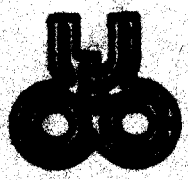

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SURVEY, ECOLOGY, AND SYSTEMATICS OF THE UPPER POTOMAC ESTUARY BIOTA: AUFWUCHS MICROFAUNA PHASE III

BY

DONALD M. SPOON

JULY, 1982

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Interaction of Zooplankters and Blue-green Algal Blooms Under Organic and Thermal Pollution in a Bench Scale Model Potomac River

by

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Project No. A005 D.C.

The work upon which this publication is based was supported by the Office of Water Research and Technology, U. S. Department of the Interior, under the provisions of Public Law 88-379, as amended.

Agreement No. 14-34-001-7018

Water Resources Research Center
The University of the District of Columbia
Washington, D. C. 20008

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ACKNOWLEDGEMENTS

The senior investigator would like to thank Dr. Robert Bradford, the former director of the D.C. WRRC, and Lisa Harris, the former administrative assistant, for their valuable assistance during the progress of this research. Dr. Mamadou H. Watt, the director of the D. C. WRRC, deserves special thanks for his assistance in the completion of this technical report. The experiments in this report were carried cut and written up with the following student investigators:

John Kolieng, Richard Nosal, David Bliss, John Quinn and Paul Brown. Also thanks go to Dee Hill, Gladys Kelly and Carmen Felix for preparing the final manuscript. The chairman of the Biology Department, Dr. George B. Chapman, is thanked for his continued support of this project which was carried out in the observatory hill research cottage.

ABSTRACT

These studies utilized two adjacent bench scale rivers, each composed of four interconnected 24-gallon aquaria, stimulating summer flow and temperature (30^{o}C) of the fresh water estuarine Potomac River from Hains Point to Piscataway Creek. Initially all eight aquaria were seeded with the filamentous blue-green alga, Anabaena. To all aquaria was added a mixture of Daphnia, copepods; and ostracods from a swamp near Little Falls. (Preliminary studies showed these zooplankters could remove the Anabaena.) The experimental side received activated sludge from Blue Plains Sewage Treatment Plant, while the control received dechlorinated tap water. Temperature, D.O., pH, phosphate, nitrate, and transmittance were determined. In experiment I, we obtained a pronounced algal bloom on the experimental side over the ten day run with reduction of zooplankters and their grazing. In experiment II, with sewage added to both sides, we used a heat shock of 10 C in a bypass simulating the conventional power plant on the experimental side causing decreased zooplankters and increased algal growth.

INTRODUCTION

There are several ways to create a model of a flowing system like the Potomac River. The most obvious is to sample the river itself, thus creating a model whose realism is limited by the extent and kind of sampling employed. From such sampling, one can determine which are the most significant parameters to consider as contributing to the general condition or health of the river.

With sufficient sampling knowhow, a mathematical model can be devised based on a limited number of parameters. (The Annapolis Field Station of the E.P.A. has made such a sophisticated mathematical model of the Potomac Rivera (Hetling, 1969).

A third modeling method is to create a bench-scale practical model of the Potomac River. The Corps of Engineers has attempted this for the entire Chesapeake Bay, including the Potomac River, yet their relatively enormous model is designed for hydrological and not biological studies.

A major advantage of studying and sampling a bench-scale model over the real river is that a control and experimental bench-scale model can be operated concurrently and select parameters can be isolated and their influences determined.

The obvious disadvantage of the bench-scale river model is that, like the mathematical model, it must be less complex than the real river. The section of the Potomac River from Key Bridge to Piscataway Creek has a complexity of interacting inputs including the main channel flow, Anacostia River, numerous side streams, storm sewer runoff, power plant coolant water, and numerous sewage effluents including the giant Blue Plains Sewage Treatment Plant. From the aspect of hydrology and biology, the main channel flow and the Blue Plains sewage treatment plant are the most significant in formulating the design of a bench-scale experimental river.

The main channel flow varies enormously from a low of about 388 million gallons per day (mgd) to a high of 100's of billions of gallons per day. The average flow is about 7 billion gallons per day. The total effluent of the 18 sewage treatment plants in the Washington area is over 350 mgd with over 70% coming from the Blue Plains Sewage Treatment Plant.

The Potomac River (Figure A) widens below Key Bridge and further widens below Woodrow Wilson Bridge. The average depth is 10 feet, with a euphotic zone of less than 2 feet due to sediment, organic content, and plankton. Thus, the majority of the flow is out of contact with the surface where re-aeration can occur, as well as beneath the euphotic zone where more oxygen can be generated than is required for respiration. It was decided to create this condition of lowered re-aeration by joining 24-gallon all-glass aquaria containing 10 gallons of aerated water by two 50-foot lengths of 1/2-inch Tygon tubing and a half-filled unaerated 5-gallon Nalgene bottle (Figure 1). A 40-gallon common aerated reservoir represented Site 1 at Key Bridge and four interconnected aquaria represented the downriver sites, which were

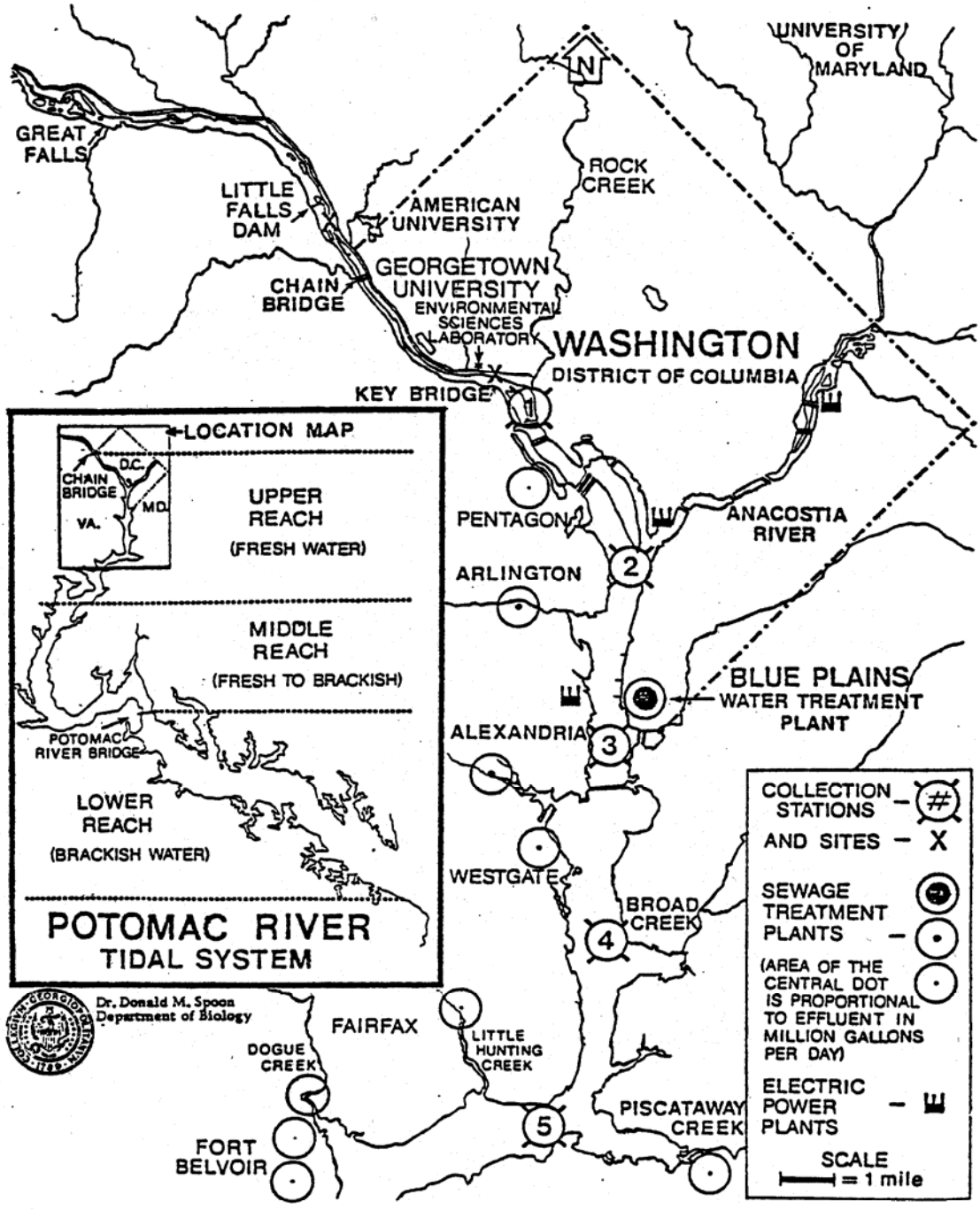


Figure A. Map of the Upper Potomac Estuary showing project sampling sites.

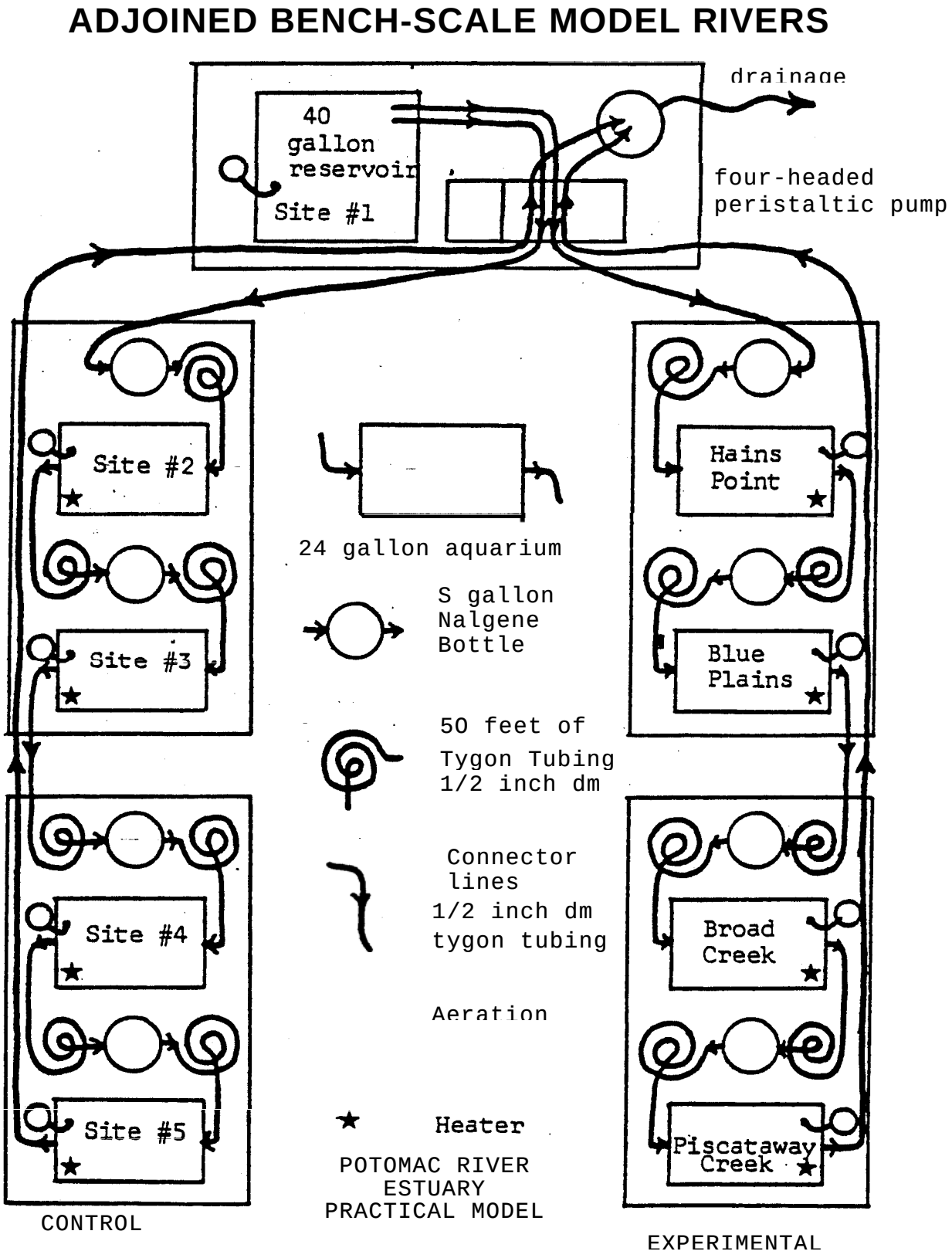


Figure 1. Diagram of the model rivers located in the Environmental Sciences Laboratory at Georgetown University, Washington, D. C.

approximately 3-1/2 miles apart: Site 2 at Hains Point, Site 3 at Blue Plains, Site 4 at Broad Creek, and Site 5 at Piscataway Creek. The common reservoir supplied identical water at the same flow rate to each of two parallel bench-scale experimental rivers composed of four interconnected aquaria.

Initially, a rather rigorous study of the volumes and flow rates through each of these four realms was considered and plans made to approximate these volumes and flow rates in each aquarium by precise elevation of the aquaria. Consideration of the Jaworski and Johnson 1969 dye study which indicated the main channel transported pollutants 100 times more rapidly than the embayments led us to discard this complication. Thus each of the four aquaria had the same volume and flow rate dictated by gravity flow based on the inflow at Aquarium 1 and outflow at Aquarium 4.

This experimental river has been used for many different studies. our studies on fish-kills is available in the D.C. Water Resources Research Center (WRRC), Report #8. In it we show that this bench-scale experimental river can be set up to simulate the characteristics, such as the dissolved oxygen sag curves, of this 15 mile section of the Potomac River.

Eutrophication in our rivers has often been connected with the increased contamination spilled into these rivers from municipal sewage treatment plants. The most outwardly apparent characteristic of cultural eutrophication is the algae bloom. Nemerow and Rand (1967) noted that indications exist that all but the largest, swiftest and coldest bodies of water may be choked with algae in the next 17 years with the present rate of the dumping of high-nutrient effluent from sewage treatment plants. Algal blooms are therefore a serious threat to a river like the Potomac which not only receives about 400 million gallons of effluent from sewage treatment but is also the main water supply to Washington, D.C.

Algal blooms occur in many different types of aquatic environments. When they occur in a waterway such as the Potomac River, the main water supply for the city of Washington, D.C., many problems can result. The process of purifying the water for drinking becomes difficult because the algae can block the filters in the filtration plants, give the water objectional odor and taste, and cause sediment in reservoirs (Skulberg 1964). Besides these problems fish kills are often associated with algal blooms.

According to Liebmann (1967), if sewage is discharged from a plant after mechanical or biological purification alone, the phosphorus and nitrate content can cause algae blooms. Liebmann contents that each inhabitant of a city produces 0.61g phosphorus by feces and 0.83g by urine $_3$each day, giving domestic sewage a mean concentration of 10g/m phosphorus and 80g/m nitrate. For these reasons indicative parameters for algae bloom experiments are quantitative tests of phosphate and nitrate content as well as algae numbers. It appears that it is the excess of these elements

along with the excess organics provided by sewage treatment effluent that is the cause of algae blooms in a river like the Potomac.

Brooke (1957) has observed that there is a seasonal variation in the dominant species in an algal bloom. Diatoms are usually dominant in the spring, green. algae in the early summer and blue greens in the late summer. Previously it was thought that the variations in the phytoplankton populations were due to changes in the availability of nutrients, with the species best adapted to use the available nutrients, becoming the dominant species.

But Fitzgerald (1961) observed algal blooms with one dominant species in sewage stabilization ponds where an abundant supply of nutrients was continuously available. For this reason there must be other factors controlling the populations of algae in aquatic environments besides nutrient availability.

In experiments by Rice (1954) it has been suggested that algae may be capable of effecting their environment by excreting substances which inhibit the growth of other algae, thus eliminating competition and allowing one dominant species to exist in any quantity. The extent to which this condition would exist in a natural environment is probably very short.

Chemicals in solution in waterways have also been considered responsible for the occurrence of algal blooms. Lackey (1964) has found that the effluent of sewage treatment plants are high in nitrates and phosphates causing algal blooms to occur. Another chemical parameter considered has been hydrogen sulfide. Lackey (1964) found that a low concentration of H S may stimulate algal activity and high concentrations of it inhibit algal growth. We too believe this to be an important factor in a river such as the Potomac which has-a large quantity of urban waste dumped into it. The breakdown of the sludge which is settled in the river may result in the production of H_2S which in turn effects the growth of the phytoplankton.

Viruses may also be significant in the control of algal populations. Shilo (1971) has shown that there are viruses known to be pathogenic to species of blue-green algae, and that these viruses are capable of reducing the quantity of blue-green algae after exposure to them. It is hoped in the near future that bacteria and viruses may provide an economical means of controlling algal blooms in natural environments. At present this type of work is limited because only a few viruses and bacteria have been isolated that are effective in reducing natural populations of blue-green algae.

Studies by Fritsch (1930) have indicated that fungi can infect blue-green algae and remove specific species of them from the plankton.- other organisms shown to have an effect on algae populations have been protozoa which prey on Anabaena, and amoeba which will ingest species of Microcystis.

Vance (1965) believes that competition between species of algae may also effect the growth rates of the different species and select for a dominant one among them.

Studies by Safferman et al. (1962) have shown that certain strains of bacteria are capable of lysing or inhibiting the growth of blue-green algae. Other studies by Vance (1966) have shown that the presence of bacteria may enhance the growth of blue-green algae because a mutualistic relationship can exist between certain species of bacteria and blue-green algae. The inhibiting effect that bacteria can have on the growth of algae is exemplified by an experiment performed by Wu et al. (1968) in which he used a strain of bacteria to eliminate a natural bloom of blue-green algae that was brought into the laboratory.

Arnold (1971) studied Daphnia pulex and its ability to consume species of blue-green algae. He suggested that zooplankters may be a significant factor in controlling populations of blue-green algae. If the zooplankters are unable to graze on predominant algal species, or the zooplankton population is depressed, grazing on the algae would cease allowing the phytoplankton to increase their population. He showed that this cladoceran preferred to ingest green algae over blue-green algae. In his experiments he found that Daphnia which were fed Anacystis nidulans had a poor survival any reproduction rate.

These rates were even lower when the food level was very high suggesting that this algae had an inhibiting effect on Daphnia. When Daphnia were fed Anabaena flos-aquae, the survival and reproduction rates were high.

Arnold suggested that copepods, rotifers and cladocerans probably utilized blue green algae more extensively than Daphnia pulex, and therefore are able to effect the population of the algae.

These experiments (I & II) dealt with the predator-prey relations of Anabaena and the zooplankters: Daphnia, Copepods, and Ostracods and a heat shock of 10^0C on algae-zooplankton interactions.

The purpose of experiment I was to determine the effects that treated sewage had on algal growth and on populations o£ zooplankters. Most studies of polluted rivers have been concerned with the direct effect popution has on algae. Such studies have usually attributed increased algal growth to the chemical constitutents such as phosphates and nitrates.

In our study we were mainly concerned with the effects pollutants have indirectly on algae through its activity directly on the zooplankteres of the river. The hypothesis with which we worked was that the increased algal growth of a polluted river was not only the result of eutrophication of the waterway by the pollutants, but was also the result of reduced grazing by zooplankters. The reduced grazing, we believe, will occur because of the effect that pollutants have upon the zooplankters. In our study, the detrimental effect the pollutants will have on the

zooplankters will be indicated by a reduced number of zooplankters in those portions of the river treated with sewage (tanks E2, E3, and E4). With these predators reduced in number the algae would then be able to increase in number, whereas the control tanks, C1, C2, C3, C4, and El, should have a smaller quantity of algae because the zooplankters will continue to graze on them, thus keeping the algal population limited. Since the sewage is added "down river" from tank El, it with Cl act as controls for the two experimental rivers.

The purpose of experiment II was to determine (under the same conditions of experiment I) whether the presence of a conventional power plant on the Potomac River would affect the predator-prey relationship between Anabaena and Daphnia, Copepods, and Ostrocods. We set ou8 the test whether a raise in temperature of the river water of 10 C as it passed through the cooling facility of the power plant would affect this predator-prey relationship and thus escalate algae blooms. It is our hypothesis that this would occur by heat-shocking the zooplankters, making them less capable of utilizing the blue greens. Intimately involved in this relationship is the presence in the river of effluent from the treatment of municipal sewage from Washington. To achieve our experiment goals we used a bench-scale model of the Potomac.

MATERIALS AND METHODS

Step 1 – Preparation of Anabaena

For the experiments a healthy stock of the blue-green algae (Anaebena) was obtained from Dr. Philip Sze of Georgetown University. The stock was maintained in a large sunlit and aerated flask for a period of approximately one year. Microscopic examination of the algae stock revealed no amoeba or flagellate contamination. A slight presence of motile bacteria was identified, but the culture was considered to be essentially healthy. In order to increase our stock of Anabaena, 250 ml of the original stock was added to each of four on- tar flasks of nutrient media giving a 4:1 dilution. Next, the flasks were equipped with aerators and the tops sealed with aluminum foil. For 24 days the cultures were irradiated with artificial light (aqualux/F20T12) after which time the flasks were thick with Anabaena. At this point, the algae stock was introduced to the experimental river system. To each of the 8 empty 24 gallon tanks of the system 250 ml of Anabaena suspension, 5 gallons of dechlorinated tap water, and 500m1 of nutrient was added. Each tank was then equipped with aerators and aquarium heaters. The temperature was maintained at approximately 30^0C for 21 days resulting in very heavy and uniform Anabaena growth.

Step 2 – Collection of zooplankton

For use as predators on the Anabaena blue-green algae, samples of zooplankton were collected from a swampy area on the Potomac River below Little Falls adjacent to the C&O canal feeder

lock. Using a 0.104 mm mesh plankton screen, 500 liters of undisturbed surface swamp water was concentrated to 10 liters. This concentrated plankton population was added to a 24 gallon aquarium containing 5 gallons of dechlorinated tap water and equipped with aerators. The water teemed with a high concentration of active microcrustacean plankters. This population was maintained for two days prior to introduction into the experimental river system.

Step 3 - Introduction of zooplankters to blue-green algae

The eight tanks of Anabaena culture were arranged into two parallel groups of four arranged in a linear manner (see fig. 2). One group was the control river consisting of tanks C1 through C4. The other 4 tanks, El through E4, made up the parallel experimental river system. The tanks in each of the two systems were successively connected by 500 foot sections of 1/2 inch diameter Tygon. Between each section of tubing was placed a 5 gallon Nalgene bottle as a sediment trap to simulate the effect of a river bottom. The tubing and bottles were covered with black plastic and aluminum foil to simulate aphotic conditions. The first tanks in each of the two systems, Cl and El, were connected by identical lengths of tubing to the same 50 gallon reservoir of dechlorinated tap water. The tubing passed through the same peristaltic pump in order to assure an identical constant rate of inflow of water from the reservoir into both river systems. Similarly, the terminal tanks in both the experimental and the control, C4 and E4, were fitted with identical lengths of tubing passing through another common peristaltic. This insured equivalent outflow. from both systems. The two pumps were adjusted to maintain a constant flow of 4 liters per day through the river systems. The heaters in each tank was calibrated to maintain a constant-30 ^{0} C. Additionally, the aerators in each tank calibrated to displace 500 ml of water from an inverted beaker in one minute. To insure uniform Anabaena concentration in each tank, 3 liters of suspension was transferred from one tank to next. Two eight-circuit transfer loops were performed in both directions.

Step 4 – Counting zooplankters

The zooplankton stock was siphoned from the appropriate tank through a 0.104 mm mesh plankton screen and rinsed three times with dechlorinated tap water. The screen was then backwashed with 4.5 liters of dechlorinated tap water. To each of the 8 tanks of the river system, 500 ml of the zooplankton suspension was added. The remaining 500 ml of suspension was concentrated to 10 mm, preserved with 15 ml of ethanol and counted.

Two different parameters were explored using the river system. In the spring of 1977, we investigated the effects of treated sewage on zooplankton grazing on Anabaena. The following spring the effects of heat shock on this same system were studied.

Experiment A

The first experiment performed was designed to determine if any of the zooplankters that were available to us would consume the algae that we were growing and of the one that did consume the algae; which ones were more efficient at it. We first collected samples of zooplankters from two different swampy areas adjacent to the Potomac River at the Little Falls feeder canal runoff. one area was labeled the north swamp. The Second was called the south swamp. The samples were each placed in a separate graduated cylinder where they sat undisturbed for approximately one week. This process allowed all the floating and suspended debris and sediment from the swamp to settle out. After this time period the zooplankton suspension were ready for our experiments.

In this initial experiment we added twelve 10 ml portions of our algal suspension to twelve petri dishes containing 16 mls of zooplankters suspension from the north swamp, south swamp, and to filtered swamp water. These dishes were kept in a room that was illuminated with an overhead fluorescent light for five days. After this time each dish was placed under a dissecting microscope in order to ascertain the amounts of algae left in each. Then all the dishes were swirled until a homogeneous suspension of algae was formed in them.

Each suspension was poured into a cuvette and read in a spectrophometer at a wavelength of 633 nm so a quantitative measure of the amount of algae present could be obtained. The blank used to obtain the zero value contained filtered swamp water. From the data obtained we selected the most efficient zooplankters and used them in the remaining experiments.

The sewage for this experiment was obtained from the activated sludge sedimentation tanks of the Blue Plains Sewage Treatment Plant and frozen into 1,333 ml cubes. Four liters of frozen sewage per day were added to tank E2 on the experimental side of the river. This was accomplished by adding a 1,333 ml cube of sewage at 8 a.m., 4 p.m., and midnight each day. To compensate for the addition of sewage on the experimental side, 4 liters of water from the reservoir were added to C2in three 1,333 ml portions on the control side. The river system was run continuously for 9 days. Physical and chemical parameters were measured on the first, second, third, fourth, fifth, and ninth days. Samples of zooplankton were collected daily.

Experiment II - The Effects of Heat Shock

For this experiment, the river system was once again set-up as described in Steps 1-4. This time 4 liters of frozen sewage were added to both sides of river system at tanks C2 and E, instead of just at E2 as before. To simulate heat shock on the experimental side of the river six lengths of Tygon tubing were

passed from tank E2, through the peristalic pump, through a hot water bath maintained at 40^0 C, and back into E2. The pump was calibrated at 4 liters a day flow. In this way, 4 liters of tank 2 was subject to a 10^oC increment each day. The river flowed for even days. Physical and chemical parameters were measured daily n all tanks. Zooplankton samples were also taken. For both' experiments measurements of phosphate levels, nitrate levels, dissolved oxygen levels, 663 nm readings for transmittance on the pectrophotometer, temperature, and pH were taken. Zooplankton samples were taken. An unmixed sample from each tank was poured into a cuvette and read at 663 nm to obtain a quantitative measure of the amount of algae present. The transmittance was recorded. The spectrophotometer was blanked with reservoir water. Temperature was read with a Tele-Thermometer. PH was measured with a Beckman Zeromatic pH meter adjusted with 7.0 pH fluid. Phosphate, nitrate, and oxygen levels were recorded using the procedure outlined in Hach's Water Analysis Handbook. Zooplankton counts were obtained by removing a 500 ml sample from each tank and. concentrating this to 10 mls with a screened sand filter and vacuum apparatus.

Fifteen mililiters of 70% isopropyl were added to each 10 ml concentrate. Counts were then performed in each tank. This was done by pouring separate samples into a petri dish and placing the dish under a dissecting microscope. Thus individual zooplankton could be counted and recorded at a later time.

In addition phosphate, nitrate, and pH measurements were performed on the sewage added to the experimental river. Each day 20 ml of reservoir water was added to each tank to compensate for the water removed during the testing.

Results of Experiment A

The purpose of experiment A was to determine which species of zooplankter that was available to us would be most efficient in consuming the species of Anabaena we were to use for the rest of our experimentation. For an indicator of the amount of algae resent in each petri dish we used the transmittance read at 33 nm on the spectrophotometer. The transmittance is inversely elated to the amount of algae present, so the most efficient zooplankter would produce the highest transmittance.

The results obtained from this experiment are shown in able A2 the 4 control dishes contained filtered swamp water and algae. This control represents the amount of algae that would have been present in all the dishes if the zooplankters were not a actor. The mean transmittance for the control dishes was 91.5%.

The set of dishes containing zooplankter from the north swamp and algae showed a mean transmittance of 95.5%. The dishes with the zooplankton from the south swamp and algae had a mean transmittance of 95.8%.

The data accumulated shows us that the control group

contained the largest amount of algae as would be expected, because the conditions were favorable for algal growth and there are no predators.

The results from the dishes containing zooplankton from the north and south swamp indicate that the zooplankters did indeed consume the algae.

Visual observations with a dissecting microscope also supported these transmittance findings. The control dishes contained dense populations of long filamentous algae whereas the dishes containing the zooplankters only had a few short filaments of algae at the end of the experiment.

This information confirms the assumption that the transmittance of these samples is inversely related to the amount of algae present and that the plankters do indeed consume the algae.

Following the criterion that the most efficient zooplankter could result in the largest transmittance, we see that the plankters from the south swamp were the most effective in reying on Anabaena.

All further experimentation involving zooplankton were performed with zooplankters collected from the south swamp.

Results of experiment I

This experiment was designed to determine if treated sewage a detrimental effect on zooplankters. This was accomplished by adding zooplankters to each tank of the experimental river, each of which had equal amounts of algae in it. Treated sewage hen added to tank E2 of the river. The river had a flow rate of four liters per day, as a result the ratio in the amount of sew age added to the flow rate was four liters to four liters.

The zooplankters used were found to be a mixture of Daphnia, copepods and ostracods, in a ratio of 1304:1379:126.

The experimental river is set up so that a slow steady flow between the tanks occur, therefore we would expect any effect that sewage has would be observed in tank E2 where it is initially added and also in tanks E2 and E4 which are down stream from tank 2.

The effect would be expected to be less in tank E3 than E2 even less in E4 because the sewage is becoming more dilute as flows down stream to the last two tanks.

The most significant and obvious result during the experiment s the differential growth of algae. Measures of the transmittance of aliquots taken from the tanks of the experimental river and read at 663 nm were used to indicate the relative ants of algae present in each tank. The amount of algae sent is inversely related to three percent transmittance. As seen from figures

As seen in figures A7, A8, and A9 these tanks had a steady increase of algae throughout the experiment. Tank E2 had the largest growth of algae as indicated by the steeply sloped range.

The variable of pH shows definite trends throughout the experiment. As seen from figures A34 through A41, tank E2 had a H that was much higher than any of the others. Tanks E3, and E4 had pHs that were slightly elevated above the others. The cause of these higher pHs is twofold. The sewage itself had a pH of 8.3 causing an elevation of the pH in the tanks. But most importantly it appears that the large quantities of algae in tanks E2, E3, and removed large amounts of carbon dioxide thus making these solutions more alkaline than the others

Figure A55, which represents zooplankter number over time for tank E2, indicates that there was a much more rapid decline in this number in E2 than in any of the other tanks. This suggests that the sewage killed the zooplankters in the initial part of the experiment in tank F2. Tanks E3 and E4 (figures A55 and A57) behaved more like the control tanks, which displayed a slow line in population number. The rapid decline in number may be absent because the sewage is diluted by the time it reaches these last two tanks. We would guess that a high concentration of sewage is needed to kill the zooplankters, but low concentrations have little effect on the zooplankters.

The results of the counts of zooplankters taken from each tank are seen in figures A50 through A57. The data indicates that during the initial stage of the experiment the number of zooplankters present in each tank fluctuated. The data also indicates, as seen in figures A50 through A57 that there was a general tendency for the number of zooplankters present to decline with time.

Results of Experiment II

This experiment was designed to see if thermal heat shock had an effect on the algae growth and zooplankton population of the river. Our hypothesis was that heat shock on the E2 tank would reduce the relative numbers of zooplankters. Reduced predation on the algae would then result in greater algae growth on the experimental (E) side of the river. At the beginning of he experiment, the algae population in each tank was equal. Algae growth was heavy and flocculent. Treated sewage was added to both rivers at E2 and C2 tanks. Heat shock occurred at the E2 tank. The flow rate was adjusted to 4 liters a day, and 4 liters of sewage were added to each river each day.

The zooplankton were a mixture of Daphnia, copepods and ostracods in a ratio of 1490:1015:935. This ratio was added to each tank as found from a count of a ninth sample after eight samples had been added. Counts were made each day. Ostracods, while in significant number, settled to the bottom of the tank, or they died since they were not seen in any count subsequent to the first sample.

The two rivers were identical except for the heat shock at The effect of the shock would be most strongly felt at E2. Possibly some effect would occur at E3 and E4.

The most significant result of the experiment was the rapid decline of zooplankters in the E2 and E4 tanks from the very first day. The total number of zooplankters in E2 on the first day was 45. In E4 there were 23. The numbers in E2 rapidly declined from 45 to 20 to 8 in three days. The numbers in E4 stayed at around 20 for three days and then leveled off at around 10 for the remaining four days. Conversely, the C2 tank with no heat shock d -a first day count of 75 zooplanktors and dropped to 35 and 30 for the first three days. The C4 tank had a higher initial count (65) as compared to the E4, but this summarily dropped off.

The low counts in the E2 tank can be deliberately attributed the heat shock. The low count in the E4 tank can be attributed an inadvertent rise in temperature of the tank to 38^{o}C on the first night. This was quickly corrected, but the heat had a direct effect on the initial zooplankter count. After the temperature was stabilized, the counts leveled off.

The zooplankton counts for the remaining tanks showed a slow steady decline. Figures B50, B51, B52, B53, B54, and B56 have very similar shaped graphs. Figures 55 and 57, E2 and E4 respectively, are different. Figures B55 shows the same shape as the other graphs, but the counts are much lower. Figure B57 shows in almost even graph. The zooplankton population in E4 was smaller after first day, and the remaining zooplankters seemed to enjoy a higher survival rate. This was probably due to lesser competition.

The next most important parameter, differential algae growth, was measured from aliquots taken from each tank in the experimental river and read at 653 nm on the spectrophometer. The relative amount of algae could be shown as a function of the percent transmittance. The relationship would be inverse. The presence of algae would color the water and reduce transmittance. The lower the transmittance, the more algae present. Table B8 and figures B34 and B38 indicate that El and Cl tanks had practically identical algae growth. This was as expected since they were entical in handling. A comparison of table B8 and figures B35 d B39 show a larger algae growth in E2 as compared to C2. Figure B39 shows a more precipitous drop. The E2 tank had a final transmittance (on the seventh day) of 93%. The experimental side f the river generally had a lower transmittance for the third and fourth tanks than did the control side of the river. This can be seen in figures B36 and B37 as compared to figures B40 and B41. This increased growth can be attributed to decreased number of predators, the zooplankton.

The levels of phosphate and nitrate varied over the course of seven days.

As can be seen in figures B2 through B17; there is no definite trends in the relative concentrations of phosphate and nitrate in any of the tanks. Since the Hach Tests only measure the amount of nitrate and phosphate in solution, it can be assumed that these were taken out into the biomass at a steady rate. New phosphate and nitrate was added to the river ach day in the form of sewage.

Since each tank should have gotten approximately the same amount of nitrate and phosphate, an increase in the amount of either in free solution would probably indicate a decrease in the amount of algae growth. There was an overall trend as indicated figures B2 through B9 for an increase phosphate level on the sixth and seventh day. This occurred in all tanks. In the same time, nitrate levels did not change significantly. This was probably due to the disproportionate amount of phosphate and nitrate in the treated sewage.

In both years (figures B2-9) phosphate levels were insignificantly higher in the sewage than in the tank water. Nitrate levels in the sewage were high than the tank but to not Ch a great degree. Phosphate, it would seem then, is taken up a greater degree by the algae. The increased phosphate levels the sixth and seventh day might have been indicative of upcoming change in the algae.

As shown in figures B42-49 all the temperatures in all tanks throughout the experiment stayed between 29°ºC and 31 ºC. This indicates that greater algae growth could not have occurred in some tanks rather than others due to uneven temperature. The rate and number of fluctuations on the graph is also similar for all tanks. For these reasons we do not see tank temperature in this experiment as a significant variable in algae growth.

The only trend seen in pH data in figures B26-33 is a rise in e pH of C2, C3, C4 and E2, E3, E4 after the addition of the sewage early in the experiment. This behavior must be due to the alkalinity of the sewage, the pH of which was 8.2 for our experiment. Therefore, pH was also not a significant factor in differences in algae growth from tank to tank.

Because figures B18-21 are nearly identical, dissolved oxygen must also have not been a significant factor in the variation of algae growth from tank to tank. The only trend apparent is the crease in dissolved oxygen levels in the second and fourth days e to increased algae numbers after the addition of the sewage.

BIBLIOGRAPHY

Arnold, D. E. 1971. Ingestion, assimilation, survival and reproduction by Daphnia pulex fed seven species of blue-green algae. Limnol. Oceanogr. 1906-920.

Brooke, A. J. 1957. Water Blooms. New Biology 23;86-101.

Fitzgerald,. G. P. 1961. Stripping effluents of nutrients by biological means. Algae and Metropolitan Wastes Trns. 1960 Seminar, R. A. Tuft SEC, US P.H.S. Tech. Report W61-3.

Fritsch, F . E. 1903. Two fungi, parasitic and species of Tolypothrix (Resticularia nodosa, Dang. and R. boodlei n. sp.) Ann. Sot. 17:649-664.

Hach Chemical Company. 1973. Water Analysis Handbook.

Hetling, Leo. J. 1969. The Potomac Estuary Mathematical Model. Tech. Report # 7. Annapolis Field Office. E.P.A. Region III.

Jaworski, N. A. and J. H. Johnson, Jr. 1969. Potomac- Piscataway dye releases and wastewater assimilation studies. Tech. Report # 19. Annapolis Field Office. E.P.A. Region III.

Lackey, J. B. 1964. The ecology of Plankton Algae. In: Algae and Man. Ed. D. F. Jackson. Plenum Press, New York 1964. pp. 707-220.

Rice, T. R. 1952. Biotic influences affecting population growth of planktonic algae. Fish. Bull. U.S. 54:227-245.

Safferman, R. S. and M. C. Morris. 1962. Evaluation of Natural Products for Algicidal properties. Appl. Microbiol. 10:289-292.

Shilo, M. 1970. Lysis of blue-green algae by a myxobacter. J. Bact. 104:453-461.

Spoon, D. M. 1976. Survey, ecology, and systematics of the upper Potomac estuary biota: aufwuchs microfauna. Phase II. WRRC Report # 8. Washington Tech. Inst. Washington, D.C. 20008.

Skulberg, O. M., 1964 Algal Problems Related to the Eutrophication of European Water Supplies, and a Bio-Assay Method to Assess Fertilizing Influences of Pollution on Inland Waters. In: Algae and Man Ed. D. F. Jackson. Plenum Press. New York 1964. p-.262-299.

Vance, B. D. 1966. Sensitivity of Microcystis aeruginosa and other blue-green algae and associated ~bacteria o selected antibiotics. J. Phycol. 2:125-128.

Wu, B., M. K. Hamdy and H. B. Howe, Jr. 1968. Antimicrobial activity of a myxobacterium against blue-green algae. Bact. Pro. p. 48.

EXPERIMENT I

(Spring, 1977)

10	ml	of	1.0% KN03 solution
10	ml	of	0.1% K_2HP04 solution
50	ml	of	soil/water extract (see below)
970	ml	of	deonized water

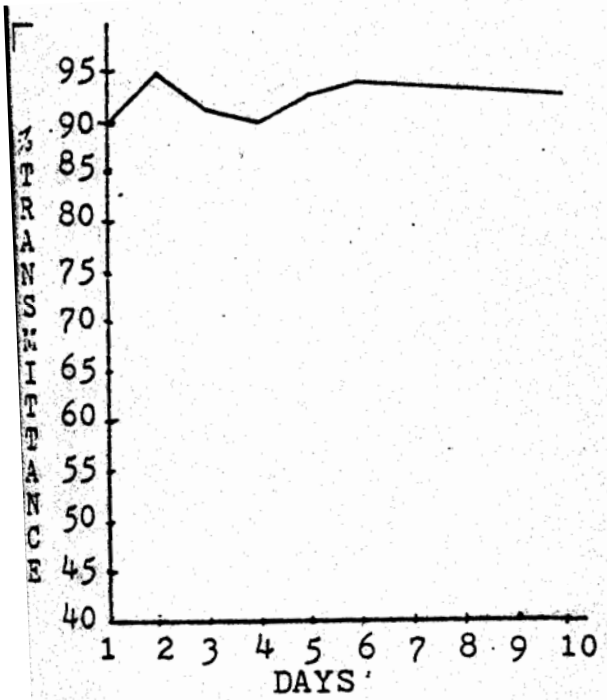
1040 ml total

Soil/water extract: In 250 ml flask, place spatula of Ca C03, cover with half inch of garden soil, fill with deonized water, autoclave, decant off liquid

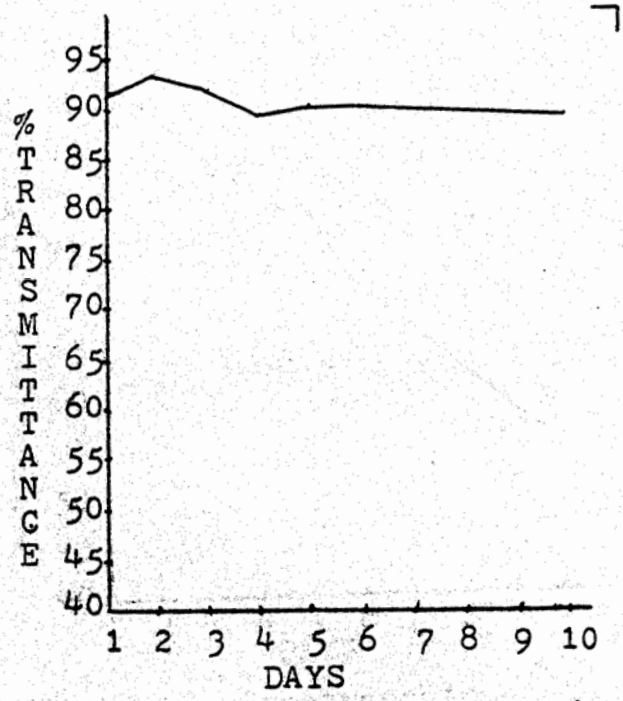
Table A1. Nutrient Culture Medium for Blue-Green Algae

South Swamp	North Swamp	Filtered Swamp Water
98.0	99.5	92.0
98.0	93.5	95.0
91.0	96.0	91.0
100.0	93.0	88.0
mean=96.8	mean=95.5	mean=91.5

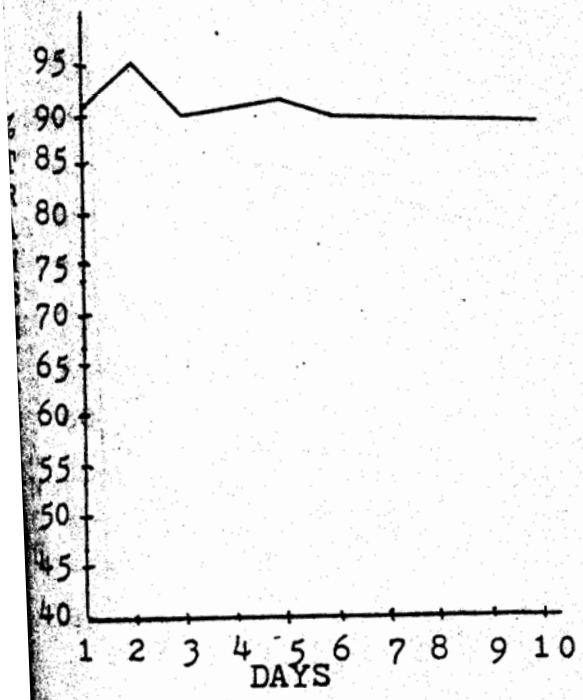
Table A2. Results of the spectrophometric readings at 633 nm. of the Petri dishes containing Anabaena and zooplankters from the north or south swamp, plus readings of the control which contained Anabaena and filtered swamp water. All readings are percent transmittance and were taken for Part I.



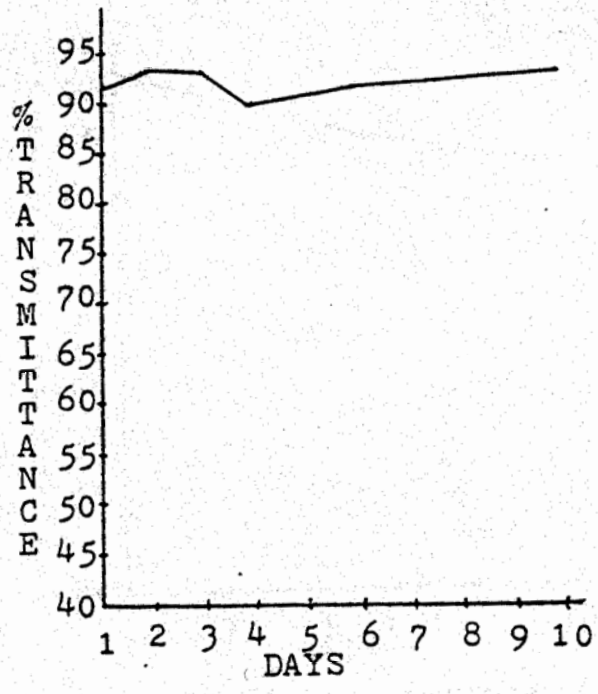
FigureA2. Spectrophotometric readings for C_1 Tank



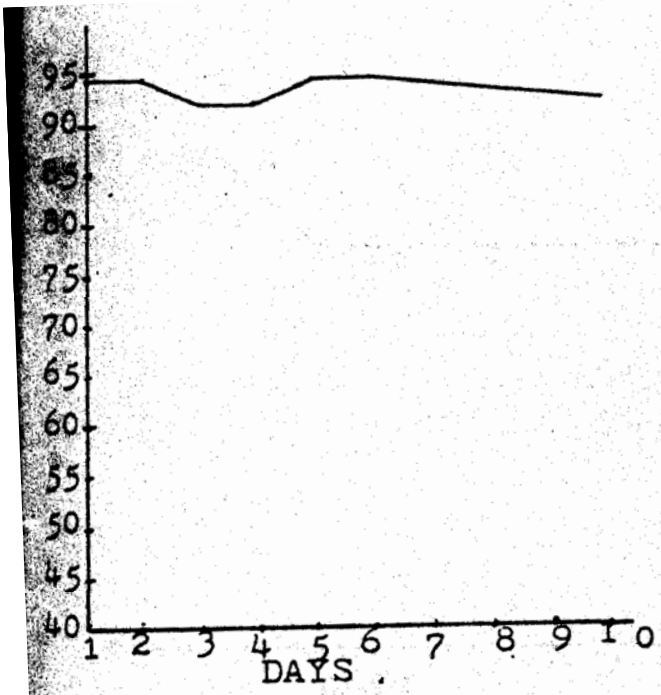
FigureA3. Spectrophotometric readings for C_2 Tank



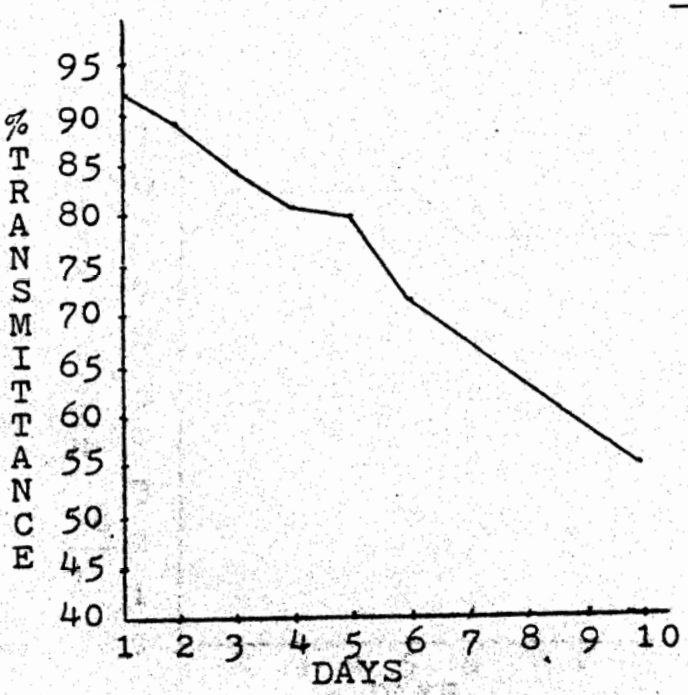
igureA4. Spectrophotometric readings for C_3 Tank



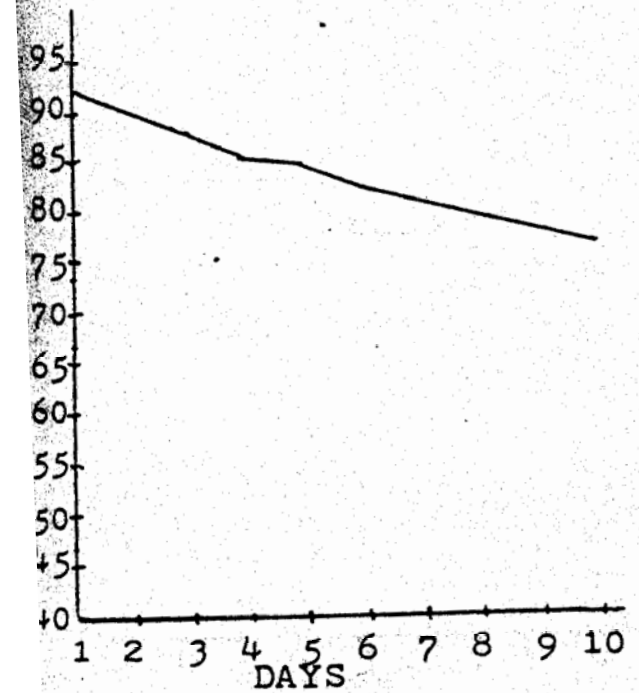
FigureA5. Spectrophotometric readings for C_4 Tank



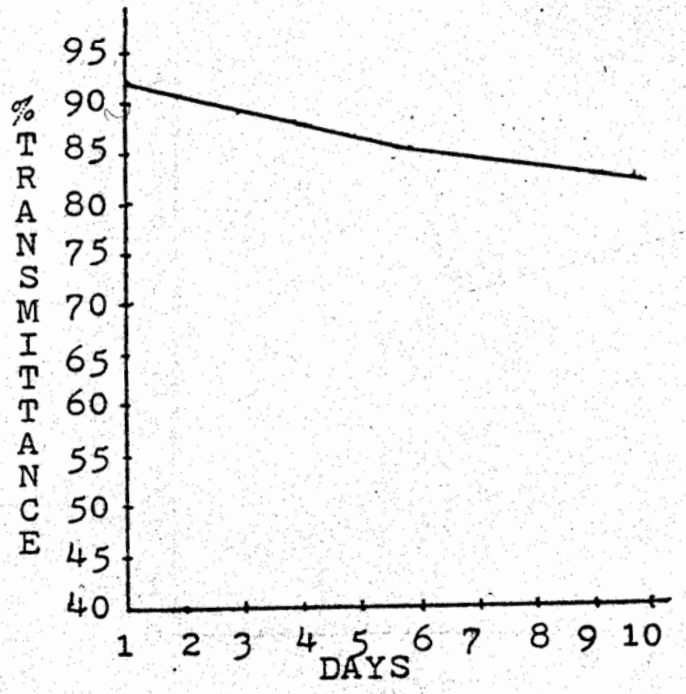
FigureA6. Spectrophotometric readings for E_1 Tank



FigureA7. Spectrophotometric readings for E_2 Tank



.gureA8. Spectrophotometric readings for E_3 Tank



FigureA9. Spectrophotometric readings for E_4 Tank

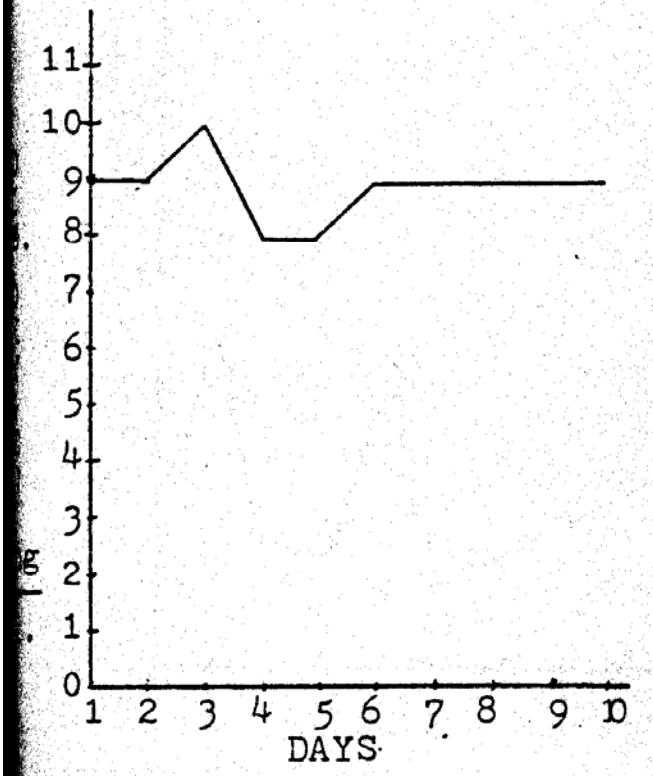


Figure A14. Dissolved Oxygen readings for E_1 Tank

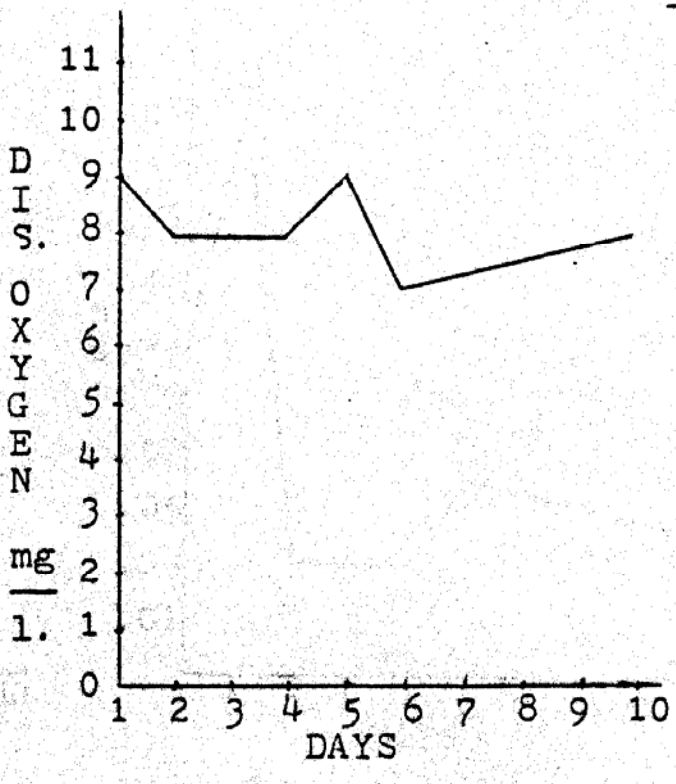


Figure A15. Dissolved Oxygen readings for E_2 Tank

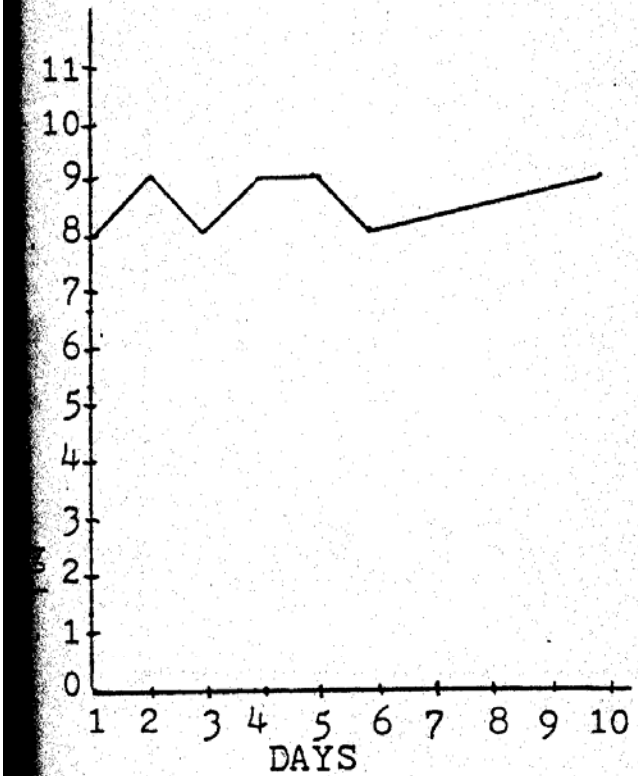


Figure A16. Dissolved Oxygen readings for E_3 Tank

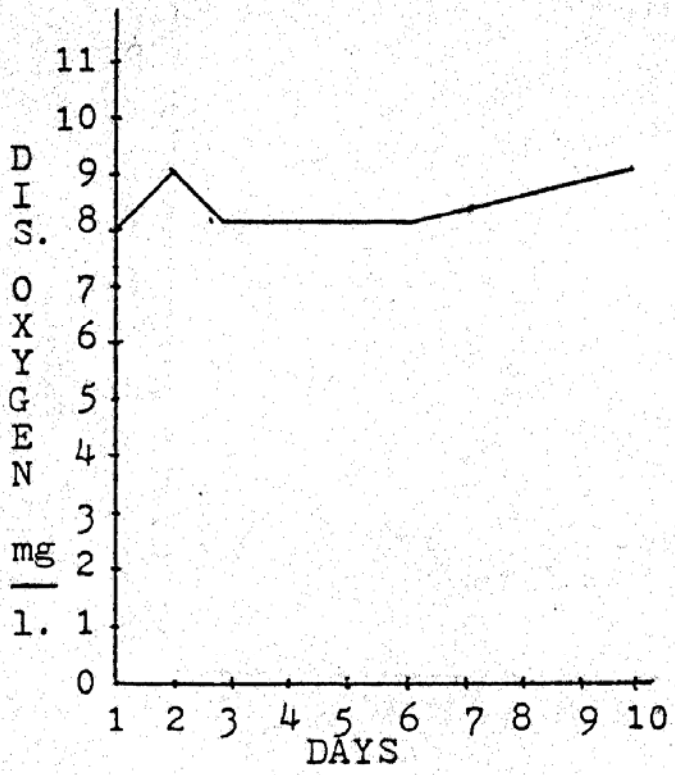


Figure A17. Dissolved Oxygen readings for E_4 Tank

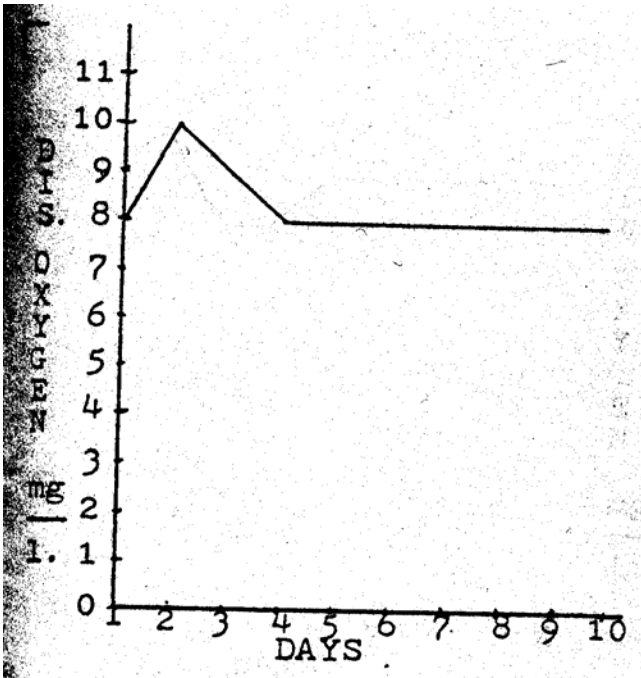


Figure A10. Dissolved Oxygen readings for C_1 Tank

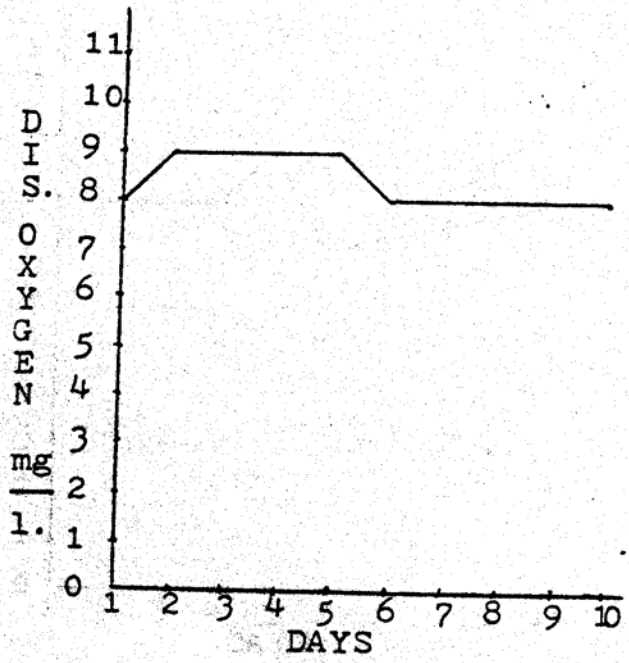


Figure A11. Dissolved Oxygen readings for C_2 Tank

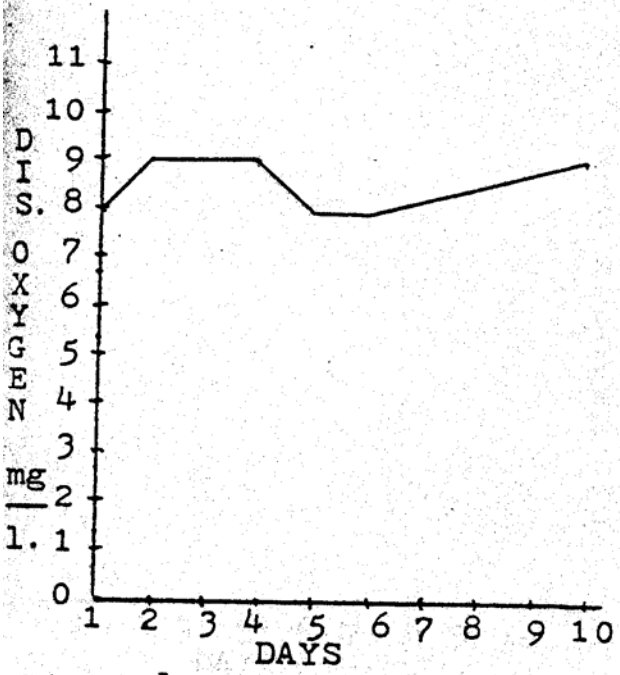


Figure A12. Dissolved Oxygen readings for C_3 Tank

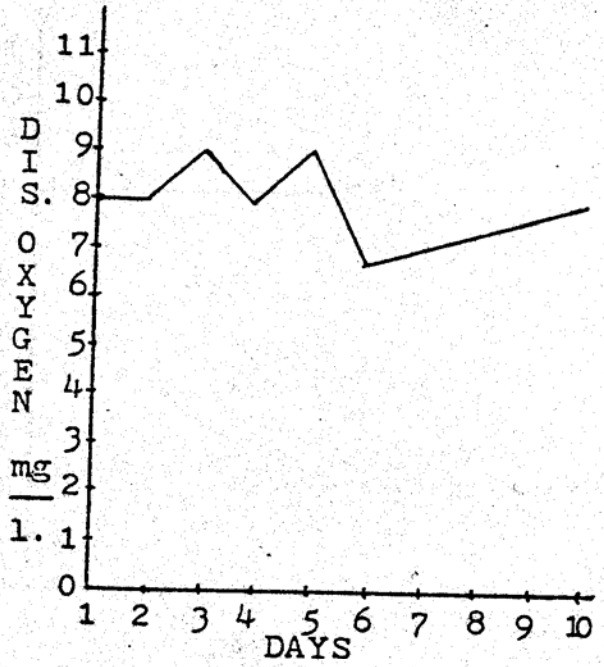


Figure A13. Dissolved Oxygen readings for C_4 Tank

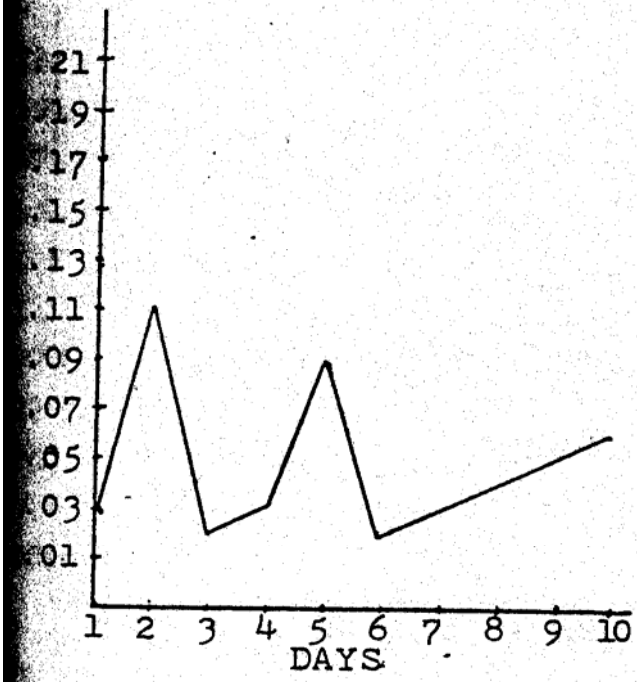


Figure A18. Phosphate levels in the C_1 Tank

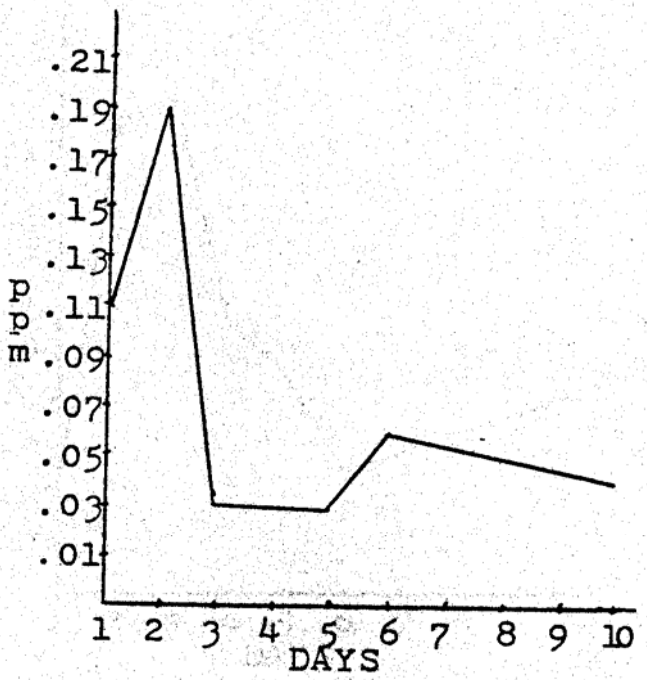


Figure A19. Phosphate levels in the C_2 Tank

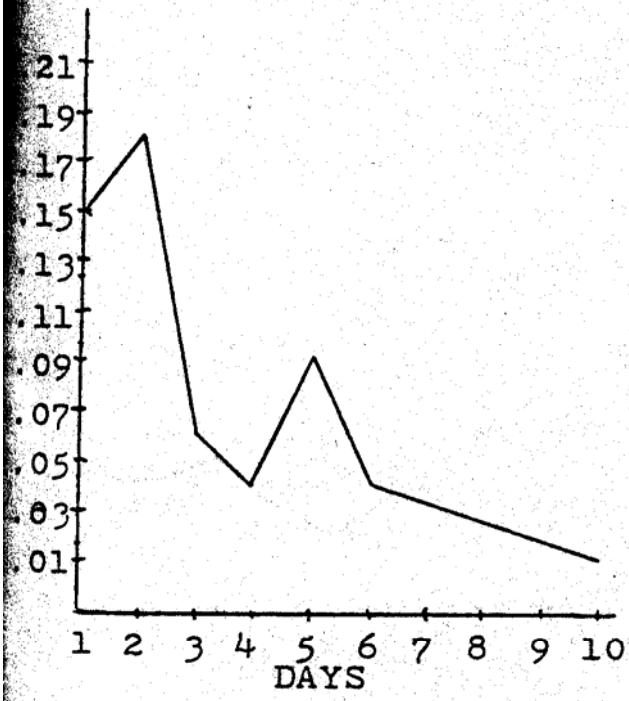


Figure A20. Phosphate levels in the C_3 Tank

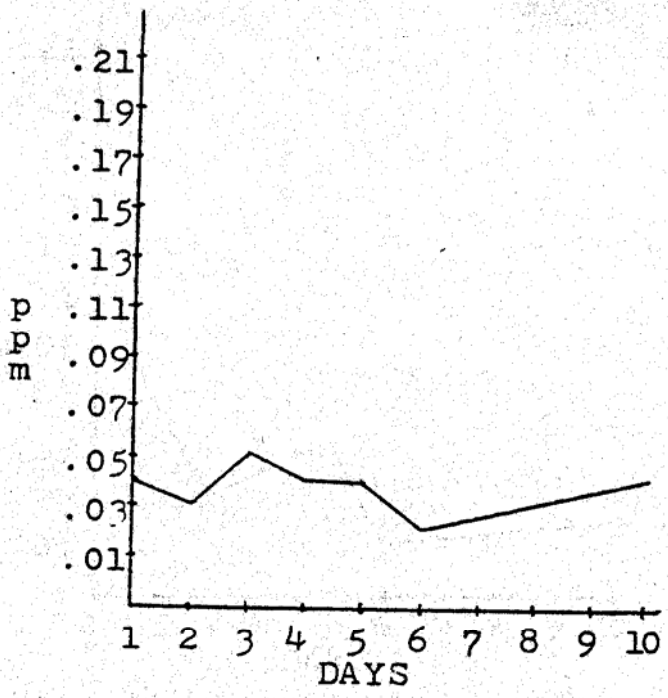


Figure A21. Phosphate levels in the C_4 Tank

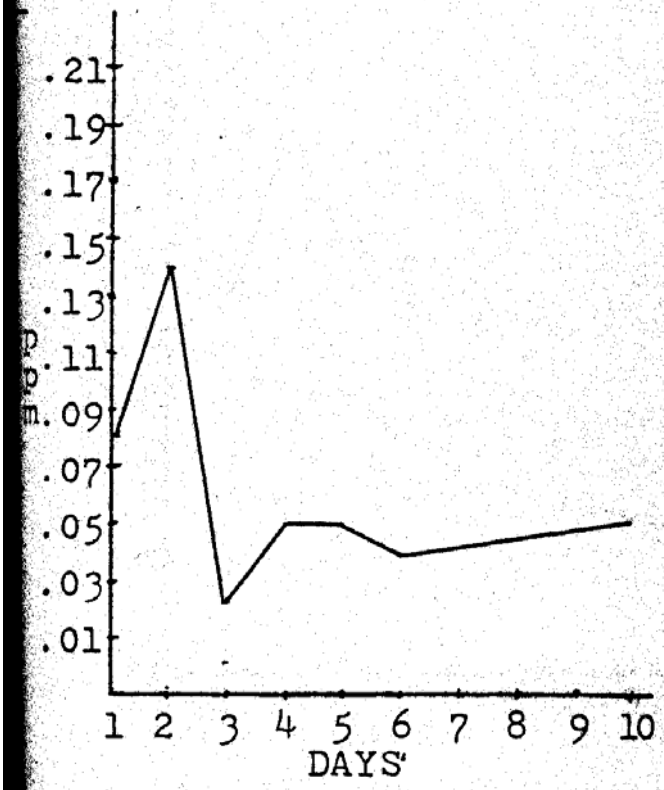


Figure A22. Phosphate levels in the E_1 Tank

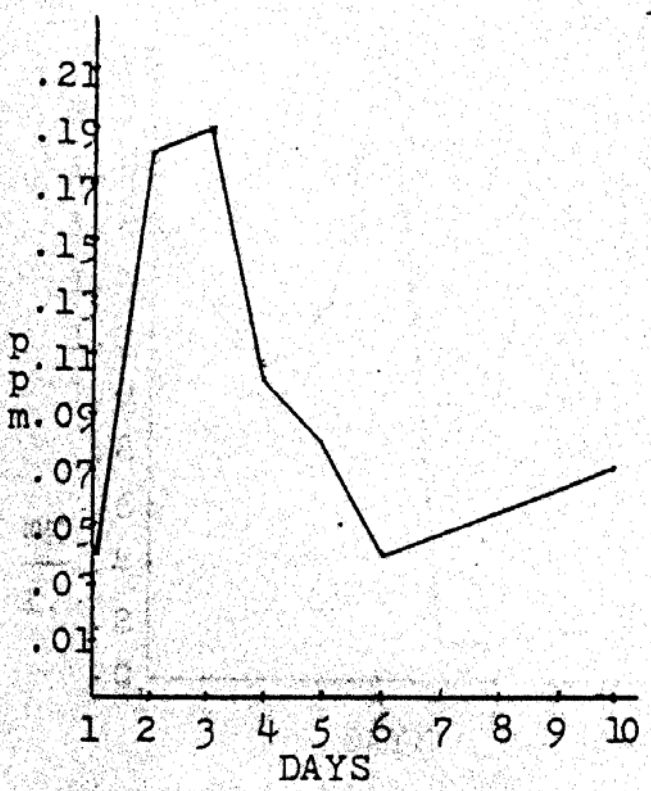


Figure A23. Phosphate levels in the E_2 Tank

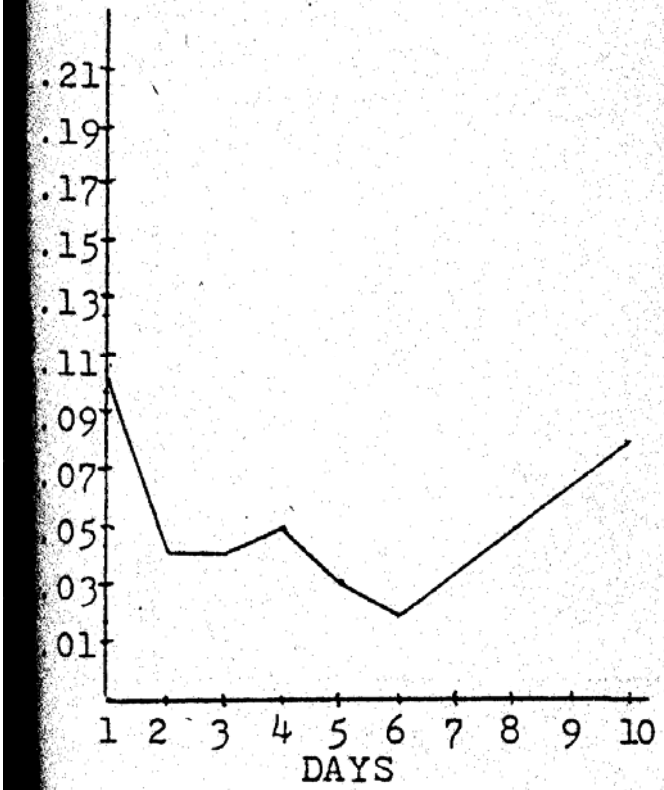


Figure A24. Phosphate levels in the E_3 Tank

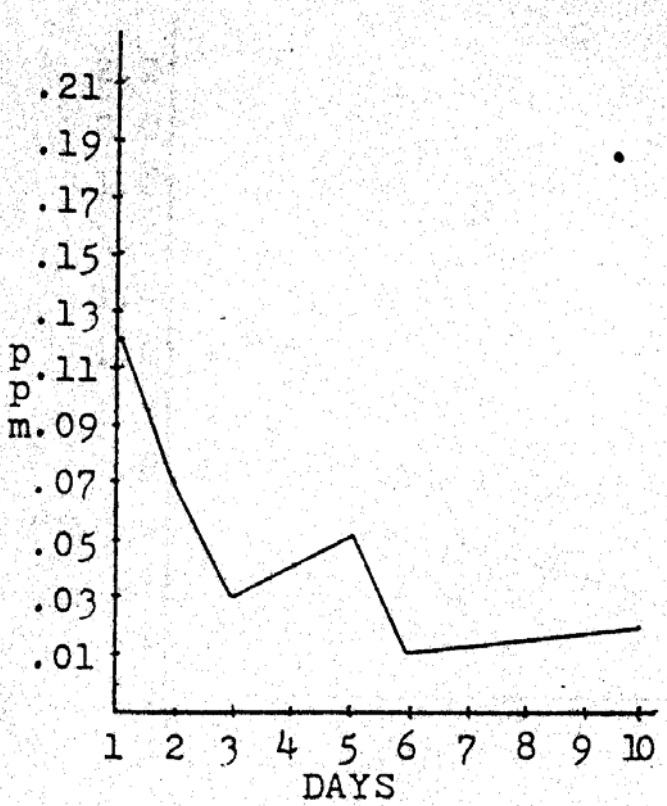


Figure A25. Phosphate levels in the E_4 Tank

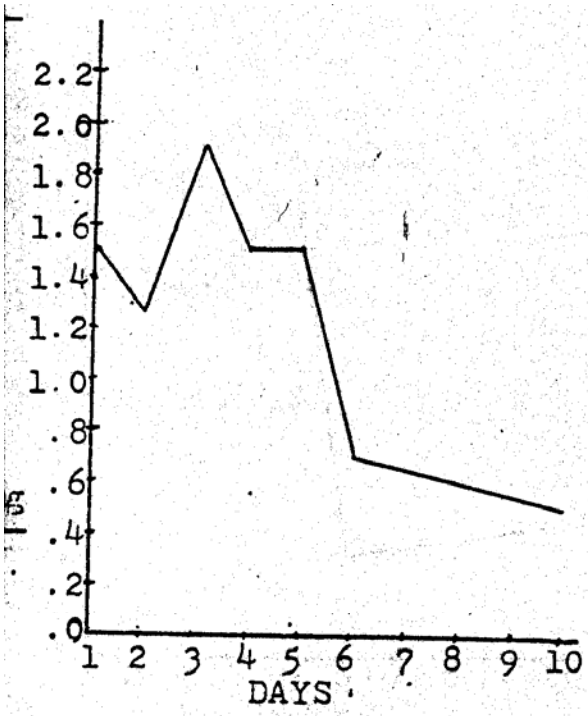


Figure A26. Nitrate levels in the C_1 Tank

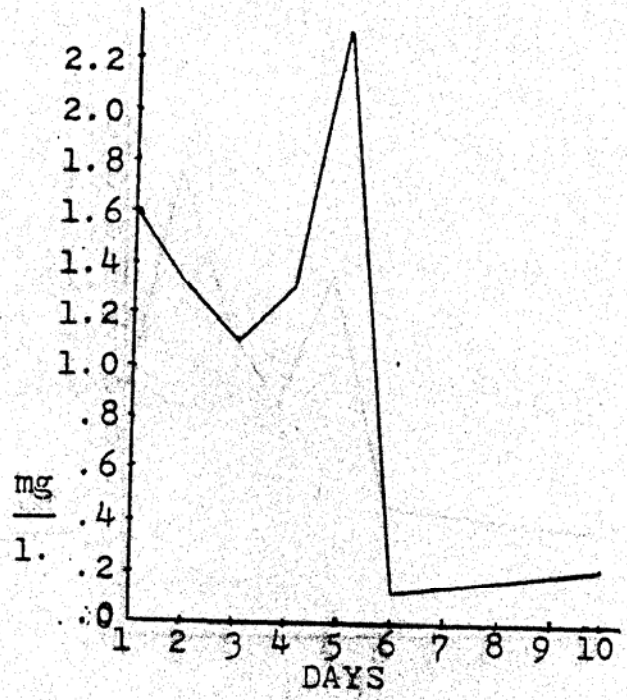


Figure A27. Nitrate levels in the C_2 Tank

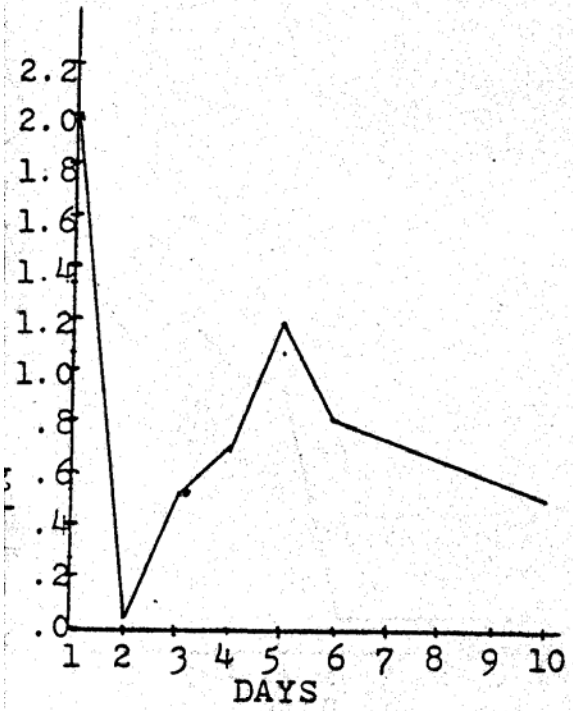


Figure A28. Nitrate levels in the C_3 Tank

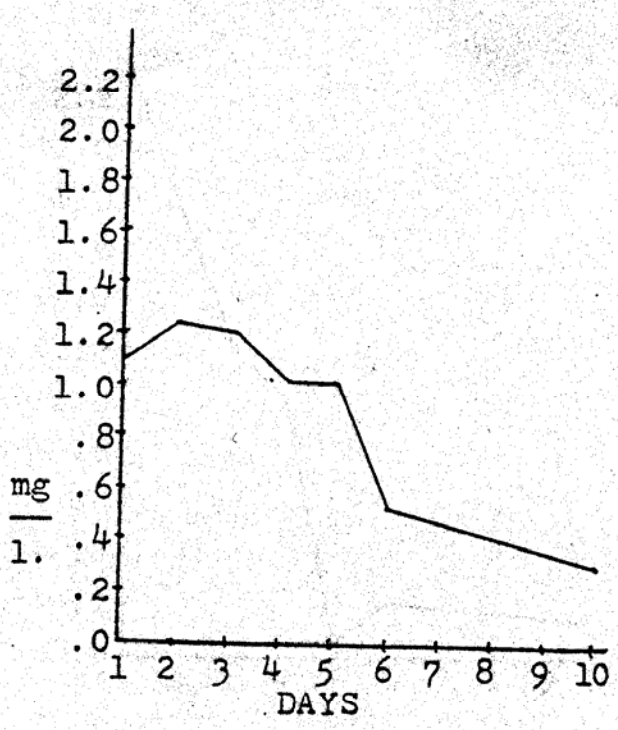


Figure A29. Nitrate levels in the C_4 Tank

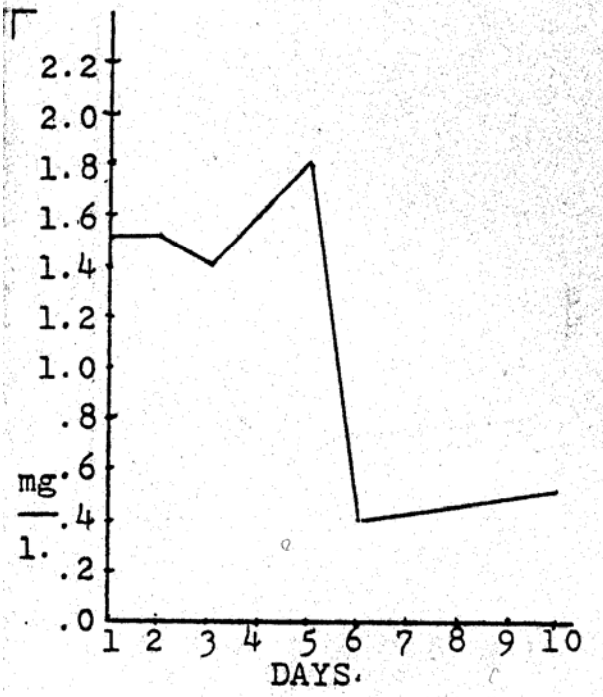


Figure A30. Nitrate levels in the E_1 Tank

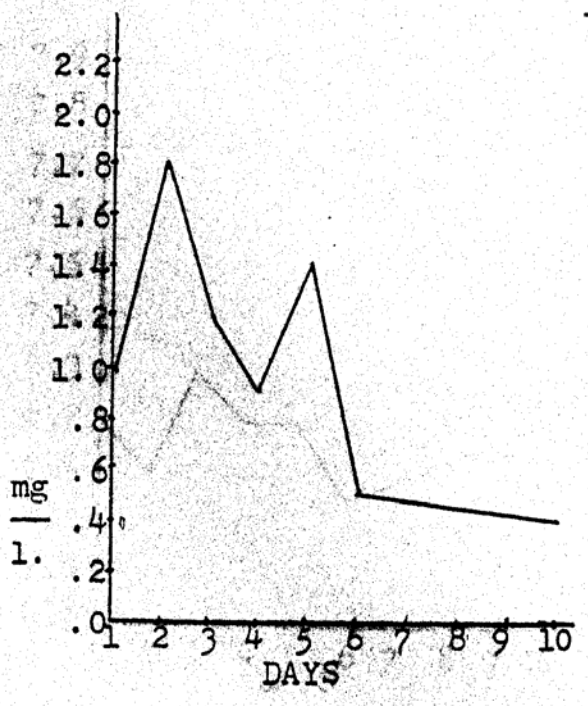


Figure A31. Nitrate levels in the E_2 Tank

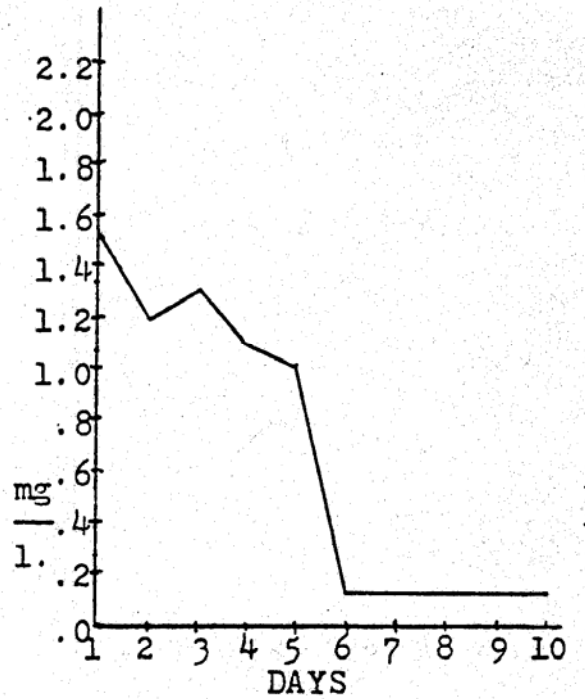


Figure A32. Nitrate levels in the E_3 Tank

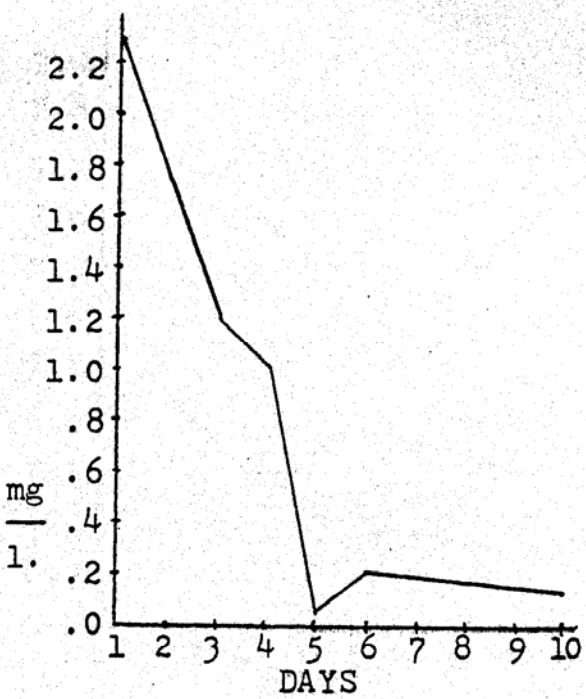


Figure A33. Nitrate levels in the E_4 Tank

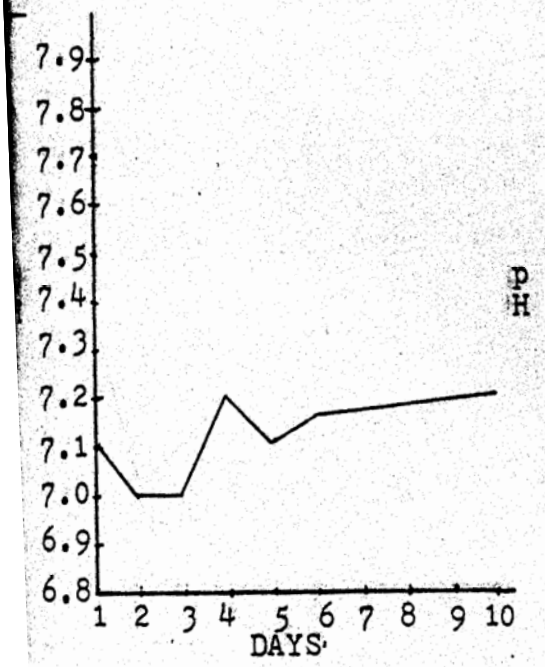


Figure A34. pH levels in the C_1 Tank

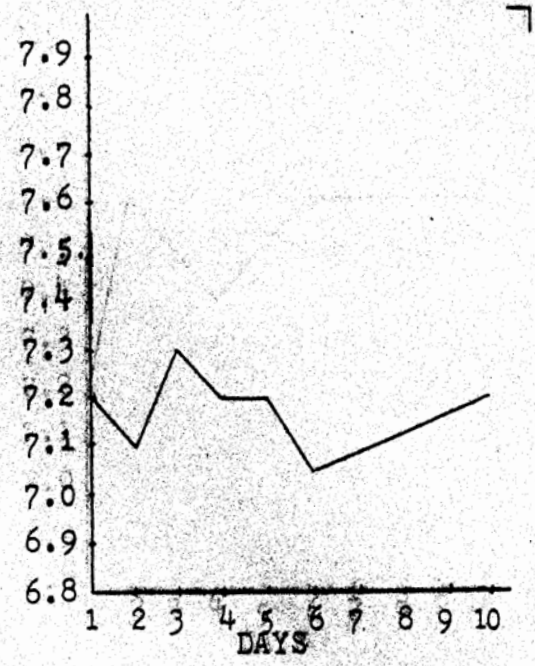


Figure A35. pH levels in the C_2 Tank

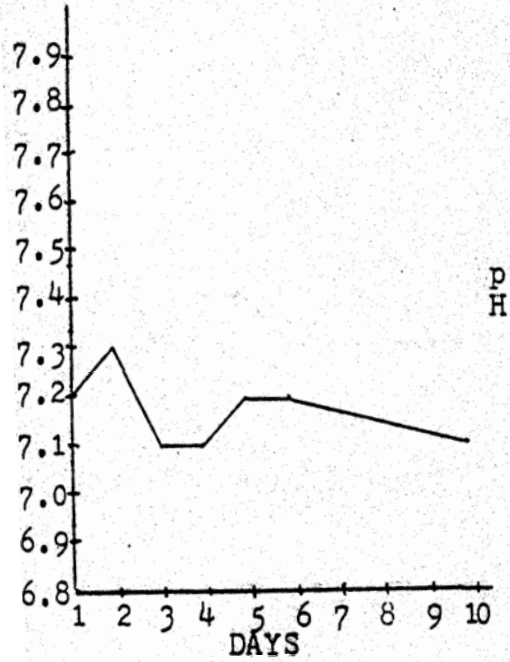


Figure A36. pH levels in the C_3 Tank

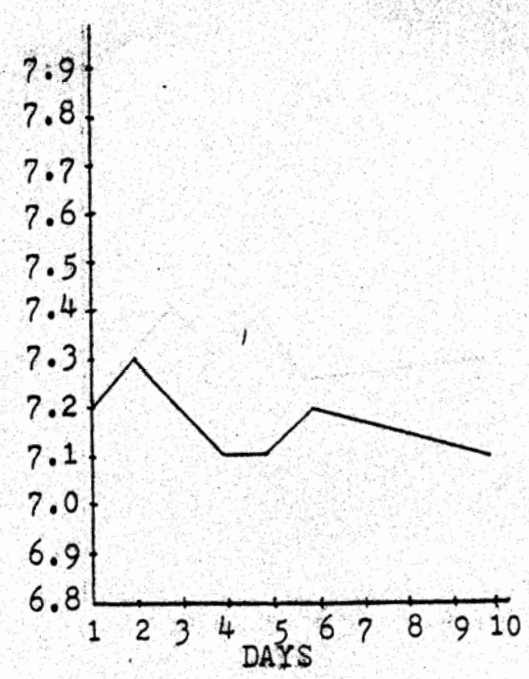


Figure A37. pH levels in the C_4 Tank

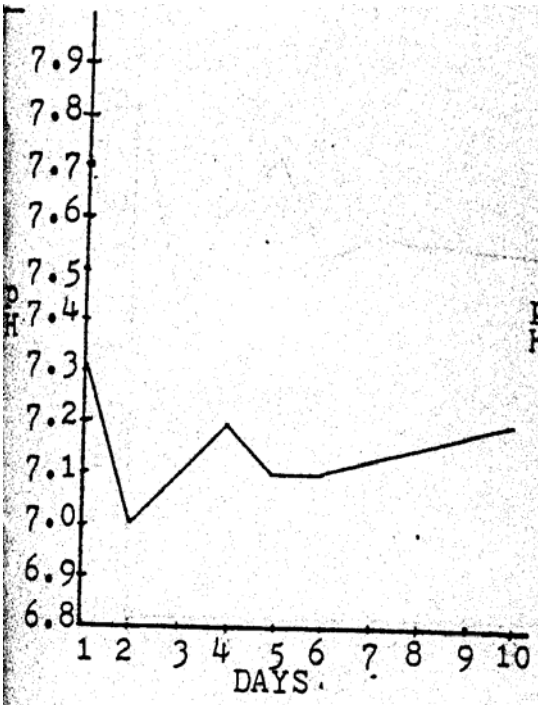


Figure A38. pH levels in the E_1 Tank

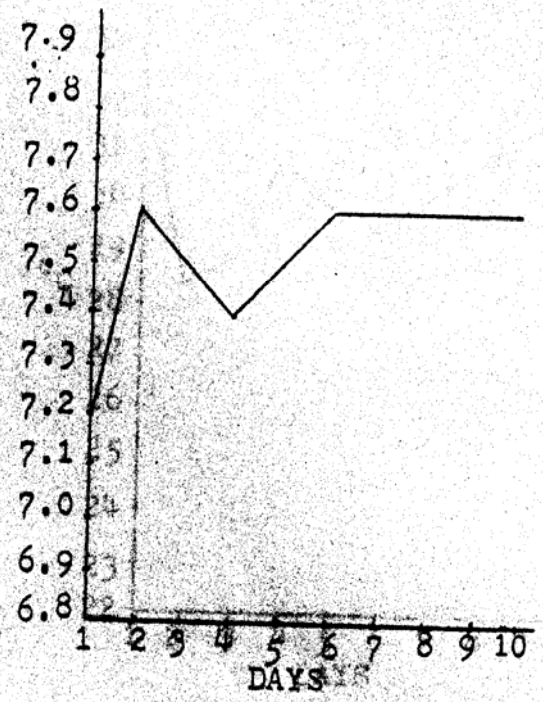


Figure A39. pH levels in the E_2 Tank

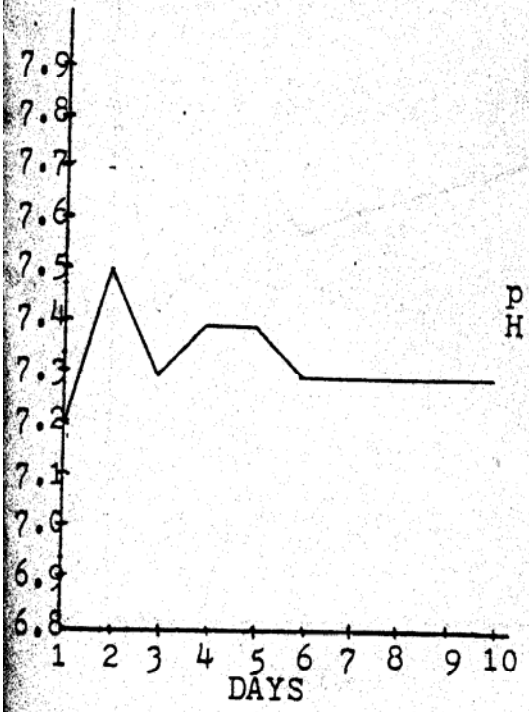


Figure A40. pH levels in the E_3 Tank

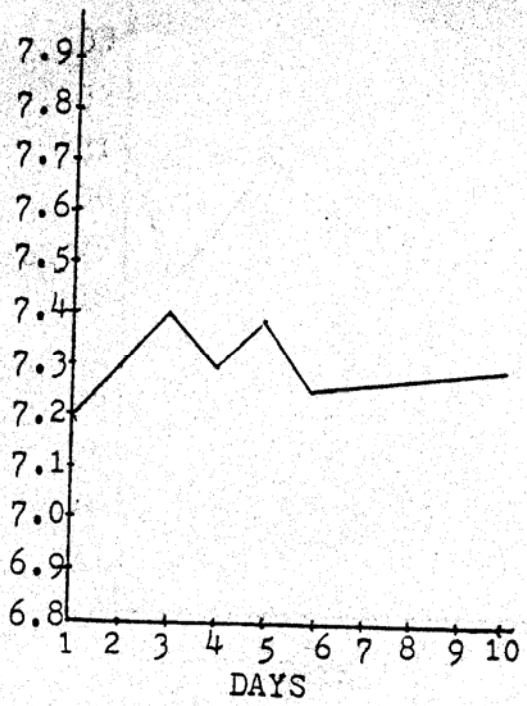


Figure A41. pH levels in the E_4 Tank

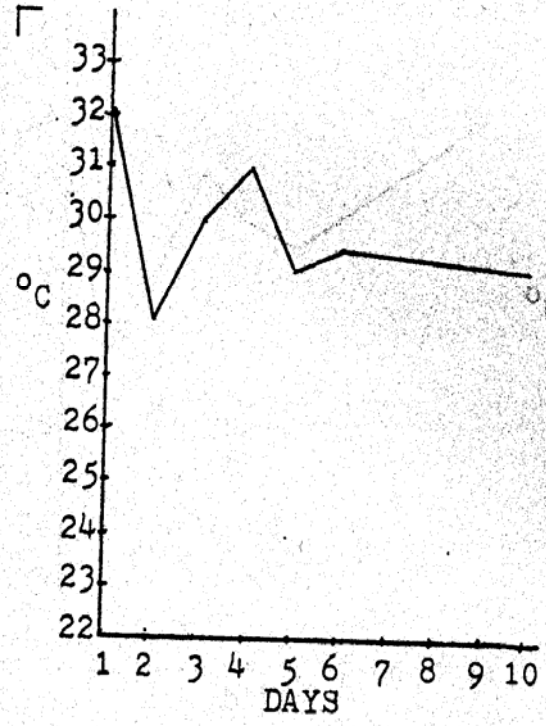


Figure A42. C_1 Tank temp.

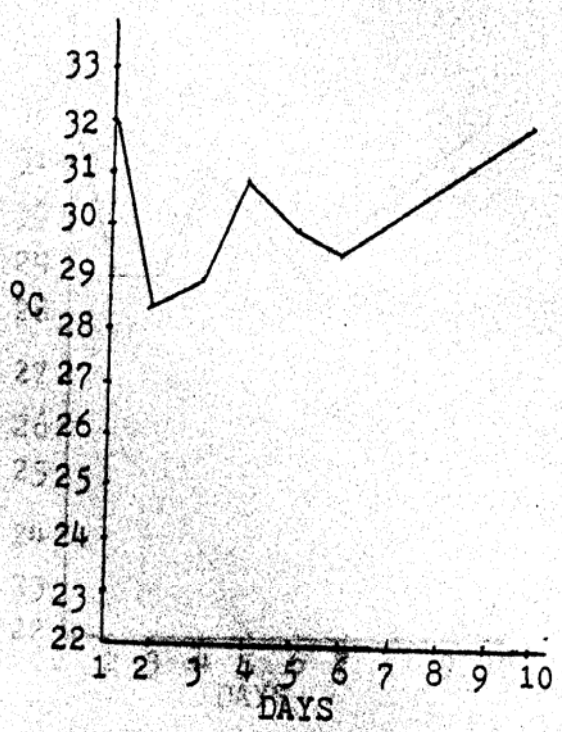


Figure A43. C_2 Tank temp.

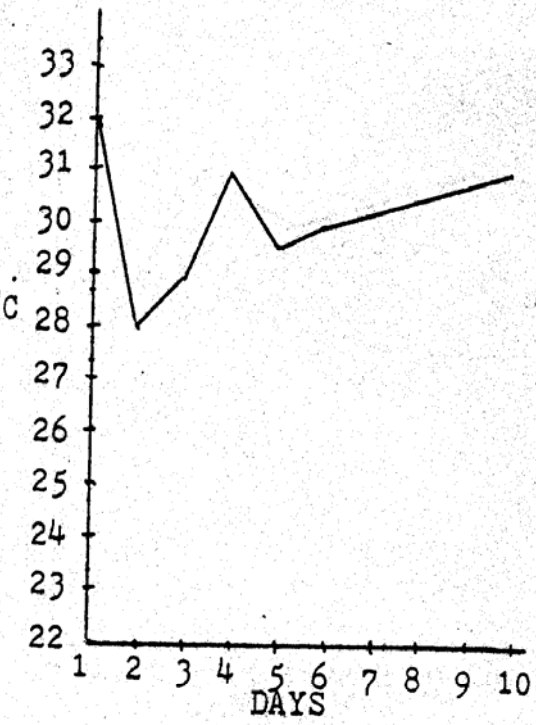


Figure A44. C_3 Tank temp.

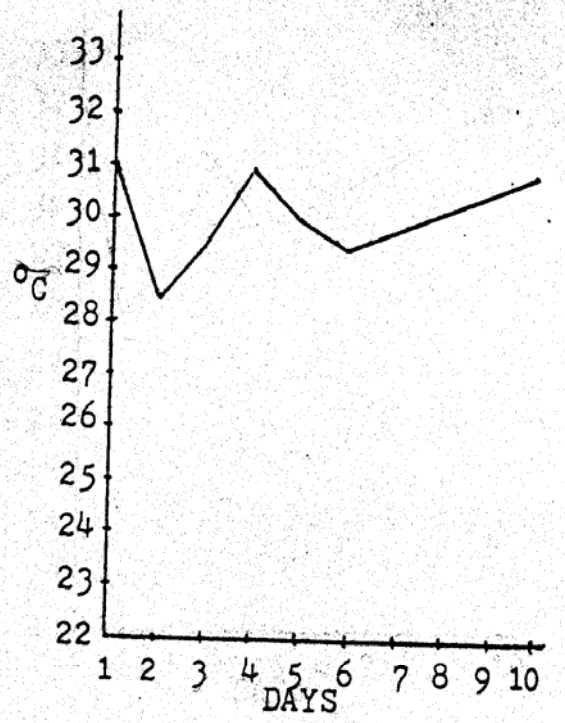


Figure A45. C_4 Tank temp

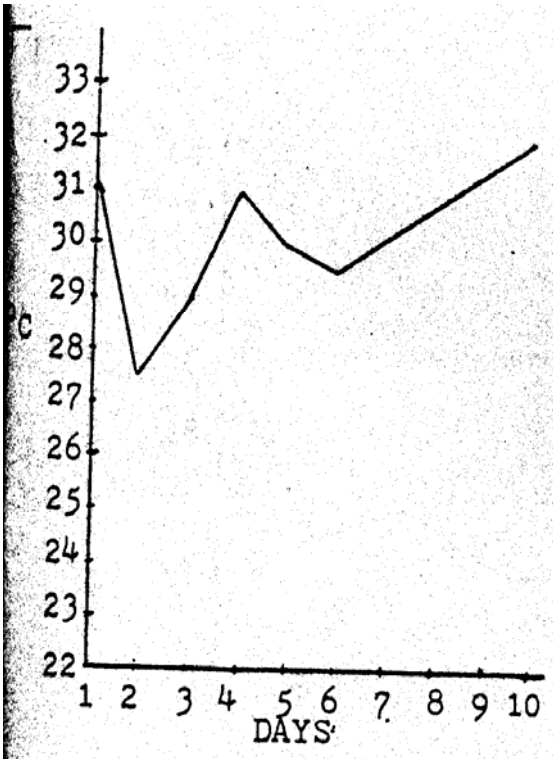


Figure A46. E_1 Tank temp.

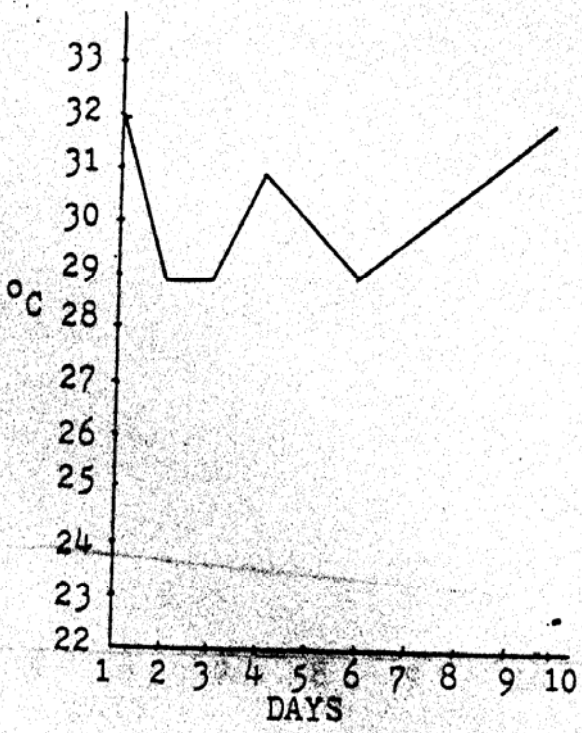


Figure A47. E_2 Tank temp.

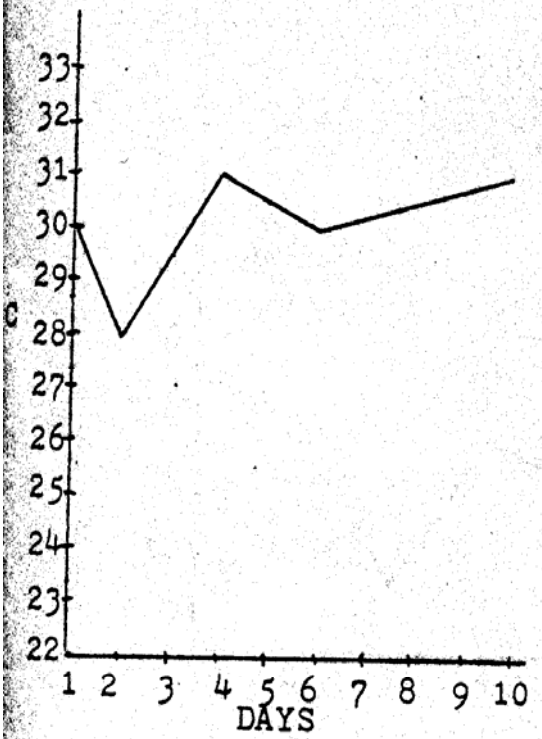


Figure A48. E_3 Tank temp.

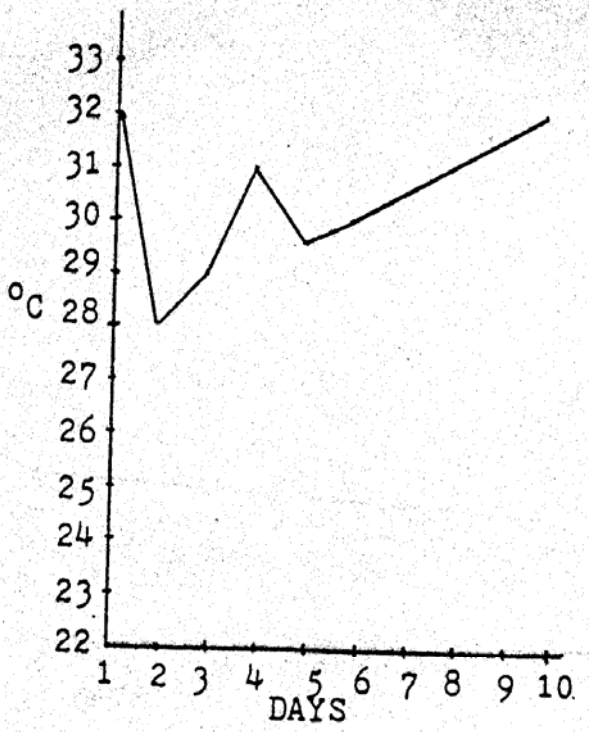


Figure A49. E_4 Tank temp.

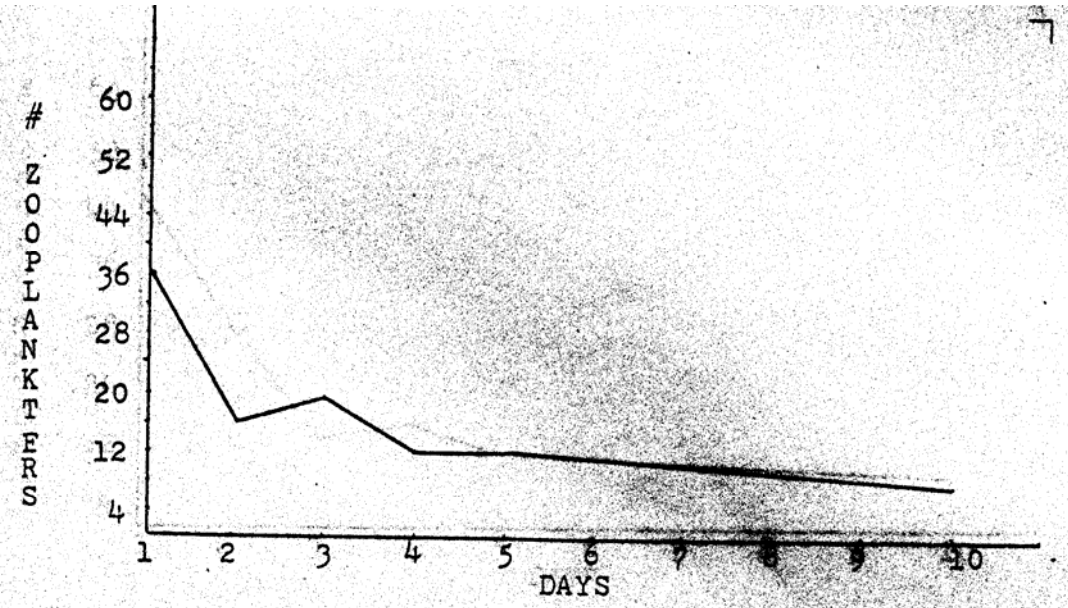


Figure A 50. Relative amounts of zooplankters in Tank C_1

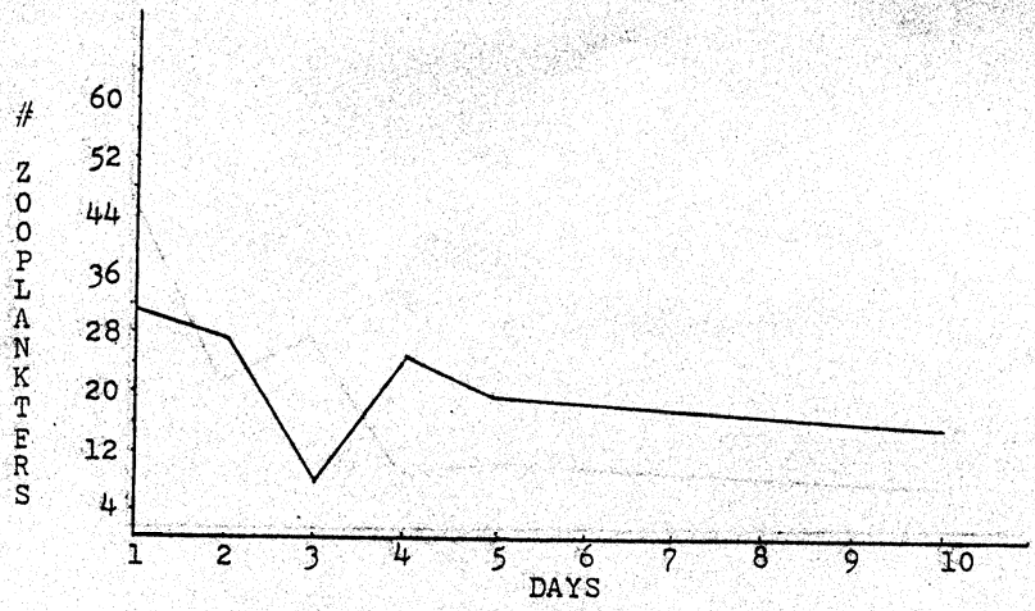


Figure A 51. Relative amounts of zooplankters in Tank C_2

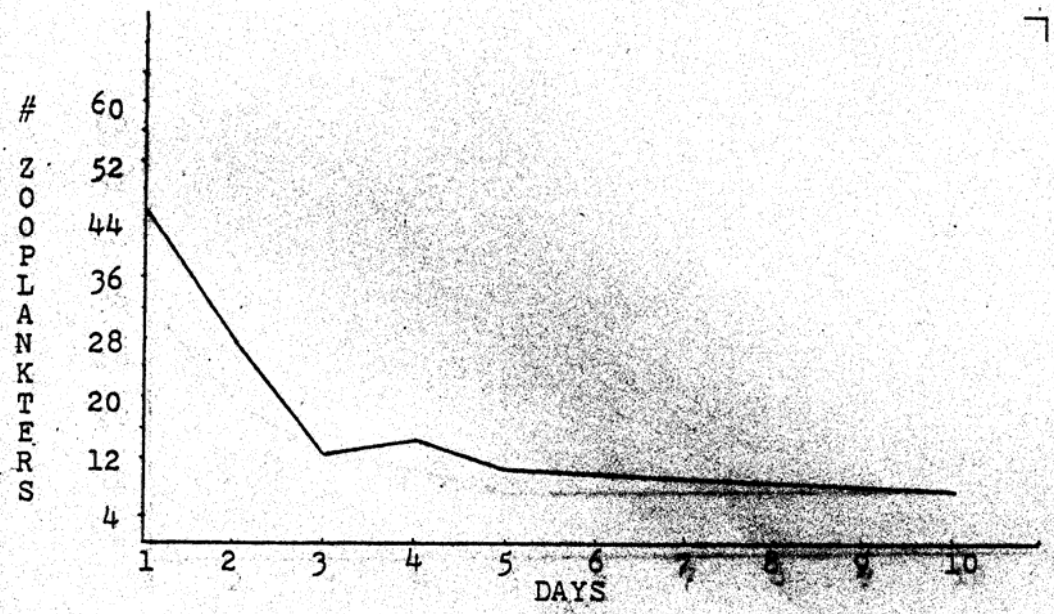


Figure A52. Relative amounts of zooplankters in Tank C_3

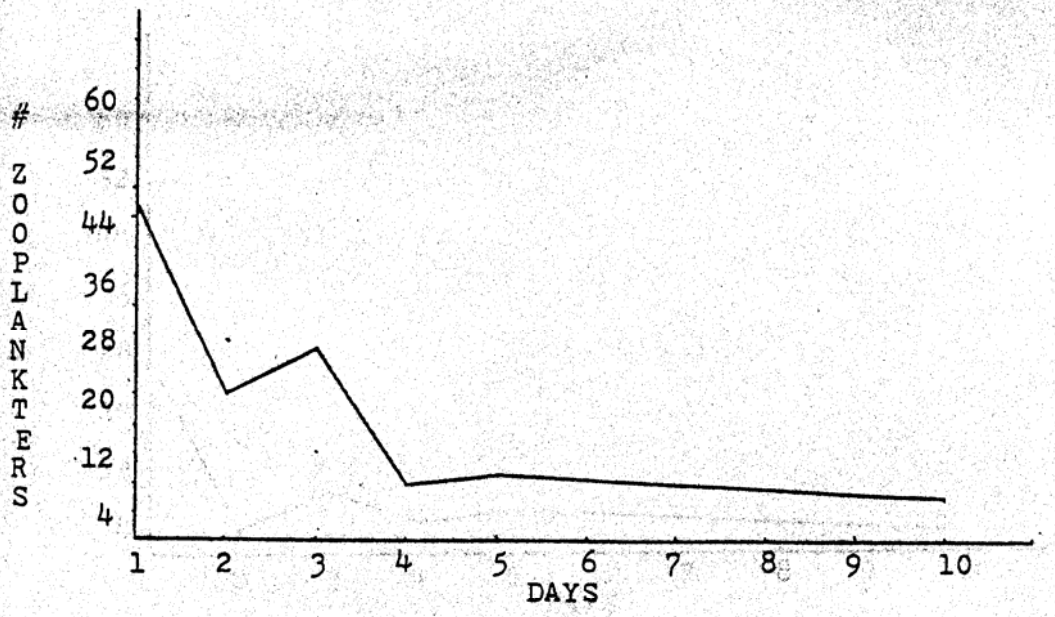


Figure A53. Relative amounts of zooplankters in Tank C_4

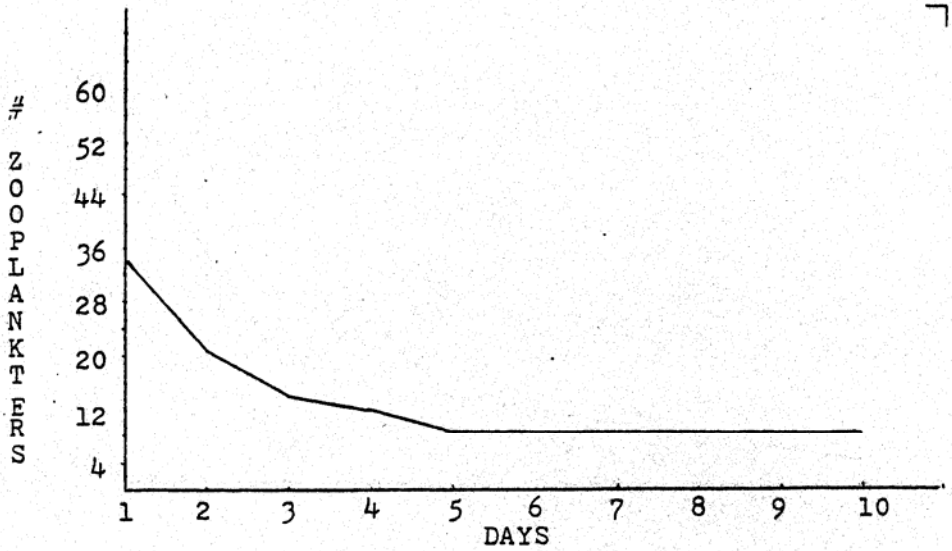


Figure A54. Relative amounts of zooplankters in Tank E_1

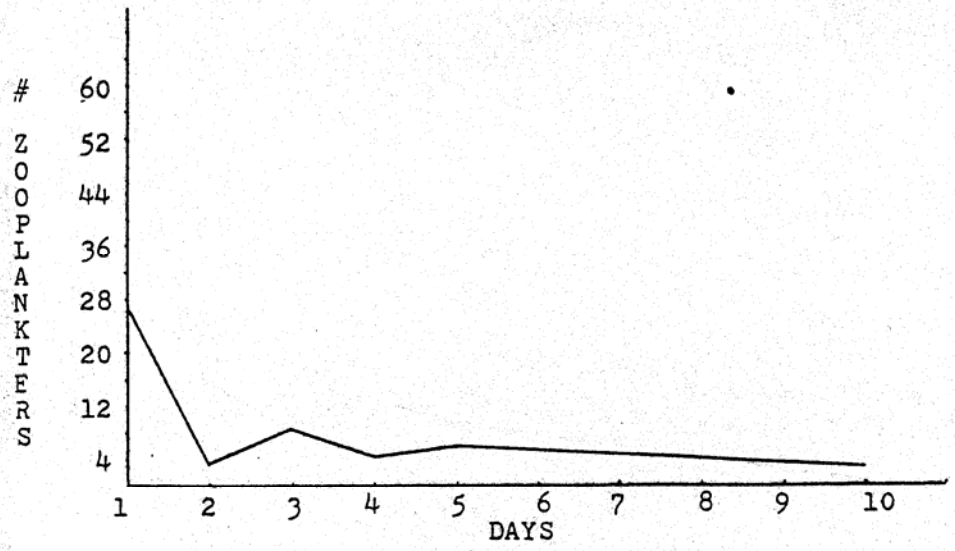


Figure A55. Relative amounts of zooplankters in Tank E_2

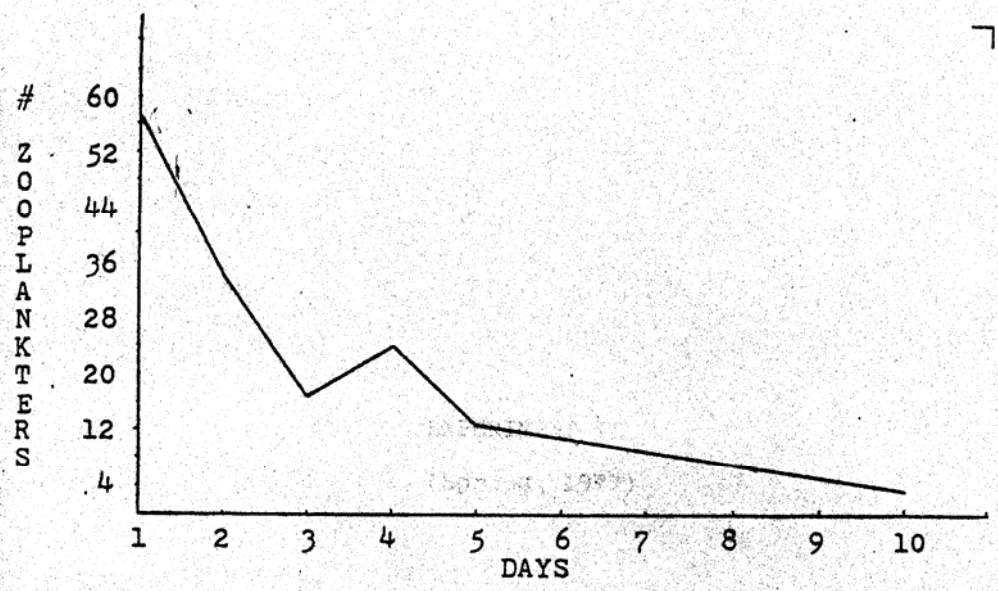


Figure A56. Relative amounts of zooplankters in Tank E_3

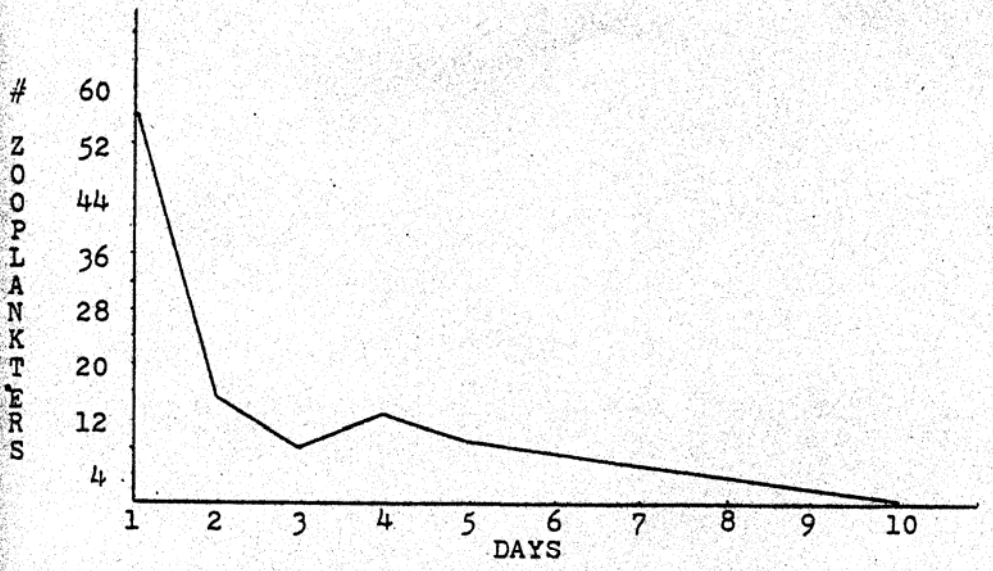


Figure A57. Relative amounts of zooplankters in Tank E_4

EXPERIMENT II

(Spring, 1977)

10	ml	of	1.00- KNO_3 solution
10	ml	of	0.1% $K2HPO_4$ solution
50	ml	of	soil/water extract (see below)
970	ml	of	deonized water
1040 ml total			

Soil/water extract: In 250 ml flask, place spatula of $CaCO_3$, cover with half inch of garden soil, fill with deonized water, autoclave, decant off liquid.

TableBl. Nutrient Culture Medium for Blue-Green Algae

Initial Readings Taken on E_1

pH 7.05 Temp. 31^0

Nitrate 0. 0 3

Phosphate 1.0

%Transmittance - 99.5%

BOD - 10 mg/L.

TableB2. Initial Measurement of Parameters
E1 After Mixing and Prior to Start of the River

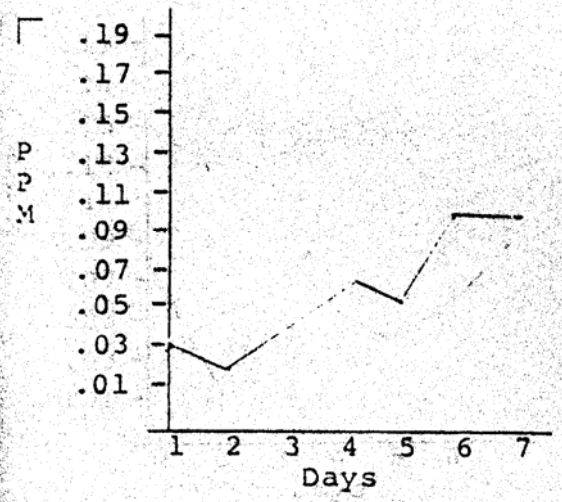


Figure B2. Phosphate Levels in C_1 Tank

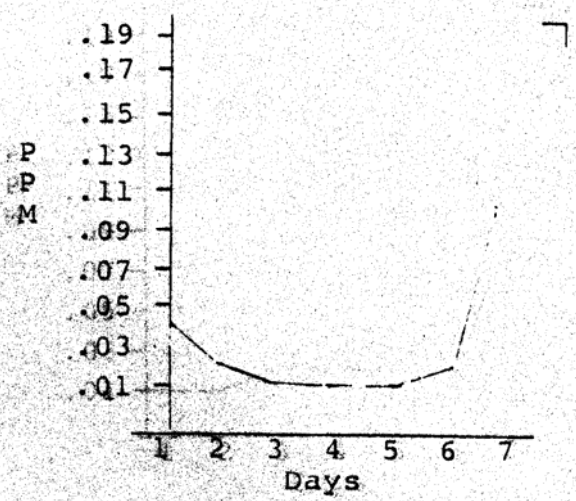


Figure B3. Phosphate Levels in C_2 Tank

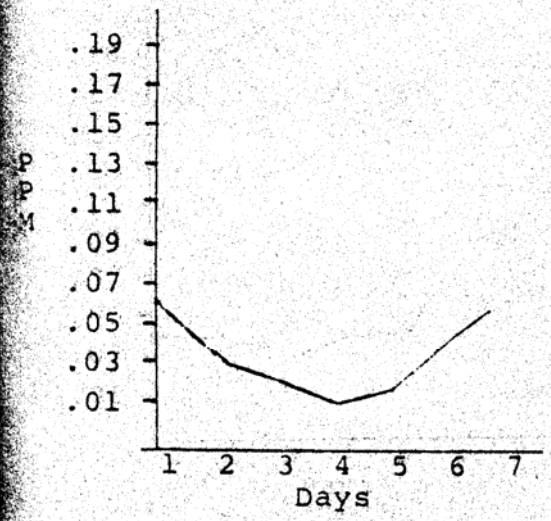


Figure B4. Phosphate Levels in C_3 Tank

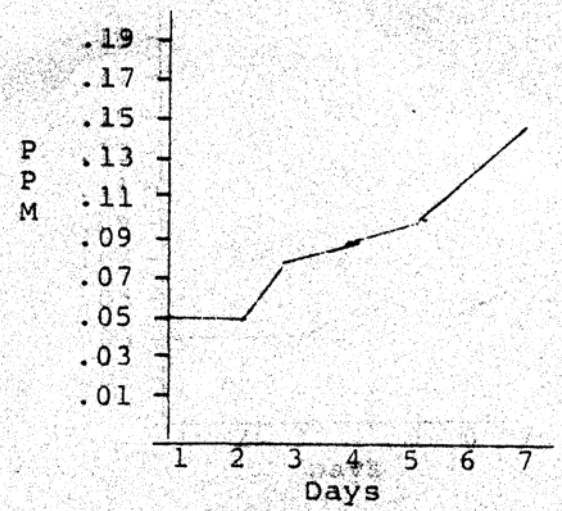


Figure B5. Phosphate Levels in C_4 Tank

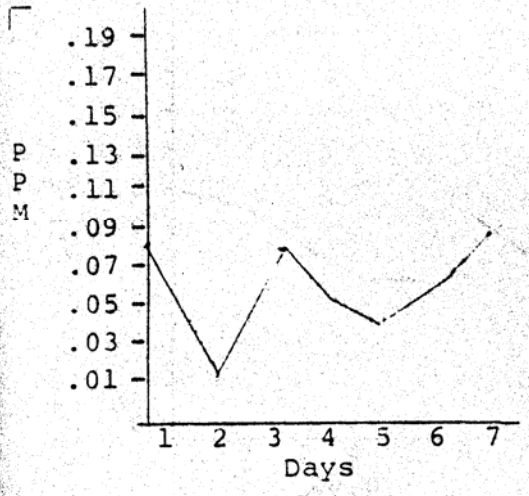


Figure B6. Phosphate Levels in E_1 Tank

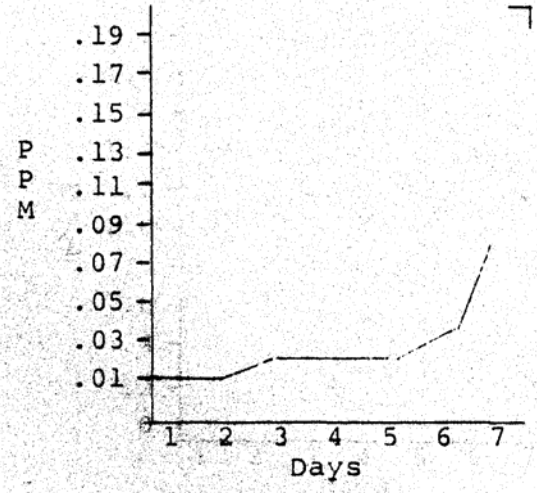


Figure B7. Phosphate Levels in E_2 Tank

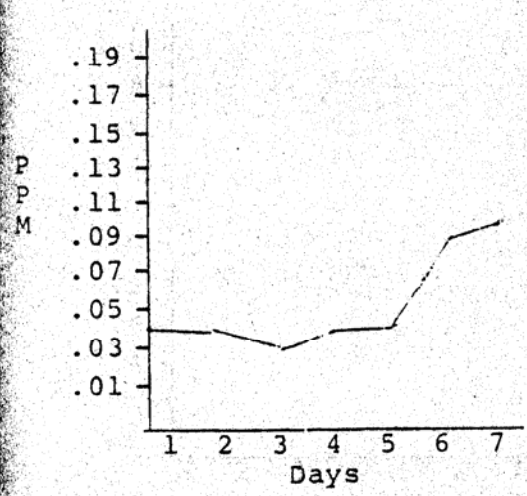


Figure B8. Phosphate Levels in E_3 Tank

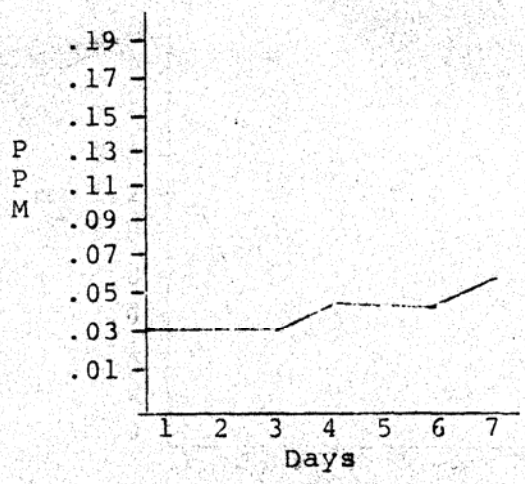


Figure B9. Phosphate Levels in E_4 Tank

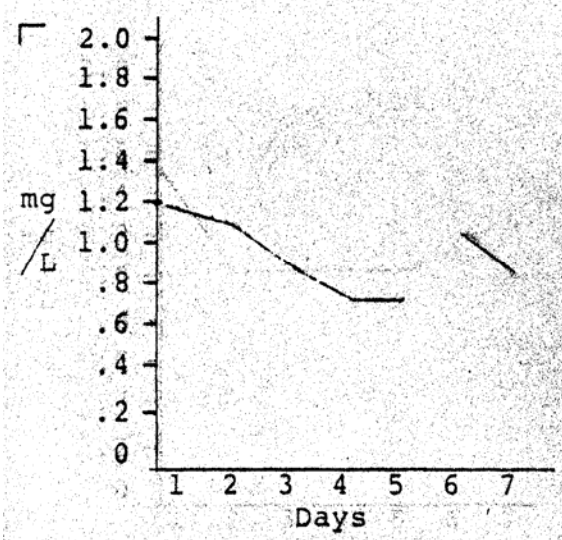


Figure B10. Nitrate Levels in C_1 Tank

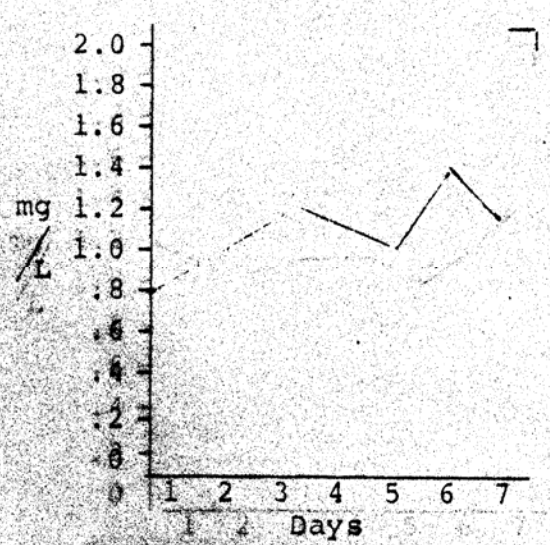


Figure B11. Nitrate Levels in C_2 Tank

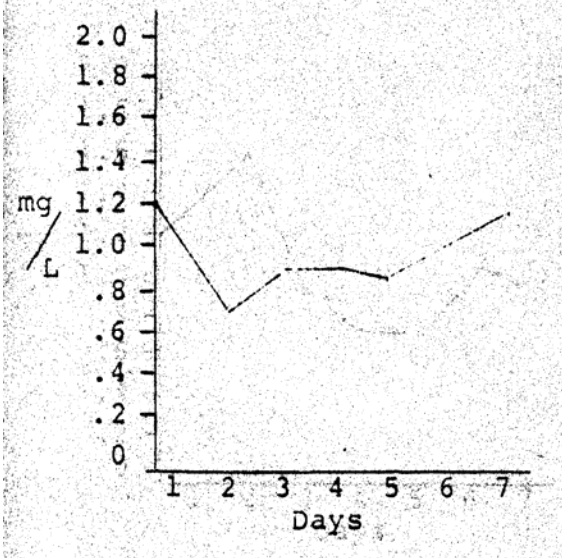


Figure B12. Nitrate Levels in C_3 Tank

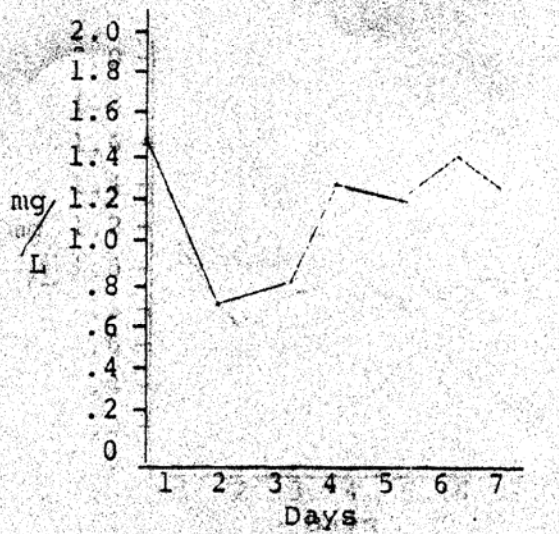


Figure B13. Nitrate Levels in C_4 Tank

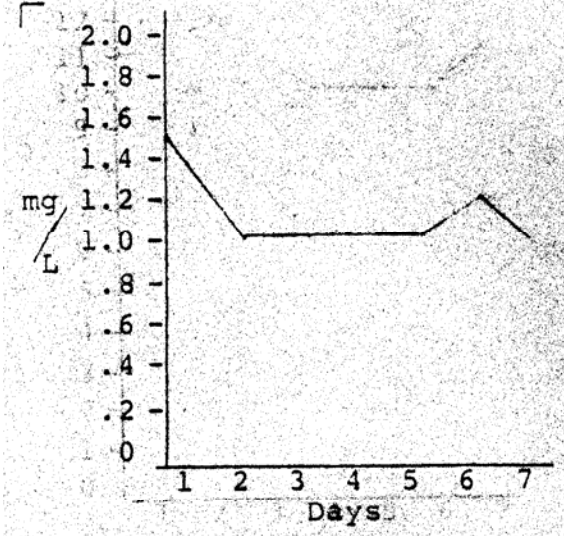


Figure B14. Nitrate Levels in E_1 Tank

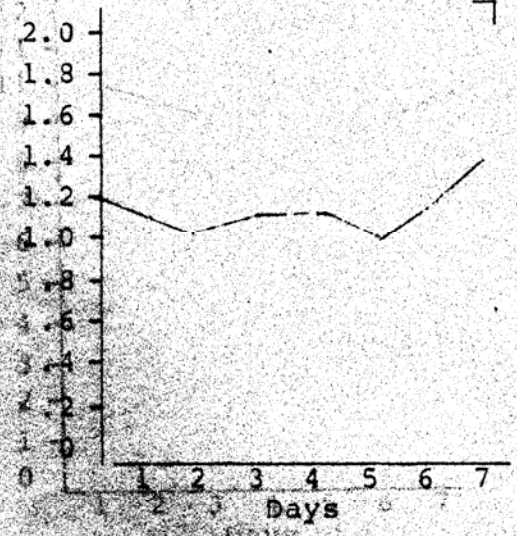


Figure B15. Nitrate Levels in E_2 Tank

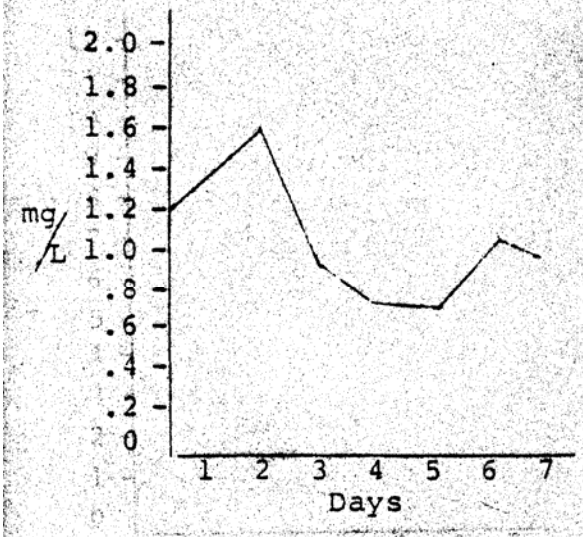


Figure B16. Nitrate Levels in E_3 Tank

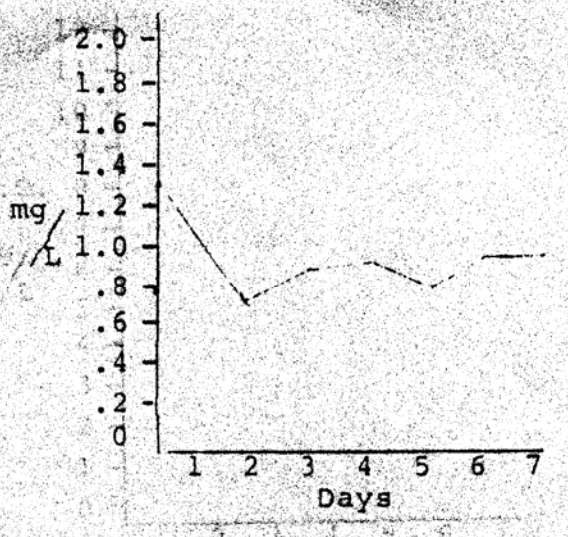
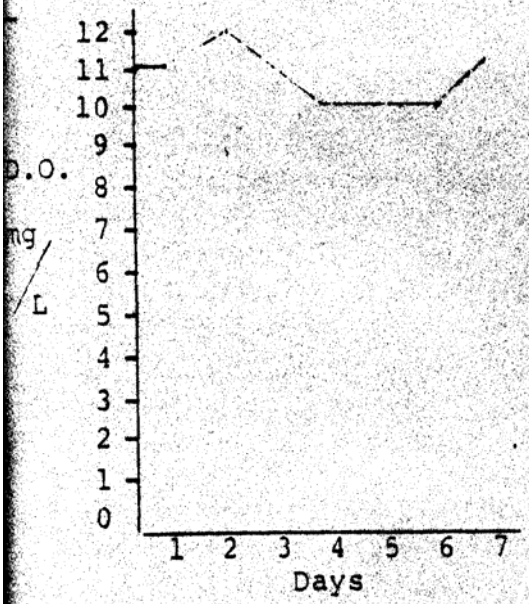
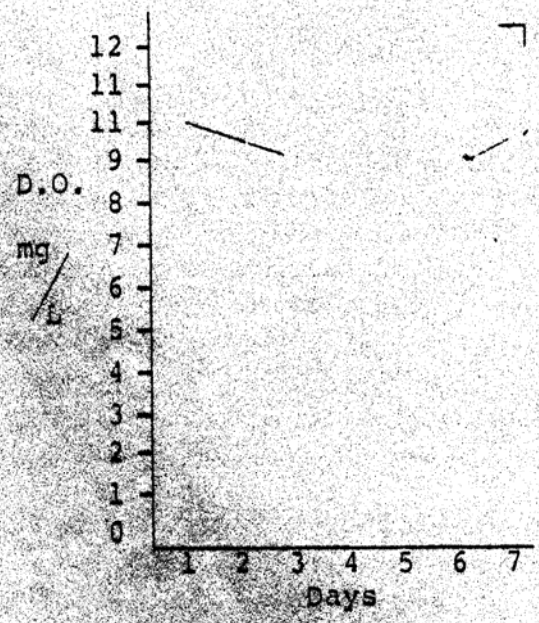


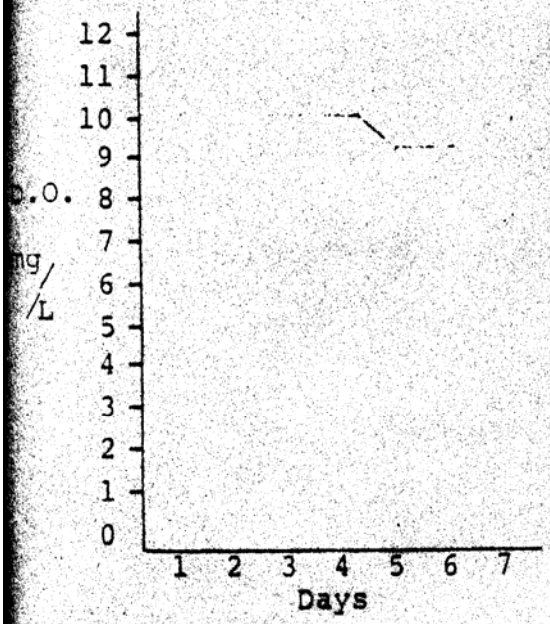
Figure B17. Nitrate Levels in E_4 Tank



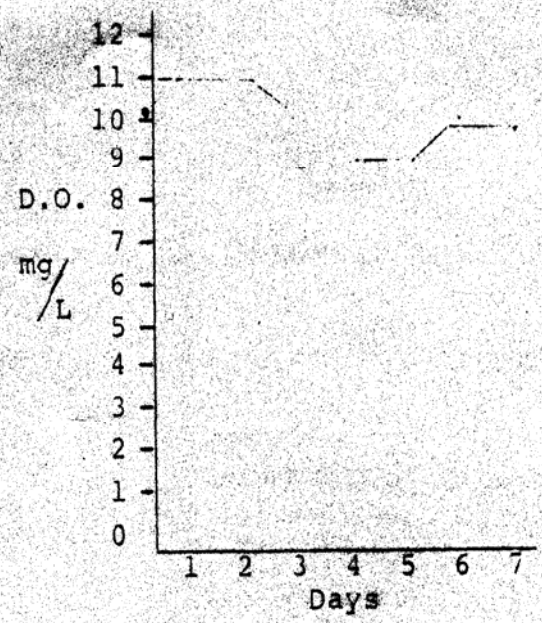
FigureB18. Dissolved O_2 in C_1 Tank



FigureB19. Dissolved O_2 in C_2 Tank



FigureB20. Dissolved O_2 in C_3 Tank



FigureB21. Dissolved O_2 in C_4 Tank

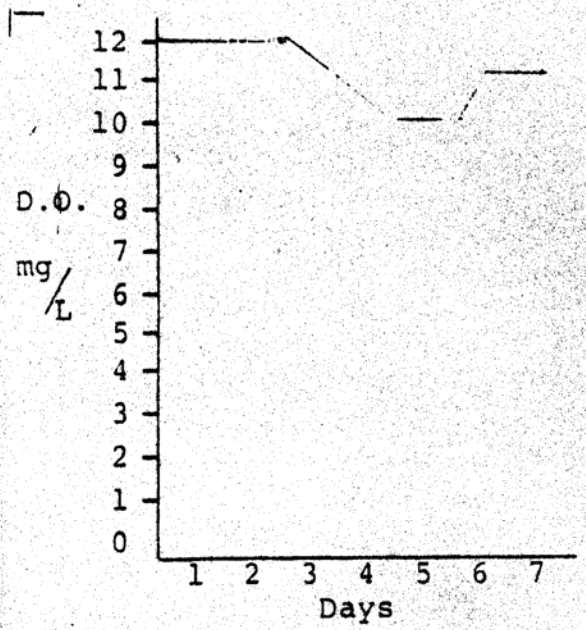


Figure B22. Dissolved O_2 in E_1 Tank

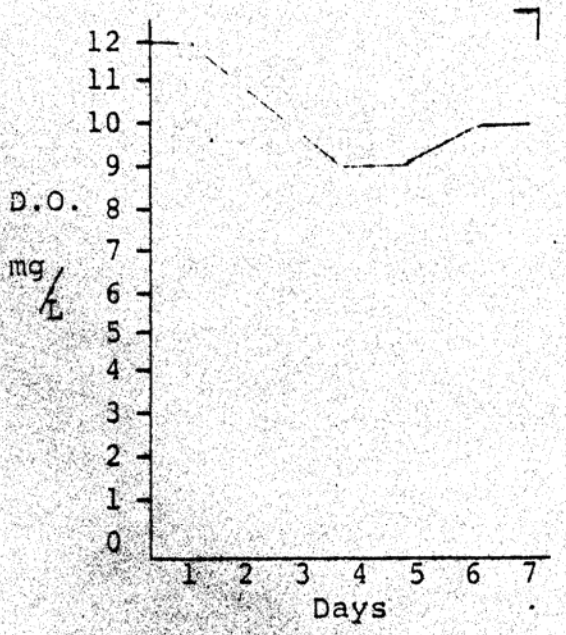


Figure B23. Dissolved O_2 in E_2 Tank

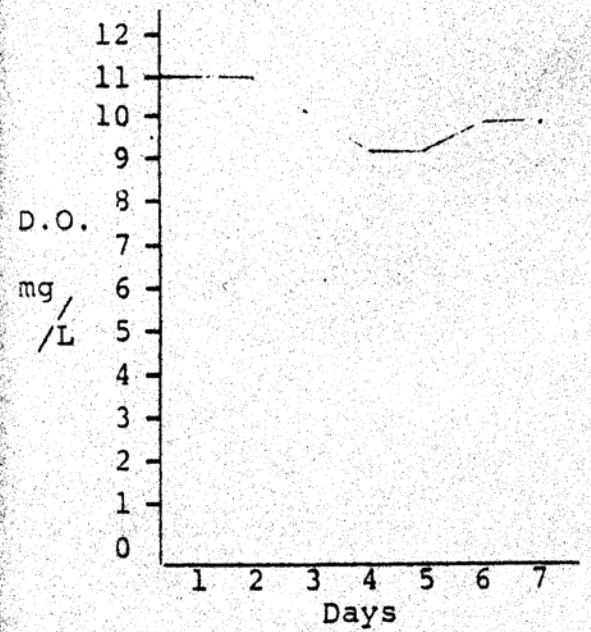


Figure B24. Dissolved O_2 in E_3 Tank

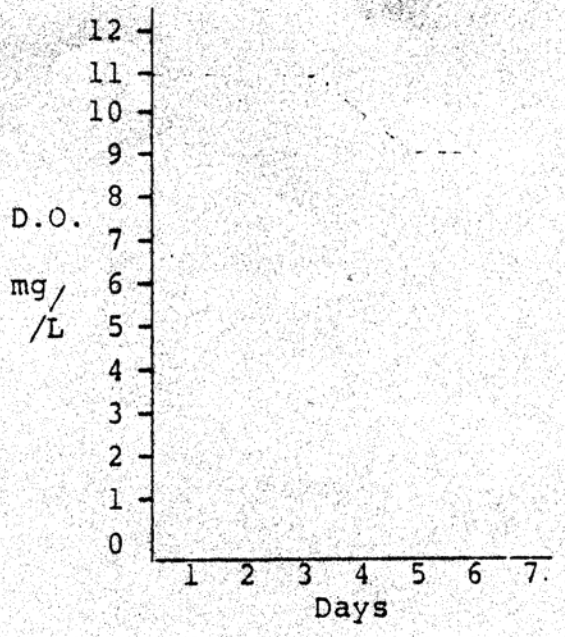


Figure B25. Dissolved O_2 in E_4 Tank

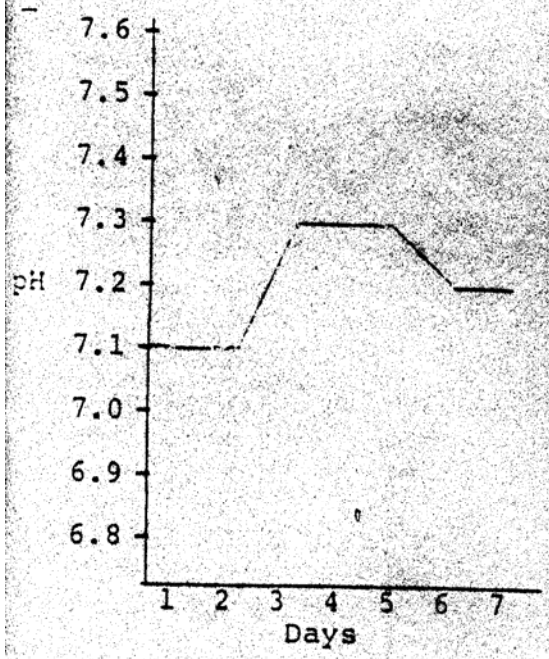


Figure B26. PH of C_1 Tank

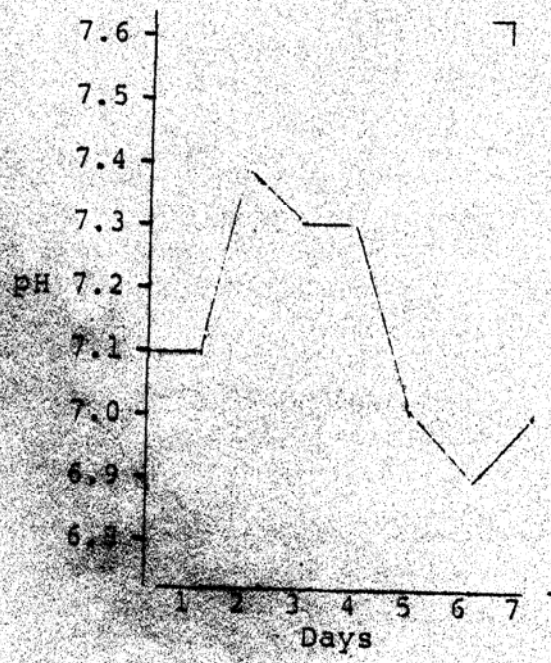


Figure B27. PH of C_2 Tank

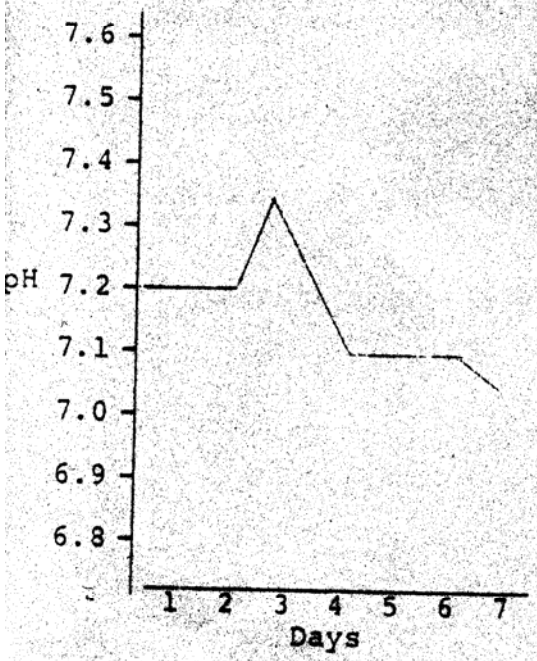


Figure B28. pH of C_3 Tank

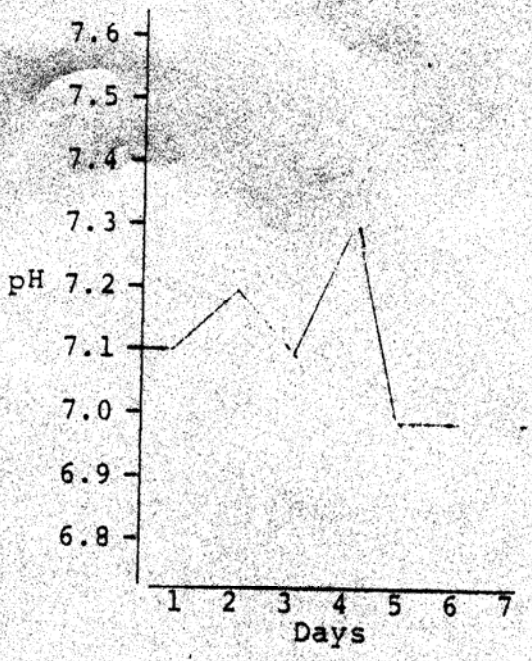


Figure B29. pH of C_4 Tank

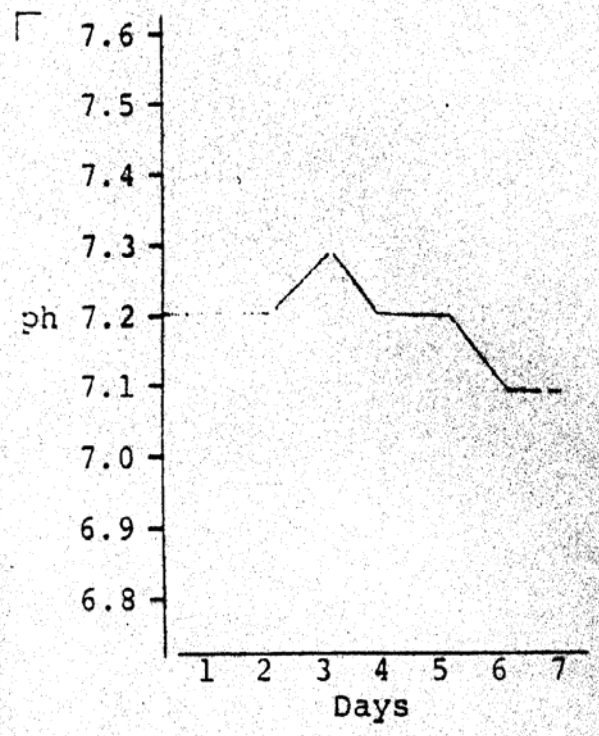


Figure B30. pH of E_1 Tank

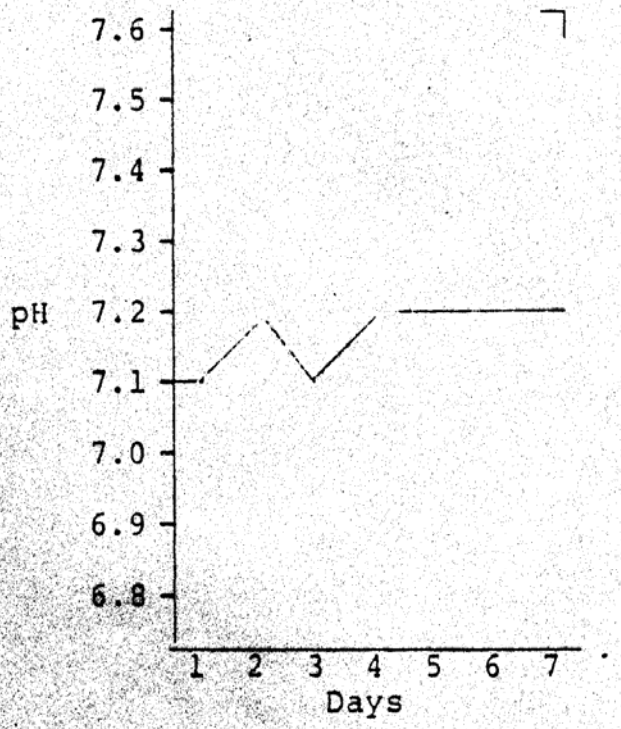


Figure B31. pH of E_2 Tank

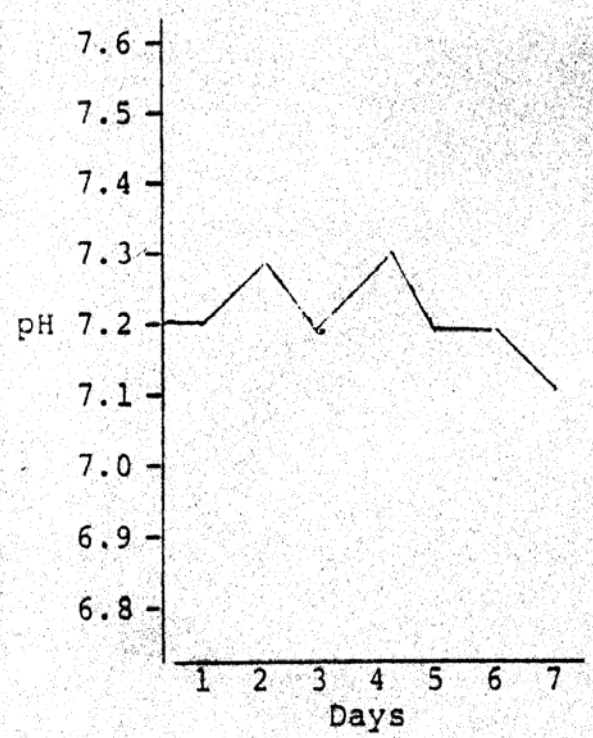


Figure B32. pH of E_3 Tank

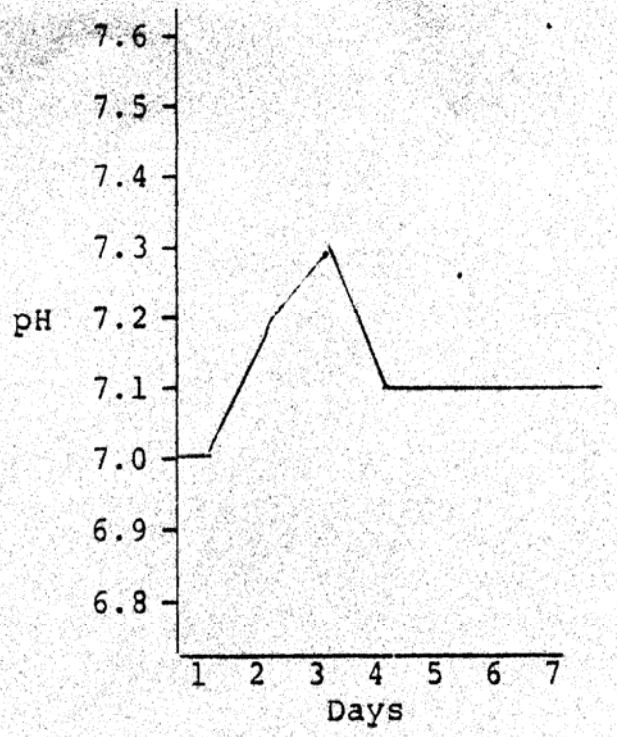


Figure B33. pH of E_4 Tank

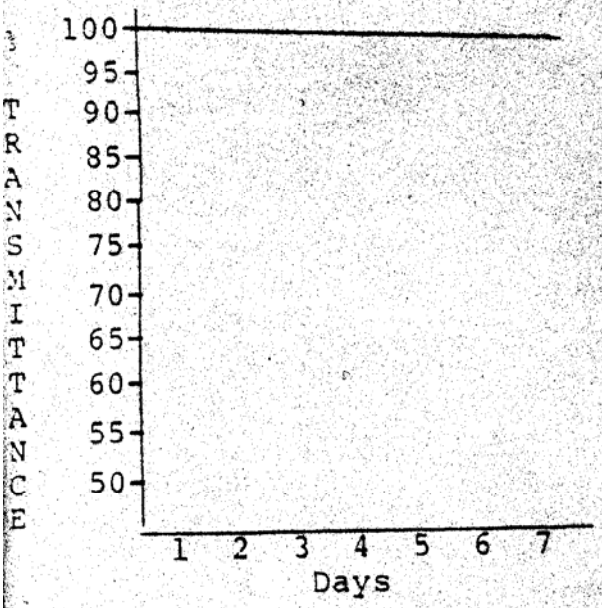


Figure B34. Spectrometric Readings for C_1 Tank

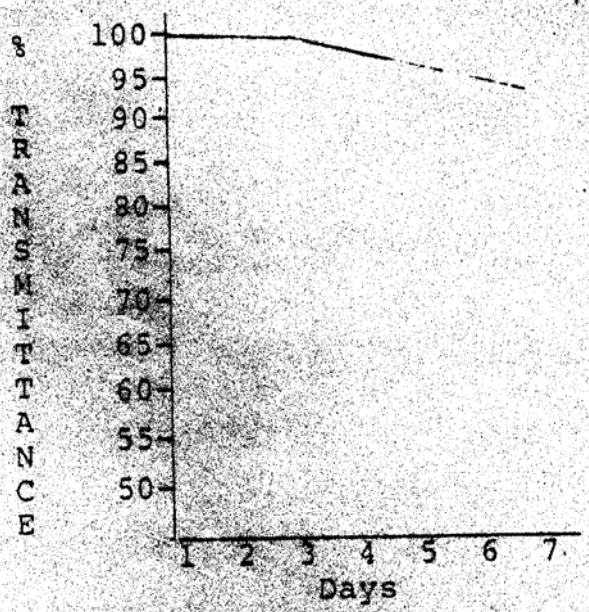


Figure B35. Spectrometric Readings for C_2 Tank

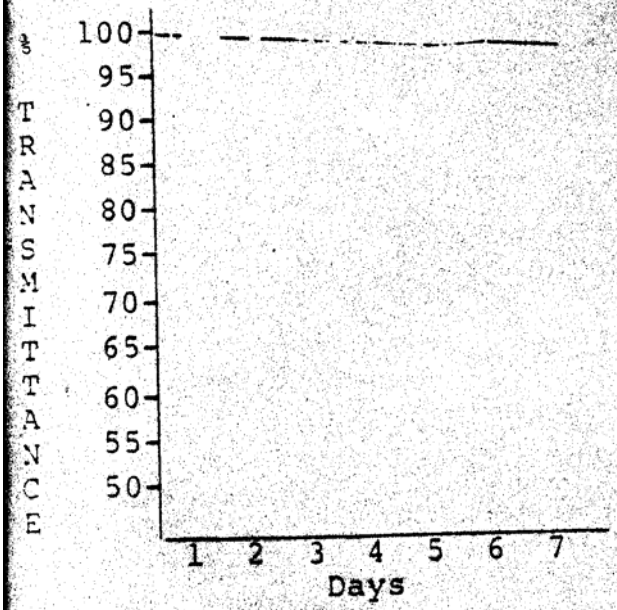


Figure B36. Spectrometric Readings for C_1 Tank

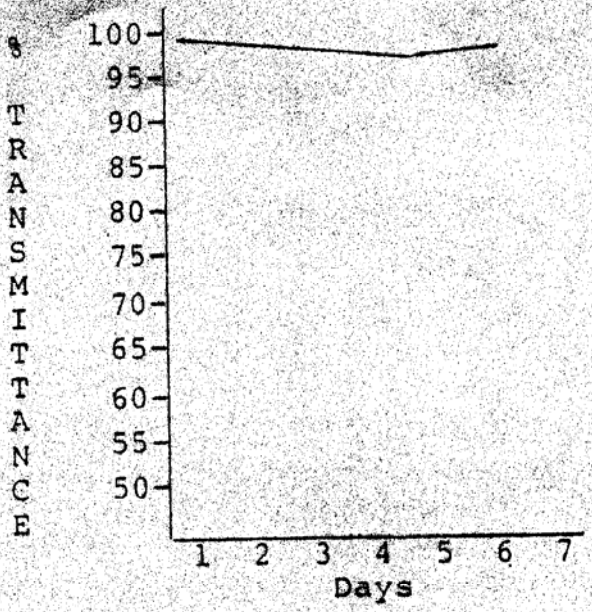


Figure B37. Spectrometric Readings for C_4 Tank

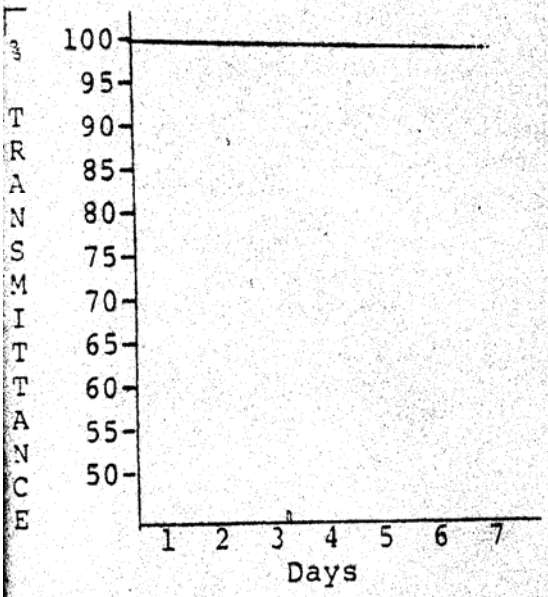


Figure B38. Spectrometric Readings for E_1 Tank

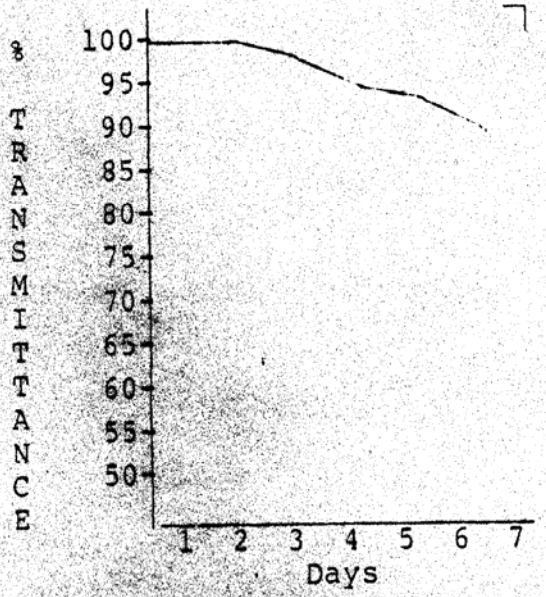


Figure B39. Spectrometric Readings for E_2 Tank

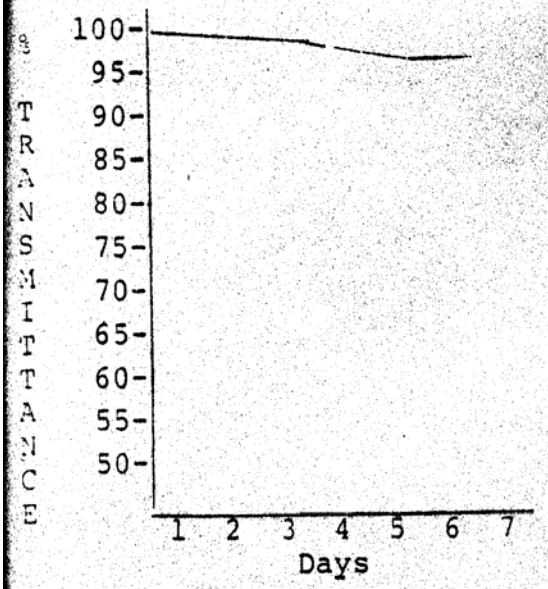


Figure B40. Spectrometric Readings for E_3 Tank

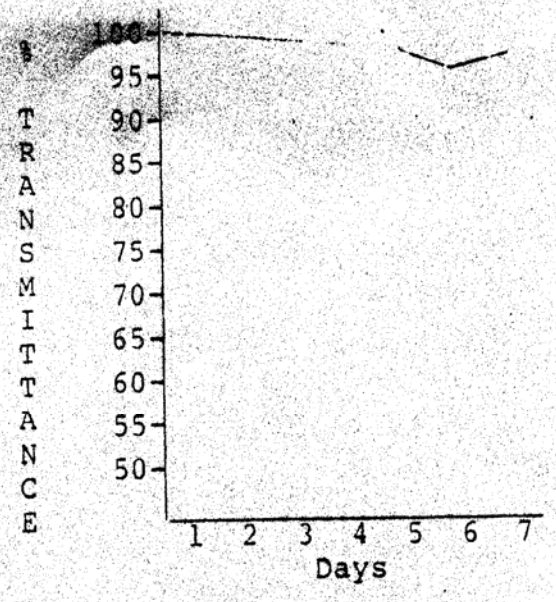


Figure B41. Spectrometric Readings of E_4 Tank

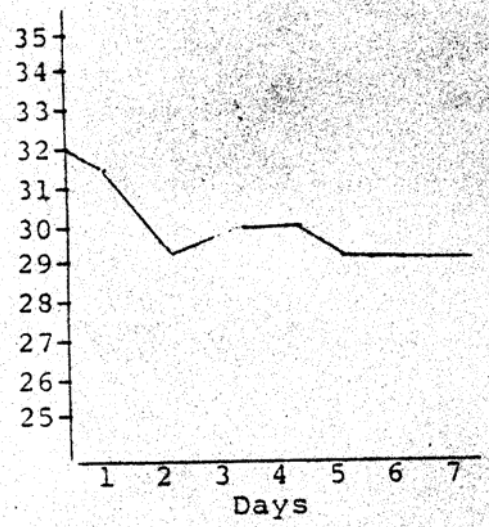


Figure B42. C_1 Tank Temp.

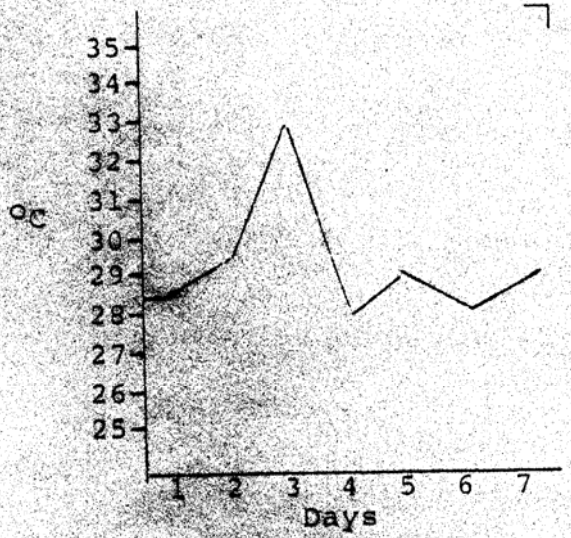


Figure B43. C_2 Tank Temp.

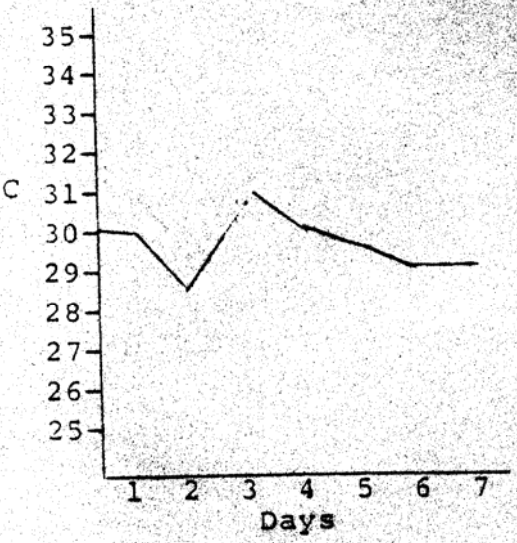


Figure B44. C_3 Tank Temp.

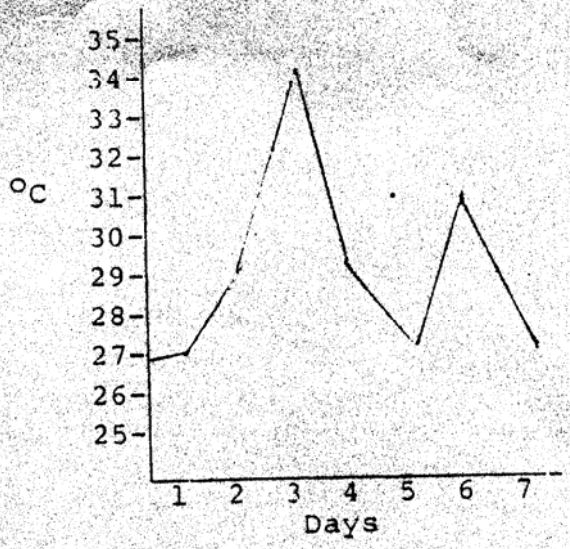


Figure B45. C_4 Tank Temp.

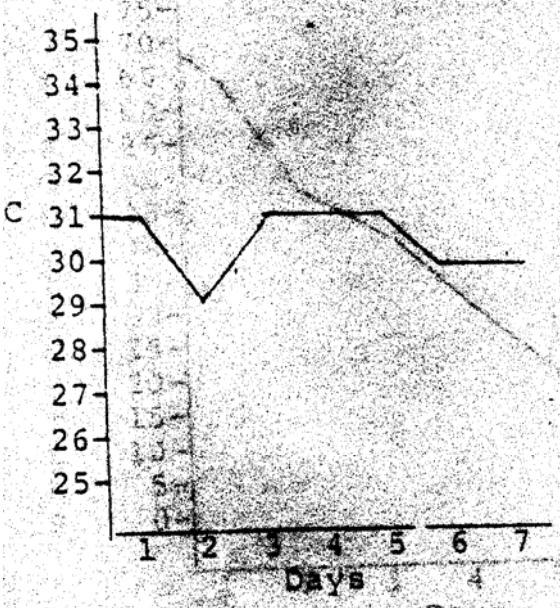


Figure B46. Tank Temp E_1

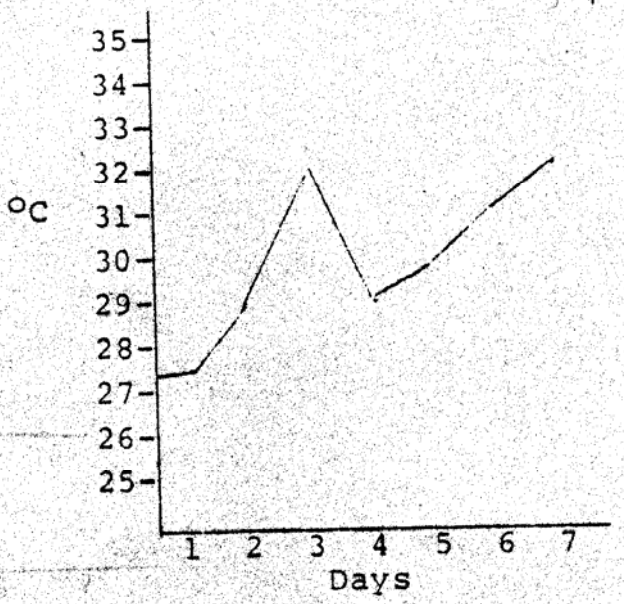


Figure B47. Tank Temp E_2

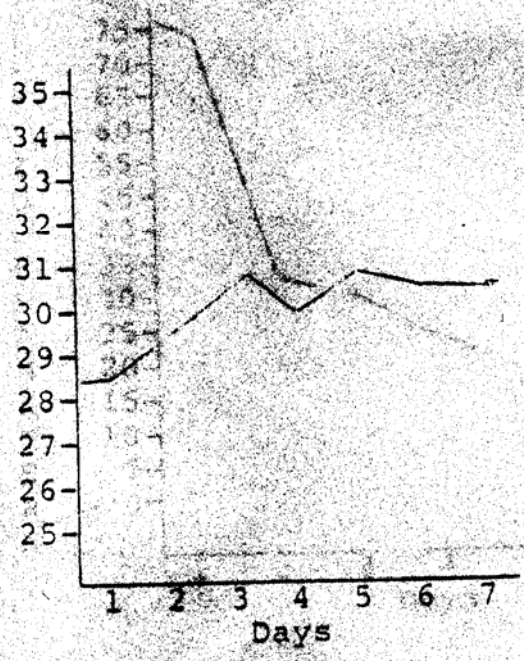


Figure B48. Tank Temp E_3

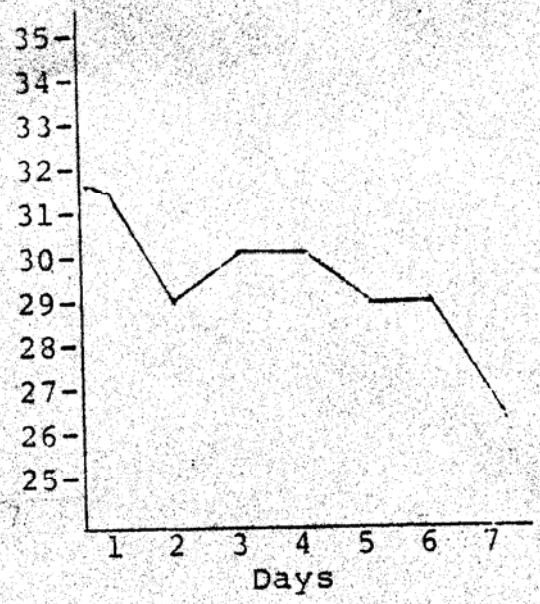


Figure B49. Tank Temp E_4

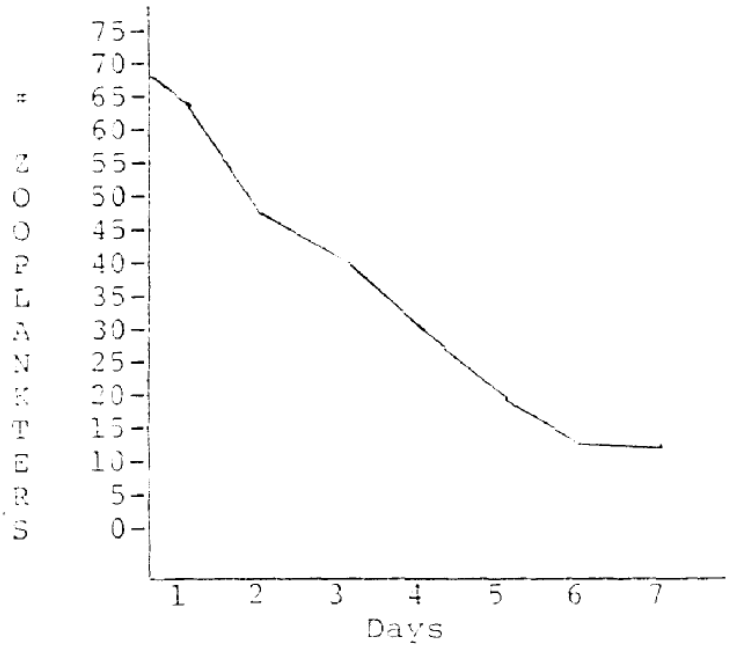


Figure 50. Relative Amounts of Zooplanters in Tank C_1

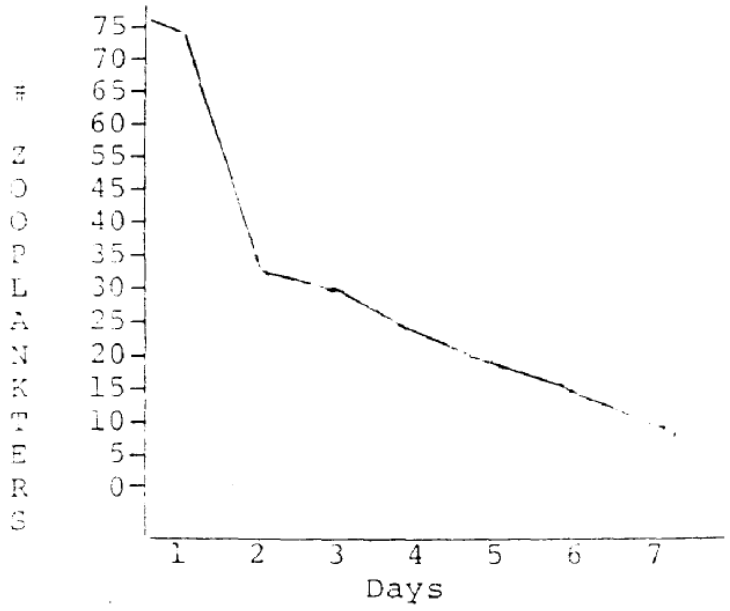


Figure 51. Relative Amounts of Zooplankters in Tank C_2

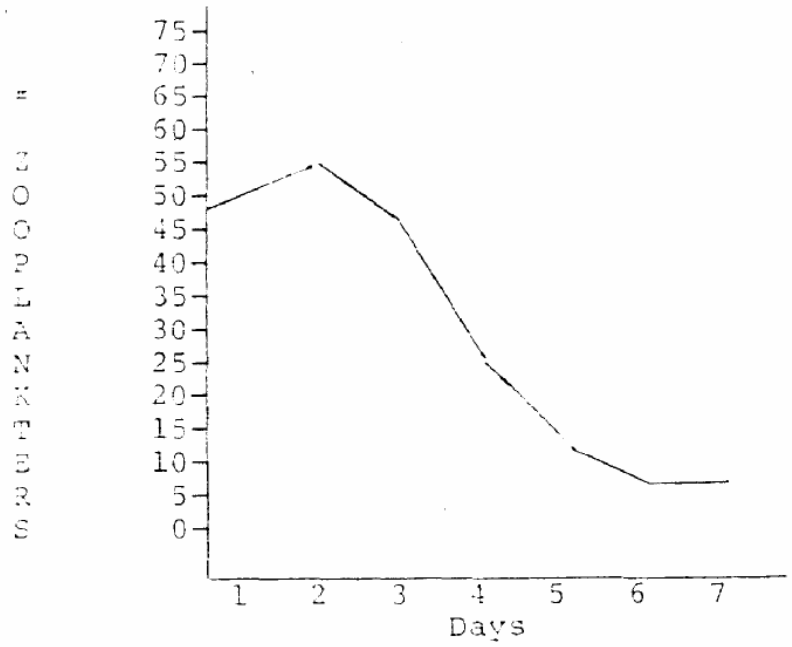


Figure 52. Relative Amounts of Zooplankters in Tank C_3

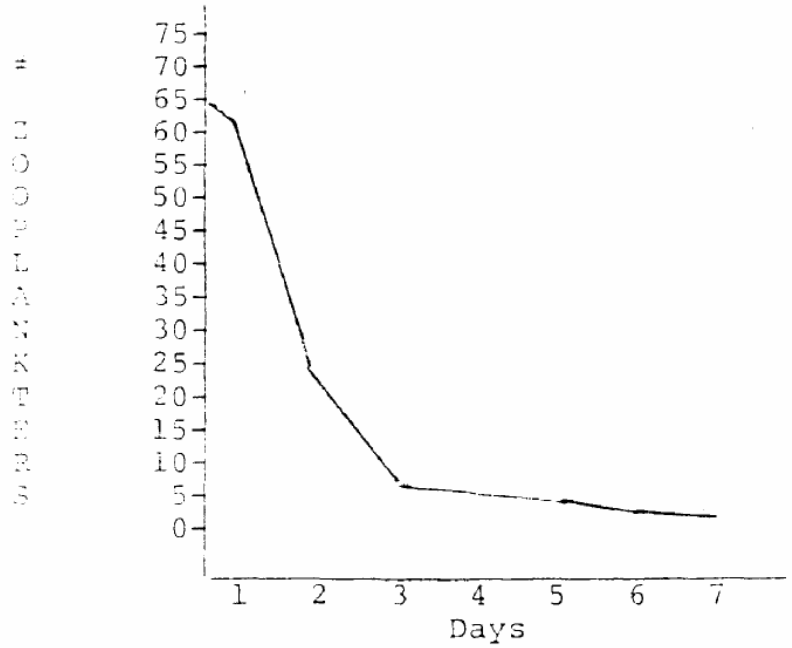


Figure 53. Relative Amounts of Zooplankters in Tank C_4

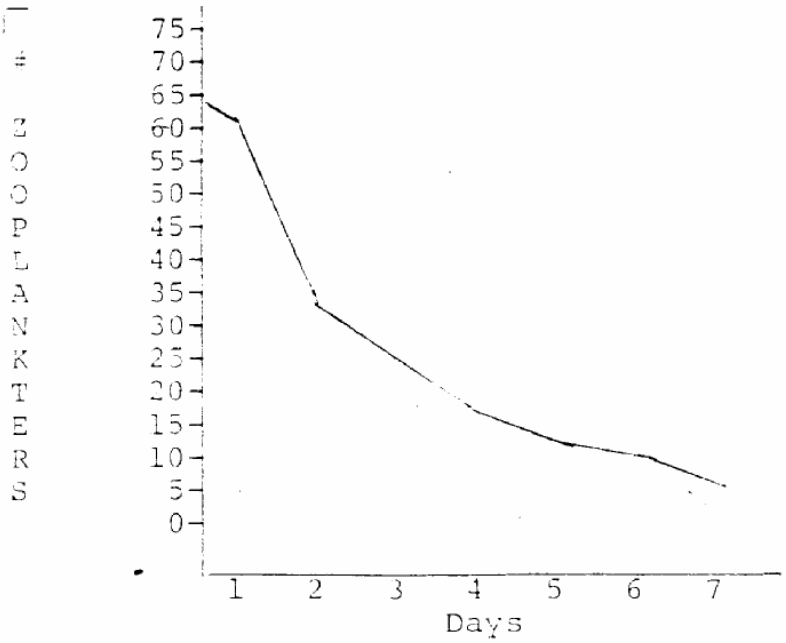


Figure 54. Relative Amounts of Zooplankters in Tank E_1

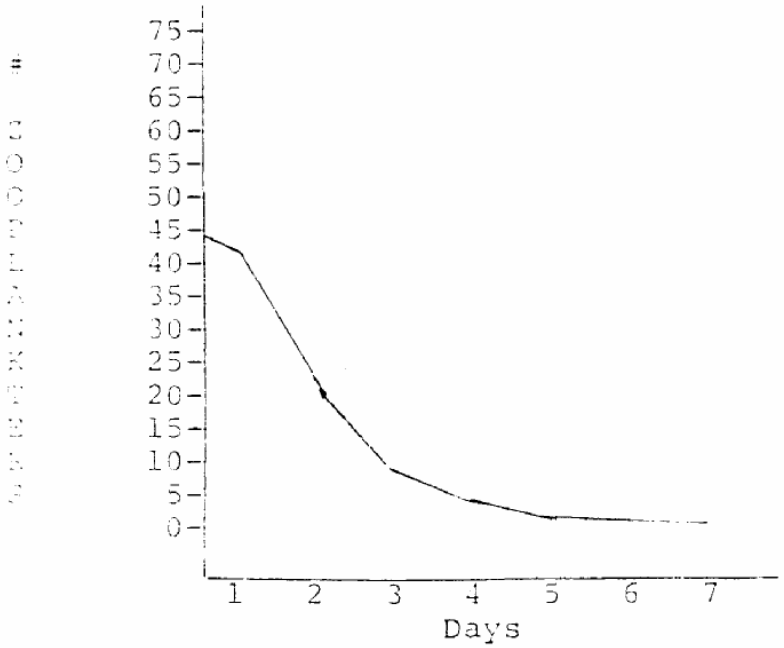


Figure 55. Relative Amounts of Zooplankters in Tank E_2

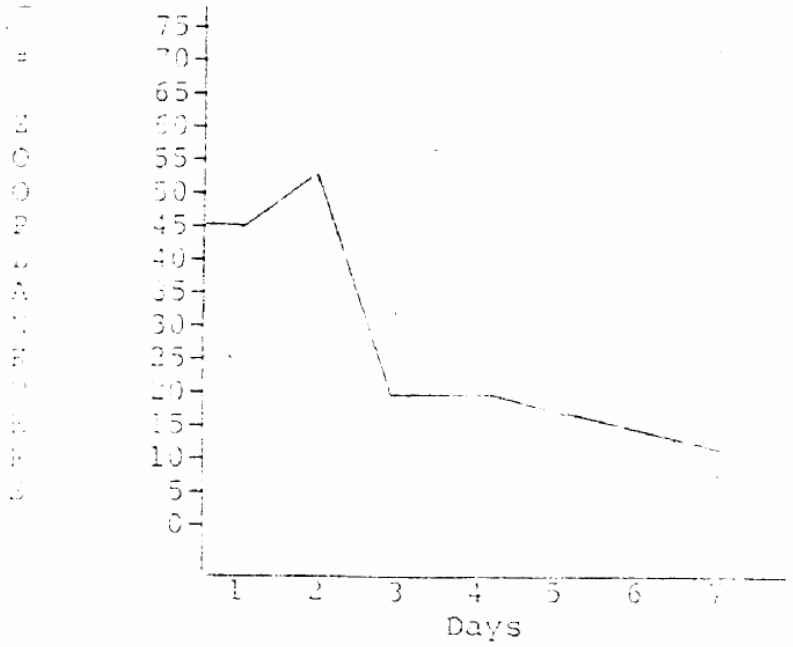


Figure 56. Relative Amounts of Zooplankters in Tank E_3

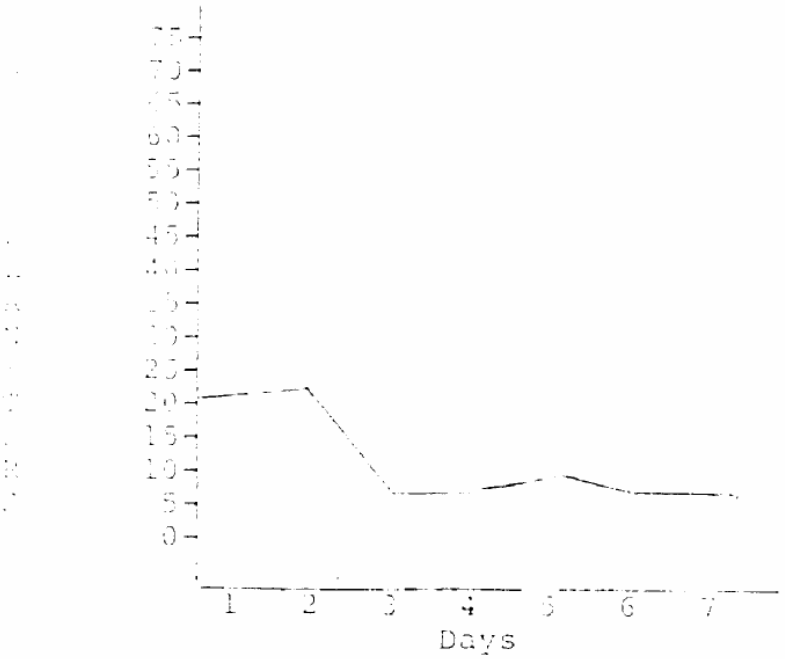


Figure 57. Relative Amounts of Zooplankters in Tank E_4