# THE TRANSLOCATION OF DDT FROM HYDROSOILS AND ITS ACCUMULATION AND DEGRADATION IN THE BIOTA

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Ronald Clifford Waybrant

1969

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ABSTRACT

THE TRANSLOCATION OF DDT FROM HYDROSOILS AND ITS ACCUMULATION AND DEGRADATION IN THE BIOTA

By

Ronald Clifford Waybrant

The manner and degree of DDT translocation from a pond bottom material into the pond biota was studied. Three levels of DDT in the hydrosoil were prepared and examined, each in a separate pond and each one representing a level of DDT which can be found in the natural environment.

The amount of DDT translocated from the hydrosoil was dependent upon the insecticide concentration in the hydrosoil. A logrithmic relationship between the concentration of DDT in the hydrosoil and the accumulation of DDT in the fish, microcrustaceans, and periphyton was found.

DDD became the major degradation product of DDT and was transported throughout the aquatic environment. DDE was relatively unimportant as a breakdown product of DDT.

The initial displacement of DDT from the bottom material was into the water, which resulted in an accumulation of DDT by the aquatic organisms as they removed DDT from the water. The insecticide accumulation in the periphyton

depends upon the concentration present in the water. It was found that a DDT concentration of ten ppm in the hydrosoil will cause constant mortality of the fish.

DDT and its metabolites were continuously recycled in the aquatic environment and were not inactivated after three months.

# THE TRANSLOCATION OF DDT FROM HYDROSOILS AND ITS ACCUMULATION AND DEGRADATION IN THE BIOTA

By

Ronald Clifford Waybrant

A THESIS

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# INTRODUCTION

Since the introduction of DDT as an insecticide during World War II, the use of organic pesticides has increased enormously. Such compounds when applied to foliage, soil, or water courses may be expected to move with rainfall and runoff into lakes and streams, provided the compounds are sufficiently resistant to degradation by physical and biochemical action.

The toxicity of organic pesticides to fish and other aquatic organisms has been documented in the literature. Graham (1960), Warner and Fenderson (1962), and Kirswell and Edwards (1967) reported fish kills caused by forest spraying with DDT. Young and Nicholson (1951) cite stream fish kills and relate them to applications of organic insecticides. Sublethal amounts of pesticides may result in egg or fry mortality (Burdick et al., 1964). Hitchcock (1965), Schoenthal (1964), and Jones and Moyle (1963) indicated population changes of aquatic invertebrates resulting from environmental treatments with DDT.

Croker and Wilson (1965) studied the kinetics and effects of DDT in a tidal marsh ditch and found that DDT was dispersed to all aspects of the environment. DDT was

removed from the water in five days, yet it was still transported to and from the vegetation, fish, and sediments for the next ten weeks.

In the water DDT is attracted to surfaces due to its hydrophobic nature (Bowman, 1964), and this attraction can result in an accumulation of DDT on pond and stream bottoms. Hindin, May, and Dunstan (1964) demonstrated that the Entiat River contained 18,000 times more DDT in the bottom soil than in the flowing water. When the bottom is sandy or pervious the pesticide may penetrate into or through the substrate. If the bottom is rich in organic material the pesticides can leave the water and adsorb on the organic surface or in the organic matter. DDT may attach to silt and suspended solids, organic or inorganic, in the water (Fredeen, Arnason, and Berck, 1953).

DDT in water is taken up by living organisms and is effectively removed from the water. Microscopic plants and higher aquatic vegetation accumulate large amounts of DDT from water (Meeks and Peterle, 1965). Aquatic animals both large and small, similarly tend to remove DDT from water as has been described by Cope (1965) in his work with C-14 labeled DDT.

DDT is strongly adsorbed from an aqueous dispersion by soils. Weidhass, Bowman, and Schmidt (1961) showed that from two different volumes of water the same percentage loss of C-14 labeled DDT, 78%, occurred in twenty-four hours.

The distribution changes, with time, of DDT at 0.02 ppm in the water was not affected by large differences in pH, total solids, or chloride content.

Harris (1959) found that DDT was biologically active in wet clay or mineral soils and that wet muck or organic soils reduced the bioactivity of DDT. Bowman, Schecter, and Carter (1965) studied the behavior of DDT in various soil types and found that DDT was not leached from soils with water, whether the soils were mineral or organic. DDT has been found to persist in various soils at least one to three years after application (Edwards, 1964). Since DDT has been found to be biologically active in wet soils, to persist for long periods of time in all soils, and since it is not readily leached from the soil by water, the hydrosoils of lakes and streams could essentially be an important reservoir of DDT.

The bioactivity of DDT in the hydrosoils was indicated by Hickey, Keith, and Coon (1966) in a study of pesticides in a Lake Michigan ecosystem. They found DDT in the bottom sediments of Green Bay averaging about 0.014 ppm and a composite Lake Michigan bottom sample to have 0.05 ppm DDT. Crustaceans had 0.41 ppm and alewives had 3.4 ppm of DDT. The concentrations in the organisms were believed to arise from the DDT levels in the bottom sediments. Hence, bottom sediments of an aquatic ecosystem may be an important reservoir of active pesticides.

This study was designed to investigate the potential and degree of DDT transport to the biota from a wide range of hydrosoil concentrations. The available information indicates that DDT is accumulated in the bottom sediments, and presents a potential hazard to aquatic organisms.

Three levels of DDT in the hydrosoil were used, each in a separate pond and each representing a level of DDT which may be found in the hydrosoils of a natural environment. This required the use of four ponds which would be insecticide free with the exception of the hydrosoil, which would be homogeneous in composition and insecticide distribution.

Four artificial ponds were used, one as a control and three with prepared DDT concentrations in the hydrosoil. Three levels of DDT were chosen, 0.05 ppm, 1.0 ppm, and 10.0 ppm. These were picked to span a wide range of insecticide contamination of natural soils and to check the possibility of differential transport of DDT to the pond biota which is dependent on the DDT concentration in the hydrosoil. Fish, periphyton, microcrustaceans, and water were added to the four artificial ponds and sampled along with the hydrosoil at regular intervals for a period of twelve weeks.

The artificial ponds were located at the Fisheries and Wildlife field laboratory on the Michigan State University Agricultural Experiment Station at Lake City, Michigan. A small creek, Mosquito Creek, has been dammed and is used to maintain water levels in four farm ponds which have

been previously designated as Ponds A, B, C, and D (Sohacki, 1968). The artificial ponds were placed in Pond A and the water for these ponds was taken from the adjacent pond, Pond B. All fish, periphyton, and microcrustaceans used in this study were taken from these four farm ponds.

The analyses for DDT and its metabolites in all samples were made by gas chromatography, using a MicroTek 220/DP floor model, dual column gas-liquid chromatograph. The instrument was equipped with two columns packed with 3% SE-30 on 60-80 mesh Gas Chrom Q. The column operating temperature was 180° C and the carrier gas flow was 70 ml/minute of nitrogen.

One column is connected to a Dohrman microcoulometric unit which was operated at a range of 200 ohms, an oxygen flow of 25 ml/minute, and a combustion tube temperature of 830° C.

The other column leads to a parallel plate electron capture detector. This detector utilizes a pulse mode power supply, which has a pulse rate of 100, a pulse width of one microsecond and a power supply of 50 volts. The argon-methane scavenger gas flow through the detector is 60 ml/minute.

Two Honeywell Brown Electronik recorders, equipped with disc chart integraters were used with both analysis systems.

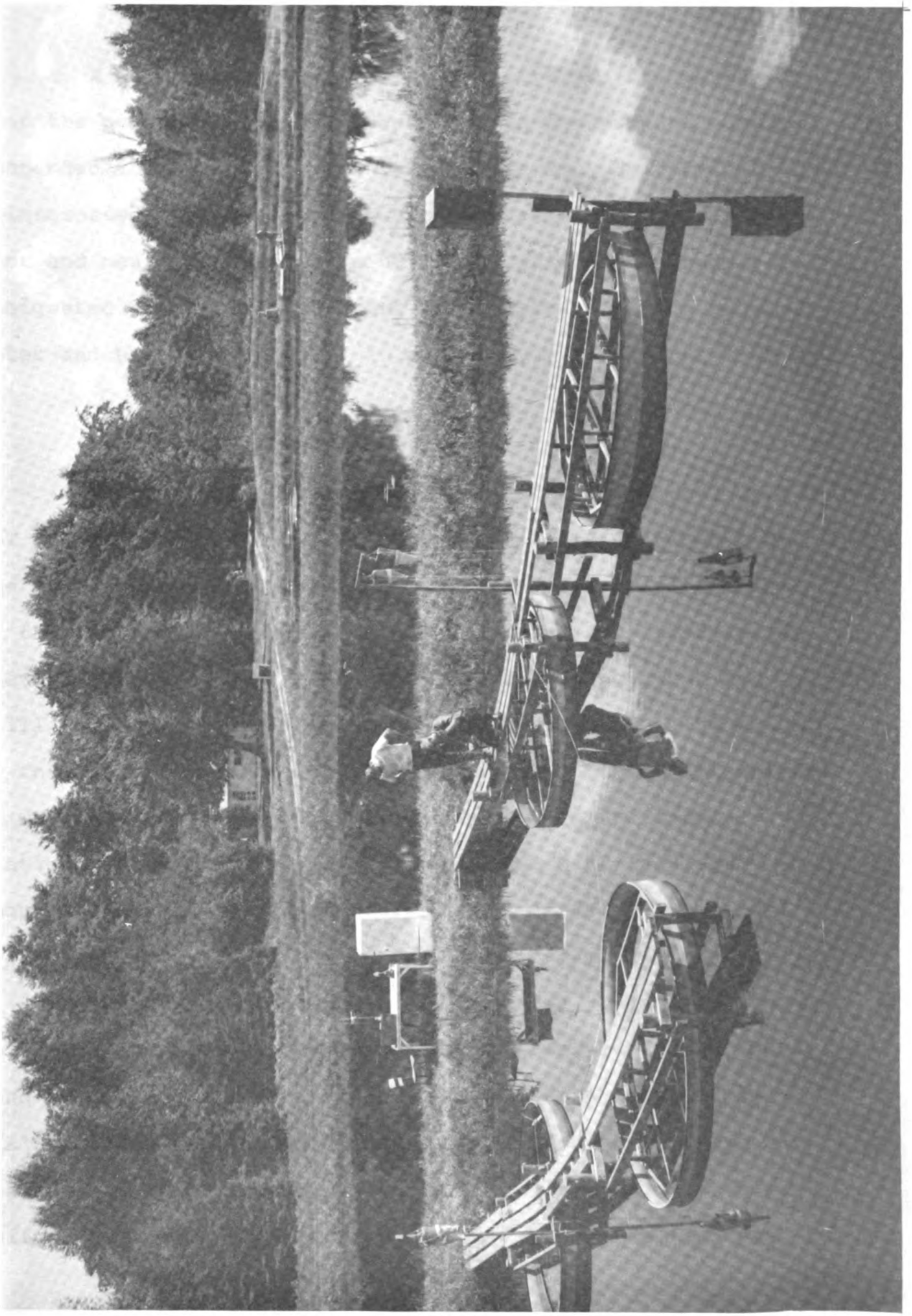
## PREPARATION OF THE STUDY PONDS

### Artificial Ponds

The artificial ponds were circular plastic lined swimming pools, ten feet in diameter and four feet deep. These four pools were placed off shore in a large farm pond (Figure 1). The farm pond kept the artificial ponds the same temperature and the artificial ponds received an equal amount of light and rain, yet each pond remained a closed system within itself.

The four ponds had been used to study the transport of DDT the previous summer which necessitated a cleanup of these ponds before they could be used again. This was done by removing all the water, the top layers of sand and organic matter from the bottom, and scrubbing down the sides of the pond. No detergent was used as this could leave residues that interfere with operating a gas chromatograph. It was assumed that any DDT that had adsorbed onto the sides of the ponds the previous summer would be tightly held to the plastic and would not interfere with this study. However, because the ponds had been used before, the pond that was the control the previous summer was used as a control the second summer.

Figure 1. A photograph, looking to the east, showing the four farm ponds at the Lake City Experiment Station and the four artificial pools set up in Pond A.



A wooden hexagonal frame was built for the ponds to keep the periphyton sheets (described in the methods section) suspended in the water. When the sheets were attached, a crisscrossed mesh was formed which had a grid size of one foot and nearly filled the pond. This grid was numerically designated as rows and columns to facilitate taking random water and bottom samples.

## Bottom Material

Three levels of DDT based on a dry weight ratio of DDT to sand were used: 0.05 ppm, 1.0 ppm, 10.0 ppm of DDT. The lowest level, 0.05 ppm, was selected to approximate some of the low contamination levels found in natural hydrosoils. The two remaining levels are considerably higher, but are still reasonable levels to study as DDT concentrations within this range and higher have been reported in the literature. It was thought that a ten fold difference in DDT concentrations between the two high level ponds would be high enough to show different transfer rates of DDT to the biota of a farm pond.

The formulation and the addition of the coated hydrosoils was approached ina similar manner for each pond. Each pond received two inches of bottom sand which amounted to six hundred kilograms of dry sand. Ungraded mortar sand was graded just prior to adding the insecticide to give a more uniform bottom material and to dry the sand. The sand was

assumed dry when it flowed easily and showed no tendency to stick together.

The addition of DDT to six hundred kilograms of sand posed a real problem. A homogeneous distribution of DDT was needed and six hundred kilograms of sand had too large a volume to be mixed all at once. To overcome this problem a base mixture was prepared. The required amount of DDT was dissolved in one liter of acetone and a few milliliters of food-coloring, and mixed with forty kilograms of sand. The food-coloring dyed the mixture a dark green and gave a good indication of how well the acetone mixture had mixed with the sand.

The base mixture was then added to the rest of the sand in a mortar box and mixed well with rakes and a hoe. The food-coloring in the base mix indicated the homogeneity of the final mixture. Because of the large volume of sand only one third of the sand was mixed at a time. The final mixture was added to the ponds with a wheelbarrow and shovels.

## Water

A plastic sheet was laid on the sand to prevent excessive stirring of the sand when the water was added. The water was taken from an adjacent farm pond and pumped into the artificial ponds using a gasoline powered Homart pump. The water was not filtered in any way to remove plankton or

algae because a natural pond situation was being sought. The adjacent farm pond had never been treated with DDT and pretreatment samples showed it to be free of insecticides. No turbidity was noticed in the ponds after a few hours.

The water levels in the artificial ponds were maintained three inches above the water level of the pond in which they were setting. Twice during the summer the water levels in the artificial ponds evaporated down to nearly equal the water level of the outside farm pond, and each time about three inches of water from the adjacent farm pond was added. The extra water in the artificial ponds was deemed necessary to keep them stable.

## Periphyton

Periphyton was added to the ponds as the primary producer utilizing the available solar radiation and dissolved nutrients. It can easily be grown on artificial substrates and removed for measurement (Kevern, 1962). In this study, plastic sheets with a large surface area were used to obtain the large amounts of periphyton required for analysis of its DDT content.

The periphyton sheets were added immediately after filling the ponds with water. The periphyton sheets were clear vinyl plastic strips, thirty-one inches long and ten inches wide. They had one half cup of clean sand stapled in a fold of the lower three inches of the sheet to hold it

vertical in the water. These sheets were seeded in the adjacent farm pond for ten days prior to adding them to the study ponds. A total of sixty periphyton sheets were added to each pond in a mesh like pattern, one foot by two feet, which encompassed the whole pond. The sheets were numbered consecutively and then randomly sampled.

Midway through the summer ten more periphyton sheets were set in the study ponds, without seeding them first. This was done to check the possibility of DDT recycling through the ponds.

## Fish and Microcrustaceans

The addition of fish and microcrustaceans completed the preparation of the study ponds. The fish were added fourteen hours after the water was added to the ponds. Four hundred green sunfish, Lepomis cyanellus Rafinesque, between one and three inches long, were seined from the adjacent ponds and added to the artificial ponds, one hundred fish per pond.

About five hours after the water had been added to the ponds, several tows with a plankton net were made and the microcrustaceans were added to the ponds. That same evening microcrustacean light traps (Baylor and Smith, 1953), had been set out in the four large farm ponds and the captured microcrustaceans added in the morning.

Midway through the summer on August 20, fifty pumpkinseeds, Lepomis gibbosus (Linnaeus), ranging from one to three inches long, were added to each pond to check for recycling of DDT and its metabolites.

## Temperature

A Taylor Water Thermograph was installed in one of the ponds to record a daily water temperature. Because the artificial ponds were placed in the same farm pond all ponds were assumed to have the same water temperature.

## SAMPLING SCHEDULE

The artificial ponds were designated by letters. The control pond was designated as pond C, the pond with 0.05 ppm DDT in the bottom as pond L (Low), the pond with 1.0 ppm DDT in the bottom as pond M (Medium), and the pond with 10.0 ppm DDT in the bottom as pond H (High).

The first samples were taken from all ponds twelve to twenty hours after the ponds were completely set up. The control pond samples were used as the initial treatment samples. This first sample was taken on July 9 and was designated as the 7/9 sample. The next three sample periods were spaced one week apart and were designated by the date they were taken, as the August 1 sample was recorded as the 8/1 sample. From August 1 to September 9, the sampling periods were ten days apart, and the last sampling period, on September 30, was twenty-one days after the previous sample. All samples are designated by the date of the day they were taken.

# METHODOLOGY

## Water

Column water samples were taken from each of the four ponds during a sampling period. The water sampler was a glass tube which could be stoppered at either end with corks. The glass rod was lowered into the water, a cork inserted in the top of the column, and the tube was raised until the bottom of the tube was just beneath the water surface. Then a cork was placed in the bottom of the tube securing the column of water. The water thus attained was drained into a glass water sample bottle. Two 1-liter water samples were taken at each sampling period. The locations of the water columns sampled were randomly picked. The water samples were refrigerated until they were extracted.

The extraction procedure involved partitioning each liter of water with one hundred ml of purified 30-60$^{o}$ petroleum ether for five minutes. The aqueous portion was discarded, and the petroleum ether was dried with anhydrous sodium sulfate. The petroleum ether was concentrated on a rotary vacuum evaporator and transferred to a graduated centrifuge tube for analysis on the gas chromatograph. The

method was 77% efficient with a relative standard deviation of 8.5%.

## Fish

Obtaining a fish sample from each of the four ponds proved to be the most difficult sample to get, as there was room for the fish to hide and they soon became trap shy. Overall, glass minnow traps, wire minnow traps, and a variety of hand nets were used to collect the fish. Two fish from each pond were kept at each sampling period, and were immediately frozen and stored for analysis.

The fish were analyzed on a wet weight basis only. Each fish was weighed while still frozen, put into a glass mortar, and diced with a scalpel. It was ground and dried with granular anhydrous sodium sulfate and a little sand. When the sample appeared to be dry, it was extracted three times with a 20 mls portion of 6% ethyl ether in petroleum ether, for a total of sixty mls.

The total extractant was then added to a prewet standard Mills Florisil column (Mills, 1959), and eluted with 250 mls of 6% ethyl ether in petroleum ether. All fractions were collected in a round bottom flask, evaporated to a workable volume, and transferred to a graduated centrifuge tube for analysis on the gas chromatograph.

The efficiency of extraction was 87% $\pm$ 1.5% for DDE, 91% $\pm$ 3% for DDD, and 81% $\pm$ 2.5% for DDT.

## Microcrustaceans

The microcrustaceans were captured with a light trap at night (Baylor and Smith, 1953). These traps were handmade light traps powered by a six volt car battery, and utilized the principles that amber light attracts certain microcrustaceans and blue light repels them. A plankton net could not be used because of the small size of the ponds and the periphyton sheets hanging in the ponds. The traps generally caught enough microcrustaceans to allow analysis for DDT. The samples were concentrated with a Foerst plankton centrifuge, placed in a plastic vial and frozen until analysis.

The microcrustacean extraction procedure was essentially the same as the procedure used with the fish. Due to the small size and amounts of microcrustaceans, a procedure for obtaining an equivalent wet weight was developed. The microcrustaceans were centrifuged in a graduated centrifuge tube and an estimate of the wet weight was obtained by assuming that one cubic centimeter of microcrustaceans was equal to one gram of microcrustaceans. The microcrustaceans were ground and extracted with 6% ethyl ether in petroleum ether, and the ether was eluted through a Florisil column in the same manner, and with the same efficiencies of extraction as the clean up procedure for the fish.

## Periphyton

The periphyton was sampled by removing two of the sixty periphyton sheets present. Each of the sheets in a pond was given a number from one to sixty and the samples were randomly chosen by number. The sheets were removed from the water, placed in a plastic bag and frozen until analysis.

The extraction of DDT and its metabolites from periphyton was complex and inefficient; recoveries were: DDE, 69.04% $\pm$ 2.05%; DDD, 91.97% $\pm$ 3.61%; and DDT, 81.18% $\pm$ 3.48%.

The sheets were scraped with rubber scrapers and rinsed with distilled water. The excess water was filtered off in a Buchner funnel, and the periphyton weighed. The weighed periphyton was placed in a glass mortar with granular sodium sulfate and sand, and extracted three times with acetonitrile. The volume of acetonitrile used was dependent upon the amount of periphyton. Ten ml of acetonitrile was used for the first gram or less and five ml for each additional gram. After extraction the acetonitrile was placed into a separatory funnel.

The total volume of acetonitrile was partitioned with one half its volume of petroleum ether for one minute and drained into a second separatory funnel. The acetonitrile was again partitioned with one half its volume of petroleum ether and allowed to stand. The petroleum ether

in the first funnel and its rinse were added to the second separatory funnel.

The acetonitrile was drained back into the first separatory funnel. A recovery partition with acetonitrile against the petroleum either was made and this was added to the acetonitrile in the first separatory funnel. The petroleum ether was then discarded.

The acetonitrile was then solivated with ten times its volume of 1% sodium sulfate in water. Petroleum ether, equal to the total volume of acetonitrile used, was added and partitioned for five minutes. The aqueous layer was discarded and the petroleum ether dried with anhydrous sodium sulfate.

NuChar Attaclay was added to the petroleum ether, approximately one tenth of a gram for every gram of periphyton to remove the phytopigments. The liquid was filtered through a filter bed of anhydrous sodium sulfate into a round bottom flask, evaporated to five ml, and transferred to a graduated centrifuge tube for analysis.

## Hydrosoil

The hydrosoil was sampled with pre-placed, randomly distributed bottom samplers. These samplers, seventy-five per pond, had been placed according to random numbers, flat on the pond bottom before the sand was added. A sampler consisted of a plastic bag attached to a circular steel band,

ten centimeters in diameter. A wire loop protruded above the sand and was attached to the steel band so the sampler could be located and removed. When sampled, the wire loop pulled the steel band up through the hydrosoil cutting a core sample into the plastic bag as it came up. These samples were easy and fast to take, and the samplers protected the plastic bottoms of the artificial ponds from being punctured.

Three samples were taken from each pond at a sampling period, and were immediately frozen and stored.

The procedure for extracting DDT and its metabolites from the hydrosoil required first a dry weight, so the samples were dried in an oven at fifty degrees Centigrade. When they were dry a weighed subsample was placed in a one liter Erlenmeyer flask, and soaked in 150 mls of 20% ethyl ether in petroleum ether for twenty-four hours.

The ether was drawn off into a separatory funnel and the sand rinsed with an additional 100 mls of 20% ethyl ether in petroleum ether. The ether left in the sand was washed out with 100 mls of distilled water and added to the separatory funnel. The water was discarded and the remaining ether dried with anhydrous sodium sulfate. The ether was concentrated with a rotary vacuum evaporator to ten milliliters and eluted through a Mills Florisil column with 250 mls of 6% ethyl ether in petroleum ether. The recovery

efficiencies were 86.0% $\pm$ 1.0% for DDE, 92.0% $\pm$ 3.0% for DDD, and 77.5% $\pm$ 3.0% for DDT.

## RESULTS AND DISCUSSION

### Water

The amount of DDT in the water varied considerably from pond to pond (Figure 2, and Table 1). In pond H, the highest level pond, the amount of dissolved DDT approached its saturation level of 1.2 ppb (Bowman, 1960). This was the highest concentration that any pond reached, and after reaching this peak the DDT concentration tapered off during the rest of the study period. DDT was detected in the water at every sampling period in pond H.

DDD, a degradation product of DDT, was detected in trace amounts in pond H in the first two sampling periods. At the end of fifteen days the DDD concentration in the water was up to 0.14 ppb and by twenty-two days it had reached 0.6 ppb. For the rest of the study period the DDD concentration fluctuated about a mean of $0.5243 \pm 0.0234$ ppb. This did not decline in the fall as the DDT concentration did. After the first week DDE was detected in trace amounts in all water samples.

The medium level pond, pond M, had its highest level of DDT in the first days sample. It maintained this approximate level until the cold water temperature set in near

Figure 2. The average concentrations of insecticide in the water showing and comparing the fluctuations and changes with time in each pond.

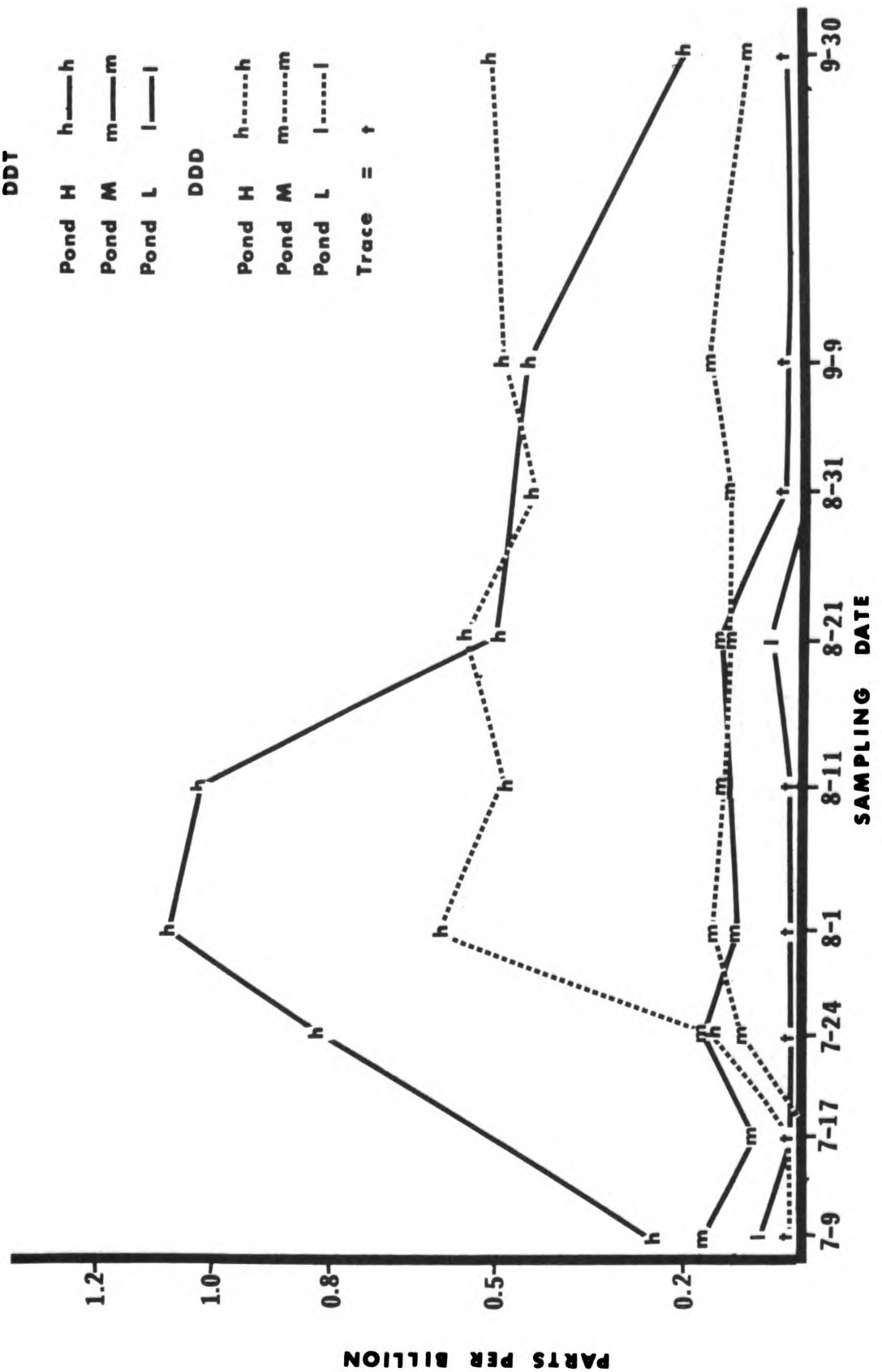

DDT
Pond H h—h
Pond M m—m
Pond L l—l
DDD
Pond H h·····h
Pond M m·····m
Pond L l·····l
Trace = t
PARTS PER BILLION
1.2
1.0
0.8
0.5
0.2
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE

Figure 2

Table 1. The average concentration of insecticide in the water of all the ponds at each sampling period.

| Sampling Dates | DDT | DDD | DDE | Total Insecticide |
|---|---|---|---|---|
| | (parts per billion) | | | |
| | | Pond C | | |
| No detectable insecticides were found in this pond. | | | | |
| | | Pond L | | |
| 7/9 | 0.054 | N.D. | N.D. | 0.054 |
| 7/17 | Tr | N.D. | N.D. | Tr |
| 7/24 | Tr | N.D. | N.D. | Tr |
| 8/1 | Tr | N.D. | N.D. | Tr |
| 8/11 | Tr | N.D. | N.D. | Tr |
| 8/21 | 0.0285 | N.D. | N.D. | 0.0285 |
| 8/31 | N.D. | N.D. | N.D. | N.D. |
| 9/9 | N.D. | N.D. | N.D. | N.D. |
| 9/30 | N.D. | N.D. | N.D. | N.D. |
| | | Pond M | | |
| 7/9 | 0.1593 | N.D. | N.D. | 0.1593 |
| 7/17 | 0.0663 | N.D. | N.D. | 0.0663 |
| 7/24 | 0.1573 | 0.0950 | Tr | 0.2523 |
| 8/1 | 0.1053 | 0.1442 | Tr | 0.2495 |
| 8/11 | N.C. | 0.1250 | Tr | N.C. |
| 8/21 | 0.1296 | 0.1244 | Tr | 0.2540 |
| 8/31 | Tr | 0.1140 | Tr | 0.1140 |
| 9/9 | Tr | 0.1510 | Tr | 0.1510 |
| 9/30 | Tr | 0.0765 | Tr | 0.0765 |
| | | Pond H | | |
| 7/9 | 0.231 | Tr | N.D. | 0.231 |
| 7/17 | N.C. | Tr | N.D. | N.C. |
| 7/24 | 0.8196 | 0.140 | Tr | 0.9596 |
| 8/1 | 1.0813 | 0.602 | Tr | 1.6833 |
| 8/11 | 1.016 | 0.504 | Tr | 1.5200 |
| 8/21 | 0.528 | 0.572 | Tr | 1.100 |
| 8/31 | N.C. | 0.440 | Tr | N.C. |
| 9/9 | 0.4514 | 0.500 | Tr | 0.9514 |
| 9/30 | 0.1825 | 0.528 | Tr | 0.7105 |

Tr - trace amount
N.D. - not detected
N.C. - not calculated

the end of August, about fifty days after the experiment was started. In the colder water the DDT was detected only in trace amounts.

DDD was first detected in the water of pond M after fifteen days, at nearly 0.10 ppb. The DDD level then remained constant at this level, 0.1218 $\pm$ 0.0099 ppb, in all following water samples. Again the DDD levels did not decline with the onset of cold water temperatures as the DDT concentrations did.

The lowest level pond, pond L, had water with a DDT concentration of 0.054 ppb in the first days sample. DDT was then detected in trace amounts for most of the summer with the exception of the forty-second day sample, where 0.0285 ppb was found. However, this is just within the range that DDT can be detected in the water and quantified. With the occurrence of cold water in the fall no DDT was detected in the water of this pond. DDD or DDE was not detected in this pond.

The control water samples from pond C never contained detectable insecticides.

Column water samples were taken and compared to water-soil interface samples, and no differences were found. This is probably due to the shallow depth of the ponds where winds and temperature changes could easily mix the water.

The analysis of water for DDT and its metabolities was hindered by the presence of an artifact which interfered

with DDT. Because of this the water data were not complete, and the data recorded have been checked on the microcoulometric gas chromatograph when possible or on another gas chromatograph which had a column containing QF-1 as its liquid phase. Some of the samples were saponified with alcoholic potassium hydroxide and the resulting DDE peak was quantified as DDT.

In general the water data seems to indicate that the amount of DDT translocated to the water is temperature dependent, because the DDT concentrations decline when the water gets cold. DDD concentrations did not decrease as the water temperatures decreased so DDD in water may not be temperature dependent.

DDD can be expected to occur in these pond waters, as Miskus, Blair, and Cassida (1965) found that natural lake waters can degrade DDT to DDD. So in this case the DDD could have come from the degradation of the DDT dissolved in the water or it may have come from another source, such as being translocated to the water from the bottom where DDD was found to be present. However, sampling was not extensive enough to show this to occur one way or the other.

## Fish

With one hundred fish in a small system like the artificial ponds, the food supply became a problem. After the first week, at the eight day sample, no microcrustaceans

were trapped in any of the artificial ponds. It was believed that the small size of the fish allowed them to feed heavily on the microcrustaceans and they had virtually eliminated their food supply. Because of this the fish were then fed ground food pellets, two to three grams per pond. By feeding the fish, transport and concentration of DDT by the food chain was probably reduced. The majority of the fish remained healthy for the study period. Throughout the summer six fish were observed to have died in the control pond and four of these died during the first week.

All fish analyzed contained DDT and its two main metabolites, DDD and DDE (Table 2). The level of these insecticides in the control fish remained about the same, $0.2018 \pm 0.0102$ ppm total insecticide, on a wet weight basis, throughout the study period. The metabolite and DDT to total insecticide ratios remained nearly constant in the control with only a slight increase in the DDD ratio at the end of the summer.

The slight fluctuations in the total insecticide content in the control fish on a part per million basis can be explained by virtue of growth and weight changes. As the summer progressed and growth occurred, the parts per million of insecticide in the fish decreased because they gained weight faster than they could accumulate the DDT that was available only in trace amounts in their environment. When the cold weather occurred in the fall and they were no longer

Table 2. The average insecticide content of the fish samples taken at all sampling periods from all ponds.

| Sampling Dates | DDT | DDD | DDE | Total Insecticide |
|---|---|---|---|---|
| | (parts per million) | | | |
| | | Pond C | | |
| 7/9 | 0.0706 | 0.0180 | 0.0955 | 0.1841 |
| 7/17 | 0.0756 | 0.0242 | 0.0970 | 0.1968 |
| 8/21 | 0.0536 | 0.0275 | 0.0675 | 0.1486 |
| 8/31 | 0.0754 | 0.0385 | 0.1087 | 0.2226 |
| 9/30 | 0.0881 | 0.0446 | 0.0857 | 0.2184 |
| | | Pond L | | |
| 7/9 | 0.0806 | 0.0127 | 0.0870 | 0.1804 |
| 7/17 | 0.1146 | 0.0385 | 0.0906 | 0.2437 |
| 7/24 | 0.0928 | 0.0275 | 0.0903 | 0.2106 |
| 8/1 | 0.0865 | 0.0509 | 0.0853 | 0.2228 |
| 8/21 | 0.1045 | 0.1295 | 0.1043 | 0.3383 |
| 8/31 | 0.0967 | 0.1519 | 0.0997 | 0.3483 |
| 9/9 | 0.2636 | 0.2875 | 0.2347 | 0.7858 |
| 9/30 | 0.0881 | 0.3005 | 0.1021 | 0.4907 |
| | | Pond M | | |
| 7/9 | 0.3725 | N.D. | 0.2505 | 0.6230 |
| 7/17 | 0.4058 | 0.1173 | 0.3408 | 0.8639 |
| 7/24 | 1.0077 | 0.2755 | 0.5252 | 1.8084 |
| 8/1 | 1.2525 | 0.5175 | 0.3500 | 2.1192 |
| 8/11 | 2.0307 | 1.1300 | 0.7501 | 3.9108 |
| 8/21 | 3.5490 | 2.9415 | 1.3088 | 7.7993 |
| 8/31 | 1.1741 | 1.7159 | 0.8883 | 3.7783 |
| 9/9 | 3.9543 | 3.3637 | 2.3244 | 9.6424 |
| 9/30 | 2.3785 | 8.3565 | 1.4268 | 12.1618 |
| | | Pond H | | |
| 7/9 | 0.1936 | 0.1071 | 0.5534 | 0.8541 |
| 7/17 | 1.0472 | 0.4816 | 0.2750 | 1.8038 |
| 7/24 | 2.1313 | 0.3444 | 0.3204 | 2.7961 |
| 8/1 | 11.5348 | 1.1595 | 1.2137 | 13.9080 |
| 8/11 | 15.3679 | 4.0453 | 2.9798 | 22.3929 |
| 8/21 | 9.7812 | 4.6605 | 1.9465 | 16.3883 |
| 8/31 | 13.0416 | 7.4071 | 2.6384 | 23.0871 |
| 9/9 | 9.9403 | 10.6777 | 2.9705 | 23.5884 |
| 9/30 | All fish had died. | | | |

N.D. - no insecticide detected

fed, the fish may have had a weight loss which would raise the ratio of insecticide in the fish.

The fish in the treated ponds contained more insecticide than the control fish. The differences in levels of DDT and its metabolites on a part per million basis with the fish's wet weight were tested with the Mann-Whitney U Test to find the probability of the distribution. The null hypothesis was: there are no differences in insecticide levels between the control and the treated ponds. The Mann-Whitney U Test was used because the samples were from different ponds and they did not have homogeneity of variances.

The two high level ponds, ponds H and M, were completely different from the control and had a probability of 0.001 or less that they were not different from the control. When pond L was tested with the control, DDT and DDD levels were significantly different at a 0.95 level of significance. The DDE distribution of ponds L and C had a probability of occurrence of 0.311 which is not significant.

DDT and its metabolites in the fish were compared on an interpond basis (Figure 3) by plotting the percentage of the total insecticide that DDT and each of its metabolites comprised in all ponds with time. All the treated ponds appeared to follow the same trends while the control pond, pond C, remained relatively constant.

Initially DDT accounted for the largest portion of the total insecticide present, but as time went on DDD

Figure 3. The insecticide makeup of the fish in the study ponds based on the individual percentages of the total insecticide that DDT, DDD, and DDE comprise at each sampling period.

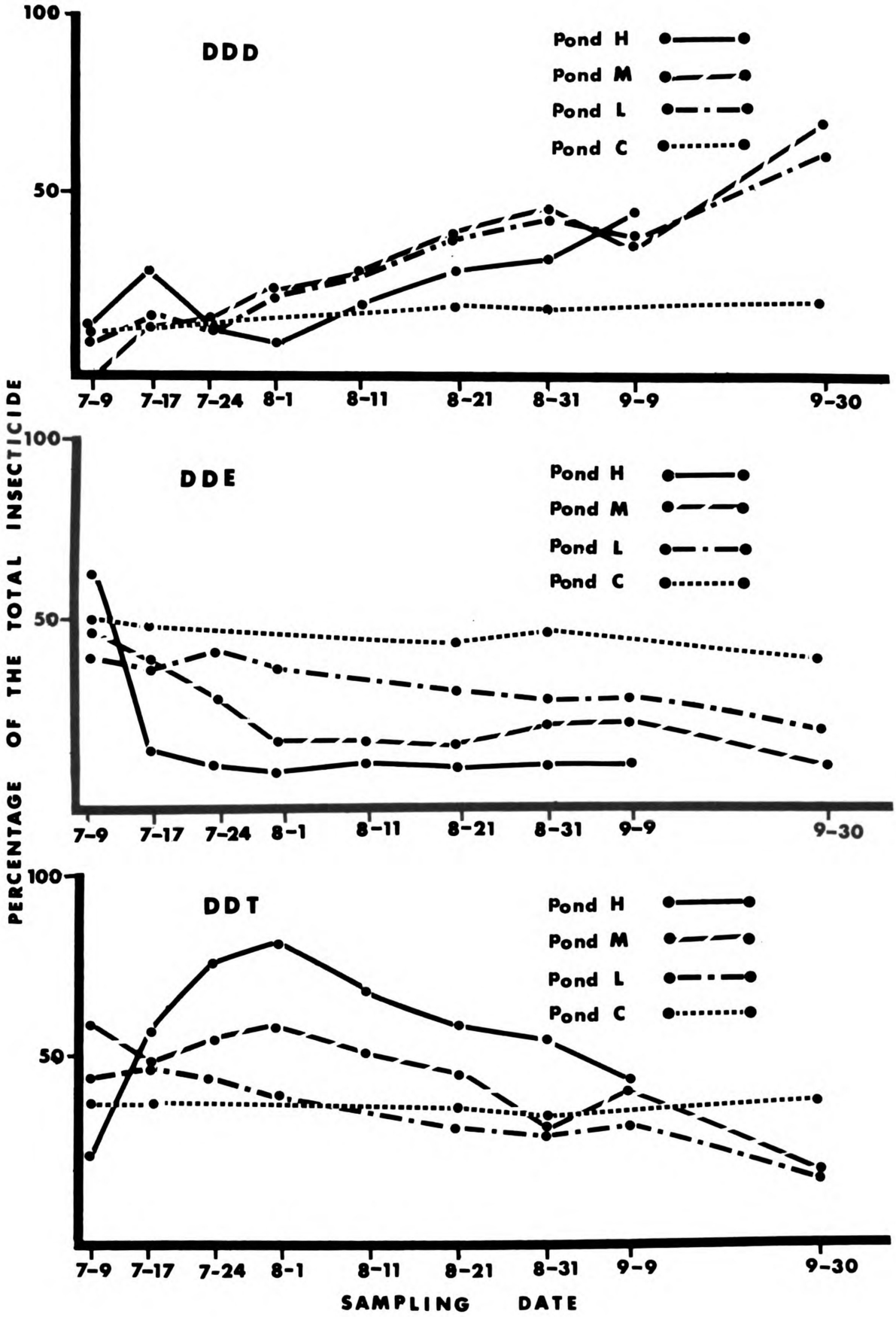

DDD
Pond H
Pond M
Pond L
Pond C
100
50
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
DDE
Pond H
Pond M
Pond L
Pond C
100
50
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
PERCENTAGE OF THE TOTAL INSECTICIDE
DDT
Pond H
Pond M
Pond L
Pond C
100
50
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE

Figure 3

replaced DDT as the most abundant compound present. DDD increased from approximately 10% to 55% of the total insecticide present. The percentage of DDE decreased as uptake of DDT occurred and thereafter remained as a nearly constant percentage of the total insecticide. This shows clearly that insecticide changes do occur with time, however DDE did not become a major factor as a degradation product of DDT.

All the treated ponds followed the same general pattern of change when compared to the control pond, even though there were large differences between the hydrosoil insecticide concentrations in the ponds. The main effect of increased hydrosoil concentrations appears to be in the degree of change when compared to the control rather than the type of change. This is shown in Figure 3 when the changes in the low level pond lie closer to the control than the changes in the high level pond.

The uptake, concentration, and change of DDT and its metabolites in the fish of each individual pond was studied (Figures 4, 5, 6, and 7) by plotting DDT, DDD, DDE, and total insecticide in the fish of each pond on the same graph. In all treated ponds DDT increased and plateaued faster than its metabolites. DDE seemed to follow the DDT changes in the treated ponds and the control pond. By the end of the study DDD had become the most abundant metabolite present and, after sixty days, all ponds showed it to have equalled or surpassed the DDT concentrations in the fish. Initially,

Figure 4. The semi-log plot of the average total insecticide concentrations in the fish at each sampling period of each study pond.

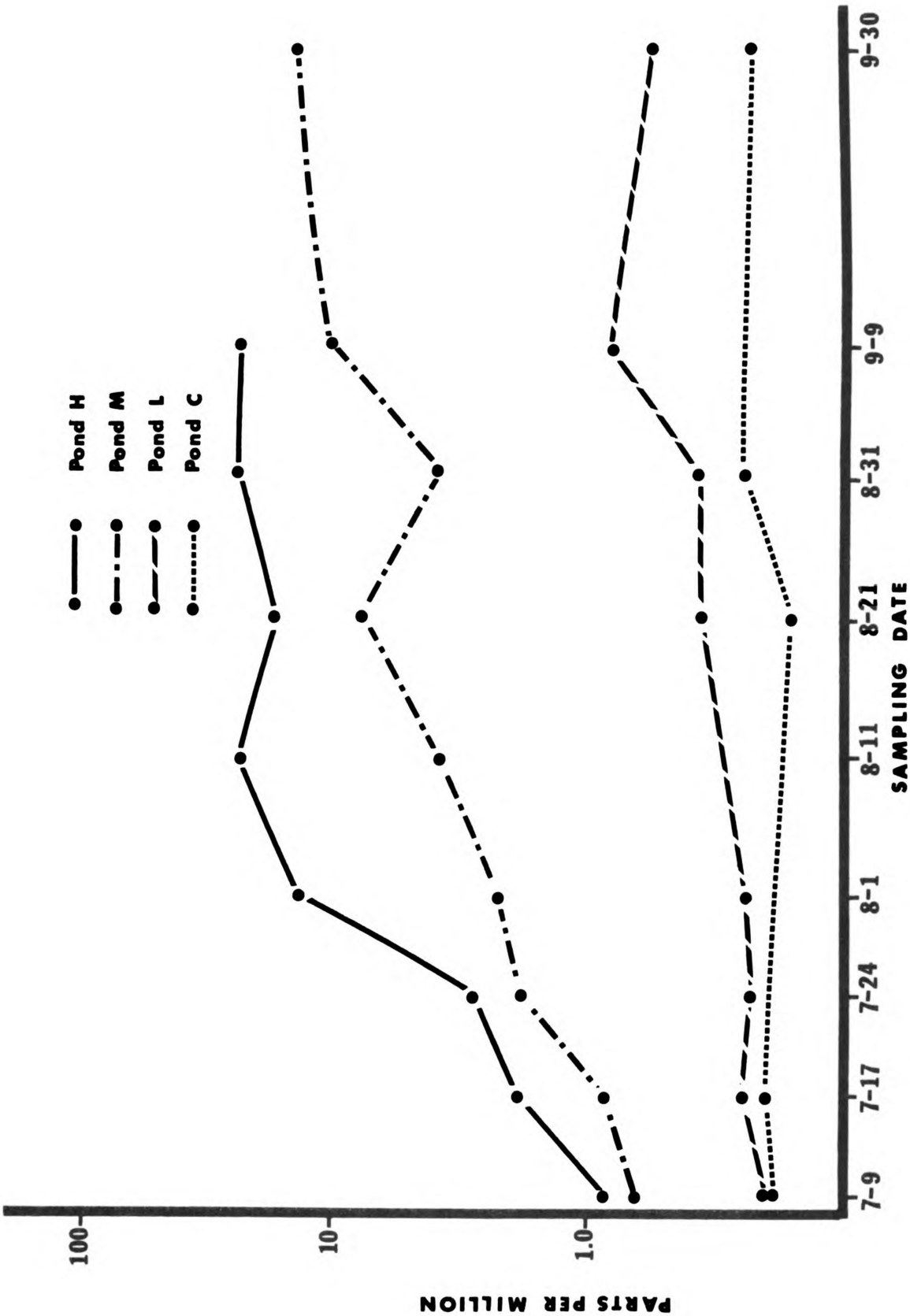

Pond H
Pond M
Pond L
Pond C
100
10
1.0
PARTS PER MILLION
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE

Figure 4

Figure 5. The semi-log plot of the average DDT concentrations in the fish at each sampling period of each study pond.

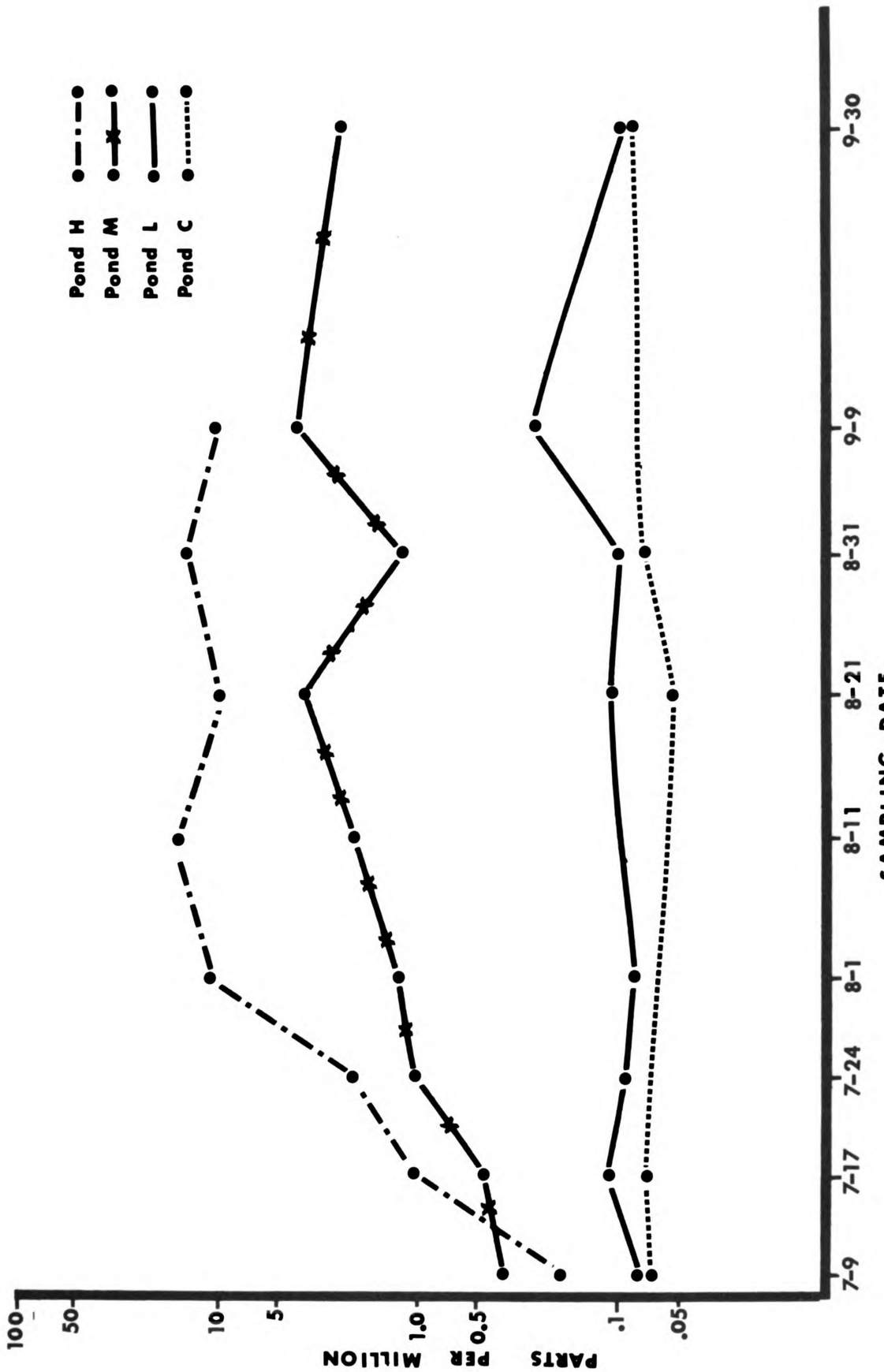

Pond H
Pond M
Pond L
Pond C
100
50
10
5
1.0
0.5
.1
.05
PARTS PER MILLION
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE

Figure 5

Figure 6. The semi-log plot of the average DDD concentrations in the fish at each sampling period of each study pond.

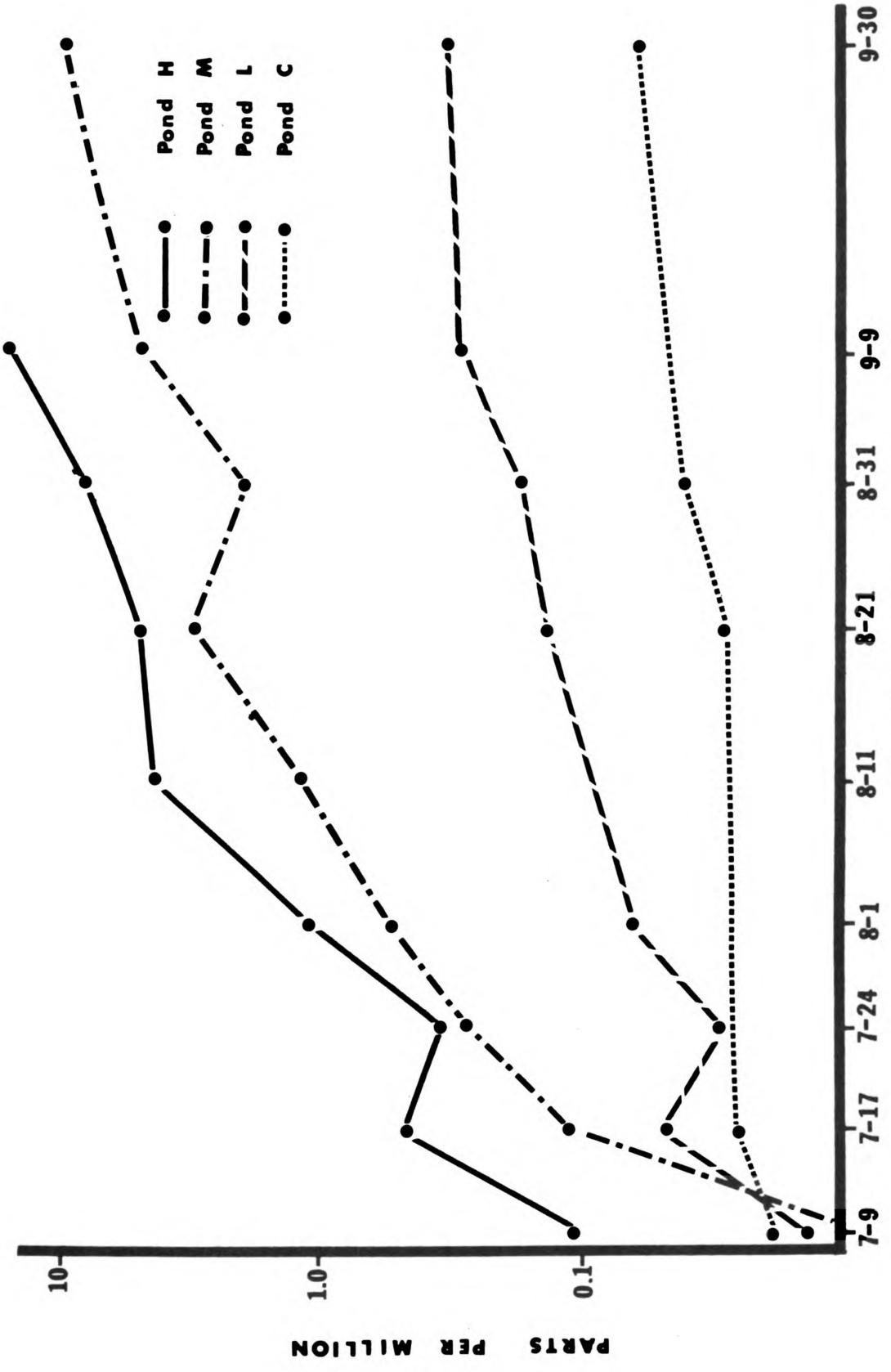

Pond H
Pond M
Pond L
Pond C
10
1.0
0.1
PARTS PER MILLION
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE

Figure 6

Figure 7. The semi-log plot of the average DDE concentrations in the fish at each sampling period of each study pond.

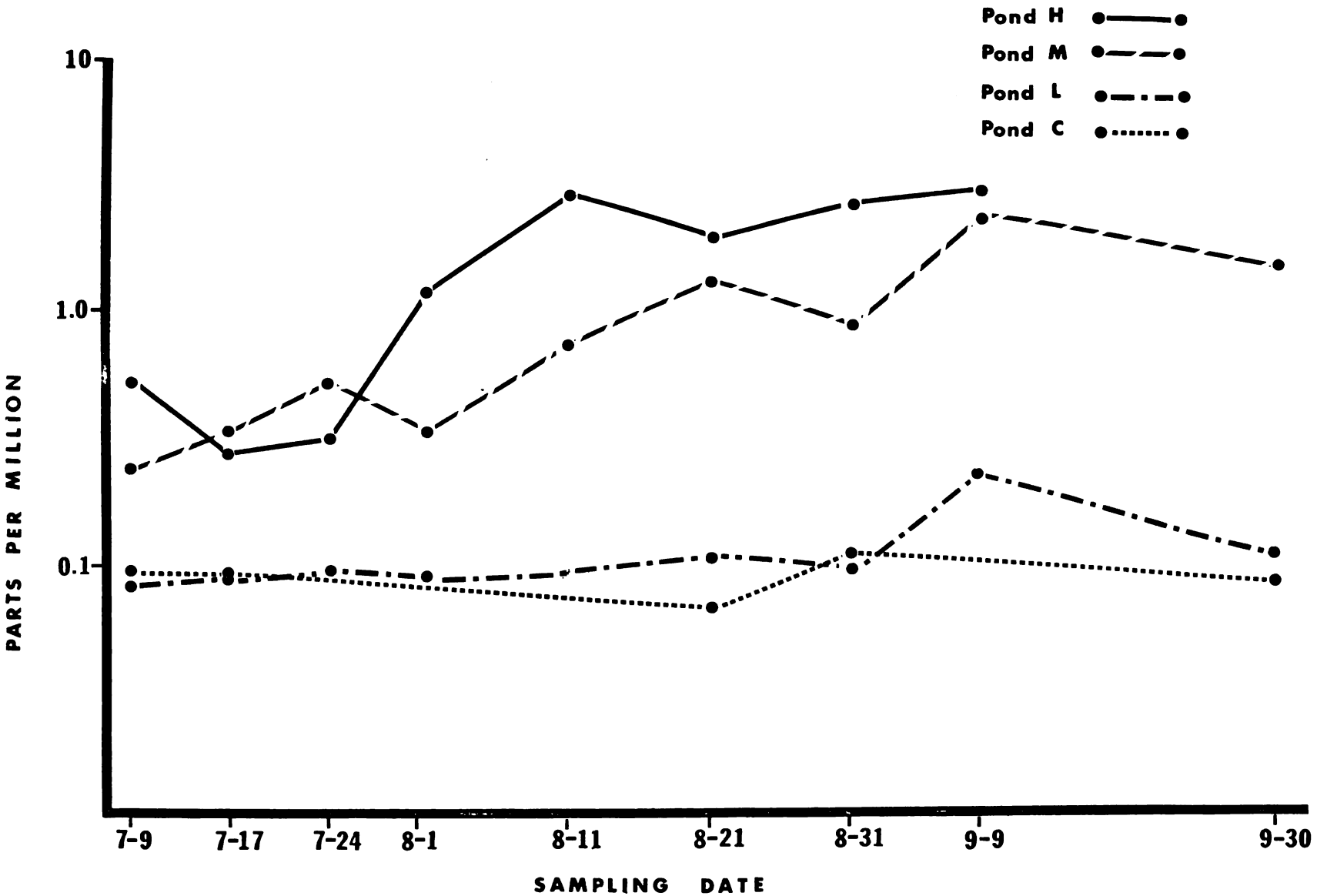

Pond H
Pond M
Pond L
Pond C
10
1.0
0.1
PARTS PER MILLION
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE

Figure 7

in all ponds, DDD was the lowest product, so low that it was not even detected in pond M. This could indicate that the DDD came from a source other than DDT degradation in the fish. The conversion of DDT to DDD was reported by Miskus, Blair, and Cassida (1964) to occur in lake water and with reduced prophorins. Wedemeyer (1967) said Aerobacter aerogenes, a common bacteria in surface water (Carpenter, 1961), could dechlorinate DDT to DDD. Finley and Pillmore (1963) demonstrated that the conversion of DDT to DDD can also occur in animal tissue, so it seems likely that the large accumulation of DDD in the fish is a result of the amount degraded in the fish and the amount translocated from sources outside the fish. Hunt (1960) showed that DDD was easily transported throughout an aquatic environment.

The route of insecticide transport is not well known, and the data from this experiment does not provide any definite answers even though there is evidence that all the insecticide in the fish could have entered across the gills.

The amount of water that must be passed over the gills to meet a fish's oxygen requirements was calculated using the oxygen requirement value given in Prosser and Brown (1961) as the milliliters of oxygen per gram wet weight per hour for freshwater fish, the efficiency of oxygen extraction from the water passing over the gills, and the oxygen concentration in the water of the artificial ponds.

This value was 0.8724 liters of water per gram wet weight of fish per day.

By assuming one hundred percent removal of insecticide from the water passing over a fish's gills, the amount of water required to give the fish a given quantity of insecticide can be calculated, if the insecticide concentration in the water is known. When the micrograms of insecticide in the fish at forty-three days was divided by the average insecticide concentration in the water during the first forty-three days of the experiment, the liters of water necessary to give the fish the given amount of insecticide was found. This value was divided by the days which gave the results of 0.3390 liters per gram per day required in pond H fish, 0.8462 liters per gram per day for pond M fish, and 0.1942 liters per gram per day for pond L fish.

The liters per day of water required to supply the individual amounts of DDT, DDD, and DDE were calculated, but these had approximately the same proportional difference between ponds as the total insecticide values had. So the difference in water requirements between the high, medium, and low ponds cannot be attributed to the different proportions of DDT, DDD, and DDE present between the ponds. Hence it appears that differential uptake between the different compounds does not exist.

Since the water requirement data are essentially estimates no definite statements regarding the differences

between ponds can realistically be made. However the data does indicate that the amount of insecticide accumulated in the fish could have been the result of transfer across the gill membranes, and accumulation from the food chain is not required to explain the concentrations present in the fish after forty-three days.

Fish mortalities were recorded in the daily observations only when the fish were found dead. There was consistent mortality only in the highest concentration pond, pond H. After one week twelve percent of these fish had died, all with DDT poisoning symptoms. As a result of this consistent mortality there were no fish left in pond H at the last sampling period.

The major dieoff began on July 14 in pond H, and the fish were fed in an attempt to curb the mortality as there appeared to be a food shortage in the ponds. This mortality occurred between July 14 and July 16, which followed a rapid temperature drop (Figure 8). Eaton and Sternburg (1967) reported that a negative temperature coefficient for the appearance of DDT poisoning symptoms is a phenomena associated with DDT poisoning in cockroaches. As the temperature decreases, the severity and numbers of DDT induced trains of nervous impulses increase, while raising the temperature decreases or even abolishes the number of impulse trains. If this phenomena can be carried over to fish by a similar action mechanism of DDT poisoning, it is likely that a

Figure 8. The plot of the water temperature in pond H in degrees F from July 13 to July 20 is compared to the numbers of dead fish and fish with DDT induced convulsions.

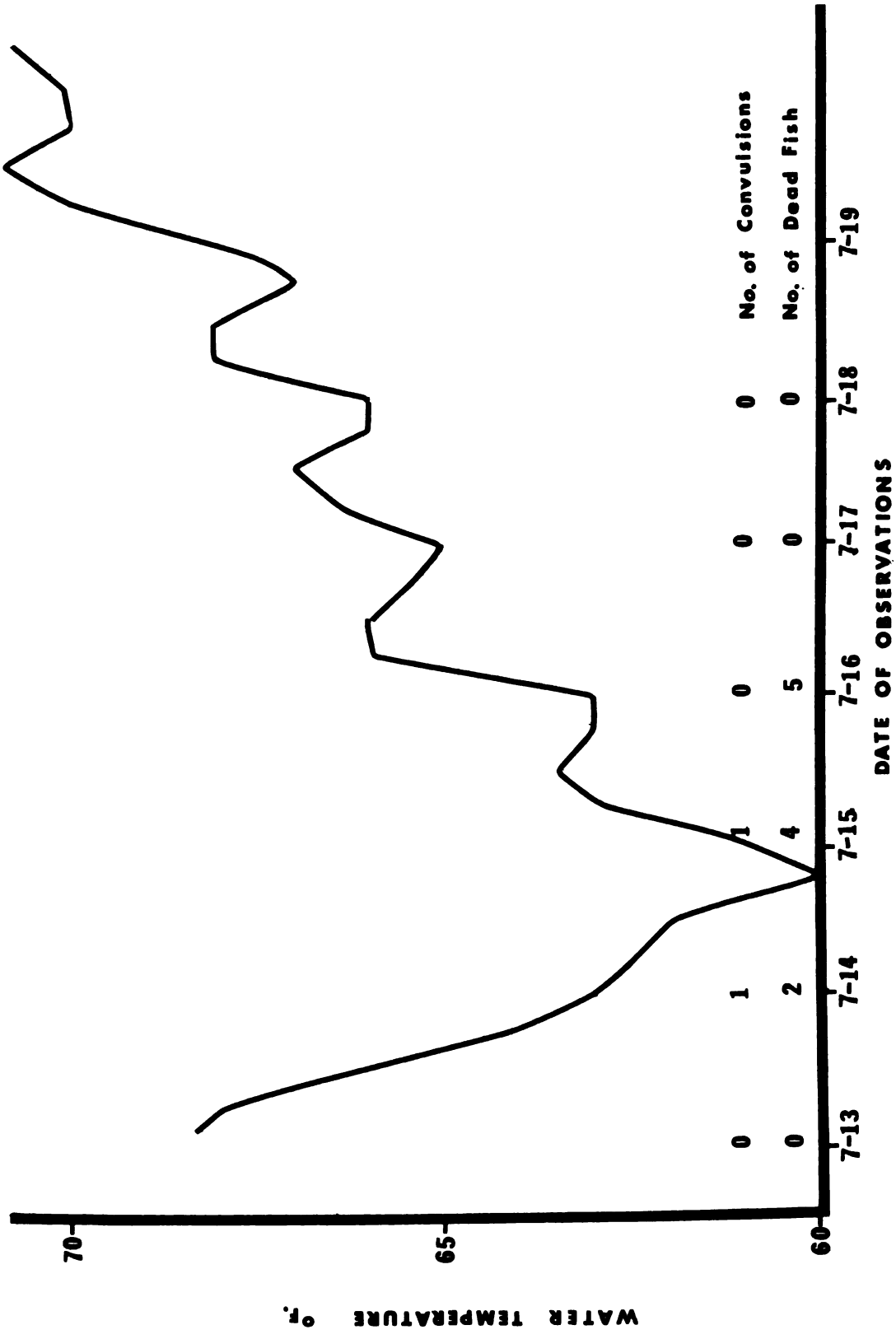

WATER TEMPERATURE °F.
70
65
60
7-13
7-14
7-15
7-16
7-17
7-18
7-19
DATE OF OBSERVATIONS
No. of Convulsions
No. of Dead Fish
0 1 1 0 0 0
0 2 4 5 0 0

Figure 8

decrease in the temperature of the pond water could induce DDT poisoning of the fish. This appears to be the case at hand, as the DDT was actively present because uptake was still occurring and there was a probable food shortage at the time of the mortality.

While other temperature drops occurred over the rest of the study period they were not accompanied with a food shortage and no major mortalities occurred. Also the insecticides present later in the summer were not predominately DDT, but included a higher percentage of DDT degradation products. By midsummer the insecticides were probably stored in the fats and lipids where they would be somewhat protected during times of stress. Hence, the insecticides later in the summer were not easily accessible to cause poisoning of the central nervous system.

On August 20, forty-five days after the study was initiated, small pumpkinseeds, Lepomis gibbosus, one to three inches long were added to the ponds to check for continuous cycling or recycling of DDT and its metabolites. In pond L only DDD differed significantly from insecticide levels in the control fish. The DDD concentration in pond L fish was approximately ten times the DDD concentration in the control fish (Table 3). In ponds M and H the DDT, DDD, and the DDE concentrations were higher than the concentrations in the control fish. Again as in pond L, the DDD

Table 3. The average insecticide content of the recycled fish at each sampling period from each pond.

| Sampling Dates | DDT | DDD | DDE | Total Insecticide |
|---|---|---|---|---|
| | | (parts per million) | | |
| | | Pond C | | |
| 8/31 | 0.0274 | 0.0253 | 0.0744 | 0.1271 |
| 9/30 | 0.0572 | 0.0369 | 0.0795 | 0.1736 |
| | | Pond L | | |
| 8/31 | 0.0340 | 0.0865 | 0.1301 | 0.2505 |
| 9/9 | 0.0513 | 0.0946 | 0.1465 | 0.2924 |
| 9/30 | 0.1697 | 0.4123 | N.C. | 0.5820 |
| | | Pond M | | |
| 8/31 | 0.1787 | 0.7341 | 0.1741 | 1.0869 |
| 9/9 | 0.2971 | 1.2140 | 0.2600 | 1.7711 |
| 9/30 | 0.1536 | 1.7860 | 0.2577 | 2.1973 |
| | | Pond H | | |
| 8/31 | 0.3095 | 3.4480 | 1.2096 | 4.9671 |
| 9/9 | 2.6160 | 7.5060 | 1.0326 | 11.1546 |

N.C. - not calculated

concentrations in ponds M and H increased faster than DDT or DDE.

The uptake of total insecticide that occurred in the pumpkinseeds with time was compared with the uptake that occurred in the original fish with time (Figure 9). The uptake patterns were quite similar for each pond, which is significant when the fact that a different species of fish was used and that the recycling experiment took place in late summer and early fall when the weather conditions were different. The water temperatures were colder during the recycling experiment and the insecticide levels in the water were lower. Thus, because the initial uptake of insecticides by the pumpkinseeds was equal to or greater than the initial uptake of the original fish it appears that the uptake of DDT and its metabolites by these fish is not completely dependent upon the insecticide concentrations in the water.

In general it was found that a DDT concentration in a sandy hydrosoil of 10.0 micrograms per gram will cause constant fish mortality. There were no effects noticed on the fish of the other treated ponds, although analysis showed that DDT and its metabolites were found to be continuously recycled in the aquatic environment and it was not inactivated with time.

Figure 9. The initial uptake of insecticide that occurred in the recycled fish is compared to the initial uptake of insecticide by the original fish.

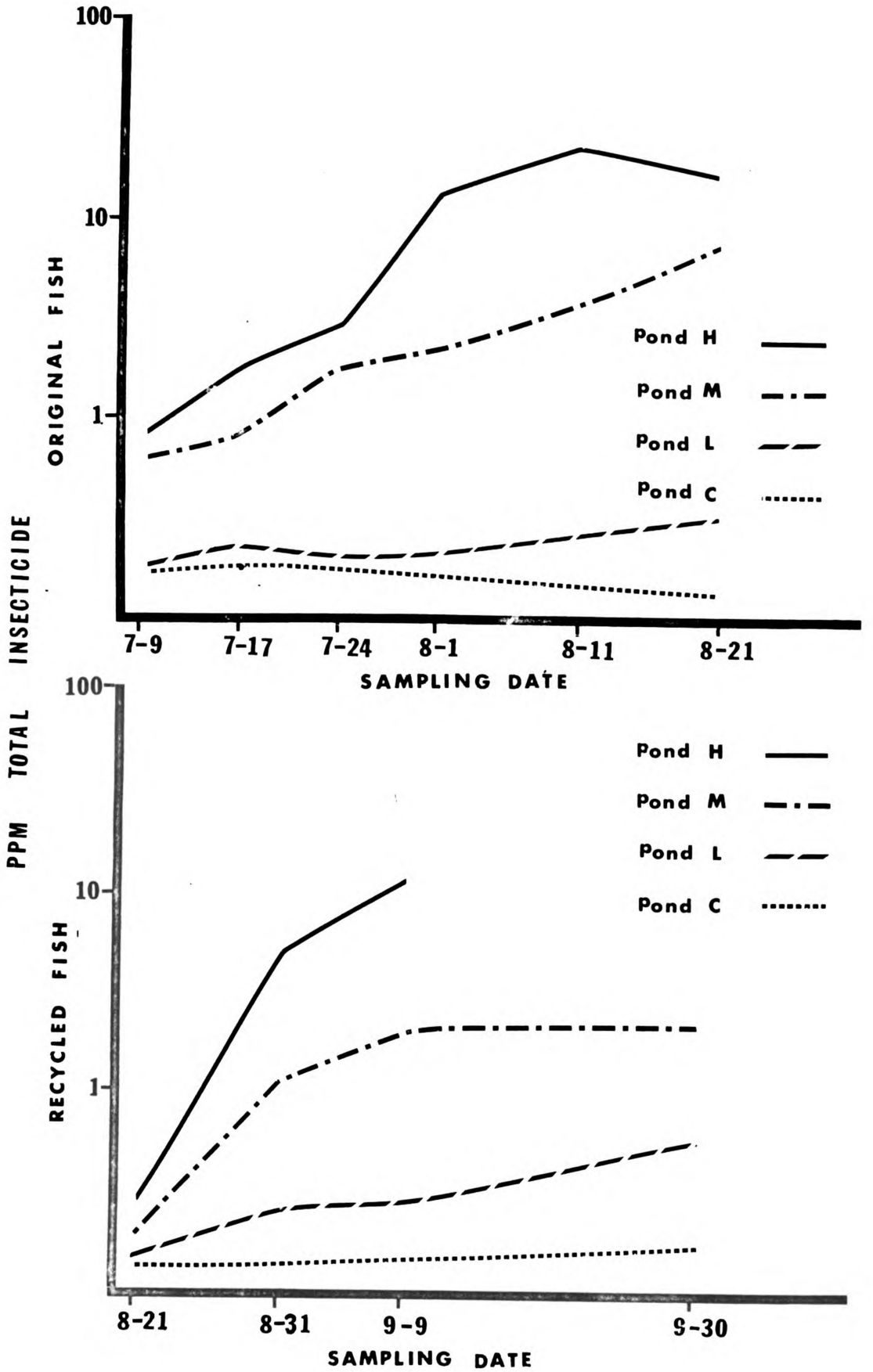

PPM TOTAL INSECTICIDE
ORIGINAL FISH
100
10
1
Pond H
Pond M
Pond L
Pond C
7-9
7-17
7-24
8-1
8-11
8-21
SAMPLING DATE
RECYCLED FISH
100
10
1
Pond H
Pond M
Pond L
Pond C
8-21
8-31
9-9
9-30
SAMPLING DATE

Figure 9

## Microcrustaceans

All the microcrustaceans of the treated ponds contained DDT and each of its metabolites, while the microcrustaceans from the control pond contained no detectable insecticides (Table 4). The lowest level pond, pond L, contained DDT, DDD, and DDE in concentrations that were detectable but not always measureable. This is a function of the small amounts of microcrustaceans that were caught which could not give enough insecticide to measure when they were extracted.

The study of microcrustaceans is not complete due to the fact that the microcrustacean population was kept very low by the large numbers of fish in the ponds. The microcrustaceans were caught with light traps at the first sample period. When the traps were set in the experimental ponds for the second sample one week later, no microcrustaceans were captured. So more microcrustaceans were trapped and netted from the adjacent ponds and added to the study pools. After seven days there were still only small amounts of microcrustaceans captured so more microcrustaceans were added to the ponds. In addition the fish were fed ground food pellets. During the rest of the study period microcrustaceans were always captured, but not always in amounts large enough to measure the DDT content. The controls never contained detectable levels of insecticides so it logically

Table 4. The insecticide content in the microcrustaceans from all ponds.

| Sampling Dates | DDT | DDD | DDE | Total Insecticide |
|---|---|---|---|---|
| | (parts per million) | | | |
| | | Pond L | | |
| 7/9 | tr | tr | tr | tr |
| 8/11 | 0.0792 | 0.0752 | 0.0779 | 0.2323 |
| 8/31 | tr | tr | 0.1410 | 0.1410 |
| 9/9 | tr | tr | tr | tr |
| | | Pond M | | |
| 7/9 | 0.5610 | 0.9815 | 0.5359 | 2.0784 |
| 8/11 | 1.0736 | 0.8648 | 0.4683 | 2.4067 |
| 8/31 | 0.1514 | 1.1920 | 0.3608 | 1.6968 |
| 9/9 | 0.2726 | 0.7405 | 0.2870 | 1.3001 |
| | | Pond H | | |
| 7/9 | 2.3570 | 1.4340 | 0.8972 | 4.6882 |
| 8/11 | 2.8901 | 1.2910 | 1.3760 | 5.5591 |
| 8/31 | 3.2800 | 5.0372 | 1.0540 | 9.3712 |
| 9/9 | 1.5800 | 2.3629 | 0.6905 | 4.6324 |
| | | Pond C | | |
| All samples contained no detectable levels of insecticide | | | | |

tr - trace of insecticide

follows that none of the three additions of microcrustaceans contained any insecticides.

The samples analyzed do not represent a population that has lived in the ponds the duration of the study period because of the many additions of microcrustaceans to the ponds at different times. The low microcrustacean populations and hand feeding the fish reduced the accumulative effect of food chain transport of DDT to the fish. However, some of the initial uptake of DDT would be a result of the fish consuming all the available microcrustaceans in the first week, as the microcrustaceans had DDT concentrations of 2.357 ppm in pond H and 0.5610 ppm in pond M after one day in the ponds (Table 4).

Overall there was not much change in the insecticide levels in the microcrustaceans with time (Figures 10 and 11). A graph of the percent of the total insecticide that either DDT, DDD, or DDE makes up with time (Figure 12) shows that the DDE comprised a nearly constant percentage of the total insecticide, about 20%. When the DDT percentage decreased, the DDD percentage increased and compensated for the DDT decrease. This could indicate that DDD is the main degradation product of the microcrustaceans or it could indicate the available insecticide ratio in the bottom material because the microcrustaceans do spend the daylight hours on the bottom. Hunt (1960) indicated that zooplankton or microcrustaceans might have the ability to accumulate DDT from

Figure 10. The change and fluctuation of insecticides in the microcrustaceans of study pond H with time.

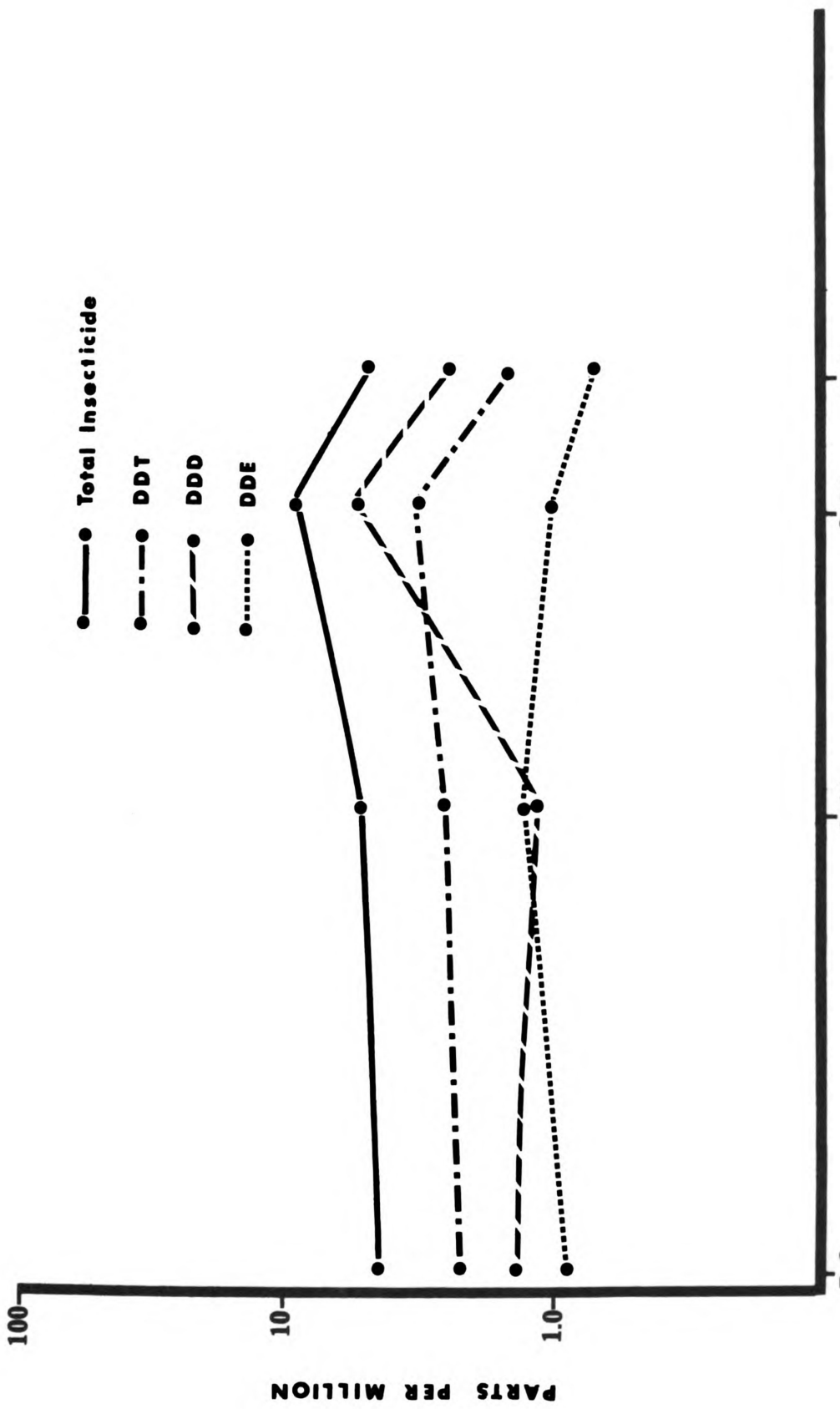

Total Insecticide
DDT
DDD
DDE
100
10
1.0
PARTS PER MILLION
7-9
8-11
8-31
9-9
SAMPLING DATE

Figure 10

Figure 11. The change and fluctuation of insecticides in the microcrustaceans of study pond M with time.

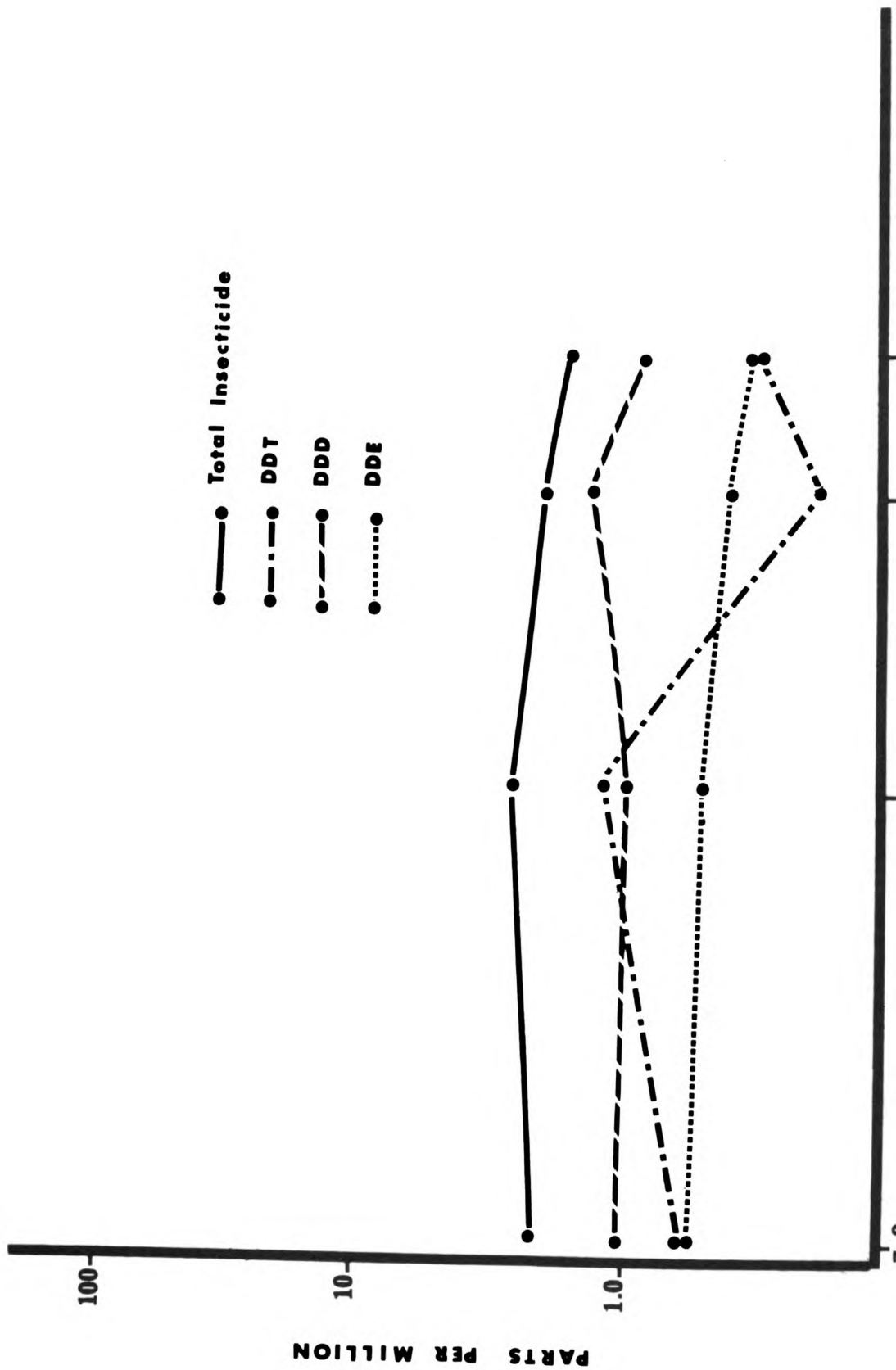

Total Insecticide
DDT
DDD
DDE
100
10
1.0
PARTS PER MILLION
7-9
8-11
8-31
9-9
SAMPLING DATE

Figure 11

Figure 12. The percentage of the total insectide that DDT and each of its metabolites comprise in the microcrustaceans at each sampling period.

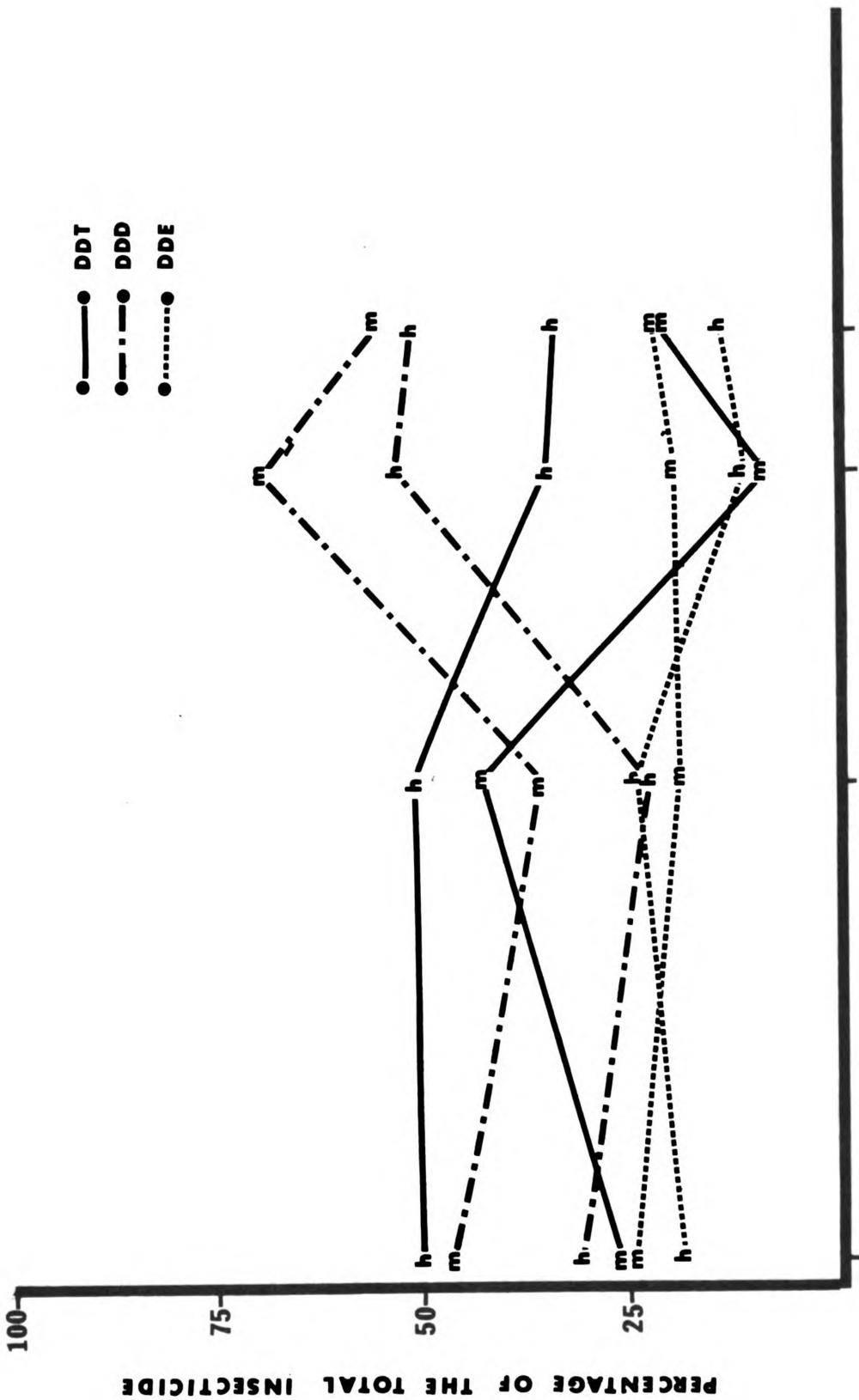

DDT
DDD
DDE
PERCENTAGE OF THE TOTAL INSECTICIDE
100
75
50
25
7-9
8-11
8-31
9-9
SAMPLING DATE
h
m

Figure 12

the bottom, and to distribute it to higher organisms. Jones and Moyle (1962) showed population changes of zooplankton as a result of farm pond treatment with DDT so the zooplankton are affected by concentrations of DDT.

It seems likely that the insecticide makeup of the hydrosoil and the ability of the microcrustaceans to dechlorinate DDT to DDD will be reflected in the DDD accumulation in the microcrustaceans, but more extensive research will have to be done to separate these two factors of DDT and DDD accumulation.

## Periphyton

The periphyton from each pond showed considerable variation in DDT content, but the DDT concentration in the periphyton of each pond was found to be significantly different from all other ponds at a 0.95 level of significance. The Mann-Whitney U Test was used to calculate these probabilities.

The variable results of the periphyton study are due to the large weight differences of the periphyton samples. The large samples were consistently lower in their DDT content (per unit weight) than the small samples. The weights of the samples were influenced by the large amounts of floating algae that accumulated in the study ponds, which were abundant enough to shield out one half to three fourths of the available light. The algae did not float continuously

so the amount of area shaded was not constant and this would have its effect on the periphyton growth rates.

The amounts of insecticide found in the periphyton were quite variable so two insecticide to periphyton ratios were used. These were based on the premises that DDT may be adsorbed as a function of growth which would be measured by weight, or that DDT may be adsorbed as a function of the exposed surface area available for periphyton growth which would be measured in square meters.

The parts per million of insecticide in the periphyton was based on a wet weight of the periphyton (Table 5). The total micrograms of insecticide present was calculated to give the value, total micrograms per square meter of available surface area (Table 6). These two values were compared for ponds H, M, and L (Figures 13, 14, 15). It can be seen in ponds H and M that initially the total amount of insecticide increased while at the same time the parts per million based on the wet weight decreased. This is due to the DDT not being adsorbed proportionally to an increase in weight of the periphyton, nor is it adsorbed strictly as a function of the available surface area. Thus DDT uptake by the periphyton appears to be a function of both growth and exposed surface area.

When the insecticides in each individual pond were compared (Figures 13, 14, 15) showing the uptake of DDT and its metabolites by the periphyton with time, it can be seen

Table 5. The average insecticide content of the periphyton samples taken at each sampling period from all ponds.

| Sampling Date | DDT | DDD | DDE | Total Insecticide |
|---|---|---|---|---|
| | (parts per million) | | | |
| | | Pond C | | |
| 7/9 | 0.0533 | N.D. | N.D. | 0.0533 |
| 7/24 | 0.0832 | N.D. | N.D. | 0.0832 |
| 8/11 | 0.0392 | N.D. | N.D. | 0.0392 |
| 9/9 | 0.0152 | N.D. | N.D. | 0.0152 |
| 9/30 | 0.0195 | N.D. | N.D. | 0.0195 |
| | | Pond L | | |
| 7/9 | 0.0534 | N.D. | N.D. | 0.0534 |
| 7/17 | 0.1266 | N.D. | N.D. | 0.1266 |
| 7/24 | 0.1021 | N.D. | N.D. | 0.1021 |
| 8/1 | 0.0741 | N.D. | N.D. | 0.0741 |
| 8/11 | 0.1004 | N.D. | N.D. | 0.1004 |
| 8/21 | 0.1371 | Tr | Tr | 0.1371 |
| 8/31 | 0.0455 | Tr | Tr | 0.0455 |
| 9/9 | 0.0221 | Tr | Tr | 0.0221 |
| 9/30 | 0.0387 | Tr | Tr | 0.0387 |
| | | Pond M | | |
| 7/9 | 0.4341 | N.D. | N.D. | 0.4341 |
| 7/17 | 0.2118 | Tr | Tr | 0.2118 |
| 7/24 | 0.1255 | Tr | Tr | 0.1255 |
| 8/1 | 0.5339 | Tr | Tr | 0.5339 |
| 8/11 | 0.4481 | 0.1557 | Tr | 0.6038 |
| 8/21 | 0.1109 | 0.0731 | Tr | 0.1840 |
| 8/31 | 0.1210 | 0.1138 | Tr | 0.2348 |
| 9/9 | 0.0294 | 0.0461 | Tr | 0.0755 |
| 9/30 | 0.1174 | 0.1420 | Tr | 0.2598 |
| | | Pond H | | |
| 7/9 | 0.5463 | Tr | N.D. | 0.5463 |
| 7/17 | 0.2735 | N.D. | N.D. | 0.2735 |
| 7/24 | 0.8259 | Tr | N.D. | 0.8259 |
| 8/1 | 1.0760 | Tr | Tr | 1.0760 |
| 8/11 | 0.7259 | 0.2554 | Tr | 0.9813 |
| 8/21 | 0.2605 | 0.1571 | Tr | 0.4176 |
| 8/31 | 0.5787 | 0.4396 | Tr | 1.0183 |
| 9/9 | 0.6173 | 0.7366 | Tr | 1.3539 |
| 9/30 | 0.2317 | 0.3534 | Tr | 0.5851 |

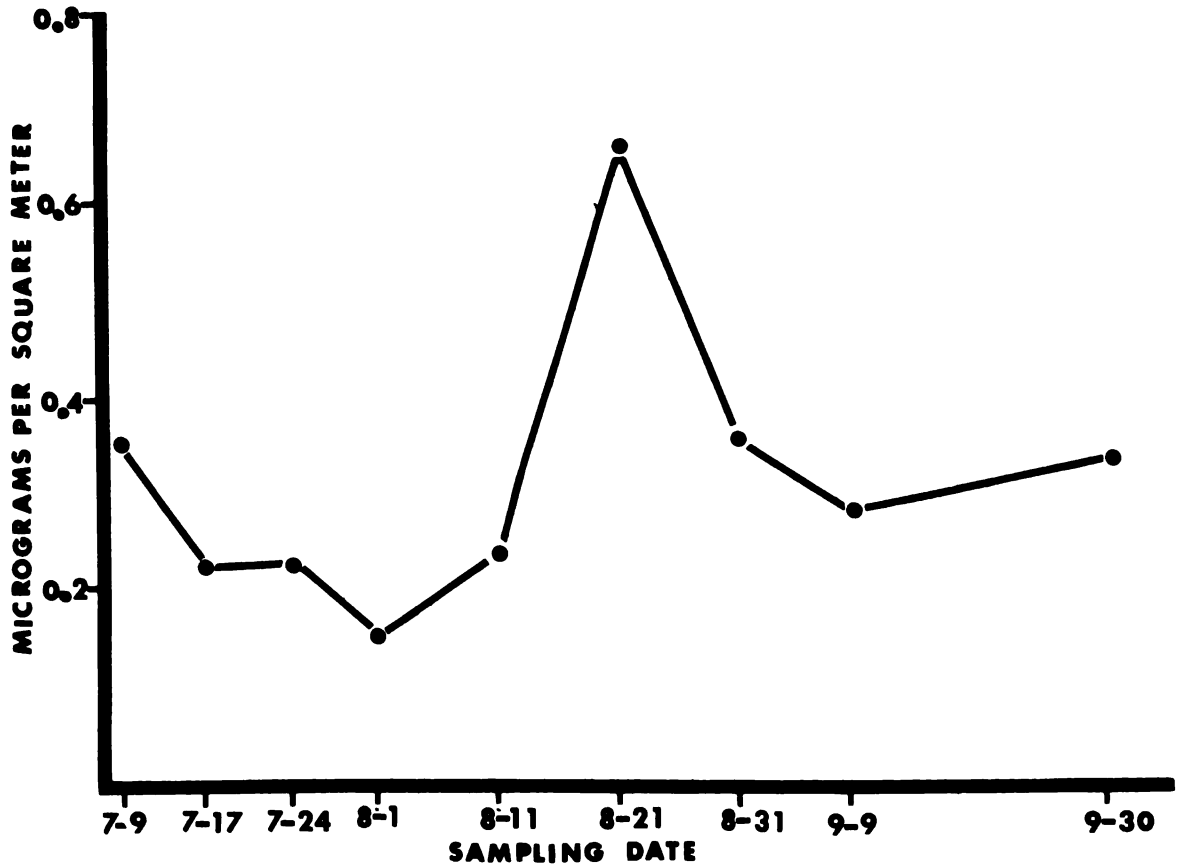
Tr - trace of insecticide
N.D. - no insecticide detected

Table 6. The average insecticide content of the periphyton samples taken at each sampling period from all ponds.

| Sampling Dates | DDT | DDD | DDE | Total Insecticide |
|---|---|---|---|---|
| | (micrograms per square meter) | | | |
| | | Pond C | | |
| 7/9 | 0.1126 | N.D. | N.D. | 0.1126 |
| 7/24 | 0.2270 | N.D. | N.D. | 0.2270 |
| 8/11 | 0.1022 | N.D. | N.D. | 0.1022 |
| 9/9 | 0.0844 | N.D. | N.D. | 0.0844 |
| 9/30 | 0.1916 | N.D. | N.D. | 0.1916 |
| | | Pond L | | |
| 7/9 | 0.3551 | N.D. | N.D. | 0.3551 |
| 7/17 | 0.2233 | N.D. | N.D. | 0.2233 |
| 7/24 | 0.2272 | N.D. | N.D. | 0.2272 |
| 8/1 | 0.1510 | N.D. | N.D. | 0.1510 |
| 8/11 | 0.2324 | N.D. | N.D. | 0.2324 |
| 8/21 | 0.7657 | Tr | Tr | 0.7657 |
| 8/31 | 0.3473 | Tr | Tr | 0.3473 |
| 9/9 | 0.2742 | Tr | Tr | 0.2742 |
| 9/30 | 0.3228 | Tr | Tr | 0.3228 |
| | | Pond M | | |
| 7/9 | 0.9168 | N.D. | N.D. | 0.9168 |
| 7/17 | 1.8116 | Tr | Tr | 1.8116 |
| 7/24 | 1.5508 | Tr | Tr | 1.5508 |
| 8/1 | 1.9375 | Tr | Tr | 1.9375 |
| 8/11 | 0.9943 | 0.2931 | Tr | 1.2874 |
| 8/21 | 1.3917 | 0.9175 | Tr | 2.3093 |
| 8/31 | 0.6041 | 0.5684 | Tr | 1.1725 |
| 9/9 | 0.4274 | 0.6701 | Tr | 1.0976 |
| 9/30 | 0.4865 | 0.5903 | Tr | 1.0769 |
| | | Pond H | | |
| 7/9 | 0.7205 | Tr | N.D. | 0.7205 |
| 7/17 | 2.6045 | N.D. | N.D. | 2.6045 |
| 7/24 | 4.1179 | Tr | N.D. | 4.1179 |
| 8/1 | 2.5516 | Tr | Tr | 2.5516 |
| 8/11 | 1.7716 | 0.6234 | Tr | 2.3950 |
| 8/21 | 2.2277 | 1.3460 | Tr | 3.5689 |
| 8/31 | 1.6252 | 1.2347 | Tr | 2.8599 |
| 9/9 | 1.6637 | 1.9855 | Tr | 3.6492 |
| 9/30 | 1.5077 | 2.3114 | Tr | 3.8075 |

Tr - trace of insecticide
N.D. - no insecticide detected

Figure 13. The composition and changes with time of insecticides in the periphyton of pond L on a part per million basis and on a total micrograms per square meter basis.


0.8
0.6
0.4
0.2
MICROGRAMS PER SQUARE METER
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE

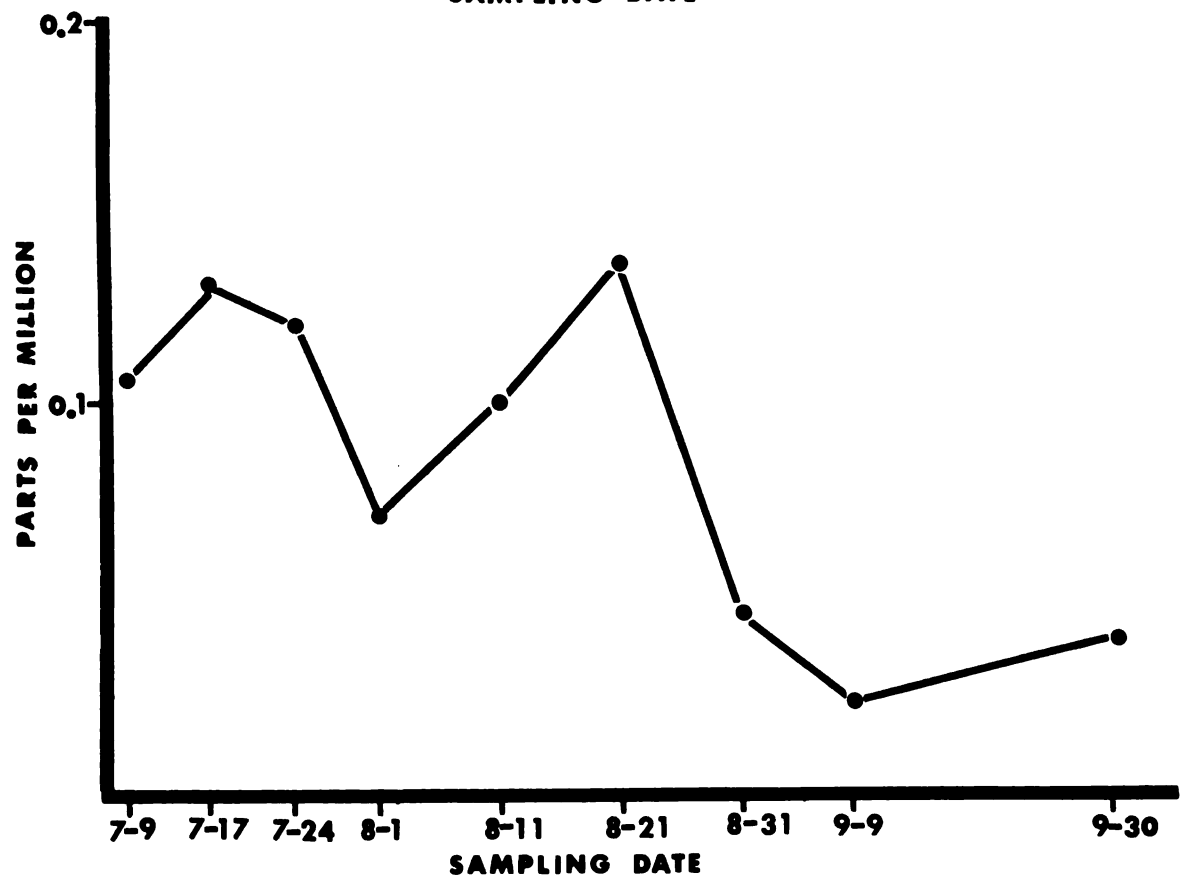
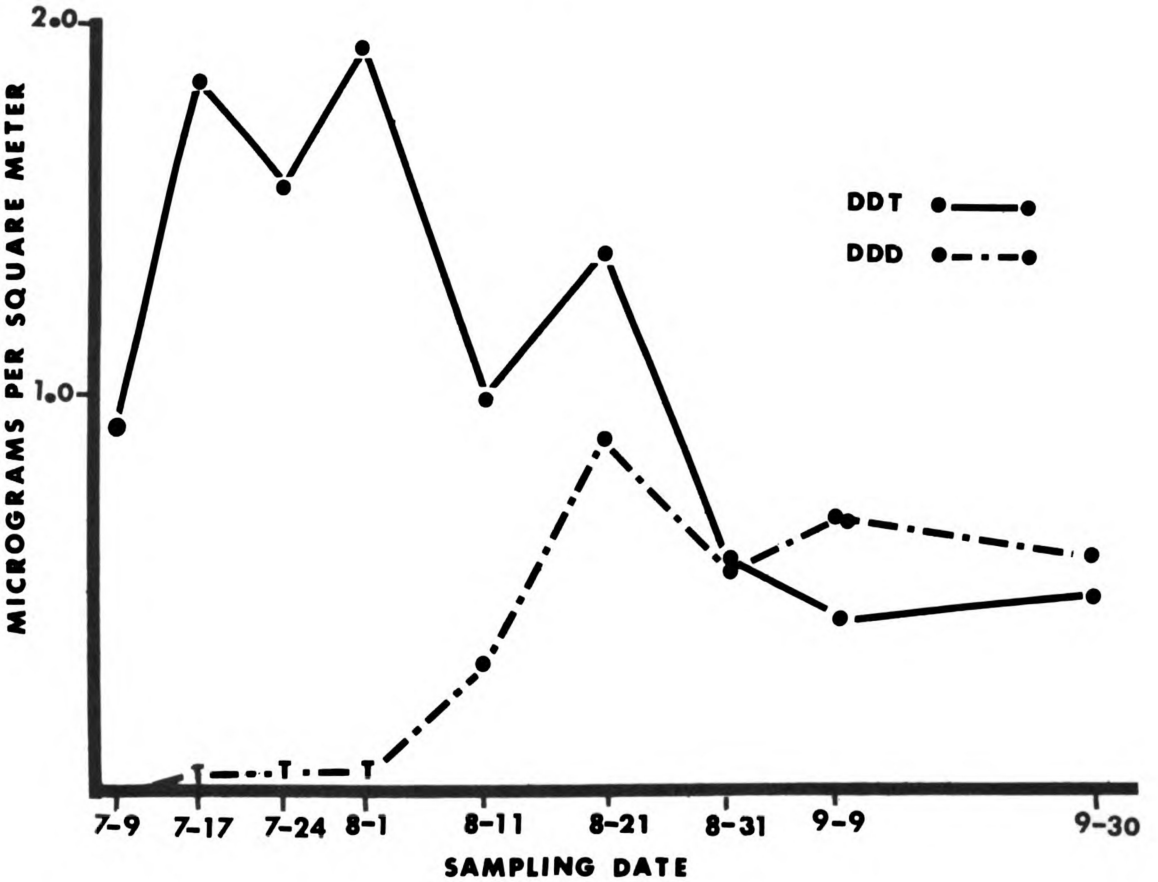

0.2
0.1
PARTS PER MILLION
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE

Figure 13

Figure 14. The composition and changes with time of insecticides in the periphyton of pond M on a part per million basis and on a total micrograms per square meter basis.


2.0
1.0
MICROGRAMS PER SQUARE METER
DDT
DDD
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE

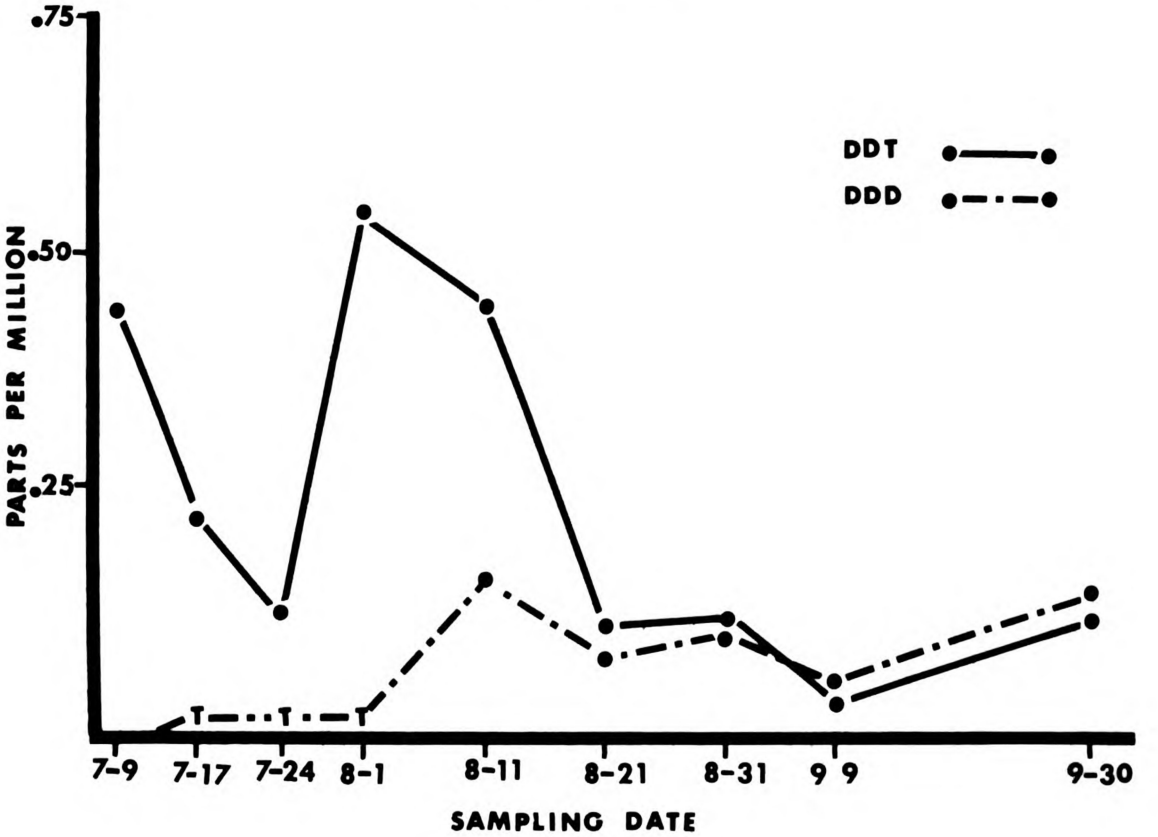

.75
.50
.25
PARTS PER MILLION
DDT
DDD
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9 9
9-30
SAMPLING DATE

Figure 14

Figure 15. The composition and changes with time of the insecticides in the periphyton of pond H on a part per million basis and one a total micrograms per square meter basis.

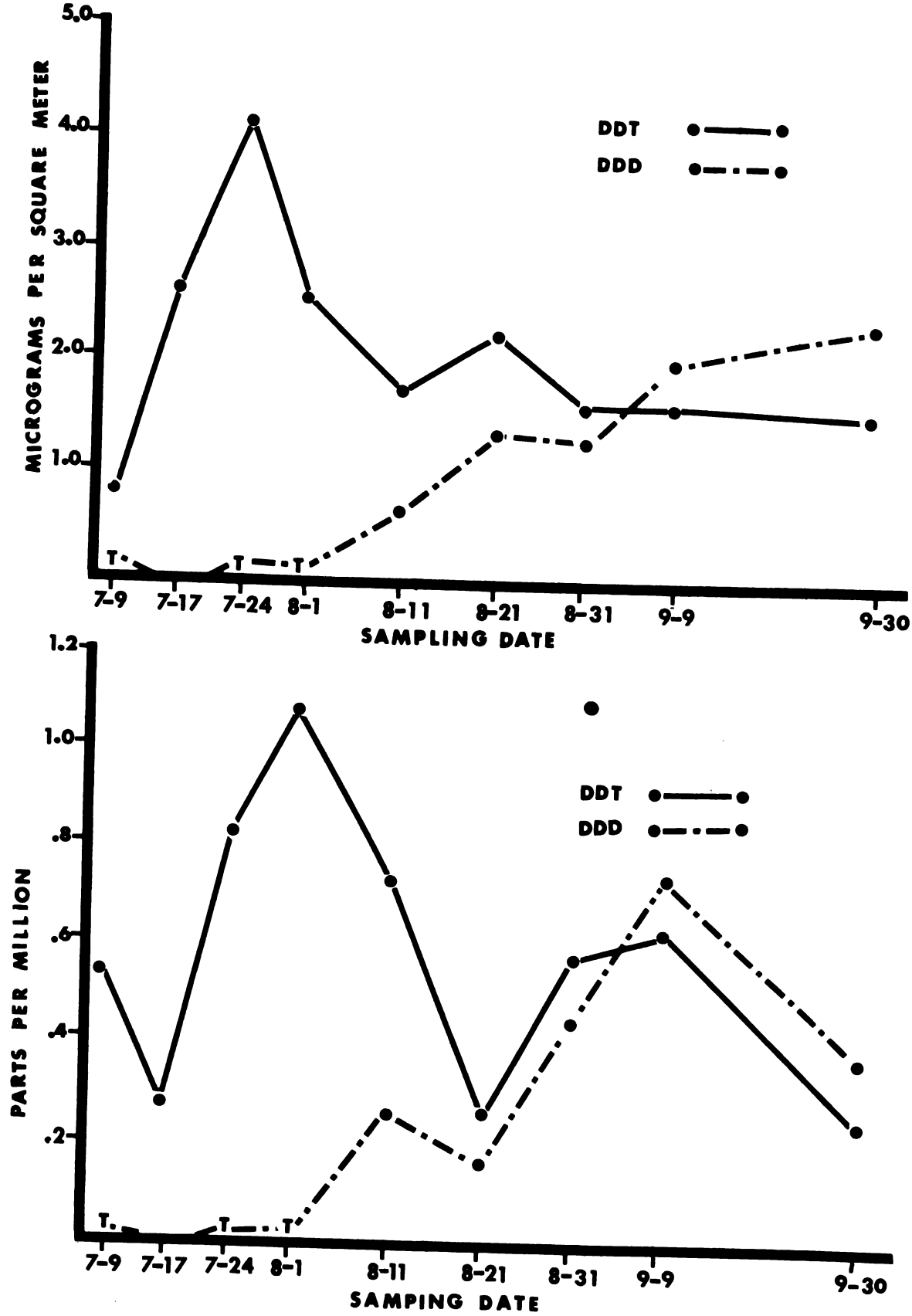

MICROGRAMS PER SQUARE METER
5.0
4.0
3.0
2.0
1.0
DDT
DDD
T
T
T
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPLING DATE
PARTS PER MILLION
1.2
1.0
.8
.6
.4
.2
DDT
DDD
T
T
T
7-9
7-17
7-24
8-1
8-11
8-21
8-31
9-9
9-30
SAMPING DATE

Figure 15

that all ponds did not contain measurable amounts of DDD until the 8/11 sampling period, thirty-two days after the study was started. Since this was well after DDD became prevalent in the water it is not likely that the algae is a basic bactor in the DDD transformation in the ponds, and that the DDD occurring in the periphyton is not a result of the periphyton actively degrading the DDT to DDD. Only trace amounts of DDE were detected in any of the ponds so the route of DDT degradation, if it occurs, is not towards DDE.

Recycling of DDT and its metabolites was checked by adding new unseeded periphyton sheets to all ponds on the forty-fifth day of the study, on August 20. Because this was the late summer, the algae did not grow fast or become very abundant. The final day of sampling, two sheets from each pond were taken and combined into one sample for analysis. The results (Table 7), show that DDT and DDD were recycled to the periphyton in the two high concentration ponds. Pond L periphyton differed from the control only because a trace of DDD was detected.

These results indicate that DDT and its metabolites are not readily recycled in the periphyton. The original periphyton accumulated DDT quite readily and by the end of one week it had relatively large quantities of DDT present. During the period when the original periphyton was accumulating DDT there was also an abundant amount of DDT in the water. However, the recycling experiment was made in the

Table 7. The insecticide concentrations of the recycled periphyton from all ponds.

| Sampling Dates | DDT | DDD | DDE | Total Insecticide |
|---|---|---|---|---|
| | (parts per million) | | | |
| | | Pond C | | |
| 9/30 | 0.0454 | N.D. | Tr | 0.0454 |
| | | Pond L | | |
| 9/30 | 0.0540 | Tr | Tr | 0.0540 |
| | | Pond M | | |
| 9/30 | 0.1313 | 0.2598 | Tr | 0.3911 |
| | | Pond H | | |
| 9/30 | 0.1973 | 0.6044 | Tr | 0.5029 |
| | (micrograms per square meter) | | | |
| | | Pond C | | |
| 9/30 | 0.0813 | N.D. | Tr | 0.0813 |
| | | Pond L | | |
| 9/30 | 0.0870 | Tr | Tr | 0.0870 |
| | | Pond M | | |
| 9/30 | 0.3582 | 0.7086 | Tr | 1.0668 |
| | | Pond H | | |
| 9/30 | 0.3197 | 0.9790 | Tr | 1.2957 |

Tr - trace of insecticide
N.D. - no insecticide detected

late summer after the DDT levels in the water had decreased. Because the recycled periphyton did not accumulate DDT whereas the original periphyton did, it appears that the transport of DDT or DDD is dependent upon the insecticide concentrations in the water.

Meeks and Pterle (1965), using Cl-36 labeled DDT, found that there is rapid uptake of DDT from the water by algae. Since there was DDT in the water of the experimental ponds, the route of transport to the algae was probably by way of the water.

## Hydrosoil

The prepared bottom concentrations of DDT in sand were not as accurate as expected (Table 8). This ruled out budget analyses, and short term changes could not be seen. The bottom samples were analyzed at four regular spaced intervals of the study period, which would show the major changes in the hydrosoil insecticide concentration and in the DDT and metabolite composition of the hydrosoil.

In ponds M and L, there was a definite decrease in the amount of measured insecticide in the bottom (Figure 16). The highest level pond, pond H, did not show this tendency nearly as well, but this can be explained by virtue of the larger amounts of insecticide present in pond H masking the changes presented by displacement and loss of DDT and its metabolites.

Table 8. The insecticide concentrations in parts per million, based on the dry weight of the bottom material in each of the artificial ponds.

| Sampling Dates | DDT | DDD | DDE | Total Insecticide |
|---|---|---|---|---|
| | (parts per million) | | | |
| | | Pond C | | |
| 7/9 | 0.00168 | N.D. | N.D. | 0.00168 |
| 8/31 | 0.00256 | N.D. | N.D. | 0.00256 |
| | | Pond L | | |
| 7/9 | 0.0468 | N.D. | 0.0017 | 0.0485 |
| 8/11 | 0.0268 | 0.0084 | 0.0016 | 0.0368 |
| 8/31 | 0.0252 | 0.0082 | 0.0015 | 0.0349 |
| 9/30 | 0.0068 | 0.0075 | 0.0003 | 0.0146 |
| | | Pond M | | |
| 7/9 | 0.9676 | N.D. | N.D. | 0.9676 |
| 8/11 | 0.2821 | 0.0656 | 0.0190 | 0.3667 |
| 8/31 | 0.2721 | 0.1039 | 0.0022 | 0.3782 |
| 9/30 | 0.2212 | 0.1372 | 0.0016 | 0.3600 |
| | | Pond H | | |
| 7/9 | 8.5710 | N.D. | N.D. | 8.5710 |
| 8/11 | 6.1500 | 0.2709 | 0.2152 | 6.6525 |
| 8/31 | 9.6649 | 0.1418 | 0.2415 | 10.0482 |
| 9/30 | 5.5851 | 0.4717 | 0.1055 | 6.0623 |

N.D. - no insecticide detected

Figure 16. The insecticide concentrations, in parts per million, based on the dry weight of the bottom material in each of the artificial ponds, are compared on a semi-log scale.

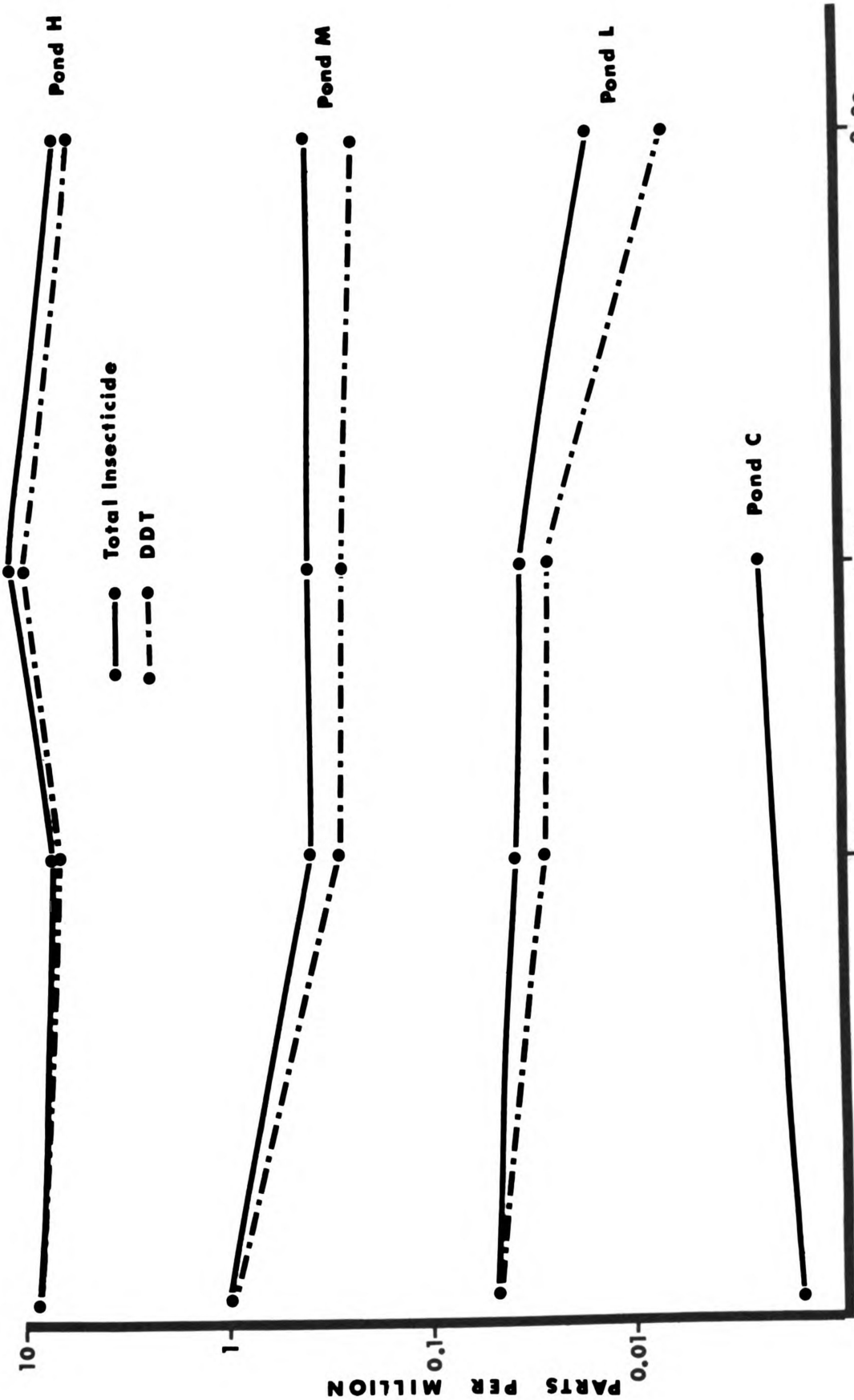

Pond H
Pond M
Pond L
Pond C
Total Insecticide
DDT
10
1
0.1
0.01
PARTS PER MILLION
7-9
8-11
8-31
9-30
SAMPLING DATE

Figure 16

All ponds indicated that DDD was the major degradation product of DDT. The two low level ponds showed a decrease in the DDT percentage of the total insecticide which corresponded to a similar increase in the DDD percentage of the total insecticide (Figure 17). Pond H again did not show this tendency as clearly as the two low ponds, but by the end of the summer the DDD level was four times that of the DDE. While pond H did not show the differences in percentage units, the amount of DDT actually converted to DDD in pond H was much higher than it was in the other ponds. The reason this change did not show up in the percentage units equal to the other ponds, could be a saturation of the mechanism of degradation, whether it is by bacteria (Wedemeyer, 1967) or by reduced porphorins (Miskus et al., 1965). While DDE was present and comprised a nearly constant percentage of the total insecticide in all ponds, it did not assume the importance that the DDD did as a breakdown product of DDT.

The fact that DDD is a major breakdown product of DDT, and increases with time, and that it is transported to and concentrated in the biota of an aquatic environment is significant. Hunt (1960) showed that DDD was mobile in an aquatic environment and it is harmful to aquatic organisms.

DDT was detected in all the bottom samples. The Lake Michigan study by Hickey, Keith, and Coon (1966) found DDT in Lake Michigan bottom materials and indicated that the DDT

Figure 17. The percentage of the total insecticide that DDT and each of its metabolites comprise in the bottom material of the treated ponds.

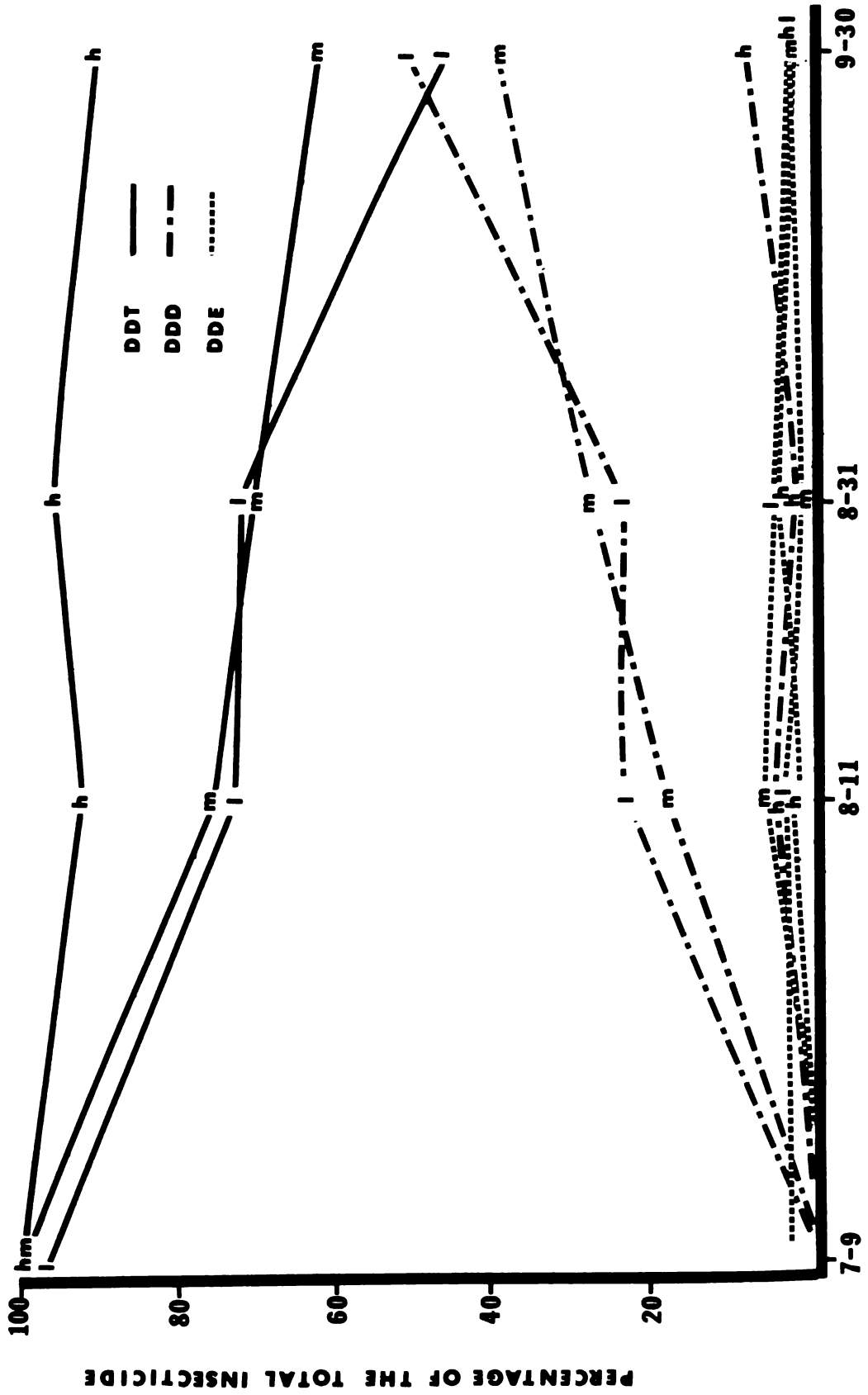

PERCENTAGE OF THE TOTAL INSECTICIDE
100
80
60
40
20
DDT
DDD
DDE
7-9
8-11
8-31
9-30
SAMPLING DATE

Figure 17

in the bottom material could be transported to the biota of an aquatic environment. Translocation of DDT was found to occur in this study and points to the importance of the hydrosoil as a reservoir of stable and biologically active pesticides.

## General Results

A comparison of the level of insecticide in the hydrosoil and the levels of insecticide in the fish, microcrustaceans, and periphyton was made. It was found that a log-log plot with the bottom insecticide concentration on the x axis and the insecticide concentrations of the biological parameters on the y axis, the results resembled a straight line (Figure 18). Regression analyses were made and the slopes and correlation coefficients were compared (Table 9). The reference lines drawn in Figure 18 were calculated from the means of x, the means of y, and the slopes.

The concentration values used for making these calculations were taken from the last five sampling periods, with the exception of the bottom. The prepared concentrations were used because the total insecticide in the bottom remained nearly constant. The concentrations of the last five sampling periods give a good estimate of the highest levels of insecticide that each of the parameters of the pond attained at the given bottom concentration, as the concentration

Table 9. The linear regression data for the log-log plots with the parts per million total insecticide in the hydrosoil on the x axis and the parts per million total insecticide of the fish, periphyton, or microcrustaceans on the y axis.

| | Fish | Microcrustacean | Periphyton |
|---|---|---|---|
| $Log_{10}$ mean x..... | 9.9073 | 10.1398 | 9.8996 |
| $Log_{10}$ mean y..... | 10.6172 | 10.2805 | 9.2649 |
| Slope..... | 0.7317 | 0.6039 | 0.5190 |
| Correlation Coefficient..... | 0.9644 | 0.9759 | 0.8948 |

Logrithms have all had 10 added to them, which needs to be subtracted when taking the anti-log.

Figure 18. The log-log plots of the parts per million total insecticide in the fish, periphyton, and microcrustaceans against the parts per million total insecticide in the bottom material.

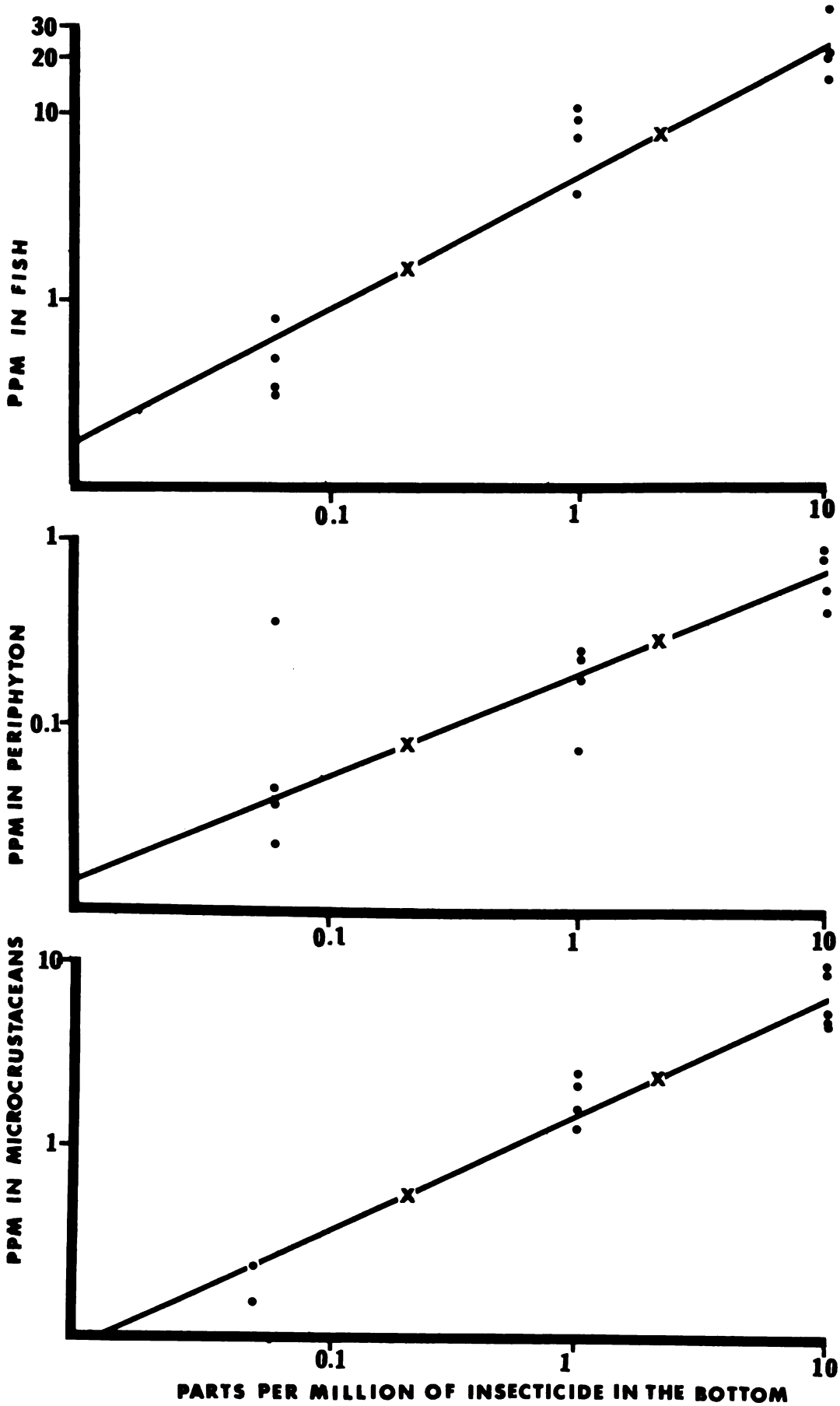

30
20
10
1
PPM IN FISH
0.1
1
10
1
0.1
PPM IN PERIPHYTON
0.1
1
10
10
1
PPM IN MICROCRUSTACEANS
0.1
1
10
PARTS PER MILLION OF INSECTICIDE IN THE BOTTOM

Figure 18

for each parameter appeared to be somewhat stable for the last fifty days.

The microcrustaceans had the highest correlation coefficient for the logrithmic relationship to the bottom concentrations. This could be due to the diurnal movements of the microcrustaceans, where they come into contact with the bottom during daylight hours.

The logrithmic relationship found shows that the uptake of DDT by the pond biota from a contaminated hydrosoil is not directly proportional to the hydrosoil concentration. It indicates that DDT is more efficiently transported from the hydrosoil to the biota at low concentrations than at high concentrations. However, the final levels showed no overlap.

The fact that the relationship exists, introduces the possibility of predicting uptake of insecticide from contaminated hydrosoils, using other, more complex relationships that may be found to exist for various types of aquatic situations.

## SUMMARY

This study showed definite biological uptake of DDT from a hydrosoil containing DDT, and that this uptake occurs at very low DDT concentrations in the hydrosoil. Therefore when an aquatic environment is to be checked for DDT contamination, the common procedure of analyzing the water for its DDT content is not a good indicator of contamination if it alone is used.

The fish, periphyton, and microcrustaceans were found to accumulate DDT which originated in the bottom material. The amounts of DDT translocated from the bottom material were dependent upon the concentration of DDT in the hydrosoil. Initially the translocation of DDT was from the hydrosoil into the water. The concentrations of DDT in the periphyton appeared to be dependent upon the DDT concentrations in the water, while the route of DDT transport to the fish and microcrustaceans was not clearly defined in this study.

As the study progressed DDD assumed a major role in the metabolism of DDT in a small pond, as it became the predominant degradation product of DDT and was transported throughout the aquatic environment.

A logrithmic relationship between the concentration of DDT in the hydrosoil and the accumulation of DDT in the fish, microcrustaceans, and periphyton was found. Even though this relationship may not be valid for other aquatic systems, it does indicate that a definite relationship between the biota of an aquatic environment and a hydrosoil containing DDT may exist in many aquatic systems.

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