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ELF Communications System Ecological Monitoring Program: Slime Mold Studies - Final Report

Eugene Goodman
Ben Greenebaum

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We have previously shown that continuous exposure of the slime mold Physarum polycephalum to extremely low frequency (ELF) electromagnetic fields (EMF) simulating those generated by the Navy's ELF communication system (then Project Sanguine) could depress the rate of respiration, and lengthen the mitotic cell cycle. (Goodman et al. 1976, 79, Greenebaum et al. 1982). In a series of experiments beginning in 1981 and ending in 1987, we sought to determine whether exposing Physarum to the field environment around the Wisconsin Transmitting Facility (WTF) could induce an altered physiological state. A laboratory component was also included to help verify methodology and to supplement studies performed at the WTF.

Initially, the experimental effort was directed to devising methods to maintain axenic Physarum cultures under ambient environmental conditions. This involved using growth chambers

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that admit the electric field or current density from the surrounding environment; the cultures were returned to the laboratory for analysis. The successful methods placed the organism on an agar bed inside double containment and introduced the samples to be assayed into shaken liquid culture medium upon arrival at the laboratory. Both WTF-generated electromagnetic fields and background strengths were measured with the help of IITRI at study locations near the antenna, at the west ground of the WTF antenna, as well as at control sites. Suitable locations that met the project's electromagnetic environmental criteria (see Appendix I) were selected. During the 1981-1984 period, the WTF was intermittently energized.

Sufficient data were collected and analyzed on both laboratory and WTF-field cultures during the 1985, 1986, and 1987 growing seasons. The end points measured were the respiration rate and ATP levels. Tests of additional variables such as replications of assay measurements and the time interval between the end of exposure and the time of assay were also made and used to reduce the variability of the final data sets. A univariate ANOVA procedure was employed to suit the nature of the data.

The data show that neither EMF exposure conditions at the antenna or the ground sites induced any consistent, statistically significant differences in either Physarum's respiration rate or ATP levels. In general, data obtained from the laboratory and the WTF are in agreement. In contrast, previous laboratory experiments using submerged shake cultures and field intensities 5 to 10 times stronger than those encountered at the WTF, showed significant decreases in both parameters. These results are taken as indicating that the laboratory component is a reasonable predictor of potential field effects.

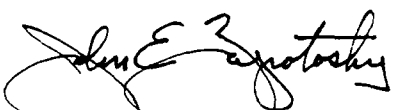
FOREWORD

This study of slime mold physiology constitutes one of several projects in the ELF Ecological Monitoring Program. The purpose of the program is to examine for possible electromagnetic effects to resident biota from the operation of the U.S. Navy's Extremely Low Frequency (ELF) Communications System. IIT Research Institute (IITRI), a not-for-profit organization, has been contracted by the Space and Naval Warfare Systems Command (SPAWAR) to provide engineering support and to manage the program. Mold physiological studies were conducted under subcontract arrangements between IITRI and the University of Wisconsin-Parkside (UWP).

These studies by the UWP were selected for funding by IITRI through a peer-reviewed, competitive bidding process in 1982. Physiological data were collected over the period 1982-1987. During 1988 and 1989, the researchers completed their analytical efforts and prepared this final report.

This report documents the results and conclusions of the UWP researchers based on data collected over the term of the study. A draft manuscript of the text was reviewed by several peers with research experience in mold physiology, statistics, and electromagnetics. The authors considered and addressed peer critiques prior to providing this report to IITRI for publication and the report presented here is without further change or editing by IITRI or SPAWAR.

Respectfully submitted,
IIT RESEARCH INSTITUTE



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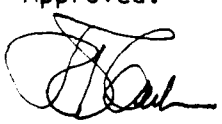

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FINAL REPORT

Eugene M. Goodman

Biomedical Research Institute
University of Wisconsin-Parkside
Kenosha, Wisconsin 53141

Subcontract #E6549-84-C009

"ELF Communications Systems Ecological Monitoring Program"

The Effects of Exposing the Slime Mold *Physarum polycephalum* to Electromagnetic Fields

August 1988

Eugene M. Goodman
Principal Investigator

Ben Greenebaum
Co-Investigator

Gary Goetz
Assistant Chancellor,
Administrative Affairs

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GLOSSARY-ACRONYMS

Respiration:	The utilization of oxygen by cells to obtain energy.
QO_2	The rate of oxygen utilization: ul of oxygen consumed/minute/mg protein.
Antenna ground:	A conducting connection between the transmitting antenna and the earth.
Axenic culture:	Growth of a single organism (slime mold) in the absence of contaminating organisms such as bacteria, fungi, etc.
Macroplasmodium:	A multinucleated (>10^7 nuclei) mass of protoplasm visible to the eye; the entire structure is delimited by a plasma membrane. In the laboratory it is usually maintained on a solid substrate such as agar or filter paper.
Microplasmodium	A small multinucleated (~10^4 nuclei) mass of protoplasm delimited by a plasma membrane. In the laboratory it is maintained in submerged liquid shake culture
Cell cycle:	The number of hours between successive divisions of the nucleus.
WTF:	Wisconsin Transmitting Facility.
EMF:	Electromagnetic Fields.
ELF:	Extremely Low Frequency
ATP:	Adenosine triphosphate; used for energy in the cell; its synthesis generally requires O_2
ANOVA	Analysis of Variance
UWP	University of Wisconsin-Parkside
M_I	The first nuclear division after a microplasmodia have been placed on filter paper and nutrient media added the cells are now referred to as macroplasmodia
M_{II}	The second nuclear division after microplasmodia have been placed on filter paper and nutrient media added
E	Electric field component of an electromagnetic field
H or B Field	The magnetic field component of an electromagnetic field

J Field	Current Density
A Site	Antenna Site at WTF
G Site	Ground Site at WTF
C Site	Control Site at WTF
Heterkaryons	fusing microplasmodia from control Physarum and EMF-exposed microplasmodia

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ABSTRACT

We have previously shown that continuous laboratory exposure of the slime mold Physarum polycephalum to extremely low frequency (ELF) electromagnetic fields (EMF) simulating those generated by the Navy's ELF communication system (then Project Sanguine) could depress the rate of respiration, and lengthen the mitotic cell cycle (Goodman et al.1976, 79, Greenebaum et al.1982). In a series of experiments beginning in 1981 and ending in 1987, we sought to determine whether exposing Physarum to the field environment around the Wisconsin Transmitting Facility (WTF) could induce an altered physiological state. A laboratory component was also included to help verify methodology and to supplement studies performed at the WTF.

Initially, the experimental effort was directed to devising methods to maintain axenic Physarum cultures under ambient environmental conditions. This involved using growth chambers that admit the electric field or current density from the surrounding environment; the cultures were returned to the laboratory for analysis. The successful methods placed the organism on an agar bed inside double containment and introduced the samples to be assayed into shaken liquid culture medium upon arrival at the laboratory. Both WTF-generated electromagnetic fields and background strengths were measured with the help of IITRI at study locations near the antenna, at the west ground of the WTF antenna, as well as at control sites. Suitable locations that met the project's electromagnetic environmental criteria (see Appendix I) were selected. During the 1981-1984 period, the WTF was intermittently energized.

Sufficient data were collected and analyzed on both laboratory and WTF-field cultures during the 1985, 1986, and 1987 growing seasons. The end points measured were the respiration rate and ATP levels. Tests of additional variables such as replications of assay measurements and the time interval between the end of exposure and the time of assay were also made and used to reduce the variability of the final data sets. A univariate ANOVA procedure was employed to suit the nature of the data.

The data show that neither the EMF exposure conditions at the antenna or the ground sites induced any consistent, statistically significant differences in either Physarum's respiration rate or ATP levels. In general, data obtained from the laboratory and the WTF are in agreement. In contrast, previous laboratory experiments using submerged shake cultures and field intensities 5 to 10 times stronger than those encountered at the WTF, showed significant decreases in both parameters. These results are taken as indicating that the laboratory component is a reasonable predictor of potential field effects.

I. INTRODUCTION

A. BACKGROUND: In 1972 a laboratory based research program was initiated to investigate the potential bio-effect(s) of weak electromagnetic fields (EMF's) that might be associated with the Navy communication system, then Project Sanguine. The system as originally conceived would emit modulated, ELF EMF's centered at 45 and 75 Hz. In the original design, the field strengths in the ground near the antenna were expected to be a maximum of 0.07 V/m and 20 uT (0.2G). Our initial laboratory experiments (1972-1978) were designed to examine potential weak-field effects at 45-75 Hz using both sinusoidal and modulated frequencies. Field intensities ranged from 0.5 to 1.0 V/m and 0.03mT to 0.2mT. Most of the experiments were performed at intensities ten times stronger (0.7 V/m, 0.2mT) than the proposed maximum fields generated by the antenna.

The slime mold Physarum polycephalum was selected as the experimental organism in part because its natural habitat is the forest floor where it functions in recycling organic material. In the initial laboratory experiments, both the temperature and electromagnetic field exposures were held constant. Microplasmodia exposed to continuous EMF's for extended periods of time exhibited a lengthened mitotic cycle and decreased respiration rate. These effects are briefly described below and have been recently summarized in a review (Goodman et al.;1988).

1. Mitosis: Cultures of Physarum maintained in suspension exist as small multinucleated, syncytial masses of protoplasm called microplasmodia. When microplasmodia in the log phase of growth are placed on filter paper, they coalesce forming a large multinucleate single cell called a macroplasmodium. Following coalescence (45 to 60 minutes), growth medium is added and a relatively synchronous mitosis (M_I,mitotic index of 85-90%) is observed within 5 - 6 hours (Rusch, 1970). A second mitosis (M_{II}) occurs approximately 8 hours later and is virtually synchronous (mitotic index >95%).

Four suspension cultures were maintained in a control incubator (no EMF exposure) and 4 cultures were kept in an EMF-exposure incubator. For a given mitosis experiment, cultures from each environment were randomly selected for use as starting inocula. The experimental endpoint in the latter experiments was the number of hours required for macroplasmodia (10 EMF-exposed and 10 non-exposed controls) to reach metaphase of the second mitosis (M_{II}).

Microplasmodia continuously exposed to 45-75 Hz, 0.035 - 0.7V/m and 0.04 - 0.2mT showed a significant Lengthening of their mitotic cell cycle that ranged from 8-15% (Goodman et al.; 1976, 1979,1988). Virtually all of our EMF experiments were continued for 180 days or more. The paired-t test and the Wilcoxin signed-rank tests were used to assess the significance of a given exposure regimen. If EMF exposure produced a "significant effect" (after about 180 days), a new set of cultures (4) was introduced into the EMF environment and the experiment was repeated. A conclusion that an EMF effect had occurred was only accepted if a significant difference could be reproduced with the new set of cultures. Since the exact point that an EMF-effect was induced could not be precisely determined, we retrospectively used the arbitrary criterion that the onset occurred about the time that significant

differences in the mitotic cycle ($p < .05$) were obtained for three consecutive experiments.

In a corollary experiment, heterokaryons were formed by mixing different proportions of EMF-exposed (75 Hz, 0.7V/m, 0.2mT) and non-exposed control microplasmodia at the time stationary cultures were formed. The data show that the ensuing mitotic delay was a function of the amount of EMF-exposed microplasmodia in the heterokaryon-mix (Goodman et al.;1988). A lengthening of the mitotic cycle was also observed when either electric fields (75 Hz, 0.7 V/m) or magnetic fields (75 Hz. 0.2mT) were individually applied (Goodman et al.;1979).

In one experiment, continuous exposure to 75 Hz, 0.2 mT, 0.7 V/m for 5 years neither increased nor decreased the magnitude of the delay once it had been induced. However, if the altered microplasmodia were removed from their EMF environment and returned to a control environment, the lengthened mitotic cycle slowly (3-4 weeks) returned to control levels. These data are interpreted as showing that EMF's have not induced a permanent alteration in the cell and that the field-induced perturbation is reversible (Goodman et al.; 1988).

2. Respiration: In many experiments, the same EMF-exposed microplasmodia used in the mitosis experiments were used to examine the cell's respiration rate or QO_2 (ul O_2 consumed/min/mg protein). The combined Electric and Magnetic fields (E + B) (75Hz, 0.7V/m, 0.2mT) significantly decreased respiration about 16% whereas the E-only (75Hz, 0.7V/m), or B-only (75Hz, 0.2mT) fields depressed the QO_2's 9 % and 8 % respectively. In agreement with the mitosis data, a prolonged exposure period (~ 180 days) was required to significantly depress the cell's QO_2. In the respiration experiments, statistical significance was established using the paired-t and Wilcoxin signed ranks tests.

3. ATP: The effects of combined E + B fields (60 Hz, 0.2mT, 1.0 V/m) and individual E (60 Hz, 2.0V/m), and B (60 Hz, 0.2 mT) fields on ATP levels in Physarum have also been examined (Marron et al.; 1986). In all experiments a statistically significant decrease in the cell's ATP level (8-11%) was observed.

II. CURRENT PROGRAM

The hypothesis tested in this program was that exposure to weak electromagnetic field generated by the WTF antenna would both induce observable physiological alterations in Physarum. In the research program, Physarum was directly exposed to EMF's generated by the WTF antenna a laboratory simulation approximating the EMF's at the WTF ground site.

III. PROCEDURES

A) WTF FIELD STUDIES:

In these studies specially constructed chambers were employed to maintain the Physarum in an axenic state while simultaneously exposing them to the ambient environment of the WTF. This in-situ procedure allows close

monitoring of the mold's physiological characteristics while reducing the possibility that extraneous variables from contaminating organisms such as soil fungi and bacteria complicating the results. Ambient electrical fields and currents were supplied to the chambers from a set of collector electrodes; each electrode was buried in the soil one meter from the chamber (see Appendix I). In the last year of the study, a microprocessor controlled monitoring system was installed to provide hourly data on electric field exposure, current density (J) exposure and soil temperature.

1. CONTROL AND EXPOSURE SITES: Three sites were used at the WTF, one control and two exposure sites (see Fig.1). One exposure site was located near the west ground of the antenna (7G3), a second exposure site (7A2) was located below the overhead cables of the antenna. The control site (7C1) was located more than 7 miles east of the nearest antenna element. The control and ground sites were selected at the beginning of the program in 1982 (see Appendix I for details about exposure). The antenna site (7A2) was selected in 1984.

Cultures were maintained and exposed in autoclavable polyethylene chambers (7" x 4" x 2 1/4") with a tight fitting top; two stainless steel electrodes were placed 6" apart and about 1/4" from the bottom of the chamber. The growth chambers were placed inside a protective chamber (10" x 10" x 20"); a tight fitting lid provided a reasonably waterproof environment. A 1/2" vent pipe was attached to the lid of the outer chamber to facilitate gas exchange. On several occasions during the early field studies, the vent pipes were separated from the outer chamber, presumably by animals. When the latter occurred, the macroplasmodia were generally contaminated and backup cultures were used. The protective chamber containing the growth chamber was placed in a hole about 20" x 20" x 20"; two, 8" square copper collector plates were buried 1 meter from each hole along a line with the predominant electric field. Ambient electric fields were brought to the growth boxes by buried wire leads that ran from the collector plates to a plug on the outer wall of the protective chamber. The ambient B fields generated by the WTF were monitored at yearly intervals; no special techniques were required to insure that Physarum was receiving the appropriate B-field exposure. To protect the exposure system from foraging animals, each outer chamber was covered with a plywood board.

2. CULTURE MAINTENANCE: The nature of our biochemical assessments required that the WTF field-exposed cultures be maintained in an axenic state. This requirement necessitated several modifications to our normal laboratory protocols. One of the most important changes was the need to maintain the cells on an agar substrate rather than in liquid medium. Macroplasmodia were grown on 150 ml of half-strength growth medium (Daniel and Baldwin, 1964) containing 3% agar and supplemented with 2 g of sterile rolled oats. The growth medium was prepared at University of Wisconsin Parkside (UWP); growth chambers containing solidified medium were placed in sterile plastic bags and transported to the exposure site. At the WTF, growth the chambers were placed in a Plexiglas hood fitted with a two banks of timer-controlled uv lights for one week. The ultraviolet lights were turned on for 10 minutes every night. These precautions allowed us to identify growth boxes that had become contaminated during transport and decreased the chance of EMF exposed cultures through contamination.

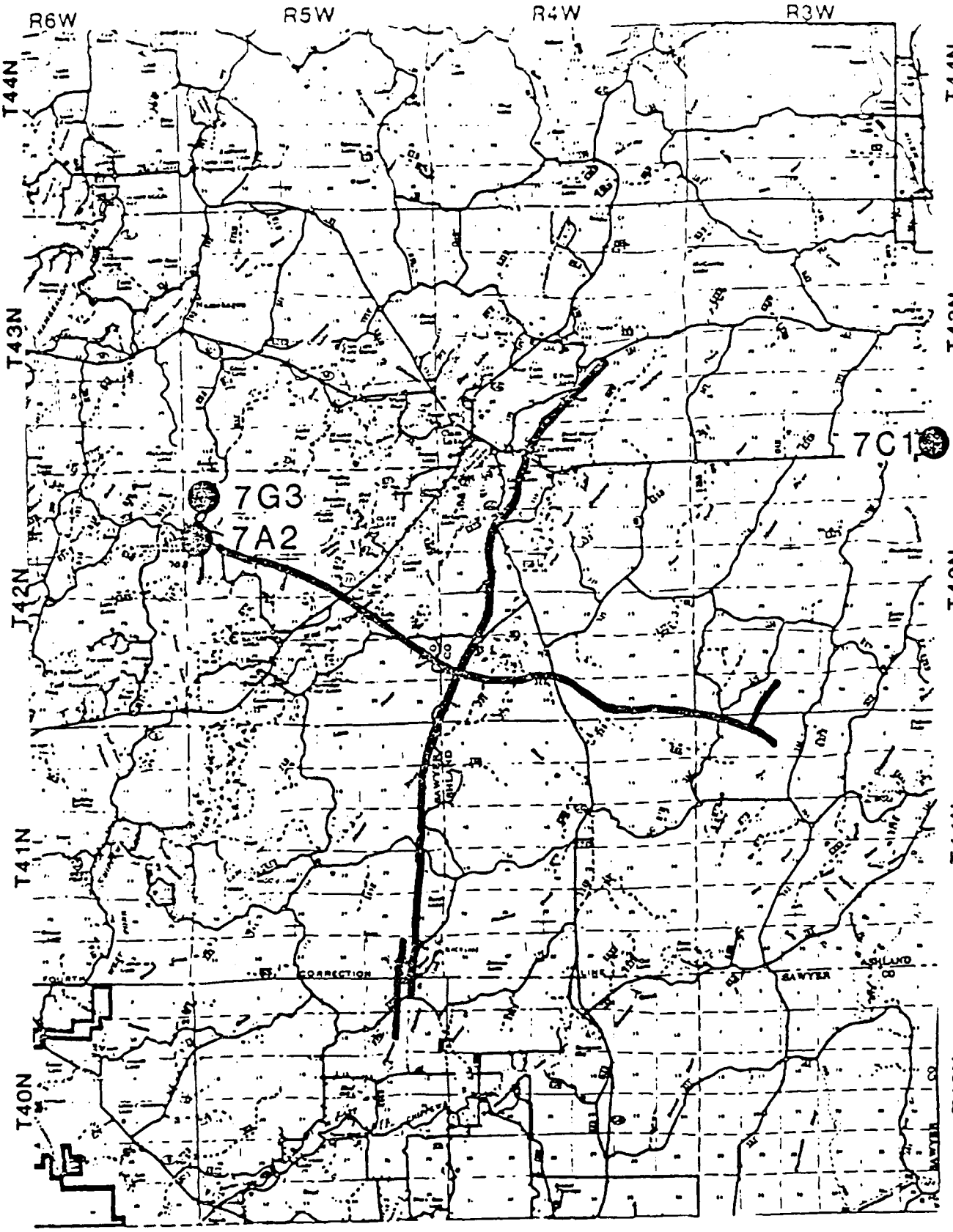

FIGURE 1.

LOCATION OF TEST AND CONTROL SITES

Map of the Clam Lake area showing the location of the Control (7C1), Ground (7G3) and the Antenna Sites (7A2). The position of the WTF antenna is shown in bold outline.

3. **WTF FIELD TRANSFER OF MACROPLASMODIA:** (1) Each protective chamber was disconnected from its collector plate (generally after making field measurements) and brought to the mobile lab where they were washed to remove mud and debris. (2) The growth chambers were removed from the protective containers and their outer surfaces thoroughly cleaned using a disposable wipe saturated with Zorbicide. (3) The growth chambers were then placed in a laminar flow hood and a 2.5 cm^2 piece of macroplasmodium was removed from the outer edge of the growing culture and transferred to a growth chamber containing new medium. (4) Following transfer of the culture, the new growth chamber was then placed back in its protective container, the electrode leads reconnected, and the chambers were returned to the field. To monitor daily temperature fluctuations at each site, a Dickson temperature recorder was placed in one of the chambers. The transfer process described above is summarized in Figure 2.

4. **CONVERSION OF MACROPLASMODIA TO MICROPLASMODIA:** During 1987, the growth chambers containing macroplasmodia were placed in a sterile autoclave bag and returned to UWP in a canvas suitcase. At the airport security station, cultures were subjected to manual examination rather than the x-ray scanners. Upon returning to the lab, macroplasmodia from each chamber were scraped from the agar surface and placed in 125 ml Erlenmeyer flasks containing 25 ml of half-strength growth medium; the flasks were then placed on a shaker (see Fig.2). Generally, the constant overnight agitation of the shaker reduced the macroplasmodium to the smaller units referred to as microplasmodia. The next morning, microplasmodia were harvested by centrifugation, washed and resuspended in sterile distilled H_2O and transferred to full-strength growth medium. Microplasmodia from WTF treatment and control sites were grown on the same shaker in the laboratory. In most cases ATP and QO_2 analyses were performed within 40 to 72 hours after removing the cultures from the WTF.

Once a suspension culture had reached vigorous growth, a 0.2 ml aliquot from each control and experimental site was pipetted to Petri plates (containing 1/2 strength agar - growth medium); the plates were then placed in an incubator maintained at 26°C. These cultures were used as backups on the ensuing weekly trip in the event a particular site had a contamination problem. If these cultures were used as backups, they would have been out of the WTF-EMF environment a total of 7 days

Prior to 1987, cultures were placed in half-strength liquid growth medium immediately following their sub-culture to new boxes at the WTF. The suspension cultures were then transported back to the laboratory and placed on a shaker. The 1987 method of introducing macroplasmodia into liquid media after returning to the laboratory appears to facilitate more rapid microplasmodia formation. One explanation for the latter observation is that cultures maintained in liquid medium require vigorous shaking for aeration; plasmodia in transit may have been subjected to an oxygen stress that manifested itself in a longer transition period. If the latter suggestion is correct, it may be that EMF effects have been masked by the larger and more immediate O_2 stress.

5. **EMF EXPOSURE:** The field study was designed so that Physarum was exposed to the same EMF environment it would encounter in its natural habitat in either

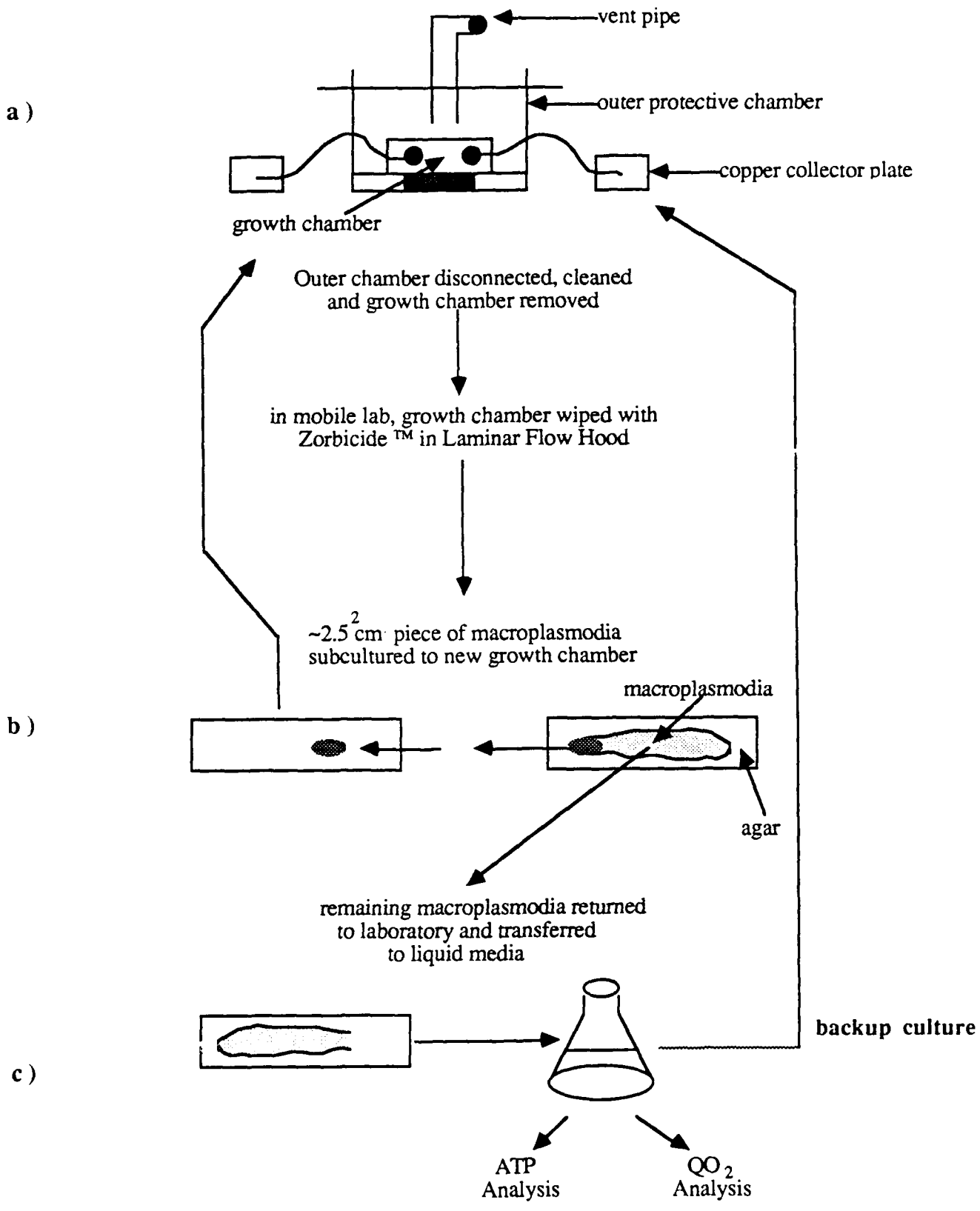

Figure 2. WTF Subculture Procedure

a) A schematic view of a growth chamber inside its outer protective chamber. Macroplasmodia are exposed to either a control or test field using this system. b) to transfer the plasmodium, a 2.5 cm^2 piece of agar is excised from the periphery of a growing culture and placed on new growth medium. c) the remaining macroplasmodium is placed in liquid growth medium and shaken until microplasmodia are formed. Microplasmodia is the growth form used to assess the cell's ATP and QO_2 content.

the soil or on surface litter (see Appendix I for additional details). This requirement was complicated by the fact that a mismatch existed between the conductivities of the soil and those of the culture medium. To correct this problem, external control circuitry (designed by IITRI) was used to match either the electric field (E) or the electrical current (J) to that of the soil. Information on the EMF exposure protocols can also be found in Haradem et al.; 1988.

Physarum macroplasmodia were exposed to either an electric field (E) or an electric current (J) matched to either the electric field or current present in the soil at each site. Magnetic field exposure was the same as that found above the soil surface and was a function of the particular exposure site. EMF's at the experimental sites were predominately those produced by the WTF although an ambient field was also present. At the control sites, the EMF's were primarily the result of ambient 60 Hz fields and were about 10 fold less than at the treatment sites.

Generally, field studies at the WTF were initiated in the latter part of May and continued through the middle of October. In 1987, cultures were placed in their WTF sites on May 23 (Day-1); they were subcultured to new growth medium on a weekly basis until October 17 (Day-148).

E and J measurements were routinely made by UWP personnel both before and after cultures were transferred during the 1986 and 1987 field seasons. In July of 1987, E and J fields as well as temperature were recorded on an hourly basis at all sites. Protocols for the EMF measurements since the beginning of the project are summarized in Haradem et al.;1988.

6. **TEMPERATURE:** Temperature at each site was estimated by placing a battery operated Dickson monitor inside the protective chamber of one of the cultures at each site. The monitors were calibrated in the lab prior to their use in the field. The accuracy of the monitor is plus or minus 1 °F, full scale. Except when water got into the chamber, the recorders performed satisfactorily. The mean weekly temperature was calculated by averaging the daily high and low temperatures at each study site. A summary of temperatures at each site during the 1987 field season is shown in Figure 3. Hourly recordings of soil temperature (beginning in July 1987) were also made by IITRI using data loggers.

B) PROTOCOLS

1. **RESPIRATION:** A respiration measurement was made by placing a 1.0 ml aliquot of microplasmodia and 2.0 ml of previously aerated growth medium into a water jacketed reaction vessel (YSI model 53) maintained at 25.8 °C. The system was closed by placing a calibrated oxygen probe into the reaction vessel, and allowing it to equilibrate for 5 min. Oxygen consumption was measured over the next 2-3 minute period. Triplicate respiration measurements were made on each microplasmodial culture. In some cases insufficient microplasmodia only allowed us to perform duplicate measurements. After withdrawing a sample for analysis, the flask was returned to the shaker. To facilitate normalization of the data, microplasmodia were removed from the vessel after completing the respiration measurement, the yellow pigment was

1987 FIELD DATA

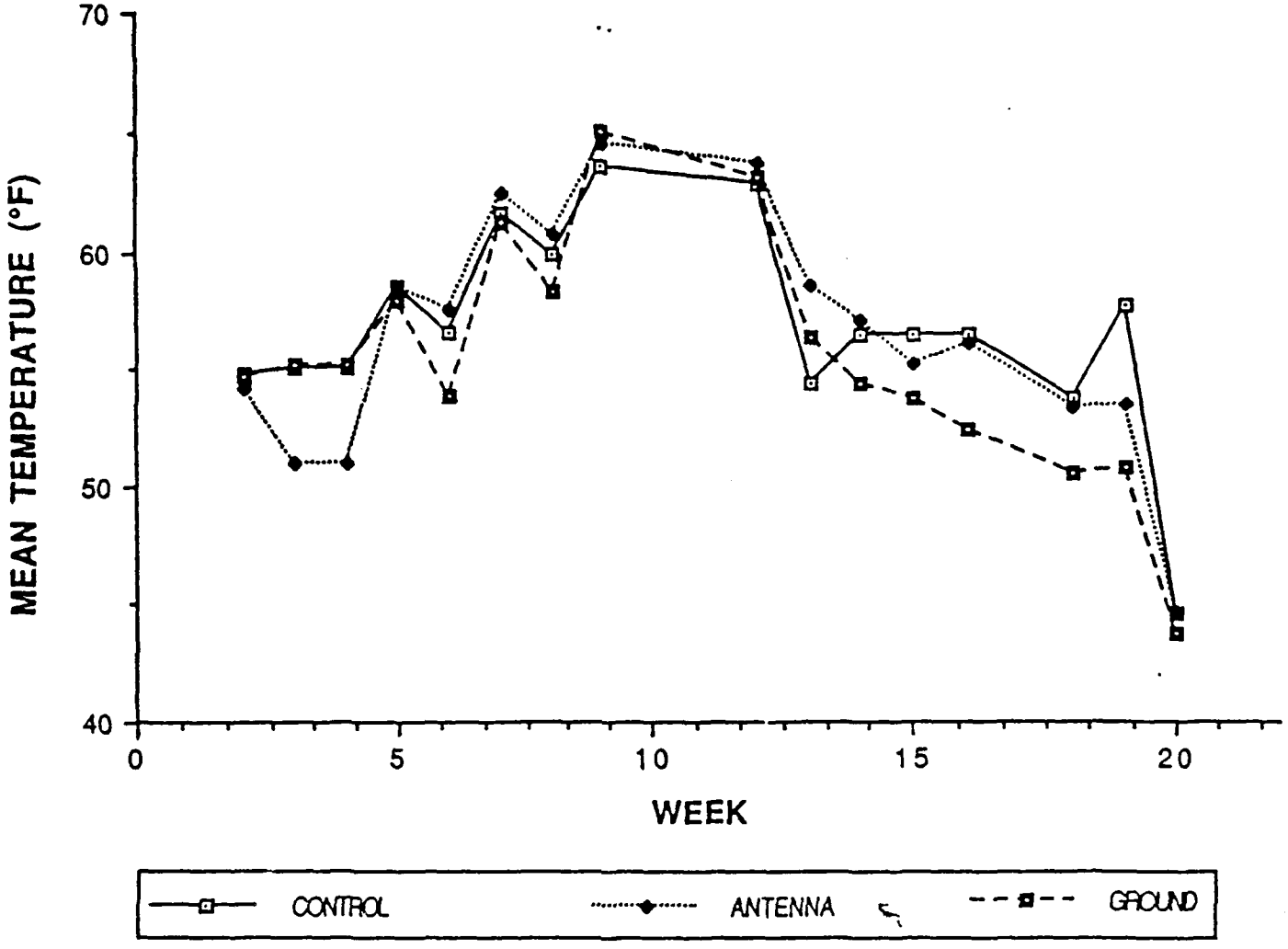

FIGURE 3.

WTF TEMPERATURE SUMMARY

A composite of the average weekly temperature at each test site at the WTF for the 1987 field season. Macroplasmodia were placed at their appropriate sites in May, 1987 and removed in October, 1987. Some intersite temperature differences are evident during the first and last quarter of the exposure period.

removed by washing in a trichloroacetic acid-acetone-water solution (5%-50%-45%); the pellet was dissolved in 0.5N NaOH. Duplicate Biuret assays were performed on each sample to estimate their protein content. A new standard curve was constructed for each protein data set. The rate of oxygen consumption is expressed as the QO_2 (µl O_2 oxygen consumed /min /mg protein).

2. ATP: To extract ATP from microplasmodia, duplicate 1.0 ml samples were removed from the shake flasks and placed in tared polycarbonate tubes containing 2.5ml Tris-borate buffer (pH 9.2) had been brought to 98 °C in a boiling water bath. The tubes were capped with a marble and the ATP was extracted for 15 min. Following extraction, the tubes were removed, wiped to remove exterior moisture and weighed. The weight was used to ascertain the final volume of the extract. The extracts were centrifuged at 84,000g. The ATP content of the supernatant was assayed using the luciferin-luciferase procedure; light emission was measured in a Packard Picolite luminometer. To determine the protein content, 0.5ml NaOH was added to solubilize the pellet and duplicate Biuret assays were performed. The ATP content is expressed as nM ATP /mg protein.

C) LABORATORY STUDIES:

Physarum macroplasmodia were grown on the same media and in the same growth chambers as described above for the WTF field studies. Cultures were maintained in Warren Sherer incubators at UW-P in Kenosha WI. Macroplasmodia were transferred on a weekly schedule and then immediately placed in suspension culture. The laboratory handling protocol differed somewhat from the way WTF exposed cells were processed. In the latter case, there was a 9 hour lag period (travel time) between the time cells were transferred to new growth medium at the WTF and their introduction into suspension culture in the lab. In contrast, lab exposed macroplasmodia were immediately introduced into liquid culture following transfer. QO_2 and ATP analyses were performed as described above. The cultures inside the incubator were kept in darkness until the time of transfer.

1. EMF EXPOSURE: In the laboratory, EMF-exposed macroplasmodia (4) were subjected to a 76 Hz modulated waveform (76 Hz_{mod}) and 0.17.5 µT magnetic field in 1985 and 1986; in 1987, the B field was decreased to 0.7 µT in 1987(see Appendix I). The E field intensities were not changed during the 1985-87 period. Two of the growth chambers were exposed to an electric field (800mV/m) and two chambers were subjected to a current density of 0.002 A/m^2 (10mV/m electric field intensity). These exposure intensities are similar to the average E and J intensities found at the WTF ground site. The laboratory simulation differed from the actual WTF field exposure in that the E and J field intensities were constant over an entire exposure period. In other words we did not attempt to duplicate the daily fluctuations in field levels that occurred at the G site. Control cultures were maintained in a separate incubator to which no fields were applied. A description of the laboratory simulators and field generating apparatus is found in Appendix I.

2. TEMPERATURE: To approximate the temperature changes encountered during a field season, the EMF-exposure and control incubators were adjusted to the

average weekly temperature encountered at the WTF ground site the previous week. Thus, in the laboratory simulations, the temperature was maintained at a constant value for a week whereupon it was changed to correspond to the average temperature at the ground site the previous week .

D) DATA ANALYSIS

A. Laboratory: The laboratory data were used to evaluate the experimental protocols and to consider the effect of the exposure variables without the confounding influence of environmental fluctuations. The results of these analyses provided the basis for the subsequent evaluation of the field data.

Data on cultures maintained and exposed in the laboratory were examined using analysis of variance techniques (ANOVA). The independent variables used in the ANOVA included:

- replication of measurements [REP]
- period of time in microplasmodia suspension culture [HOURS]
- intensity of EM exposure [EXP]
- period of EM exposure [weeks]

The dependent variables were the cells' ATP levels and respiration rates (QO_2).

The ANOVA compared E-field exposure to control with respect to each of the independent variables. Similarly, an ANOVA compared current density exposure to control for the same effects. The absence of significant correlations among these independent variables indicate they are independent measures and appropriate for use in these ANOVA's.

B. WTF Field Study: As one would expect, field conditions produced more variability in ATP and QO_2 levels than we observed in the laboratory. Temperature was recorded as a means of controlling for some of this variability. Further, the seasonal drift or change in temperature made this a potentially confounding variable in the analysis of EMF effects. High, low, and weekly average temperatures were calculated for each site based upon daily temperature measurements. Of these, weekly average temperatures bore the highest correlation with both ATP levels and respiration rates (QO_2) see Appendix 2. This relationship did not demonstrate a significant lag. A linear regression procedure evaluating ATP and QO_2 as a function of weekly average temperature was performed. The residuals from this regression were used in subsequent analyses to control for temperature effects (Tables 5 and 7).

The impact of the electromagnetic field on Physarum metabolism was evaluated in two ways. The first approach utilized a multivariate analysis of variance (ANOVA) to consider the relationship between the proximity to the antenna and the culture's metabolism. These ANOVA's included the following

independent variables:

. exposure type [TYPE]

. exposure site [SITE]

. period of exposure [WEEKS]

As in the laboratory analyses, QO_2 and ATP were the dependent variables. Exposure type [TYPE] refers to either the E-fields (E) or current density (J) exposure regimen. These two variables have been matched to the E and J fields of the surrounding soil. To avoid confusion with the laboratory exposure regimens (EXP) a new descriptor was selected. Exposure site [SITE] refers to the specific location of the WTF cultures, i.e. antenna, ground and control and to the EM exposure found at these sites. Separate analyses were performed for each of the two metabolic outcome variables, ATP and QO_2.

On the tenth week of field exposure in 1987, back-up cultures were used to replace some contaminated cultures in the field. Analyses of the lab data suggested that time out of an EMF environment may alter the characteristics of the culture. Therefore, in addition to the multivariate analysis of variance, an ANOVA was performed which excluded data points for the month following the introduction of back-up cultures. In addition, use of a backup culture for electric field exposure raises the possibility that the two exposure sites were not equivalent. To investigate this possibility, the data were evaluated twice, once with the sites considered separately, and once with them combined.

As a second approach to the evaluating the relationship between exposure variables, values derived from hourly measurements of E-fields, J-fields and temperature were included in a separate analysis. These data were combined with the ATP and respiration measurements by averaging the data for each of the three exposure variables at each site over the periods between metabolic measurements. In other words, the exposure associated with a given metabolic measurement of a culture was defined as the average temperature, E-field and J-field as measured at that site since the previous measurement. A multivariate linear regression procedure was used to predict ATP levels and respiration rates as a function of temperature, E-field and J-field exposure. The number of weeks of exposure was also included as a predictor variable. Pearson moment correlations were also calculated to compare the two sets of exposure measurements (see Appendix IIB).

IV. RESULTS

A) Laboratory: Tables 1 and 2 presents the descriptive statistics and ANOVA's for the 1987 laboratory data. The data show that the length of time Physarum microplasmodia remain in the ambient lab environment (out the applied EMF) can significantly effect the QO_2 (Table 1). A review of the means (Table 2) suggest that the respiration rates decline with time (a least significant difference analysis bears this out). ATP levels are not significantly affected by time out of the field but do tend to show an increase as measurements are replicated. The replication effect does not interact significantly with exposure type. In light of this, the small effect of

Table 1

Analysis of 1987 Lab Data for Effects of Replication and Time in the Lab

Variable	QO_2	ATP
EXP	0.61	0.32
REP	0.93	0.52
HOURS	0.005	0.82
EXP*REP	1.00	0.46
EXP*HOURS	0.93	0.91
REP*HOURS	1.00	0.47
EXP*REP*HOURS	1.00	0.67

The 1987 lab data were analyzed to consider four variables; the effect of replicating measurements (REP), the length of time a culture was maintained out of the EMF environment (HOURS), the type of exposure (EXP), and the weeks of exposure (WEEKS). An analysis of variance (ANOVA) was performed to examine the effects of replication (REP) and HOURS along with their exposure (EXP) interactions.

To evaluate the appropriateness of an ANOVA for this an subsequent analyses, autocorrelations for the independent variables of the laboratory data were evaluated. The absence of significant correlations ($p < 0.5$) among the independent variables indicate they are independent measures and appropriate for use in ANOVA; the dependent variables were ATP and QO_2.

Table 2

Descriptive Statistics Associated with Replication and Hour Effects

		ATP (nM ATP/mg protein)	QO_2 (μ/O_2 consumed/ min/mg protein
REPLICATION:			
ATP	1	15.3 ± .77	0.728 ± 0.026
	2	16.8 ± .68	0.720 ± 0.026
	3		0.725 ± 0.025
HOURS:			
ATP	48	15.7 ± 1.31	0.806 ± 0.042
	72	18.2 ± 2.41	0.719 ± 0.044
	96	15.7 ± 0.577	0.644 ± 0.039

Duplicate and triplicate samples taken during 1987 were respectively run on the dependent variables ATP and QO_2 after 48 hours in liquid suspension culture. The effect of replication and hours out of the field are examined. The analyses show that the mean for one dependent variable (QO_2) is changing with the number of hours in submerged shake culture (i.e. time out of the EMF). As a result, only the first sample (48 hrs) was used in subsequent statistical analyses.

replication was not judged to be sufficient to preclude averaging the replicated values. Because the time a culture is in the laboratory has a significant effect, only the first set of measurements recorded after microplasmodia formation were used in the analyses. All data in this report (1985, 1986 and 1987) only used the first set of data (~48 hours) acquired after conversion to microplasmodia.

An analysis of the effect of WEEKS and EXPOSURE in the laboratory is shown in Table 3. The culture parameters changed significantly over time for all variables however, this change did not seem to follow any ordered pattern (see Figures 4 and 5). Although this finding argues against the presence of a cumulative effect for length of exposure, it does indicate that significant fluctuations occurred from one week to the next under laboratory conditions. The level of exposure was not significant for any of the outcome variables.

The results of earlier laboratory experiments in this program (1982-86) present a somewhat more confusing picture. This is in part a result of numerous changes in exposure, growth, and handling protocols many, of which were designed to more closely mimic the WTF field component of the study. Because of extensive protocol changes over the years, only the 1985 and 1986 laboratory data were re-analyzed. This decision was based on the fact that the exposure levels were reasonably similar during this period as were most of the growth and handling protocols. A summary of the QO_2 and ATP laboratory data from 1985, 1986 and 1987 is shown in Table 4. At the intensities used in this study (76 $Hz_{(mod)}$ 17.5 uT (or 0.7μT) and either 10mV/m or 800mV/m) no statistically significant differences between EMF-exposed and control (non-exposed) cells were observed. In examining the data, there appears to be general agreement in the respiration rates observed in 1985 and 1987; however, the QO_2's for 1986 were lower by 20-30% (see Table 4). In contrast to the respiration data, ATP results from 1985 and 1986 both show higher ATP levels (about 25%) than were obtained in 1987.

B) WTF: The analyses of the 1987 field data are shown in Tables 5 and 6. If one examines the two, E-field data sets (from each site) separately, only weeks of exposure has a significant effect. The effect of <u>time</u> is strongly significant for respiration ($p=.0001$), but not significant ($p=.07$) for ATP levels. No case shows a steady change in either QO_2 or ATP with time indicating that the electromagnetic exposure did not have a cumulative effect (Figs.9-12). The interaction of SITE and WEEK is significant for QO_2 ($p=.02$). The only effect of SITE is seen in the combined exposure data for ATP levels. A least significant difference analysis of the means indicates that the average ATP level from E-field exposure was greater at the antenna site than at either the control or ground site (Table 6). In contrast, analyses of ATP levels at the J sites indicate that the ATP levels are similar at the antenna and control site but are depressed at the ground site.

ANOVA's of the 1985 and 1986 data also show a significant effect of time in the field (Table 5). In addition a significant interaction for SITE (not found in 1987) was also observed. In 1986 significant interactions between the TYPE of exposure and SITE-WEEK were found for QO_2; the latter was also significant for ATP. In 1985, neither of these interactions (TYPE, SITE-

Table 3

Results (p values) for ANOVA of EXPOSURE and WEEK Effects in laboratory data for 1987

Variable	QO_2	ATP
EXP	0.61	0.32
WEEK	0.001	0.0002
HOURS	0.005	0.82
EXP*WEEK	1.00	0.64
EXP*HOURS	0.93	0.91

The 1987 lab data were analyzed to consider the length of time a culture was maintained out of the EMF environment (HOURS), the type of exposure (EXP), and the weeks of exposure (WEEKS). An analysis of variance (ANOVA) was performed to examine the interactions between length of exposure and type of exposure (EXP * WEEK) and the number of hours out of the exposure and type of exposure (EXP * HOURS).

1987 LAB DATA
(E + J)

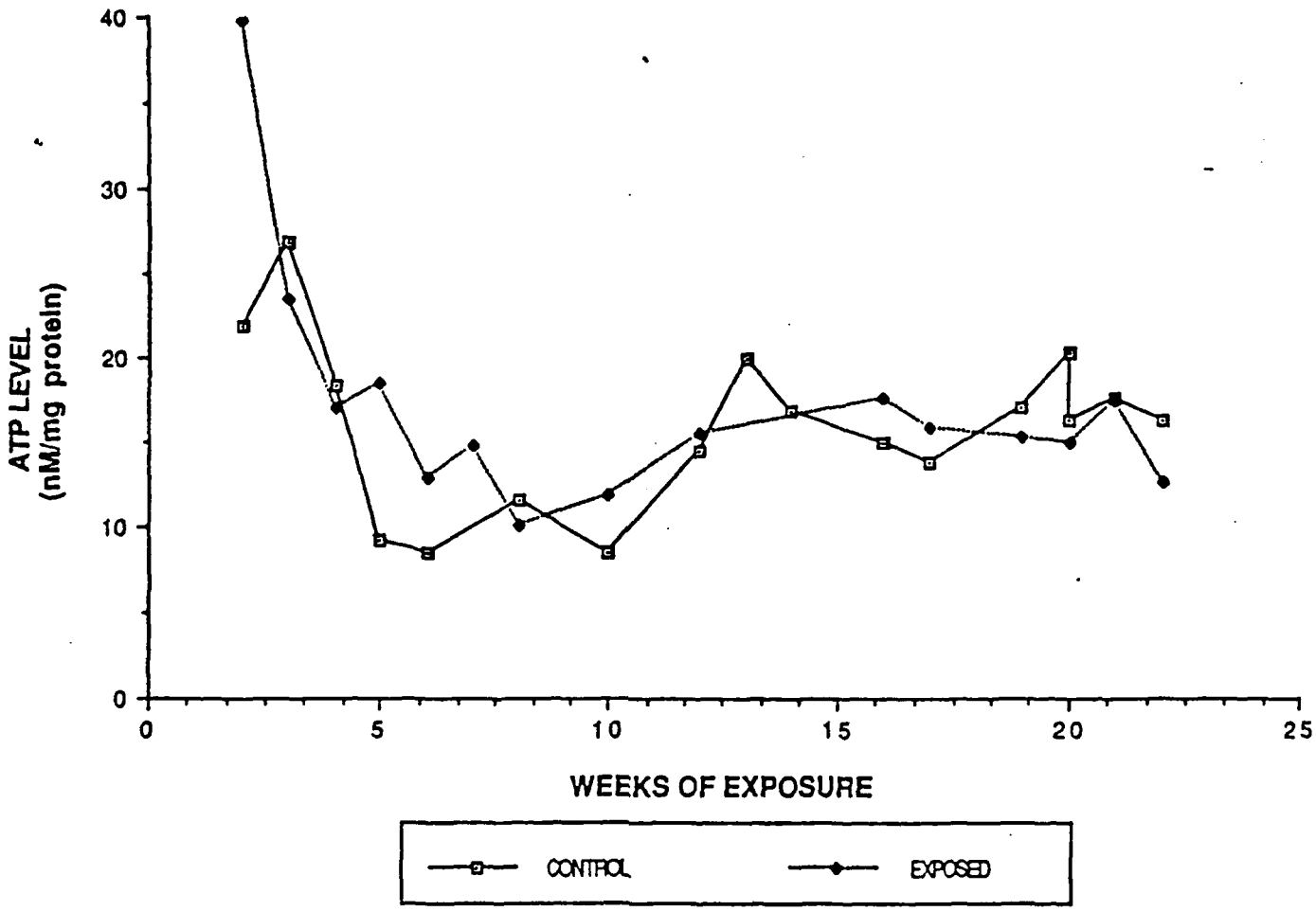

FIGURE 4.

SUMMARY OF ATP LEVELS IN MACROPLASMODIA EXPOSED IN THE LAB

The ATP level (nM/mg protein) in macroplasmodia exposed to field intensities approximating those at the WTF ground site. In this figure the data from both the current density and electric field exposures have been combined; field exposures were held constant whereas the temperature was changed once a week to equal the average at the WTF ground site.

1987 LAB DATA
(E + J)

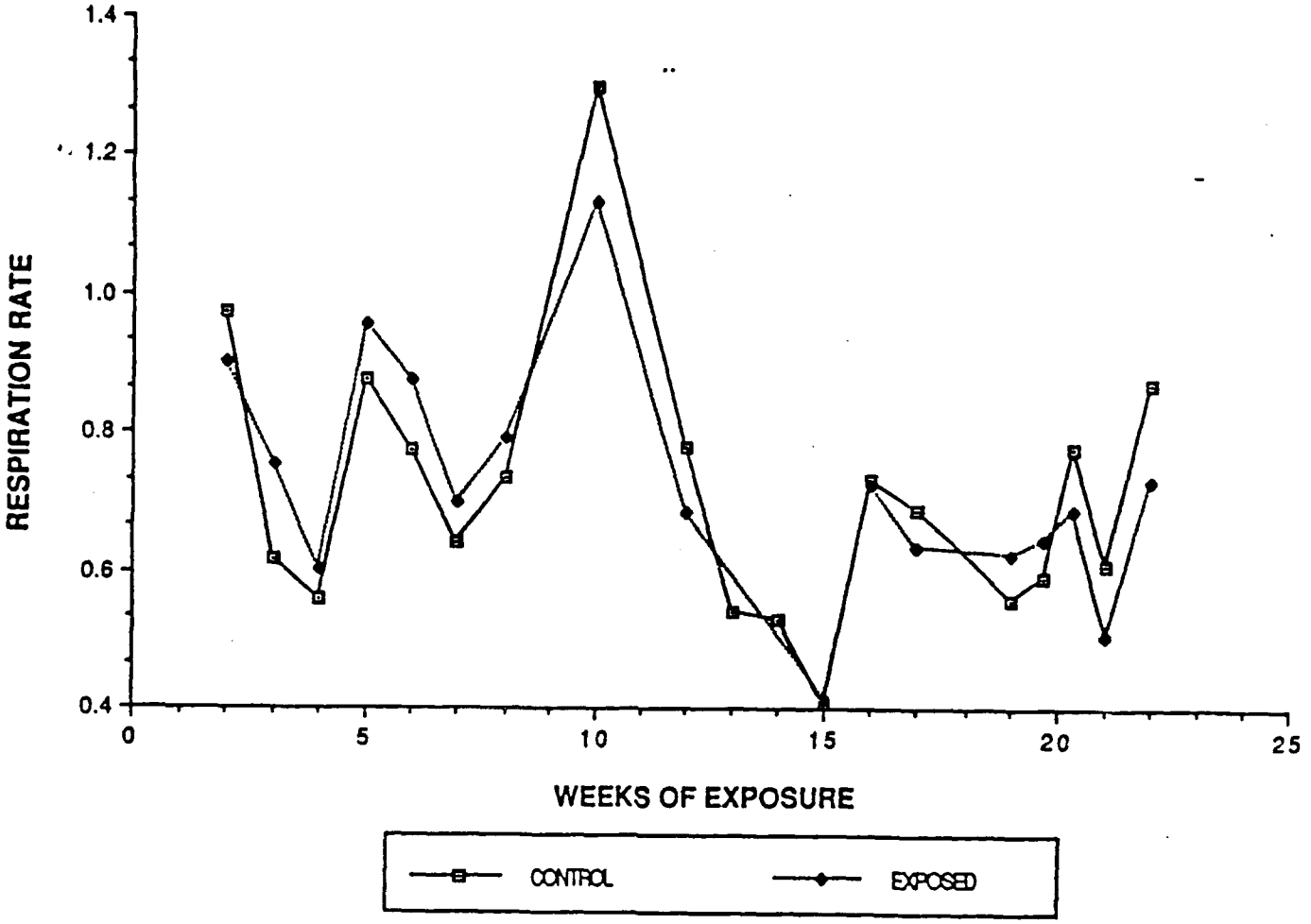

FIGURE 5.

SUMMARY OF QO_2 LEVELS IN MACROPLASMODIA EXPOSED IN THE LAB

The respiration rate (µl O_2 consumed/min/mg protein) in macroplasmodia exposed to field intensities approximating those at the WTF ground site. In this figure the data from both the current density and electric field exposures have been combined; field exposures were held constant whereas the temperature was changed once a week to equal the average at the WTF ground site.

TABLE 4

DATA SUMMARIES OF MEAN ATP AND QO_2 LEVELS FOR LABORATORY CULTURES (1985–1987)

Year	Parameter	CONTROL	EXPOSURE CONDITIONS		DECREASED TEMPERATURE
		No field	E	J	(20°C)
			Electric Field	Current Density	No Field
1985	QO_2 [1]	.68 ± .03	.66 ± .04	.71 ± .04	.68 ± .03
	ATP [2]	21.6 ± 2.1	17.7 ± 2.1	21.4 ± 2.8	22.1 ± 2.6
1986	QO_2	.56 ± .02	.51 ± .02	.52 ± .02	.56 ± .02
	ATP	20.7 ± 1.9	23.3 ± 2.1	22 ± 1.2	25.2± 1.8
1987	QO_2	.73 ± .04	.72 ± .03	.72 ± .03	
	ATP	15.9 ± 1.1	15.5 ± .99	16.2 ± .99	

The data from the 1985 and 1986 laboratory study were maintained at a constant 26°C. Also included in the table are the effects on ATP and QO_2 levels of *Physarum* macroplasmodia maintained as agar cultures at a decreased temperature (20°C) prior to microplasmodia formation and growth at 26°C. It is evident that decreased temperature had little long-term effect on either respiration or ATP content. In 1987, the EMF-expsoure and control incubators were adjusted to the average weekly temperature encountered at the WTF ground site the previous week. Thus in the laboratory, the temperature was maintained at a constant value for a week whereupon it was changed to correspond to the average temperature at the ground site the previous week.

1. µl O_2 consumed/min/mg protein

2. nM ATP/mg protein

± = Standard error of the mean

Table 5

Summary Analysis (p values) of WTF Field Data (1985–1987)

		Site	Type	Week	Site*week	Site*type
1985	QO_2	.56	.65	.02	.51	.44
	ATP	.09	.11	.0001	.70	.27
1986	QO_2	.0001	.0001	.005	.0001	.70
	ATP	.004	.18	.001	.01	.18
1987	QO_2	.32	.26	.0001	.26	.29
	ATP	.01	.01	.068	.02	.16

QO_2 and ATP are the dependent variables. Exposure type [TYPE] refers to either the E-fields (E) or current density (J) exposure regimen. These two variables are matched to the E and J fields of the surrounding soil. Exposure site [SITE] refers to the specific location of the WTF cultures, i.e. antenna, ground and control and to the EM intensity found at these sites.

Correlations were evaluated for the field data along with lagged cross-correlations with three temperature variables. Adjustment for the association of temperature with the two outcome variables was performed using a simple linear regression procedure. The residuals from this regression served as the independent variables for a multivariate ANOVA. This ANOVA included exposure type (TYPE), exposure site (SITE), and weeks of exposure (WEEKS) and their two-way interactions as predictor variables with ATP and QO_2 as separate outcome variables. Type refers to either the E-field (E) or current density (J).

TABLE 6

THE MEANS OF THE DEPENDENT VARIABLES (ATP AND QO_2) FROM THE THREE WTF SITES OBTAINED DURING THE 1987 FIELD SEASON

TYPE	SITE	ATP nM ATP/mg protein	QO_2 µl O_2 consumed/min/ mg protein
E-FIELD:			
	CONTROL	13.3 ± 1.15 a)	.780 ± .038
	GROUND	14.3 ± .924	.793 ± .038
	ANTENNA	16.0 ± 1.37	.762 ± .032
C-DENSITY:			
	CONTROL	15.0 ± .953 b)	.794 ± .044
	GROUND	13.3 ± .739	.712 ± .051
	ANTENNA	15.5 ± 1.14	.702 ± .032

a) the means and standard deviation at the three matched E-field sites
b) the means and standard deviation at the three matched current density sites (J)

1987 IITRI EXPOSURE DATA

(E-culture #1)

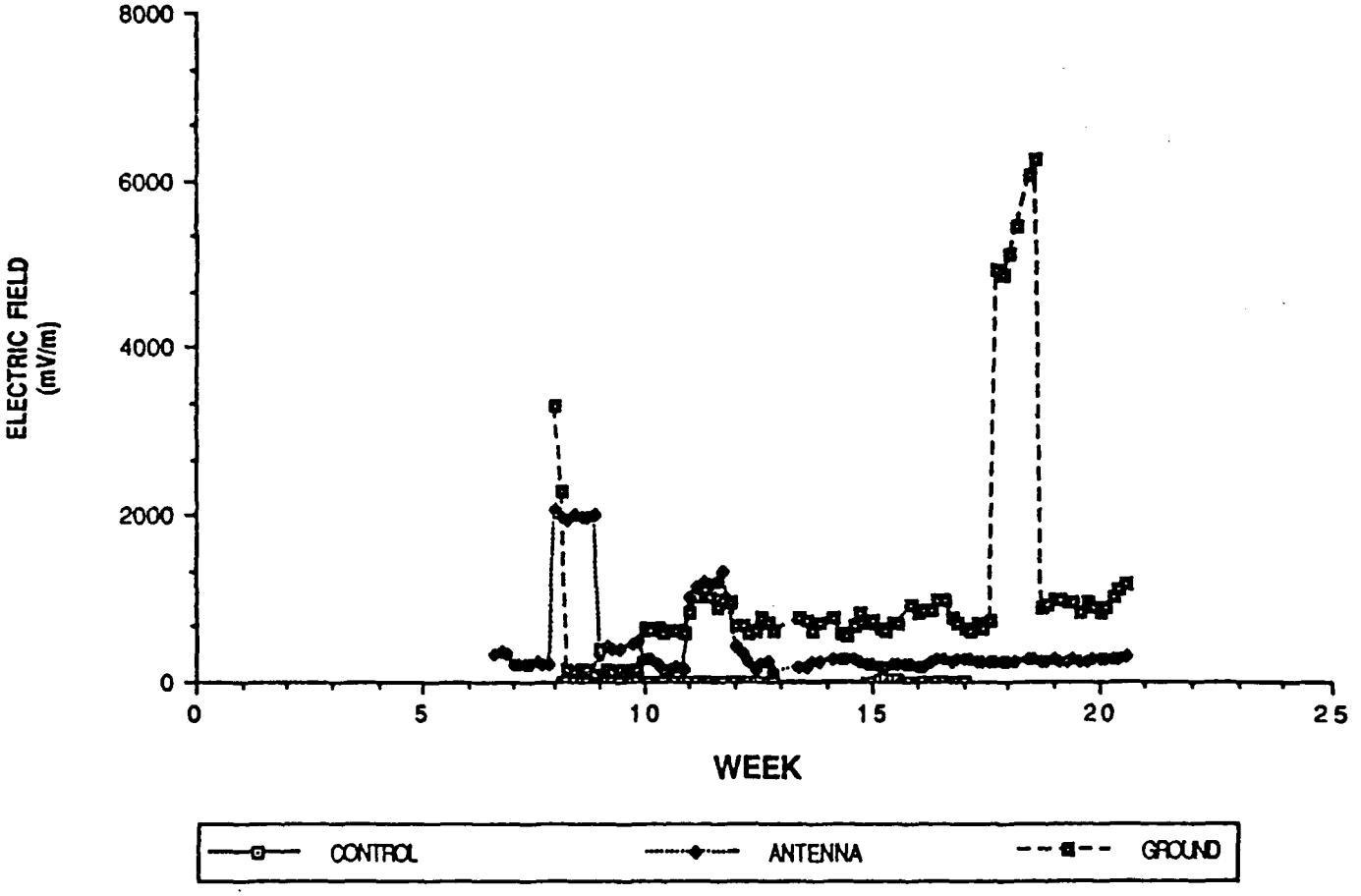

FIGURE 6.

SUMMARY OF ELECTRIC FIELD EXPOSURE AT CHAMBER #1 AT THE WTF

A weekly summary of the electric field exposure chamber number 1 (matched E-field) at the Control, Antenna and Ground sites. These data were acquired using the data loggers installed by IITRI in July of 1987. Increases in the E-field at both the Antenna and Ground sites are evident between weeks 8–9 and 11–12; an increase at the ground site occurred between week 17.5–18.5.

1987 IITRI EXPOSURE DATA

(E-culture #2)

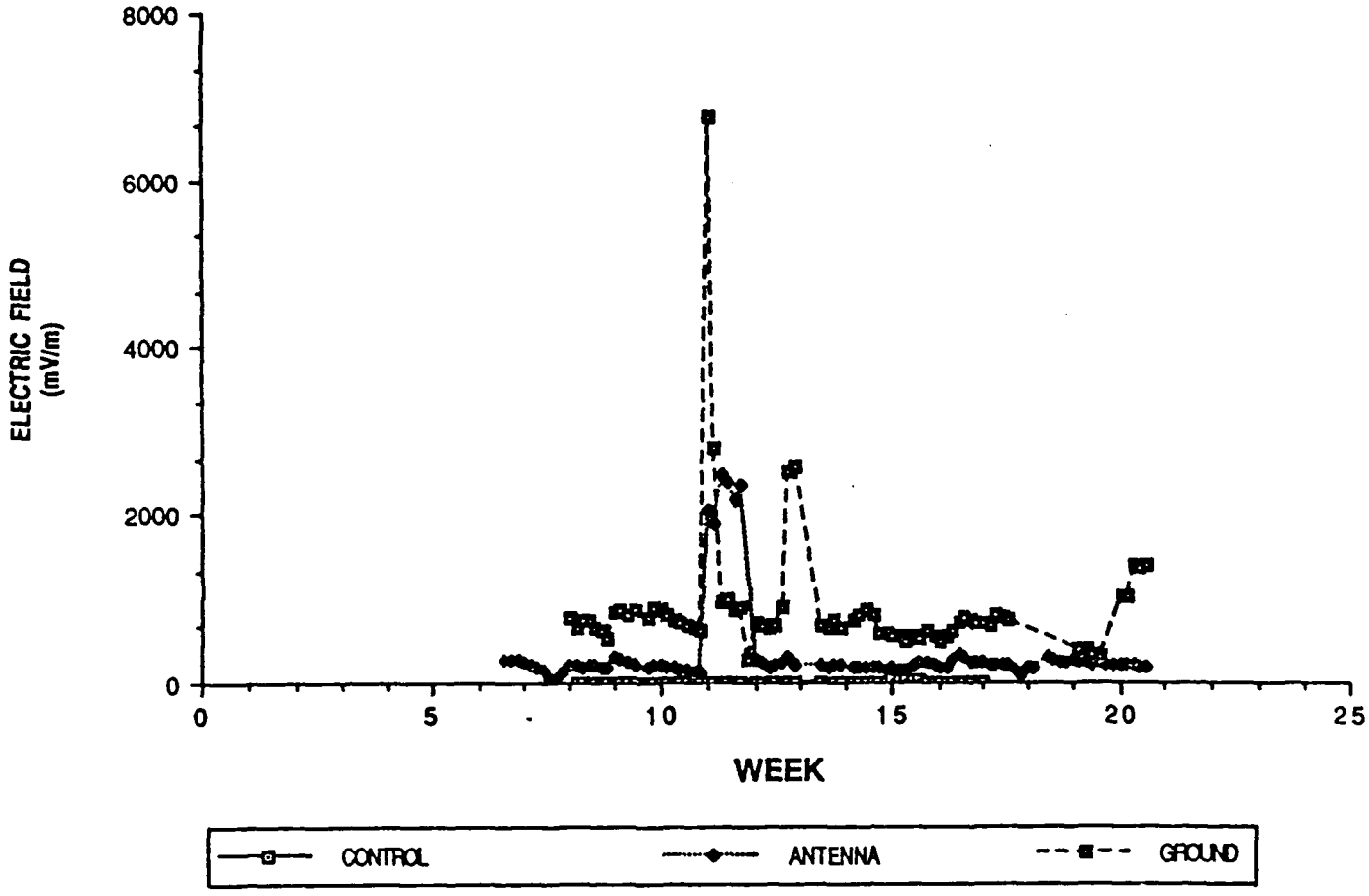

FIGURE 7.

SUMMARY OF ELECTRIC FIELD EXPOSURE AT CHAMBER #2 AT THE WTF

A weekly summary of the electric field exposure chamber number 2 (matched E-field) at the Control, Antenna and Ground sites. These data were acquired using the data loggers installed by IITRI in July of 1987. Increases in the E-field at both the Antenna and Ground sites are evident between weeks 11–12; an increase at the ground site occurs between week 12.5–13.5.

1987 IITRI EXPOSURE DATA

(J-CULTURE #3)

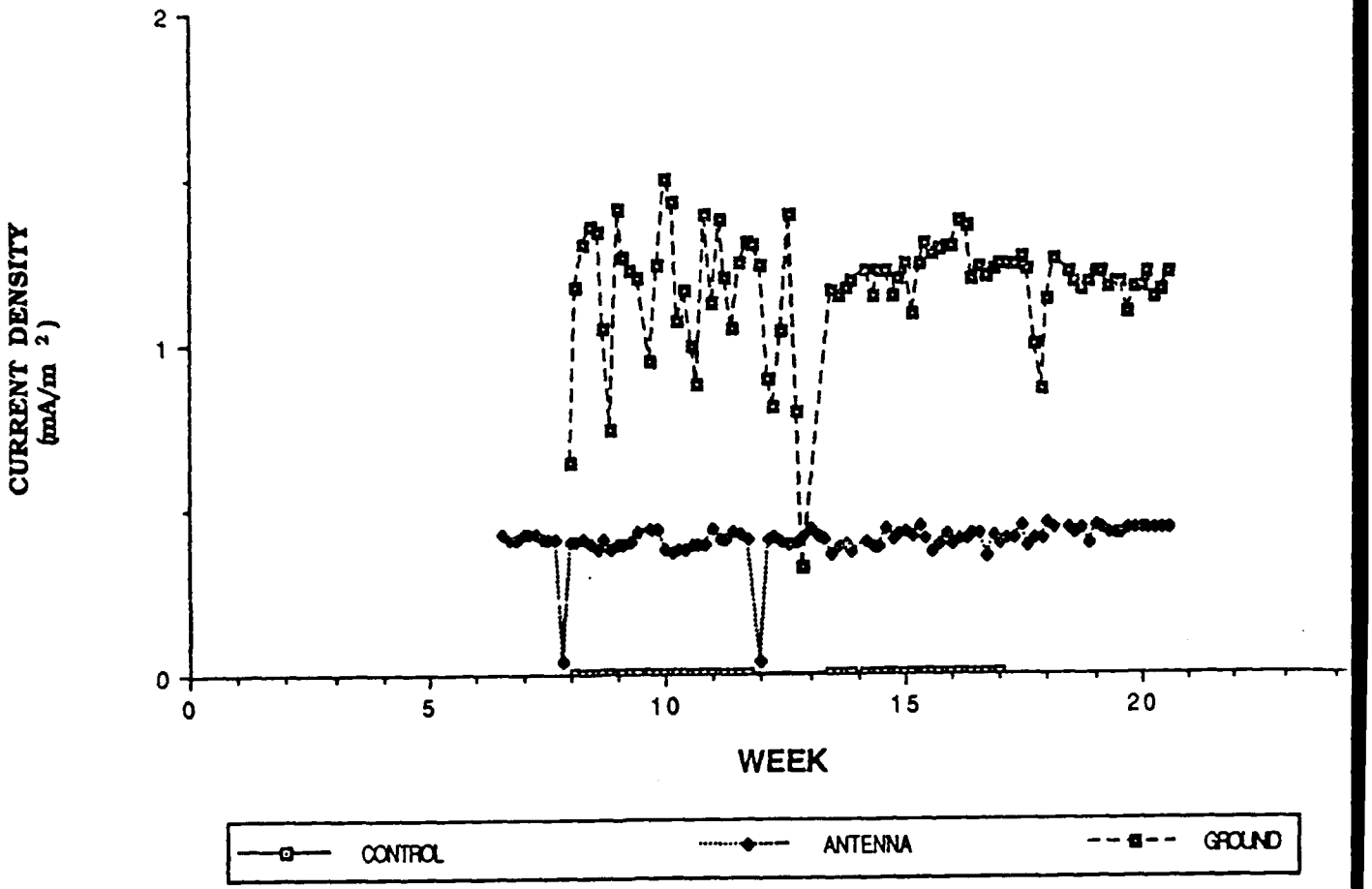

FIGURE 8.

SUMMARY OF ELECTRIC FIELD EXPOSURE AT CHAMBER #3 AT THE WTF

A weekly summary of the current density exposure chamber number 3 (matched J-field) at the Control, Antenna and Ground sites. These data were acquired using the data loggers installed by IITRI in July of 1987. Note that the ground site appears more variable during the first half of the exposure season.

1987 WTF DATA

(E only)

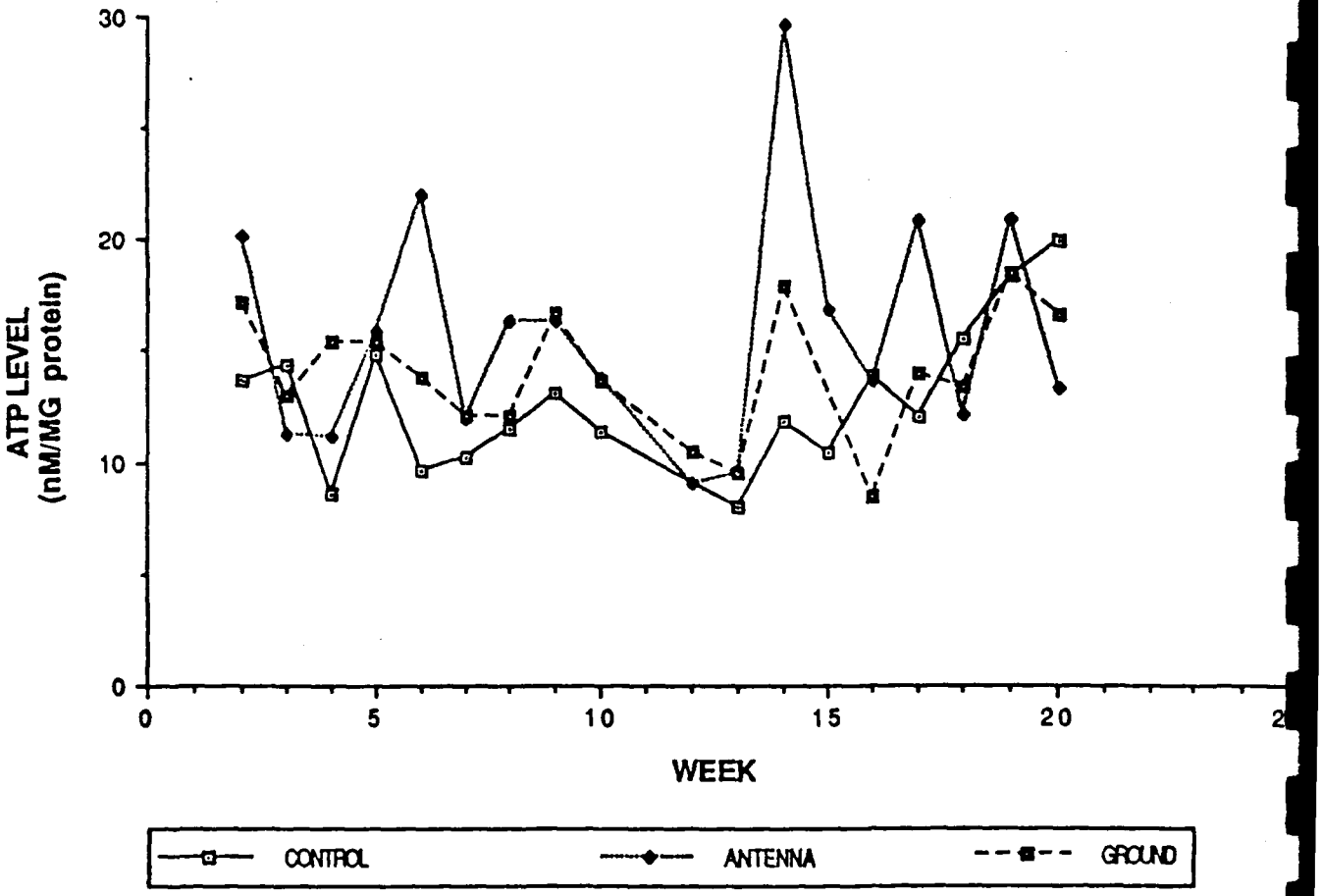

FIGURE 9.

SUMMARY OF ATP LEVELS FROM E-FIELD EXPOSURE AT THE WTF

A weekly summary of the ATP levels (nM ATP/mg protein) in plasmodia exposed to a matched electric field (E-field) at the WTF. In general, the ATP levels appear to track together, although digressions are most evident during the early and later exposure periods.

WEEK) were significant. Overall, no consistent pattern emerges from this analysis.

The multivariate regression using the continuous exposure variables (for all data) found no relationship between the exposure variables and the metabolic outcome variables. No independent variable was a significant predictor of ATP levels; only the length of time in the field showed a significant relationship to respiration (p=.0001). This regression is shown in Appendix II A.

The correlation analysis supported the results of the regression analysis. The only significant correlation for the outcome variables was .32 for the respiration rate and temperature and -.42 for respiration rate and weeks of exposure. Because temperature and weeks of exposure are highly collinear (r=0.91) it is difficult to determine the relative importance of the two factors. No other independent variables were significantly correlated with the dependent variables.

The IITRI field exposure data for each site and each exposure chamber are plotted in a weekly summary in Figures 6-8. In general the two E-field sites track together although there are some dramatic differences in field intensities during weeks 8-9, 11-13 and 17.5-18.5. The current density exposure at the ground site shows more variation than the antenna site in the early part of the season (~week 8-12) whereupon, the exposure appears to stabilize.

A non-statistical examination of the summary data (Fig.9) suggests that cultures at the A site tend to exhibit higher ATP levels for E-field exposure whereas ATP levels at the G site generally track between the C and A sites (Fig.9). These observations are corroborated by the means for ATP (Tables 6,7). In contrast, the respiration data for both E and J exposures appear to track independently with no immediate evidence of the interrelationships seen with E field exposure (Figs 11,12).

Another aspect of interest in the 1985 and 1986 data sets was an apparent closer tracking of the experimental parameters with time at each site. For example, when increases or decreases were noted in either QO_2 or ATP, they tended to occur at all sites (Figs.13-20). This tracking phenomenon is not evident in the 1987 data sets.

C. OTHER FACTORS:

1. Temperature: The ANOVA's show that both temperature and fields clearly change with time. The temperature variations are directly related to both the extremes associated with the day/night cycles and seasonal cycles (Figs.3,21 and 22). To address the changes associated with temperature during the field season, temperature was included as a covariate in the ANOVA procedures.

2. Time: The question of whether a culture changes after it has been returned to the laboratory has been examined. The data (Table 1.) indicate that QO_2 is apparently more sensitive to the number of hours away from the exposure

1987 WTF DATA

(J only)

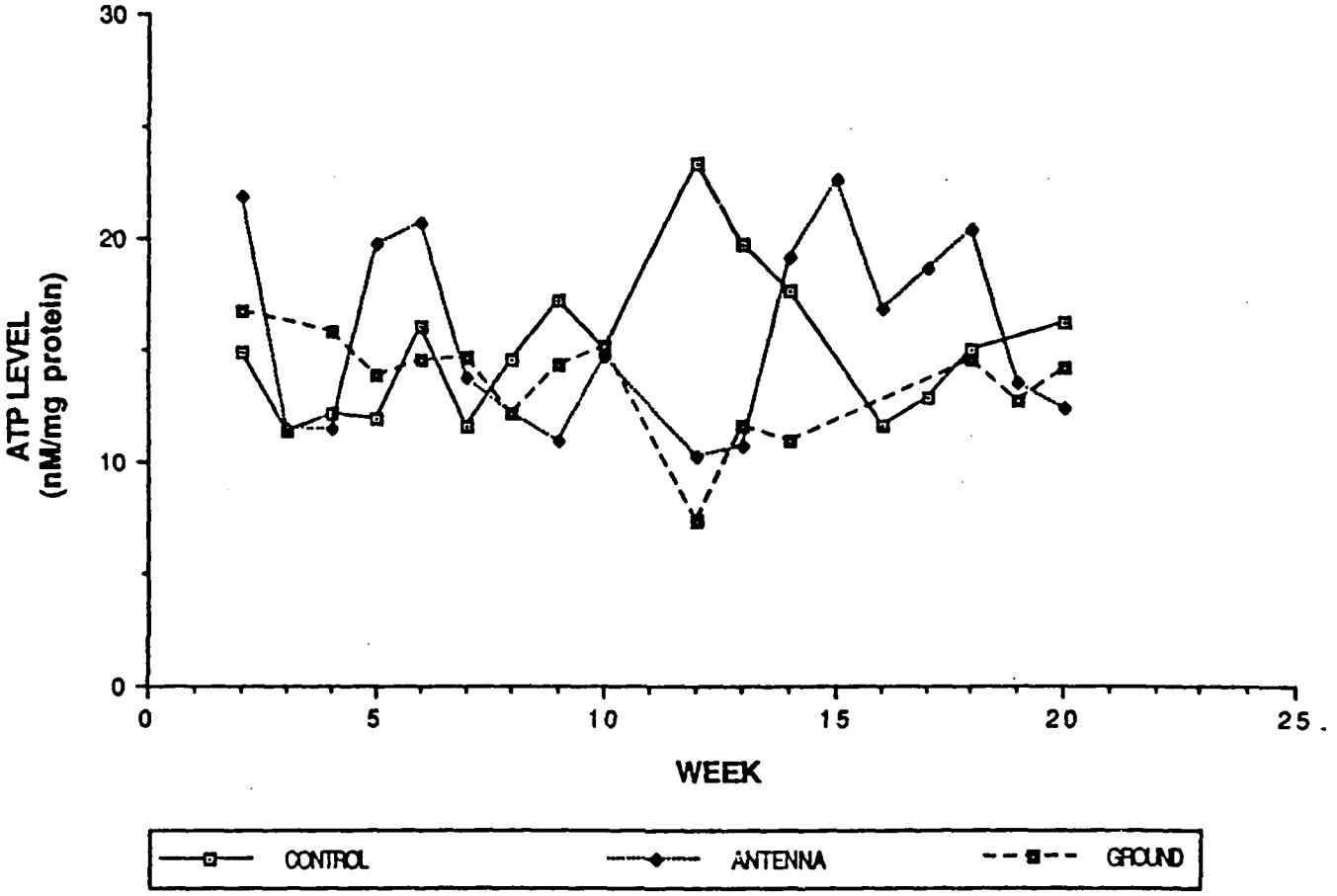

FIGURE 10.

SUMMARY OF ATP LEVELS FROM J-FIELD EXPOSURE AT THE WTF (1987)

A weekly summary of the ATP levels (nM ATP/mg protein) in plasmodia exposed to a matched current density (J-field) at the WTF. Each test site appears to show somewhat more independence with little coordinate tracking evident.

1987 WTF DATA

(E only)

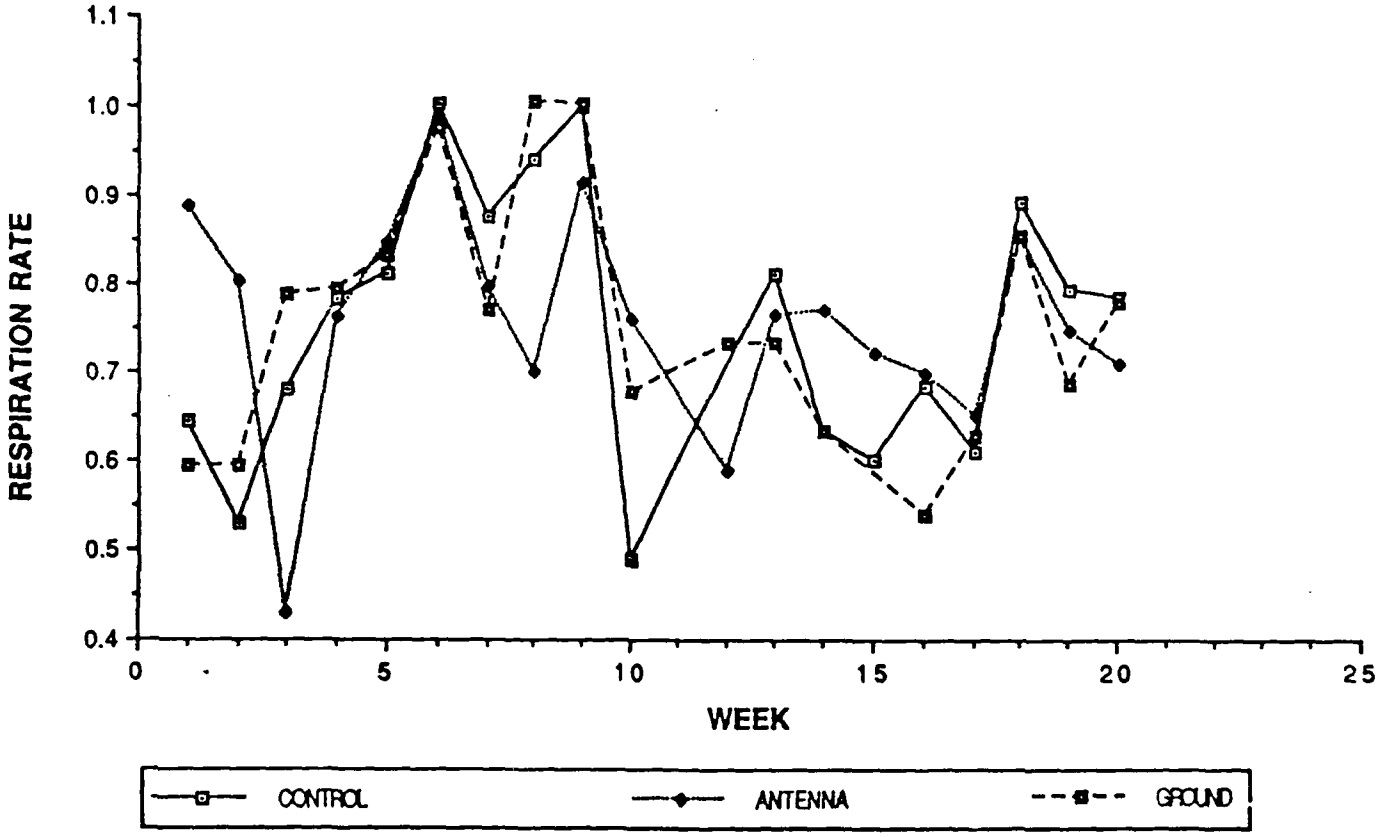

FIGURE 11.

SUMMARY OF RESPIRATION RATES FROM E-FIELD EXPOSURE AT THE WTF (1987)

A Weekly summary of the respiration rates or QO_2's (μl O_2 consumed/min/mg protein) in plasmodia exposed to a matched electric field (E-field) at the WTF. In general, the QO_2 levels appear to track together, although digressions are evident.

1987 WTF DATA

(J only)

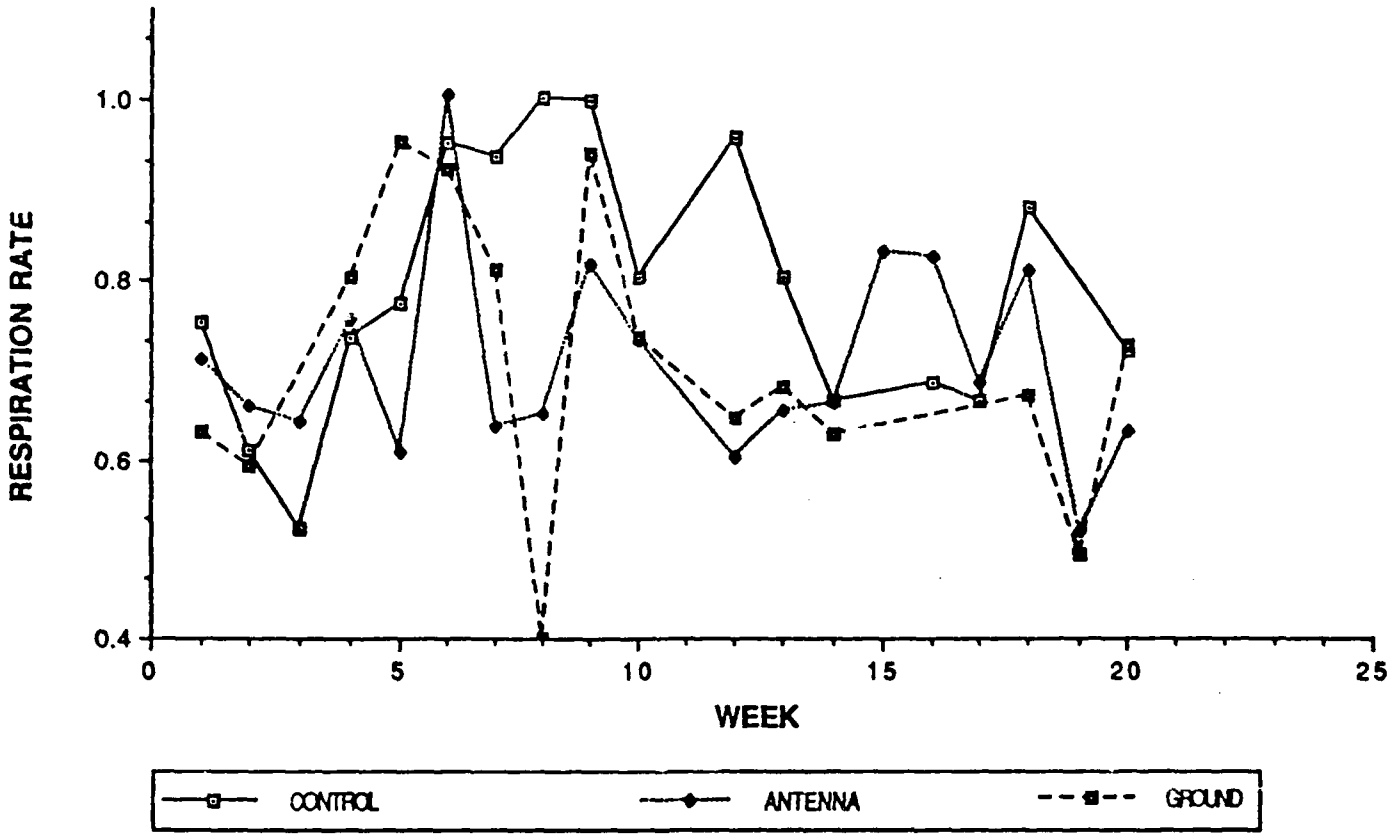

FIGURE 12.

SUMMARY OF RESPIRATION RATES FROM J-FIELD EXPOSURE AT THE WTF (1987)

A weekly summary of the respiration rates or QO_2's (μl O_2 consumed/min/mg protein) in plasmodia exposed to a matched current density (J-field) at the WTF. In general, the QO_2 levels do not track together.

TABLE 7

DATA SUMMARIES OF MEAN ATP AND QO_2 LEVELS IN CULTURES EXPOSED AT THE WTF SITES 1985–1987

		CONTROL SITE		ANTENNA SITE		GROUND SITE	
Year	Parameter	E-Field	J	E-Field	J	E-Field	J
1985	QO_2 [1]	.55 ± .05*	.59 ±.05*	.61 ± .07	.61 ± .04	.60 ± .04	.64 ± .04
	ATP [2]	23.2 ± 4.6	22.0 ± 2.7	33.5 ± 5.2*	23.3 ± 3.1	18.6 ± 3.1	22.3 ± 4.7
1986	QO_2	.76 ± .13*	.59 ± .02*	.58 ± .01*	.55 ± .03*	.56 ± .03*	.51 ± .04*
	ATP	22.6 ± 3.1*	22.7 ± 2.3*	20.0 ± 1.7	18.8 ± 1.6	19.4 ± 1.9	19.3 ± 1.4
1987	QO_2	.75 ± .04	.79 ± .03	.76 ± .03	.71 ± .03	.76 ± .03	.71 ± .04
	ATP	12.8* ± .80	15.1 ± .83	15.9 ± 1.3*	15.7 ± 1.0	14.0 ± .71	13.5* ± .65

Comparisons in a given year should be made between similar exposure conditions, i.e. compare E vs E and J vs J. An asterisk indicates a significant difference ($p < .05$) within a set.

1) μl O_2 consumed/min/mg protein

2) nM ATP/mg protein

Values following ± are standard error of the mean

E = Electric field
J = Current density

1986 WTF DATA

(E only)

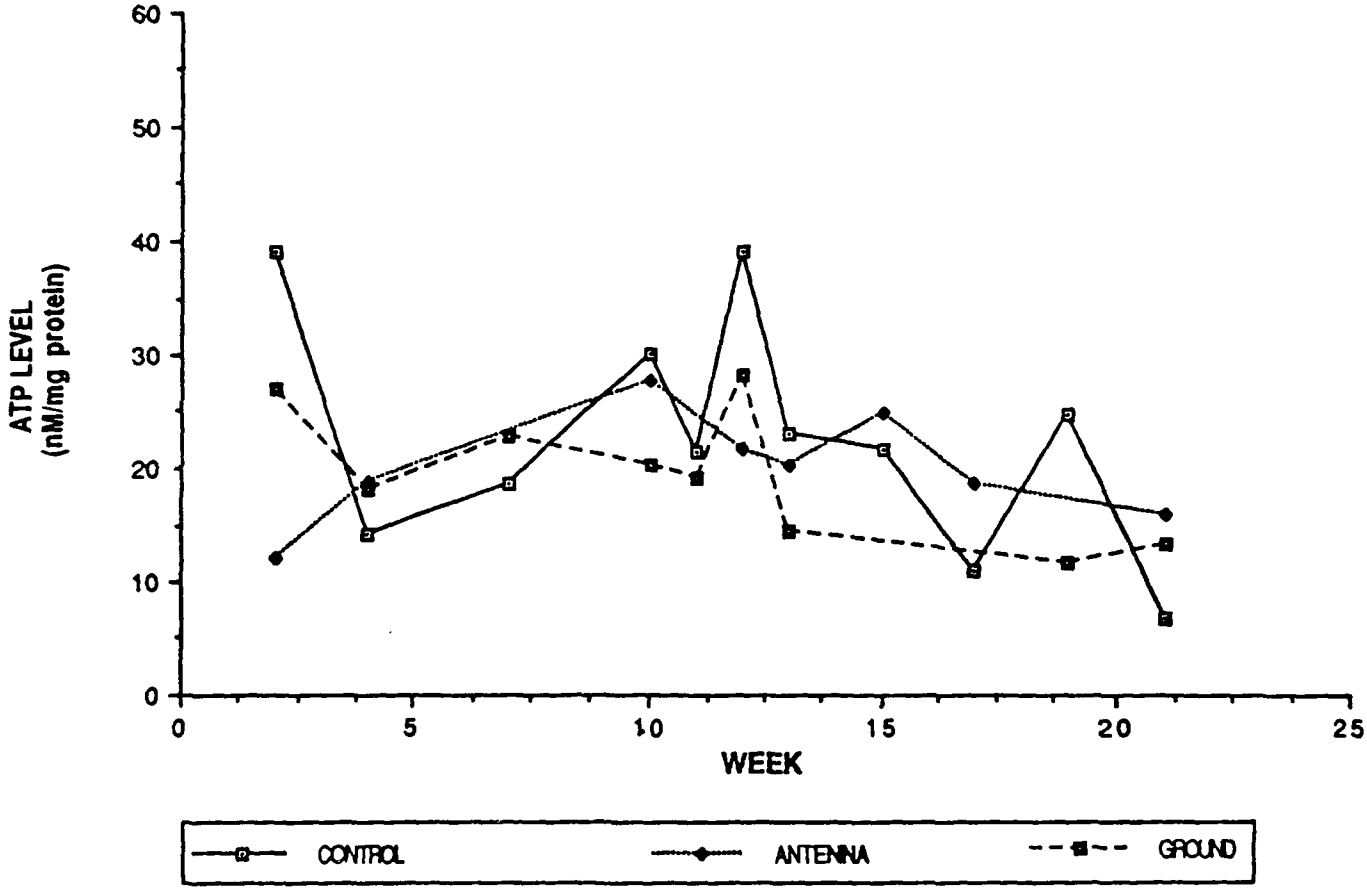

FIGURE 13.

SUMMARY OF ATP LEVELS FROM E-FIELD EXPOSURE AT THE WTF (1986)

A weekly summary of the ATP levels (nM ATP/mg protein) in plasmodia exposed to a matched electric field (E-field) at the WTF. In general, the ATP levels appear to track together, although digressions are most evident during the early and later exposure periods. It should be noted that the 1986 field season has fewer data points at the end of the season because trips to recover cultures were made biweekly.

1986 WTF DATA

(J)

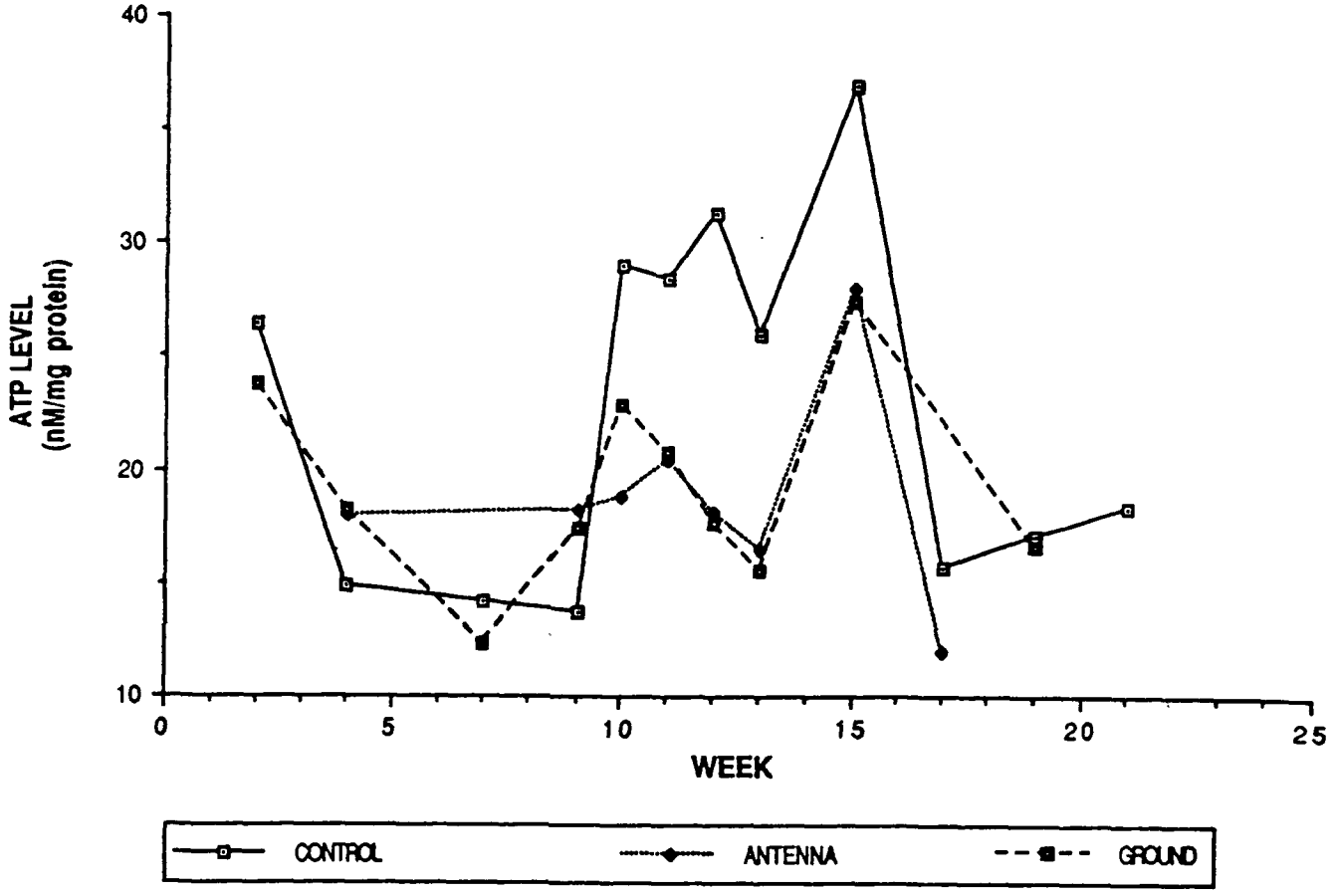

FIGURE 14.

SUMMARY OF ATP LEVELS FROM J-FIELD EXPOSURE AT THE WTF (1986)

A weekly summary of the ATP levels (nM ATP/mg protein) in plasmodia exposed to a matched current density (J-field) at the WTF. Each test site appears to show somewhat more independence with little coordinate tracking evident. It should be noted that the 1986 field season has fewer data points at the end of the season because trips to recover cultures were made biweekly.

1986 WTF DATA

(E cultures)

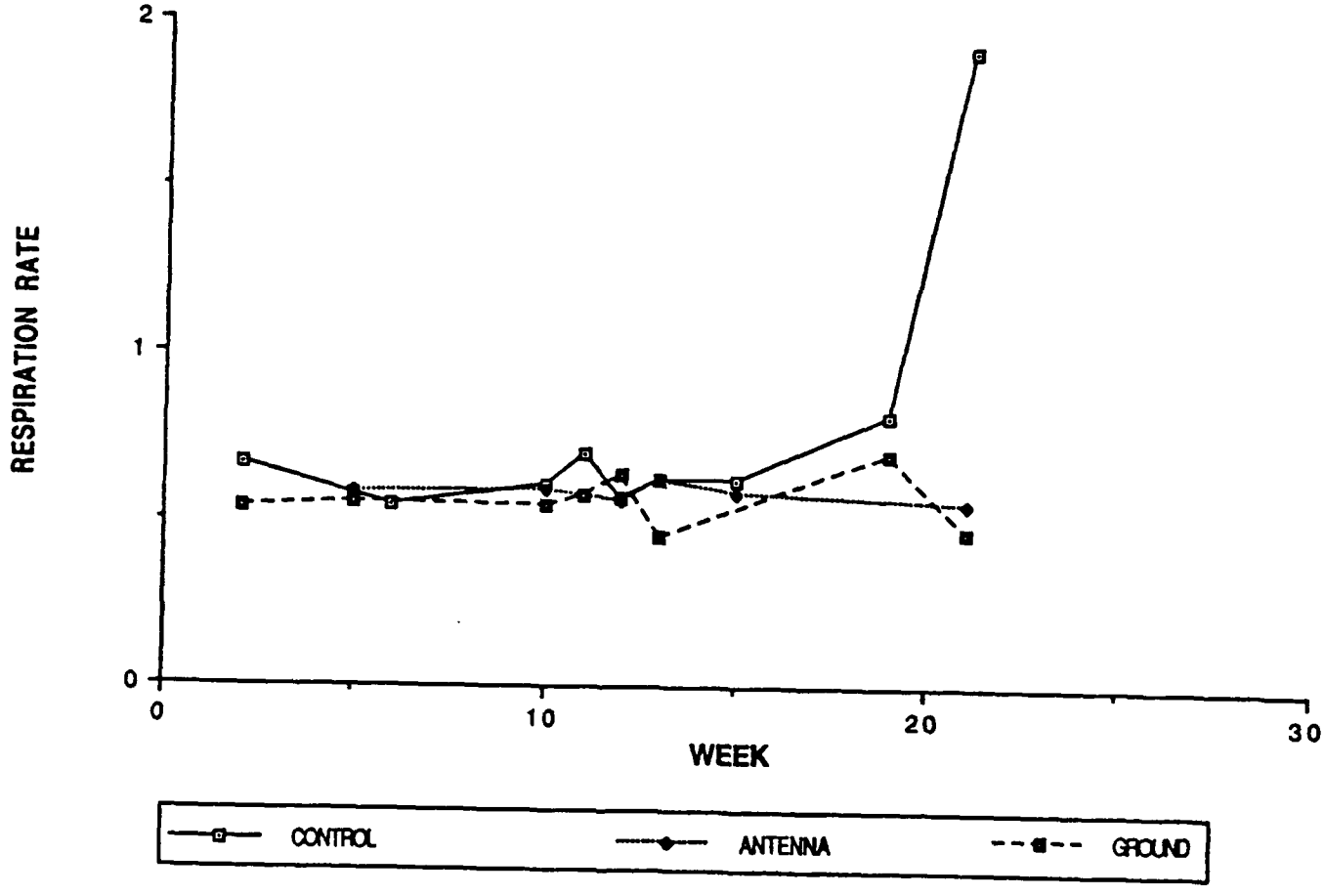

FIGURE 15.

SUMMARY OF RESPIRATION RATES FROM E-FIELD EXPOSURE AT THE WTF (1986)

A weekly summary of the respiration rates or QO_2's (μl O_2 consumed/min/mg protein) in plasmodia exposed to a matched electric field (E-field) at the WTF. In general, the QO_2 ;levels appear to track together showing small variations with the exception of the dramatic increase in the control QO_2 at the end of the field exposure period. It should be noted that the 1986 field season has fewer data points at the end of the season because trips to recover cultures were made biweekly.

1986 WTF DATA

(Current density)

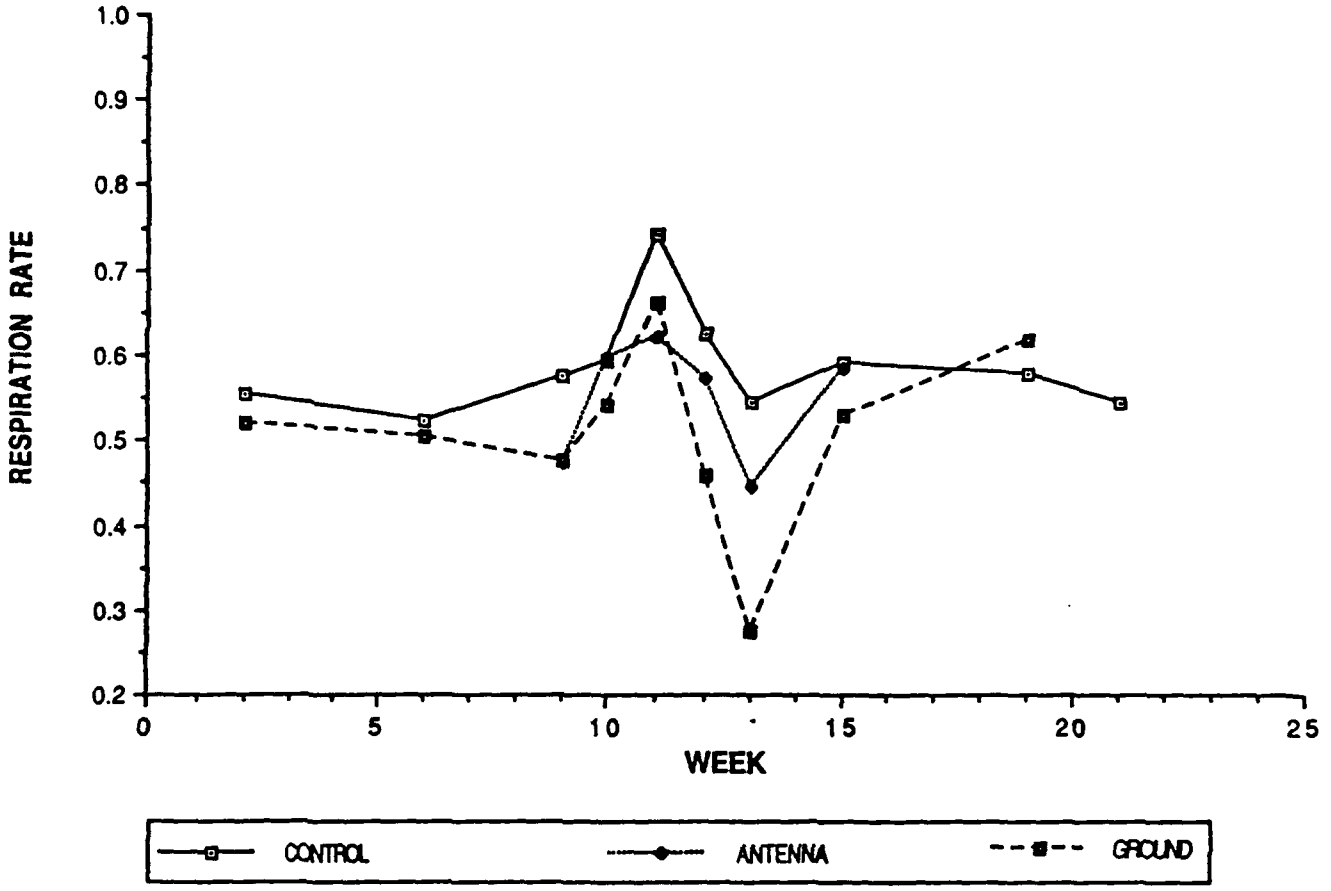

FIGURE 16.

SUMMARY OF RESPIRATION RATES FROM J-FIELD EXPOSURE AT THE WTF (1986)

A weekly summary of the respiration rates or QO_2's (μl O_2 consumed/min/mg protein) in plasmodia exposed to a matched current density (J-field) at the WTF. In general, the QO_2 ;levels appear to track together. It should be noted that the 1986 field season has fewer data points at the end of the season because trips to recover cultures were made biweekly.

1985 FIELD DATA

(Electric field exposure)

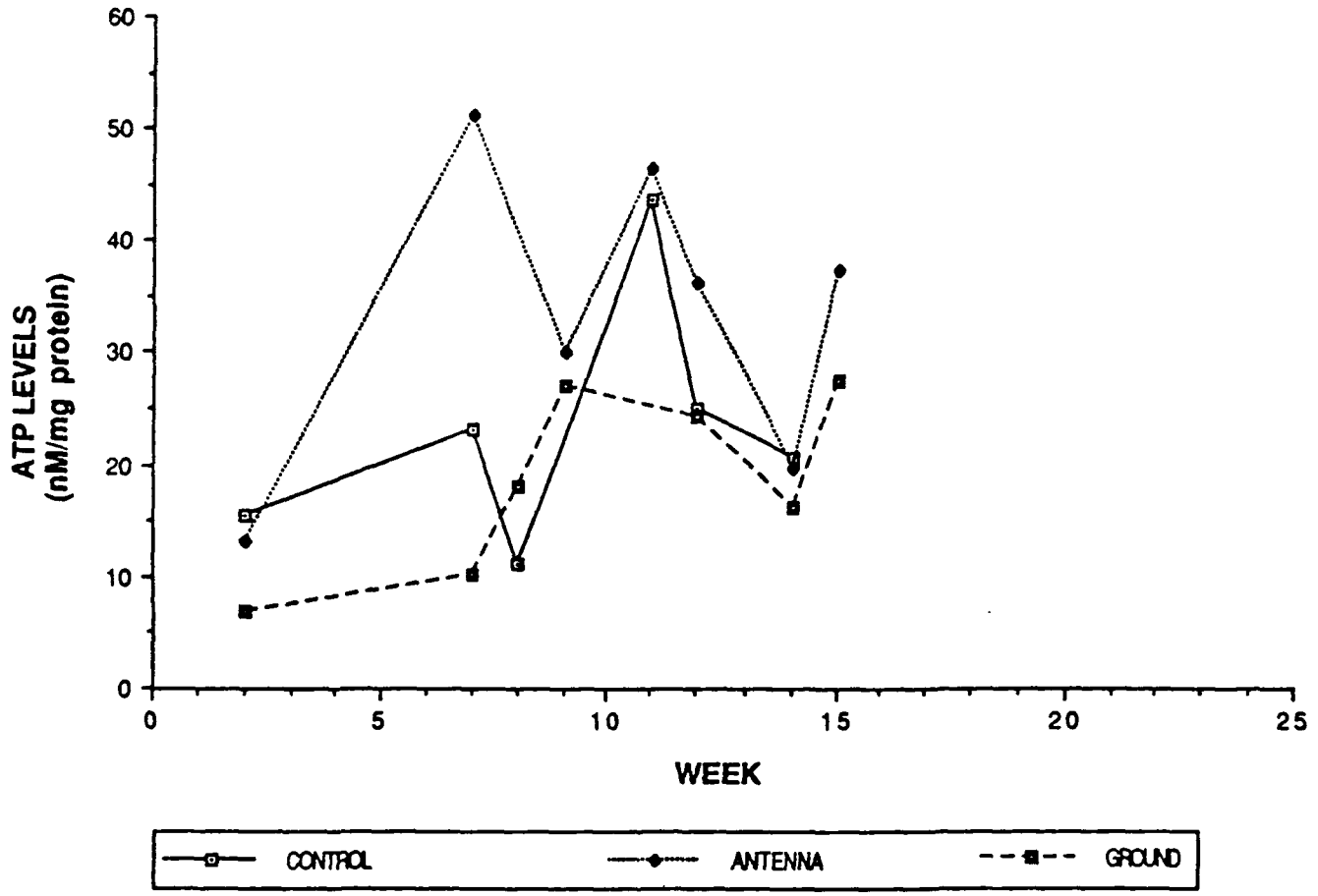

FIGURE 17.

SUMMARY OF ATP LEVELS FROM E-FIELD EXPOSURE AT THE WTF (1985)

A weekly summary of the ATP levels (nM ATP/mg protein) in plasmodia exposed to a matched electric field (E-field) at the WTF. In general, the ATP levels appear to track together. There are fewer data points because we were examining the feasibility of including an analysis of ATP in our protocol during 1985.

1985 WTF DATA

(Current density cultures)

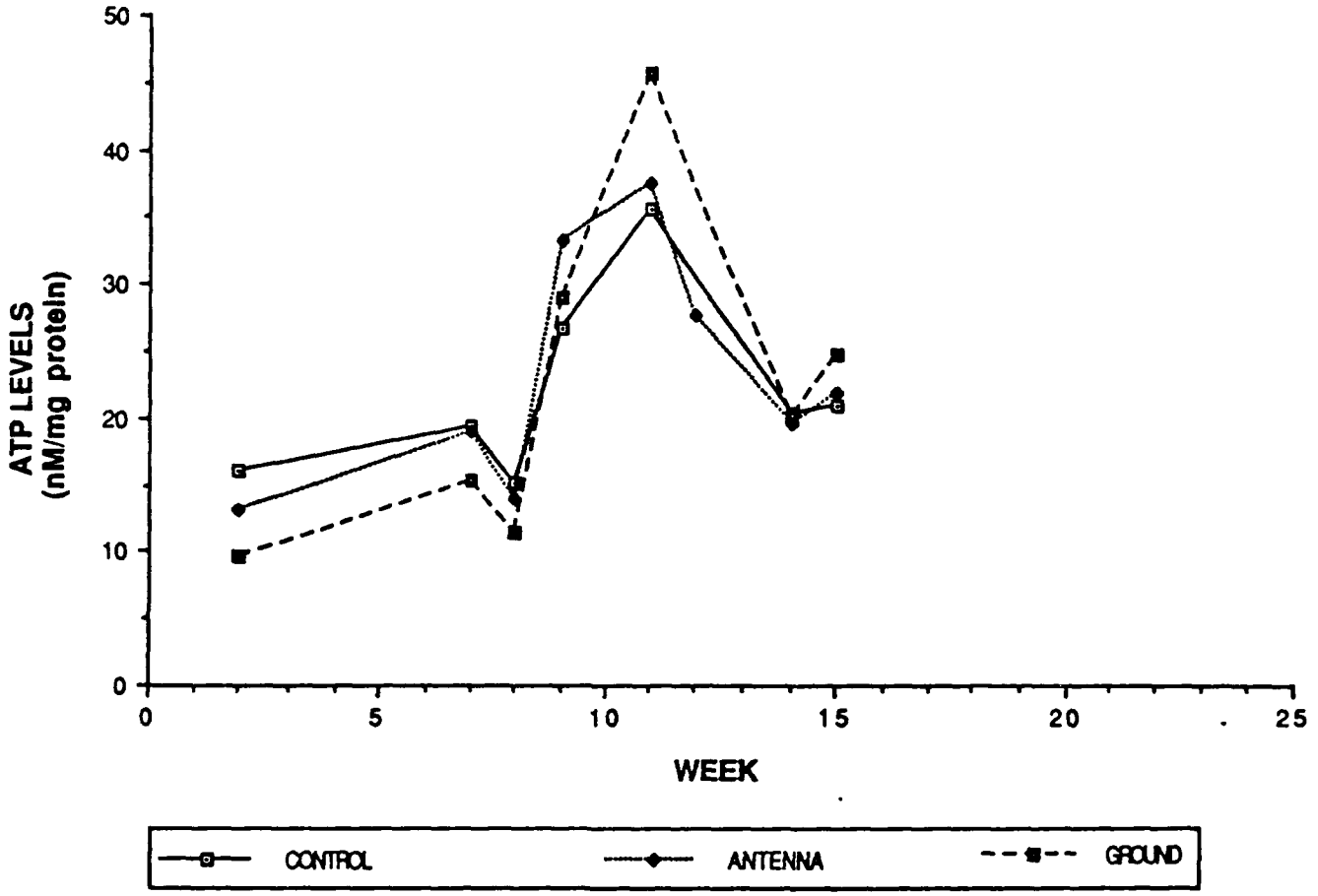

FIGURE 18.

SUMMARY OF ATP LEVELS FROM J-FIELD EXPOSURE AT THE WTF (1985)

A weekly summary of the ATP levels (nM ATP/mg protein) in plasmodia exposed to a matched current density (J-field) at the WTF. The ATP levels at each site track together. There are fewer data points because we were examining the feasibility of including an analyses of ATP in our protocol during 1985.

1985 WTF DATA

(Electric field)

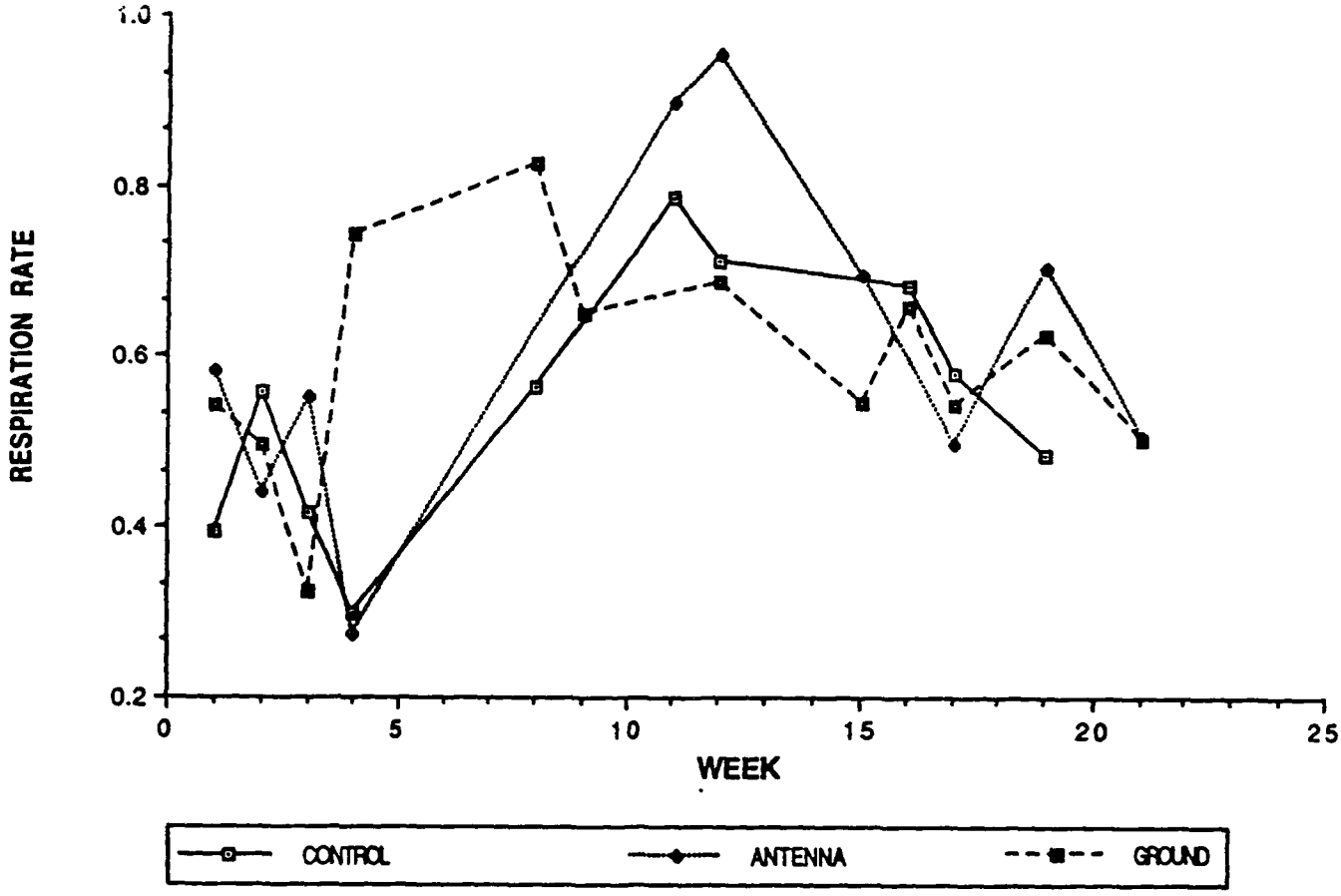

FIGURE 19.

SUMMARY OF RESPIRATION RATES FROM E-FIELD EXPOSURE AT THE WTF (1985)

A weekly summary of the respiration rates or QO_2's (μl O_2 consumed/min/mg protein) in plasmodia exposed to a matched electric field (E-field) at the WTF. It should be noted that the 1985 field season has fewer data points at the end of the season because trips to recover cultures were made biweekly.

1985 WTF DATA

(Current density)

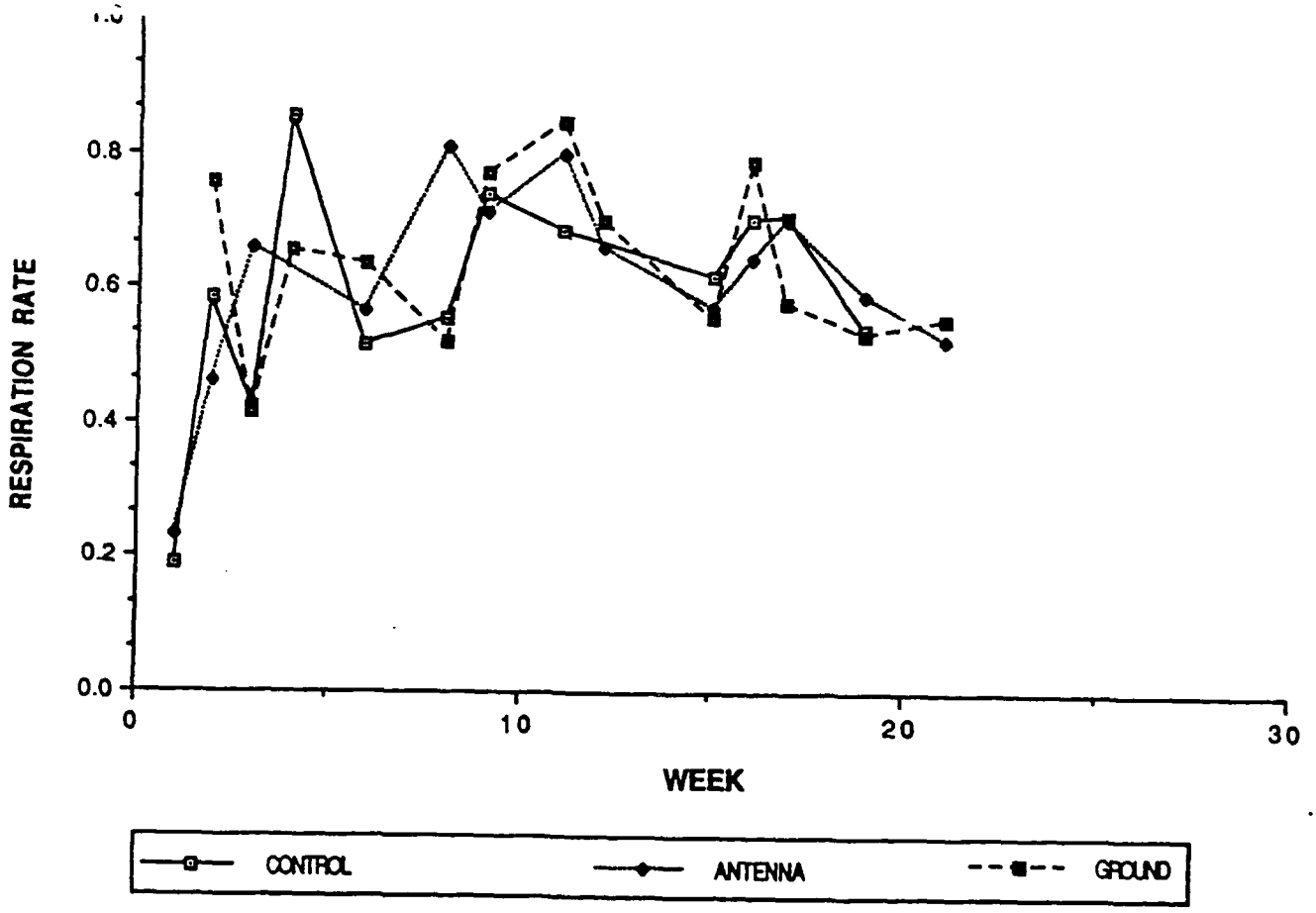

FIGURE 20.

SUMMARY OF RESPIRATION RATES FROM J-FIELD EXPOSURE AT THE WTF (1985)

A weekly summary of the respiration rates or QO_2's (μl O_2 consumed/min/mg protein) in plasmodia exposed to a matched current density (J-field) at the WTF. In general, the QO_2 ;levels track closer than the comparable E-field data (Fig. 19).

1985 WTF DATA

(Electric field)

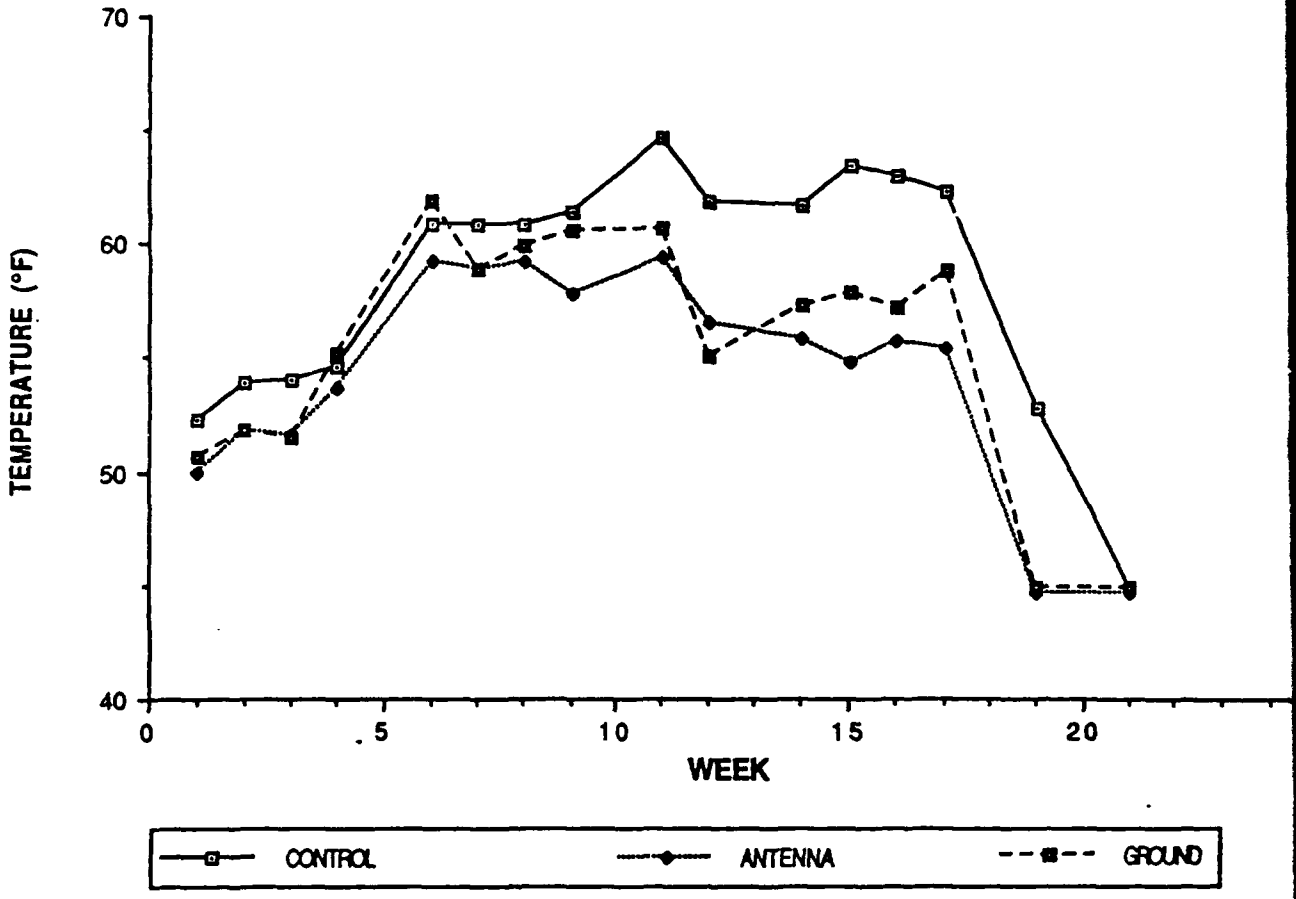

FIGURE 21.

SUMMARY OF TEMPERATURE AT THE EXPERIMENTAL AND CONTROL SITES (1985)

A weekly temperature summary at the Control, Antenna and Ground sites. A clear difference between the control and the two experimental sites emerges at week 11 and continues through the end of the field season.

1986 WTF DATA

(Current density)

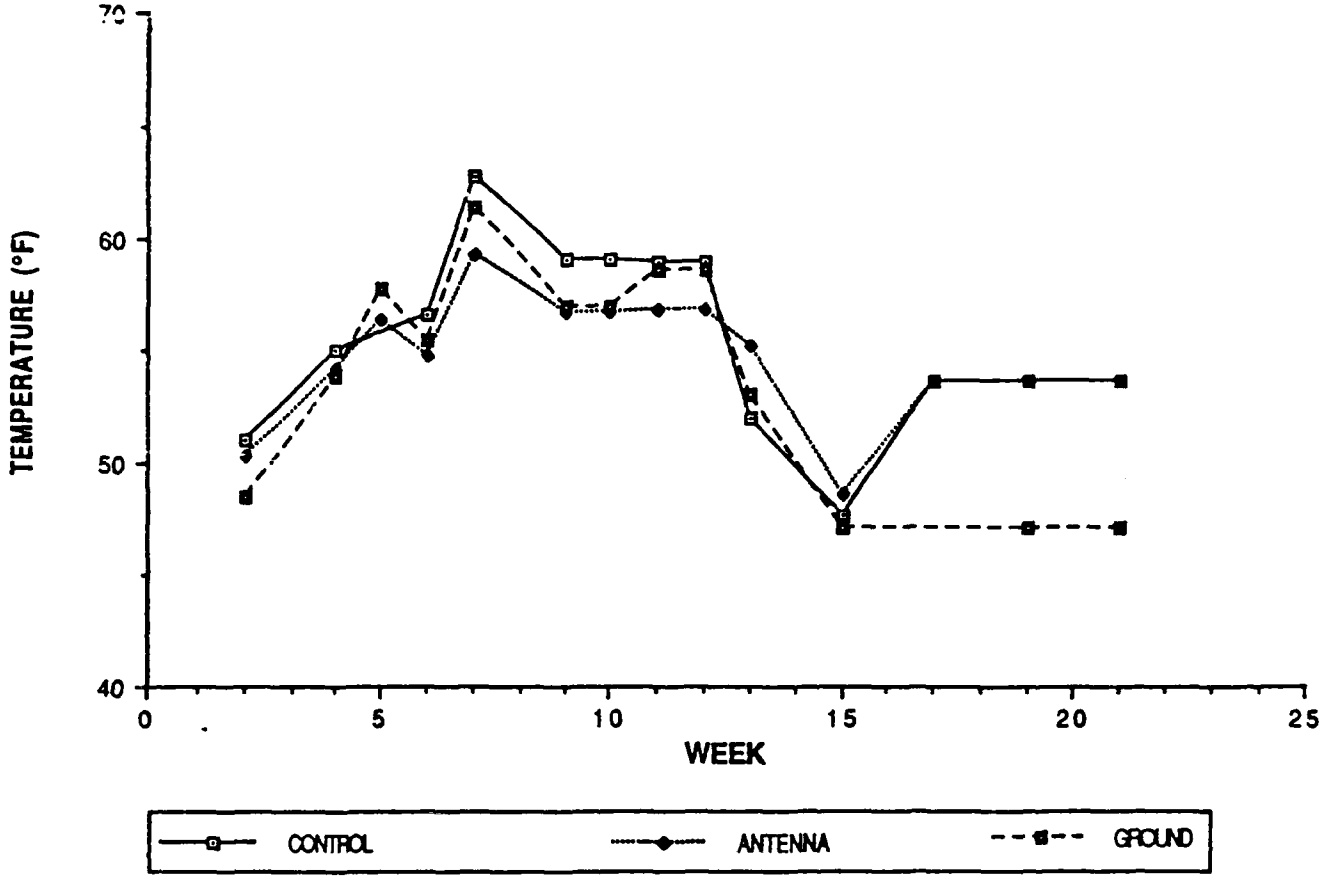

FIGURE 22.

SUMMARY OF TEMPERATURE AT THE EXPERIMENTAL AND CONTROL SITES (1986)

A weekly temperature summary at the Control, Antenna and Ground sites. The control site has a slightly higher temperature between weeks 7–12 whereupon the sites appear to track together.

environment than is ATP. That is, the QO_2 was significantly different at 48 72 and 96 hours. For this reason, only the first set of experimental data (48 hours after returning to UW-P) was used to test for potential EMF effects. The data obtained from the 1986 data set suggest that the cultures may be displaying a cumulative response with field exposure. These interactions are not evident in either the 1985 or 1987 data sets.

3. Exposure: One of the more difficult aspects to deal with in the field experiments involves the data obtained from continuous monitoring of the sites during 1987. These data show that the E and J fields were more dynamic than had been initially envisioned. Scrutiny of the data show both hourly and daily fluctuations in both E and J exposure; these fluctuations may in part be related to the changing conductivity of the soil. The conductivity changes can be related to the degree of moisture in the soil at any given time. Although the electric field components are constantly changing, the magnetic field exposure will remain essentially unchanged unless the antenna power is changed. One major difference between the 1987 exposure data and previous years is the paucity of information involving the E and J exposure in the time interval between culture transfers.

V. DISCUSSION

The data from both the laboratory and field components of this study show that the cultures are changing with time. These changes appear to be independent of the type of EMF exposure (E or J), the intensity of exposure (C, A, or G), or the seasonal temperature variations. One explanation for the change in the culture is that Physarum may be undergoing senescence, a phenomenon described by Hu et al.; 1985, and Clark, 1984. Senescence in Physarum has only been observed when cultures are maintained on agar for prolonged periods (>8weeks). This aging process can be reversed, up to a point, if the macroplasmodia (growing on agar) are converted to microplasmodia in shake suspension cultures. For unknown reasons, senescence does not occur in cultures maintained as microplasmodia. It is noteworthy that all of our previously published studies involving EMF effects on Physarum (where bioeffects were observed) used only the microplasmodial form. This is reinforced in early laboratory experiments in this program in which a 14 hour on/10 hr off cycle was used to mimic the early duty cycle of the antenna. These cultures were only grown as microplasmodia at about the same field strengths as used in 1985 and 1986. The latter experiments did find an alteration in the QO_2 levels with weak field exposure (Goodman et al. 1984). In the current study (1985-87), macroplasmodia were maintained on agar for both "long-term" (~17 weeks) field and laboratory experiments. As a result senescence may have inadvertently become an uncontrolled variable in the study. If the latter suggestion is correct, than it might explain some of the variability encountered in both ATP levels and respiration rates. For example, if upon returning the macroplasmodium to a microplasmodium (in a submerged shake flask liquid environment) the senescence process is either stopped or altered, then we might also be encountering these physiological changes in addition to any EMF effect. In view of the relatively small effects (10-15%) we previously observed on ATP and QO_2 levels (Goodman et al; 1988), any effect from WTF exposure might therefore be lost in the increased variability of the system.

Although there were substantial differences between the field and laboratory exposure regimens with respect to changing the WTF field intensities and temperature, these variables appeared to have had little impact on the final outcome of the experiments in a given year. For example, in 1987 the ATP levels and QO_2's were similar for both the lab (control and exposed) and field sites (control and G). These similarities are less evident in the 1985 data however; with the exception of the C site, the lab and field data are in reasonably close agreement.

The daily variability in EMF field exposure and temperature is easily seen by even a cursory examination of the data acquired by the IITRI data loggers. In these printouts, small changes can be seen virtually each hour the data was acquired (see Appendix III). In contrast, cultures in the lab were exposed to a constant electromagnetic field and relatively long term changes (weekly adjustments) in temperature. Despite these differences, both data sets suggest no extensive bio-effects occurred as a result of the weak field exposures employed in this study.

The question of why we were unable to detect a difference in the lab or field studies when we reported differences in our earlier published work may be explained in two ways. First, as noted above the original studies only employed submerged microplasmodia and experiments were always continued for longer time periods (>180 days). In this program, weather conditions in northern Wisconsin prevented experiments from being carried out for more than about 140 days. That is, if we attempted to start too early in May, the cultures would not grow because of the cold; the same rationale holds for growth during October. Secondly, most of the original lab experiments were performed at electromagnetic field intensities 5 to 10 times higher than were used in the current program. Finally, as discussed above, the culture methods were also substantially different. It is possible that the length of the cell cycle that occurs under different growth conditions may impact on the susceptibility of Physarum. For example, continuous exposure of microplasmodia with a cell cycle of about 8 hours may be more sensitive to EMF-perturbations than macroplasmodia (growing on agar) and whose cell cycle of about 12 to 15 hours (Goodman and Ritter 1969).

Examination of the 1985/86 laboratory data show reasonably good agreement in their level of ATP but not in their QO_2's; no consistently significant differences were found in either parameter. A comparison of the 1987 laboratory data with these earlier experiments also showed no differences, although the mean values for ATP in 1987 were lower than those in 1985/86. In contrast, the QO_2's for 1985 and 1987 were similar while those in 1986 were considerably lowered. One possible explanation for the variation in these data may be related to a small handling difference introduced in 1987 that was not employed in the other years. The change involved replacing the microplasmodia back onto the shaker after each measurement. This might have resulted in better aeration and thus higher values for the QO^2's, although it would appear to contradict the results obtained for ATP.

A somewhat different problem is encountered in the field data in which the 1985 (intermittent exposure) and 1987 both showed no differences in their ATP content or respiration rate. In contrast, the QO_2 and ATP data for macroplasmodia exposed at the WTF in 1986 both show significant decreases in their respective parameters. One possible though unsupported suggestion to explain these differences is that the conductivity of the soils may have been different. For example, it is a fact that the region had substantially less

rainfall during the 1986 field season. With decreased soil moisture, conductivity would decrease and the cells would be thus be exposed to lower E-fields.

VI. CONCLUSIONS:

1. The slime mold Physarum polycephalum was grown on agar for about 140 days during which time it was exposed to both the ambient electromagnetic fields at the Wisconsin Transmitting Facility and in the laboratory at field intensities similar to the ground site at the WTF. Although some differences were observed, the data show that both exposure conditions failed to induce any reproducible, statistically significant differences in either the cell's respiration rate or its ATP levels.

2. In general, data obtained from both the laboratory and the WTF are in agreement. These results are taken as indicating that the laboratory component is a reasonable predictor of potential field effects. It is also noteworthy, that the field and lab values obtained for Physarum's QO_2 and ATP were similar to those we have previously published.

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APPENDIX I.

ELECTROMAGNETIC FIELD ENVIRONMENT

EMF EXPOSURE

WTF ELECTROMAGNETIC FIELD ENVIRONMENT:

Because of the uncertainty as to whether the important electromagnetic field component was the magnetic field, electric field, electric current, or a combination of these three, the WTF field sites and exposure parameters were selected in an attempt to sort out this question. Sites were chosen in consultation with IITRI staff and in accord with the specifications of the overall ecological monitoring program protocols. The ambient fields from the 60 Hz power grid and other sources were to be no stronger than 1/10 the strength of the WTF fields at either the antenna or ground site and the ambient and WTF fields at the control site were to be within a factor of 10 of the ambient fields at the two exposure sites. (Exhibits from Haradem et al. 1988, and Brosh et al. 1985).

To prevent contamination by airborne molds and bacteria, it was necessary to grow the Physarum cultures in a sterile container that could not be penetrated by the external electric fields without the use of pickup electrodes and wires. The external magnetic fields easily penetrate the materials of the boxes and growth medium without distortion. Since Physarum grows naturally in moist, rotting leaves or fallen branches in the woods of northern Wisconsin, we exposed the growing macroplasmodia to fields and currents equivalent to those in the top layers of the earth. Thus the cells growing at the various sites were subjected to a realistic exposure. However, the conductivity of the culture medium is greater than that of the earth (and probably greater or at best equal to than that of the rotting leaves), so that establishing in the culture medium the electric field strength of the earth will establish a higher current density than that in the earth. Since it is unclear from the literature whether electric field strength or current density is the appropriate measure of electric field exposure two types of experiments were performed at each site. In one type cultures were exposed to electric fields and in the other to current densities equal to those in the nearby earth. Control circuitry and operational protocols to allow this matching were designed and added to the external exposure containers by IITRI (Ex.3 from Haradem et al. 1988).

In 1985 and thereafter, the electric field and current density in the earth were matched in separate chambers. Planar electrodes were placed in the earth about 2 m apart along the direction of maximum electric field strength and connected to circuitry in the culture chamber containers that allowed adjustment of the applied field strength or applied current density to that measured in the surrounding earth at the time of transfer of the cultures (see Ex. 3). These adjustments were made each week, except when the antenna was not operating at the time of transfer.

In the fall of 1982 the investigators and an IITRI team began to measure the electromagnetic environment of potential test sites. Two control, two ground, and one antenna site were subjected to careful field analyses. In 1983, one control (IITRI designation 7C1, (see Figure 1, report), one antenna (7A1), and one ground site (7G3) were remeasured; and pits for the culture chambers were dug, electrodes buried, and wires run from the electrodes to the pits to connect to the chambers. In 1984, the antenna site was found to have an ambient 60 Hz field environment that was slightly too large, and it was replaced by site 7A2. Sites 7A2, 7G3, and 7C1 were used for the remainder of the project.

During the 1983 and 1984 field seasons, the electric field across the culture chambers was generated by a pair of planar copper electrodes in the earth, separated by a distance of about 30 cm and oriented perpendicular to the electric field lines. These buried electrodes were connected directly to the culture chamber electrodes. Field strengths are summarized in Exhibit 4. (from Haradem et al. 1988).

During the early field years (1981-84), most of the field efforts were directed towards developing both techniques to maintain the culture in an axenic state in the forest, and protocols to handle the cultures before and after exposure to weak fields. Tables 1.1A, 1.1B and Exhibit 5 (Haradem et al.,1988) summarize the antenna operation for the summer months of 1986-1988. Since the Antenna and Ground sites lie along the East-West leg, periods when only the North-South leg was energized have been omitted from the table; in general this leg was energized by itself less often than the East-West one. In 1986 and 1987, the antenna was operating most of the time with both legs energized. Most of the remaining time in both years was spent with the antenna fully off, though a percentage point or two of the time had only one leg on. In 1985 as well, with the exception of July, another 27% of the time was spent with only the East-West leg (along which the antenna and ground sites lay) energized. Prior to 1985, the majority of the time was spent with neither leg energized (Haradem et al. 1988). It is interesting to note that the number of times the operating condition of the antenna were adjusted in 1986 greatly exceeded that of 1987, while the number of adjustments in 1985 with both legs on was also smaller than the number of 1986 adjustments in that configuration. There was a large number of additional adjustments in 1985 with only the East-West leg on, although most of these were in May and June when the leg was operating only for very short periods of time.

LABORATORY ELECTROMAGNETIC FIELD ENVIRONMENT:

1987: Both ambient magnetic fields inside the laboratory incubators and those generated by the exposure apparatus were assessed by IITRI and monitored periodically by the investigators. As a result of 1987 measurements of the applied and ambient fields in the laboratory, several adjustments in the applied fields were made to ensure that the environment of the laboratory cultures was as close as possible to that at the WTF ground site. The choice was made to emulate the ground site because that site had the highest electric field, and until very recently the general wisdom in much of the bioelectromagnetic community has been that the electric, rather than the magnetic fields are the element of chief concern.

In the year ending October 30, 1987, the lab exposures used applied fields that differed from those described in the 1984 IITRI report, although the ambient fields remained the same. Applied magnetic fields continued to be monitored by the UWP team using both the Bell Model 640 Hall-Effect Gaussmeter and a newly acquired Monitor Industries (Boulder, Colorado) Model 42A-1 gaussmeter. The Monitor gaussmeter operates on the principle of detecting the voltage induced in a 15 cm dia. coil, and is sensitive to 5×10^{-8} T (full-scale reading of most sensitive scale). The two gaussmeters were cross-calibrated at the 0.1 mT level. The Monitor gaussmeter has a flat frequency response in the ELF region and reads total rms field strength; the signal waveform was always displayed on an oscilloscope to check its frequency and determine whether there were significant higher harmonics. Electric fields continued to be applied using the technique of applying a voltage directly across the culture medium; the field strength in this instance can be directly

calculated. Earlier reports have discussed our direct measurements of electric field strength that have confirmed these calculations.

In 1986 and 1987 the incubator simulating WTF exposure continued to be used for exposures to a 76 Hz MSK-modulated signal produced by the standard IITRI-supplied generator and amplified by the Elgar amplifiers. The control incubator for these exposures was located in the adjoining room; dummy loads to simulate the electric field-exciting network were connected to the control cultures' growth chambers. For 1987, fields in the exposure chamber were adjusted to simulate the fields at the ground test site of the Wisconsin Test Facility. The 76 Hz MSK magnetic field was 0.7 (+_ 0.1) uT. Two rectangular (18 x 9.5 cm) culture chambers were used, with electric potential applied across parallel electrodes at opposite ends of the chamber to create an electric field parallel to the long dimension. In one chamber the culture was exposed to an electric field equal in magnitude to that in the earth at the WTF; in the other the exposure was to a current density equal to that in the earth at the WTF. The difference in conductivity between earth and the culture medium meant that both electric field and current density in the earth could not be duplicated simultaneously. The situation in the laboratory duplicated the situation in the test chambers at the WTF, where separate chambers were set up at the earths electric field intensity and at the earth's current density. The electric field-matching chamber was set to 0.8 V/m (0.16 A/m^2); the current density-matching one to 0.01 V/m (0.002 A/m^2).

Ambient and applied fields in the control and exposure incubators may be taken from the above-mentioned 1987 and previous IITRI measurements (Tables 2 and 3). The field measurements were taken prior to laboratory rearrangements to mitigate stray fields from another exposure apparatus. The ambient magnetic field, including crosstalk, in WTF test chamber at the location of the exposed culture boxes ($/B^2/$) was 0.1 uT; since the harmonic content was quite small, the frequency was almost entirely 60 Hz. In the control incubator, the ambient magnetic field intensity was 0.5 uT, again essentially all at 60 Hz.

An additional ambient field measurement was made at the moving table of the shaker used to convert macroplasmodia to microplasmodia. Since this position was near another exposure incubator some crosstalk from its 60 Hz magnetic field generating coils was detected; the addition of magnetic shielding material reduced the fields by a factor of two to a level of 0.15 uT. In addition the shaker motor generated a 0.3 uT magnetic field at essentially 60 Hz. The net magnetic field magnitude was measured to be 0.3 uT. It should be emphasized that exposed and control cultures experienced these background fields equally and simultaneously during the time when they were being grown in suspension culture to prepare them for the QO_2 and ATP assays.

Prior Years: From the beginning of the current program (7/81) through February 1984 the laboratory incubator exposures levels were those previously used in other laboratory experiments, that is, 0.1 mT magnetic fields and 1.0 V/m electric fields using a 75 Hz cw sinusoidal signal. In 1984, the signal was changed to a 76 Hz_{MSK} modulated field at 0.1mT and 1.0 V/m; the signal generator was supplied by IITRI. In May, 1985 the field levels were changed to approximate the highest levels of each type of field found at any of the WTF sites. The resulting laboratory exposure conditions were therefore based on realistic field exposure conditions, but were a hybrid of the field situation exposed cultures to a more severe field combination than would be

found in the field. The rationale for these field settings was that if no laboratory results were detectable at these exposure levels, it would not be reasonable to think that field results were within the range of detectability of our techniques. The field intensities used were a magnetic field of 17.5 uT similar to that found at the antenna (A) site and an electric field 0.8 V/m or a current density of 0.002 A/m^2 similar to the field/current density level found at the ground (G) site. As noted above, the magnetic field was decreased for 1987.

These field levels were monitored periodically by the experimental team using the Hall-effect probe discussed above. In addition, IITRI staff measured fields, field uniformity, and background fields in 1984 and, as noted above, in 1987. (J. Gauger, personal communication).

TABLE I-1 A

SUMMARY OF WTF OPERATIONS WITH BOTH ANTENNAE ENERGIZED

		May	June	July	Aug.	Sept.	Monthly Avg.
1985	A.	0.08	0.38	0.31	0.10	0.23	0.22
	B.	55	69	15	46	20	41
1986	A.	0.89	0.89	0.93	0.85	0.94	0.90
	B.	112	72	66	42	63	71
1987	A.	0.96	0.96	0.94	0.79	0.97	0.92
	B.	13	14	24	23	13	17

A = Fraction of all possible hours WTF operated with both antennae energized in each month.

B = Number of changes of operating mode with both antennae powered (e.g., switch-on and changes in phase frequency, intensity, etc.). Excludes switch-off or changes with only one antenna powered.

TABLE I-1 B

SUMMARY OF WTF OPERATIONS WITH EAST-WEST ANTENNA ONLY ENERGIZED

		May	June	July	Aug.	Sept.	Monthly Avg.
1985	A.	0.09	0.04	0.27	0.02	0.01	0.09
	B.	143	273	31	59	45	100
1986	A.	0.01	0.00	0.00	0.00	0.01	0.00
	B.	65	32	20	5	8	26
1987	A.	0.02	0.01	0.01	0.00	0.00	0.01
	B.	67	6	9	5	3	18

A = Fraction of all possible hours WTF operated with only East-West antenna energized in each month.

B = Number of changes of operating mode with only East-West antenna powered (e.g. switch-on and changes in phase frequency, intensity, etc.). Excludes switch-off or changes with both or with only North-South antenna powered.

Table I–2

Summary of Laboratory Exposure Conditions

Year	Freq. (Hz)	Applied Magnetic Field (mT)	Applied Electric Field (V/m)	Applied Current Density (A/m2)	Ambient Magnetic (60 Hz) (mT)		Ambient Electric (60 Hz) V/m)	
					Exposed	Control	Exposed	Control
1981–83	75cw	0.1	1.0	n/a	*	*	*	*
1984	76msk[a)]	0.1	1.0	n/a	0.0003	0.0002	0.0005	0.0001
1989–June '87	76 msk	0.0175	0.8	0.002	*	*	*	*
from June '87	76 msk	0.0007	0.8	0.002	0.0001	0.0005	*	*

a) "Minimum shift keying"

* Not measured in these years

EXHIBIT 1 - 1

EM EXPOSURE CRITERIA

Because the electromagnetic (EM) intensity and operational characteristics required to produce a bioeffect are not known, EM exposure criteria were established to assist investigators in selecting study sites. The exposure criteria ensure that the 76 Hz EM fields at a test site are significantly larger than the 76 Hz EM fields at the control site, the 60 Hz fields at the test site, and the 60 Hz fields at the control site. In addition, the exposure criteria verify that there is not a substantial difference in the ambient 60 Hz EM field between the test and control sites.

The EM exposure criteria used in site selection are expressed in equation form as follows:

$$T\ (76\ Hz) / C\ (76\ Hz) > 10 \qquad (1)$$

$$T\ (76\ Hz) / T\ (60\ Hz) > 10 \qquad (2)$$

$$T\ (76\ Hz) / C\ (60\ Hz) > 10 \qquad (3)$$

$$0.1 < T\ (60\ Hz) / C\ (60\ Hz) < 10 \qquad (4)$$

where: T (76 Hz) = Test site exposure due to ELF system
T (60 Hz) = Test site exposure due to power lines
C (76 Hz) = Control site exposure due to ELF system
C (60 Hz) = Control site exposure due to power lines

Based on the exposure assessment, each possible test and control site pairing was classified as acceptable, conditionally acceptable, or unacceptable. These categories are defined as follows:

> Acceptable. A test/control site pair was placed in this category if it satisfied all four EM exposure inequalities for each of the EM fields applicable to the study. For example, the small mammals and nesting birds studies would be concerned with both the soil and air electric fields as well as the magnetic fields. The soil arthropods and earthworms studies, however, would not be concerned with the electric field in the air, since this field terminates at the earth's surface and would not be expected to impact biota existing in the soil or litter layer.

Conditionally Acceptable. A test/control site pair was placed in this category if it approached, but did not meet, the criteria for acceptability. This category was established since the EM exposure criteria were not rigidly defined. The assumption that a difference of one order of magnitude or more would constitute a significant difference between test and control sites was chosen for these studies, but without knowing what effects will be experienced, if any. It is difficult to define this difference a priori. Furthermore, the EM field measurements themselves encompass a certain degree of error, as do any physical measurements.

Unacceptable. A test/control site pair was placed in this category if it neither satisfied the criteria for acceptability nor qualified for conditional acceptability.

EXHIBIT 2-1

TABLE G-3. EXTERNAL ELECTRIC FIELD INTENSITIES AND MAGNETIC FLUX DENSITIES[1,2]
Slime Mold Studies

Site No.	Meas. Pt.	Meas. Yr.	Transverse E Field (Air), V/M		Longitudinal E Field (Earth), mV/M		Magnetic Flux Density, mG	
			76 Hz	60 Hz	76 Hz	60 Hz	76 Hz	60 Hz
7A1	1	1982	--	--	188.0	0.092	184.0	0.033
7A1	1	1983[a]	--	--	157.0	0.116	163.0	0.077
7A1	1	1983[b]	--	--	164.0	0.13	149.0	0.019
7A1	1	1984	0.17	<0.001	156.0	0.11	153.0	0.030
7A2	1	1984	--	--	204.0	0.035	45.0	0.002
7A2	1	1984	0.035	<0.001	239.0	0.052	314.0	<0.001
7C1	1	1982	--	--	1.8	0.062	0.026	0.002
7C1	1	1983	--	--	1.9	0.070	0.025	<0.001
7C1	1	1984	--	--	2.2	0.099	0.025	<0.001
7G3	1	1983	--	--	1860.0	0.091	5.2	<0.001
7G3	1	1984	1.5	<0.001	1510.0	0.13	5.6	0.001

[1] Values shown are magnitudes determined as the square root of the sum of the squares of the orthogonal field components measured.

[a] Before hole was dug.

[b] After hole was dug.

TABLE G-4. RATIO MAGNITUDES FOR EXTERNAL FIELDS[1]
Slime Mold Studies

Compared: Site No.	Transverse E Field (Air)				Longitudinal E Field (Earth)				Magnetic Flux Density			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
7A1/7C1	170	170	170	1.000	71	1418	1576	1.111	6120	5100	153000	30.000*
7A2/7C1	>35	>35	>35	1.000	>93	>3923	>2061	0.354-0.525	>1800	>22500	>45000	1.000-2.000
7G3/7C1	1500	15000	15000	1.000	686	11615	15253	1.313	224	5600	5600	1.000

[1] $R_1 = \frac{T(76\ Hz)}{C(76\ Hz)}$, $R_1 \geq 10$

$R_2 = \frac{T(76\ Hz)}{T(60)}$, $R_2 \geq 10$

$R_3 = \frac{T(76\ Hz)}{C(60)}$, $R_3 \geq 10$

$R_4 = \frac{T(60)}{C(60)}$, $0.1 \leq R_4 \leq 10$.

When a range of values were available for calculating a given ratio, the ratio range was calculated as follows:

High Limit ---> Maximum Numerator Value/Minimum Denominator Value
Low Limit ---> Minimum Numerator Value/Maximum Denominator Value

* Does not meet the exposure criteria.

EXHIBIT 2-3

TABLE G-5. CULTURE CHAMBER AND EARTH ELECTRIC FIELD INTENSITIES (76 Hz)
Slime Mold Studies

Site No.	Chamber	Meas. Yr.	Chamber Voltage, mV	Chamber E Field, mV/m	Longitudinal E Field (Earth)[1], mV/m
7A1	West(A)	1984	4.8	30.97	166.25±14.93
	East(B)		13.5	87.10	
7A2	West	1984	3.0	19.35	221.50±24.75
	East		4.2	27.10	
7C1	#1	1984	0.030	0.19	1.97±0.21
	#2		0.010	0.06	
7G3	North	1984	14.5	93.55	1,685±247.49
	South		16.7	107.74	

[1] Mean ± standard deviation for 1982-1984 data, see Table 1.

The electromagnetic field exposure criteria were also applied to the electric field inside the chamber by computing the field intensity ratios utilized in mathematical representation of exposure criteria (see Section 2.1). The results are of this effort illustrated in Table G-6.

The investigator can pair either of the test sites with the control site and have an acceptable site pair in terms of EM exposure.

EXHIBIT 2-4

TABLE G-6. EXPOSURE RATIO FOR CULTURE CHAMBER
Slime Mold Studies

Compared: Chamber No.	Electric Fields[1] R1	R2	R3	R4
7A1 West(A)/7C1#1	160	4800	4364	0.909*[1]
7A1 West(A)/7C1#2	480	4800	9600	2.000
7A1 East/7C1#1	450	13500	12273	0.909*
7A1 East/7C1#2	1350	13500	27000	2.000
7A2 West/7C1#1	100	3000	2727	0.909*
7A2 West/7C1#2	300	3000	6000	2.000
7A2 East/7C1#1	140	4200	3818	0.909*
7A2 East/7C1#2	420	4200	8400	2.000
7G3 North/7C1#1	483	14500	13182	0.909*
7G3 North/7C1#2	1450	14500	29000	2.000
7G3 South/7C1#1	557	16700	15182	0.909*
7G3 South/7C1#2	1670	16700	33400	2.000

[1] $R_1 = \frac{T(76\ Hz)}{C(76\ Hz)}$, $R_1 \geq 10$

$R_2 = \frac{T(76\ Hz)}{T(60)}$, $R_2 \geq 10$

$R_3 = \frac{T(76\ Hz)}{C(60)}$, $R_3 \geq 10$

$R_4 = \frac{T(60)}{C(60)}$, $0.1 \leq R_4 \leq 10$.[1]

When a range of values were available for calculating a given ratio, the ratio range was calculated as follows:

High Limit ---> Maximum Numerator Value/Minimum Denominator Value
Low Limit ---> Minimum Numerator Value/Maximum Denominator Value

* Does not meet the exposure criteria.

[1](Note: These asterisks are in error according to the criteria.)

EXHIBIT 3-1

EXPOSURE SETUP PROTOCOLS FOR SLIME MOLD STUDIES

MATCHED ELECTRIC FIELD PROTOCOL

(1) Measure maximum electric field in soil with 1 meter probe, E.

(2) Multiply electric field value by 0.2 to determine the minimum required chamber voltage, V_{CH} (min).

$$V_{CH} \text{ (min)} = E \times 0.2 \text{ (volts)}$$

(3) Locate collector electrodes in line with maximum electric field in the earth and spaced far enough apart to generate a voltage across a 1000 ohm resistor that is greater than or equal to V_{CH} (min) (see Figure L-4).

(4) Measure and record electrode spacing and open circuit (no load) voltage, V_{OC}.

(5) Connect test chamber to electrodes. Connect voltmeter to measure voltage across test cell, V_{CL} (see Figure L-5). Adjust variable resistor (Pot) so that the voltage across the test cell is equal to V_{CL} as determined by the formula:

$$V_{CL} = E \times 0.155 \text{ (volts)}$$

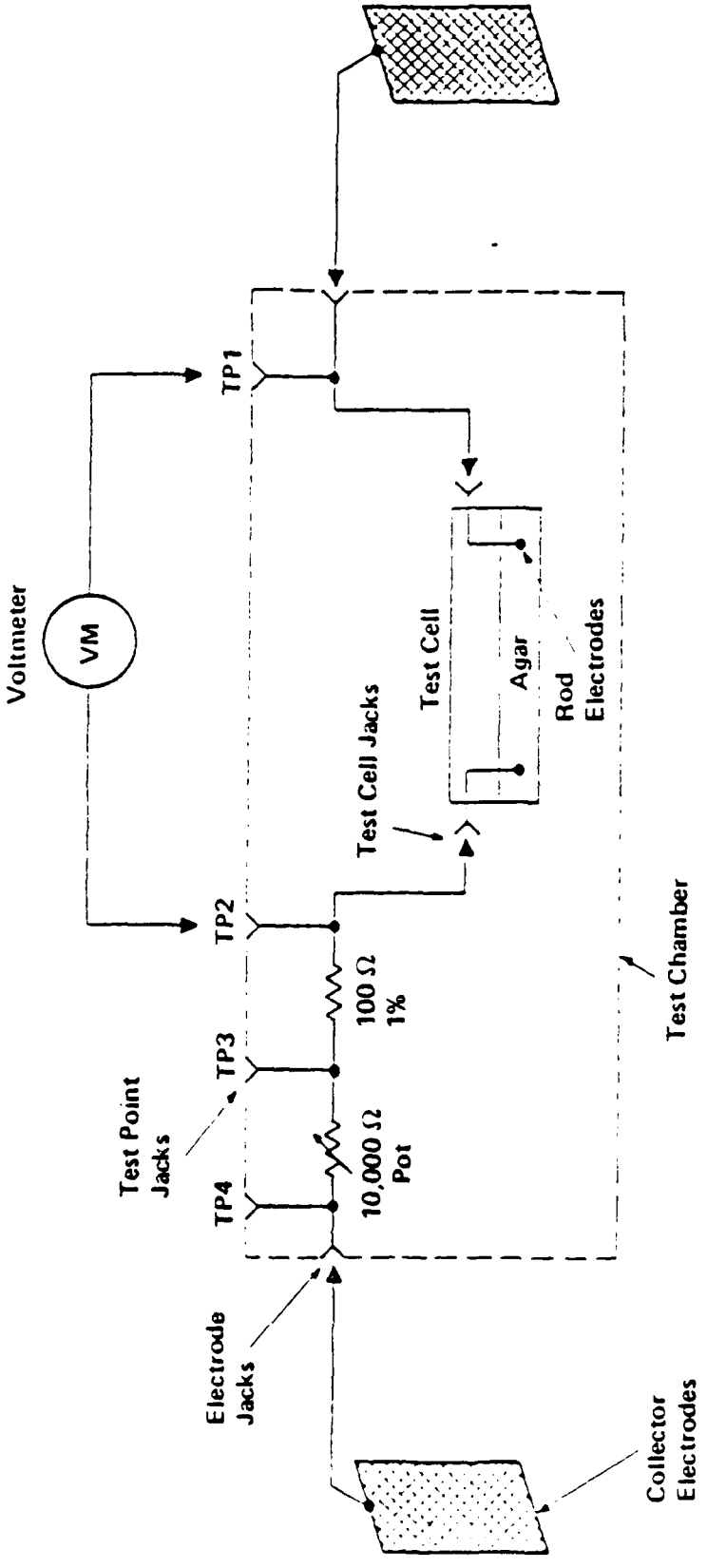
(6) Measure and record the voltage across the 100 ohm series resistor, V_R (see Figure L-5). This allows calculation of the cell current and current density.

(7) Measure and record the voltage between the electrodes, V_{CH}, with the test chamber connected and adjusted as per Step 5.

MATCHED CURRENT DENSITY PROTOCOL

(1) Measure maximum electric field in soil using 1 meter probe, E.

(2) Locate collector electrodes in line with maximum electric field with a separation of 1 meter.



Test Number	Connect Voltmeter Across
4 V_{OC}	Collector Electrodes
5 V_{CL}	TP1 – TP2
6 V_R	TP2 – TP3
7 V_{CH}	TP1 – TP4

FIGURE L-5. TEST CHAMBER HOOKUP FOR THE SLIME MOLD STUDIES MATCHED ELECTRIC FIELD PROTOCOL.

(3) Measure exact electrode spacing and open circuit (no load) electrode voltage, V_{OC}. Measured voltage should be within a few percent of that measured in Step 1. If not, correct electrode spacing as appropriate.

(4) Connect current-limiting test chamber (see Figure L-6) to electrodes. Place the current limit select switch to the 500 kilohm position (500 K).

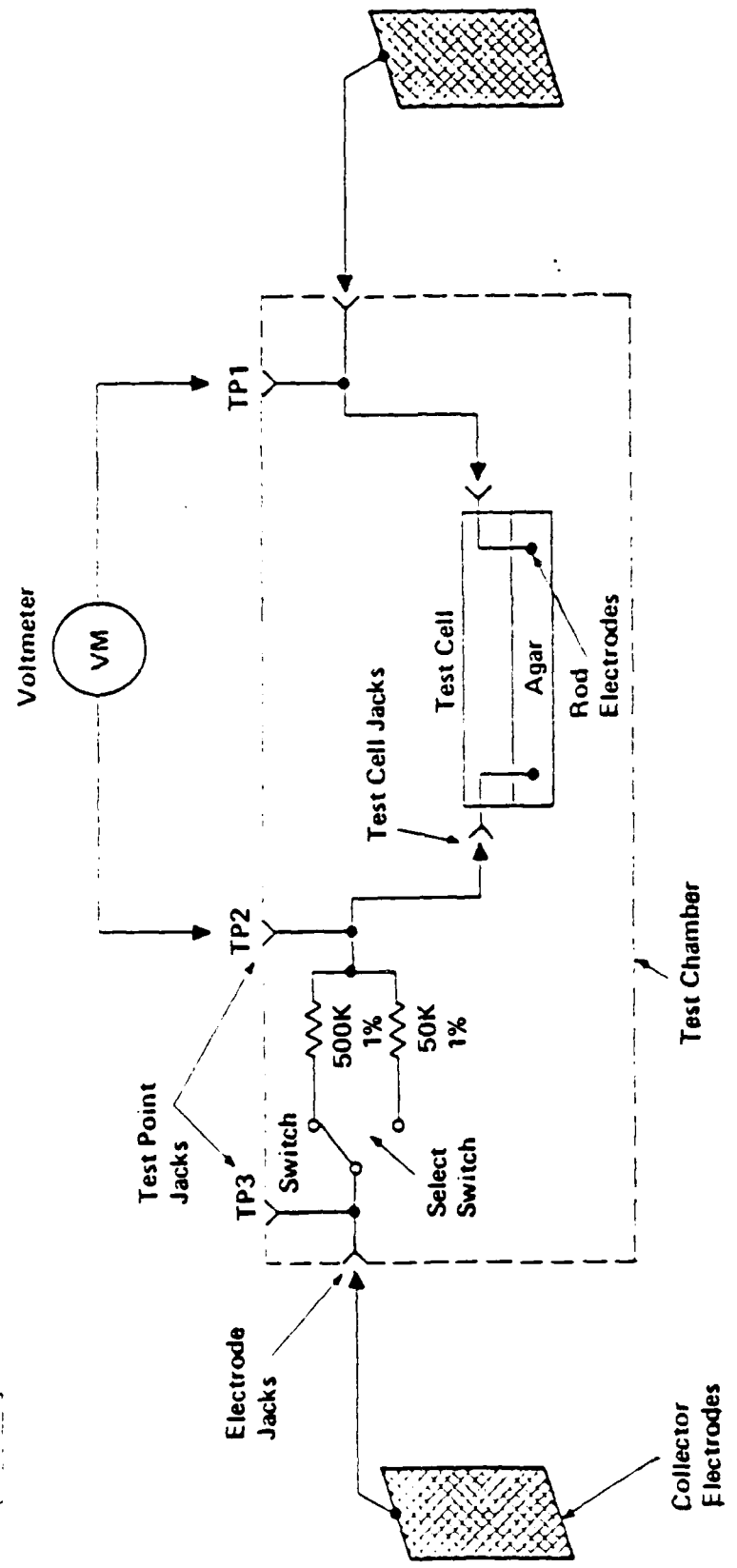
(5) Measure and record the voltages across the test cell, V_{CL}, the resistor, V_R, and the test chamber, V_{CH}, using the test point jacks (see Figure L-6 for test point numbering).

The voltages across the resistor and across the test chamber should be close in value to V_{OC} from Step 3.

$$V_R = V_{CH} = V_{OC}$$

The voltage across the test cell will be much lower, and can be estimated as:

$$V_{CL} = 1.6 \times 10^{-3} \times V_{OC} \text{ (volts)}.$$



To Measure Voltage Across	Connect Voltmeter Between
V_{CL} Test Cell V_R Resistor V_{CH} Test Chamber	TP1 – TP2 TP2 – TP3 TP1 – TP3

FIGURE L-6. TEST CHAMBER HOOKUP FOR THE SLIME MOLD STUDIES MATCHED CURRENT DENSITY PROTOCOL.

EXHIBIT 4 - 1

SLIME MOLD STUDIES

On 20 and 21 August 1987, IITRI field crews made ELF electromagnetic (EM) field measurements at nine measurement points at a total of two test sites and one control site for the slime mold studies. The study sites and measurement points within the study sites were unchanged from 1986.

The positions of the three sites relative to the WTF are shown on the composite map in Figure G-1. The site numbers listed on the map are those used by IITRI. Table G-1 provides a cross-reference of IITRI site numbers, investigator site names, and township, range, and section numbers for the sites.

TABLE G-1. SITE NO. CROSS-REFERENCE
Slime Mold Studies

IITRI Site No.	Investigator's Site Name	Location		
		Township	Range	Section(s)
7A2	Antenna No. 2	T42N	R5W	7
7C1	Control No. 1	T43N	R2W	31
7G3	Ground No. 3	T42N	R5W	7

The slime mold studies have been designed to monitor for ELF EM field exposure effects on the respiration and mitosis of the slime mold, Physarum polycephalum. The electric and magnetic fields in the earth are considered important EM factors influencing soil biota. The electric field in the air is not expected to have a significant impact on the objectives of these studies.

Several of the above objectives require the use of buried culture chambers at the study sites. These chambers are used to match the internal EM fields of the cultures to those present in the surrounding earth.

Tables G-2, G-3, and G-4 present a summary of 76 Hz transverse electric field intensities, longitudinal electric field intensities, and magnetic flux densities, respectively, as measured at the study sites. Data for 1987 are

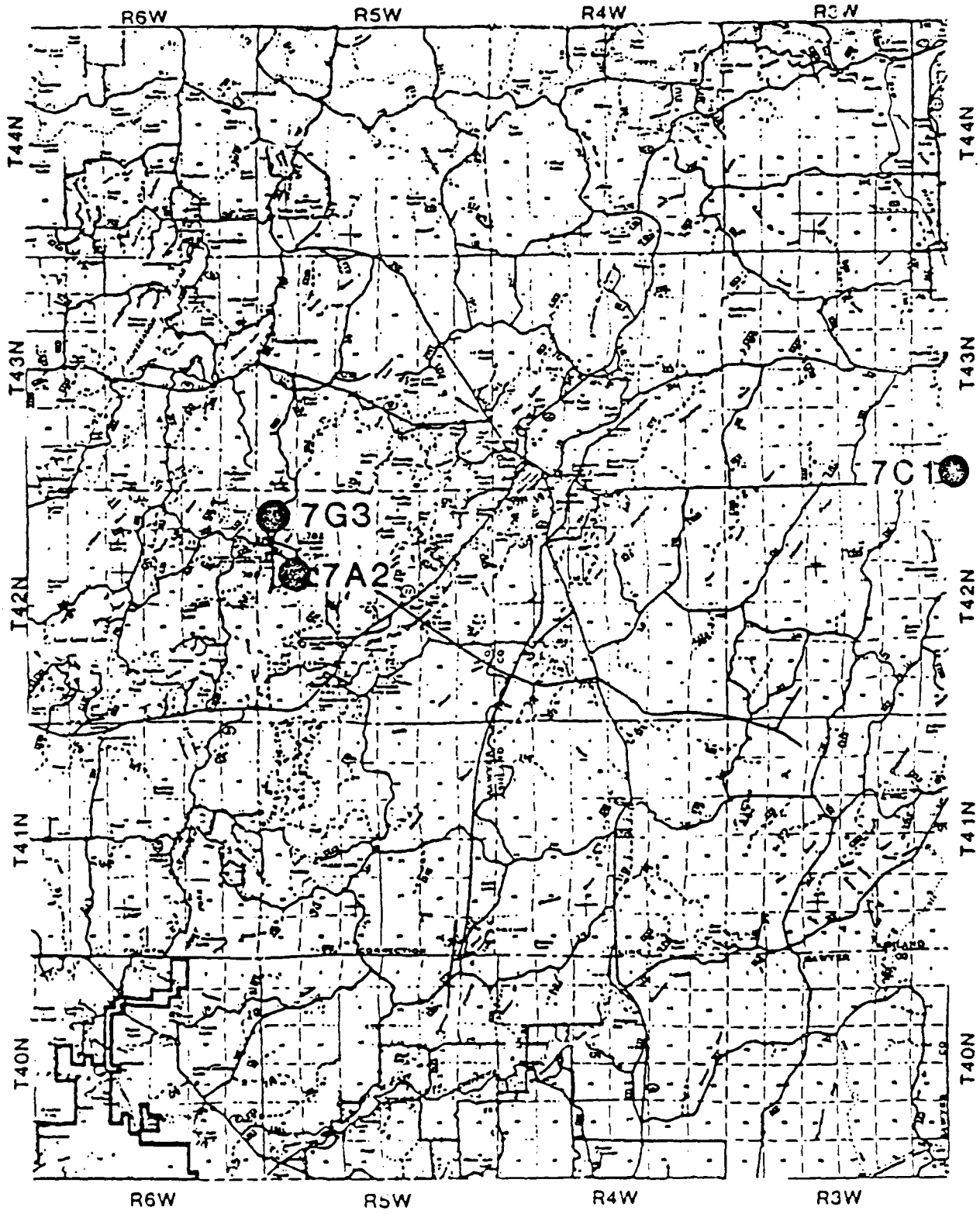


FIGURE G-1. POSITIONS OF SLIME MOLD STUDY SITES RELATIVE TO WISCONSIN TRANSMITTING FACILITY ANTENNA ELEMENTS.

TABLE G-2. 76 Hz TRANSVERSE ELECTRIC FIELD INTENSITIES (V/m) Slime Mold Studies

Site No., Meas. Pt.	1982 NS	1982 EW	1983 NS	1983 EW	1984 NS	1984 EW	1985 NS	1985 EW	1985 B(-75)	1986 B(-75)	1987 B(-75)
7A2-C	-	-	-	-	0.004	0.031	/	/	/	/	/
7A2-N	-	-	-	-	-	-	/	/	/	/	/
7A2-S	-	-	-	-	-	-	/	/	/	/	/
7C1-C	/	/	/	/	/	/	/	/	/	/	/
7C1-N	-	-	-	-	-	-	/	/	/	/	/
7C1-S	-	-	-	-	-	-	/	/	/	/	/
7G3-C	-	-	/	/	0.014	1.49	/	/	/	/	/
7G3-N	-	-	-	-	-	-	/	/	/	/	/
7G3-S	-	-	-	-	-	-	/	/	/	/	/

NS = north-south antenna.
EW = east-west antenna.
B = both antennas.
- = site not established.
/ = data not taken.

TABLE G-3. 76 Hz LONGITUDINAL ELECTRIC FIELD INTENSITIES (mV/m) Slime Mold Studies

Site No., Meas. Pt.	1982 NS	1982 EW	1983 NS	1983 EW	1984 NS	1984 EW	1985 NS	1985 EW	1985 B(-75)	1986 B(-75)	1987 B(-75)
7A2-C	-	-	-	-	3.9,4.1	200,240	/	189,210	200,220	240	207
7A2-N	-	-	-	-	-	-	/	210	/	220	205
7A2-S	-	-	-	-	-	-	/	200	/	156	169
7C1-C	0.95	0.97	0.96	0.96	1.11	1.13	0.90	/	1.17	1.20	1.04
7C1-N	-	-	-	-	-	-	1.03	/	/	1.38	1.41
7C1-S	-	-	-	-	-	-	1.00	/	/	1.35	1.20
7G3-C	-	-	18	1840	13.3	1460	/	610,780	570	1000	498
7G3-N	-	-	-	-	-	-	/	910	/	960	660
7G3-S	-	-	-	-	-	-	/	980	/	1170	601

NS = north-south antenna.
EW = east-west antenna.
B = both antennas.
- = site not established.
/ = data not taken.

EXHIBIT 4 - 4

TABLE G-4. 76 Hz MAGNETIC FLUX DENSITIES (mG)
Slime Mold Studies

Site No., Meas. Pt.	1982		1983		1984		1985			1986	1987
	NS	EW	NS	EW	NS	EW	NS	EW	B(-75)	B(-75)	B(-75)
7A2-C	-	-	-	-	0.045,0.051	44	/	44	44	40	42
7A2-N	-	-	-	-	-	-	/	/	/	42	45
7A2-S	-	-	-	-	-	-	/	/	/	39	41
7C1-C	0.012	0.014	0.012	0.013	0.011	0.013	/	/	0.016	0.016	0.017
7C1-N	-	-	-	-	-	-	/	/	/	0.016	0.016
7C1-S	-	-	-	-	-	-	/	/	/	0.016	0.017
7G3-C	-	-	0.060	5.2	0.078	5.6	/	3.2	3.1	3.9	3.03
7G3-N	-	-	-	-	-	-	/	/	/	4.0	3.2
7G3-S	-	-	-	-	-	-	/	/	/	3.9	3.1

NS = north-south antenna.
EW = east-west antenna.
B = both antennas.
- = site not established.
/ = data not taken.

given for each of the three currently active sites. Where available, 76 Hz data from previous years are presented for reference.

A data logger was installed at each of the slime mold study sites during the week of 6 July 1987. These instruments measure and record the drive voltage and current for each culture chamber on an hourly basis. Monitoring of the culture chambers continued until the end of the field season in mid-October. Results of the measurements have been provided to the principal investigator of the slime mold studies, but are not included in this report because of their length.

Comparison of the data in the tables indicates that there were no significant changes in the 76 Hz EM field intensities at either the antenna or control site in 1987. At the ground site, however, the EM fields were reduced from the 1986 measurements and were at approximately the levels recorded in 1985. Previous changes in the ground site fields were attributed to rebalancing of the west ground terminal segment currents. However, no rebalancing of currents was made on this segment of the west ground between the 1986 and 1987 measurements. It is therefore likely that the observed EM field variations are the result of changes in soil moisture and conductivity and their effect on ground current distribution in the ROW adjacent to the study site.

Measurements of 60 Hz ambient EM fields could not be conducted at the WTF in 1987 as in previous years, because of its full-time modulated signal operation. 60 Hz EM field data from 1984 and early 1985 were given in the 1985 annual measurement report.*

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*ELF Communications System Ecological Monitoring Program: Electromagnetic Field Measurements and Engineering Support--1985. IIT Research Institute Technical Report E06549-24, September 1986, 48 pp. plus appendixes.

TABLE M-6. 1985 WTF OPERATIONS SUMMARY: NORTH-SOUTH AND EAST-WEST ANTENNA ELEMENTS SIMULTANEOUSLY
[Hours of Operation]

Frequency, Hz	Month												Annual Totals
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	
Mode: Modulated Signal[a]													
44	0.23	--	--	1.97	0.46	0.05	29.73	--	--	--	--	--	32.44
76	0.70	--	7.62	15.01	45.98	261.68	118.66	74.91	161.36	451.69	657.61	730.96	2526.18
78	--	--	--	0.99	--	0.05	73.94	--	0.43	--	--	--	75.41
Subtotals	0.93	--	7.62	17.97	46.44	261.78	222.33	74.91	161.79	451.69	657.61	730.96	2634.03
Mode: Unmodulated Signal													
76	0.34	--	10.16	90.06	10.35	10.70	--	1.63	4.82	3.40	0.61	--	132.07
80	--	--	--	9.80	--	0.98	--	--	--	1.72	--	--	12.50
Subtotals	0.34	--	10.16	99.86	10.35	11.68	--	1.63	4.82	5.12	0.61	--	144.57
Other[b]	0.13	--	25.48	43.63	0.31	0.22	10.87	--	0.03	--	--	--	80.67
Totals	**1.40**	--	**43.26**	**161.46**	**57.10**	**273.68**	**233.20**	**76.54**	**166.64**	**456.81**	**658.22**	**730.96**	**2859.27**
Changes in Operational Mode	13	0	14	60	55	69	15	48	20	54	38	38	424

[a]Frequencies listed refer to the center frequency of modulation.

[b]Denotes short periods of time at other frequencies or urdesignated operation.

TABLE M-5. 1985 WTF OPERATIONS SUMMARY: EAST-WEST ANTENNA ELEMENT ONLY
[Hours of Operation]

Frequency, Hz	Month												Annual Totals
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	
Mode: Modulated Signal[a]													
44	--	--	3.15	3.07	1.34	1.62	--	--	0.34	--	--	--	9.52
76	0.85	--	7.30	14.82	20.69	1.28	142.27	2.23	2.17	3.02	0.03	--	194.66
78	--	--	1.64	0.93	--	--	--	--	0.32	--	--	--	2.89
Subtotals	0.85	--	12.09	18.82	22.03	2.90	142.27	2.23	2.83	3.02	0.03	--	207.07
Mode: Unmodulated Signal													
76	1.08	2.06	8.94	0.61	40.81	12.87	37.19	10.98	4.76	28.76	3.31	--	151.37
80	--	--	1.50	2.55	3.77	6.55	--	--	1.86	0.13	--	--	16.36
Subtotals	1.08	2.06	10.44	3.16	44.58	19.42	37.19	10.98	6.62	28.89	3.31	--	167.73
Other[b]	0.10	--	5.86	3.94	0.90	5.26	19.13	0.19	1.16	--	--	--	36.54
Totals	**2.03**	**2.06**	**28.39**	**25.92**	**67.51**	**27.58**	**198.59**	**13.40**	**10.61**	**31.91**	**3.34**	--	**411.34**
Changes in Operational Mode	46	1	74	80	143	223	31	59	45	22	3	0	727

[a]Frequencies listed refer to the center frequency of modulation.

[b]Denotes short periods of time at other frequencies or undesignated operation.

TABLE M-9. 1986 WTF OPERATIONS SUMMARY: NORTH-SOUTH AND EAST-WEST ANTENNA ELEMENTS SIMULTANEOUSLY
[Hours of Operation]

Frequency, Hz	Month												Annual Totals
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	
Mode: Modulated Signal[a]													
76	672.44	633.09	600.27	595.68	656.56	641.07	689.94	633.20	675.58	688.85	696.57	679.21	7862.46
Subtotals	672.44	633.09	600.27	595.68	656.56	641.07	689.94	633.20	675.58	688.85	696.57	679.21	7862.46
Mode: Unmodulated Signal													
72	0.00	0.00	0.00	0.00	0.89	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.89
76	0.15	0.07	7.54	0.00	0.25	0.00	0.00	0.00	0.00	1.80	0.44	0.00	10.25
80	0.00	0.00	0.00	0.00	0.51	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.58
Subtotals	0.15	0.07	7.54	0.00	1.65	0.00	0.00	0.00	0.00	1.87	0.44	0.00	11.72
Other[b]	0.00	0.00	0.00	0.00	1.48	0.00	0.00	0.00	0.33	0.00	0.00	0.34	2.15
Totals	**672.59**	**633.16**	**607.81**	**595.68**	**659.69**	**641.07**	**689.94**	**633.20**	**675.91**	**690.72**	**697.01**	**679.55**	**7876.33**
Changes in Operational Mode	45	54	69	61	112	72	66	42	63	62	45	61	752

[a]Frequencies listed refer to the center frequency of modulation.

[b]Denotes short periods of time at other frequencies or undesignated operation.

TABLE M-12. 1987 WTF OPERATIONS SUMMARY: NORTH-SOUTH AND EAST-WEST ANTENNAS SIMULTANEOUSLY
[Hours of Operation]

Frequency, Hz	Month												Annual Totals
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	
Mode: Modulated Signal[a]													
76	712.70	651.08	658.35	697.30	715.76	691.87	700.54	584.40	697.31	724.71	703.15	695.82	8232.99
Subtotals	712.70	651.08	658.35	697.30	715.76	691.87	700.54	584.40	697.31	724.71	703.15	695.82	8232.99
Mode: Unmodulated Signal													
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
76	1.77	0.00	0.00	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.53	2.76
80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03
Subtotals	1.77	0.00	0.00	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.56	2.79
Other[b]	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38
Totals	**714.85**	**651.08**	**658.35**	**697.30**	**716.22**	**691.87**	**700.54**	**584.40**	**697.31**	**724.71**	**703.15**	**696.38**	**8236.16**
Changes in Operational Mode	32	10	11	10	13	14	24	23	13	10	12	12	184

[a]Frequencies listed refer to the center frequency of modulation.

[b]Denotes short periods of time at other frequencies or undesignated operation.

Table 1

Slime Mold Studies
Matched Current Density Chambers

	Magnetic Flux Density mG		Electric Field In Earth mV/m		Culture Cell E-Field mV/m		Culture Cell Current Density mA/m^2	
	76 Hz	60 Hz	76 Hz	60 Hz	76 Hz	60 Hz	76 Hz	60 Hz
Lab Test Chamber	205	2.7	N/A	N/A	7.7	0.065	2.6	N/M
WTF Ground Site (7G3-N)	4.0	0.001	960	0.113	9.2	0.00116	2.1	0.00024
Lab Control Chamber	0.066	3.1	N/A	N/A	Cell Not Connected		Cell Not Connected	
WTF Control Site (7C1-N)	0.016	0.001	1.38	0.060	0.0113	0.00019-0.00045	0.0028	0.00015

N/A = Not Applicable
N/M = Not Measured

EXHIBIT 6-1

Table 2

SLIME MOLD STUDIES
MATCHED E-FIELD CHAMBERS

	Magnetic Flux Density mG		Electric Field In Earth mV/m		Culture Cell E-Field mV/m		Culture Cell Current Density mA/m^2	
	76 Hz	60 Hz	76 Hz	60 Hz	76 Hz	60 Hz	76 Hz	60 Hz
Lab Test Chamber	209	4.1	N/A	N/A	870	0.49	N/M	N/M
Ground Site 7G3-C	3.9	0.001	1000	0.113	900	0.094	230	0.028
Ground Site 7G3-S	3.9	0.001	1170	0.113	860	0.103	167	0.029
Lab Control Chamber	0.066	3.1	N/A	N/A	Cells Not Connected		Cells Not Connected	
Control Site 7C1-C	0.016	0.001	1.20	0.060	2.5	0.32	0.11-1.00	0.060
Control Site 7C1-S	0.016	0.001	1.35	0.060	1.74	0.32	0.11-1.28	0.096

N/A = Not Applicable

N/M = Not Measured

EXHIBIT 6-2

APPENDIX II. A

A multivariate regression using the continuous exposure variables ..[TYPE], [SITE], and [WEEKS]. The analyses found no relationship between the exposure variables and the metabolic outcome variables (ATP and QO_2). No independent variable was a significant predictor of ATP levels; only the length of time in the field showed a significant relationship to respiration (p=.0001).

BACKWARD ELIMINATION PROCEDURE FOR DEPENDENT VARIABLE ATP

WARNING: 60 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 0 ALL VARIABLES ENTERED R SQUARE = 0.04693794 C(P) = 5.00000000

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	53.00519219	13.25129805	0.91	0.4621
ERROR	74	1076.25600085	14.54400001		
TOTAL	78	1129.26119304			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	15.30393524				
TEMP	-0.05031472	0.11364199	2.85099435	0.20	0.6592
CDAVG	0.00150067	0.00973281	0.34576307	0.02	0.8779
EFAVG	0.00040556	0.00120369	1.65102380	0.11	0.7371
WEEKS	0.07772951	0.27210082	1.18685048	0.08	0.7759

BOUNDS ON CONDITION NUMBER: 6.166262, 64.09004

STEP 1 VARIABLE CDAVG REMOVED R SQUARE = 0.04663175 C(P) = 3.02377359

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	52.65942912	17.55314304	1.22	0.3072
ERROR	75	1076.60176392	14.35469019		
TOTAL	78	1129.26119304			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	15.12419264				
TEMP	-0.04740575	0.11133324	2.60259296	0.18	0.6715
EFAVG	0.00052972	0.00088880	5.09892188	0.36	0.5530
WEEKS	0.08218952	0.26879233	1.34212484	0.09	0.7606

BOUNDS ON CONDITION NUMBER: 6.057903, 39.23856

STEP 2 VARIABLE WEEKS REMOVED R SQUARE = 0.04544325 C(P) = 1.11605390

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	51.31730428	25.65865214	1.81	0.1708
ERROR	76	1077.94388876	14.18347222		
TOTAL	78	1129.26119304			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	17.90909675				
TEMP	-0.07845989	0.04534279	42.46797076	2.99	0.0876
EFAVG	0.00056642	0.00087539	5.93819239	0.42	0.5196

BOUNDS ON CONDITION NUMBER: 1.006612, 4.026448

BACKWARD ELIMINATION PROCEDURE FOR DEPENDENT VARIABLE ATP

STEP 3 VARIABLE EFAVG REMOVED R SQUARE = 0.04018478 C(P) = -0.47565455

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	45.37911190	45.37911190	3.22	0.0765
ERROR	77	1083.88208114	14.07639066		
TOTAL	78	1129.26119304			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	18.22513599				
TEMP	-0.08063769	0.04502270	45.37911190	3.22	0.0765

BOUNDS ON CONDITION NUMBER: 1, 1

ALL VARIABLES IN THE MODEL ARE SIGNIFICANT AT THE 0.1000 LEVEL.

SUMMARY OF BACKWARD ELIMINATION PROCEDURE FOR DEPENDENT VARIABLE ATP

STEP	VARIABLE REMOVED	NUMBER IN	PARTIAL R**2	MODEL R**2	C(P)	F	PROB>F
1	CDAVG	3	0.0003	0.0466	3.02377	0.0238	0.8779
2	WEEKS	2	0.0012	0.0454	1.11605	0.0935	0.7606
3	EFAVG	1	0.0053	0.0402	-0.47565	0.4187	0.5196

BACKWARD ELIMINATION PROCEDURE FOR DEPENDENT VARIABLE QO2

WARNING: 60 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 0 ALL VARIABLES ENTERED R SQUARE = 0.21635096 C(P) = 5.00000000

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	0.31249887	0.07812472	5.11	0.0011
ERROR	74	1.13190823	0.01529606		
TOTAL	78	1.44440710			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	1.19044661				
TEMP	-0.00307557	0.00368542	0.01065262	0.70	0.4067
CDAVG	0.00002569	0.00031564	0.00010132	0.01	0.9354
EFAVG	0.00004643	0.00003904	0.02163652	1.41	0.2381
WEEKS	-0.02225029	0.00882425	0.09725133	6.36	0.0138

BOUNDS ON CONDITION NUMBER: 6.166262, 64.09004

STEP 1 VARIABLE CDAVG REMOVED R SQUARE = 0.21628082 C(P) = 3.00662392

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	0.31239755	0.10413252	6.90	0.0004
ERROR	75	1.13200955	0.01509346		
TOTAL	78	1.44440710			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	1.18736974				
TEMP	-0.00302577	0.00361013	0.01060269	0.70	0.4046
EFAVG	0.00004855	0.00002882	0.04283525	2.84	0.0962
WEEKS	-0.02217394	0.00871594	0.09768907	6.47	0.0130

BOUNDS ON CONDITION NUMBER: 6.057903, 39.23856

STEP 2 VARIABLE TEMP REMOVED R SQUARE = 0.20894031 C(P) = 1.69978869

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	0.30179486	0.15089743	10.04	0.0001
ERROR	76	1.14261224	0.01503437		
TOTAL	78	1.44440710			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	0.93374086				
EFAVG	0.00004637	0.00002863	0.03939478	2.62	0.1096
WEEKS	-0.01551013	0.00356411	0.28471657	18.94	0.0001

BOUNDS ON CONDITION NUMBER: 1.016952, 4.067807

BACKWARD ELIMINATION PROCEDURE FOR DEPENDENT VARIABLE QO2

STEP 3 VARIABLE EFAVG REMOVED R SQUARE = 0.18166629 C(P) = 2.27527444

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	0.26240008	0.26240008	17.09	0.0001
ERROR	77	1.18200702	0.01535074		
TOTAL	78	1.44440710			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	0.93897511				
WEEKS	-0.01476525	0.00357128	0.26240008	17.09	0.0001

QO2 decreases with time

BOUNDS ON CONDITION NUMBER: 1, 1

ALL VARIABLES IN THE MODEL ARE SIGNIFICANT AT THE 0.1000 LEVEL.

SUMMARY OF BACKWARD ELIMINATION PROCEDURE FOR DEPENDENT VARIABLE QO2

STEP	VARIABLE REMOVED	NUMBER IN	PARTIAL R**2	MODEL R**2	C(P)	F	PROB>F
1	CDAVG	3	0.0001	0.2163	3.00662	0.0066	0.9354
2	TEMP	2	0.0073	0.2089	1.69979	0.7025	0.4046
3	EFAVG	1	0.0273	0.1817	2.27527	2.6203	0.1096

APPENDIX II. B

The results of a correlation analysis performed on the WTF field data. The only significant correlation for the outcome variables was .32 for the respiration rate and temperature and -.42 for respiration rate and weeks of exposure. Because temperature and weeks of exposure are highly collinear (r=0.91) it is difficult to determine the relative importance of the two factors. No other independent variables were significantly correlated with the outcome variables

APPENDIX II. B

VARIABLE	N	MEAN	STD DEV	SUM	MINIMUM	MAXIMUM
TEMP	93	53.83848590	9.51935461	5006.97918857	33.56600000	70.49840000
E	92	183.09250000	240.33532582	16844.51000000	0.06000000	903.02000000
CD	89	53.04038184	69.61559778	4720.59398333	0.00170000	311.00010000
TEMPMEAN	135	56.52189888	5.45172869	7630.45634921	41.71428571	65.64285714
CDAVG	87	51.51321888	60.44455156	4481.65004280	0.00000000	241.00000000
EFAVG	84	328.55862615	488.05023865	27598.94139660	0.00000000	2691.16666667
WEEKS	116	12.92859545	4.70065603	1499.71707233	5.28571429	20.57142857
WEEK2	116	189.05426328	122.78827401	21930.29454023	27.93877551	423.18367347

PEARSON CORRELATION COEFFICIENTS / PROB > |R| UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

	TEMP	E	CD	TEMPMEAN	CDAVG	EFAVG	WEEKS	WEEK2
TEMP	1.00000	0.07110	0.15571	0.80983	0.04406	-0.08497	-0.90638	-0.92830
	0.0000	0.5959	0.2802	0.0001	0.6853	0.4422	0.0001	0.0001
	93	58	50	89	87	84	92	92
E	0.07110	1.00000	0.81622	-0.05430	0.91018	0.71123	0.07786	0.07389
	0.5959	0.0000	0.0001	0.6072	0.0001	0.0001	0.5097	0.5315
	58	92	79	92	55	53	74	74
CD	0.15571	0.81622	1.00000	0.06232	0.86960	0.58895	-0.01891	-0.03197
	0.2802	0.0001	0.0000	0.5618	0.0001	0.0001	0.8793	0.7973
	50	79	89	89	48	46	67	67
TEMPMEAN	0.80983	-0.05430	0.06232	1.00000	-0.06166	-0.17032	-0.69947	-0.76835
	0.0001	0.6072	0.5618	0.0000	0.5798	0.1309	0.0001	0.0001
	89	92	89	135	83	80	112	112
CDAVG	0.04406	0.91018	0.86960	-0.06166	1.00000	0.68609	0.03219	0.03246
	0.6853	0.0001	0.0001	0.5798	0.0000	0.0001	0.7686	0.7667
	87	55	48	83	87	84	86	86
EFAVG	-0.08497	0.71123	0.58895	-0.17032	0.68609	1.00000	0.12496	0.13428
	0.4422	0.0001	0.0001	0.1309	0.0001	0.0000	0.2603	0.2262
	84	53	46	80	84	84	83	83
WEEKS	-0.90638	0.07786	-0.01891	-0.69947	0.03219	0.12496	1.00000	0.98785
	0.0001	0.5097	0.8793	0.0001	0.7686	0.2603	0.0000	0.0001
	92	74	67	112	86	83	116	116
WEEK2	-0.92830	0.07389	-0.03197	-0.76835	0.03246	0.13428	0.98785	1.00000
	0.0001	0.5315	0.7973	0.0001	0.7667	0.2262	0.0001	0.0000
	92	74	67	112	86	83	116	116

APPENDIX III.

A 24 hour summary of temperature and EMF Data obtained from IITRI data loggers at 3 WTF sites.

APPENDIX III.

A typical 24 hr examples of temperatures and electromagnetic fields at the A, C, and G sites using the IITRI-supplied data loggers. The tables show data collected at each site on 7/26/87.

LEGEND

YR.....year
MO.....month
DY.....day
HR.....hour
MN.....minutes
BAT.....battery check (Volts)
TEMP.....temperature (^{o}C)
NE,CE,& SE.....electric field in the north, center and south exposure boxes (mV/m)
NJ, CJ, & SE...current density north, center and south exposure boxes(mA/m^2)

APPENDIX III pp. 2 - 4

Data Logger Record from Site 7A2

YR	MO	DY	HR	MIN	BAT (V)	TEMP (C)	NE (mV/m)	NJ (mA/ m^2)	CE (mV/m)	CJ (mA/ m^2)	SE (mV/m)	SJ (mA/ m^2)
87	7	25	16	25	12.0	18.6	1903.2	16.7	200.0	59.4	1.0	0.37
87	7	25	17	25	12.0	18.8	1774.2	16.7	193.5	59.4	1.0	0.48
87	7	25	18	25	12.0	18.6	1774.2	16.7	196.8	58.3	1.0	0.44
87	7	25	19	25	12.0	18.6	2000.0	16.7	196.8	57.8	1.0	0.37
87	7	25	20	25	12.0	18.4	1774.2	16.7	196.8	58.3	1.0	0.37
87	7	25	21	25	12.0	18.0	1709.7	16.7	196.8	57.8	1.0	0.42
87	7	25	22	25	12.0	17.8	1903.2	16.7	196.8	57.2	1.0	0.42
87	7	25	23	25	12.0	17.6	2096.8	16.7	196.8	57.8	1.3	0.39
87	7	26	0	25	12.0	17.2	1774.2	16.7	193.5	57.2	1.0	0.37
87	7	26	1	25	12.0	16.7	2129.0	16.7	196.8	56.1	1.0	0.40
87	7	26	2	25	12.0	16.1	1967.7	16.7	196.8	56.1	1.0	0.39
87	7	26	3	25	12.0	15.9	2129.0	16.7	200.0	56.1	1.0	0.40
87	7	26	4	25	12.0	15.7	1774.2	16.7	203.2	56.1	1.3	0.41
87	7	26	5	25	12.0	15.3	2129.0	16.7	200.0	55.6	1.0	0.39
87	7	26	6	25	12.0	15.3	2129.0	16.7	200.0	56.1	1.0	0.42
87	7	26	7	25	12.0	15.3	2129.0	16.7	203.2	55.6	1.3	0.40
87	7	26	8	25	12.0	15.7	2129.0	16.7	200.0	55.0	1.3	0.42
87	7	26	9	25	12.0	15.9	2096.8	16.7	200.0	55.0	1.0	0.39
87	7	26	10	25	12.0	16.5	1806.5	16.7	193.5	53.9	1.3	0.40
87	7	26	11	25	12.0	16.7	1967.7	16.7	190.3	53.9	1.0	0.48
87	7	26	12	25	12.0	17.2	1935.5	16.7	187.1	53.9	1.0	0.38
87	7	26	13	25	12.0	17.6	1838.7	16.7	187.1	53.3	1.0	0.42
87	7	26	14	25	12.0	18.4	1838.7	16.7	183.9	53.9	1.0	0.39
87	7	26	15	25	12.0	18.4	2000.0	11.1	180.6	53.3	1.0	0.39
87	7	26	16	25	12.0	18.2	1935.5	11.1	177.4	52.8	1.0	0.40
87	7	26	17	25	12.0	18.0	2000.0	16.7	174.2	51.7	1.0	0.37
87	7	26	18	25	12.0	17.9	1774.2	11.1	177.4	52.2	1.0	0.44
87	7	26	19	25	12.0	17.8	1903.2	16.7	174.2	51.7	1.0	0.38
87	7	26	20	25	12.0	17.4	1935.5	16.7	174.2	50.6	1.0	0.48
87	7	26	21	25	12.0	16.3	1806.5	16.7	177.4	50.6	1.0	0.39
87	7	26	22	25	12.0	15.3	2129.0	16.7	177.4	49.4	1.0	0.41
87	7	26	23	25	12.0	14.6	2096.8	16.7	177.4	48.9	1.0	0.42
87	7	27	0	25	12.0	13.9	2096.8	16.7	177.4	48.3	1.3	0.42

Data Logger Record from Site 7G2

YR	MO	DY	HR	MN	BAT (V)	TEMP (C)	NE (mV/m)	NJ (mA/ m^2)	CE (mV/m)	CJ (mA/ m^2)	SE (mV/m)	SJ (mA/ m^2)
87	7	25	18	37	12.0	22.8	4.5	1.02	161	0	o77	183
87	7	25	19	37	12.0	22.2	4.8	1.12	194	0	677	178
87	7	25	20	37	12.0	21.3	4.2	1.70	226	6	806	172
87	7	25	21	37	12.0	20.7	4.8	1.77	129	0	677	183
87	7	25	22	37	12.0	19.7	5.2	1.13	161	11	516	172
87	7	25	23	37	12.0	18.8	4.8	1.76	129	11	581	183
87	7	26	0	37	12.0	18.4	5.2	1.78	65	6	677	178
87	7	26	1	37	12.0	17.6	5.2	1.77	0	6	516	189
87	7	26	2	37	12.0	17.2	5.5	1.62	65	11	677	178
87	7	26	3	37	12.0	16.7	5.5	1.74	0	0	452	178
87	7	26	4	37	12.0	15.8	5.2	1.63	65	6	613	178
87	7	26	5	37	12.0	15.7	5.8	1.01	0	17	548	178
87	7	26	6	37	12.0	15.5	5.5	1.07	0	6	484	183
87	7	26	7	37	12.0	15.8	6.1	1.07	0	11	613	178
87	7	26	8	37	12.0	16.3	6.1	1.07	0	11	516	172
87	7	26	9	37	12.0	16.9	5.8	1.07	161	6	613	183
87	7	26	10	37	12.0	21.3	4.8	1.00	161	11	677	172
87	7	26	11	37	12.0	22.8	3.9	0.89	258	0	806	167
87	7	26	12	37	12.0	22.4	4.8	0.74	194	6	774	167
87	7	26	13	37	12.0	23.3	4.5	1.64	161	6	677	161
87	7	26	14	37	12.0	21.9	4.2	0.77	194	0	677	167
87	7	26	15	37	12.0	22.4	3.5	0.69	194	6	774	167
87	7	26	16	37	12.0	24.7	2.9	0.70	194	11	903	156
87	7	26	17	37	12.0	24.5	1.9	0.54	97	0	677	167
87	7	26	18	37	12.0	22.8	3.2	0.69	129	11	677	161
87	7	26	19	37	12.0	21.3	3.9	0.80	226	0	484	178
87	7	26	20	37	12.0	19.5	3.9	0.78	258	0	581	183
87	7	26	21	37	12.0	18.0	4.5	0.73	65	11	516	178
87	7	26	22	37	12.0	16.5	4.8	0.73	65	11	452	178
87	7	26	23	37	12.0	14.9	4.8	0.72	97	11	387	167
87	7	27	0	37	12.0	15.1	5.2	0.71	0	6	484	172
87	7	27	1	37	12.0	13.5	4.8	0.71	0	6	452	167
87	7	27	2	37	12.0	12.8	4.8	0.71	32	0	484	178

Data Logger Record from Site 7C1

YR	MO	DY	HR	MN	BAT (V)	TEMP (C)	NE (mV/m)	NJ (mA/ m^2)	CE (mV/m)	CJ (mA/ m^2)	SE (mV/m)	SJ (mA/ m^2)
87	7	25	17	22	12.0	17.6	0.00	0.00233	0.65	0.00	1.29	0.56
87	7	25	18	22	12.0	17.6	0.00	0.00233	0.65	0.00	1.29	0.56
87	7	25	19	22	12.0	17.6	0.00	0.00222	0.65	0.00	1.29	0.56
87	7	25	20	22	12.0	17.4	0.00	0.00222	0.65	0.00	1.29	0.56
87	7	25	21	22	12.0	17.2	0.00	0.00211	0.65	0.00	1.29	0.56
87	7	25	22	22	12.0	16.9	0.00	0.00222	0.97	0.00	1.29	0.56
87	7	25	23	22	12.0	16.7	0.00	0.00211	0.97	0.00	1.29	0.56
87	7	26	0	22	12.0	16.3	0.00	0.00222	0.65	0.00	0.97	0.56
87	7	26	1	22	12.0	15.9	0.00	0.00233	0.65	0.00	1.29	0.56
87	7	26	2	22	12.0	15.7	0.00	0.00222	0.65	0.00	1.29	0.56
87	7	26	3	22	12.0	15.5	0.00	0.00233	0.65	0.00	1.29	0.56
87	7	26	4	22	12.0	15.1	0.00	0.00222	0.97	0.00	1.29	0.56
87	7	26	5	22	12.0	14.7	0.00	0.00233	0.97	0.00	0.97	0.56
87	7	26	6	22	12.0	14.4	0.00	0.00222	0.65	0.00	1.29	0.00
87	7	26	7	22	12.0	14.3	0.00	0.00233	0.97	0.00	1.29	0.56
87	7	26	8	22	12.0	14.4	0.00	0.00267	0.97	0.00	1.29	0.56
87	7	26	9	22	12.0	14.6	0.00	0.00256	0.65	0.00	1.29	0.56
87	7	26	10	22	12.0	14.9	0.00	0.00256	0.65	0.00	1.29	0.56
87	7	26	11	22	12.0	15.3	0.00	0.00244	0.65	0.00	0.97	0.56
87	7	26	12	22	12.0	16.1	0.00	0.00267	0.65	0.00	1.29	0.56
87	7	26	13	22	12.0	16.7	0.00	0.00256	0.65	0.00	0.97	0.56
87	7	26	14	22	12.0	16.7	0.00	0.00244	0.65	0.00	0.97	0.56
87	7	26	15	22	12.0	16.9	0.00	0.00244	0.65	0.00	0.97	0.56
87	7	26	16	22	12.0	17.0	0.00	0.00244	0.65	0.00	0.97	0.56
87	7	26	17	22	12.0	17.2	0.00	0.00244	0.65	0.00	0.97	0.56
87	7	26	18	22	12.0	17.0	0.00	0.00244	0.65	0.00	0.97	0.56
87	7	26	19	22	12.0	16.7	0.00	0.00233	0.65	0.00	0.97	0.56
87	7	26	20	22	12.0	16.1	0.00	0.00222	0.65	0.00	0.97	0.56
87	7	26	21	22	12.0	15.3	0.00	0.00222	0.65	0.00	0.97	0.56
87	7	26	22	22	12.0	14.7	0.00	0.00244	0.65	0.00	0.97	0.56
87	7	26	23	22	12.0	14.3	0.00	0.00233	0.65	0.00	0.97	0.56
87	7	27	0	22	12.0	13.7	0.00	0.00222	0.65	0.00	0.97	0.56
87	7	27	1	22	12.0	13.1	0.00	0.00233	0.65	0.00	0.97	0.56