operated intermittently (1989) or continuously (1990-1993) by the Navy.

16
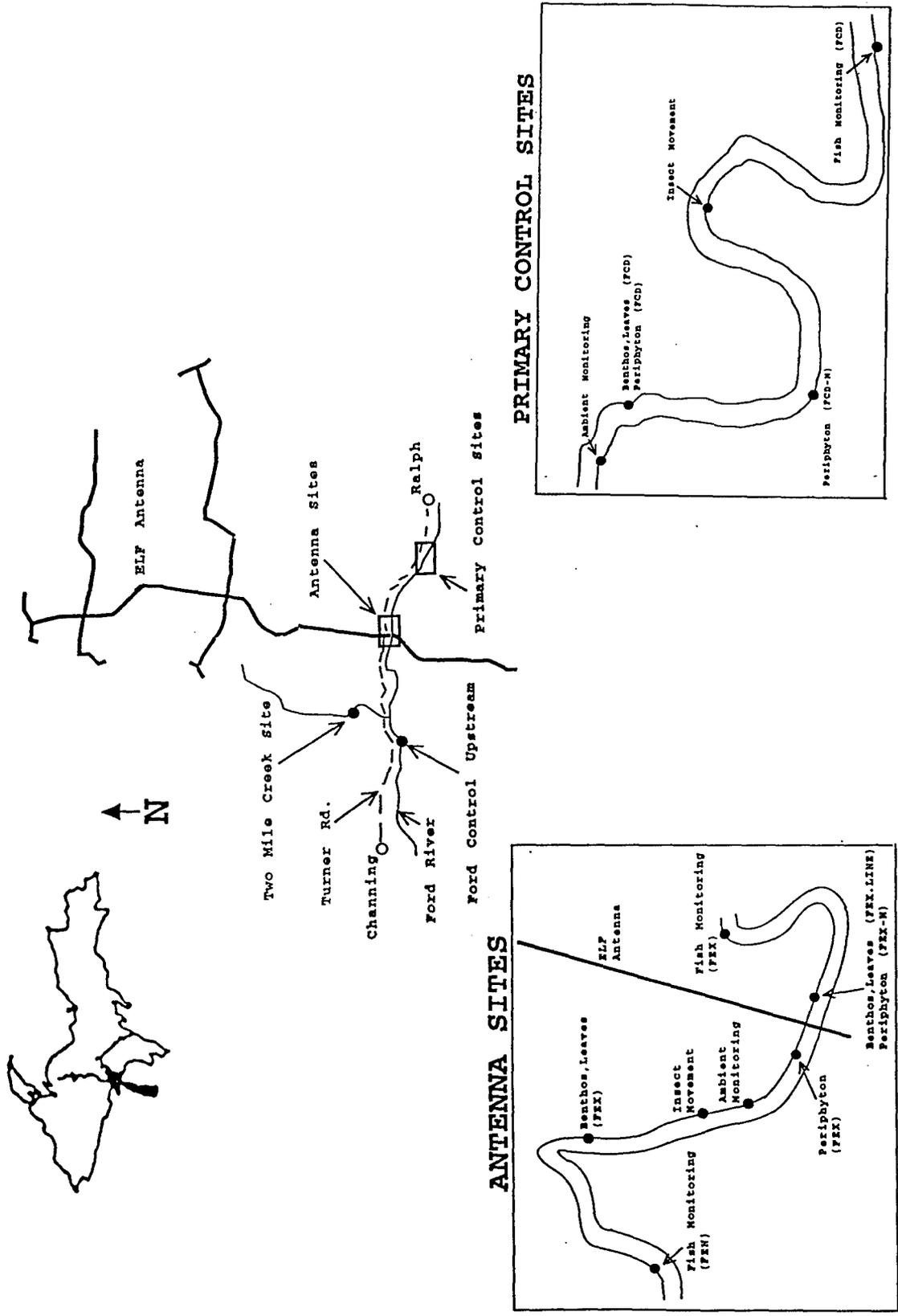
Figure VI.1   ELF Research Sites on the Ford River, Dickinson County, Michigan.

17

The ELF antenna was first tested using low (4-6)
amperage in 1986. Amperage was increased to 15 amps for
testing in 1987 and to 75 amps in 1988. Full-current (150
amps) testing began in April 1989 and continued through
October 1989. Since October 1989, the antenna has been
operational at full power except for periods of maintenance.
Tables VI.1 and VI.2 show earth electric field intensities,
magnetic flux densities and intersite ratios from 1986-1993
at the four periphyton sites. The primary control site
(FCD) and the primary antenna site (FEX) have been used
throughout the project. Because the intersite ratio in
electric field exposure at these sites was less than the 10-
fold minimum ratio called for in the initial study protocol
(see IITRI Technical Report DO6209-1), two additional
periphyton (benthic algal) sites were added in 1990 (FCD-N
and FEX-N, FEX-N for the benthic algal studies is
essentially the same as FEX.LINE for the insect studies) to
achieve the desired differences in exposure (Table VI.2).
Since data were not collected for the before period for
these new sites, primary results still rely on the original
control (FCD) and antenna (FEX) sites. Sites used for fish
and insect monitoring and movement studies are also shown on
Figure VI.1.

Table V1.1 76Hz Earth Electric Field Intensities (mV/m) at the Four Study Sites and the Ratios between the Paired Antenna and Control Sites. Data were Collected by IITRI Personnel.

Site	1986^	1987^	1988^	1989	1990	1991^	1992	1993
FEX	1.62	6.1	3.1	65.0	61.0	55.0	66.0	60.0
FCD	----	----	7.1	11.9	12.3	11.5	10.3	9.2
FEX-N	----	----	----	----	85.0	71.0	79.0	70.0
FCD-N	----	----	----	----	8.0	7.0	7.1	7.3
FEX:FCD	----	----	4.4	5.5	4.9	4.8	6.4	6.5
FEX-N:FCD-N	----	----	----	----	10.6	10.1	11.1	9.6

^ The electric field intensites reported for these years are associated solely with the North-South leg of the ELF antenna, the leg closest to the Ford River study sites and the largest contributor to total electromagnetic radiation at the study sites.

19

Table VI.2   76Hz Magnetic Flux Densities (mG) at the Four Study Sites and the Ratios between the Paired Antenna and Control Sites. Data were Collected by IITRI Personnel.

Site	1986^	1987^	1988^	1989	1990	1991^	1992	1993
FEX	0.31	1.20	5.50	12.70	11.40	13.50	11.20	11.40
FCD	----	----	0.02	0.04	0.04	0.05	0.04	0.04
FEX-N	----	----	----	----	19.10	19.90	21.00	19.10
FCD-N	----	----	----	----	0.04	0.04	0.04	0.04
FEX:FCD	----	----	250	334	317	300	303	317
FEX-N:FCD-N	----	----	----	----	516	463	553	516

^The magnetic flux densities reported for these years are associated solely with the North-South leg of the ELF antenna, the leg closest to the Ford River study sites and the largest contributor to total Electromagnetic radiation at the study sites.

VII. OVERALL AND SPECIFIC TASK OBJECTIVES

OVERALL OBJECTIVE

The major objective of this study was to determine the effects of low level, long term electromagnetic fields and gradients produced by the ELF Communication System on aquatic plant and animal life in streams.

SPECIFIC TASK OBJECTIVES

Periphytic Algal Studies

The objectives of the benthic algal studies were:
(1) to quantify any changes in diatom density, diversity, evenness, cell volume, and biovolume that occurred as a result of ELF electromagnetic fields.
(2) to quantify any changes in chlorophyll a and organic matter biomass standing crop and daily accumulation rates that occurred as a result of ELF; and
(3) to quantify any changes in community primary production and respiration that occurred as a result of ELF electromagnetic fields.

Aquatic Insect Studies

The objectives of the studies of aquatic insects were:
(1) to quantify any changes in organic matter processing rates that occurred as a result of ELF;
(2) to quantify changes in species richness, individual abundances, and species diversity of aquatic insect communities associated with leaf pack and inorganic stream bottom substrates that occurred as a result of ELF; and
(3) to quantify trophic, behavioral, and community level changes in selected species of aquatic insects from an array of functional feeding groups (grazers, collectors, etc.) that occurred as a result of ELF.

Fish Studies

The objectives of the studies of the fish were:
(1) to quantify any changes in the seasonal movement patterns and abundance of the mobile fish community that occurred as a result of ELF; and
(2) to quantify any changes in the rate of brook trout movement through the ELF corridor that occurred as a result of ELF electromagnetic fields.

VIII. PROGRESS BY WORK ELEMENT

Task One, Element 1: Ambient Monitoring

Introduction

Many environmental factors such as light availability, water quality, temperature, land use in the watershed and many other factors influence aquatic communities and may have differential impacts along a river's course. We chose to measure the parameters that were called for in the original request for proposal or, based on our experience, were most likely to affect the biota of the Ford River. The experimental design for biotic monitoring of ELF electromagnetic radiation effects called for matched sites with the antenna site near or under the ELF antenna and the control site far enough away to receive an order of magnitude less earth electric and magnetic flux exposure once the antenna became operational. Our goal was to select sites that were characterized by identical chemical, physical and biological conditions prior to antenna operation. Of course, identical sites do not exist, but we were able to document the fact that there were no significant differences in any of the monitored parameters between the antenna and control sites or that those differences that did exist were small and probably not significant biologically. The following is a summary of the more important findings from ambient monitoring. Detailed 28 day summary statistics for all monitored parameters were summarized in the 1993 annual report.

Objectives and Rationale

The Ambient Monitoring Program had two primary objectives:

(1) to provide the background data on physical and chemical parameters needed to correlate observations on biological community dynamics with environmental parameters; and

(2) to determine whether or not observed changes in biological parameters were related to physical and chemical changes in the Ford River rather than ELF electromagnetic radiation.

The parameters monitored were chosen based on their importance to periphyton (benthic algae), aquatic insects or fish, the biotic components monitored. For instance, various major plant nutrients such as soluble reactive phosphorus, inorganic nitrogen species and silica were chosen because of their importance to benthic algal (periphyton) production and thus trophic level dynamics. Parameters known to influence insects and fish such as water temperature, dissolved oxygen, discharge and turbidity, were also monitored. A number of

parameters were also monitored to offer general indices to site productivity and water quality. These included alkalinity, hardness, specific conductance and chloride.

Materials and Methods

Detailed materials and methods were given in the annual report for this project (AE-143 for 1993) and will not be given in detail here. A summary of these procedures is given below. Unless specified, procedures used were those recommended by A.P.H.A. (1985) or earlier editions and/or by U.S. EPA (1979).

A number of physical and chemical parameters were monitored continuously each year from mid April through October (mid-May through August in 1992 and 1993) at ambient monitoring stations at the control (FCD) and antenna (FEX) sites (Figure VI.1). The stations automatically logged on Omnidata data pods (Model DP 211) the following parameters:

(1) Photosynthetically active solar radiation (PAR) (both below water and above) was measured using Li-Cor Model LI-192SB underwater quantum sensors. These data were taken in the most open location that could be found on the bank near the monitoring station for above water and at a point in the center of the river for underwater PAR. These data are not at the exact location of the algal sampling location, and comparisons between the two sites were not possible for these parameters due to uncontrolled, differential shading of the sensors. These data only provide a general index of PAR availability at these sites.

(2) Dissolved Oxygen (DO) was monitored using L. G. Nestor Model 8500 portable dissolved oxygen meters with general purpose submersible probes.

(3) pH was measured using the Altex (Beckman) Monitor II System with specially built long term, gel-filled submersible pH probes from Fisher Scientific.

(4) Air and water temperature were monitored using thermistors.

Water depth was continuously monitored using Stevens Type F strip chart recorders. The depth data were used to calculate discharge using stage height-discharge least squares regressions. Stage-discharge relationships were determined for each station using Teledyne Gurley pygmy or Price-type current meters using the velocity area technique (Gregory and Walling, p. 129).

Data from the data pods were transferred from the EPROM chips in the data pods to diskettes using an Omnidata Model

23

217 reader and an Apple II plus computer. Data, accumulated daily at 30 minute intervals, were read and summarized every two weeks throughout the April to October period.

All automatically acquired data were checked and calibrated by manually determining each parameter at least twice per week. Water samples were taken once per week for determination of turbidity, alkalinity, hardness, and specific conductance following methods recommended in Standard Methods (A.P.H.A. 1985 or earlier versions of this manual). Twice per week, samples were taken and frozen for later determination of total phosphorus, soluble reactive phosphorus (samples were placed on ice after collection and were filtered within 3-5 hours of collection), nitrate-N, nitrite-N, ammonium-N, organic-N (total Kjehldahl N minus ammonium), chloride, and dissolved silicate-Si (Si samples were refrigerated instead of being frozen since freezing can cause interference with this procedure). The N, P, Si, and Cl samples were analyzed in the winter months using a Technicon AutoAnalyzer following methods recommended by U.S. EPA (1979).

During winter months, samples were taken at one month intervals for all of the parameters discussed above from 1983 through the winter of 1986-1987. This interval was decreased to once every other month in 1987-1988 and once every 6 weeks in 1988-1989 and 1989-90. Winter sampling was discontinued completely following October 1991 due to financial and logistical constraints. Total P, Cl, and total N analyses were eliminated as part of the negotiated phase back of these studies during 1991-1993.

Land use in the Ford River Watershed was analyzed in 1992 to determine what the major land uses were and if significant land use changes had occurred during the course of this study. Land use changes were extracted from the Michigan Resource Information System (MIRIS) using Geographic Information Systems (GIS) technology. The MIRIS included land use inventory data for 1979 and 1986. Changes over this time period were documented. Changes from 1986 to 1993 were not available, but general observations were included.

Before-After, Control-Impact (BACI) analyses (Stewart-Oaten et al. 1986) were used to determine if between site differences in ambient monitoring parameters were the same between the before and after antenna operation periods. Please see the more detailed rationale and procedures for the use of this statistic in the Materials and Methods section of Task two, Element 2. Differences between means for the before and after periods were accepted as significant using unpaired t-tests at the $p > 0.05$ level. The before period was from April 1984 to October 1985 prior to the antenna ever being turned on even for low amperage testing. The after period was from June 1989 to August 1993 after testing and operation

of the antenna began at 150 amps. The 1986 to 1988 period was
a period of low amperage testing of the antenna. Data for
this period is presented graphically as a transition period
but are not included in statistical comparisons between
sites. In previous reports, between site comparisons were
made using paired t tests. Paired t tests were used in this
report to compare consistent differences between sites to see
if these differences were significant at the $p<0.05$ level,
since BACI analyses only test for changes in the differences
between sites between the before and after periods. BACI
analyses were used to test differences between sites in
ambient monitoring parameters for the ice-free period for the
before and after years to be consistent with the analyses of
the periphyton in Task two, Element 2 in the final report.

Earth electric and magnetic fields were monitored by
IITRI engineers. Readers are referred to their reports for
techniques used (contact IIT Research Institute, 10 West 35th
Street, Chicago, Il 60616).

Results and Discussion

Land Use Changes

Results of this analysis showed that forests were the
predominant land use in the Ford River Watershed in Dickinson
County with almost 90 % of land use being in deciduous,
coniferous or wetlands forest (Table 1.1). There were few
changes from 1979 to 1986 in most categories with the largest
decrease in land area being in coniferous forest and the
largest increase in land area being in cleared land (Table
1.1). Even these changes were relatively minor and were
located several kilometers from our research sites. Personal
observations and discussions with the local Michigan
Department of Natural Resources forester suggested that the
area is subjected to routine timber harvest where small
patches (less than 20 ha) are clear-cut on a 40-50 year
harvest cycle. This harvesting was underway prior to the
start of these studies and continued through the study
period. The harvested area quickly revegetated from stump
sprouting and did not involve large areas in any particular
year. There has been little change in land use over the
course of the study, either for the before data as documented
with the GIS analysis of the MIRIS data base or since that
time based on extensive travel and personal observations in
the watershed. Thus, any long term changes in the biota are
unlikely to be related to changes in land use in the
watershed.

Field Chemistry

The differences in means for the before period were not
significantly different from the difference in means for the

25

Table 1.1   Land use changes in the Ford River watershed
             in Dickinson County from 1979 to 1986 obtained
             from BSTATS in ERDAS.

Land Use	Percent Cover 1979	Percent Cover 1986	Change
Deciduous Forest	41.40	40.80	-0.60
Coniferous Forest	38.30	37.25	-1.05
Wetlands Forest	10.36	10.26	-0.10
Herbs/Shrubs	5.89	5.73	-0.16
Agriculture	1.27	1.10	-0.17
Non-Forested Wetlands	0.91	0.91	0.00
Barren, Open, or Cleared	0.14	1.55	1.41
Other Terrestrial*	0.32	0.31	-0.01
Aquatic	2.11	2.10	-0.01

* = Residential, Commercial, Utility, and Extractive

after period for any of the field chemistry parameters monitored according to BACI analyses except for dissolved oxygen (Table 1.2). Differences in dissolved oxygen between the before and after periods between the antenna and control site were slight (within 0.5 mg/L, Figure 1.1). The dissolved oxygen values at each site were never more than 1 or 2 mg/L below saturation and remained well above the 5 mg/L needed to maintain trout populations in good condition (Mckee and Wolf 1963). Concentrations of the other field chemistry parameters monitored (conductivity, pH, alkalinity, turbidity and water hardness) were similar between the control (FCD) and antenna (FEX) sites. Significant differences (paired t tests, $p < 0.05$) that did exist between the two sites were small and consistent from the before to after periods.

Water hardness, alkalinity and turbidity were consistently, significantly slightly higher at the downstream site than they were at the upstream site (paired t tests) for both the before and after periods. The differences between the sites in these three parameters may be due to the expected increase in the load of dissolved ions and suspended particles in the downstream direction. Such increases are found because of inputs from groundwater, surface runoff, and in-stream processing of organic matter coupled with concentration due to evapotranspiration. The differences in hardness and alkalinity were small and were well within the range of variation that occurs between peak runoff and baseflow periods. Hardness and alkalinity for both sites were typical of a hardwater stream and were well above known threshold limits for biota (Mckee and Wolf 1963). Higher turbidity could lower PAR received by periphyton which could ultimately lower production, but the turbidity at both sites was very low (less than 3 NTU's even during runoff events), so this is unlikely to be the case. pH and conductivity levels were not significantly different ($p < 0.05$) between the two sites.

Nutrient Chemistry

Values for the nutrient chemistry parameters were similar at the control and antenna sites for the 1983-1993 interval (see graphs of data in 1993 annual report). Paired t tests demonstrated that there were no significant differences for six of the nine parameters monitored (nitrate-N, ammonium-N, soluble reactive P (SRP), total P, silicate-Si, and inorganic-N). Differences in the other three (nitrite-N, total organic-N, and chloride) were significant but were small and consistent from the before to the after period, except for nitrate-N. According to BACI analyses, differences in mean concentration of inorganic nitrogen between the sites changed significantly between the before and after periods (Table 1.2). Inorganic nitrogen is the sum of nitrate-N, nitrite-N and ammonium and should be a good

27

Table 1.2   Summary of BACI Statistics for Chemical and Physical
             Parameters at Control (FCD) and Experimental (FEX) sites.
             The comparisons were made using months in which the Ford
             River was free of ice cover (April - October). Before period
             = 1984-1985, After period = 1989-1993.

Parameter	N	BACI P values	Power[t] (.50)	Power (.20)
Conductivity	50	0.151	99	99
Discharge[§]	38	NS	99	99
Dissolved Oxygen	50	0.028[*]	99	99
Inorganic Nitrogen[§]	49	<0.05[*]	99	54
pH	50	0.462	99	99
Silica	49	0.445	99	99
Soluble Reactive Phosphorus	46	0.455	99	85
Water Hardness	48	0.589	99	99
Water Temperature	50	0.073	99	99

- The following were log (x+1) prior to BACI analysis: dishcharge, water temperature
- [t] power to detect a difference equal to 50% or 20% respectively of before period grand mean.
- * $P < 0.05$
- [§] t statistic corrected for positive serial correlation.
- Alkalinity and turbidity datasets were not analyzed due to the lack of an additive relationship between site and time in the before period
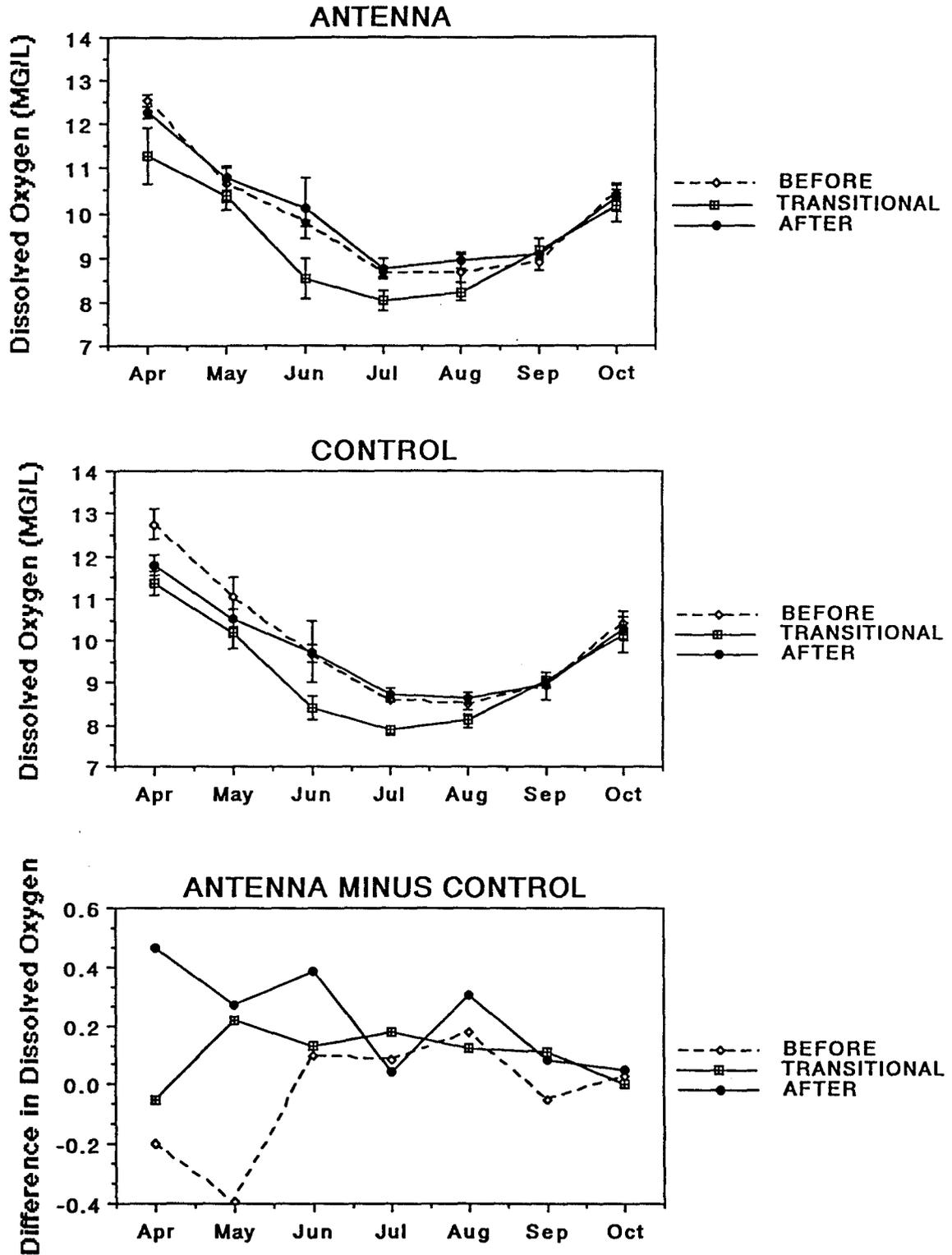
Figure 1.1 Dissolved Oxygen at the Antenna (FEX) and Control (FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before = Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.
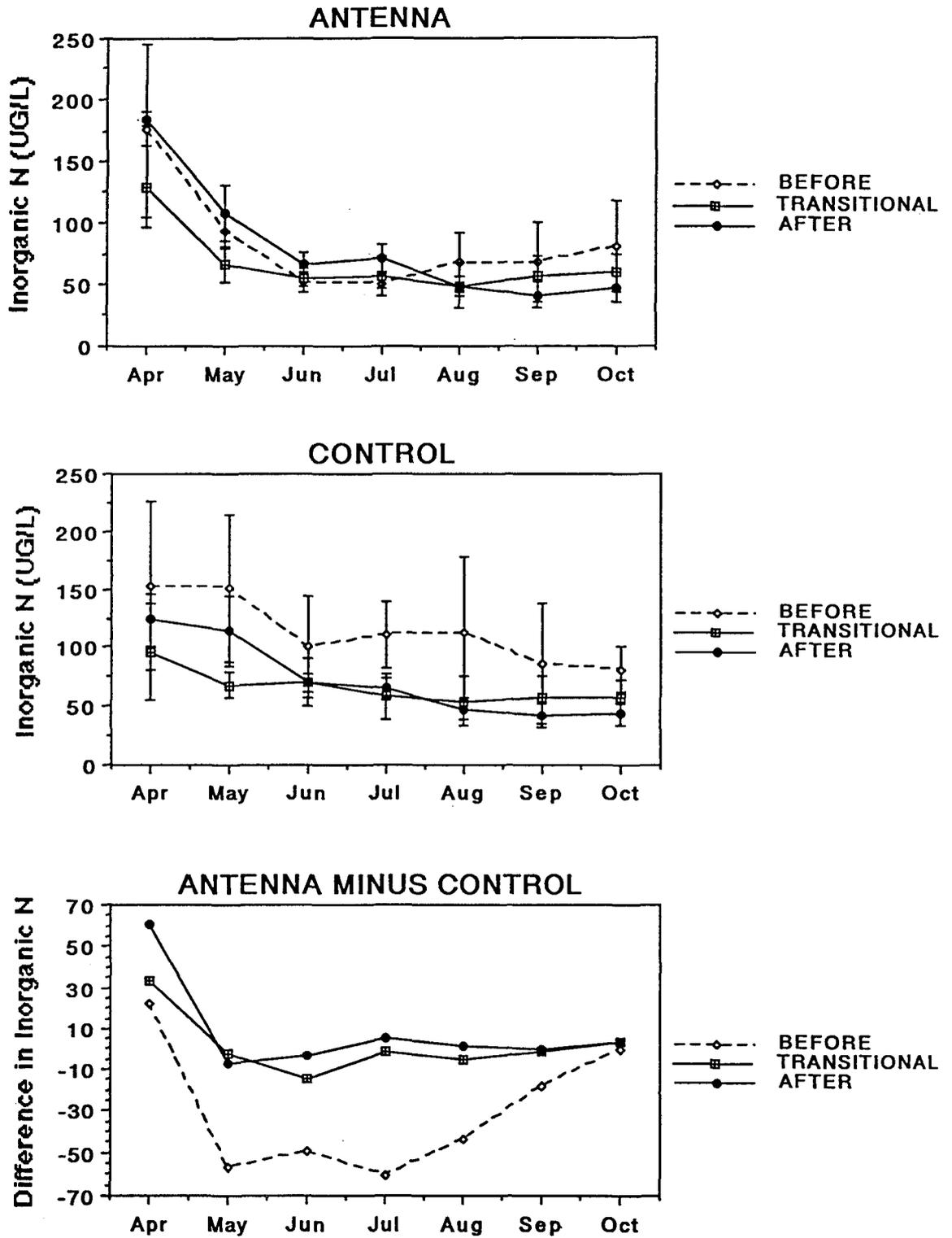
Figure 1.2 Inorganic Nitrogen at the Antenna (FEX) and Control (FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before = Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.
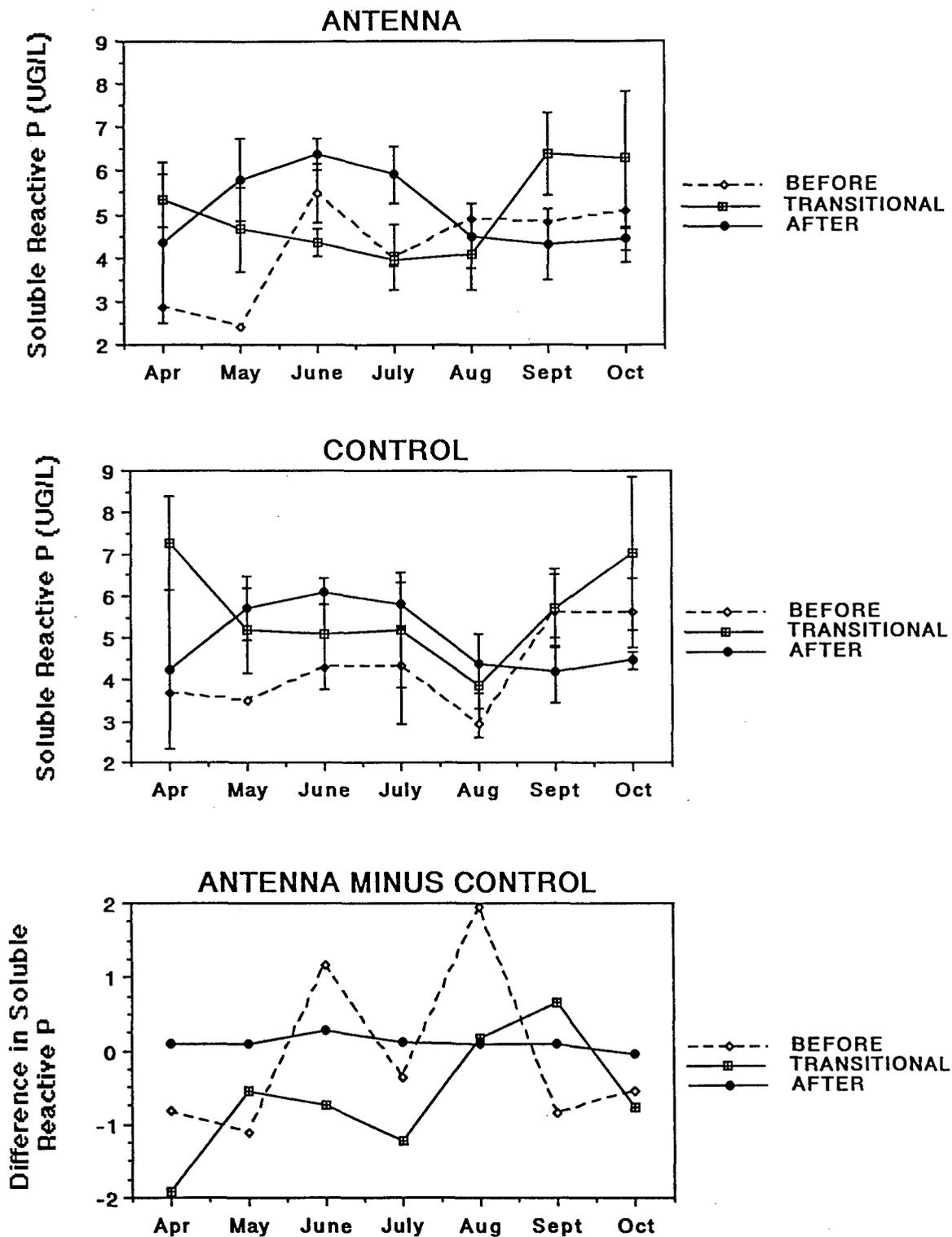
Figure 1.3 Soluble Reactive Phosphorus at the Antenna (FEX) and Control (FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before = Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.

31

indicator of the amount of nitrogen available to algae in the Ford River. Monthly mean values were 50 to 60 µg N/L higher at the control site than at the antenna site in the before period (Figure 1.2), due to unexpectedly high values for nitrate-N in 1985. This difference disappeared in the transition (antenna testing) and after (antenna operational) years with inorganic-N being almost identical at both sites from May to October (Figure 1.2) Inorganic-N was also not significantly different (p<0.05, paired t test) at the control and antenna site in 1984, the first of the two control years. The similarity in inorganic N between the two sites for 1984 and from 1986 through 1993 resulted in the finding of no significant difference in the two sites when the entire 1984-1993 period was compared using paired t tests. The unexpectedly greater amounts of inorganic nitrogen at the control site in 1985 may have been due to the clearcutting of the forest along the river just upstream and adjacent to the control site in 1985. This forest practice is known to lead to high nitrate losses in the first year or so after cutting for some northern hardwood forests similar to the forests along the Ford River (Bormann and Likens 1979).

Differences in soluble reactive phosphorus (SRP) between the control and antenna site were not significantly different between the before and after antenna operation periods according to BACI analyses (Table 1.2). Even so, a graphical summary of its temporal dynamics at both the control and antenna sites (Figure 1.3) is presented, since it is an important nutrient used by periphyton. SRP was similar between sites during the before operation and after ELF antenna operation periods (Figure 1.3) with mean differences between sites never exceeding 2 µg P/L. Mean concentrations at both sites varied from 2 to 8 µg P/L (Figure 1.3). These levels are very near the analytical limit of detection using autoanalyzer technology, and 2 µg/L is well within the range expected from analytical error.

Physical and Meteorological Parameters

Physical and meteorological parameters were similar in magnitude at the control and antenna sites during the 11 year study period. Exceptions were below water and above water photosynthetic active radiation (PAR). PAR was highly variable, but one site was not consistently higher than the other (see 1993 Annual Report). PAR was monitored at the ambient monitoring stations, since cable limitations made recording PAR at the exact locations of the diatom collectors impossible. Thus, these data were only used as an index of available PAR at each site, not as a measure of absolute amounts of PAR received by algae at the monitoring sites and not for intersite comparisons.

32

Discharge was consistently and significantly (paired t test, p<0.05) slightly higher at the control site relative to the antenna site (Figure 1.4), especially during May, June and July. Even though there are no tributaries entering the Ford River between the two sites, a slight increase in discharge at the downstream site was expected due to ground water inputs between the two sites during baseflow events and due to possible overland flow inputs during runoff events. Discharge between the two sites was highly correlated (R^2>0.98), and large increases in discharge due to runoff events at the upstream antenna location were followed after short lag times with similar increases at the downstream control location. Thus, runoff events large enough to scour algae and insects from the substrate were of similar magnitude or only slightly higher at the downstream site (Figure 1.4). More importantly, these differences were consistent for the before and after antenna operational periods except during May and October (Figure 1.4). Differences during these two months apparently accounted for the significant differences between the means for control and antenna sites between the before and after periods according to BACI analyses (Table 1.2).

There were no significant differences in water temperature between the before and after periods between the antenna and control sites (Table 1.2, Figure 1.5) at the p<0.05 level but differences were significant at p=0.07. Water temperature was significantly higher (paired t-tests, p < 0.05) at the antenna site than at the control site during the study period, but the difference between the two sites was small (less than 0.6 °C except for April and October in the transitional period) (Figure 1.5).

Summary

Data for all chemical and physical parameters demonstrated that the experimental and control sites were very well matched. For the majority of the parameters, there were no significant differences between sites. When significant differences did occur between the chemical constituents, they usually involved a slight increase in a downstream direction. This trend of slight increase from the upstream site to the downstream site for hardness, alkalinity, turbidity and organic nitrogen may be related to accumulation of dissolved load in a downstream direction. These differences were consistent for the before and after antenna operation periods. Other parameters shown to have slight, but significant differences between sites were dissolved oxygen, water temperature, and chloride. The impact of these minimal differences between the sites will be discussed as appropriate in the rest of the report.
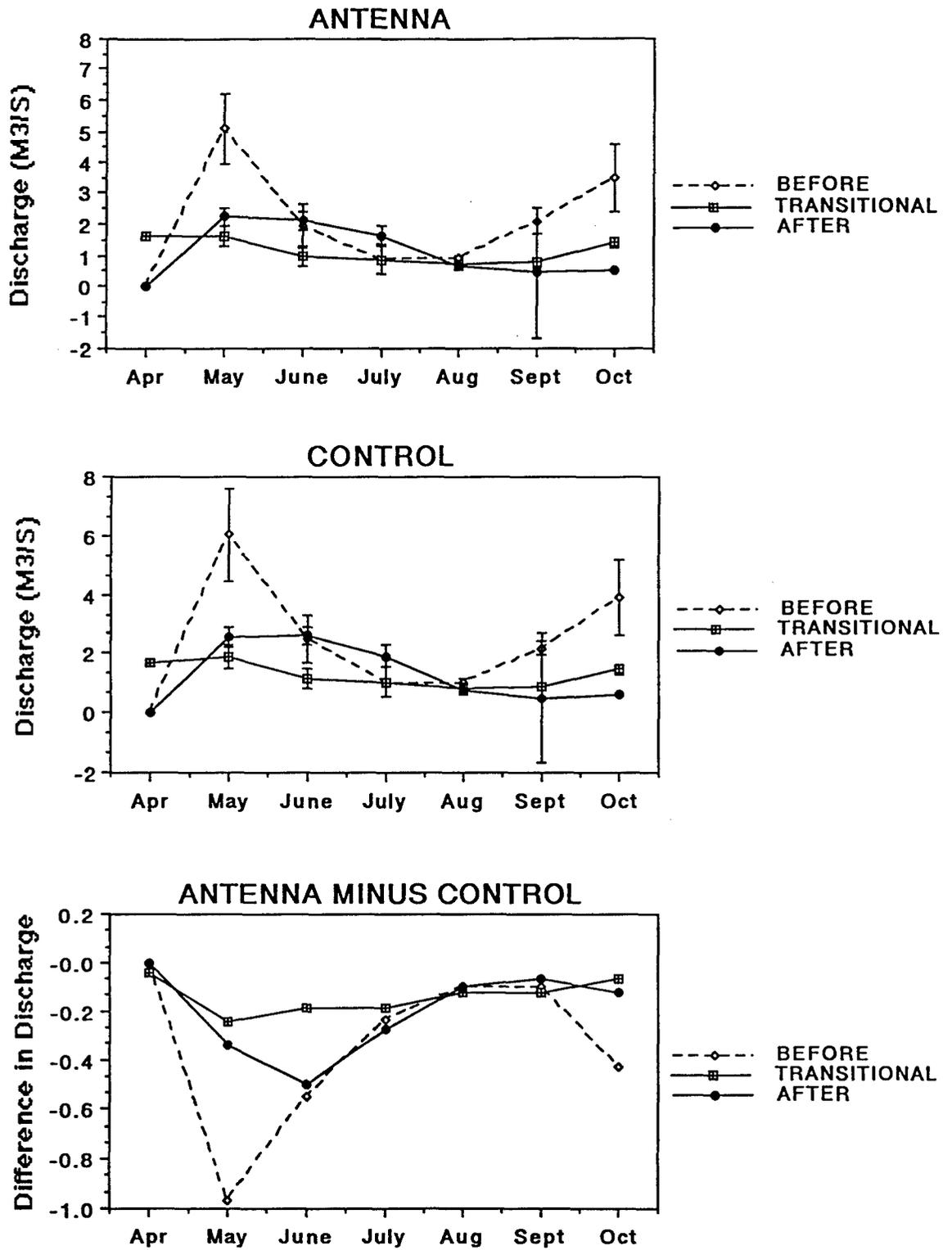
Figure 1.4 Discharge at the Antenna (FEX) and Control
(FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before =
Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.
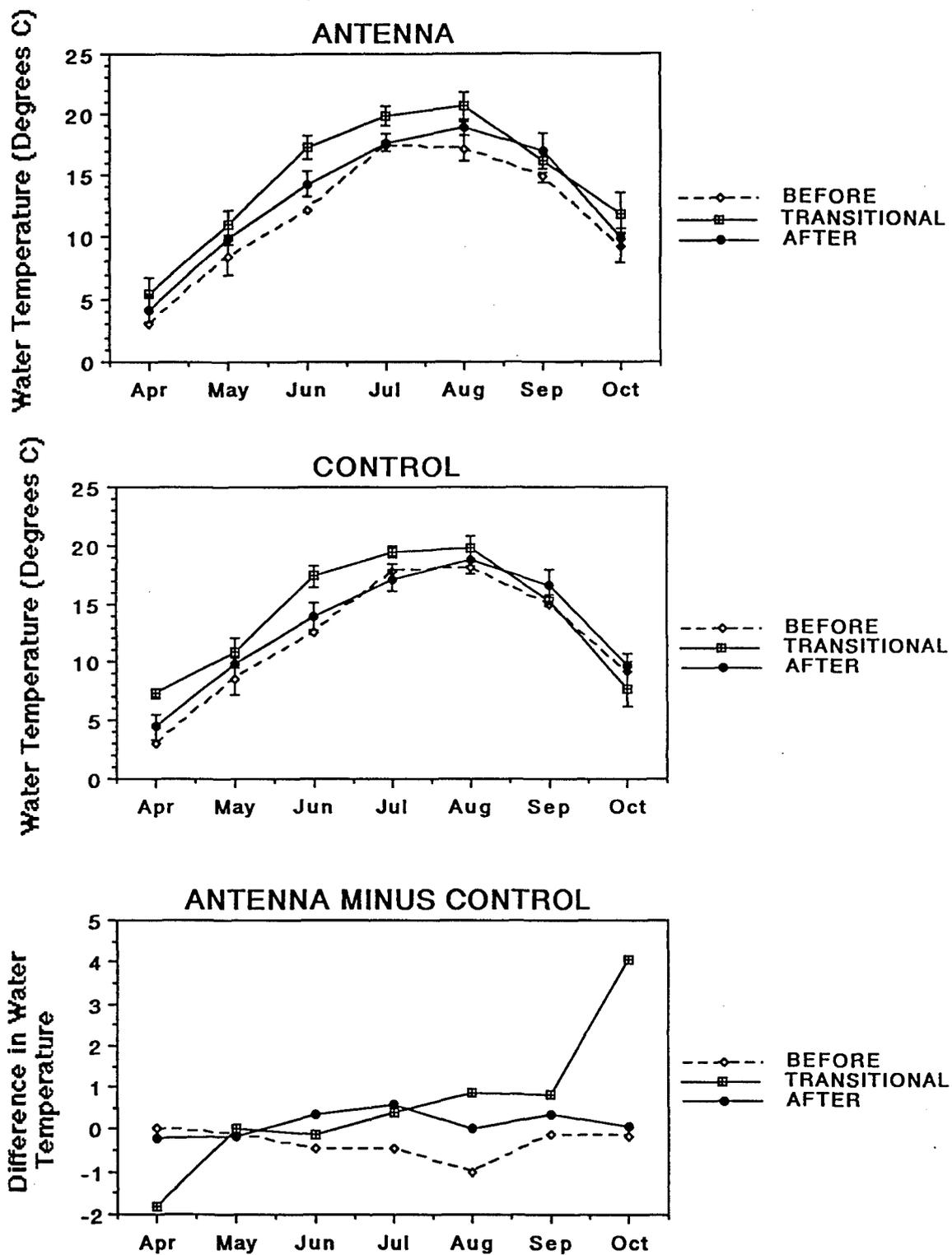
Figure 1.5 Water Temperature at the Antenna (FEX) and Control
(FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before =
Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.

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Task Two - Periphyton Studies(Elements 2 And 3)

Task 2, Element 2:   Benthic Algal (Periphyton) Responses to ELF Electromagnetic Exposure

Introduction

Benthic algal communities are usually the dominant primary producers in mid-order streams (Vannote et al. 1980). At the initiation of this study in 1982, there was little, if any, evidence in the literature concerning the effects of ELF electromagnetic fields on algal species or communities. Blakemore (1975) had established that a species of magnetotactic bacteria in marsh sediments migrated along magnetic lines. Frankel et al. (1979) had confirmed these findings and confirmed that magnetite in these bacteria was the substance responsible for the magnetotactic behavior. Blakemore et al. (1980) established that orientation along magnetic lines resulted in downward migration of the bacteria in both the southern and northern hemispheres. Later, Torres de Araujo et al. (1986) reported that an euglenoid alga from a coastal swamp in Brazil contained magnetite and exhibited magnetotactic behavior. No other species of algae is known to exhibit such behavior to our knowledge.

Magnetic fields may also increase the mobility of some species of algae. Smith et al. (1987) documented increased mobility of the diatom Amphora coffeaeformis when exposed to magnetic fields at 16 and 48 Hz. Mobility increased relative to the control at a flux density as low as 0.050 G and peaked at 0.209 G. No increased mobility was documented at 32 and 64 Hz.   Smith et al. (1987) suggested that the mobility effect was mediated by transport of calcium ions across the diatom's cell membrane. Reese et al. (1991) confirmed the basic findings of Smith et al.'s experiment. However, Parkinson and Sulik (1992) were unable to reproduce Smith et al.'s results. These findings along with the cellular impacts documented by Bawin et al. (1975) and Blackman et al. (1982). suggested that algal communities might respond to increased exposure to electromagnetic fields once the U.S. Navy's communication antenna became operational. To our knowledge, no field based studies of the effects of ELF electromagnetic fields on algal communities have been conducted. Thus, we began background studies of benthic algal communities in the Ford River in Dickinson County in the upper peninsula of Michigan in 1982 in order to obtain pre-operational data that could be compared to post-operational data after the antenna was activated.

Objectives

The objective of the benthic algal studies was to determine whether or not 76 Hz ELF electromagnetic fields

produced by the antenna would have a detectable effect on benthic algal communities and populations using a variety of measurements that could be related to growth, production and diversity of the algal communities. Specific objectives were:

(1) to compare changes in: (a) algal cell density and algal biovolume (an estimate of algal biomass) and (b) standing crop and accumulation rates of chlorophyll a and ash-free dry weight organic matter at the control and antenna sites before and after antenna operation as a method of detecting differential changes in algal community function related to ELF antenna operation.

(2) to quantify changes in relative abundance of dominant species, species diversity and species evenness at the control and antenna sites before and after antenna operation to detect any differential changes in community composition related to antenna operation.

(3) to quantify changes in algal primary productivity and community respiration at the control and antenna sites before and after antenna operation as a means of detecting differential responses in productivity of the algal communities related to antenna operation.

Methods

We conducted colonization and initial monitoring studies of algae in the Ford and nearby rivers in 1982 (Oemke and Burton 1986). Algal communities similar in composition to the communities on nearby undisturbed cobble on the stream bed developed on microscope slides oriented vertically into the current in riffles near the streambed in 21 to 28 days. Species composition on these slides reflected composition of the "mature" algae on nearby cobble, and differences in community composition between riffle and pool habitats had disappeared after 21 to 28 days (Oemke and Burton 1986). Thus, we elected to use microscope slides as our sampling unit and sampled these slides after 14 days for accumulation rates of chlorophyll and organic matter dry weight (during the period of exponential increase in cell density on the slides) and after 28 days for measures of community composition of the "mature" algal community. Over 90 % of the cells on these slides were diatoms, and this dominance by diatoms was also characteristic of communities on nearby cobble. Therefore, all of our cell count based statistics (e.g. diversity, evenness, relative abundance, etc.) were based on counts of the diatom community on microscope slides only.

The microscope slides used for sampling diatoms were held vertically in plexiglass slide holders attached to

bricks that were placed on the stream bed. There was only 3-5 mm between the microscope slides. Grazing insects such as *Glossosoma nigrior* were seldom found on these slides, while they were common on nearby substrates. This sampling design minimized grazing effects on the benthic algal communities on the slides, since only small grazers had access to slide surfaces.

After the exact planned location of the antenna became known in 1983, permanent study sites were established near the proposed antenna crossing (FEX) and at a downstream site (FCD) far enough away to, hopefully, be exposed to at least an order of magnitude less electric and magnetic field exposure once the antenna became operational (Figure VI.1). After antenna operation began, IITRI engineers found that exposure between these two sites did not meet the 10 fold difference criterion for ground electric fields (the antenna site has received from 4.9 to 6.5 greater exposure than the control site since the antenna became operational). Thus, we added a new antenna and a new control site in 1990 (FEX-N and FCD-N, respectively) near the old sites (Figure VI.1) but just far enough away to meet the criterion of a 10 fold difference (it has ranged from 9.6 to 11.1 times greater exposure at the antenna site compared to the control site) in ground electric field exposure between the two sites. Note that FEX-N is essentially the same site as the FEX.LINE site used for insect studies. Magnetic field exposure between the original two sites ranged from 300 to 334 fold greater exposure at the antenna site compared to the control site, while differences at the new sites have ranged from 463 to 553 fold greater exposure at the new antenna site compared to the new control site. Because we did not have pre-operational data for the new sites, we continued to monitor all parameters at the old sites as well except for primary productivity and community respiration measurements which were moved to the new sites. All these sites were selected in open areas to maximize exposure to sun so that light exposure between sites was similar. The location of slide holders on the bottom of the stream was adjusted weekly within a 10 m reach of the stream using a current meter to insure that all slides were exposed to similar flow rates and water depths.

Detailed analytical methods have been reported in each of the 11 annual reports submitted to date and will not be repeated here. In general, techniques used were techniques recommended in Standard Methods (APHA 1985 or earlier editions). However, diatom counts were conducted on diatoms scraped from air dried slides and cleaned for counting using the methods of Van der Weff (1955). Details of counting and calculation of community indices are given in the annual reports and in Oemke and Burton (1986). Calculations of Shannon-Wiener diversity (H') followed Southwood (1978), while evenness (J') was calculated following Pielou (1969, p.

233). Cell volume was determined by measuring lengths, widths, and depths of diatoms and using appropriate geometric formulae based on shell shape.

A Before and After Control and Impact (BACI) sampling design (Stewart-Oaten et al. 1986) was used with unpaired t-tests to determine if differences in biological, physical and chemical parameters were the same between before and after periods. The BACI design was adopted to cope with the problem of pseudoreplication (Hurlbert 1984) that arises from having upstream-downstream experimental and control sites on the same river. The BACI design achieves replication by sampling repeatedly through time. Benthic algal samples were taken every 28 days during the ice-free season (April-October) each year. From 1983 through 1991, samples were also routinely taken during the winter. These samples were characterized by smaller numbers but greater diversity of diatoms and low chlorophyll a and organic matter standing crops. The winter samples were more variable than ice-free samples and were eliminated from the experimental design because of this high variability and difficulty in collection of the data. The data set in this final report was restricted to the ice-free periods for the BACI analysis in order to maintain consistency between before and after periods. Additional periods analyzed in previous reports included all data (winter and summer) and periods from June to October when diatom counts were dominated by *Cocconeis placentula* and *Achnanthes minutissima*. These analyses were eliminated from the report in order to maintain consistency between periods (Smith et al. 1993) while maximizing the total number of samples included in the analyses.

The before period used in these analyses included the ice-free seasons for 1984 and 1985. In previous reports, the 1983 data were also included in this before period. However, fewer samples were taken in 1983, and these samples were taken from both riffle and pool areas of the stream. Starting in 1984, all samples were taken from riffles only and slide holders were placed in areas with almost identical current velocities at each site using a current meter. This careful matching of current velocity was not done in 1983. Thus, 1983 data have been eliminated from final analyses. The ice-free seasons of 1986, 1987 and 1988 included some testing of the antenna at low amperage (4-6 amps in 1986, 15 amps in 1987 and 75 amps in 1988 - see Tables 1.2, 1.3). Operation of the antenna at 150 amps began in 1989, and the ice-free seasons from 1989 to 1993 are included in the after period. Data from the testing period (1986-1988) are included in graphs of the results but are not included in the BACI analyses.

The rationale for use of BACI and our preliminary experiences with BACI procedures have been published in

40

previous annual reports and in two papers (Burton et al. 1992; Eggert et al. 1992). The BACI procedure can demonstrate that the mean of the impact-control difference before manipulation is/is not different from the mean of the impact-control difference after manipulation of the impact site has taken place. However, it cannot attribute the change in the means of the impact-control differences before and after manipulation to the applied manipulation (Cooper and Barmuta 1993; Eggert et al. 1992) and must be used in conjunction with biological arguments where alternative explanations of the change are explored (Stewart-Oaten et al. 1992). Where differences are detected, alternative explanations and biological arguments as to why (or why not) the effects are (or are not) likely to be caused by ELF electromagnetic exposure will be presented. In earlier reports, Random Intervention Analysis (RIA) (Carpenter et al. 1989) was also used, since the authors of this technique argued that it did not require normality and was less sensitive to serial correlation than was BACI. Recently, Stewart-Oaten et al. (1992) argued that the RIA analysis offers little advantage over BACI, since the BACI analysis is not affected very much by non-normal data unless sample sizes are very small. Sample sizes for this study were large enough that either statistic should be valid, and BACI analyses have been used exclusively in this report.

The unpaired t-test in a BACI design requires that several assumptions be met (Stewart-Oaten et al. 1986, Stewart-Oaten et al. 1992). These assumptions are: (1) time and location effects are additive, (2) successive samples are independent of one another (this assumption was met by using new microscope slides every 28 days and measuring parameters on them every 14 (chlorophyll a and ash free dry weight organic matter accumulation) or 28 days (all other parameters) and (3) data are normally distributed with equal variance both within and between before and after periods.

There are two categories of non-additive data. The first involves data in which the between site difference increases with the mean of the two sites in some cyclical fashion. The second is where between site differences increases or decreases steadily over time. The Tukey test for additivity was used to check for the first type of additivity using the raw between site differences in the before period. When the test failed, the log (x+1) transformation usually produced additive data. Possible trends in the before period were regressed by regressing differences in cumulative days. If transformation failed to correct either type of additivity problem, the data sets (two cases) were not analyzed further.

Positive serial correlation between successive samples in the before period was detected using the Durbin-Watson test (Durbin and Watson 1951) as recommended by Stewart-Oaten

et al. (1986). This test presents special problems when data sets contain time gaps. Stewart-Oaten's (1986) method for creating null distributions for the Durbin-Watson test was followed and involved using a linear algebra Gausse program. When serial correlation was found, the t statistic in the unpaired t-test was adjusted using a correction factor based on the magnitude of the Prais-Winsten estimate of the autocorrelation coefficient (Stewart-Oaten 1986, Bence et al. 1994 and J. R. Bence, personal communication).

T-tests can be sensitive to unequal variances both between and within time periods. Welch's t-test was used to overcome the problem of unequal variances between the before and after time periods, since it uses separate variances from the before and after periods rather than pooling the variance. The Welch t-test is robust to cases where variance differs markedly within or between periods, and this is not necessarily true for conventional t-tests (Stewart-Oaten et al. 1992). The normality of the deviations of the observed differences from the mean difference was not assessed, since t-tests are affected very little by non-normality (Stewart-Oaten et al. 1992).

The BACI procedure requires multiple years of before and after data. Before data are only available from the original sampling sites (FEX and FCD). Thus, comparison between these original sites were used to detect changes that might be related to ELF exposure even though the experimental site (FEX) only received 4.9 to 6.5 times greater earth electric field exposures than did the control site. Paired t-tests between the two new sites (FEX-N and FCD-N) were used as independent checks to see if patterns of differences detected between the original sites showed up or were enhanced at the new sites with greater differences in ELF exposure (Tables VI.1, VI.2).

Results

This final report will include a detailed summary of results from the overall study. Actual data summaries and graphs of means for data collected for each 28 day sampling period throughout the study have already been reported in the most recent and previous annual reports for this project and will not be repeated here. These reports have been included as Section H of the Annual Compilation of Reports of the Navy ELF Communications Ecological Monitoring Program submitted to the Navy by IIT Research Institute (10 West 35th Street, Chicago, IL 60616-3799) and are unclassified and available directly from IIT Research Institute or from the National Technical Information Service in Springfield, Virginia. The final annual report submitted in 1994 includes overall summaries of all parameters.

Comparisons of biological parameters between the control and antenna sites for all biological parameters monitored for ice-free months show that chlorophyll *a* 28 day standing crop, chlorophyll *a* daily accumulation rate, organic matter 28 day standing crop and organic matter daily accumulation rate have shown changes potentially related to ELF exposure (Table 2.1). Differences in the means of diatom cell density between the control and antenna site are not shown to be significantly changed between the before and after periods by BACI analysis at the $p<0.05$ level but are different at the $p<0.1$ level ($p=0.09$) despite poor power to detect differences for this parameter (Table 2.1). Chlorophyll and organic matter biomass and accumulation rates and diatom cell density should be correlated with each other, since they are related measures associated with the number of cells produced on the slides over time. Chlorophyll *a* is a measure of photosynthetic pigment production (accumulation rates) or chlorophyll biomass (28 day standing crop data) produced by the entire algal community. Diatom cell density is a measure of accumulation of diatom cells on the microscope slides over the 28 day period, and these diatoms make up more than 90 % of the algae that are producing the chlorophyll on the slides. A reviewer suggested that increases in chlorophyll *a* be put on a per cell basis by dividing chlorophyll by cell density. This conversion would be very crude, since chlorophyll was extracted from one set of slides while counts were only done on diatoms rather than on all algae and were done on completely separate slides from those used for chlorophyll extraction. Thus, the suggested conversion is unjustified. AFDW organic matter daily accumulation rate and standing crop data are partially functions of increased algal accumulation on slides but are also affected by bacterial growth on slides as well as some settling of organic matter on the slides from the water column (settling should be minimal due to the vertical exposure of the slides). The same reviewer suggested that a chlorophyll *a*/organic matter ratio be calculated. This calculation is again not feasible with any precision, since organic matter was measured on different slides than those used for chlorophyll analysis. The related measures of chlorophyll standing crop and daily accumulation rate, organic matter standing crop and daily accumulation rate and cell density all suggest that there has been some change in accumulation of algae and organic matter on slides at the antenna site that may be related to ELF electromagnetic radiation exposure.

Biovolume is calculated from cell volume and cell numbers (density) and is a crude measure of biomass. It should also be correlated with chlorophyll *a* and organic matter standing crop and daily accumulation rate following the same logic used above. Biovolume did not differ significantly between the control and antenna sites for the before and after periods (Table 2.1). Average individual diatom cell volume showed no significant difference for

43

Table 2.1 Summary of BACI Statistics for Biological Parameters at Control (FCD) and Experimental (FEX) sites. The comparisons were made using months in which the Ford River was free of ice cover (April - October). Before period = 1984-1985, After period = 1989-1993.

Parameter	N	BACI P values	Power[†] (.50)	Power (.20)
Organic Matter Standing Crop	48	0.021*	99	42
Organic Matter Daily Accumulation	46	0.000**	93	34
Chlorophyll a Standing Crop[§]	50	<0.05*	99	43
Chlorophyll a Daily Accumulation	46	0.000**	40	12
Density	50	0.094	31	12
Cell Volume	50	0.609	99	99
Biovolume	50	0.133	95	37
Diversity	50	0.681	99	99
Evenness	50	0.365	99	99
Achnanthes minutissima relative abundance	47	0.605	99	92
Cocconeis placentula relative abundance	46	0.108	99	76

-The following were log (x+1) prior to BACI analysis: chlorophyll a
standing crop, cell volume, biovolume; The following were arcsin √x
transformed: *Achnanthes minutissima* relative abundance, *Cocconeis placentula* relative abundance.
-† power to detect a difference equal to 50% or 20% respectively of before period grand mean.
- * P<0.05, ** P<0.01
-§ t statistic corrected for positive serial correlation.

control and antenna sites for the before and after periods (Table 2.1).

Algal production and biofilm (algae, bacteria, protozoa and other small animals on the surface of stones) respiration on the bottom of the stream were the most direct measurements of function that were measured. BACI analyses of means of impact-control differences in production/respiration ratios did not differ significantly between the control and antenna sites for the before and after periods for antenna operation ($p=0.59, N=48$). The mean of net primary production (NPP) was always closely matched between sites for any particlular year but varied from 39 ± 8 mgO_2/m^2 and 42 ± 8 mgO_2/m^2 at the control and antenna sites in 1992 respectively to 115 mgO_2/m^2 and 114 ± 5 mgO_2/m^2 at the control and antenna sites in 1993 respectively. Respiration varied just as widely from year to year but was not significantly different between sites for any of the before and after years using paired t-tests ($p<0.05$). The before years of 1984 and 85 were characterized by mean NPP and respiration values (means of 98 and 50 versus 95 and 35 mgO_2/m^2 at the control and antenna sites respectively) that were within the range of values recorded for the control and antenna sites in the after years. Since these measurements were quite labor intensive, fewer data points were available for production and respiration than for other functional based parameters such as chlorophyll accumulation rates. Even so, the closely matched values obtained for each year at the antenna and control sites indicated that no change in these parameters could be attributed to ELF electromagnetic fields. These parameters did vary substantially from year to year and from date to date within a year.

Between mid-June and early October each year, the diatom community was dominated by two species, *Cocconeis placentula* and *Achnanthes minutissima*. *A. minutissima* was a dominant member of the community throughout the year. *C. placentula* was dominant only in the mid-June to October period and had a higher relative abundance based on numbers than did *A. minutissima*. *C. placentula* had an even greater abundance based on biomass than did *A. minutissima* since it was about 1.5 times larger larger volumetrically than was *A. minutissima*. Thus, this mid-summer time period was referred to as a *Cocconeis placentula* dominant period in earlier reports. This period tended to be a period of high chlorophyll production and a period of low variability for diatom count based statistics compared to calculations based on the entire ice-free period. The ice-free period included periods of cool water and high current velocity and discharge for spring run-off events and cool, rainy periods that tended to be characteristic of autumn. Less variance for the *C. placentula* dominant period resulted in greater statistical power to detect ELF related changes than was true for other

45

seasons. However, this gain due to lower variance was more than offset by fewer numbers of samples compared to using all samples from the ice-free months. Thus, all parameters for this final report were analyzed using data from all ice-free months, and BACI analyses reported for the *Cocconeis placentula* dominant period in past reports are not included in this report.

Patterns of changes for most of the above parameters were examined by graphing mean monthly values for each parameter for the before period (1984 and 1985), for the transition period when testing of the antenna at less than full power was taking place (1986-88) and for the after period when the antenna was being tested at full power for several hours per week (May-September,1989) or operated continuously at full power (since October 1989) (Figures 2.1 to 2.5).

Chlorophyll *a* standing crop was about 1 mg/m^2 higher from June to September at the control site than at the antenna sites in the before period (Figure 2.1). This pattern changed after antenna testing began with the antenna site having consistently higher values from June to September than did the control site (Figure 2.1). Note that this June to September period coincides with the *Cocconeis placentula* dominant period discussed above. Peak differences for the transition and after years occurred in either July (transition years) or August (after years) with chlorophyll *a* at the antenna site being more than 3 mg/m^2 higher than at the control site (Figure 2.1). Interestingly, the increased chlorophyll standing crop during the June to September period showed up during the transition (testing) period at the antenna site with increases even during the first year of low amperage testing at 76 Hz (Figure 2.1 and see Figure 2.1 in the 1993 annual report). The magnetic flux exposure at the antenna site for the 4 amp testing at 76 Hz in 1986 was only 0.31 mG (0.001 mG at the control), and it increased to 1.20 mG (0.005 mG at the control) during 15 amp testing in 1987 and to 5.5 mG (0.022 mG at the control) during 75 amp testing in 1988 (Table VI.2). Magnetic flux exposure at full 150 amp operation varied from 10.6 to 13.5 mG during 1989 through 1993 (0.035 to 0.045 mG at the control site). Earth electric field exposure at 76 Hz at the antenna site increased from 1.62 mV/m in 1986 to 6.1 in 1987 to 31 in 1988 and varied 55 to 66 mV/m during full operation from 1989 through 1993; exposure at the control site increased from 0.33 mV/m in 1986 to 1.33 in 1987 to 7.1 in 1988 and varied from 10.3 to 12.3 mV/m during full operation (Table VI.1). Thus, if the change in chlorophyll *a* standing crop is a response to magnetic flux exposure from the ELF antenna, the threshold for this effect must be between 0.045 mG and 0.31 mG, and the response does not appear to increase as magnetic flux increases. Earth electric fields at the control site were greater during full

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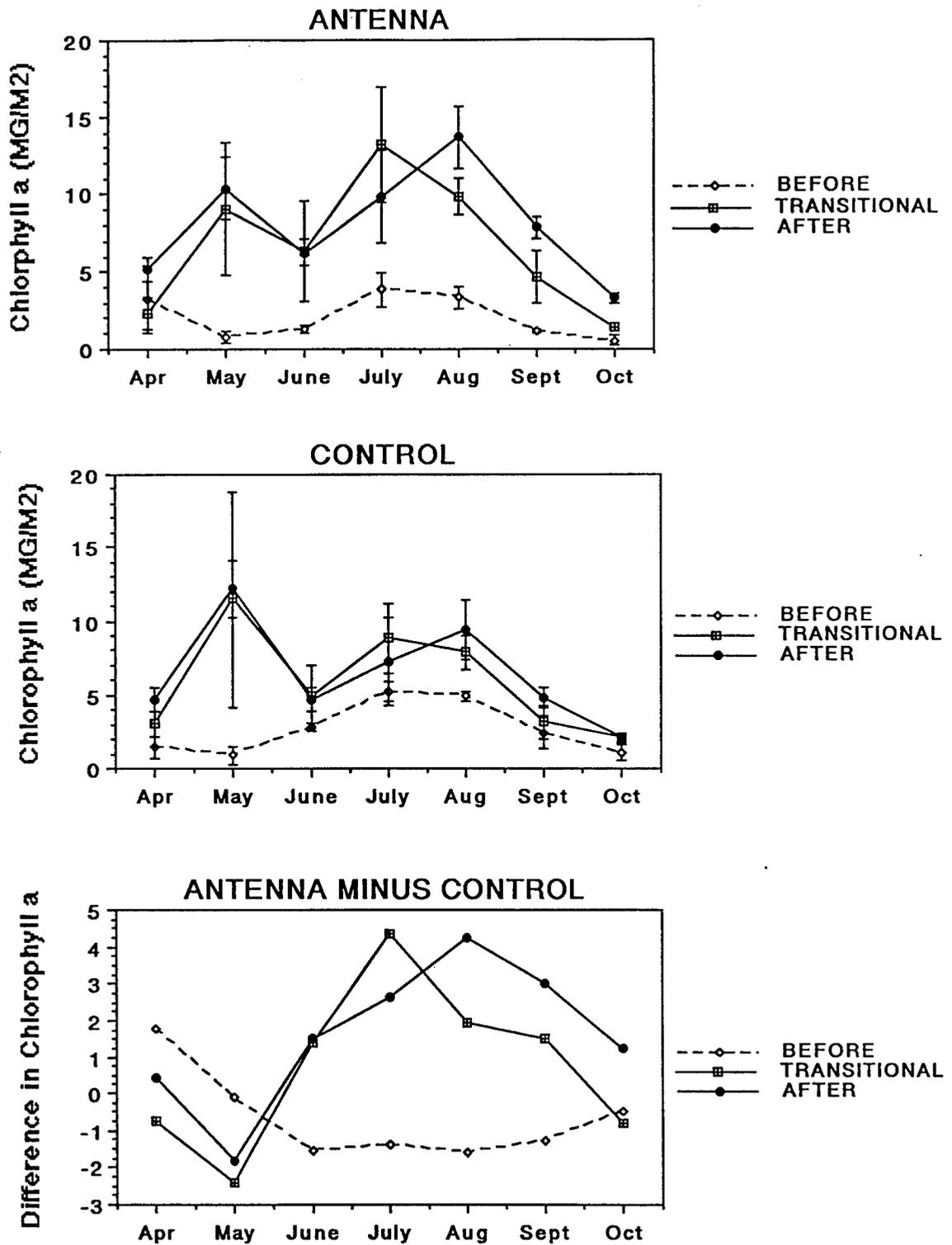
Figure 2.1 Chlorophyll a Standing Crop at the Antenna (FEX) and Control (FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before = Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.
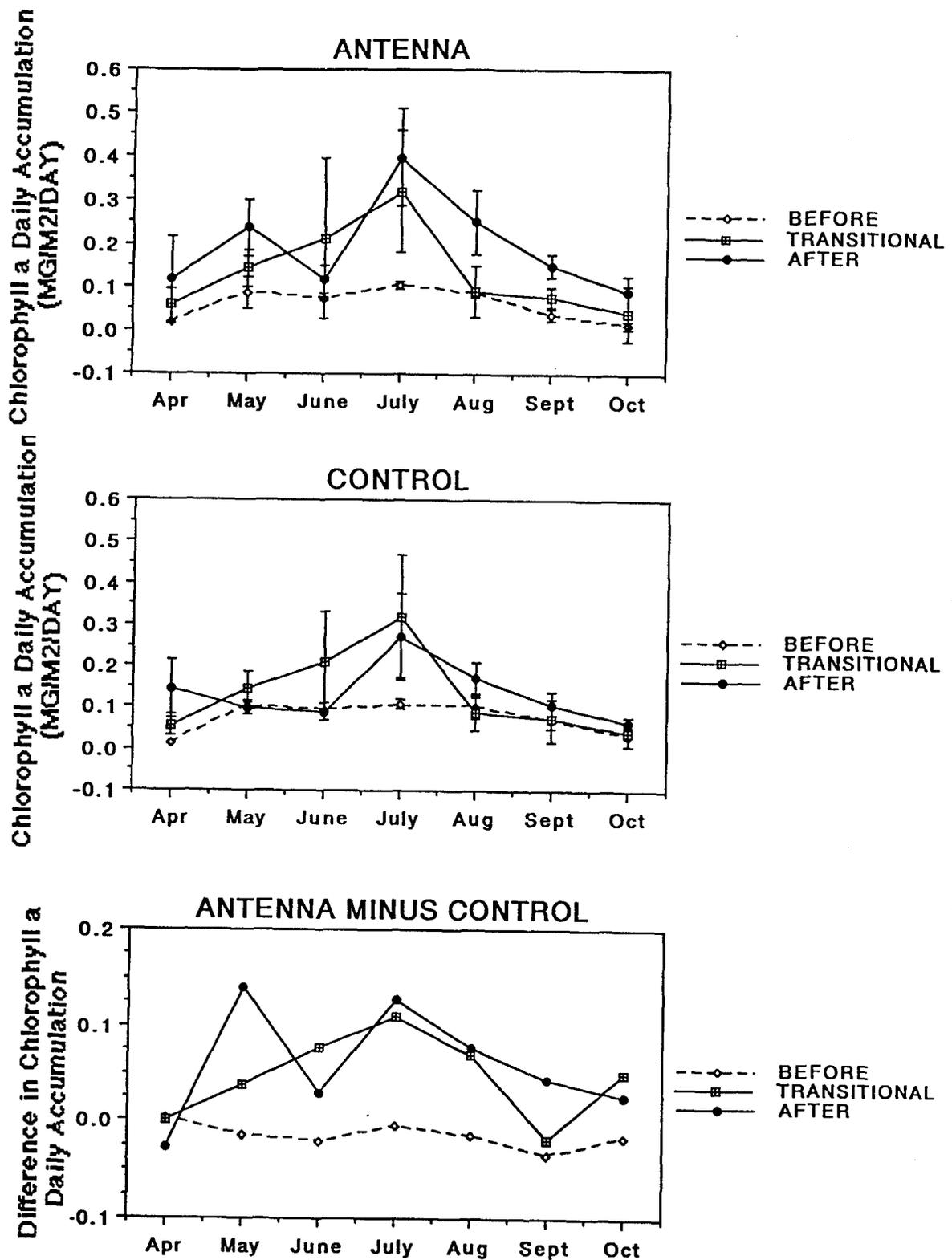
Figure 2.2 Chlorophyll a Daily Accumulation at the Antenna (FEX) and Control
(FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before =
Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.

operation than they were at the antenna site during antenna testing in 1986-88, perhaps indicating that increased chlorophyll during 1986-88 at the antenna site was related to magnetic field exposure and was not related to exposure to the electric fields.

The change in differences in the means of chlorophyll *a* daily accumulation rates detected by BACI (Table 2.1) between the before and after periods was the result of greater increases in daily rates of accumulation of chlorophyll *a* at the antenna site after the antenna became operational than at the control site (Figure 2.2). There was no significant difference in chlorophyll *a* daily accumulation rates between the control and antenna sites for the before period (overall mean of 0.07 ± 0.01 mg/m^2/day for the control versus 0.06 ± 0.01 mg/m^2/day for the antenna site), while daily accumulation rates were greater at the antenna site than at the control site for the after period (0.14 ± 0.02 for the control and 0.21 ± 0.03 for the antenna site). This increase in chlorophyll *a* daily accumulation rates parallels the changes seen in chlorohyll *a* standing crop (Table 2.1, Figure 2.1). Accumulation rates were determined from one set of slides after 14 days of exposure, while standing crop was determined from totally different slides after 28 days of exposure. Therefore, these two parameters were independent measures of changes in chlorophyll *a*. Both sets of data suggest that exposure to ELF electromagnetic radiation may have stimulated algae to produce more chlorophyll *a* at the antenna site especially during the *Cocconeis placentula* dominant period from June to September.

Patterns in organic matter 28 day standing crops and daily accumulation rate patterns (Figures 2.3, 2.4) differed somewhat from the patterns exhibited by chlorophyll *a* (Figures 2.1, 2.2). As was true for chlorophyll *a*, organic matter standing crop was greater at the control site than at the antenna site during the before period and was greater at the antenna site than at the control site for the after period (Figure 2.3). However, maximum differences occurred in September and October rather than in July and August as had been true for chlorophyll *a* standing crop (Figure 2.1). Organic matter standing crop increased at the antenna site for the transition and after periods compared to the before years while remaining similar to before period values at the control site (Figure 2.3). The increase at the antenna site also occurred for chlorophyll *a* but peaked in July and August rather than in September and October. Organic matter daily accumulation rates (an independent measure of organic matter since they were determined from different slides than were standing crop data) also increased at the antenna site during the transitional and after periods from July to October while remaining similar to before operation values at the control site (Figure 2.4). The patterns for chlorophyll *a* and

49
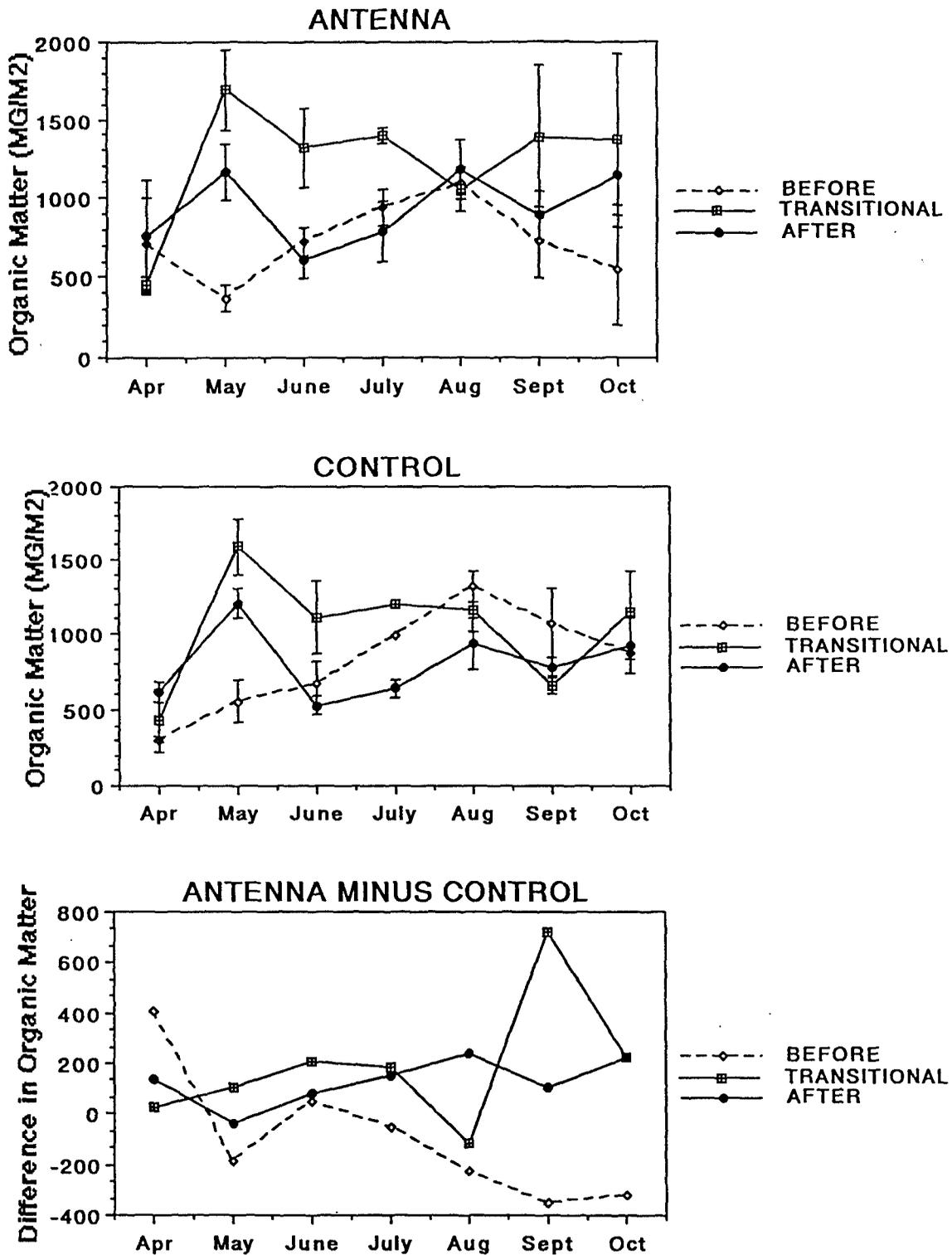
Figure 2.3 Organic Matter Standing Crop at the Antenna (FEX) and Control (FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before = Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.
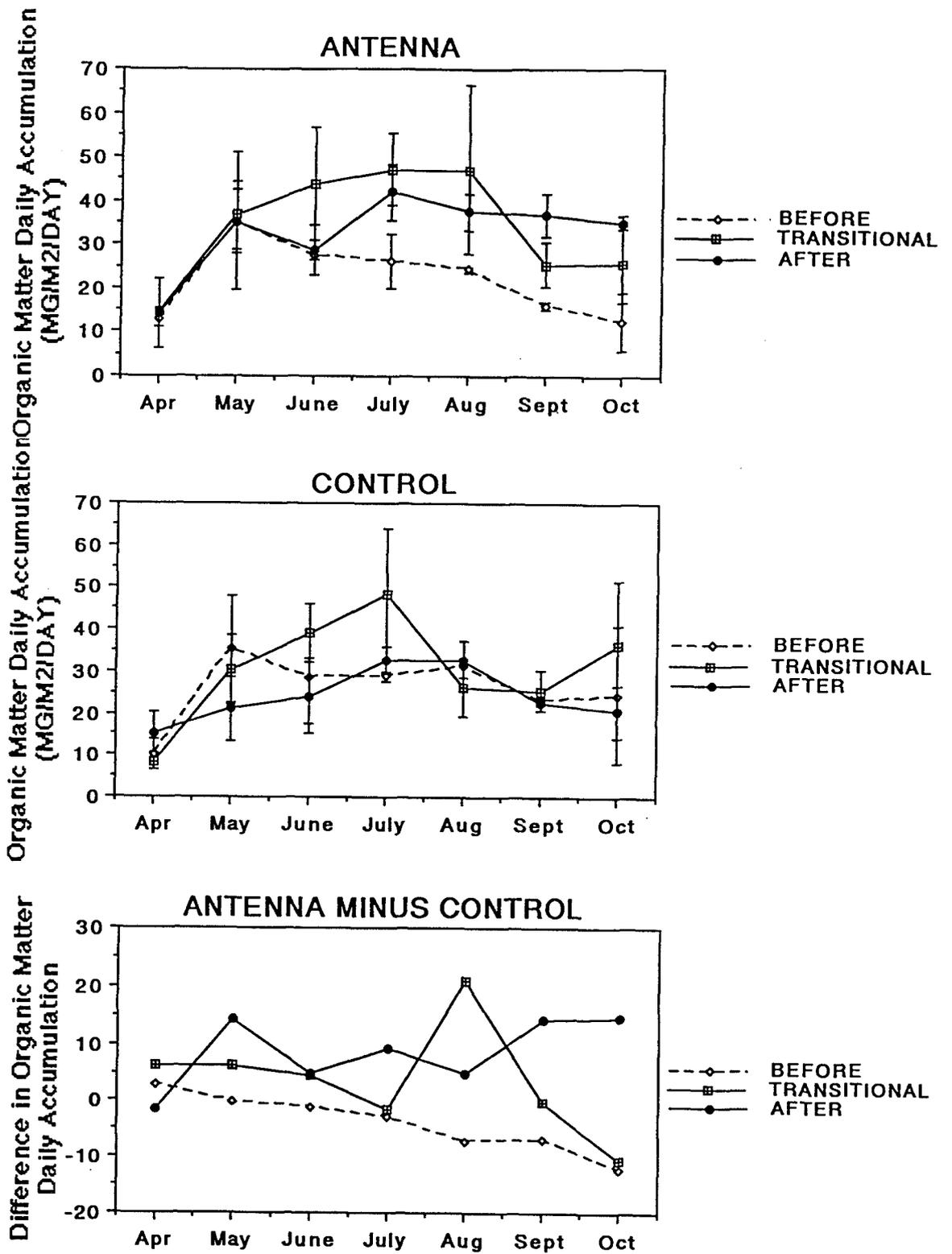
Figure 2.4 Organic Matter Daily Accumulation at the Antenna (FEX) and Control (FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before = Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.

organic matter standing crop and daily accumulation rates all are consistent with stimulation of these parameters for the June to October period at the antenna site during the transition (antenna testing) period with these increases continuing through the operational period (after years).

Diatom cell density differences between the antenna and control sites before antenna operation were negligible (Figure 2.5). Differences in diatom cell densities for the transition and after years occurred primarily in May and June (Figure 2.5) rather than in the June to September period where maximum differences occurred for chlorophyll a and organic mattter standing crops and daily accumulation rates (Figures 2.1-2.4). These differences in cell density did not follow a consistent pattern of more cells at the antenna site after antenna testing and operation began with fewer cells at this site compared to the control for the before period as did chlorophyll a and organic matter. Rather, the antenna site had fewer cells/m^2 in May than did the control site for the after period and more in June and July, while there were more cells/m^2 at the antenna site for May-July during the transitional period. These inconsistent patterns coupled with lack of differences according to BACI analyses at the $p<0.05$ level suggest that no ELF effect can be demonstrated for this parameter.

Minimum detectable differences (MDD) for all of the algal parameters for the ice-free ("summer") season were reported in the most recent annual report. The parameters discussed above that showed differences in before and after data according to BACI and RIA analyses were characterized by high minimum detectable differences using paired t-tests that ranged from 26 % for AFDW organic matter biomass to 33.5 % for chlorophyll biomass to 51 % for density. The mean detectable difference for cell volume was 24 %; it was 59 % for biovolume. Differences that may be related to ELF were detected for organic matter and chlorophyll a but were not detected for cell density and biovolume, perhaps because MDD's were higher for the latter two parameters. Power analyes calculated on the complete data set also suggest poor power to detect differences in cell density (Table 2.1).

Diversity (H') and evenness (J'), the two descriptive measures of community structure, had the lowest minimum detectable differences of 9 and 6 % respectively and excellent power to detect differences of even 20 % between means (Table 2.1). Means of differences between the control and antenna sites were not significant between the before and after antenna operation periods for diversity and evenness (Table 2.1).

Diversity and evenness were higher at both sites for the before period than they were for the transition and after
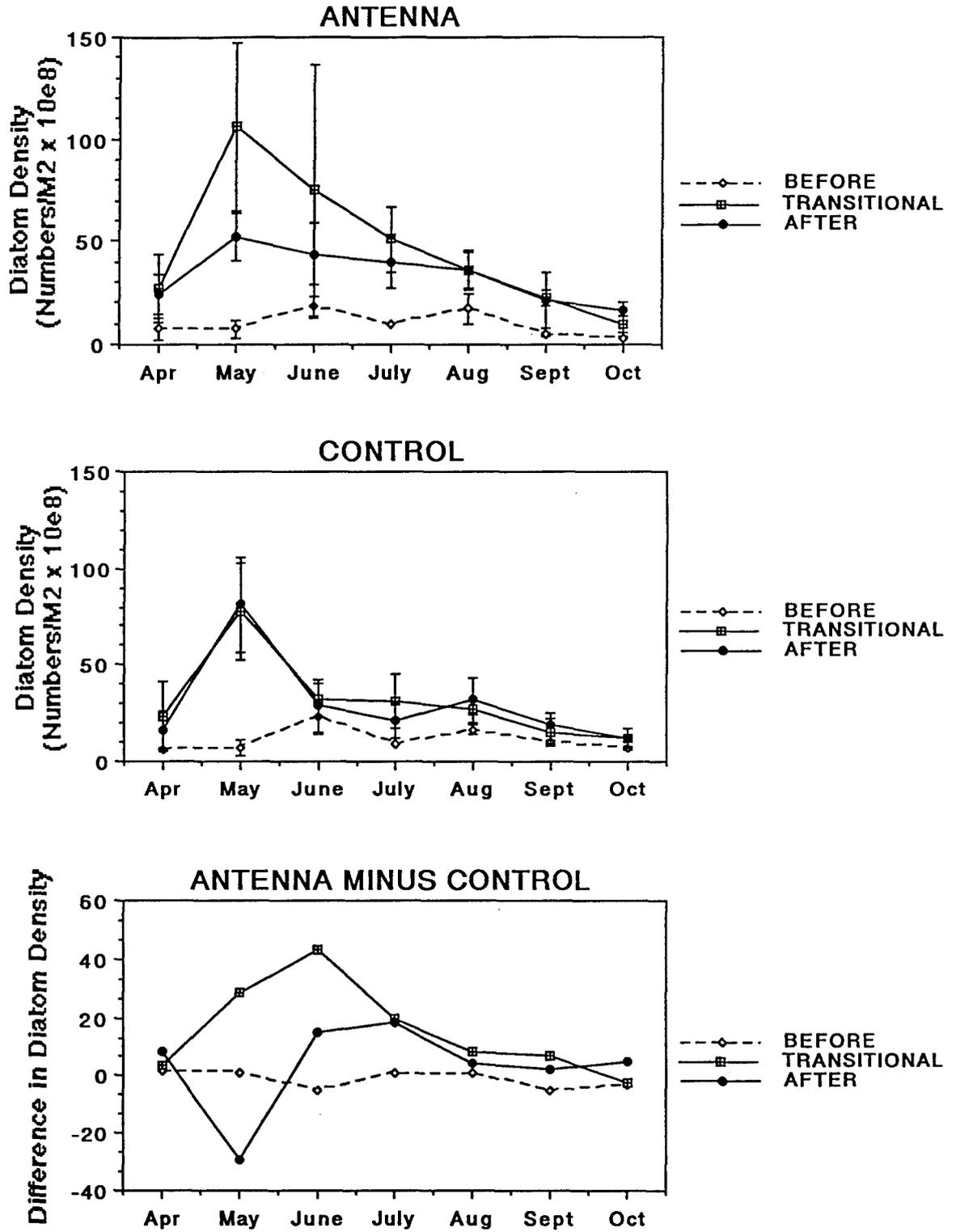
Figure 2.5 Diatom Cell Density at the Antenna (FEX) and Control (FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before = Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.

54
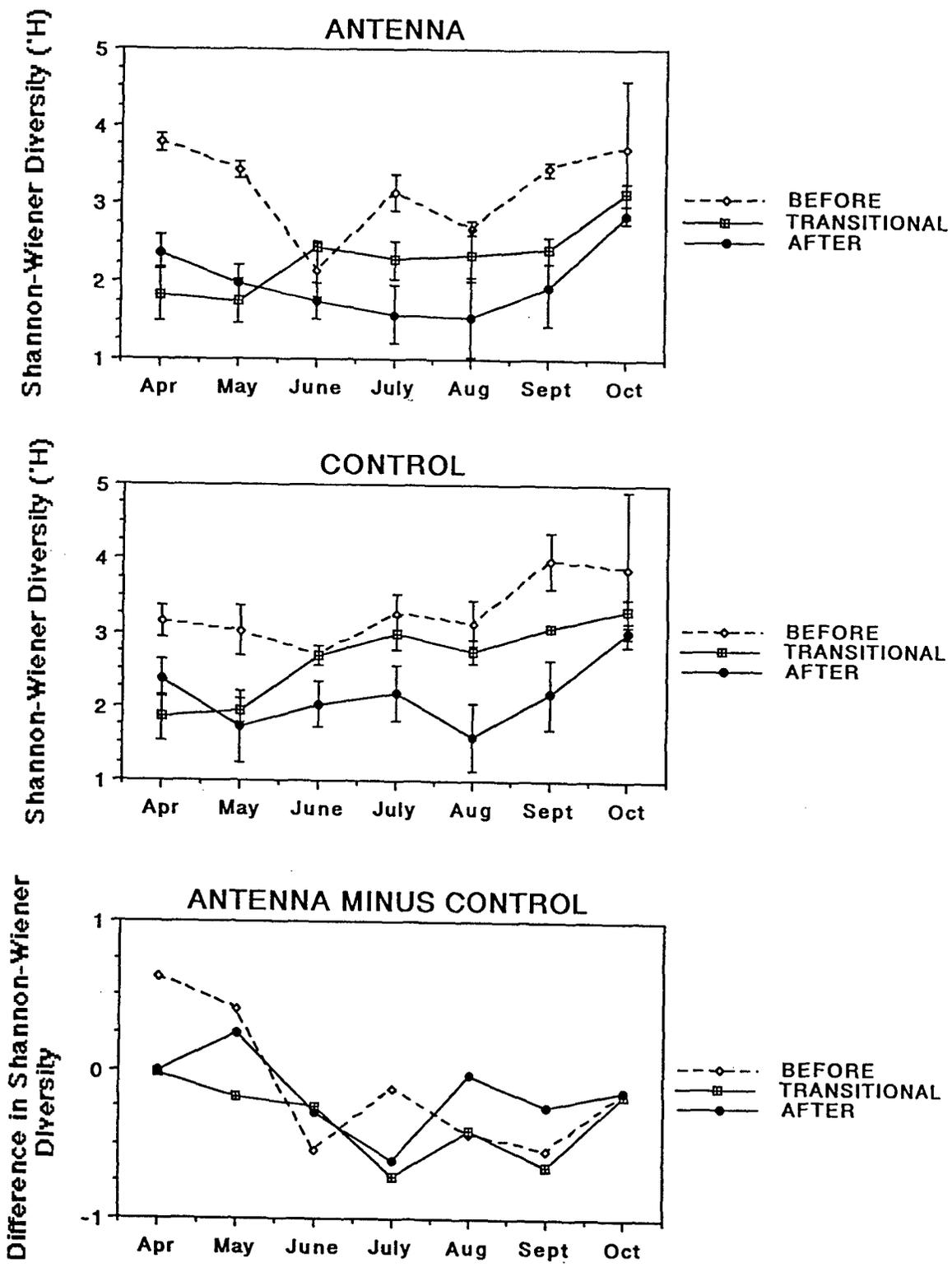
Figure 2.6 Diatom Species Diversity at the Antenna (FEX) and Control
(FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before =
Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.

periods (see Figure 2.6 for diversity; evenness followed a similar trend). There was a change in personnel responsible for diatom counts in the transition period (May 1986-October 1989) with all counts after September 1987 done by a different person than were counts done before this time. The differences in diversity and evenness may be related to this personnel change, but this seems unlikely since the largest drop in diversity and evenness occurred during July and August of 1991, 1992 and 1993 (Figure 2.9 in the 1993 annual report) well after the change in personnel had taken place. Also, there was a downward trend in diversity and evenness thoughout the study with the transition period (May, 1986 to May 1989) being intermediate in diversity and evenness compared to the after period (1989-1993) (Figure 2.6; also compare graphs of the 28 day exposure data in Figures 2.9 and 2.10 in the 1993 annual report (Annual Report AE-142)). If real, these data suggest a change in the environment that affected both sites equally as far as diversity and evenness were concerned. Both sites were exposed to greater 76 Hz earth electric and magnetic fields after the antenna became operational even though the antenna site had much greater exposure (Tables VI.1, VI.2). If the changes are related to ELF exposure, the threshold for response to magnetic exposure would have to be below the 0.001 to 0.022 mG of magnetic flux exposure and/or the threshold for response to earth electric fields would have to be below the 0.33 to 7.1 mV/m earth electric field received at the control site in the transition years. There would also have to be no linear, dose related response. Trends at both sites were the same despite a 4.9 to 6.5 fold increase in earth electric field exposure and 300-334 fold increase in magnetic flux exposure at the antenna site as compared to the control site in the after years (Tables VI.1, VI.2). Therefore, parallel reductions in diversity and evenness of the diatom communities at the control and antenna sites after the antenna became operational are likely related to: (1) some environmental change other than ELF antenna operation, or (2) represent a general response to ground electric fields and magnetic flux that is not dose related or (3) are related to experimental error related to changes in personnel responsible for diatom counts. Alternative one seems most plausible given the overall pattern of decreasing diversity over time since the start of the study.

We examined differences in relative abundance for diatom species that achieved higher than 10 % relative abundance for any of the years of the study and found no significant differences in the before and after periods between the control and antenna sites (Table 2.1; Figures 2.7, 2.8). Lack of significant difference in relative abundance of dominant species along with lack of significant difference in diversity and evenness suggests that there have been no detectable effects of ELF antenna operation on community structure.
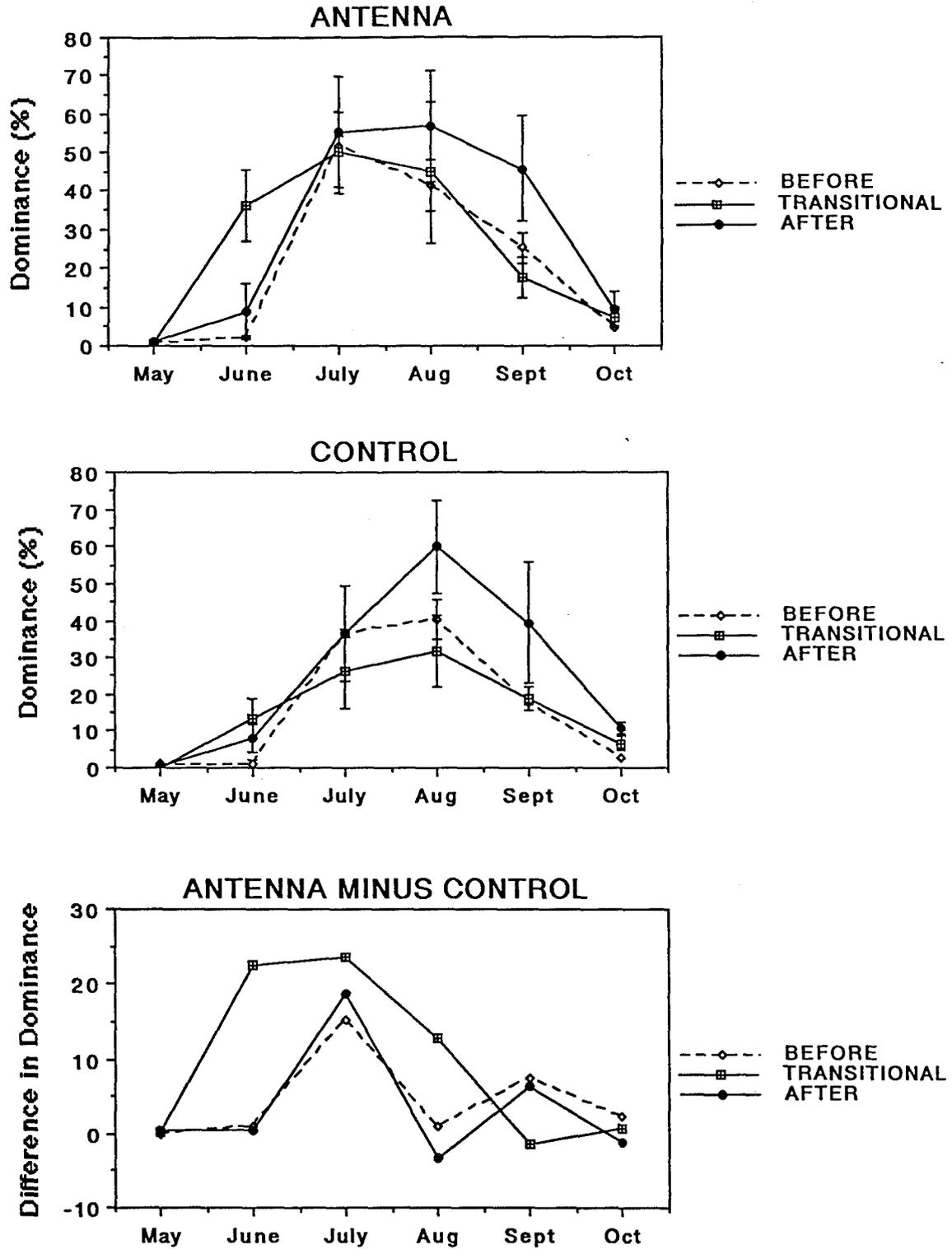
Figure 2.7 Cocconeis placentula Percent Dominance at the Antenna (FEX) and Control (FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before = Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.
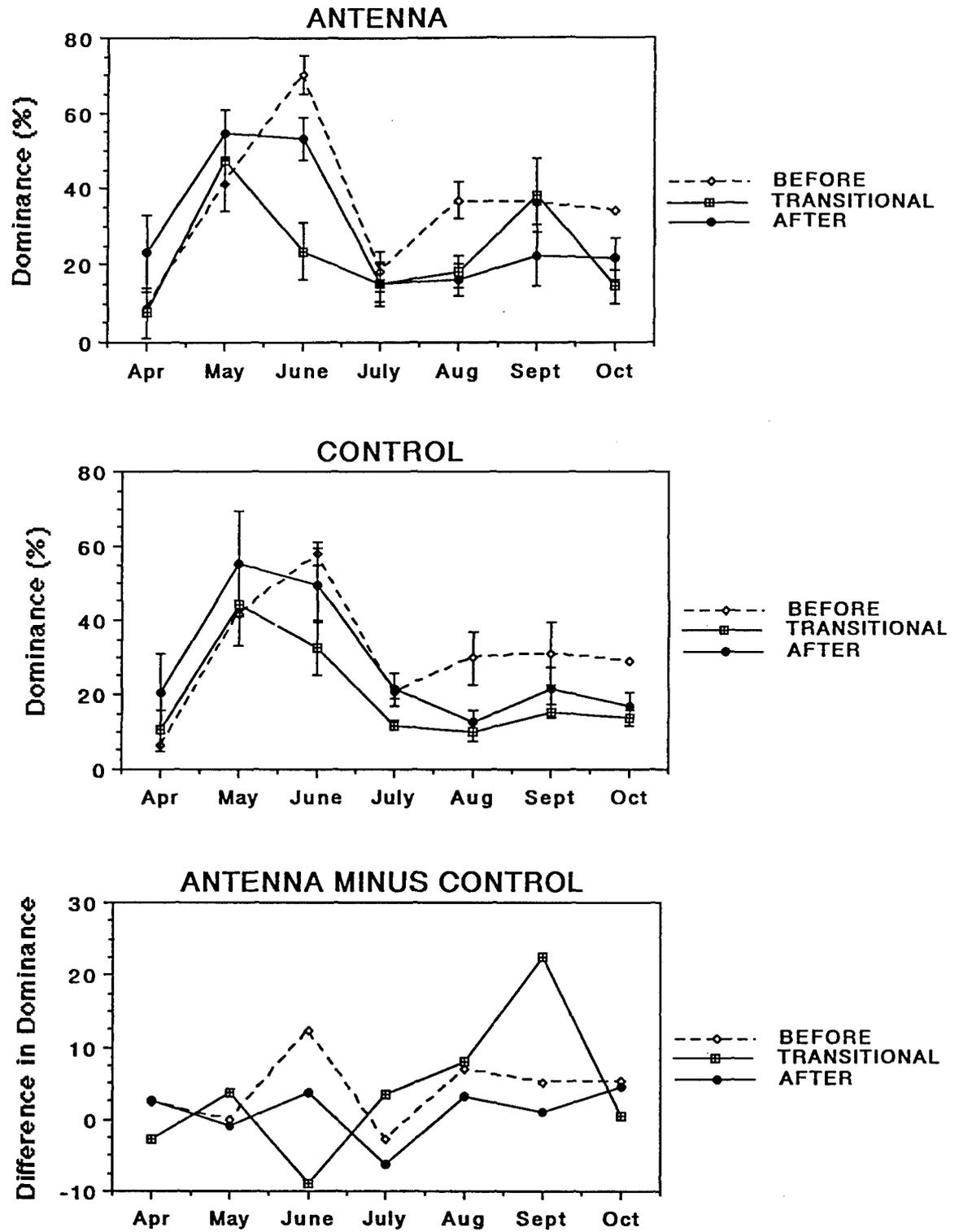
Figure 2.8 Achnanthes minutissima Percent Dominance at the Antenna (FEX) and Control (FCD) Sites and the Difference Between the Two Sites. Error Bars are ± S. E. Before = Apr 1984-Oct 1985; Transitional = Apr 1986-May 1989; After = Jun 1989-Aug 1993.

Discussion

The BACI procedure demonstrated that the mean of the impact-control difference before manipulation was different from the mean of the impact-control difference after manipulation of the impact site had taken place for chlorophyll a and organic matter standing crops and daily accumulation rates. Proof that these changes in differences detected as significant were directly related to ELF exposure would only be possible under completely controlled conditions where all environmental parameters other than ELF exposure remained constant. Since this was not (and never is) possible under field conditions, complete cause and effect proof was not possible. BACI analyses can never attribute the change in the means of the impact-control differences before and after manipulation to the applied manipulation (Cooper and Barmuta 1993; Eggert et al. 1992) and must be used in conjunction with biological arguments where alternative explanations of the change are explored (Stewart-Oaten et al. 1992). To minimize differences and to limit alternative explanations, the control and antenna sites were carefully matched on the basis of physical and chemical characteristics (see Task One, Element 1). Nevertheless, differential responses to some difference in measured or unmeasured parameters between the two sites other than ELF electromagnetic exposure could have been responsible for the observed differences in chlorophyll a and AFDW organic matter standing crops and daily accumulation rates that were observed. Alternative explanations of the results are explored below.

Correlation matrices between benthic algal parameters and physical and chemical factors indicated that water temperature, dissolved oxygen, and discharge were the most important physical parameters correlated with chlorophyll a and organic matter standing crop data (Tables 2.2, 2.3), the parameters that showed differences between the before and after periods for the control and antenna sites. Correlations with Bonferonni adjustments (Tables 2.4, 2.5) resulted in fewer significant correlations but still indicated the importance of temperature, dissolved oxygen and discharge. Temperature, discharge and dissolved oxygen are correlated parameters. Colder water contains more dissolved oxygen at saturation than does warm water. Thus, positive correlations of a biological parameter with temperature will often also result in negative correlations with dissolved oxygen (see Tables 2.2-2.5). Likewise, highest discharge often occurs during periods of snow melt or during autumn coincident with autumnal rains and lack of transpiration by deciduous vegetation. Again, positive correlations with temperature should result in negative correlations with discharge as occurred (Tables 2.2-2.5). Stepwise multiple regressions generally resulted in no model fit or suggested that water temperature or concentration of one of the

essential nutrients explained most of the variance in the data (Tables 2.6, 2.7). Therefore, water temperature or some other unmeasured parameter correlated with temperature seems to be the most important environmental variable correlated with the algal parameters. Earth electric field exposure and magnetic flux data did not explain significant amounts of the variance for the after data except for diatom cell volume at the control site (Tables 2.6, 2.7).

Water temperature for the antenna and control sites were closely matched for the before and after periods at the control and impact sites (Figure 1.5). Water temperature varied from no difference to 0.6 $^{\circ}$C warmer at the control site than at the antenna site before antenna operation began and from -0.1 to 0.5 $^{\circ}$C cooler at the control site than at the antenna site after antenna operation began (Figure 1.5). Overall, BACI analysis demonstrated that diferences in mean temperatures between the antenna and control sites for the before and after periods was only significant at $p=0.07$ (Table 1.2). Such small differences in the differences in before versus after water temperatures seem unlikely to have led to differences in chlorophyll a standing crop between the two sites as large as 3 mg Chlorophyll a/L after antenna operation began (Table 2.1, Figure 2.1)(we are aware of no specific references that would suggest that such a small difference in temperature would lead to such a large difference in chlorophyll). Nevertheless, the slight differences of 0.5 to 0.6 $^{\circ}$C between the control and antenna sites in the before and after periods (Figure 1.5) could have been a contributing factor in differences in chlorophyll a based on the generally accepted phenomenon that an increase in temperature will lead to increases in biological activity with a doubling for about every 10 $^{\circ}$C.

Discharge differences are unlikely to have caused the observed differences in chlorophyll a and organic matter standing crop and daily accumulation rates. The position of the microscope slide holders on the stream bed was adjusted so that exposure of the algal communities to current velocity was matched at the control and antenna sites even when differences in discharge occurred between the two sites (May, June and October, see Figure 1.4, Element 1). Discharge was consistently 0.1 to 0.4 m^3/sec higher at the control site than at the antenna site for most months for the before and after operation years with May, June and October values diverging even further for the before years (Figure 1.4). These differences were to be expected, since the control site was downstream from the antenna site. Groundwater and overland flow inputs between the two sites were the probable sources of the greater discharge at the downstream site. Even so, differences were relatively slight and should not have directly affected algae, since current exposure at the slide holders was matched at the two sites. The major

60

Table 2.2 Correlations Between Physical or Chemical Parameters and Benthic Algal Parameters Before Antenna Operation (June 1983 - April 1986) During Ice-Free Months. Variables Included in the Matrix are as Follows: pH, Dissolved Oxygen (DO), Inorganic Nitrogen (INO-N), Discharge (DIS), Mean Water Temperature (H_2O), Soluble Reactive Phosphorous (SRP) and Silicate (Si).

Parameter	Site	Independent Variables With Largest Correlation Coefficients					
Chlorophyll a Biomass	FEX	H_2O	0.504*	DIS	-0.496*	DO	-0.437*
	FCD	H_2O	0.728**	DO	-0.686**	DIS	-0.545*
Organic Matter Biomass	FEX	H_2O	0.509*	DO	-0.499*	DIS	-0.496*
	FCD	DO	-0.763**	H_2O	0.759**	DIS	-0.616**
Density	FEX	H_2O	0.415	DIS	-0.374	DO	-0.364
	FCD	H_2O	0.488*	DO	-0.436*	DIS	-0.375
Cell Volume	FEX	DIS	0.757**	DO	0.702**	H_2O	-0.591**
	FCD	DO	0.425*	pH	-0.415	H_2O	-0.358
Total Biovolume	FEX	Si	0.242	DO	0.215	pH	-0.187
	FCD	Si	0.313	DIS	-0.298	INO-N	-0.287
Species Diversity	FEX	SRP	-0.420	Si	0.329	DIS	0.233
	FCD	Si	0.467*	pH	0.151	SRP	-0.146
Species Evenness	FEX	SRP	-0.521*	H_2O	-0.322*	INO-N	0.313*
	FCD	Si	0.262	INO-N	-0.201	pH	0.108

*Significant at $P < 0.05$; **Significant at $P < 0.01$

Table 2.3 Correlations Between Physical or Chemical Parameters and Benthic Algal Parameters After Antenna Operation (June 1989 - Aug 1993) During Ice-Free Months. Variables Included in the Matrix are as Follows: pH, Dissolved Oxygen (DO), Inorganic Nitrogen (INO-N), Discharge (DIS), Mean Water Temperature (H₂O), Soluble Reactive Phosphorous (SRP), Silicate (Si), Electric Field Exposure and Magnetic Field Exposure.

Parameter	Site	Independent Variables With Largest Correlation Coefficients					
Chlorophyll a Biomass	FEX	H₂O	0.644**	DO	-0.540**	pH	0.254
	FCD	H₂O	0.341	DO	-0.247	INO-N	0.206
Organic Matter Biomass	FEX	DIS	-0.276	DO	-0.227	Si	0.220
	FCD	DIS	-0.266	DO	-0.177	H₂O	0.139
Density	FEX	DIS	0.405*	pH	-0.262	H₂O	0.144
	FCD	DIS	0.221	DO	0.210	SRP	-0.188
Cell Volume	FEX	H₂O	-0.534**	DO	0.305	Si	0.191
	FCD	H₂O	-0.424*	DO	0.194	DIS	-0.169
Total Biovolume	FEX	DIS	0.396	pH	-0.327	DO	0.223
	FCD	DO	0.255	DIS	0.148	SRP	-0.131
Species Diversity	FEX	H₂O	-0.459**	DO	0.417*	SRP	-0.402*
	FCD	H₂O	-0.448**	DO	0.330	INO-N	0.287
Species Evenness	FEX	H₂O	-0.457**	DO	0.418*	SRP	-0.412*
	FCD	H₂O	-0.458**	DO	0.316	INO-N	0.288

*Significant at $P < 0.05$; **Significant at $P < 0.01$

Table 2.4 Correlations Between Physical or Chemical Parameters and Benthic Algal Parameters Before Antenna Operation (June 1983-April 1986) During Ice-Free Months. Variables Included in the Matrix are as Follows: pH, Dissolved Oxygen (DO), Inorganic Nitrogen (INO-N), Discharge (DIS), Mean Water Temperature (H_2O), Soluble Reactive Phosphorous (SRP) and Silicate (Si).

Parameter	Site	Independent Variables With Largest Correlation Coefficients					
Chlorophyll a Biomass	FEX	H_2O	0.504	DIS	-0.496	DO	-0.437
	FCD	H_2O	0.728*	DO	-0.686*	DIS	-0.545
Organic Matter Biomass	FEX	H_2O	0.509	DO	-0.499	DIS	-0.496
	FCD	DO	-0.763**	H_2O	0.759**	DIS	-0.616
Density	FEX	H_2O	0.415	DIS	-0.374	DO	-0.364
	FCD	H_2O	0.488	DO	-0.436	DIS	-0.375
Cell Volume	FEX	DIS	0.757*	DO	0.702*	H_2O	-0.591
	FCD	DO	0.425	pH	-0.415	H_2O	-0.358
Total Biovolume	FEX	Si	0.242	DO	0.215	pH	-0.187
	FCD	Si	0.313	DIS	-0.298	INO-N	-0.287
Species Diversity	FEX	SRP	-0.420	Si	0.329	DIS	0.233
	FCD	Si	0.467	pH	0.151	SRP	-0.146
Species Evenness	FEX	SRP	-0.521	H_2O	-0.322	INO-N	0.313
	FCD	Si	0.262	INO-N	-0.201	pH	0.108

*Significant at $P < 0.05$; **Significant at $P < 0.01$
^Significance at alpha of .05 or .01 was adjusted by a standard bonferroni method due to the large number of comparisons being tested.

Table 2.5 Correlations Between Physical or Chemical Parameters and Benthic Algal Parameters After Antenna Operation (June 1989 - Aug 1993) During Ice-Free Months. Variables Included in the Matrix are as Follows: pH, Dissolved Oxygen (DO), Inorganic Nitrogen (INO-N), Discharge (DIS), Mean Water Temperature (H_2O), Soluble Reactive Phosphorous (SRP), Silicate (Si), Electric Field Exposure and Magnetic Field Exposure.

Parameter	Site	Independent Variables With Largest Correlation Coefficients					
Chlorophyll a Biomass	FEX	H_2O	0.644**	DO	-0.540	pH	0.254
	FCD	H_2O	0.341	DO	-0.247	INO-N	0.206
Organic Matter Biomass	FEX	DIS	-0.276	DO	-0.227	Si	0.220
	FCD	DIS	-0.266	DO	-0.177	H_2O	0.139
Density	FEX	DIS	0.405	pH	-0.262	H_2O	0.144
	FCD	DIS	0.221	DO	0.210	SRP	-0.188
Cell Volume	FEX	H_2O	-0.534	DO	0.305	Si	0.191
	FCD	H_2O	-0.424	DO	0.194	DIS	-0.169
Total Biovolume	FEX	DIS	0.396	pH	-0.327	DO	0.223
	FCD	DO	0.255	DIS	0.148	SRP	-0.131
Species Diversity	FEX	H_2O	-0.459	DO	0.417	SRP	-0.402
	FCD	H_2O	-0.448	DO	0.330	INO-N	0.287
Species Evenness	FEX	H_2O	-0.457	DO	0.418	SRP	-0.412
	FCD	H_2O	-0.458	DO	0.316	INO-N	0.288

*Significant at $P < 0.05$; **Significant at $P < 0.01$
^Significance at alpha of .05 or .01 was adjusted by a standard bonferroni method due to the large number of comparisons being tested.

Table 2.6 Stepwise Multiple Regression Results During the Ice-Free Months for the Period Prior to Antenna Activation (June 1983 - April 1986). Independent Variables Included in the Matrix are as Follows: Discharge (DIS), Mean Water Temperature (H_2O), Inorganic Nitrogen (INO-N), Soluble Reactive Phosphorus (SRP), Silicate (Si), Dissolved Oxygen, and pH.

Parameter	Site	R^2 of Model	Factors in Model & % Variance Explained by Each		
Chlorophyll a Biomass	FEX*	0.359	H_2O (96%)	INO-N (4%)	
	FCD	0.647	H_2O (79%)	DIS (21%)	
Organic Matter Biomass	FEX*	NMF^			
	FCD	0.651	H_2O (82%)	Si (18%)	
Cell Density	FEX*	NMF			
	FCD	NMF			
Diatom Cell Volume	FEX	0.682	DIS (84%)	SRP (16%)	
	FCD*	0.296	pH (51%)	INO-N (49%)	
Total Biovolume	FEX	NMF			
	FCD	NMF			
Species Diversity	FEX	0.380	H_2O (45%)	SRP (30%)	Si (25%)
	FCD	NMF			
Species Evenness	FEX	NMF			
	FCD	NMF			

*Indicates log transformed
^Indicates No Model Fit

Table 2.7   Stepwise Multiple Regression Results During the Ice-Free Months for the Period After Antenna Activation (June 1989 - Aug 1993). Independent Variables Included in the Matrix are as Follows: Discharge (DIS), Mean Water Temperature (H_2O), Inorganic Nitrogen (INO-N), Soluble Reactive Phosphorus (SRP), Silicate (Si), Dissolved Oxygen (DO), pH, Earth Field Exposure and Magnetic Field Exposure (MFE).

Parameter	Site	R^2 of Model	Factors in Model & % Variance Explained by Each			
Chlorophyll a Biomass	FEX*	NMF^				
	FCD	NMF				
Organic Matter Biomass	FEX*	NMF				
	FCD	NMF				
Cell Density	FEX*	0.472	Si	(59%)	DIS	(41%)
	FCD	0.235	DO	(79%)	H_2O	(21%)
Diatom Cell Volume	FEX	NMF				
	FCD*	0.213	MFE	(66%)	H_2O	(34%)
Total Biovolume	FEX	0.650	SRP	(36%)	DIS	(29%)	Si	(22%)
	FCD	NMF				
Species Diversity	FEX	0.227	SRP	(54%)	INO-N	(46%)
	FCD	NMF				
Species Evenness	FEX	0.236	SRP	(59%)	INO-N	(31%)
	FCD	NMF				

*Indicates log transformed
^Indicates No Model Fit

differences in discharge occurred in May, June and October (Figure 1.4), and discharge between the two sites was quite similar in July and August, the months where maximum differences in chlorophyll a standing crops and daily accumulation rates occurred.

Dissolved oxygen (DO) levels in the Ford River differed only slightly between the antenna and control sites (Figurfe 1.1). DO levels were always near 100 % saturation or were just slightly under saturated for both sites. Therefore, the differences in the means of DO between control and antenna sites between the before and after periods (Table 1.2, Figure 1.1) are not likely to have caused the differential responses for chlorophyll a and organic matter that occurred for the after and transition periods.

Nutrient differences were another group of parameters that could have resulted in changes in means between the antenna and control sites between the before and after periods. The two major nutrients most likely to stimulate plant growth were inorganic N and soluble reactive P (SRP). SRP concentrations were not significantly different between the two sites for the before and after periods (Table 1.2, Figure 1.3), but there were significant differences in inorganic-N concentrations between the before and after period for the control and antenna sites (Table 1.2, Figure 1.2). Inorganic N was 50 µg/L higher at the control site than at the antenna site during May-July for the before period but was about equal at both sites for the after period (Figure 1.2). This difference was primarily due to increased nitrate-N at the control site during 1985, perhaps due to clear-cutting of a small patch of forest near this site (see Element 1). There were no significant differences in chlorophyll standing crop between these two sites in 1985 according to paired t-tests even though summer values were slightly higher at the control site. This same pattern of higher chlorophyll standing crop at the control site occurred in 1984, the other pre-operational year when nitrate-N levels at the two sites were similar (it also occurred in 1983 when nitrate levels were similar; these data are not included in the analyses because of changes in procedures between 1983 and the rest of the study).

The results from experiments conducted in 1986 on nutrient limitation of benthic algae in this river suggested that adding either N or P alone caused no stimulation of chlorophyll a standing crop, while addition of both at the same time caused stimulation (Burton et al. 1991). No such simultaneous increase in both N and P occurred for the antenna site as compared to the control site for the before or after period (Figures 1.2, 1.3). Inorganic N was higher at the control site than at the antenna site for the before period because of the increase in nitrate-N in 1985, but there were no substantial differences in inorganic-N between

the two sites for these months after antenna testing and operation began (Figure 1.2). There were no trends apparent in SRP concentrations between the sites (Figure 1.3). Thus, stimulation of chlorophyll a production by nutrient increases seems to be an unlikely explanation for the stimulation of chlorophyll a and organic matter standing crop and daily accumulation rates that occurred at the antenna site following onset of antenna testing and operation (Figures 2.1, 2.2).

No differential change in physical or chemical factors between the two sites other than ELF electromagnetic radiation exposure can be correlated with the increases in chlorophyll a and organic matter biomass and daily accumulation rates that occurred after testing and operation of the antenna began with the possible exception of slight changes in water temperature. Even though it is not possible to prove conclusively with field data that the increases in chlorophyll a and organic matter biomass and daily accumulation rates are a direct consequence of ELF electromagnetic radiation exposure, such exposure seems to be the most likely explanation.

Biological interactions could also have led to the increases in chlorophyll a and organic matter biomass and daily accumulation rates. For example, grazing insect biomass could have been reduced by ELF exposure leading to increased algal and organic matter standing crops. This explanation seems unlikely for three reasons. First, the slide holders were designed so as to minimize exposure to larger cased grazers that move along the substrate and graze rock surfaces. The cased caddisflies, *Glossosoma nigrior* and *Protoptila* sp, the most common insect grazers in the stream, were seldom ever observed on periphyton microscope slides. These microscope slides were in plexiglass slide holders that were attached to bricks and were held vertically into the current with only 2-3 mm space between the slides. Second, the insect grazers including the two caddisflies above and three mayflies that may include diatoms in their diet were the more common members of the collector-gatherer functional feeding group used in analysis of ELF effects in Task 3. There were no differences in the differences in the means for the before and after antenna operational periods for this functional feeding group (Tables V2 and 3.2). Third, total insect mass, the major insect parameter that changed from the before to the after periods according to BACI analyses (see Task 3), was regressed against periphyton biomass (see Task 3). Positive correlations occurred for spring and autumn (Figure 3.4) but not for the summer months when differences between sites in chlorophyll a and organic matter standing crop and daily accumulation rates were most consistent.

Direct impacts on the benthic algae by fish are unlikely for two reasons. First, there are no true grazing fish such

as *Campostoma anomalum*, the stoneroller, in the Ford River, and biomass of omnivores such as the white sucker did not change significantly from 1983 to 1993 (see Task 4, Figure 4.4). Second, no major change in total biomass or biomass of any of the five most common species of fish changed significantly from before to after ELF operational years (see Task 4, Figures 4.3, 4.4). Most fish in the river are insectivores or piscivores, and changes in their biomass, had they occurred, would have been more likely to impact the insects with potential cascade effects on the algae. No such effect was evident as discussed above.

No alternative explanation for the increases in chlorophyll *a* and organic matter biomass and daily accumulation rates related to biological interaction or to differential responses to the measured physical or chemical changes seem plausible. Either some unmeasured parameter was responsible or the most plausible and parsimonious explanation for the increases in chlorophyll *a* and organic matter biomass and daily accumulation rates is that operation of the ELF antenna caused these increases.

If ELF exposure does actually stimulate increases in chlorophyll *a* and organic matter standing crop and daily accumulation rates during the period of June-September as the data indicate, what is the biological significance of this finding? Should stimulation of algal production be viewed as positive or negative? These increases are not accompanied by changes in algal community structure as measured by relative dominance of the dominant algae or by diversity and evenness of the diatom community. No increases in grazing insect biomass were found to accompany this increase in chlorophyll or organic matter (see Task three, this report). Spatial studies to determine how far downstream stimulation occurred were not conducted. Even if we assume that the effects are observable half the distance to the control site (about 8 km by air and probably twice that along the river), the fact that no effects on algal community structure could be documented and that few effects on higher trophic levels occurred (see Tasks 3 and 4, the insect and fish sections of this report) suggest that these effects have little impact on ecosystem structure or function.

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Task 2, Element 3 - Effects of Insect Grazer Populations on Periphyton Communities

This element was eliminated following the 1989 field season, since effects were determined to be too variable and inconsistent from year to year to be useful in detecting ELF effects. Efforts previously spent on this element were used for the periphyton studies at two additional sites for Element 2. Work on Element 3 led to the following manuscript:

Burton, T. M., M. P. Oemke, and J. M. Molloy. 1994. Effects of grazing by the trichopteran, *Glossosoma nigrior*, on diatom community composition in the Ford River, Michigan. Proc. 11th International Diatom Symposium, San Francisco. Mem. Calif. Acad. Sci. (In press).

No effects of grazers on chlorophyll *a* or organic matter standing crop or daily accumulation rate was demonstrated in these studies. In some years, species composition was significantly changed; in other years no such changes occurred. See the above reference for details.

Task Three, Element 4: Species Richness and Biomass of Stream Insects From Natural Substrates in Riffles

Introduction

There is considerable controversy over whether extremely low frequency electromagnetic fields (ELF) affect organisms (A.I.B.S. 1985, Brodeur 1989, Hutchison 1992, Ahlbom and Feychting 1993). Experimental studies on terrestrial organisms exposed to weak electromagnetic fields have reported loss of coordination (Watson 1988), increased incidence of rearing activity (Rudolph et al. 1985), impedance of navigation (Tenforde 1989), and alterations of life history patterns (Walters & Carstensen 1986). Other researchers suggest little or no biological effects from exposure (Sheppard & Eisenbud 1977, Ganskopp et al. 1991, Stout 1992). Some aquatic organisms, for example, bacteria, algae, and elasmobranch fishes, are known to possess magnetite, a biogenic compound responsible for geomagnetic sensitivity, which allows them to respond behaviorally to weak electromagnetic fields (Frankel & Blakemore 1989, Kirschvink 1989). If aquatic insects contain magnetite crystals, the insects may be able to respond behaviorally or physiologically to ELF fields. Only one study concerning possible behavioral effects by ELF fields on stream insects has been done(Stout 1992). We designed this field study to determine whether there were ELF effects on the community structure of insects living in stream benthos.

The composition of benthic insect communities may also be modified by a variety of naturally occurring biotic and abiotic factors. Biotic factors, especially predation, have received much attention (Peckarsky & Dodson 1980, Gilinsky 1984, Power 1990). Others have demonstrated, however, that benthic community structure, particularly in lotic systems, is often governed by physical factors (Stout 1982, Scullion & Sinton 1983, Duncan & Blinn 1989) that may influence the intensity of biotic interactions (Hemphill & Cooper 1983, Feminella & Resh 1990). High discharge events can scour the benthic substrata and create barren surfaces for colonization and recruitment of epilithic algae and insects. Current velocity also directly redistributes stream bottom particles, altering the recolonization patterns of insect communities (Rabeni & Minshall 1977). In the northern United States, stream discharge levels and water temperatures vary across seasons. Therefore, separation of natural physical events, anthropogenic factors, and unexplained variability in insect life cycles requires long-term monitoring (McElravy et al. 1989).

The goal was to determine whether ELF fields affected aquatic insects in stream benthos over and above the effects of

natural physical phenomena. Ten years (1984 - 1993) of data on insect community fluctuations were related to mean discharge, water temperatures, and ground field exposures of ELF fields. Biotic data at the two sites before ELF activation from May 1984 through May 1986 and after full operation from June 1989 through August 1993 are presented, using intervention analysis techniques. Nine biotic parameter estimates were used; namely, taxon diversity, taxon richness, taxon evenness, numbers of individuals (without chironomids), percent numerical dominance of chironomids, total insect mass, percent mass dominance of chironomids, collector-gatherer percent dominance, and predator/prey ratios. Our large data set enabled us to measure seasonal as well as year to year variability for both physical and biotic factors.

Materials and Methods

Studies included two sites on the Ford River, a hardwater stream in the western central portion of the upper peninsula of Michigan. The experimental site, called FEX (Ford Experimental) was located where the north-south arm of the ELF antenna crosses the river. FEX was approximately 7 areal km upstream from the reference site, FCD (Ford Control Downstream). The sites had similar stream widths, discharges, and water chemistries. Riparian vegetation was also similar between sites, consisting primarily of speckled tag alder (_Alnus rugosa_ [Duroy] Spreng), balm of gilead (_Populus gileadensis_ Rouleau), and red osier dogwood (_Cornus stolinifera_ Michx.).

Field Sampling

Details of the materials and methods are given in the recent Annual Report (AE-143 for 1993). A summary of the procedures appear below. Half-cylinder mesh-line (60 micron) plastic sampler baskets (18 x 28 x 10 cm) were filled with natural substrata and buried flush with the stream bottom. Seven samples were collected each month, with replacement, from each site from April through November, with five samples being selected at random for analysis. After washing and subsequent removal of large rocks, benthic samples were washed in a 60 micron mesh soil sieve to separate insects from fine substrate. Insects were identified to species or genus level, except for non-biting midges (Chironomidae) which were identified as one taxon. Chironomids comprised a considerable fraction in the samples, and identification of 1000 to 4000 larvae in each sample would have been too labor intensive (see Hilsenhoff 1981). Because they were numerically dominant and their mass comprised a proportionately large fraction of each sample, chironomids were analyzed separately. Shannon-Weiner's Taxon evenness (J') would be altered by the inclusion of chironomids

as one taxon and so numbers of chironomids were deleted from that index. Taxon richness (S') included chironomids, with chironomids being considered as one taxon. Monthly mean discharge and monthly mean water temperatures were calculated from values between sampling dates. Cumulative degree days were determined by using daily water temperature maxima and minima, with the mimimum threshold value being $2^{o}C$. Cumulative ELF ground field exposure values were taken from operational duration data from the Naval Radio Transmitting Facility at Republic, Michigan. Field electromagnetic field intensities were measured at each site once a year to insure that the fields had not changed. They were used for estimates as to 76 Hz and 60 Hz electromagnetic exposures. The data were provided by the IIT Research Institute (IITRI) in Chicago, Illinois.

Statistical Analyses

All data were grouped by season: spring (April, May), summer (June-August), and fall (September-November). Statistical analyses were performed on seasonally grouped data rather than on data grouped by year because the five samples for most biological parameters had larger coefficient of variation (CV) values for each month during the spring and fall than for the summer when water temperatures and discharge values were more stable. Effects on stream insects associated with electromagnetic fields probably would be most easily detected in the summer.

Two-way ANOVA tests were used to determine whether community indices differed between sites and years each season over ten years. Before versus after effects of electromagnetic fields, using the BACI (Before and After, Control and Impact) procedure (Stewart-Oaten et al. 1986), was used. The Before Impact data were from April 1984 through May 1986 and the After Impact data were from June 1989 through August 1993. The transitional period was excluded from intervention analyses tests because the transitional period could obfuscate any ELF effects. The BACI method uses mean values, and therefore, the often encountered problem of temporal pseudoreplication in stream studies can be avoided (sensu Hurlbert 1984). Whenever the pre-operational data failed Tukey's test for non-additivity, the BACI tests were replaced by the randomized intervention analysis, RIA (Carpenter et al. 1989).

Multiple linear regression tests were used to assess the relative contributions of natural physical factors to the variation in the biotic factors. A graphical analysis for summer insect mass values versus average discharge, cumulative water temperatures in degree days, and cumulative ELF ground field exposures was used to determine whether there were

69

changes associated with ELF activity along a 10 year continuum. Total insect mass mean values for each season were also compared with periphyton densities, using regression analyses.

Results

(Details of results appear in Element 4 of the 1993 Annual Report). Coefficients of variation computed for each sample date for the biotic estimates were generally highest in the spring and lowest in the summer. Fluctuations of those values were also greater in the spring and fall but lower in the summer. The CV for total insect mass averaged 55 percent in the spring (range 21 to 123 percent), 37 percent in the summer (range 13 to 69 percent), and 46 percent in the fall (range 16 to 103 percent). Because there were distinct seasonal differences, they were analyzed separately for all tests.

Each of the biotic estimates was tested for homogeneity of variance using the Scheffe-Box test (Sokal & Rohlf 1981). Evenness in the spring, chironomid numerical dominance in the summer, and chironomid mass dominance in the fall showed heterogeneous variances. Those were tested using the non-parametric Friedman two-way ANOVA test. The remaining seasonal data met the assumptions for parametric two-way ANOVA tests. Each parameter estimate showed significant site differences for each season, except in the spring for H', J', total insect mass, chironomid mass dominance, and predator/prey ratios (Table 3.1). Year effects were significant except for H' and J' in the spring, chironomid numerical dominance in the summer, and chironomid mass dominance in the fall. Significant interaction terms occurred for 12 of the 26 parametric tests.

Neither BACI nor RIA tests showed significant before versus after differences for H', J', S, Chironomid biomass dominance, collector-gatherer biomass dominance, or predator/prey ratios for any season (Table 3.2). Only numbers of individuals in the summer and fall, chironomid numerical dominance in the spring and fall, and total insect mass in the summer showed significant before versus after differences.

Multiple linear regression tests showed that monthly mean discharge accounted for more of the variation in the biotic estimates than did years or cumulative daily water temperatures (Table 3.3, Figure 3.1A). This was especially true in the spring when some of the highest discharges occurred. For example, Figure 3.1B shows that in May of each year, a linear regression of S' (averaged from both sites) against mean discharge had an R^2 value of 0.61 ($F_{1,8} = 12.54$, p = 0.008).

Table 3.1
2-Way ANOVA Tests: 1984 – 1993

Parameter, Season	Site	Year	Site X Yr.	D.F.
H', Spring	0.40!	7.86 n.s.	not approp.	1,9
Summer	15.78**	7.64***	2.40*	1,9,9
Fall	6.14*	13.59***	1.91	1,8,8
J', Spring	3.60!, n.s.	9.79!, n.s.	not approp.	1,9
Summer	44.77***	6.76***	3.54***	1,9,9
Fall	26.04***	7. 28***	1.80	1,8,8
S, Spring	8.62***	9.56**	1.96*	1,9,9
Summer	27.65***	12.07***	1.22	1,9,9
Fall	14.15***	21.54***	2.21*	1,8,8
# Individuals, Spg.	40.36***	10.58***	4.30***	1,9,9
Summer	223.10***	6.36***	5.43***	1,9,9
Fall	4.30***	5.43***	3.49**	1,8,8
Chiro # Dom, Spg.	20.42***	12.79***	4.65***	1,9,9
Summer	6.40!,*	10.47!	not approp.	1,9
Fall	78.77***	27.00***	1.67	1,8,8
Tot. Insect Mass, Spg.	1.58	7.71***	1.32	1,9,9
Summer	10.99***	11.38***	3.83***	1,9,9
Fall	15.93***	12.23***	4.58***	1,8,8
Chiro. Mass Dom, Spg.	3.18 n.s.	4.42***	1.48 n.s.	1,9,9
Summer	16.16***	6.86***	1.81 n.s.	1,9,9
Fall	9.00!,**	8.27!, n.s.	not approp.	1,8
Coll-Gath. Dom., Spg.	7.98*	3.50***	1.72	1,9,9
Summer	15.44***	6.27***	0.90	1,9,9
Fall	18.20***	3.52**	0.96	1,8,8
Pred/Prey Ratio, Spg.	0.02	2.72*	1.00	1,9,9
Summer	24.17***	5.07***	2.54**	1,9,9
Fall	4.18*	4.12***	1.10	1,8,8
Error D.F., Spring: 180 Summer: 280 Fall: 252				

! = Friedman 2-WAY ANOVA,   * = p < .05, ** = p < .01, *** = p < .001

Table 3.2
B.A.C.I. and R.I.A. Tests for Biotic Variables
FEX versus FCD, Spring, Summer and Fall

Parameter	Season	Trans-form	d.f.	Tukey's test	d.f.	BACI test		RIA test
Diversity	Spring	none	4	51.57***		N/A	14	0.216
	Summer	none	4	3.64	19	0.75		
	Fall	none	7	0.30	19	1.21		
Evenness	Spring	Arcsin	4	21.06***		N/A	14	0.135
	Summer	Arcsin	4	4.63	19	0.002		
	Fall	Arcsin	7	0.76	16	1.52		
Richness	Spring	none	4	7.63	12	0.50		
	Summer	none	4	6.54	19	0.20		
	Fall	none	7	1.72	16	1.29		
No. Individ.	Spring	log(X+1)	4	5.71	12	1.06		
	Summer	log(X+1)	4	135.43***		N/A	21	557.5***
	Fall	log(X+1)	7	21.17***	16	N/A	18	195.9***
Chiro. # Dom	Spring	none	4	7.70	12	3.91**		
	Summer	none	4	4.60	19	0.46		
	Fall	Arcsin	7	68.70***		N/A	21	7.04***
Total Mass	Spring	log(X+1)	4	2.41	12	0.20		
	Summer	log	4	11.79*		N/A	19	97.9***
	Fall	log(X+1)	7	3.96	16	0.95		
Chiro. Mass %	Spring	none	4	2.05	12	0.03		
	Summer	none	4	3.66	19	0.70		
	Fall	Arcsin	7	1.39	16	0.88		
Coll.-Gath. %	Spring	ratio	4	2.73	12	0.03		
	Summer	ratio	4	2.32	19	1.19		
	Fall	ratio	7	3.25	16	0.17		
Pred/Prey Ratio	Spring	log(X+1)	4	6.73	12	0.06		
	Summer	log(X+1)	4	0.72	19	0.34		
	Fall	log(X+1)	7	0.64	16	0.09		

Table 3.3. Multiple linear regressions for Biotic Estimates vs. Years, Discharge, and Cumulative Degree Days

Dependent Variables,
R^2 and Standard Partial Regression Coefficients
N/A = not appropriate

A. Spring 1984 - Spring 1993

Independent Variables	H'	J'	S'	# Individ.	Chiro. No. Dom.	Total Insect Mass	Chiro Mass Dom.	% Coll Gath. Dom.	Pred/ Prey Ratio
FEX									
R^2	N/A	N/A	0.33	0.38	0.28	0.33	0.06	0.04	0.04
Years			0.09	-0.22	0.36	-0.12	0.08	0.03	-0.16
Discharge			-0.56	-0.56	0.40	-0.50	0.23	0.06	0.05
Cum.D.Days			0.01	0.19	-0.11	0.28	0.06	-0.18	0.10
FCD									
R^2	N/A	N/A	0.26	0.33	0.18	0.12	0.03	0.01	0.07
Years			-0.11	-0.27	0.31	0.01	0.07	0.01	-0.25
Discharge			-0.48	-0.55	0.25	-0.33	0.15	0.06	-0.09
Cum.D.Days			-0.15	-0.03	-0.18	0.08	0.05	-0.08	0.08

B. Summer 1984 - Summer 1993

Independent Variables	H'	J'	S'	# Individ.	Chiro. No. Dom.	Total Insect Mass	Chiro Mass Dom.	% Coll Gath. Dom.	Pred/ Prey Ratio
FEX									
R^2	0.57	0.57	0.36	0.06	N/A.	0.14	0.05	0.17	0.01
Years	-0.01	-0.06	0.20	-0.25		0.02	-0.04	-0.05	-0.01
Discharge	-0.53	-0.47	-0.65	-0.01		-0.36	0.26	-0.09	0.01
Cum.D.Days	0.31	0.35	-0.08	-0.02		0.02	0.10	-0.43	0.06
FCD									
R^2	0.25	0.23	0.52	0.35	N/A	0.24	0.04	0.06	0.05
Years	-0.32	-0.48	0.30	0.39		0.40	-0.11	0.13	-0.20
Discharge	-0.38	0.13	-0.18	-0.53		-0.42	0.11	-0.01	-0.05
Cum.D.Days	-0.07	0.05	-0.19	-0.02		-0.20	0.21	-0.23	-0.02

C. Fall 1984 - Fall 1992

Independent Variables	H'	J'	S'	# Individ.	Chiro. No. Dom.	Total Insect Mass	Chiro Mass Dom.	% Coll Gath. Dom.	Pred/ Prey Ratio
FEX									
R^2	0.32	0.26	0.26	0.34	0.49	0.12	NA	0.04	0.03
Years	-0.10	-0.15	-0.01	-0.45	0.55	-0.27		0.10	-0.13
Discharge	-0.58	-0.55	-0.44	-0.35	-0.10	-0.38		0.23	-0.19
Cum.D.Days	0.17	-0.11	-0.23	-0.44	0.46	0.09		0.02	-0.04
FCD									
R^2	0.22	0.08	0.20	0.18	0.28	0.19		0.12	0.03
Years	0.23	0.02	0.24	0.15	0.56	0.32		-0.39	0.04
Discharge	-0.31	-0.29	-0.26	-0.29	0.07	-0.16		-0.19	-0.11
Cum.D.Days	-0.08	-0.12	0.18	0.21	0.01	-0.20		0.08	-0.09

73

TAXON RICHNESS VERSUS DISCHARGE

FEX & FCD AVERAGED, JULY 1983–JULY 1993

Figure 3.1A. Insect richness versus discharge over all seasons (FEX and FCD averaged together). 1984 -1993.

RICHNESS VS. DISCHARGE; MAY EACH YR.

AVERAGE OF FEX AND FCD, 1984–1993

Figure 3.1B. Insect richness versus discharge for **MAY** of each year (FEX and FCD averaged together). 1984 -1993.

Numbers of individuals (with chironomids excluded) as well as numerical dominance of chironomids varied greatly from sample to sample, even in the summertime. Intervention analyses showed significant before versus after differences in the summer and fall for numbers of individuals and for chironomid numerical dominance in the fall. Numbers of individuals were almost always higher at the experimental site over the years; however, peak values were lower after 1989. This biotic estimate was highly varying and no covariate stood out as explaining much of the variation. Chironomid numerical dominance was lower at both sites before ELF activation (April 1984 to May 1986) than after full operation in 1989. After full operation, chironomid numerical dominance was higher at FCD as compared with FEX. Multiple linear regressions showed that years accounted for most of the variation in the fall.

There were no significant before versus after differences for this total insect mass in the spring and fall, but they were highly significant differences in the summer months (Table 3.2). Very little of the summer variation could be accounted for by three physical factors together (Table 3.3). Even so, more of the variation was attributable to discharge than to increasing years or to cumulative degree days at each site, especially at FEX.

Because coefficient of variation values were relatively low in the summer season and a R.I.A. test showed significant before versus after differences, series of multiple linear regressions were performed for summer total insect mass, adding one year of data for each test to see whether we could graphically detect a change at one site that was not matched at the other site. Coefficient of multiple determination (R^2) values and partial regression coefficients for discharge, cumulative degree days, and E.L.F cumulative ground field exposures were plotted, with the horizontal axis being cumulative years from 1984 through 1993. The R^2 changes with increasing years at FEX and at FCD were not associated with ELF initial activation or with full operation (Figure 3.2A). It appears that ELF fields alone did not explain the variation in summer insect mass. The partial regression coefficients also showed no distinct changes over time (figures 3.2B, 3.2C). Figure 3.2B shows that discharge was consistently the most important factor at FEX, even though none of the coefficients explained much of the variation. In contrast, all three partial regression coefficients substantially contributed toward explaining the variation in summer insect mass at FCD (Figure 3.2C). Because ELF ground field exposures were more important at FCD than at FEX, we suggest that any effects those fields may have had would have been subtle.
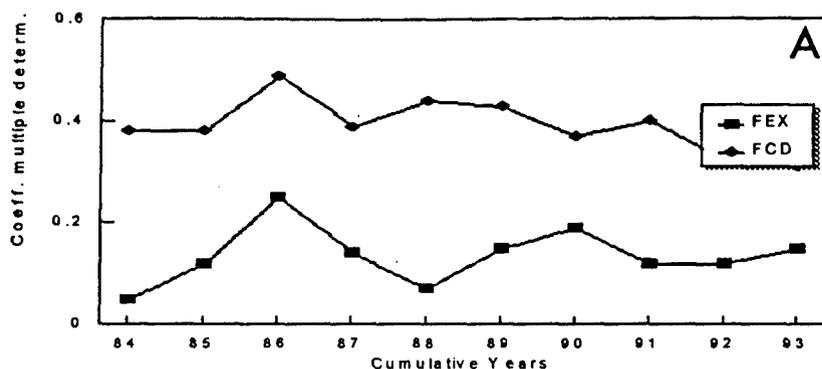
Figure 3.2A. Coefficient of multiple determination for summer insect mass vs. discharge, cum. degree days, cum. ELF exposures, accumulated from
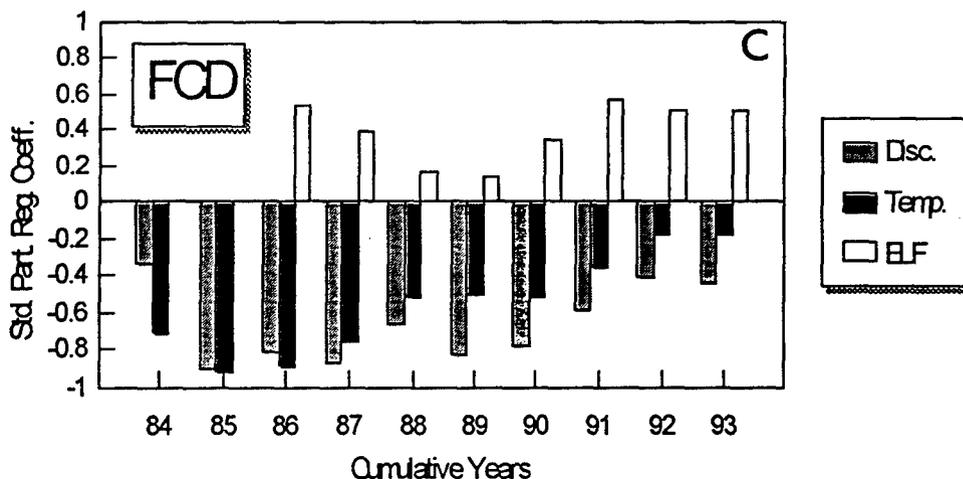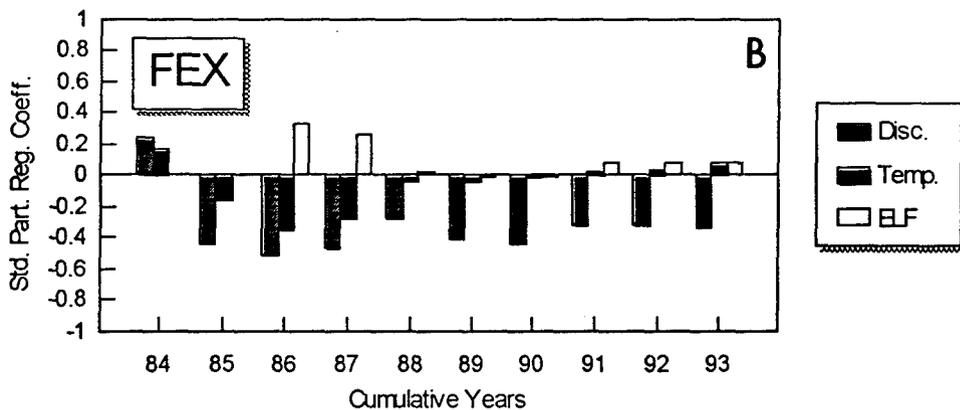
Figure 3.2B (FEX) 3.2C (FCD). Standard partial regression coefficients for summer mean insect mass vs. discharge, cum. degree days, and cum. ELF exposures, 1984-1993.

Deviations in summer insect mass with respect to three phases of the ELF study were computed: before ELF activation, during the transition phase, and after full power of ELF fields. The 10 year grand means for each month were subtracted from the monthly means for each of the phases. Deviations in mean values for those three phases are shown for FEX (Figure 3.3A) and FCD (Figure 3.3B). Before as compared with after ELF full operation mean values deviated little at FEX in the first two months of summer. Insect mass values at FEX during the transition period were much different from values either before ELF activation or after ELF full operation. The largest separation of pattern for the summers was at the reference site. At that site, insect mass was much higher after full operation, as compared with before ELF activation. This was especially true in August. The wide differences at FCD (rather than at the experimental site) appear to be the reason for significant before versus after differences in the summer.

Many of the aquatic insects in this study were collector-gatherers, that use periphyton as a food source. Mean insect total mass was correlated with mean periphyton density, discharge, and water temperatures at the sites. In the spring and fall when discharge and water temperatures fluctuated more than in the summer, significant correlations were found for these parameter estimates (Table 3.4). Regressions of insect mass versus periphyton density were significant (Figure 3.4A; p = 0.01) in the spring and fall (Figure 3.4B; p < 0.001) but not significant in the summer (p = 0.627).

Discharge was the most important physical factor contributing to the variation for the biotic variables. Over the ten year study, this physical variable showed trends that could have obfuscated detection of subtle changes, if they had existed, in the biotic variables as a consequence of ELF fields. From 1984 through 1986, spring discharges were high (Figure 3.5A) relative to later years when the ELF system was activated and then fully powered (Figure 3.6). Coincidentally, after ELF activation, spring discharge increased along with ELF field increases in duration and intensity. This relationship occurred by chance, and is a fine example of the maxim that correlation does not imply causation.

Summer discharge was usually punctuated by a large peak in June, followed by lower discharge values in July and August (Figure 3.5B). Discharges in June of 1985, 1989, 1990, 1991, and 1993 were high relative to other June discharges. The last four periods all occurred after ELF fields were at full amperage (Figure 3.6). Fall discharges (Figure 3.5C) were usually higher before ELF activation than after full operation of the fields.
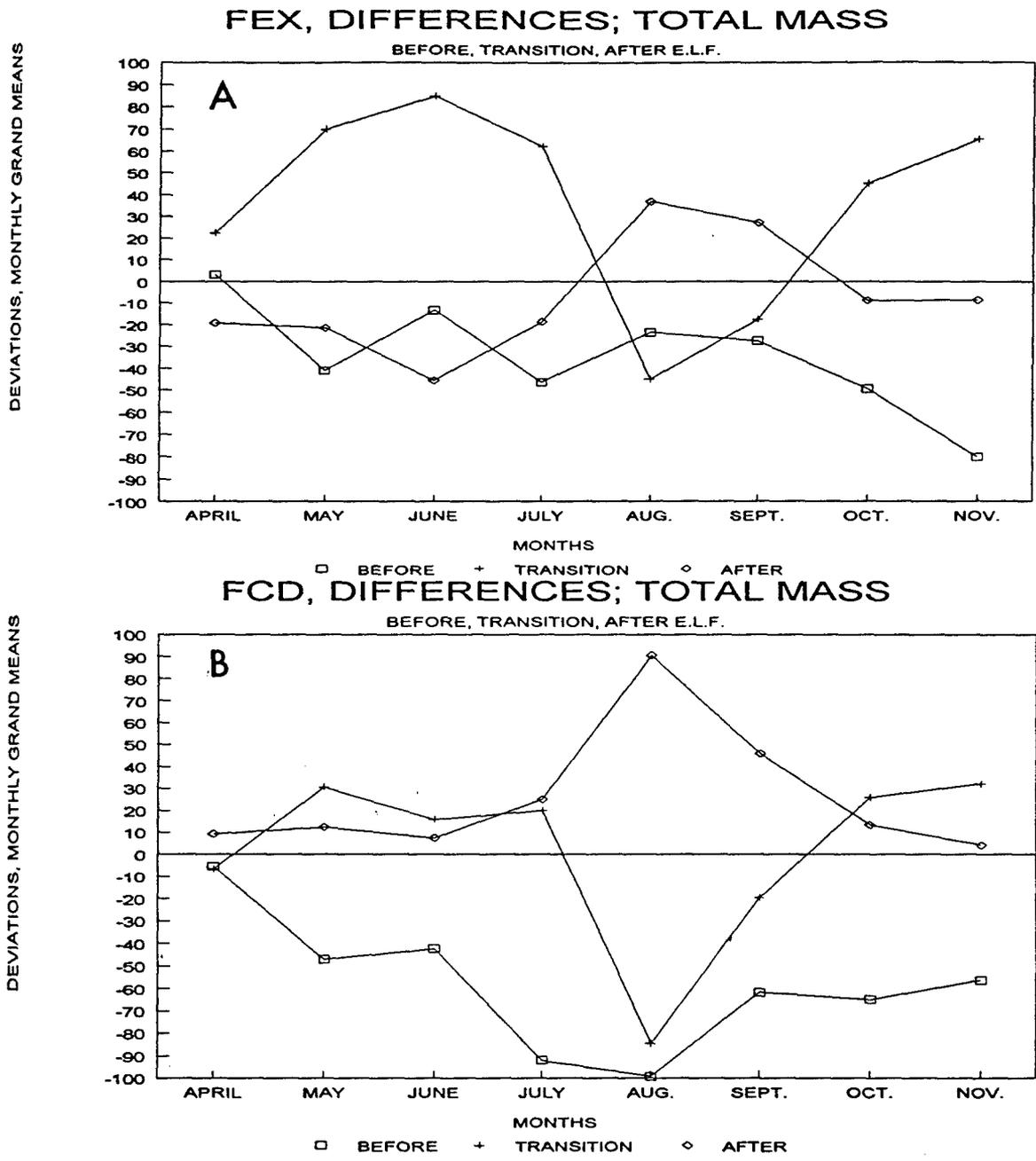
Figure 3.3A, 3.3B. Deviations in mean summer mean insect mass before ELF activation (squares), during transition (pluses), and after full activation (diamonds). 3.3A = FEX, 3.3B = FCD.

78
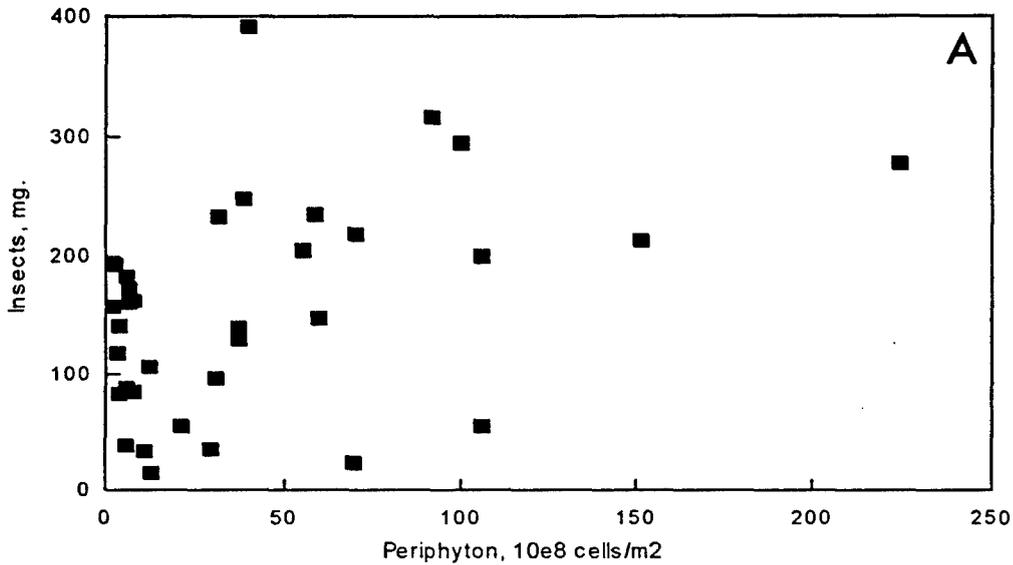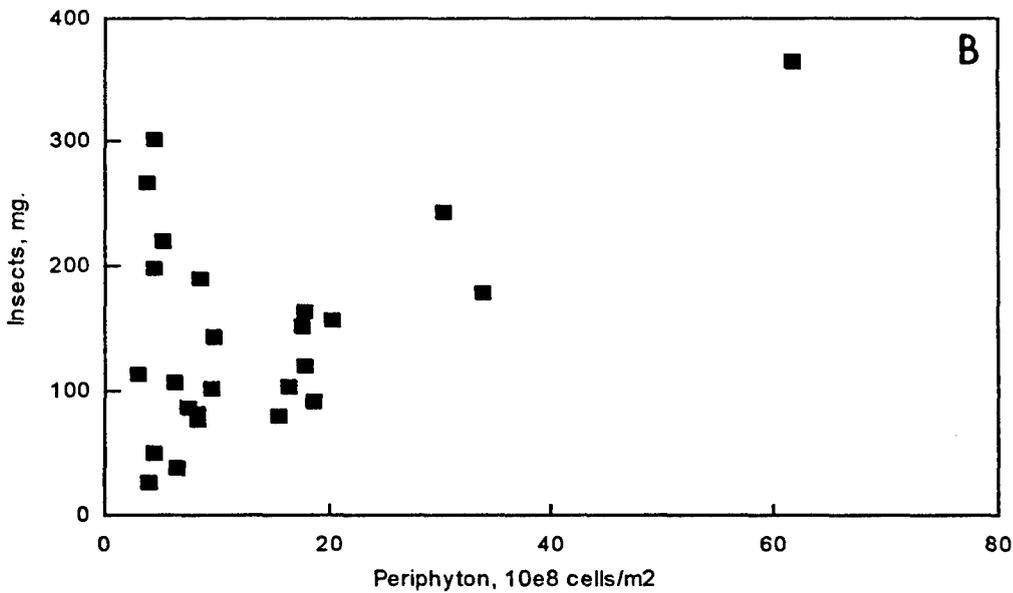
Figure 3.4,A, 3.4B. Mean total insect mass (mg) versus mean periphyton density (no./m^2 x 10^{e8}) at FEX and FCD in the spring (3.4A) and fall (3.4B)

SPRING DISCHARGE, APRIL—MAY, 1984—1993
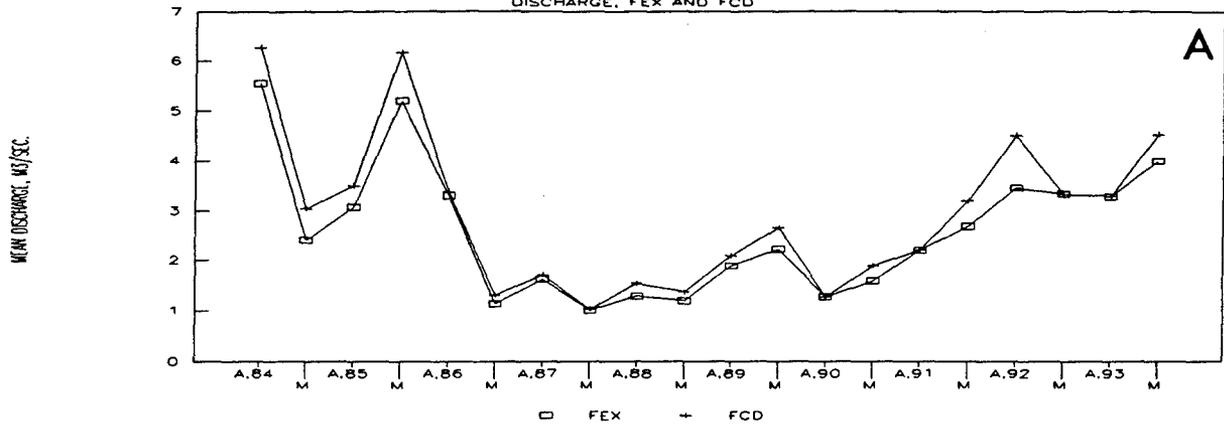
SUMMER DISCHARGE, JUNE—AUG., 1984—1993
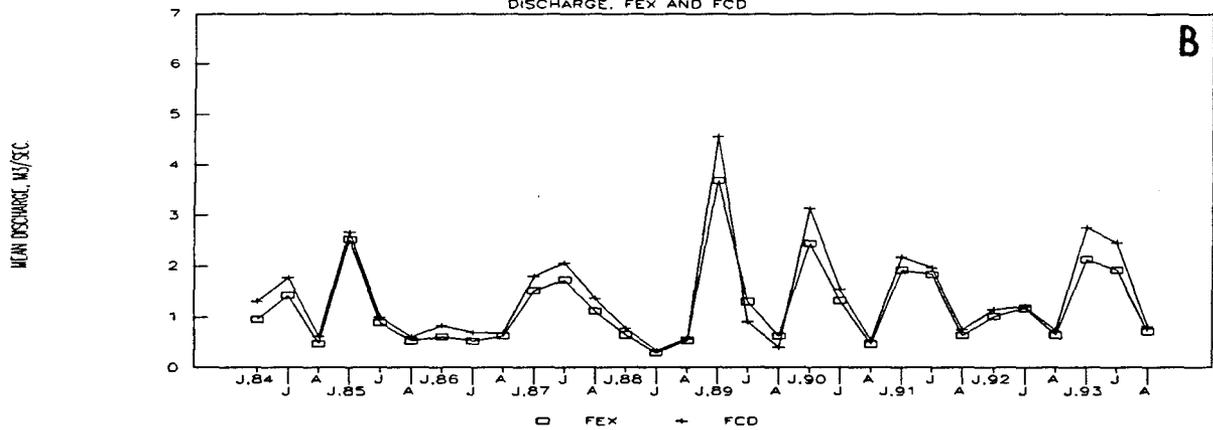
FALL DISCHARGE, SEPT. — NOV., 1984—1992
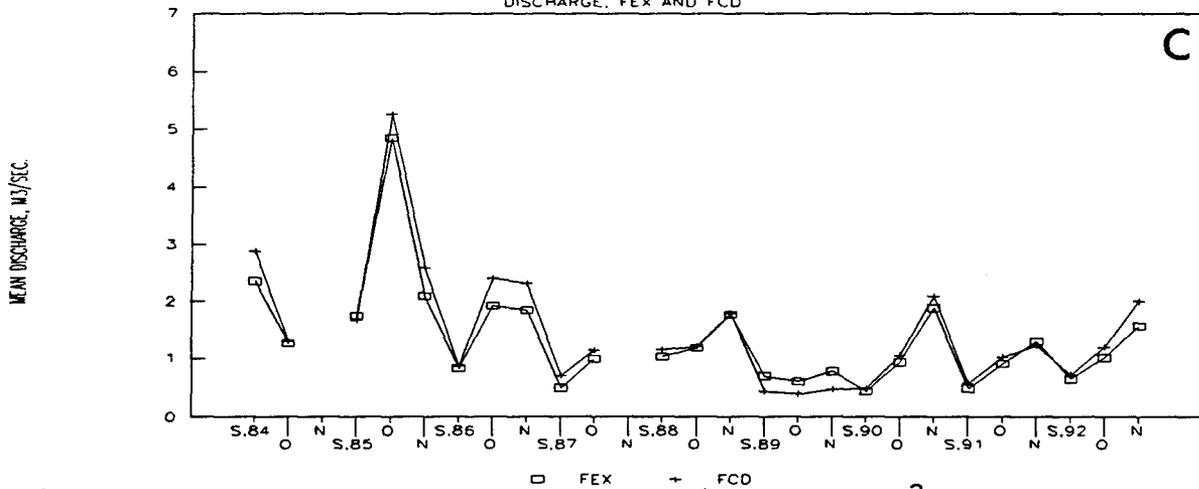
Figure 3.5A, 3.5B, 3.5C. Average discharge (m³/sec) per month at FEX and FCD. 1984-1992; **3.5A: SPRING, 3.5B: SUMMER 3.5C: FALL**
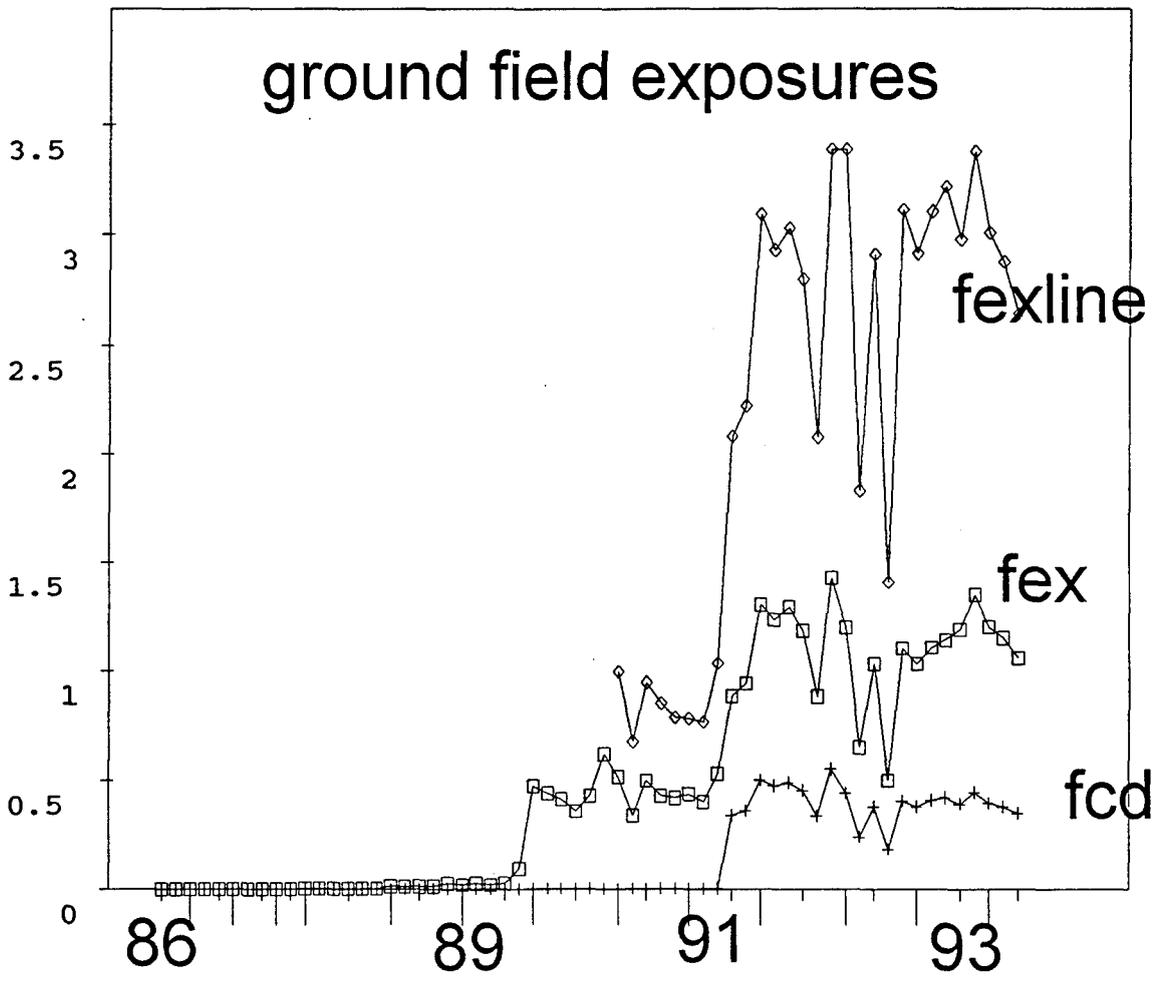
Figure 3.6. Cumulative E.L.F. ground field exposures (millivolts/day^millions) April 86 - Aug 93.

Table 3.4
Correlation Coefficients Insect Mass, Periphyton Density, Discharge and Water Temperature, 1984 – 1993

Spring (84-93)

	Insects	Periphyton	Discharge	Temp.
Insects	1.000			
Periphyton	**0.424**	1.000		
Discharge	**-0.626**	**-0.424**	1.000	
Temperature	**0.703**	**0.627**	**-0.479**	1.000

Two-tail critical value at p = .05: +,- 0.312

Summer (84-93)

	Insects	Periphyton	Discharge	Temp.
Insects	1.000			
Periphyton	0.064	1.000		
Discharge	**-0.386**	-0.093	1.000	
Temperature	**0.326**	0.008	**-0.555**	1.000

Two-tail critical value at p = .05: +,- 0.254

Fall (84-92)

	Insects	Periphyton	Discharge	Temp.
Insects	1.000			
Periphyton	**0.483**	1.000		
Discharge	**-0.351**	**-0.384**	1.000	
Temperature	0.229	**0.406**	**-0.411**	1.000

Two-tail critical value at p = .05: +,- 0.268

Boldface Numbers: significant at p < 0.05

Mean summer water temperatures over the years also showed distinct patterns (figures 3.7A, 3.7B). Deviations from the average cumulative degree days each month over the 10 years showed that water temperatures were always lower or equal to the average in the summer before ELF activation (1984, 1985). They were above average during the transition years (1986 through 1988). After full power, they were below average in four of five years. If changes in amperage, duration, and on-off surges affected the biota, detection of those changes may well be difficult because those transition years were years of high water temperatures as well.

Changes in numerical recruitment and estimates of growth rates (insect mass per unit individual) were monitored for five common species of collector-gatherers as well as for the family Chironomidae. The taxa were Paraleptophlebia mollis, Ephemerella invaria, and Ephemerella subvaria (collector-gatherer mayflies), and Glossosoma nigrior and Protoptila sp. (collector-grazer caddisflies), Paraleptophlebia mollis best fulfilled the criteria as a univoltine, numerically abundant taxon.

Data for changes in MDW/IND of P. mollis versus cumulative degrees were grouped for ANCOVA analyses to reflect three levels of ELF intensity: Before (July 1984-June 1986), intermediate intensity (July 1987-June 1989), and full operation (July 1989-June 1993). The physiological independent parameter, cumulative degree days incorporated the yearly differences in water temperatures. This species has been shown to achieve its largest size each year after 500 to 600 $^{\circ}$C have accumulated in the stream (Figure 4.25B, 1991 Annual Report). ANCOVAS were performed to determine whether there were significant slope differences between FEX and FCD for changes in MDW/IND values for each of the three ELF activity periods (Table 3.5). There were no site differences with respect to adjusted mean differences or to slope differences. Figure 3.8 shows changes in MDW/IND values for this species.

Figure 3.9A shows changes in MDW/IND values for Ephemerella invaria. This species was most abundant in the early fall when its size was small. It is a univoltine species, with its major emergence being in late spring. Ephemerella subvaria was less common than E. invaria, and therefore, there were more gaps in the data (Figure 3.9B). Final instars were collected in June of 1988 and in June 1990 through 1993. As for E. invaria, more full-sized instars were found at FEX than at FCD through the years.

83

FEX, DIFFERENCES IN CUM.DEG.DAYS
1983 THROUGH 1988, APRIL — OCTOBER
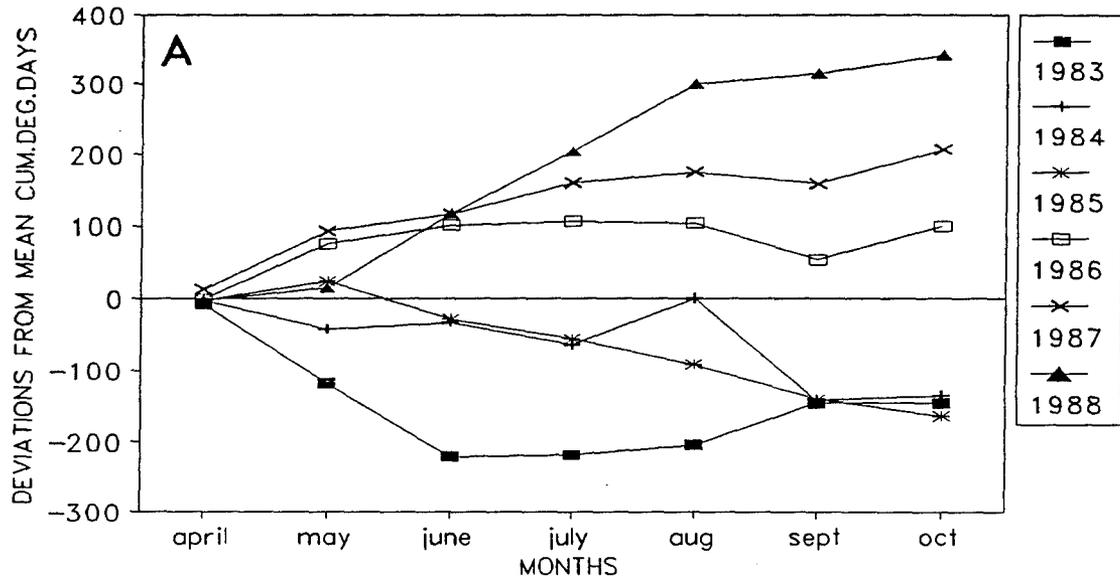
Figure 3.7A. **FEX.** Deviations in monthly mean values for cumulative degree days.
BEFORE: 1983-1985; TRANSITION: 1986-1988.

FEX, DIFFERENCES IN CUM.DEG.DAYS
1989 THROUGH 1993, APRIL — OCTOBER
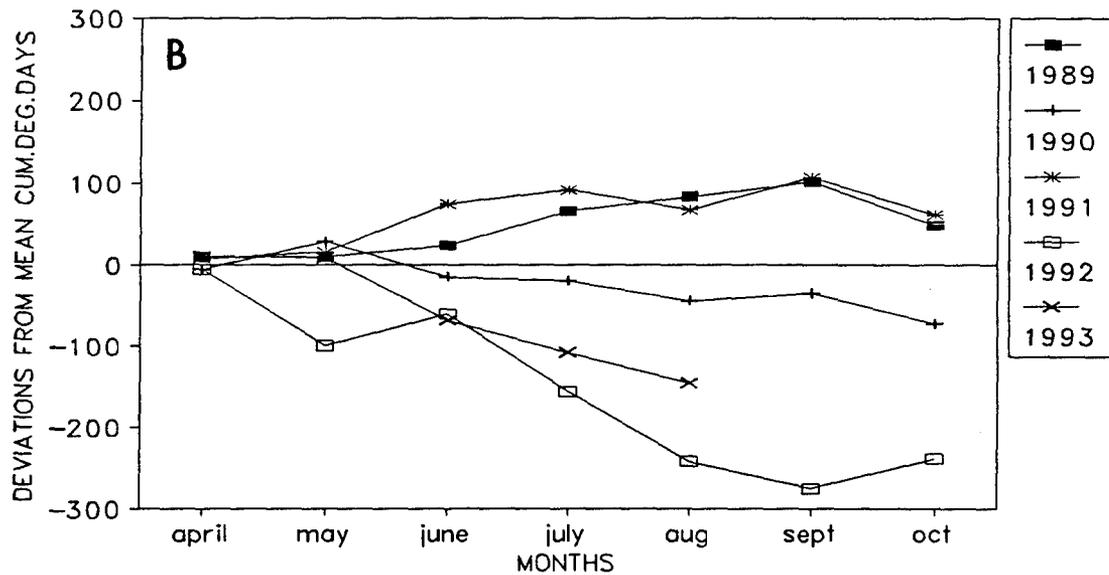
Figure 3.7B. **FEX.** Deviations in monthly mean values for cumulative degree days.
AFTER: 1989 - 1993.

Table 3.5

ANCOVAS for Changes in MDW/IND for P. mollis vs.
Cumulative Degree Days
FEX versus **FCD**: Before, Transitional, and After Impact
for E.L.F. Fields

Time Period	Adj. Means: $F_{d.f.}$ and Significance	Slopes; $F_{d.f.}$ and Significance
BEFORE E.L.F.	$F_{1,30} = 1.640$, P > .10	$F_{1,29} = 1.876$, P > .10
TRANSITIONAL	$F_{1,45} = 0.296$, P > .50	$F_{1,44} = 0.245$, P > .5
AFTER E.L.F.	$F_{1,60} = 0.013$, P > .75	$F_{1,59} = 0.003$, P > .75

P. MOLLIS AT FEX AND FCD, 1984 — 1993
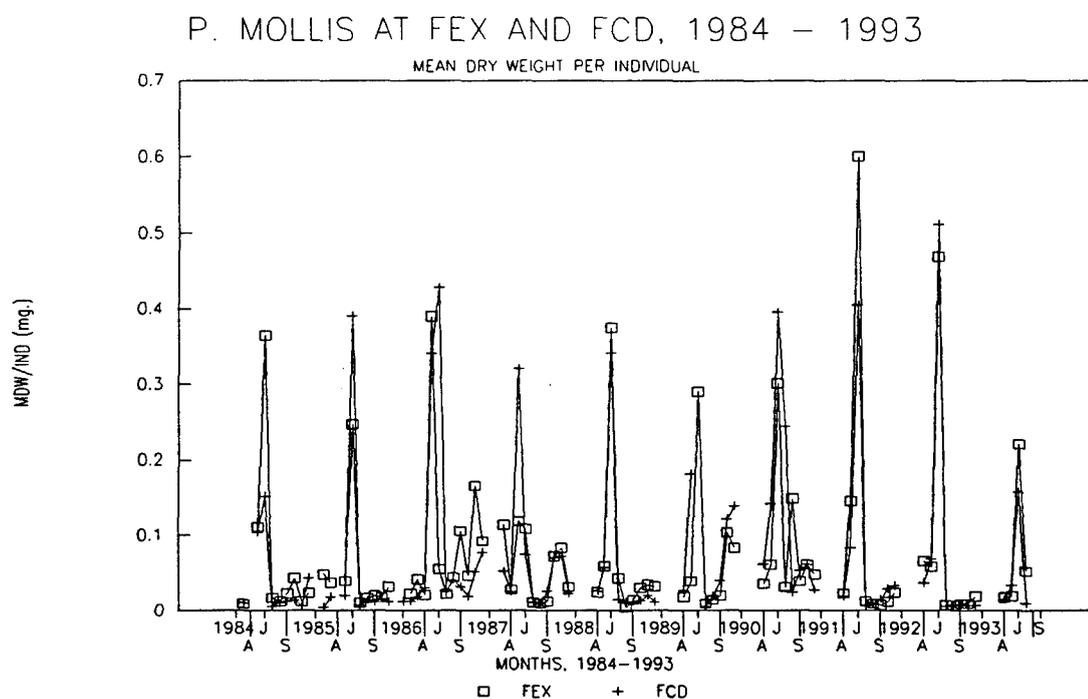
Figure 3.8. Changes in MDW/IND values for <u>Paraleptophlebia</u>
<u>mollis</u> at FEX (squares) and FCD (pluses), July 1984 –
August 1993.

E. INVARIA AT FEX AND FCD. 1984—1993

MEAN DRY WEIGHT PER INDIVIDUAL

MONTHS, 1984—1993

□ FEX   + FCD

E. SUBVARIA AT FEX AND FCD, 1984—1993

MEAN DRY WEIGHT PER INDIVIDUAL

MONTHS, 1984—1993
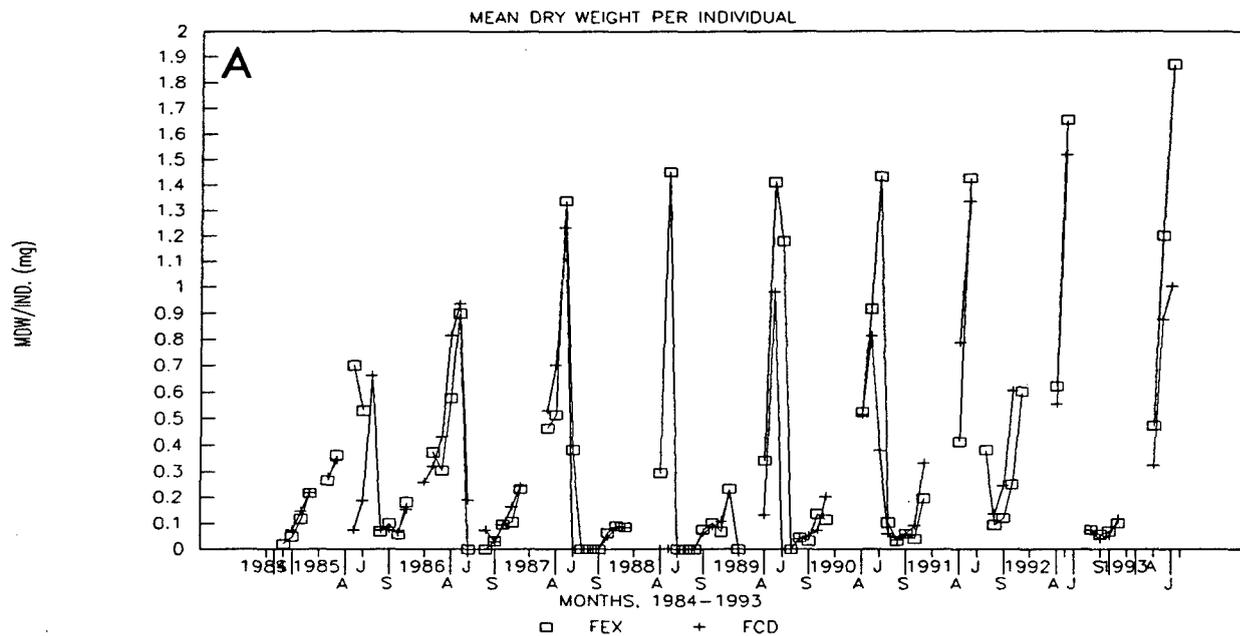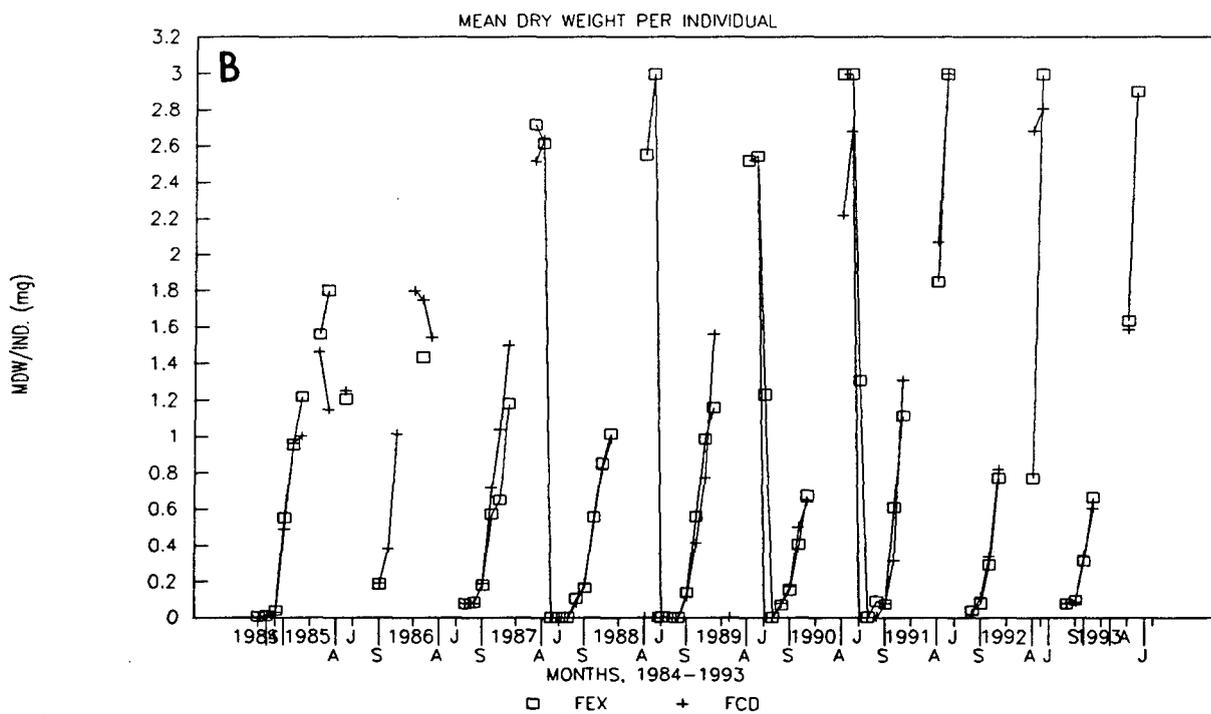
□ FEX   + FCD

Figure 3.9A, 3.9B. Changes in MDW/IND values for
Ephemerella invaria (**3.9A**) and E. subvaria (**3.9B**) at FEX
(squares) and FCD (pluses). June 1984 - July 1993.

PROTOPTILA SP.; FEX, FCD, 85–93

MEAN DRY WEIGHT PER INDIVIDUAL
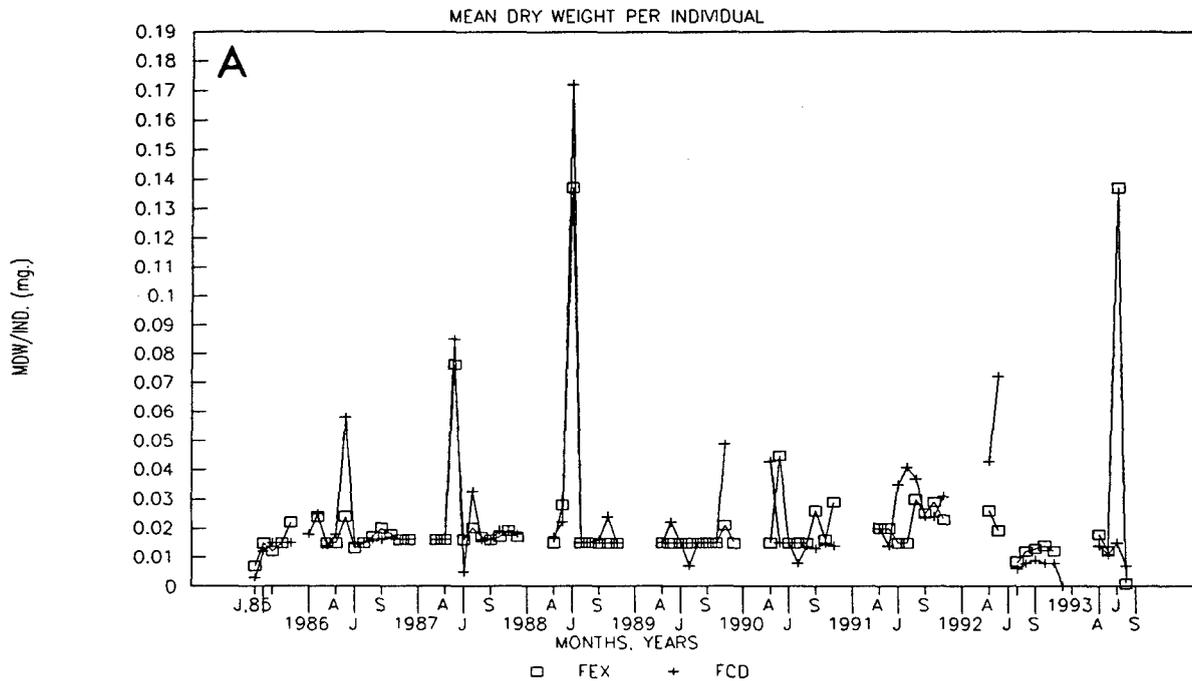
GLOSSOSOMA NIGRIOR; FEX, FCD, 85–93

MEAN DRY WEIGHT PER INDIVIDUAL
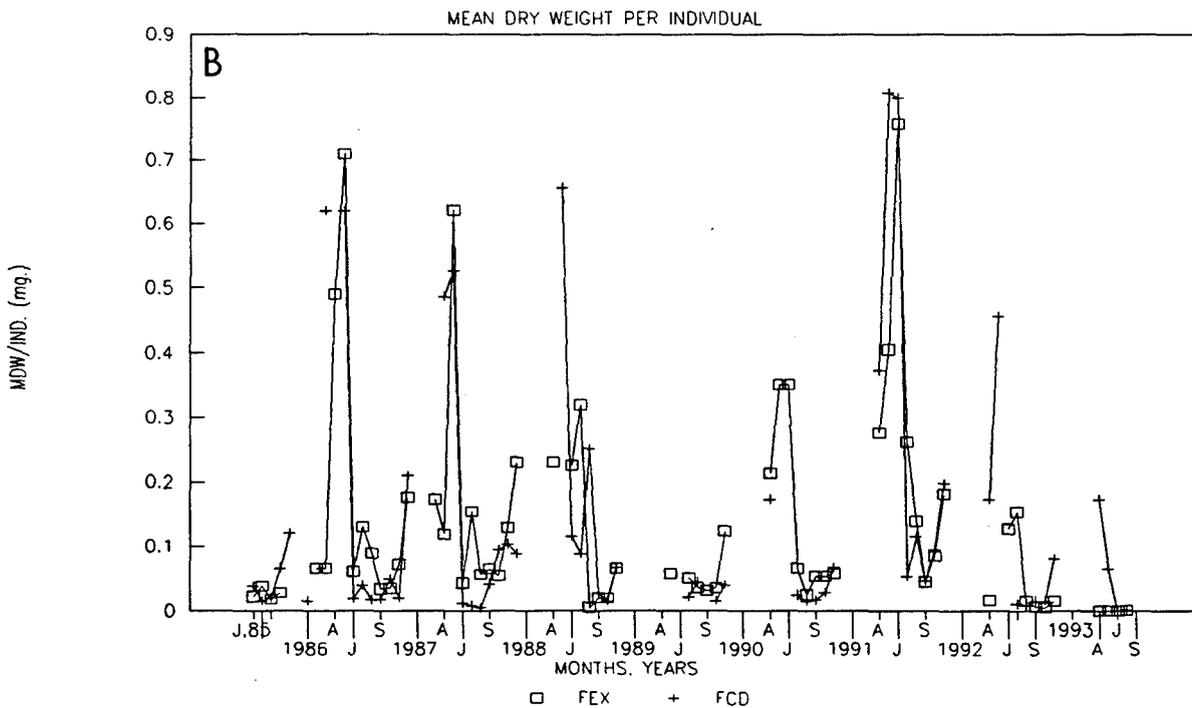
Figure 3.10A, 3.10B. Changes in MDW/IND values for **(3.10A)**
Protoptila sp. and **(3.10B)** Glossosoma nigrior at FEX
(squares) and FCD (pluses). May 1985 – July 1993.

87
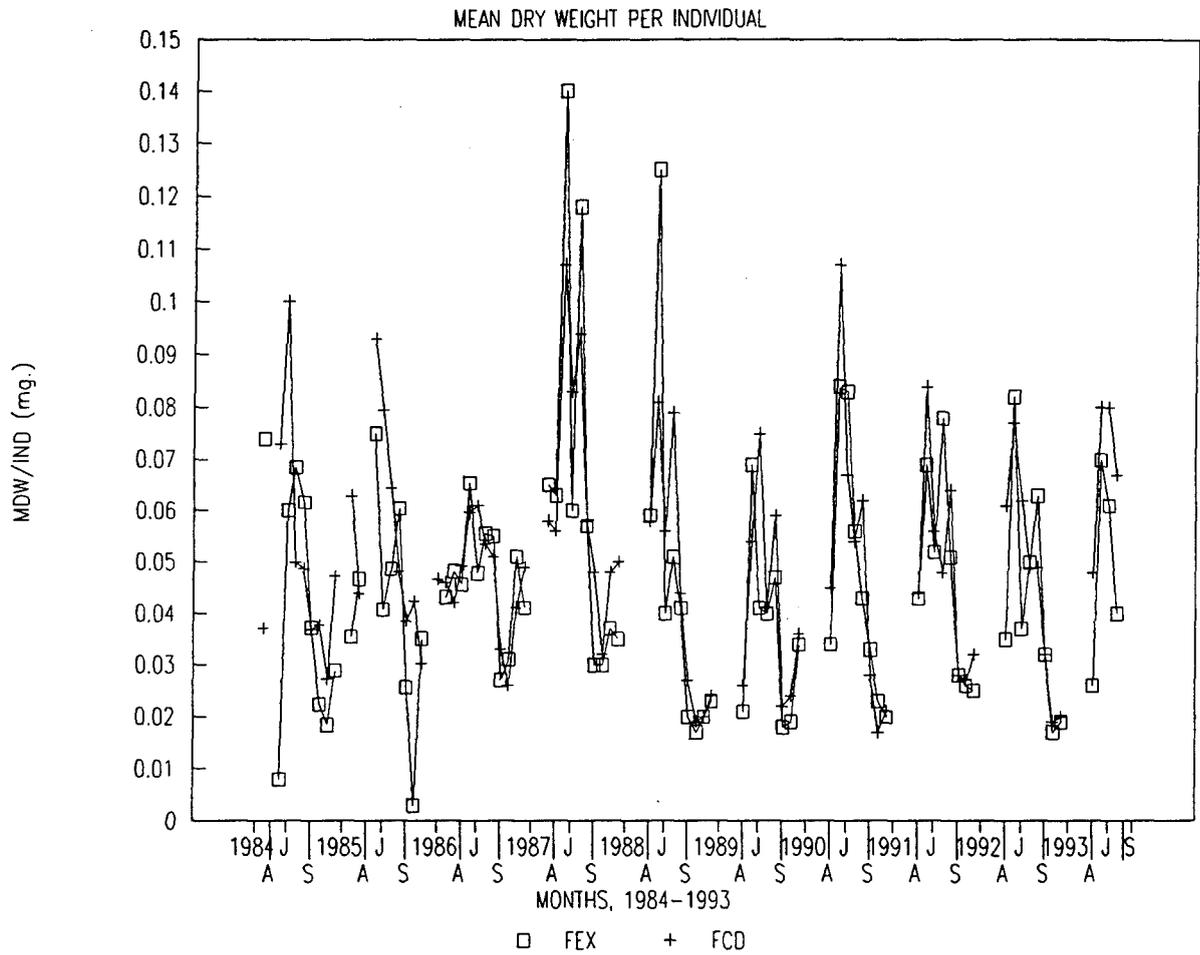
Figure 3.11. Changes in MDW/IND values for Chironomids at FEX (squares) and FCD (pluses), June 1984 - August 1993.

The young of <u>Protoptila</u> were most common during the mid-summer, just after their mid-May emergences (Figure 3.10A). In 1989, there were heavy rains in late May and June, which may have contributed to the low numbers of individuals and the lack of large individuals during that time. <u>Glossosoma nigrior</u>, which is in the same family as <u>Protoptila</u>, which was most abundant af FEX (Figure 3.10B), was monitored to facilitate research on grazers by Dr. Thomas Burton.

Chironomids were very numerous. Changes in their MDW/IND values showed a pattern (Figure 3.11). Large individuals were more abundant during the summer and smaller individuals were more abundant during the fall to early winter. Seasonal differences could reflect replacements of summer emerging species for fall growing species and/or differences in maximum size classes of different species through the year.

Discussion

Multiple linear regressions and intervention analyses provided little evidence for electromagnetic ground field intensity effects on stream insects. If ELF intensities were responsible for eliciting changes in the composition of insects, differences between FEX and FCD should have been seen after initial operation of the antenna or after elevation of intensities to full power. Intervention tests revealed few differences. Linear regression analyses consistently showed that discharge was the most important variable over the years.

Other factors such as substrate particle size, differences in beaver activity along each site, and/or in-stream biotic interactions are probably important. Trend analyses for insect mass as related to discharge, cumulative degree days, and cumulative ELF ground field exposures did not explain much of the variation in the biotic variable at FEX.

While ANOVA tests usually showed year differences for the biotic variables, those differences appeared to be related to changes in biotic and abiotic factors at the sites rather than to any ELF exposure. The substratum at FEX has become increasingly sandy since the beginning of the study, owing to beaver activity above the site, and to reduced spring spating over the last half of the study. The condition of the substratum can be as critical as current velocity in controlling insect populations (Rabeni & Minshall 1977). Diversity (Hart 1978) and abundance (Flecker & Allan 1984) of insects are heavily dependent on substrate type.

Three independent factors were used to determine the amount of explainable variation in the insect community at the experimental and reference sites. However, in most cases, less than 40 percent of the variation was explained. Biotic interactions, such as predation and competition were not monitored. Predation by insectivorous fish such as brook trout and creek chubs could have affected benthic insect distributions and abundances even though trout and chubs are not present in great numbers in the Ford River (See ELF Annual Report for Aquatic Ecosystems, 1993). This potential effect may be minimal, as in several instances, fish have been shown to have little effect on benthic invertebrate production (Allan 1982, Flecker & Allan 1984). Insect-insect interactions may be more important. Large insect predators are common at the sites in the Ford River. They include the dragonfly, <u>Ophiogomphus colubrinus</u>, the stonefly, <u>Pteronarcys dorsata</u> (Say), and the dobsonfly, <u>Nigronia serricornis</u> (Say). Biotic interactions can override abiotic controls on lotic communities (Power et al. 1988), and so the importance of the relationships among the insects themselves in the Ford River cannot be discounted. Physical factors other than the ones studed are undoubtedly affecting the insect community.

Variability in algal production could also impact the diversity and abundance of grazing insects. Periphyton density was positively correlated with insect mass in the spring and fall. The rising temperatures and spring discharges trigger growth in "spring" species of periphyton and insects. The decreasing temperatures and autumn rains also trigger "fall" species. In both seasons, the availability of periphyton can be a positive factor in the abundance of grazing insects. <u>Paraleptophlebia mollis</u> increased in size with increasing water temperatures in the spring. <u>Ephemerella invaria</u> and <u>E. subvaria</u> showed their maximum rate of increase in size with decreasing water temperatures and their sizes were inversely related to cumulative degree days (See discussion for Task 3, Element 6).

The availability of natural refugia may also be critical; refugia act as buffers against high flows, permitting faster recolonization and production by the organisms using them (Townsend 1989). The data indicate that the smaller-sized chironomids were less impacted by high flows than were the larger predators and shredders. A prior paper studying movement patterns of the predator, <u>Ophiogomphus colubrinus</u>, showed that many individuals were washed downstream during periods of heavy flows (Stout 1992).

Discharge explained more variation in the community parameters than did the other physical factors. Periods of

90

high discharge were negatively related to taxon richness, total insect mass, and to a lesser extent, taxon evenness at both FEX and FCD. Conversely, high discharge was positively related to numerical dominance and with biomass dominance of chironomids.

Investigations have shown that scouring during high discharge negatively affects stream insects. Sagar (1986) found that species diversity and abundance was inversely related to antecedent discharge in a braided New Zealand river. In that river, high diversity and high insect abundances occurred during stable winter flows, while there was low diversity and low abundances after unpredictable spring, summer, and fall flows. Numbers of individuals from two species of naucorids declined significantly after floods in a spate-prone Costa Rican stream; whereas, individuals decreased only slightly in a nearby stream that backflooded during heavy rains (Stout 1982). Rae (1990) reported an inverse relationship between species richness and discharge within an assemblage of chironomids. The effects of discharge may be augmented if flows are sufficient to scour or wash away resources such as leaves or diatoms (Anderson & Lehmkuhl 1968, Stevenson 1983). In the present study, periods of very high discharge during all seasons were consistently accompanied by concomitant decreases in richness and total insect mass. The persistence of chironomids relative to other insects even during these periods may be related to their small size and their behavior. Most species of chironomids burrow into interstices in the benthic substrata, rendering them less susceptible to the shearing action of fast-flowing water, which can easily dislodge sprawling or clinging insects (Merritt & Cummins 1984).

Extremely low frequency ground fields were not shown to affect lotic insect communities. If there were impacts, their effects would have been subtle.

91

Task Three, Element 5: Movements of <u>Ophiogomphus colubrinus</u>, a dragonfly predator

<u>Introduction</u>

Drift, a major phenomenon associated with movements of aquatic insects in streams and rivers (Muller 1974, Waters 1972, Wilzbach et al. 1986), is often monitored by the use of fixed drift nets. This technique has its limitations; only the the downstream vector can be measured, and movement rate estimates are rather imprecise. Mark-recapture techniques do not have these constraints. They can be used in studies of insect dispersion, behavioral responses to life cycle transitions, interactions among taxa, and responses to physical factors such as discharge, temperatures, or anthropogenic factors. Mark-recapture techniques do not require extensive field equipment and are, therefore, well suited for field work far distant from research facilities (Stout 1981, 1982).

Movement patterns of naiads of the dragonfly predator, <u>Ophiogomphus colubrinus</u>, were studied for five years to determine whether extremely low frequency electromagnetic fields (ELF) from a U. S. Navy communications antenna affected naiad dispersion patterns.

<u>Materials and Methods</u>

In 1983 two sites were selected for study, the Ford Experimental (FEX) and the Ford Control Downstream (FCD). Efforts were made to have the sites similar, except for ELF fields. Recapture distances extended to more than 45 m below the FEX release site and to more than 55 m below the FCD release site. Stream widths at the sites were similar, varying between nine and 11 m. Summer water velocities were also similar at low water, with the fastest areas approximating 50 cm/sec. There were no significant differences between the sites with respect to water chemistries and discharge.

In June of each year, each corner of one meter square grids was delineated with forester flags. The permanent release grid, midway between the stream banks at each site, was designated as the reference point from which all measurements were made. Current directions were taken by following the path of a floating orange placed at the middle of each upstream 1 m grid. Deapths were measured at each flag, and stream widths at 5 m intervals were recorded. Prior to each mark-recapture period, velocities were taken with a Gurley Flowmeter at 1 m intervals across the stream every 5 m downstream.

Naiads were collected with one meter square handscreens at least 500 m upstream of each release site. Animals were placed in holding pans containing stream water. Each individual was removed from the holding pan, the dorsum blotted dry and then marked with Testors enamel paint. The marked animal was placed in a dry holding pan for five minutes before water was added. After all marked animals were counted, they were placed in another holding pan with water prior to release. Animals were recaptured after 24 or after 48 hrs. On recapture days, two people with kickscreens began downstream at the farthest distance thought to be possible for the animals to travel. Work was facilitated by a moveable 2-person rubber boat.

Results

A detailed description of results appears in Stout 1992. Naiads of O. colubrinus did not move long distances and any movement only occurred in a downstream direction. Recapture success was high (Table 3.6). There were significant site differences for seven of the nine 24 hr mark-recapture studies. Animals travelled farther at FEX in 1985, the pre-operational year, and in 1989, the year in the study that experienced the highest ELF intensity, Figure 3.12. Animals moved significantly farther at FCD for the remaining years.

The average distance animals moved over the 24 hr period was regressed against discharge and ELF exposure levels. Results appear in Table 3.7. The distance the animals moved downstream was more related to discharge than to ELF cumulative exposures. ELF cumulative exposures did not add appreciably to overall regression coefficients.

There were significant site differences for distances travelled for six of the eleven 48 hr recapture experiments (Figure 3.13). In 1985 and in 1989 animals travelled significantly farther from the release sites at FEX than at FCD. In 1988 and for the first recapture series in 1989 animals moved significantly farther at FCD than at FEX. There was no pattern for distances travelled by the naiads between the sites after ELF fields were activated.

Multiple linear regressions of the average distance animals moved over 48 hr versus discharge and cumulative ELF exposures were performed. for 1985 through 1988 data and for 1989 data separately, owing to a change in technique for releasing animals in 1989. At FEX, there was no significant relationship with either independent variable (Table 3.7). At FCD, there was a significant relationship with discharge in 1985 through 1988 (p = 0.003*) but not in 1989.

93

	FEX		FCD	
	24 hr	48 hr	24 hr	48 hr
July, 1985	31.45	30.92	33.85	27.2
August	40.45		28.08	
June, 1986	43.70		49.84	
July	42.51		46.57	
August	47.81	45.87	50.12	32.89
June, 1987	55.21	46.56	54.00	49.68
June, 1988	47.23	34.90	62.23	52.00
July	73.87	47.67	67.67	51.00
June, 1989	8.01		38.46	
July		80.02		75.46
July		72.78		66.67
August		82.50		72.03
August		79.44		82.69
September		66.77		67.19

Table 3.7.   Multiple regressions for mean distance animals
moved discharge and cumulative ELF exposure at FEX and FCD

24 Hours

FEX, distance travelled	Discharge, 1985-89	ELF Exposure, 1985-89
p value	0.025*	0.516
partial r^2 value	0.593	0.073

Total r^2 = 0.60, $F_{2,6}$ = 8.84, p < 0.05

FCD, distance travelled	Discharge, 1985-89	ELF Exposure, 1985-89
p value	0.006**	0.653
partial r^2 value	0.740	0.036

Total r^2 = 0.75, $F_{2,6}$ = 4.50

48 Hours

FEX,distance	Disch., 85-88	ELF Exp, 85-88	Disch., 1989	ELF Exp., 1989
p value	0.184	0.550	0.796	0.574
partial r^2 value	0.666	0.202	0.026	0.111

1985-88 r^2=0.81, $F_{2,4}$= 4.37;   1989 r^2 = 0.19; $F_{2,5}$= 4.34

FCD,distance	Disch., 85-88	ELF Exp, 85-88	Disch., 1989	
p value	0.003**	0.086	0.391	0.579
partial r^2 value	0.994	0.835	0.250	0.114

1985-88 r^2 = 0.99, $F_{2,4}$= 369.3,p<.001
1989 r^2 = 0.49; $F_{2,5}$= 1.44

24 HR. RECAPTURES. T-VALUES. 1985-1989

FEX VS. FCD FOR DISTANCES TRAVELLED
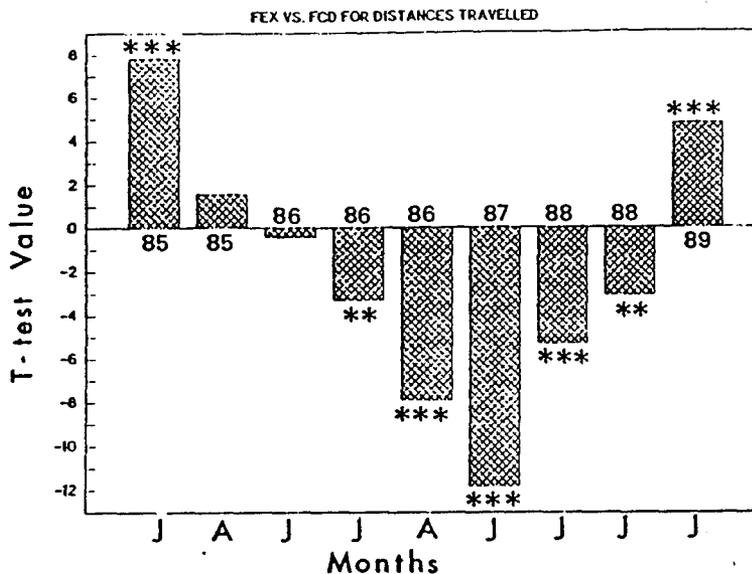
Figure 3.12. T-tests and levels of significance for site differences for distances travelled after 24 hours, 1985 - 1989. Bars above the zero line: animals moved farther at FEX. Bars below the zero line: animals moved farther at FCD. Level of significance: * <0.05, ** <0.01, *** <0.001.

48 HR. RECAPTURES. T-VALUES. 1985-1989

FEX VS. FCD FOR DISTANCES TRAVELLED
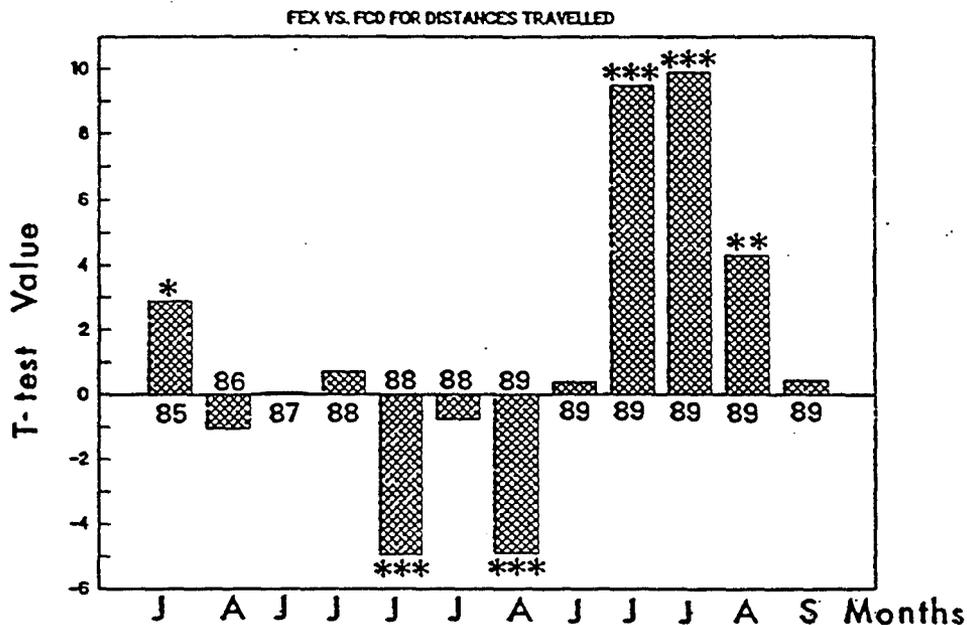
Figure 3.13. T-tests and levels of significance for site differences for distances travelled after 48 hours, 1985 - 1989. Bars above the zero line: animals moved farther at FEX. Bars below the zero line: animals moved farther at FCD. Level of significance: * <0.05, ** <0.01, *** <0.001.

Discussion

There were significant differences between sites for distances animals travelled for 13 recapture series. However, sometimes animals travelled significantly farther at FEX and other times they travelled farther at FCD. If ELF fields had affected distances travelled, there should have been consistent and increasing site differences between 1986 and 1989 as the ELF field intensities and durations increased.

ELF exposures did not explain much of the variation in distances the animals travelled for either the 24 or the 48 hr recapture experiments. Discharge explained a significant amount of the variation for the 24 hr recapture experiments at the sites. In the 48 hr tests, only one of the four tests showed a significant relationship with discharge and distance travelled; that was for FCD in the 1985 through 1988. Then, discharges were higher and varied more at FCD than at FEX. In 1989, discharges varied little at either site. One would have expected significant relationships with cumulative ELF exposures at FEX. This never occurred.

It appears that current flow affected movements of the animals soon after their release, but when the animals adjusted for longer periods after being released, their own activities appeared to override those of current flow unless large discharge events uplifted them from the substrata. In 1986, heavy rains fell 12 hr after the animals had been released at FCD. Although recapture 48 hr after release were above 30 percent, animals were recovered much farther downstream.

If ELF fields affect movements of these animals, their effects are sufficiently subtle so as to be undetectable by mark-recapture field techniques. Certainly, differences in discharge were more important in accounting for differences in distances the animals travelled. Laboratory experiments may be the only avenue to determine whether ELF fields have subtle effects on orientation by these aquatic insect predators.

Task 3, Element 6 - Leaf Litter Processing

Introduction

Processing rates of leaves incorporate the functional responses of fungi, bacteria, protozoans and leaf feeding invertebrates, especially shredding insects (Cummins et al. 1989, Petersen and Cummins 1974, Stout and Taft 1986, Stout et al. 1985). E.L.F. fields may influence some of those processors with regard to orientation, activity, or both, as many aquatic plant and animal species contain magnetite crystals (Kirschvink 1989). Some of these species, including freshwater bacteria and algae, are magnetotactic, (Tenforde 1989). It is conceivable that some aquatic species in the Ford River respond to E.L.F. fields as well as to other geomagnetic fields. If so, not only might their activities or growth rates be altered, but leaf processing rates may also be altered.

Many non-anthropogenic environmental factors can affect leaf processing rates: water temperature and flow rates (Kaushik and Hynes 1971), leaf chemistry (Iverson 1974, Stout 1989), and beaver activity (Naiman et al. 1984) may all play a role in the Ford River. Some of these factors may override any E.L.F. effects (see Tenforde 1989) or some E.L.F perturbations may themselves "...be within the ranges of disturbances that a system can experience and still function properly." (O.T.A. 1989). In either case, any potential E.L.F. effects may or may not be detectable even though coefficient of variation values for many biotic parameter estimates were very low in this study.

A number of factors can affect leaf processing rates and colonization of insects on those leaves. Examples include chemical inputs (Fairchild et al. 1984, Stout and Cooper 1983, Cairns 1985), thermal stress associated with impoundments and commerical industries (Gersick and Brusven 1981), and forest alterations (Webster and Waide 1982). As E.L.F. fields appear to be a phenomenon for which there is no analog, the foundations for decisions as to which factors may most strongly affect any organism -- intensity, duration, transient behavior -- are poorly understood (O.T.A. 1989). This problem is especially critical when studying potential effects under field conditions, where several non-anthropogenic and anthropogenic factors may interact. Considering these uncertainties, the continual monitoring of biological parameters that show low variation in time and space under field conditions is the most pragmatic approach for detecting any E.L.F. effects.

Materials and Methods

Fresh tag alder leaves were collected from one site near the control site, FCD each year. Leaves were removed from whole branches at the laboratory and weighed into individual leafpacks. Picked leaves were gently mixed prior to the construction and weighing of the leafpacks in order to reduce selection bias for any site. After leafpacks were weighed, they were taken to the field, lashed to bricks and placed in riffles at the sites.

Leafpack samples were collected from the sites six times over a three to four month period. On collection days, each leafpack was removed carefully from its brick and placed in a plastic box and the portion of the brick touching the leafpack was carefully washed over the box. After returning to the laboratory, each leafpack and container was washed over a 60 micron mesh sieve, which retained the insects. Insects were preserved in 90% alcohol. Leaves were placed in paper triangles and dried at less than 40°C for 48 hr, at which time they were weighed to the nearest 0.01 gm.

Leaf processing rates were computed as -k/day after Petersen and Cummins (1974). Processing rate values were regressed against year, cumulative degree days, and discharge values using multiple regression analyses. B.A.C.I. tests could not be performed on these data as only one value was generated each year. B.A.C.I. tests also could not be used for comparing leaf losses from each collection period, as leaf losses represented serial data. Leaves were put into the stream at a fixed input date and then collected six times over a three month period each year. Paired t-tests were used to test for for differences in -k/day values between sites over the years. Two-way ANOVAs were performed on leaf losses for leaves that had been in the stream four weeks to test for site and year differences.

Insect taxa from the leaves were determined to the lowest taxon possible. Identified insects were then measured to the nearest mm length (after Smock 1980). Taxon diversity (H'), evenness (J'), richness (S') numbers of individuals, percent numerical dominance of chironomids, and total insect mass, adjusted for leaf mass values, were computed. An attempt to analyze the mass of the shredder functional feeding group failed, owing to extremes in size classes for the few shredders on each leafpack.

98

Coefficient of variation (C.V.) values for each estimated parameter from each set of samples were obtained. A power test was used to determine if there were sufficient replicates to be confident 95% of the time that the mean varied no more than ±40% with an alpha of 0.05. (Seven replicates were sufficient if the parameter had a C.V. value of 20% or less.) The lowest C.V. values for each of the biotic parameters occurred after four week's incubation each year (see 1988 Annual Report.). The variables were analyzed with 2-Way ANOVA tests to determine site, year, or site by year differences. Multiple linear regression analyses for linear data were performed to determine their relationship with mean discharge and cumulative degree days.

Cumulative degree day and mean discharge values were computed by taking the time from Day 0 when the leaves were put in the stream each year and accumulating degree day water temperatures to the fourth week, and determining the mean discharge value during that period .

Mean dry biomass per individual (MDW/IND) values were determined for three species: Ephemerella subvaria, E. invaria, and Isoperla transmarina. Differences in growth rates (MDW/IND) between sites were analyzed with ANCOVA tests.

Results

Leaf processing rates (-k/day) were not significantly different between the FEX and FCD (Figure 3.14). In 1985 were leaves processed fast at FEX as compared with other years. Table 3.9 shows that fresh leaves at the sites were processed "fast" (after Petersen and Cummins 1974). There were no significant differences between FEX and FCD for processing rates over the years ($T_{16} = 0.530$, $p > 0.30$). [A Fisher Exact Test showed no significant difference between FEX and FEX.LINE ($T_4 = 0.12$) and between FCD and FEX.LINE ($T_4 = 0.66$).] Only in 1985 and in 1990 did -k/day values strongly deviate between FEX and FCD. In the pre-operational year of 1985 the rate was faster at FEX. In the post-operational year the rate was faster at FCD.

There were differences in discharge and in water temperatures from year to year. Figure 3.15A shows mean discharge values at FEX and FCD over the years when the leaves had been in the river for four weeks. Figure 3.15B is a plot for the amount of cumulative degrees the leaves had experienced after four weeks in the river each year.

99

The partial regression for discharge at FEX was very high (0.700). The partial regression value for years was not high (0.031), suggesting that the variation in leaf losses at or near FEX were related more to variation in discharge than to before versus after effects of ELF. At the control site, FCD, no variables including discharge, were strongly correlated with leaf losses. The location for the leafpacks at FCD may have been less subject to abrasion, activities of the insects less pronounced, or more years of data may be required before any effects of discharge can be detected.

The lowest mean to variance ratios (C.V. values) for insect community parameter estimates occurred after leaves had been in the river approximately four weeks. Data for that time period were analyzed for differences between the two sites across years. Taxon diversity (H') declined from 1984 through 1990 and then increased in 1991 and 1992. (Figure 3.16A), but coefficient of variation values remained rather steady and usually below 20 percent (Figure 6.7B, 1993 Annual Report). Two-Way ANOVA tests for taxon diversity (H') showed significant site, year, and interaction differences (Table 3.10). Diversity was higher at FCD than at FEX only in 1985 and 1991. These reversals in the usual pattern resulted in a significant interaction term. E.L.F. fields were activated in the summer of 1986 and were fully operational in the fall of 1989. The pattern between FEX and FCD during that time was similar.

Multiple linear regressions were performed for each site's data for H', J', numbers of individuals, chironomid numerical dominance and insect mass versus mean discharge and cumulative degree days, Table 3.11. (Richness was not included as the data were not linear.) Both discharge and cumulative degree days were negatively related to taxon diversity. Much of the variation in H' could be attributed by the two physical factors at FCD (R^2 = .673). Overall, cumulative degree days accounted for more of the variation than did mean discharge. Taxon evenness (Figure 3.16B) had patterns similar to those for taxon diversity (Figure 3.16A). Evenness (J') showed no significant site differences or a significant interaction term (Table 3.10). There were highly significant year differences, as one would suspect, given the days each year that the leafpack experiments were initiated. Much of the variation in J' at each site was explained by cumulative degree days (Table 3.11). Evenness was higher in 1984 and 1985 when leafpacks were placed in the stream in late September. Other years, they were put in the streams in mid-August to early September. The higher cumulative degree day values were in

1986 through 1992, with the highest values being in 1988, the year of extreme heat and low rainfall. Clearly, cumulative degree days accounted for most of the variation in J'.

Taxon richness did not show a steady decline over the years (Figure 3.17A). In fact, richness values were not similar at the two sites until 1988, afterwhich time, richness at the two sites became more similar. (Taxon richness at FEX.LINE was similar to that for the original experimental site, FEX.) Coefficient of variation values were high at FEX in 1985 (Figure 6.9B, 1993 Annual Report). From 1987 through 1992, C.V. values were below 20 percent. Table 3.10 shows that there were no significant site differences but there were significant year differences and significant interaction between years and site. FEX and FCD alternated in having the highest numbers of taxa for most years in the study. As taxon richness showed no linear pattern, no multiple linear regressions were performed on these data.

Numbers of individuals on the leaves generally increased through time (Figure 3.17B). Numbers were higher on leaves at FEX some years and were higher on leaves at FCD other years. FEX.LINE had values similar to those at FEX and FCD. Coefficient of variation values were high in 1985, 1988, and 1990 at FEX. On the other hand, C.V. values remained steady at FCD over the years (Figure 6.10B, 1993 Annual Report). FEX.LINE had values similar to those at FCD. Table 3.10 shows no significant site differences or a significant interaction term for numbers of individuals. Multiple linear regressions were performed for numbers of individuals at FEX and then at FCD versus mean discharge and cumulative degree days, Table 3.11. Discharge was negatively related to numbers of individuals, which makes logical sense. Numbers were positively related to cumulative degree days, suggesting that when the leaves were placed in the river later in the season (1984 and 1985), the leaves attracted more insects after four weeks incubation than if the leaves were placed in the stream in mid-August to early September (1986-1992). Much more of the variability in numbers of individuals was explained at FCD by the two physical variables than at FEX. However, numbers of individuals were negatively related to discharge and positively related to cumulative degree days.

Chironomid dominance increased over the years (Figure 3.18A). Coefficient of variation values were very high in 1985 at both sites, but they decreased to less than 20 percent after 1986. Chironomid dominance values showed no

101

significant site or site by year interaction differences
(Table 3.10). Because chironomid numerical dominance was
linear over time, multiple linear regressions were
performed on data from FEX and FCD, Table 3.11. Chironomid
numerical dominance was positively related to both mean
discharge and to cumulative degree days. More of the
variation in chironomid dominance was explained by
cumulative degree days than by discharge. When the leaves
were put into the river in mid-August or when the August
and September were unusually warm (1988), we found
proportionately more chironomids than other taxa. A great
deal of the variation at FCD for this biotic parameter was
explained by the two physical variables (coefficient of
multiple determination = 0.705).

Total insect mass, adjusted for leaf mass was similar
over time until 1989 when they very high at both sites
(Figure 3.18B). In 1991, total insect mass/leaf mass was
exceedingly high at FEX, primarily owing to some very large
individuals of the stonefly Acroneuria lycoria on two of
the seven leafpacks. Coefficient of variation values were
very high (Figure 6.12B, 1993 Annual Report), owing to a
few very large animals relative to many smaller animals.
There were years when C.V. values were less than 20
percent, but because the C.V. values were often high, the
probability of detecting any ELF effect on the mass of
insects is small. Two-way ANOVAS show significant year
differences between the two sites but no significant site
differences nor a significant interaction term (Table
3.10). Although insect mass adjusted for leaf mass
increased over the years, the increases were similar at the
sites. Multiple linear regressions were performed to
determine the relationship between insect mass versus mean
discharge and cumulative degree days from 1984 through
1992, Table 3.11.

Individuals of species found in sufficient numbers on
leafpacks that grew during the autumn and winter seasons
were monitored for possible changes in yearly growth rates
at FEX, FCD, and at FEX.LINE. Three species fulfilled
those criteria: Ephemerella subvaria, Ephemerella invaria
(mayfly collector-gatherers), and Isoperla transmarina (a
predatory stonefly). Changes in MDW/IND values for each
species were plotted against physiological time, cumulative
degree days. As growth was related to reductions in daily
water temperatures, cumulative degree days were used to
determine whether growth rates were correlated with
decreasing water temperatures in the fall and winter. The
fastest growth rates occurred when the fewest number of
degree days accumulated between sampling dates. By late

October through November, the waters had cooled and the leaf inputs were high for these collector-gatherers and predators. The species emerge in the late spring-early summer (See Element 4, 1993 Annual Report). They had not attained their peak growth by the end of the leafpack experiments, but their accelerated rates of growth were obvious during the leafpack studies. If ELF exposure alters growth rates, one would expect the effects to be apparent in rate changes and/or in maximum size at emergence. This study and and studies on insects in substrates were designed to determine whether there were any significant changes, owing to ELF activation.

Table 3.12A presents results for ANCOVAS for growth of E. subvaria. The covariate is chronological time. There were no significant differences in adjusted mean values for E. subvaria on fresh leaves at FEX versus FCD each year. However, slopes differences were significant in 1986, 1990, and 1992. The slopes of the MDW/IND values were higher at FCD in 1986 and 1992; in 1989, they were higher at FEX. There were significant differences between FEX and FEX.LINE with respect to adjusted means in 1990 and with respect to slopes in 1990 and 1991. In 1990 animals were larger at FEX.LINE and in 1991 they were larger at FEX.

Table 3.12B shows that there were no significant adjusted mean value or slope differences for Ephemerella invaria at FEX and FCD. There were differences between FEX and FEX.LINE in 1991; slopes were higher at FEX. It would take several years to determine whether the higher E.L.F. fields at FEX.LINE had an impact on growth of E. invaria. The data for FEX and FCD span nine years and those data give a good view as to changes in size classes for the three species we are monitoring. We have three years of data from FEX.LINE for making comparisons with it and the original experimental site, FEX.

A predatory stonefly, Isoperla transmarina, showed significant differences between FEX and FCD only once (Table 3.12C). That was in 1984 when the adjusted mean value for MDW/IND was higher at FCD. There were no significant differences between FEX and FCD after full operation of E.L.F. lines in the fall of 1989. It appears that E.L.F. fields did not affect its growth rates. There were significant slope differences between FEX and FEX.LINE in 1991. The sizes of the animals increased much faster at FEX. It appears that FEX.LINE cannot be considered as a matched site for FEX. If ELF. fields affected biotic parameters at FEX.LINE more than at the original test site, we could not know this, as we have no 'before' data there.

103

Discussion

There were no differences between FEX and FCD for leaf processing rates. Leaf processing at the new site, FEX.LINE was usually slower than leaf processing at FEX or FCD. The addition of this new site came too late in our monitoring program for conclusions to be drawn relative to ELF activity at this site.

Variation in leaf losses after four weeks' incubation at the experimental site were explained more by discharge than by years or cumulative degree days. This result does not support any notion that ELF activation affected 28 day leaf losses over the years. None of those variables explained much of the variation in leaf losses at FCD.

Coefficient of variation values for structural and community parameters of the insect community colonizing leaves were low after the leaves had been in the river four weeks. They were higher for earlier collections (Day 7, 14, and 21) and higher again for later collections (Day 50, 80). Therefore, we concentrated our statistical analyses on data from the four week incubation period. Two-way ANOVAS showed significant year differences for each of the six biotic parameters. Leafpack experiments were initiated earlier in the season in 1987 through 1992. This change in procedure probably contributed to the significant differences in years. The insect community colonizing leaves at FEX appears to be more diverse, but the higher diversity cannot be attributable to ELF fields. There were no significant site differences for evenness, richness, numbers of individuals, chironomid dominance, or for total insect mass (adjusted for leaf mass). Evenness decreased over the years until 1991 and 1992. Numbers of individuals increased over the years. Chironomid dominance and insect mass/leaf mass ratios increased over the years until 1991 and 1992.

Graphical analyses did not show that year differences were associated with ELF activation in 1986 nor from 1989 through 1992 (when the fields were at full power). Multiple linear regressions, using mean discharge and cumulative degree days as the independent variables, showed that cumulative degree days accounted for more of the variability than discharge for the six biotic parameters. (Note that discharge was more important for leaf losses and that the coefficient of multiple determination was higher at FEX.) Coefficient of multiple determination values were always higher at FCD than at FEX for each biotic parameter measured for the insect community. Apparently, the higher

104

r^2 values at FCD reflect a closer relationship to changing water temperatures and discharge, which could be related to more sandy conditions. More cobble could provide refugia for the colonizing animals during periods of spating and associated cooling of water.

ANCOVAS showed that mean sizes of three species, Ephemerella subvaria, Ephemerella invaria, and Isoperla transmarina, were similar at FEX and FCD. There were three years, however, when there were significant slope differences between the two sites (individuals of E. subvaria only). Because the sites oscillated over time with respect growth rate differences for E. subvaria, it is improbable that ELF activation had any effect. Results from the new site, FEX.LINE, were compared with those from the original site, FEX. There were significant differences in slopes and/or in adjusted mean values for E. subvaria at FEX and FEX.LINE in 1990 and 1991. There were significant slope differences for the other two species in 1991. The new site appears to be distinctly different from either the original test or control site.

Table 3.9.   Leaf Processing Coefficients (-$\underline{k}$/day) and Regression Coefficients for the slopes.   Fresh Leaves at FEX, FCD, and FEX.LINE, 1984 - 1992

Year	FEX, -k/day	FEX, r^2	FCD, -k/day	FCD, r^2	FEXLINE -k/day	FEX.LINE r^2
1984	.0151	.78	.0149	.83		
1985	.0321	.62	1.016	.47		
1986	.0099	.69	.0105	.68		
1987	.0124	.80	.0130	.74		
1988	.0145	.70	.0122	.57		
1989	.0102	.84	.0087	.74		
1990	.0091	.78	.0144	.78	.0081	.62
1991	.0147	.49	.0126	.75	.0166	.68
1992	.0074	.46	.0100	.54	.0046	.45
Mean	.0137		.0123		.0098	
S.D.	.0075		.0022		.0062	

Table 3.10. Two-Way ANOVA Tests for for Insects on Fresh Leaves After 24 to 28 Days, 1984 – 1992

Source	d.f.	SS	MSS	F value
H', Site		0.528	0.538	9.938**
Years	8	13.096	1.637	30.829***
Interaction	8	0.895	0.112	2.107*
Error	108	6.093	0.053	
Source	d.f.	SS	MSS	F value
J', Site	1	21.24	21.24	2.02 n.s.
Years	8	3118.76	389.84	37.15***
Interaction	8	107.62	13.45	1.28 n.s.
Error	108	1190.56	10.49	
Source	d.f.	SS	MSS	F value
S', Site	1	12.70	12.70	1.94 n.s.
Years	8	653.00	81.62	12.46***
Interaction	8	252.87	31.61	4.83***
Error	108	676.86	6.55	
Source	d.f.	SS	MSS	F value
No.Ind., Site	1	73201	73201	1.34 n.s.
Years	8	1677083	209635	3.50**
Interaction	8	644281	80535	1.22 n.s.
Error	108	6490664	59894	
Source	d.f.	SS	MSS	F value
Chiro Dom., Site	1	6.12	6.12	0.20 n.s.
Years	8	3404.50	425.56	14.18***
Interaction	8	480.95	60.12	2.00 n.s.
Error	108	6.09	0.05	
Source	d.f.	SS .	MSS	F value
Tot. Insect Mass Site	1	33.11	33.11	0.86 n.s.
Years	8	5596.76	699.60	18.09***
Interaction	8	357.92	44.74	1.16 n.s.
Error	108	3548.67	38.68	

Table 3.11. Multiple Linear Regressions for H', J',
Numbers of Individuals, Chironomid Numerical Dominance and
Mean Insect Mass/Leaf Mass versus Mean Discharge and
Cumulative Degree Days, 1984 – 1992

Sites, Description	Diversity	Evenness	# Individ.	Chiro. Dom.	Insect Mass
FEX					
R^2	0.380	0.467	0.187	0.247	0.310
Discharge	-.177	-.319	-.181	.406	-0.317
Cum.D.D.	-.726	-.864	.287	.683	0.288
FCD					
R^2	0.673	0.760	0.547	0.705	0.418
Discharge	-.592	-.310	-.303	.295	-0.273
Cum.D.D.	-1.094	-1.050	.500	1.010	0.430

Table 3.12.   Multiple Linear Regressions for Mean Insect Mass/Leaf Mass versus Mean Discharge and Cumulative Degree Days, 1984 - 1992

A. *Ephemerella subvaria*

F VALUES, SIGNIF.

FEX VS.FCD	ADJ.MEANS	SLOPES	FEX vs.Line	ADJ MEANS	SLOPES
1984	0.688	.001			
1985	1.951	.066			
1986	1.610	5.978*			
1987	1.761	2.299			
1988	1.797	1.555			
1989	2.169	12.356***			
1990	2.115	16.567***	1990	4.330*	34.465***
1991	0.069	0.892	1991	0.483	4.230*
1992	2.398	14.831***	1992	0.180	0.092

B. *Ephemerella invaria*

F VALUES, SIGNIF.

FEX VS.FCD	ADJ. MEANS	SLOPES	FEX vs.Line	ADJ. MEANS	SLOPES
1984	0.503	0.460			
1985	1.605	0.434			
1986	2.332	0.034			
1987	1.400	0.008			
1988	1.332	0.691			
1989	1.413	0.048			
1990	0.420	0.166	1990	0.215	0.106
1991	0.008	0.399	1991	0.246	4.349*
1992	1.033	3.572	1992	0.180	0.092

C. *Isoperla transmarina*

F VALUES, SIGNIF.

FEX VS.FCD	ADJ.MEANS	SLOPES	FEX vs.Line	ADJ MEANS	SLOPES
1984	4.329*	1.963			
1985	2.310	1.674			
1986	0.452	3.172			
1987	0.002	0.380			
1988	too few	data			
1989	0.003	2.183			
1990	0.282	0.374	1990	0.343*	0.011
1991	1.024	1.223	1991	2.284	12.215**
1992	too few	data	1992	too few	data

109

Changes in -k/day over time
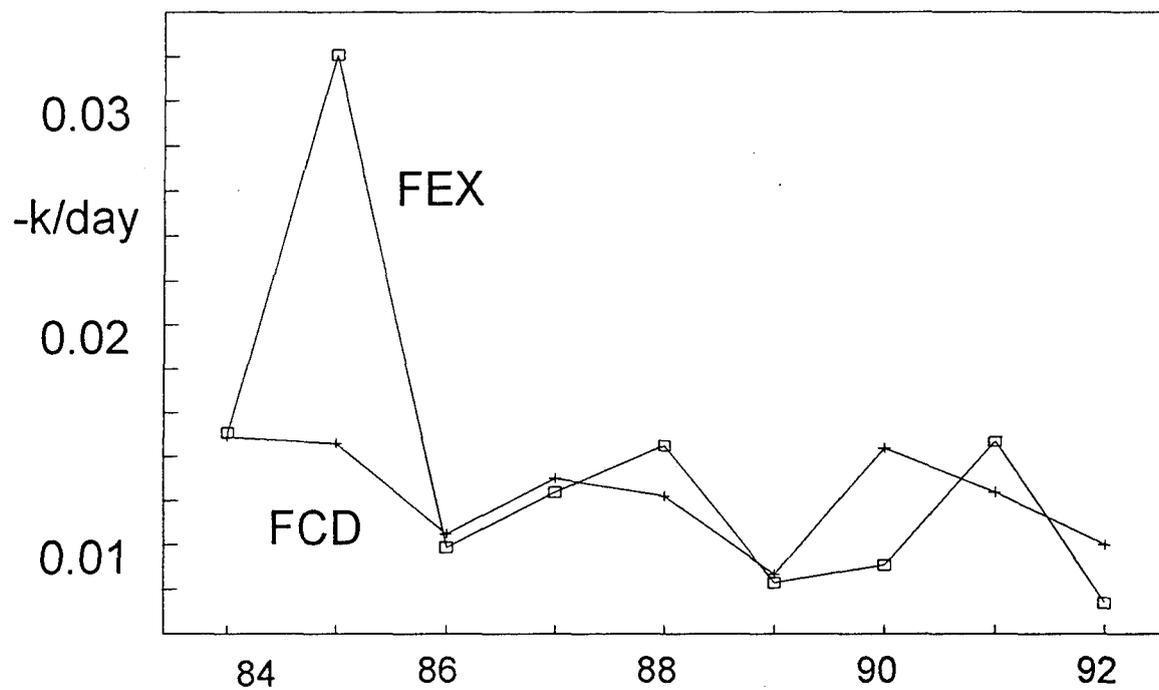
3.14. Changes in processing rates (-k/day) at FEX and FCD
       1984 - 1992.

Mean Discharge vs. Years
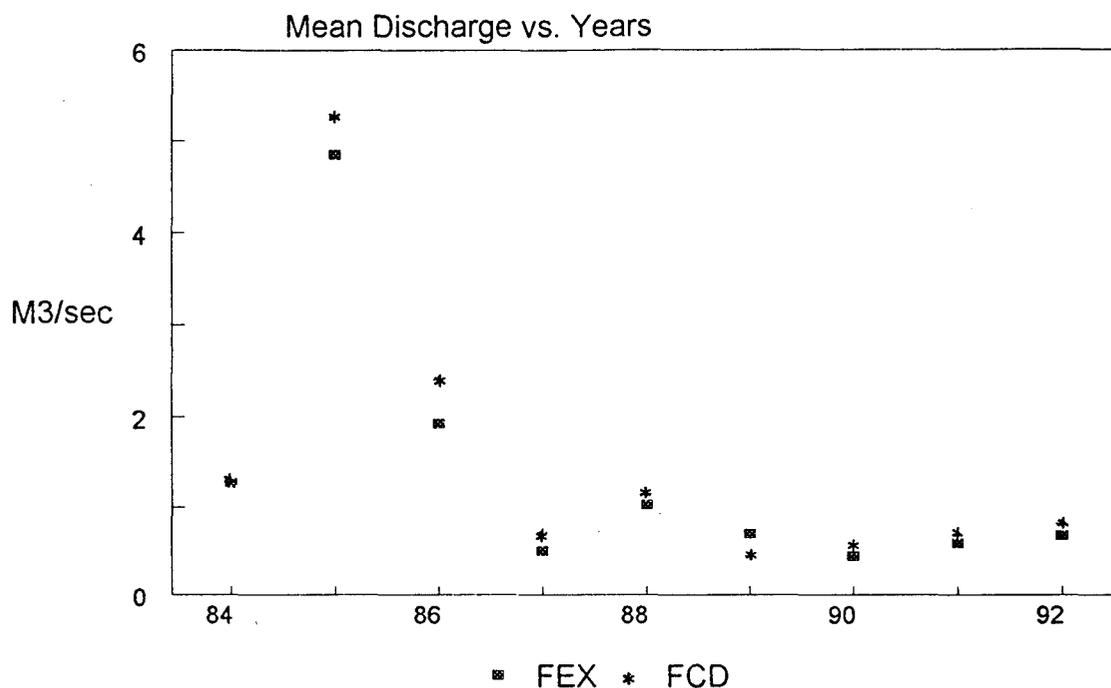
3.15A. Mean discharge at FEX and FCD each year during four week incubation period for leafpacks.

Mean Cum.Deg.Days vs. Years
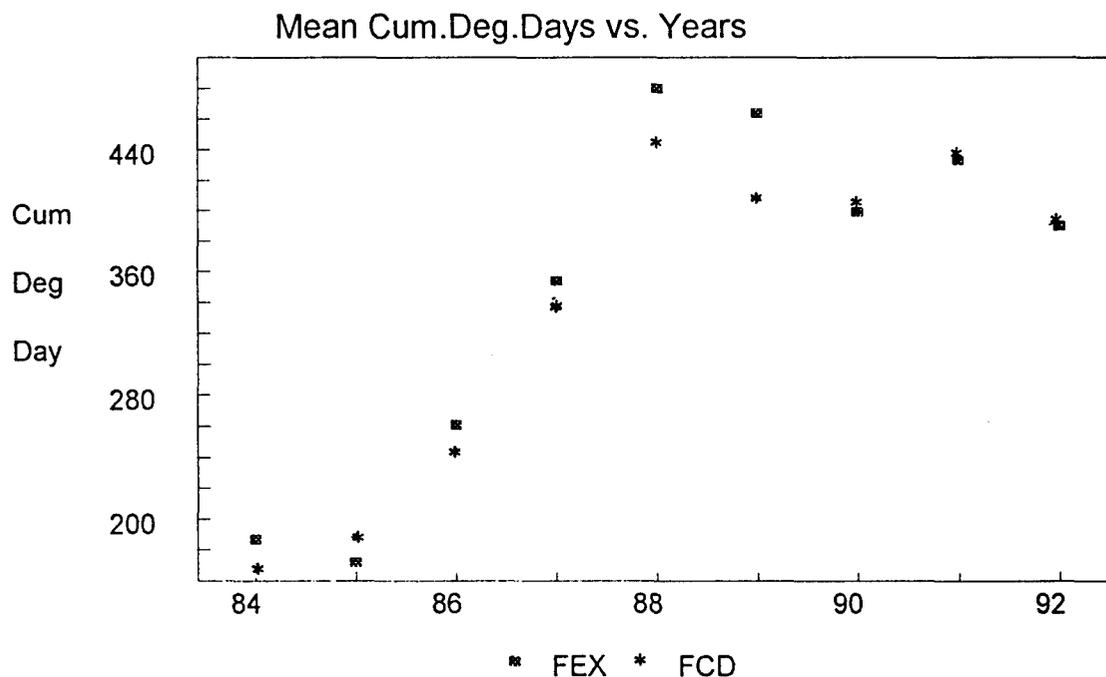
3.15B. Cumulative degree days at FEX and FCD each year during four week incubation period for leafpacks.

Mean Taxon Diversity
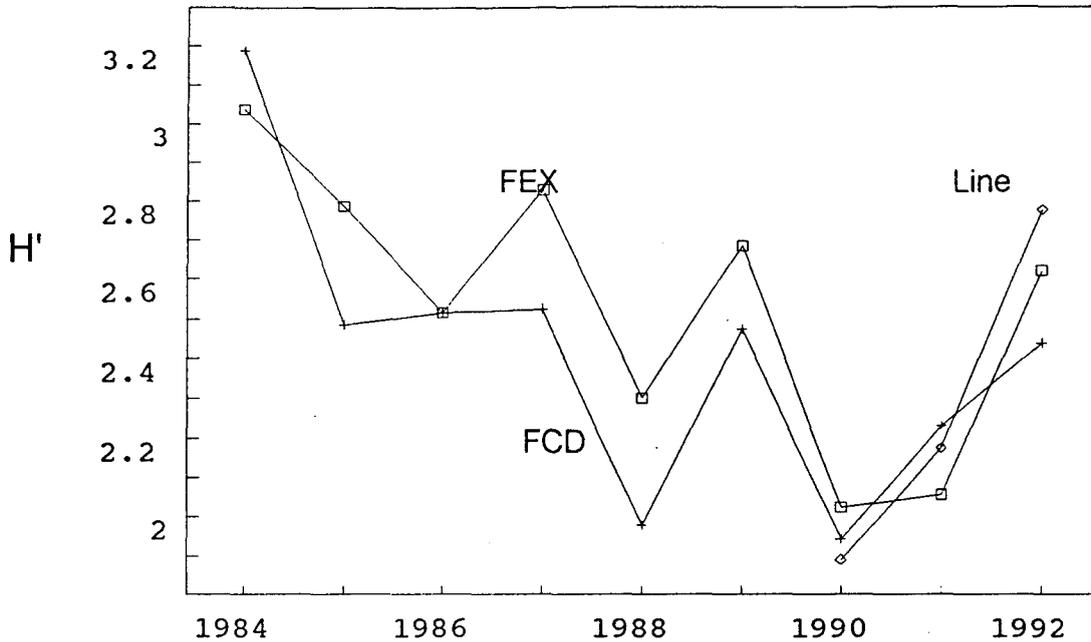
3.16A. Mean taxon diversity (H') on fresh leaves at FEX, FCD, and FEX.LINE after four weeks incubation, 1984 - 1992.

Mean Taxon Evenness, after 4 Weeks
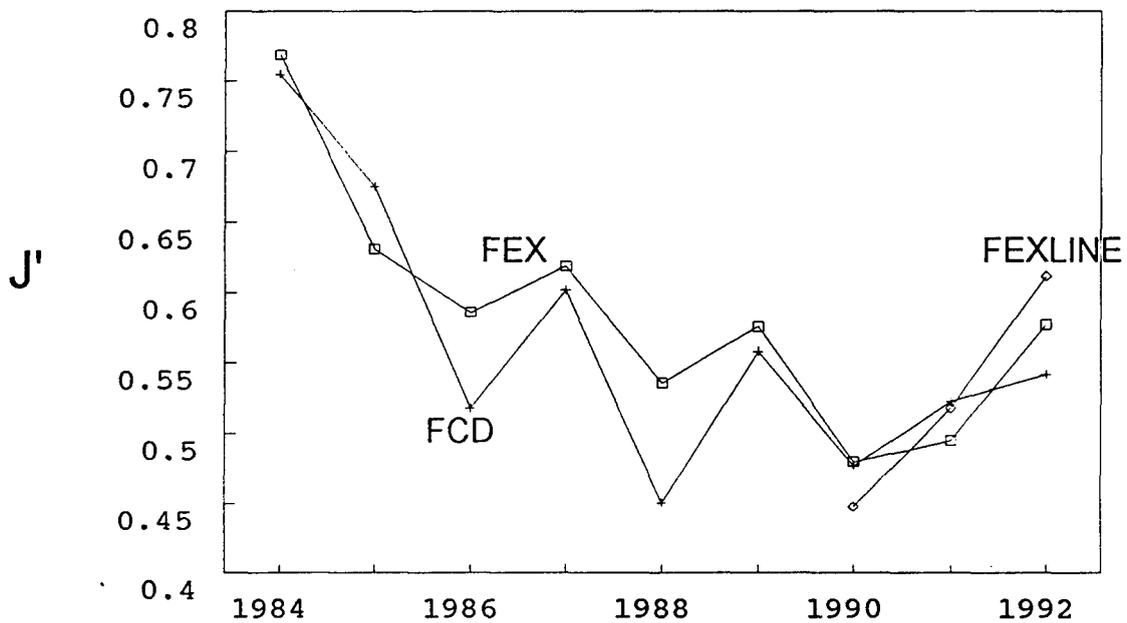
3.16B. Mean taxon evenness (J') on fresh leaves at FEX, FCD, and FEX.LINE after four weeks incubation, 1984 - 1992.

Mean Taxon Richness, after 4 Weeks
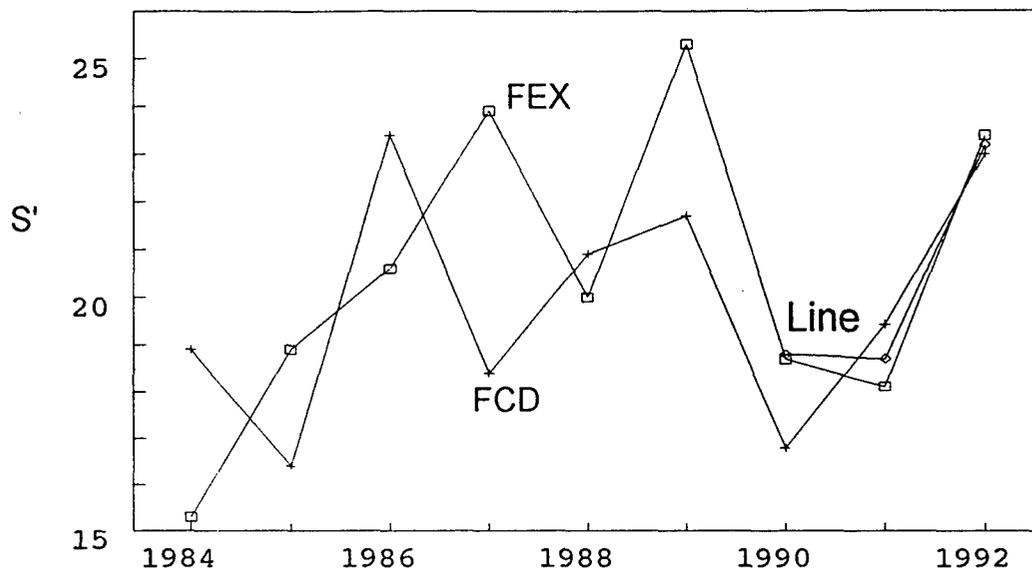
3.17A. Mean taxon richness (S') on fresh leaves at FEX, FCD, and FEX.LINE after four weeks incubation, 1984 - 1992.

Mean No. Individuals, after 4 Weeks
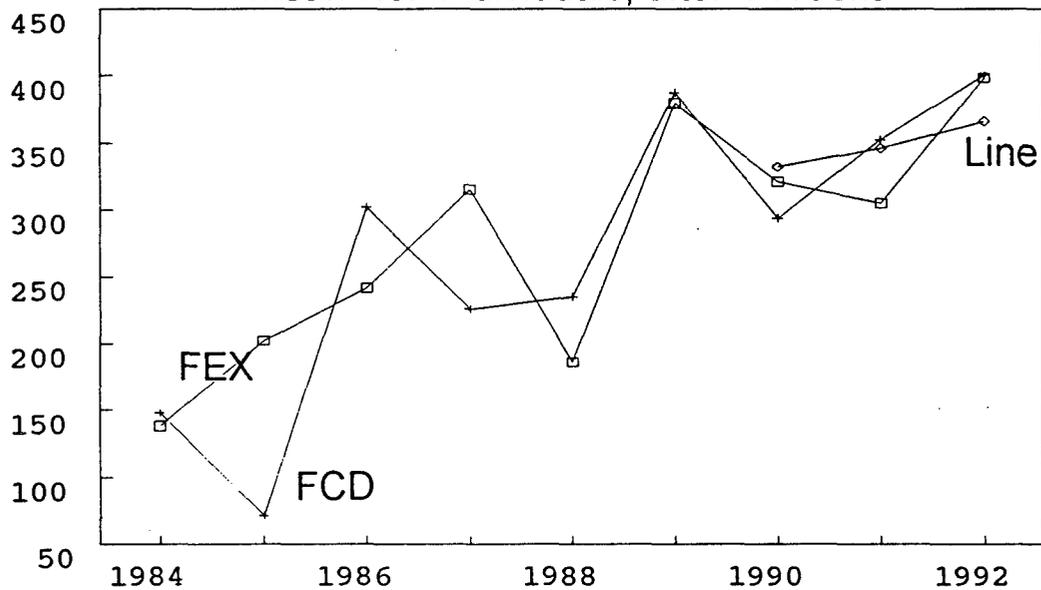
3.17B. Mean number of individuals on fresh leaves at FEX, FCD, and FEX.LINE after four weeks incubation, 1984 - 1992.

Mean Chironomid Dominance, after 4 Weeks
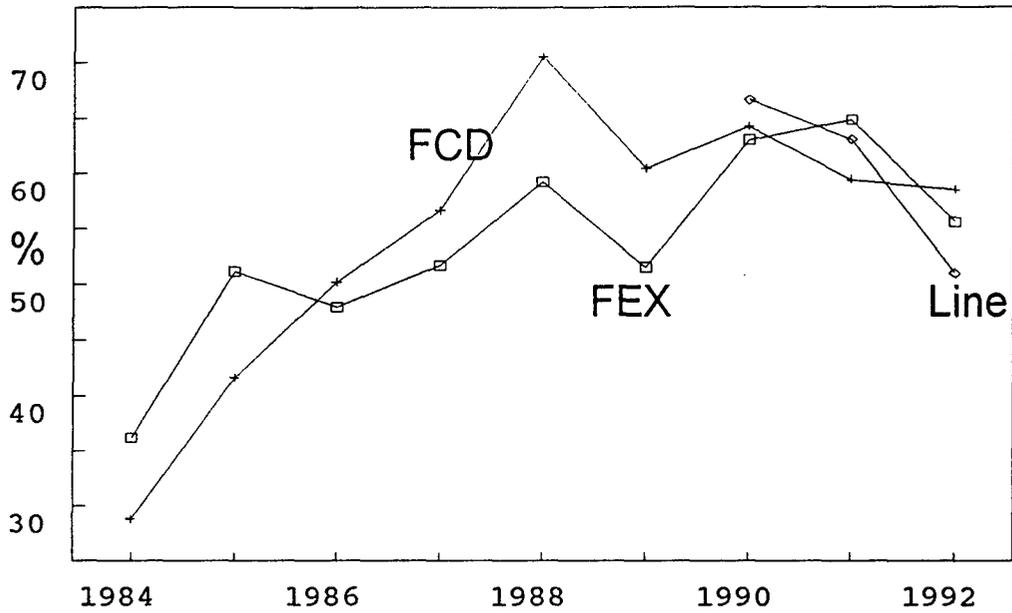
3.18A Mean chironomid numerical dominance on fresh leaves
     at FEX, FCD, and FEX.LINE after four weeks incubation,
     1984 - 1992.

Insect Mass/Leaf Mass
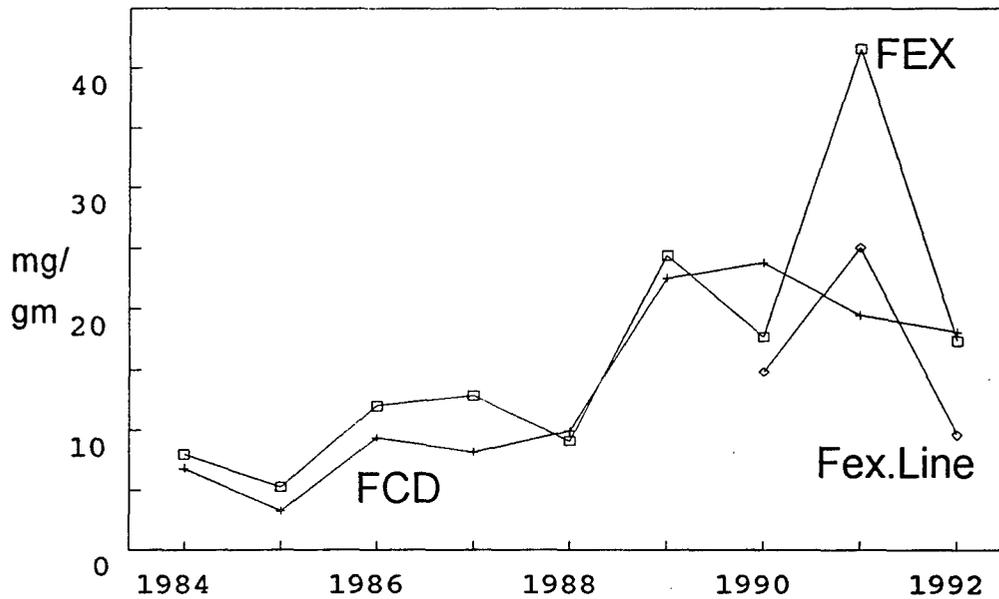
3.18B Mean total insect mass adjusted for leaf mass on
     fresh leaves at FEX, FCD, and FEX.LINE after four
     weeks incubation, 1984 - 1992.

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Task Four (Elements 7 and 8): Fish Community Composition, Abundance, and Movement

Introduction

The overall goal of this element was to determine if the operation of the ELF antenna has measurable effects on the fish community of the Ford River, Dickinson County, Michigan. In particular, we set the following objectives: 1) to determine if the fish species composition in the river changed in response to the ELF operation; 2) to determine if the abundance and biomass of individual species changed in response to the ELF operation; 3) to determine if the size structure, age and growth patterns of fish populations changed in response to the ELF operation; 4) to determine if fish condition (health) changed in response to the ELF operation; and 5) to determine if fish movement behavior changed in response to the ELF operation. No previous studies have been published on the effects of electromagnetic fields on fish communities or fish populations.

Study design

The fish community study objectives were accomplished by comparison of appropriate data sets across two comparisons: between a control site (FCD) and an experimental site (FEX), and between pre-operational (1983-1985), transitional (1986-1988) and operational (1989-1993) periods. The control site, FCD was located 14 km downstream of the point where the ELF antenna crosses the Ford River and was originally selected so as to be out of the range of detection of the ELF electromagnetic field above ambient. The experimental site, FEX was located immediately (100 m) downstream of the ELF crossing over the Ford River, and was within the measurable EM field around the ELF antenna. Comparisons of community and population parameters between sites were used as one means of assessing whether the ELF antenna had an impact. We have used a variety of statistical methods, described in more detail in subsequent sections, to determine if measurement variables differed between the two sites before and during operation of the ELF antenna.

120

Because stream ecosystems exhibit considerable natural variation in time (Hynes, 1970; Grossman et al., 1982) we also have attempted to determine ELF effects by comparison of changes from the pre-operational period to the transitional and operational periods at both sites. If ELF has impacts on the measurement variables, differences among periods at the experimental site (FEX) should differ from those at the control site (FCD). Again, several statistical methods, described later, were used to assess the period effects in measurement variables at the two sites.

Three additional sampling locations were used at different times in the study to allow more complete assessment of fish movement behavior on either side of the ELF crossing and across the ELF corridor. These were all located upstream of the ELF crossing, and included FEN, 400 m upstream of FEX and across the ELF corridor from FEX, FCU, approximately 14 km upstream of FEX on the Ford River, and TM, approximately 13 km upstream of FEX on Two Mile Creek, a spring-fed tributary of the Ford River. Data from these three sites were only used in evaluating fish movement behavior.

Materials and Methods

A. Fish community composition and population characteristics

Fish in the Ford River were captured using fyke nets fished in tandem, one facing upstream and one facing downstream, at FCD, FEX, and FEN. FCD and FEX were sampled throughout all years of the study. Sampling at FEN occurred from 1990 through 1993. In addition, wire mesh weirs were used to sample at FCU and TM; FCU was sampled from 1983 through 1990 and TM was sampled from 1984 through 1991. In most years, the gear was fished continuously from mid-May through early July; however, on several occasions discharge levels were too high for the gear to sample effectively and the gear was removed. When catch rates were low from mid-July through September or October, the gear was fished 4 days/week (deployed on Monday and removed on Friday). All gear was checked every 24 hours.

All fish were enumerated, measured for total length, weighed, and marked with a fin clip distinctive to each study site. The fish were then returned to the river upstream or downstream from the station in their original direction of travel. In addition to our study of fish community composition, we focused on population responses of the five most common species: brook trout (_Salvelinus fontinalis_), burbot (_Lota lota_), creek chub (_Semotilus atromaculatus_), common shiner (_Luxilus cornutus_), and white sucker (_Catostomus commersoni_).

Brook trout age and growth determination were estimated by use of the body-scale relationship technique described in Smale and Taylor (1987) for data from 1983-1991. Backcalculations were made using the linear technique described in Bagenal and Tesch (1978). Scales were projected onto a Summagraphics digitizing pad using a Ken-A-Vision Microprojector scope. The focus, subsequent annuli, and the outside edge of each scale were digitized and recorded on a microcomputer for determination of backcalculated length at age.

B. Fish movement

Movement patterns for the five most common species were monitored by observing the frequency of recapture of marked fish. Fish recaptured at a site other than the original marking site were measured for total length, weighed, and marked with an additional fin clip specific to the recapture site.

All brook trout were removed on a daily basis from the fyke nets and anesthetized with MS-222 at a 50 mg/l dosage as recommended by Meister and Ritzi (1958) and Schoettger and Julin (1967) to reduce handling stress. Scale samples were removed from each fish for age determination and backcalculation of growth in 1983-1991. In 1983-1985, brook trout longer than 135 mm were tagged using streamer or disk tags applied posterior to the dorsal fin. Due to a high incidence of infection in these years, strap tags were applied to the adipose fin and the operculum in 1986 and 1987 respectively. Tagged fish recaptured at the site of initial tagging and angler reports during these two years

suggested poor tag retention. In 1988 brook trout were fin clipped with a site specific mark only. In 1989 through 1993 fish greater than 140 mm were tagged using Visible Implant (V.I.) tags manufactured by Northwest Marine Technologies, while fish less than 140 mm were marked with a site specific fin clip only. The V.I. tag is inserted into clear, cartilaginous tissue posterior to the eye. Prior research has shown greater than 90% retention, less than 2% mortality, and no infection on rainbow trout in the laboratory (Stan Moberly, personal communication). After tagging, all fish were released upstream or downstream from the site in their original direction of travel.

The effects of discharge and temperature on brook trout movement at FEX and FCD were evaluated by use of ambient monitoring data collected by Dr. Thomas Burton and staff. Discharge and temperature data at FCU and TM were collected by the fisheries staff from 1984-1991. Discharge was calculated from a calibrated staff gauge at both FCU and TM on a daily basis. Temperature data were collected continuously using a calibrated max-min thermometer at TM and FCU. In addition, Ryan tempmentors and/or thermographs were deployed in 1988 through 1991 at these two sites so that temperature could be monitored on a continuous basis.

Results

A complete and detailed summary of the entire set of data on fish populations and communities in the Ford River is provided in the 1993 Annual Report. We have provided a summary description of these data in the following.

A. Electromagnetic field data

Testing of the ELF Antenna began in 1986 and continued to 1989. Thereafter, the antenna was fully operational. Therefore, we have separated data into three time periods: pre-operational (1983-1985), transitional (1986-1988) and operational (1989-1993). Summaries of the electric field intensities and magnetic flux densities at the control (FCD) and experimental (FEX) sites are provided in Tables VI.1 and VI.2.

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B. Fish community composition

The fish communities at FCD and FEX were similar throughout the study period, with FCD exhibiting a slightly more diverse fauna. A total of 29 species was collected at FCD and 24 were taken at FEX over 11 years of sampling. The median annual species richness at FCD was 17 and at FEX was 15. Species richness was greater at FCD than at FEX in all years except 1987 and 1993. The highest species richness was recorded in 1988 at both sites: 21 at FCD and 18 at FEX.

Neither site showed a distinct response in species richness to the transitional or operational periods (Figure 4.1). At FEX, species richness never dropped below the pre-operational mean, and it reached maxima of 4 and 3 species greater than the pre-operational mean in the transitional and operation periods, respectively. Richness has varied more at FCD during the transitional and operation periods than at FEX, which in part reflects the slightly greater pool of potential species at the FCD site. However, interannual changes have been concordant between the two sites. In particular, richness has declined over the last three years of the study at both sites, but it is not possible to determine whether this decline is ecologically significant or if it is a response to ELF operations. None of the five most common species has disappeared from either site.

Although species richness has not exhibited a consistent trend over the study period, species diversity, as measured by the Shannon-Weaver diversity index has consistently declined at both sites (Figure 4.2). A BACI one-way ANOVA of the log-transformed index data was used to test for differences among periods, and no difference was detected ($F_{2,8}=0.44$, $p>0.05$). However, the direction and degree of interannual change in diversity has been consistent between sites (ANCOVA, $F_{1,18}=0.71$, $p>0.05$), and has been downward since the onset of the transitional period. Furthermore, the decline in diversity from the pre-operational mean was not as great in the transitional period as in the operational period. Because richness has not shown the consistent decline exhibited by the diversity index, most of the change can be attributed to a decline in
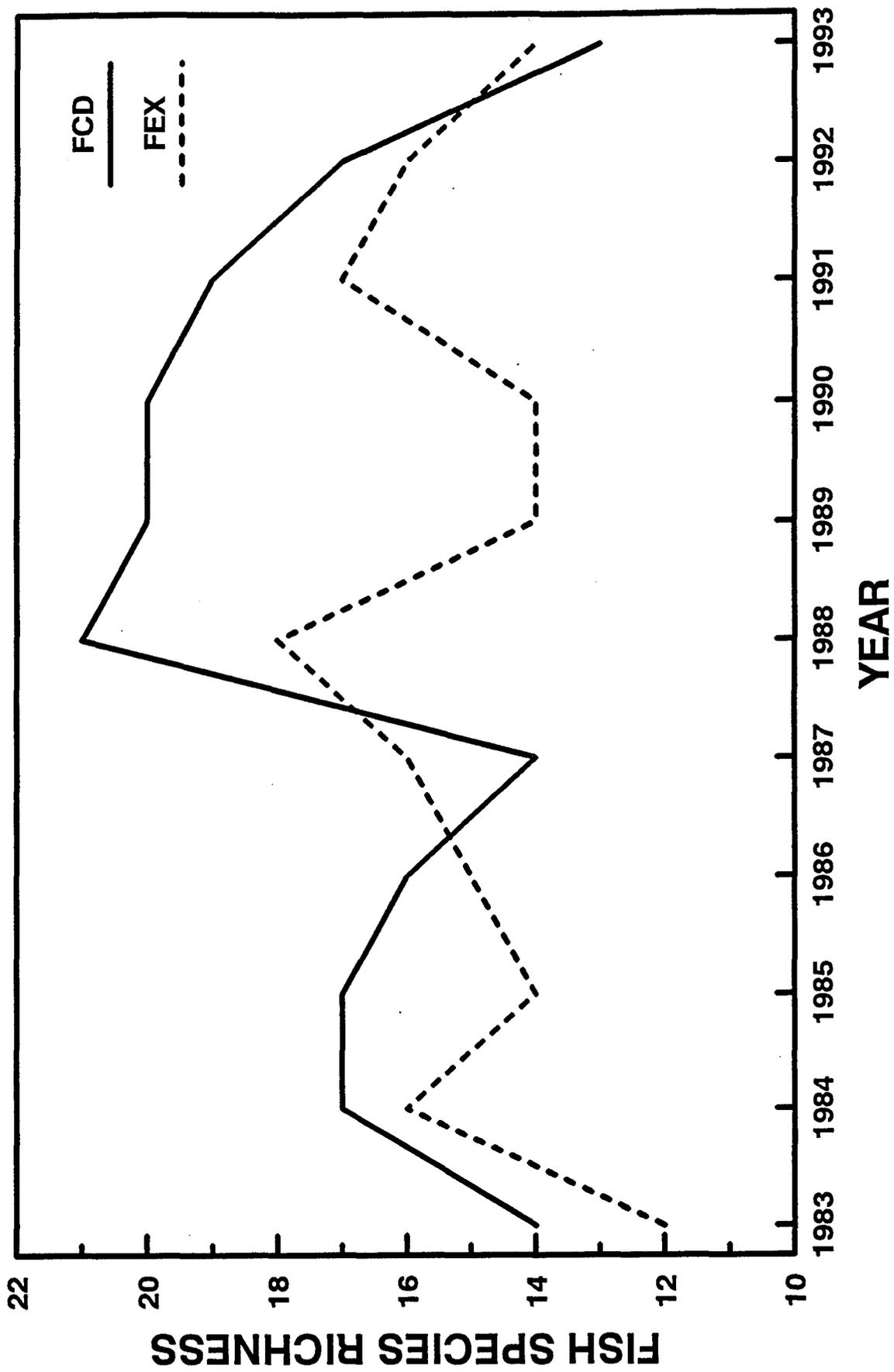
Figure 4.1   Species richness at FCD and FEX, 1983-1993.

126
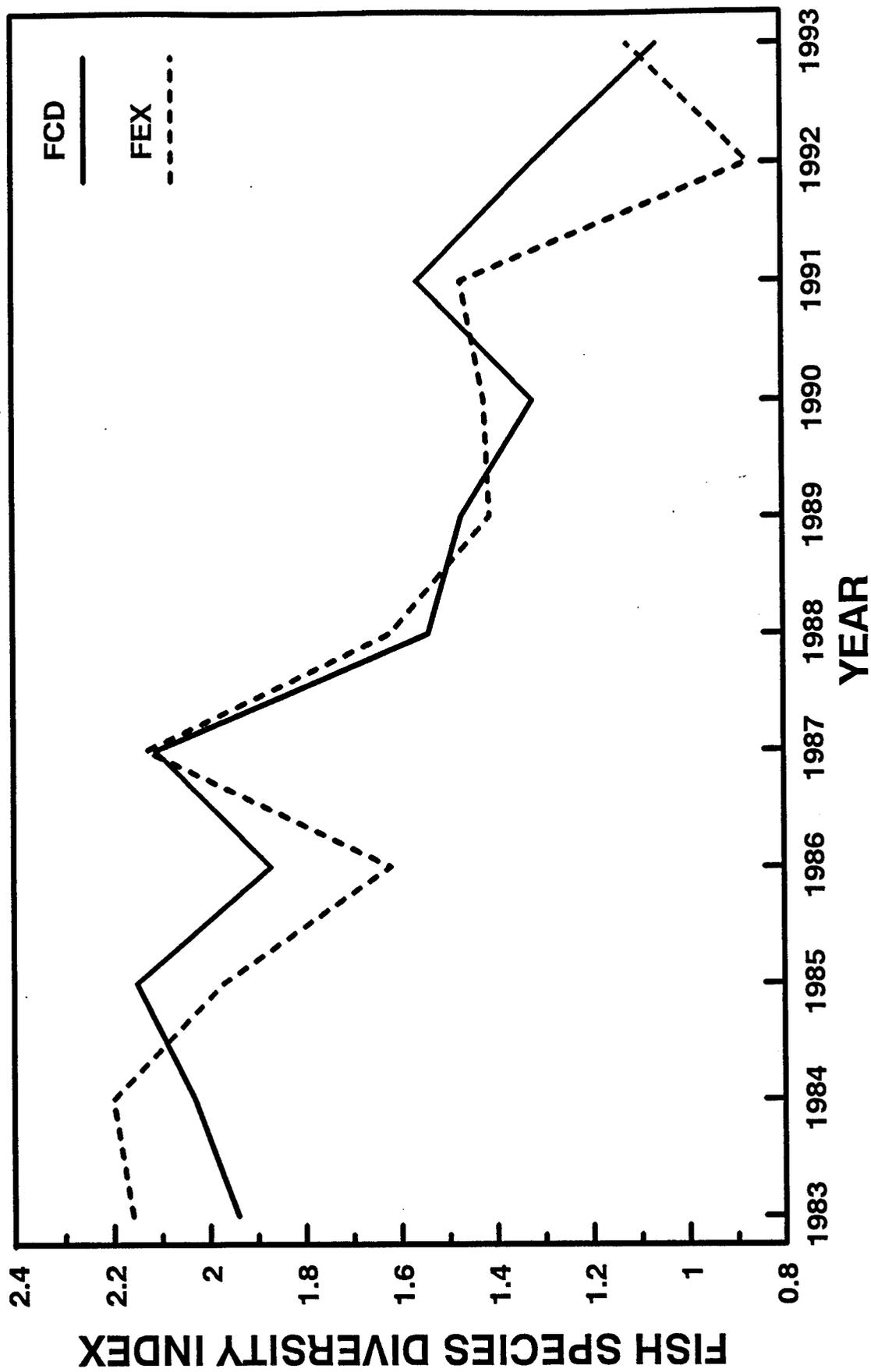
Figure 4.2   Mean daily Shannon-Weaver diversity index values at FCD and FEX,
1983-1993.

species evenness. Both communities have become
progressively more dominated by fewer species.

C. Fish species abundance and biomass

Five fish species have remained the most abundant
species throughout the study period, brook trout, burbot,
creek chub, common shiner and white sucker, although the
ranks of species abundance have changed among these five
species throughout the study period. We present abundance
data here in terms of biomass of fish caught per unit of
sampling effort because the biomass data are less variable
than counts of fish caught, and biomass integrates
information on fish numbers with fish size. Total fish
biomass at FCD has been lower than the pre-operational mean
during the transitional period and the operational period,
except for one year in the operational period, 1993 (Figure
4.3). At FEX, total fish biomass has been lower than the
pre-operational mean in two of the three transitional years
and in all of the operational years. The only year in which
the total biomass was greater than the pre-operational mean
was in 1987. However, even though the total biomass has
been lower than the pre-operational mean for most of the
transitional and operational periods, it is difficult to
attribute this decrease to an ELF effect, because the
decrease began before ELF operations began (1985). At both
sites, the deviation of total biomass from the pre-
operational mean has decreased somewhat through the
operational period. Because the responses at the control
and treatment sites have been similar during the
transitional and operational periods, the biomass data show
no indication of an ELF effect.

BACI one-way ANOVA were used to test for ELF effects on
biomass (weighted by sampling effort) for each of the five
most common species (Table 4.1). The same analyses were
reported in Table 7.7 of the 1993 Annual Report, however
that analysis did not include data from 1993 due to
malfunction of the weighing scales. We have measured and
corrected the bias in the 1993 biomass data and provide the
updated BACI ANOVA results here. None of the five common
species showed a significant effect of period on biomass.
Without the 1993 data, only burbot showed a significant

128
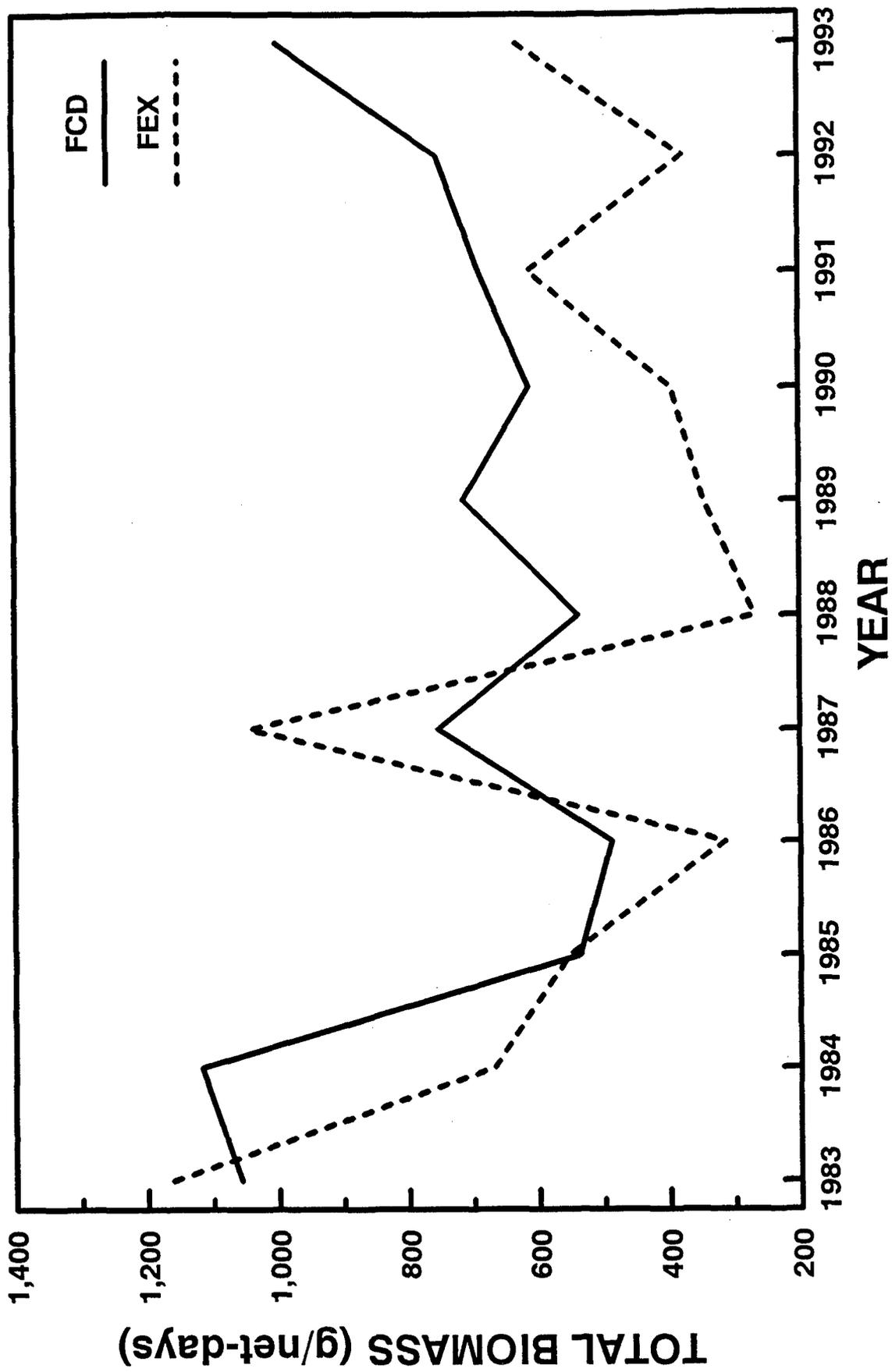
Figure 4.3   Total biomass (g/net-days) at FCD and FEX, 1983-1993.

Table 4.1 BACI one-way ANOVA on log-transformed
biomass data corrected for net-days at
FCD and FEX, 1983-1993.

Species	$F_{2,8}$	P
brook trout	0.00	1.00
white sucker	0.48	0.63
burbot	1.54	0.27
creek chub	3.24	0.09
common shiner	0.92	0.44

None significant

period effect ($F_{2,7}$ = 4.62, p = 0.053), however the extra year of data eliminated this effect in the updated analysis (Table 4.1). The descriptions that follow refer to apparent trends in the data that were not detectable with the statistical tests used.

Two species, brook trout and white sucker have exhibited a greater degree of variation in biomass over the study period than the other three species, and these two species also accounted for over 50% of the fish biomass in each year (Figure 4.4). Brook trout biomass has been lower at both sites during the transitional and operational periods than in the pre-operational period. The interannual changes in brook trout biomass have been concordant between sites.

White sucker biomass has shown a gradual increase since the end of the pre-operational period at both sites. The greatest degree of variation occurred during the pre-operational period, so it is difficult to interpret the significance of the change that has occurred during the transitional and operational periods.

Although the degree of interannual variation in mean biomass has been lower for burbot, creek chubs and common shiners, each species has exhibited slightly different trends during the transitional and operational periods. Burbot biomass has been below the pre-operational mean at both sites for all but one of the years in the transitional period and for all years in the operational period.

At both sites, creek chub biomass was slightly below the pre-operational mean during the transitional period and for all but the final year in the operational period. Common shiner biomass was slightly below the pre-operational mean during the transitional period at both sites, and was equal to or slightly greater than the pre-operational mean during the operational period at both sites.

D. Fish population size structure

For the five most common fish species, the size structure of the populations has not shown a consistent
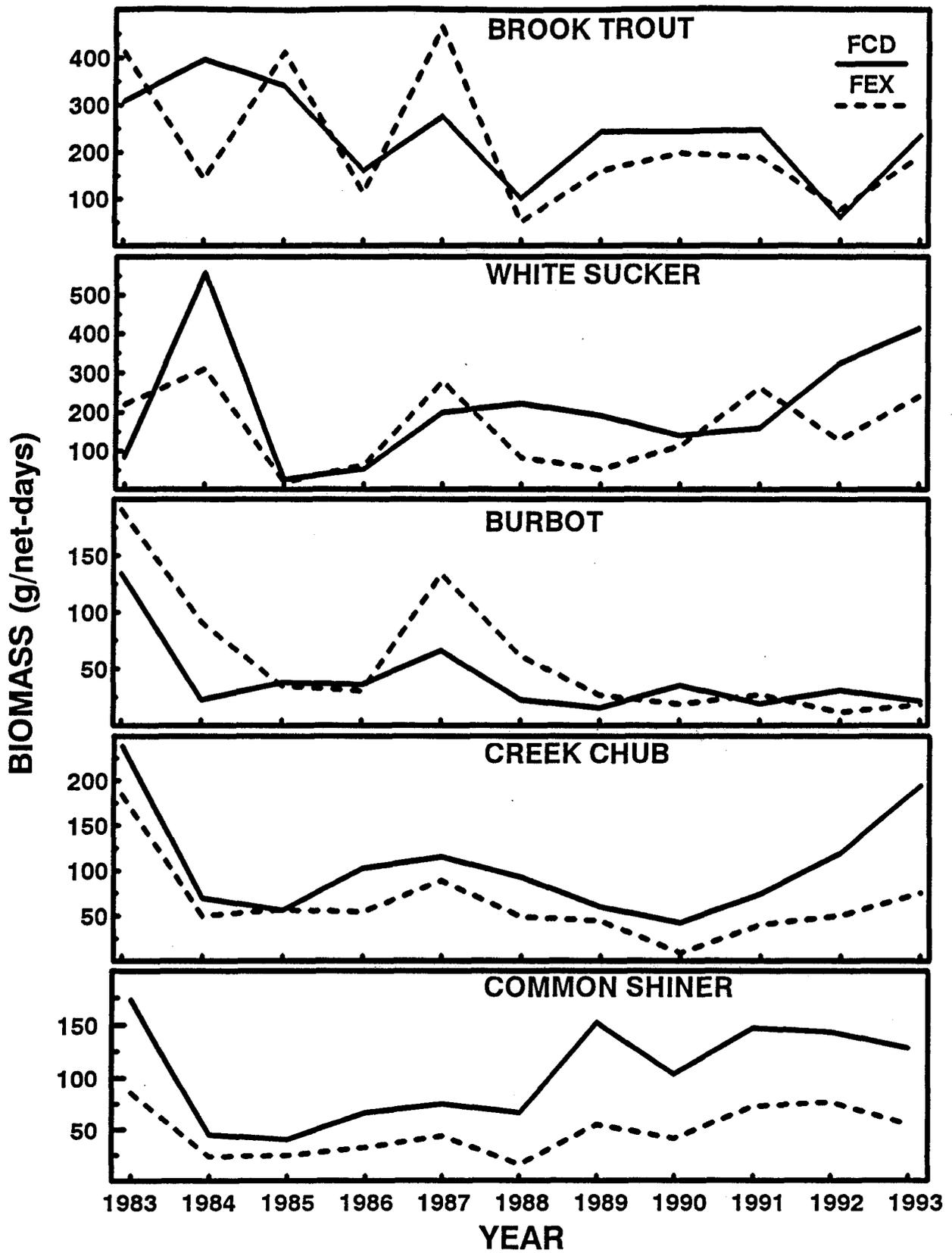
Figure 4.4   Biomass (g/net-days) for five common
            species at FCD and FEX, 1983-1993.

response among species to the effect of ELF operation either by period (pre-operational, transitional, operational) or by site (FCD, FEX). Size structure is summarized here by the mean total length of all fish captured for each species. A more detailed analysis of brook trout data follows this summary. For brook trout, the mean total length was lower than the pre-operational mean during the transitional period at FEX, but not at FCD (Figure 4.5). During the operational period, mean total length was greater than the pre-operational mean for three of five years at FCD and two of five years at FEX. These changes in mean total length represent changes in recruitment as much as changes in fish growth and survival. For example, the decrease in mean total length at FEX from 1989 to 1993 reflects an increase in the number of fish in the 100 - 200 mm size range, rather than a decrease in the number of fish greater than 200 mm total length. It appears that recruitment of small fish into the population has increased over the last five years of the study, and this accounts for the change in mean length for the population.

Mean length of white suckers deviated more from the pre-operational mean during the pre-operational period than in either of the succeeding periods (Figure 4.5). At both sites, mean length was below the pre-operational mean during the transitional period. In the operational period, mean length remained close to the pre-operational mean except in the final study year, 1993. Mean length was greater than the pre-operational mean in 1993 at both sites.

Burbot mean lengths have increased throughout the study period at both sites (Figure 4.5). Mean length of burbot has dropped below the pre-operational mean only once at FCD, in 1986, and twice at FEX, in 1986 and 1990. In the other years of operation, mean length was greater than the pre-operational mean at both sites. Creek chub mean length has remained above the pre-operational mean for all of the transitional years and for all but one of the operational years at FEX (Figure 4.5). In contrast, mean length of creek chubs has been below the pre-operational mean in all transitional years and all but one of the operational years (1993). Common shiners were larger in the transitional and operational periods than in the pre-operational period at

133
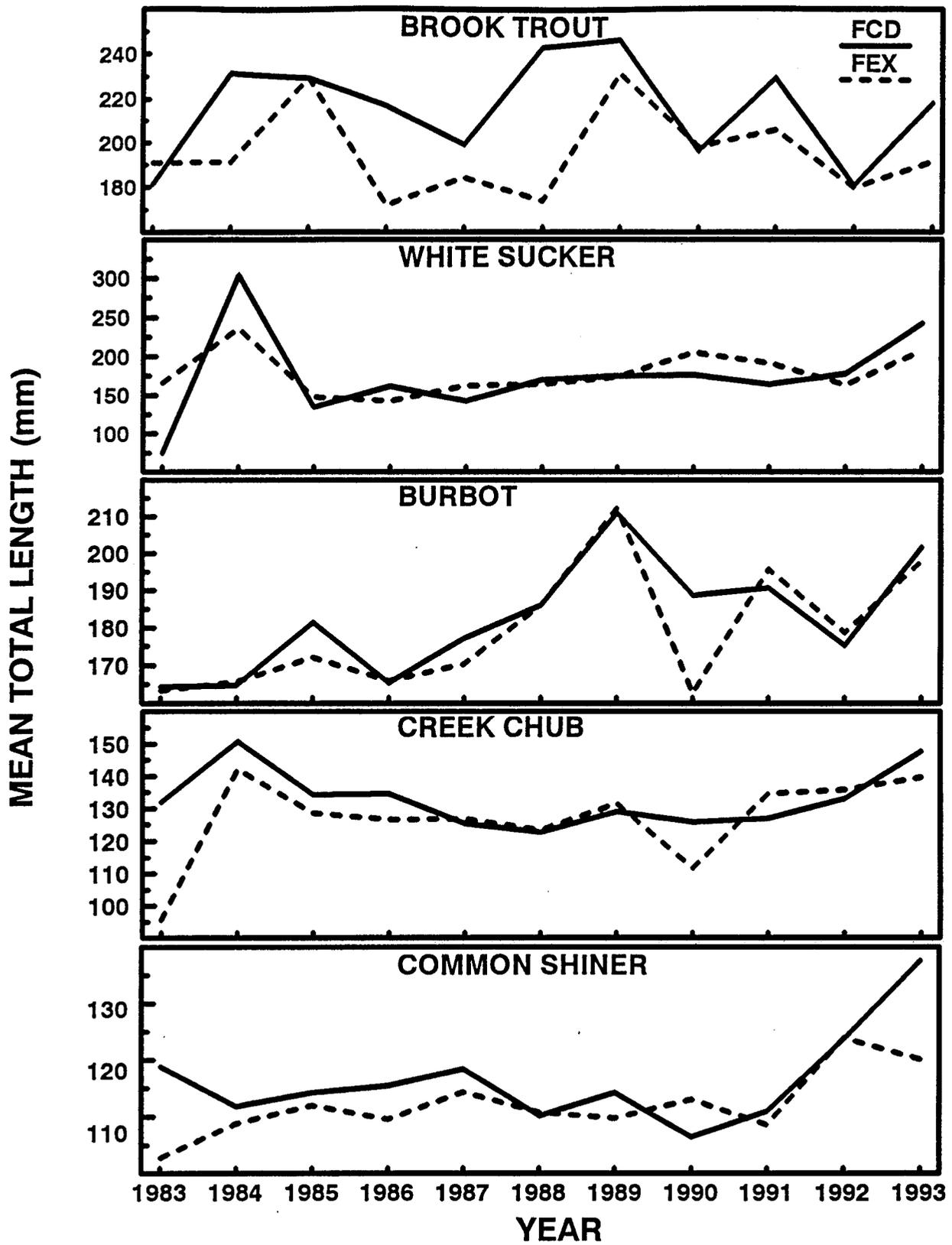
Figure 4.5   Mean total length (mm) for five common
             species at FCD and FEX, 1983-1993.

FEX (Figure 4.5). At FCD, mean length of common shiners was greater than the pre-operational mean in two of three transitional years, but only in two of the five operational years.

E. Brook trout age and growth

Age and growth analysis of Ford River brook trout has the potential to be a sensitive indicator of ELF effects. A large background of data is available on this species, and the individual fish can be aged accurately by use of scale growth characteristics. The growth analysis was performed using length frequency distributions and total length versus total scale radius regression equations developed for brook trout captured at FCD and FEX from 1983 through 1991.

Covariance analysis was used to test for differences in the slopes of regression lines between FCD and FEX in each year. Significant differences ($p < 0.05$) were found in 1984, 1986, and 1988, but the differences were not consistent among years. In 1984 and 1988, older trout were longer at FEX relative to FCD and younger trout were shorter at FEX compared to FCD. Differences between the sites were reversed in 1986. In general, patterns of brook trout growth were similar at FCD and FEX throughout the course of the study.

Covariance analysis also detected overall differences in the slopes of the regression lines among pre-operational, transitional, and fully-operational periods ($F_{5,765} = 2.35$, $p < 0.05$). A Tukey-Kramer Multiple Comparison Test (Miller 1986) identified a similar pattern for FCD and FEX (alpha=0.05): the slopes of the regression lines at each site were similar between the pre-operational and transitional periods. However, the operational period differed significantly from the first two periods. In fact, the regression lines at both sites for the operational period described increased growth at age and was the opposite of what would be expected if the antenna were having a negative effect on fish growth; decreased growth was expected if the ELF system inhibited fish from reaching cold water refuges.

135

The similar, positive responses observed at both sites suggested that a factor other than the ELF antenna was responsible for the observed changes in brook trout growth. Treml (1992) concluded that the age and size structure of Ford River brook trout was related to late spring and early summer water temperature patterns. Years with cool water temperatures were correlated with proportional increases in the abundance, total length at age, and relative growth rates of age 2 and older trout. Brook trout in the Ford River displayed relatively fast growth when compared to trout populations in Carlander (1969) despite water temperature conditions that were far from optimal (Treml 1992).

F. Fish population condition

Relative weight is an index of fish health that compares the weight of an individual fish to that expected for a fish of that length from standards based on many populations of the same species. No weight standard is available for burbot, so we present relative weight data on the other four common species, brook trout, white sucker, creek chub and common shiner.

For two of the four species, brook trout and common shiner, relative weight showed no consistent difference between FCD and FEX sites (Figure 4.6). In addition, we used analysis of covariance to test for differences in the slope of the total length - weight regression for brook trout, and found no significant effect of site on the regression slopes for all years except 1985, which was a pre-operational year (ANCOVA, p>0.05). For white suckers and creek chubs, the deviation of relative weight from the pre-operational mean at FEX was offset from FCD for most years in the transitional and operational periods (Figure 4.6). However, the only occasion when the deviations were substantially different between sites was for creek chubs in 1991, when the FEX mean was 16.5% greater than the pre-operational mean and the FCD mean was only 1.6 % greater. Subsequent to 1991, the difference in deviations from the pre-operational means were reduced for creek chubs at FEX and FCD.

136
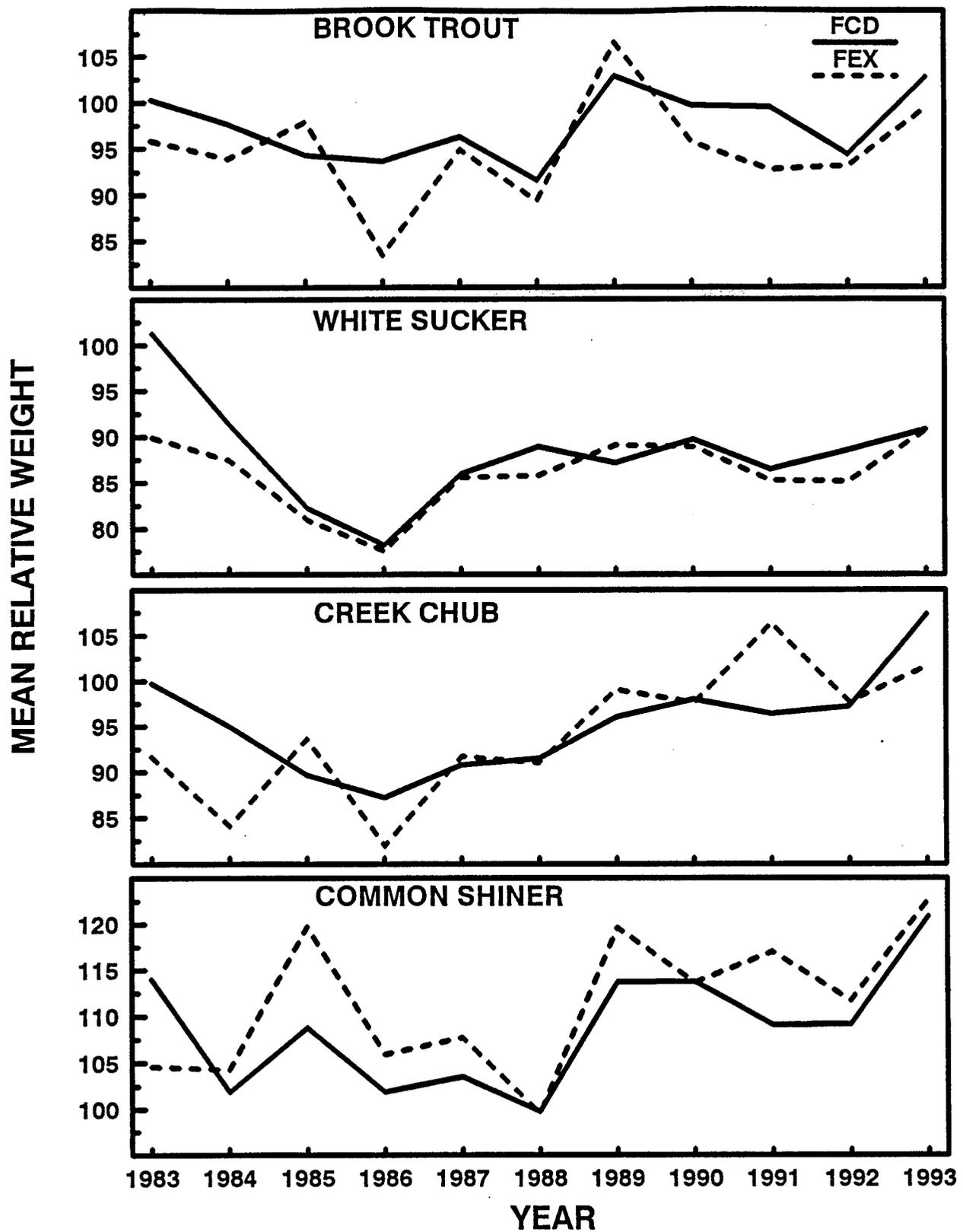
Figure 4.6   Mean relative weight (Wr) for four common
             species at FCD and FEX, 1983-1993.

For all four species, two subtle patterns are evident in the relative weight record over the sampling period (Figure 4.6). During the transitional period, the relative weight of all four species was below the pre-operational mean. White suckers showed the greatest degree of deviation from the pre-operational mean, -11.2 for both sites combined. The second pattern also holds across the four species: relative weight has increased during the operational period, such that all four species had relative weights greater than the pre-operational mean by the final year of the study (1993). White suckers showed the least degree of improvement in condition and the two minnow species showed the greatest degree of improvement. Because the period effect is mixed, i.e., reduced condition during transitional period and improved condition during the operational period, it is difficult to attribute either change to the operation of the ELF antenna. We used covariance analysis to test for differences in the regressions between total length and weight for brook trout, and found no significant effect of period (pre-operational, transitional, operational) on the regression slopes ($F_{5,1784}=1.66$, $p>0.05$).

G. Fish movement

We evaluated fish movement past the ELF electromagnetic field by comparison of recapture records of marked fish. Distinctive marks placed on fish at each site and release of fish in the direction of movement at the time of capture allowed us to count the number of fish movements that involved passage through the ELF field (e.g., from FCD to Two-Mile Creek or from FEX to FEN) and the number of fish movements that were not through the ELF field (e.g., from FEX to FCD or from FEX to Two-Mile Creek). If the ELF field inhibited the movement behavior of fish, the number of fish movements through the ELF field should be less than the number of fish movements that did not pass through the ELF field.

No marked fish were recaptured in the first year of the study, perhaps a result of the low sampling effort in that year (20 net-days per site, >40 net-days in subsequent years). In 1984, only brook trout recaptures were recorded.

In contrast to the expected effect of ELF on fish movement, we found that more fish movements of all species involved passage through the ELF field than movements that did not pass through the field (Table 4.2). The number of fish recaptures was substantially greater in the last three years of the study than in the previous years, and in all three years, the number of movements through the field was two to three times greater than the number of movements that did not pass through the field. This pattern held for brook trout movements as well as the total of all other species (Table 4.2). During the transitional period and the first two years of the operational period, the trend was reversed, in that more movements did not involve crossing the ELF field than the number of movements that did cross it. Part of this change is likely to be a result of improved capture efficiency at the FEX site in the last three years of the study. Even more important was the addition of the FEN sampling site, which was a short distance upstream of FEX (400 m), and on the opposite side (upstream) of the ELF field from FEX. This site was added in 1990, and was relatively inefficient in its first year of operation.

The greater distance between FCD and FEX compared to FEX and FEN added some bias to the comparison of the number of movements that involved crossing the ELF field to the number of movements that did not involve crossing the ELF field. However, the large number of observations of movements through the ELF field by brook trout and other species during the operational period discounts the hypothesis that fish movement is prevented by the operation of the ELF antenna.

Other factors seem to have more influence on the movement behavior of fish in the Ford River. Temperature and discharge appeared to interact in their influence on fish movement. Brook trout in particular seemed sensitive to temperature, and exhibited a greater frequency of movement between sites when temperature exceeded 16 C. All species showed a greater tendency to move between sites when discharge was high (greater than 1.5 m^3/sec).

139

Table 4.2 Number of observations of fish movement between sampling sites, divided into movements that involved crossing the antenna path across the Ford River and movements that did not involve crossing the antenna, 1983 - 1993.

YEAR	ALL SPECIES:		BROOK TROUT:		NON-TROUT SPECIES:	
	MOVES CROSSING	MOVES NOT CROSSING	MOVES CROSSING	MOVES NOT CROSSING	MOVES CROSSING	MOVES NOT CROSSING
1983	0	0	0	0	0	0
1984	50	7	50	7	0	0
1985	31	21	19	4	12	17
1986	6	15	1	1	5	14
1987	6	40	4	1	2	39
1988	3	23	1	5	2	18
1989	22	90	6	7	16	83
1990	3	38	3	26	0	12
1991	133	57	69	19	64	38
1992	129	35	12	8	117	27
1993	144	21	20	2	124	19
TOTALS:	527	347	185	80	342	267

Discussion

Of the many fish population and community parameters that were measured and tested in this study, only two showed statistically detectable results that may be attributable to ELF operations. In both cases, the differences occurred over time, and were consistent between sites. The Shannon-Weaver diversity index consistently declined over the period of the study. Although this difference was not detectable by the BACI one-way ANOVA, it did appear in a significant negative regression between Shannon-Weaver diversity and year at both sites. This occurred in spite of no trend in species richness, which suggests that evenness of fish distribution among species declined over the study period. In other words, fewer fish species accounted for more of the fish captured in late years than in early years of the study. This may have resulted from ELF effects, such as a differential change in mortality among species, or may have been caused by other factors, such as changes in stream discharge, stream temperature or other environmental variables. It is not possible to attribute this change to ELF effects alone.

The second data set that showed a significant period effect was the growth data for brook trout. In particular, the scale radius - total length regression, a measure of fish growth that integrates growth for the entire fish's life previous to capture, had a significantly greater slope in the operational period than in the pre-operational and transitional periods at both sites. This indicates that brook trout growth was better during the operational period than in the previous two periods, an effect that is contrary to the expected effect of ELF on fish growth. In particular, brook trout demonstrated a tendency to move upstream into Two-Mile Creek and other cooler habitats during summer when temperature at FCD and FEX exceeded 16 C. If ELF interfered with this movement, e.g. by preventing fish from moving past the ELF corridor, we would expect brook trout to have been stressed in the warmer Ford River temperatures and to exhibit reduced growth.

The fact that growth was better during the operational period suggests either that ELF has some less obvious direct

or indirect impact on fish growth or some other factor accounts for the improved growth, such as increased macroinvertebrate production resulting from the increased periphyton productivity, different temperature and flow conditions or changes in trout stocking practices or trout harvest practices that may have yielded improved growth of brook trout.

Several factors impair the effectiveness of this study design to distinguish between potential ELF effects and other factors on changes in fish population and community variables. One factor is the effectiveness of the control site as a true control. The degree of fish movement between FCD and upstream sampling sites suggested that the fish community of the Ford River was poorly segmented among reaches. In particular, it suggests that it is invalid to assume that ELF operations do not affect the assemblage at FCD. Furthermore, the degree of movement between sites suggests that individual fish captured at FEX may have been exposed to the ELF electromagnetic field for a period as brief as one day or as long as the fish's entire life. In effect, this makes the FCD location a site with a lower impact of ELF operations than the FEX site at best.

A second factor that influences the sensitivity of this study design is the background of temporal variation in the physical stream habitat and the influence of this variation on the fish populations in the Ford River. In particular, interannual differences in stream discharge and in stream temperature have a significant influence on fish populations in a variety of stream types (Hynes, 1970). The Ford River varies considerably among years not only in the daily mean discharge for the year and the daily mean temperature, but also in the temporal pattern of change in discharge and temperature. Because the two sites are on the same river and experience the same interannual variation in discharge and temperature, our design should help to control for this source of variation, but only if the control site is a true control site. However, because we cannot discount the possibility that our control site was influenced by ELF operations, we cannot distinguish between the effects of ELF operations and other environmental changes on fish population and community parameters. The fact that we only

142

have one replicate per year for each population and community parameter at each site further compounds our inability to determine if ELF operations affected the fish community of the Ford River because we cannot measure the degree of measurement error in these variables.

Given these constraints, if ELF operation has an effect on any of the parameters that we have measured, our study design is only likely to detect the effect if it is extreme. A subtle change in growth rates (e.g., on the order of several mm/year) or in species richness or species diversity (e.g. on the order of < 10%/year) is beyond the range of detection with this design. However, differences between periods that are entirely consistent within periods or differences between sites that are entirely consistent between periods may be attributable to ELF effects.

One of the most direct means of determining whether fish were able to detect the electromagnetic field generated by the ELF antenna and whether their behavior was affected by this was the evaluation of fish movement through the ELF field. From the first year of the study, it was apparent that fish of several species moved over distances at least as great as the distances between the sampling sites on the Ford River. Whether these movements represented regular migrations, e.g. to specific spawning areas or rearing areas in the watershed, or less regular forays in search of prey or temperature refugia, the movements appeared to be an important component of the ecology of most fish species in the Ford River. Indeed, the sampling method depended on some degree of movement of fish species, and the less mobile species, e.g. blacknose dace or mottled sculpins were probably under-represented in our samples. Interpretation of the fish movement data summarized in Table 4.2 was complicated by the different distances between sampling locations upstream and downstream of the antenna. However, perhaps the most significant result from this analysis was that fish of several fish species moved past the antenna corridor throughout the study period. Again, an extreme effect of ELF on fish behavior would have been a complete inhibition of movement through the ELF electromagnetic field. The failure to find a result this extreme and the limitations on the study design make it difficult to

conclusively state that ELF had no effect on fish movement behavior, but these factors also suggest that if the ELF operation did influence fish behavior, it was a subtle effect.

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