ESTIMATION OF BOTTOM AREA IN AN ARTIFICIAL STREAM, USING PHOSPHORUS-32

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
JOHN PAUL GIESY JR.
1971

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ESTIMATION OF BOTTOM AREA IN AN ARTIFICIAL STREAM, USING PHOSPHORUS-32

by

JOHN PAUL GIESY jr.

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
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1971

ABSTRACT

An epilithic algal community, composed mainly of diatoms, was allowed to colonize a laboratory stream, 21.9 meters in length. A phosphorus-32 tracer pulse of 7.72×10^7 cpm was added in an attempt to determine the colonizable surface area of the stream by ^{32}P sorption.

Glass substrata placed in the stream removed an average of 1677.7 cpm or 87.38 cpm per cm^2. Although the weight of algae on the artificial substrata varied from one part of the stream to another, the activity collected by the substrata did not vary significantly. This indicated that the stream bottom was uniformly covered and that the ^{32}P sorption was a surface phenomenon, not effected by the standing crop of algae, during a short contact time in the lotic system.

Of the 7.72×10^7 cpm of ^{32}P added, only 6.09×10^6 cpm or approximately 12.5% of the initial activity was removed. This coupled with a large amount of variation between the water samples taken from the stream, made it impossible to calculate an accurate change in activity from the beginning to the end of the stream. To be able to calculate a change in activity and hence the surface area, less activity must be used in any further experiments.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Productivity and standing crop of aquatic systems have always been important limnological parameters, receiving much attention. Traditionally, periphyton has been measured in terms of weight of algae per unit area because there was no way to determine the total colonizable bottom surface area of lotic systems. This relative measure is useless when calculating total production or total available biomass.

A rapid method for determining the bottom surface area and standing crop of small rocky streams, using ^{32}P, proposed by Nelson et al. (1969) found that the colonized bottom area was more than four times as great as expected from calculation from surface measurements.

Studies by Reigler (1956) showed that ^{32}P is rapidly taken up by the algae. It has also been demonstrated that ^{32}P did not occur in higher trophic levels until several days after its addition to aquatic systems (Ball and Hooper, 1961; Davis and Foster, 1958).

It has also been found that only a small amount of ^{32}P is sorbed into sediments and detritus (Nelson et al., 1969).

The rapid uptake of tagged phosphorus, especially in lotic systems where phosphorus is limiting, and the fact that little activity is lost initially to anything other than the epilithic algae, makes ^{32}P sorption a very attractive method for determining the amount of bottom area colonized by epilithic

algae.

Autoradiography of ^{65}Zn sorption by an epilithic algal growth has shown that nutrient uptake in a flowing system with short contact time is a surface phenomena, involving only the upper surface of the algal mat (Rose and Cushing, 1970).

The object of this investigation was to devise a method to determine the colonized bottom surface area of a laboratory stream with a known bottom surface area.

ARTIFICIAL STREAM

Artificial laboratory streams have been used for controlled studies of various stream parameters. Artificial streams have been used to study primary productivity (McIntire et al., 1964; McIntire and Phinney, 1965 and Kevern and Ball, 1965). Artificial streams have also been used to study metabolism of a microcosm (Odum and Hoskins, 1957) and interactions among stream organisms (Whitford et al., 1964). Modeling of aquatic populations has been done using artificial streams (Lauff and Cummins, 1964). Artificial streams have even been used as a hatchery for insect specimens (Feldmeth, 1970).

Artificial streams have been built in a variety of forms to meet the requirements of many types of experiments. Because of the need for a relatively long stream to allow for phosphorus sorption, the stream used in this study was much longer than most previous artificial streams.

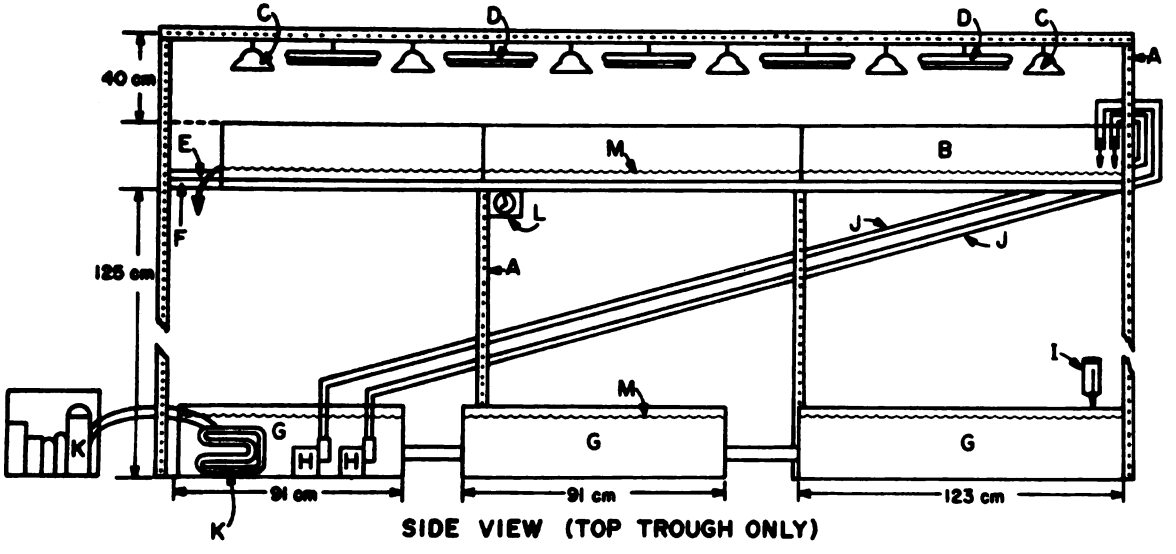
Stream bed. The stream was constructed as six, galvanized steel troughs, 3.657 m long, 20.32 cm wide and 12.70 cm deep (Table 1). These troughs were supported on an angle iron frame such that water from one trough flowed over a 1.91 cm baffle and dropped 17.78 cm into the next trough (Figures 1 and 2). To conserve space the water flow was alternated from right to left from one trough to the next, forming a total stream length of 21.94 m.

The inside of each trough was lined with heavy, black polyethylene with 1218 plexiglas cylinders each 1.9 cm high

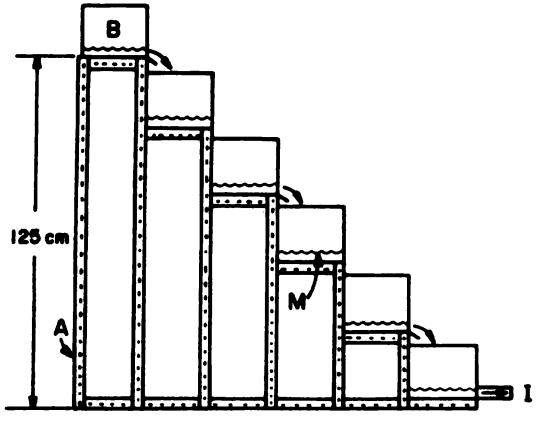
Table 1. Artificial stream dimensions and capacities.

STREAM SURFACE AVAILABLE FOR COLONIZATION	
Bottom area	44,600 cm^2
End area	155 "
Side area	13,600 "
Inside of baffles	387 "
Outside of baffles	619 "
	59,400 cm^2
Area of Plexiglas cylinders	10,200 cm^2
TOTAL surface area available for colonization	69,600 cm^2
Trough length	3.65 m
Trough width	12.70 cm
Total length	21.94 m
Water depth	3.18 cm
Cross sectional area of water in stream	40.39 cm^2
Water velocity	2.69 m/min
Rate of discharge	20.00 liter/min
Reservoir volume	135.8 liters
Stream volume	458.6 liters
Total volume	594.6 liters

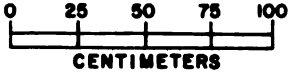
Figure 1. Diagram of artificial stream.



SIDE VIEW (TOP TROUGH ONLY)



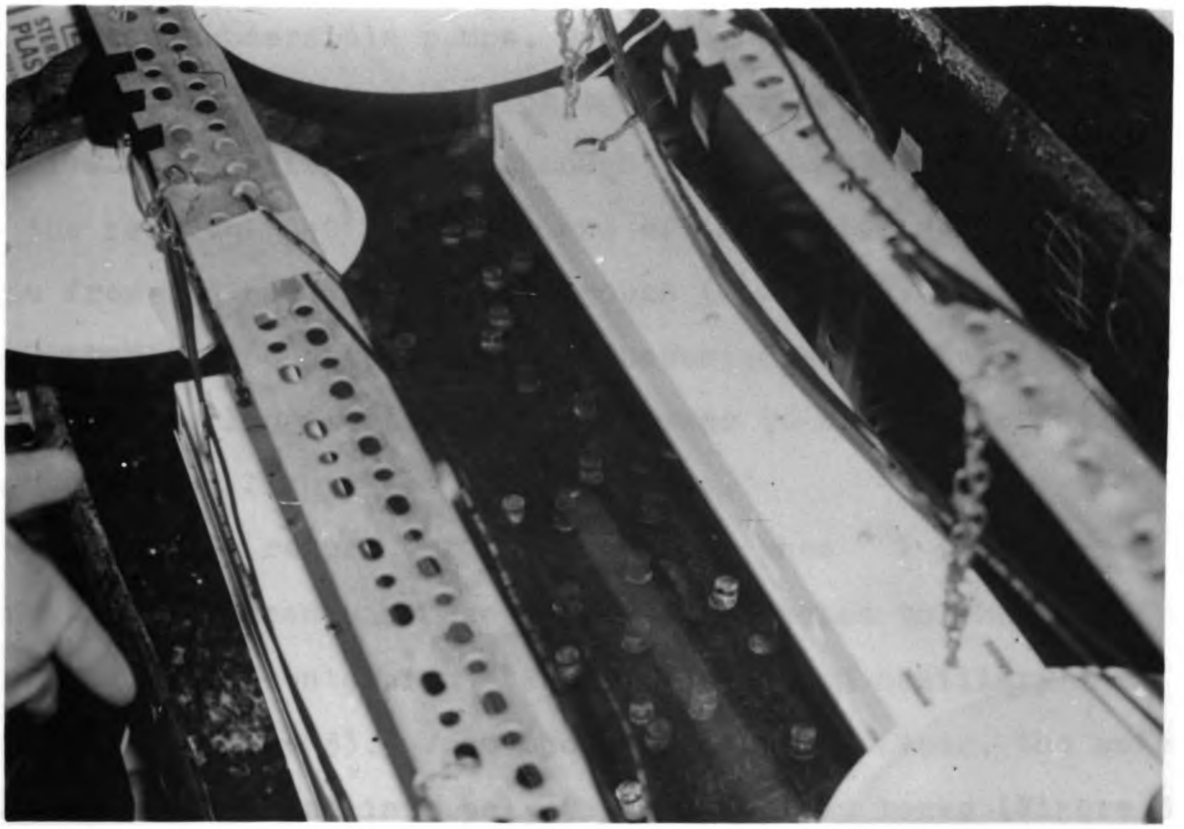
END VIEW



A. Frame
B. Trough
C. Incandescent Bulb
D. Flourescent Bulb
E. Baffle
F. Lip
G. Reservoir
H. Pump
I. Return Pipe
J. Inlet Pipe
K. Cooler
L. Timer
M. Water

Figure 2. Photographs of artificial stream: (a) complete artificial stream; (b) inside of stream trough, showing plastic lining and vertical cylinders.

a



b

and 0.635 cm in daimeter, placed semirandomly on the stream bottom to increase the surface area and provide some vertical surface for algal colonization (Figure 2b).

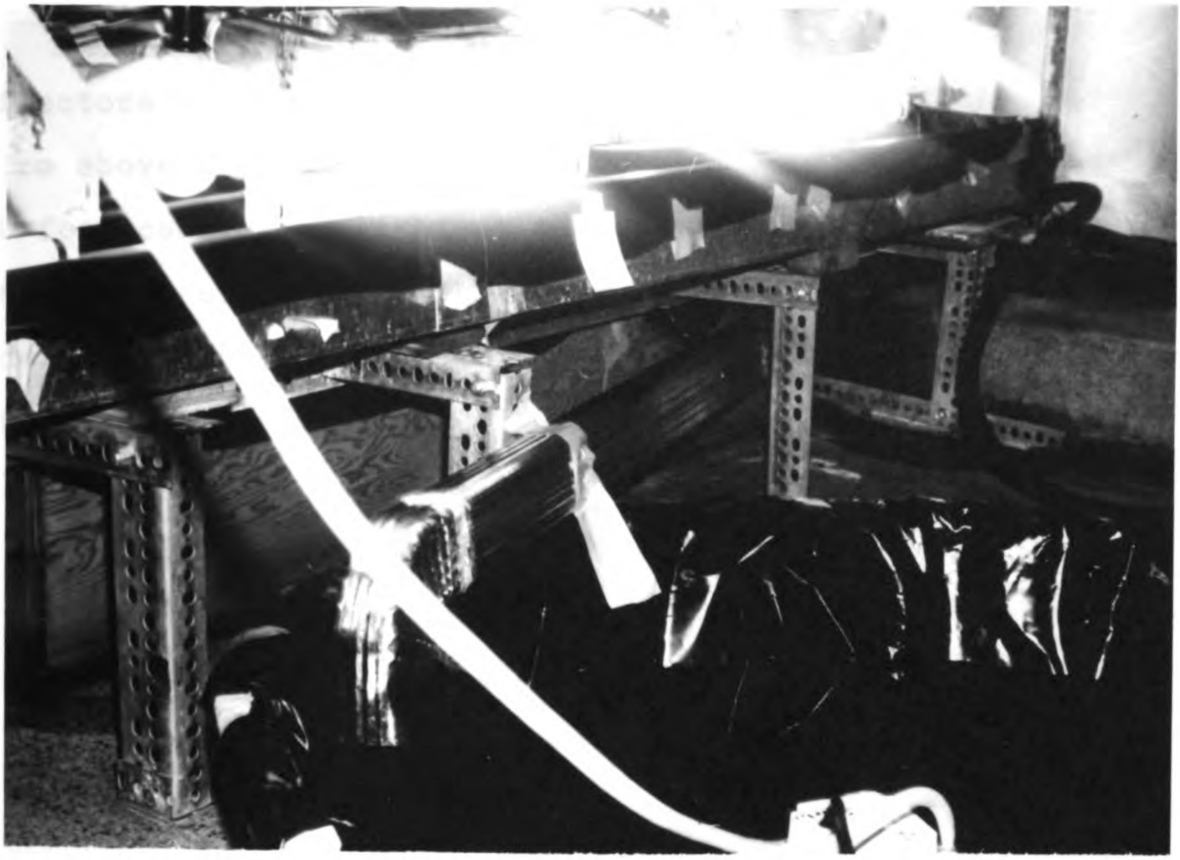
Reservoirs. The stream was a recirculating system, with water drawn from and returning to a series of three interconnected, wooden reservoirs with a total capacity of 141.2 liters. All three of the reservoir cells were sealed with epoxy paint and allowed to dry for one week. After drying, the reservoirs were flushed with running water for a day before being placed in the stream system. The reservoir cells were placed under the stream bed supports, connected with PCV pipe and covered with black polyethylene to reduce evaporational loss and keep the water from being contaminated with zinc from the water that condensed on the underside of the troughs.

Using submersible pumps, water was pumped from one reservoir through tygon tubing to the stream surface (Figure 1). After circulating through the stream, water returned to the reservoir at the other end of the series via a return pipe from the end of the last trough (Figure 1). This kept the water in the reservoirs from becoming stagnant while allowing the longest time for the water to cool, after being warmed by the lights above the stream.

The water return was built so that when ^{32}P was run through the stream a bypass could be connected to the exit pipe and water containing ^{32}P shunted into an auxiliary reservoir (Figure 3). From the auxiliary reservoir, the water and ^{32}P were pumped into polyethylene holding tanks (Figure 3b).

Figure 3. Photographs of the apparatus for catching ^{32}P after it passed through the stream: (a) auxiliary reservoir and pump; (b) polyethylene holding tanks.

a



b

Lighting. Six, 60 watt incandescent light bulbs with reflectors and five fluorescent lamps were placed alternately 21 cm above the water in each trough, providing approximately 380 foot candles of light at the water surface. Light intensity was slightly higher under the incandescent lamps than under the fluorescents. Incandescent bulbs were used in conjunction with grow-lux fluorescent bulbs to provide a balanced spectrum of light.

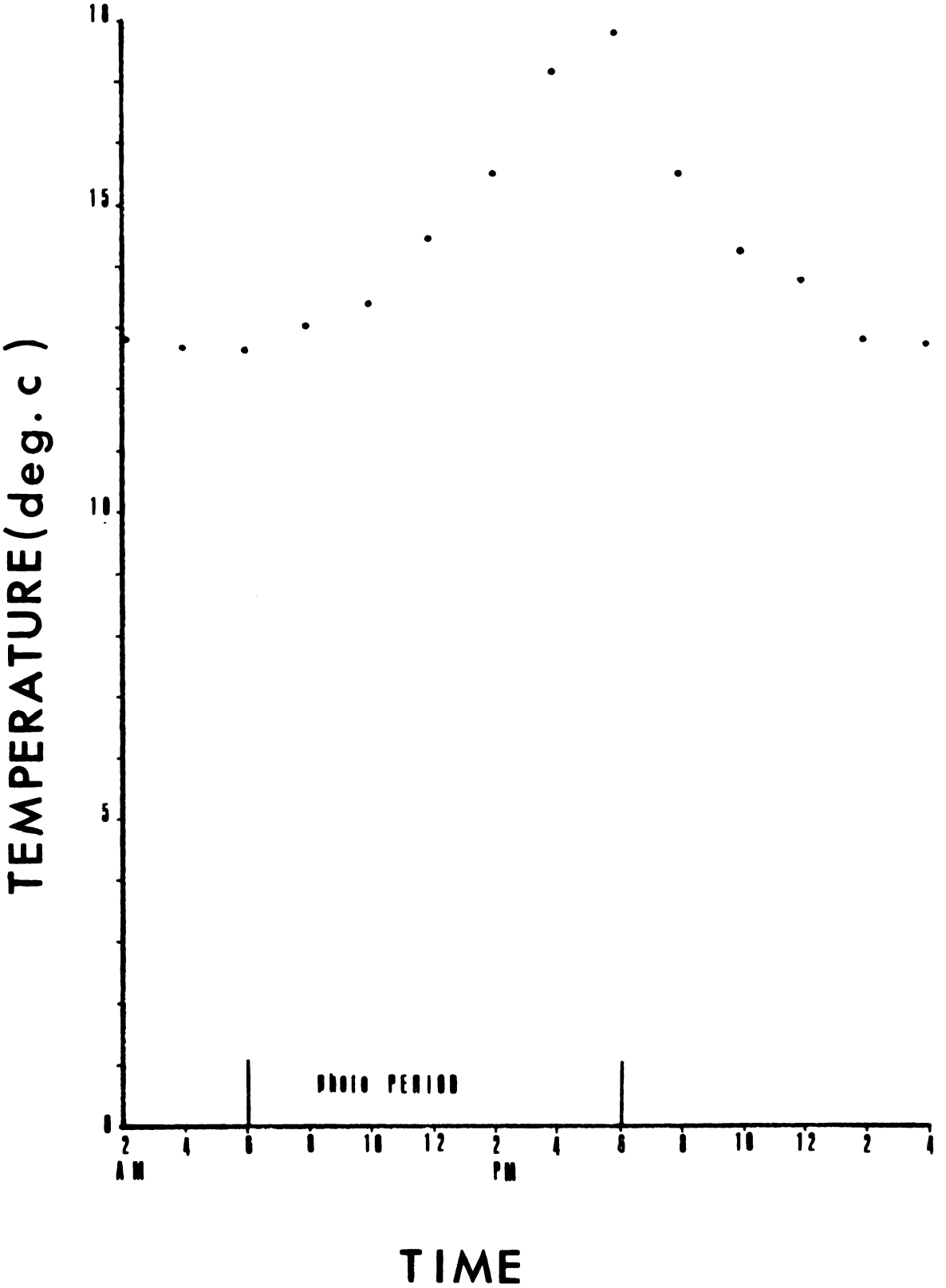
The 12-hour photoperiod, controlled by timers, was from 6:00 A.M. to 6:00 P.M. The fluorescent room lights were on constantly, resulting in a constant light intensity of 30 foot candles at the water surface during the time the stream lights were off.

Temperature control. Water temperature was reduced to between 12.5 C and 18.0 C with a cooling coil submerged in the reservoir. There was a diurnal temperature fluction of about eight degrees, due to heat input from the lights during the twelve hour photoperiod (Figure 4). The temperature cycle was very constant over the two months preceding experimentation. The temperature, measured at 2:00 P.M. each day, was constant at 15.5 C.

Water and nutrient medium. The stream was initially filled with distilled water and distilled water was used to replace water lost by evaporation.

Nutrients were added according to the Chu #10 medium, modified by Roche as described by Prescott (1951). Micronutrients not contained in the modified Chu #10 medium were

Figure 4. Diurnal temperature fluctuation of stream water.


TEMPERATURE(deg. c)
20
15
10
5
0
PHOTO PERIOD
2
4
6
8
10
12
2
4
6
8
10
12
2
4
AM
PM
TIME

added after Bonner (1952). EDTA (Ethylenidiaminetetraacetic acid) was added to keep the cationic species in solution by forming complex ions. Vitamin B^{12} and biotin were added because these are the most frequently needed organic compounds for growth of alloautotrophic algae.

Appropriate amounts of each nutrient were added initially to make up the entire system to the concentrations required by the combined media (Table 2). During the two months prior to experimentation, biotin and vitamin B^{12} were added to the stream. An additional 100 g of $Na(HCO_3)_2$ was also added.

It was not necessary to add nutrients during the two months prior to the tracer studies because algae was continually being sloughed off and decomposing at the bottom of the reservoir so that nutrients were recycled (Kevern and Ball, 1965).

Alkalinity. Total, phenolphthalein and methyl orange alkalinity were determined titrimetrically following standard methods (APHA, 1971). The alkalinity decreased slowly from the time the initial nutrients were added until the time of the tracer experiments (Figure 5).

Hardness. Total hardness, measured by EDTA titration, using erichrome black-T as indicator, was found to be constant at 20 ppm.

The hardness was kept low on purpose to reduce interference in determinations of the activity of water samples. The EDTA used to keep cationic nutrients in solution may have also reduced the measured hardness.

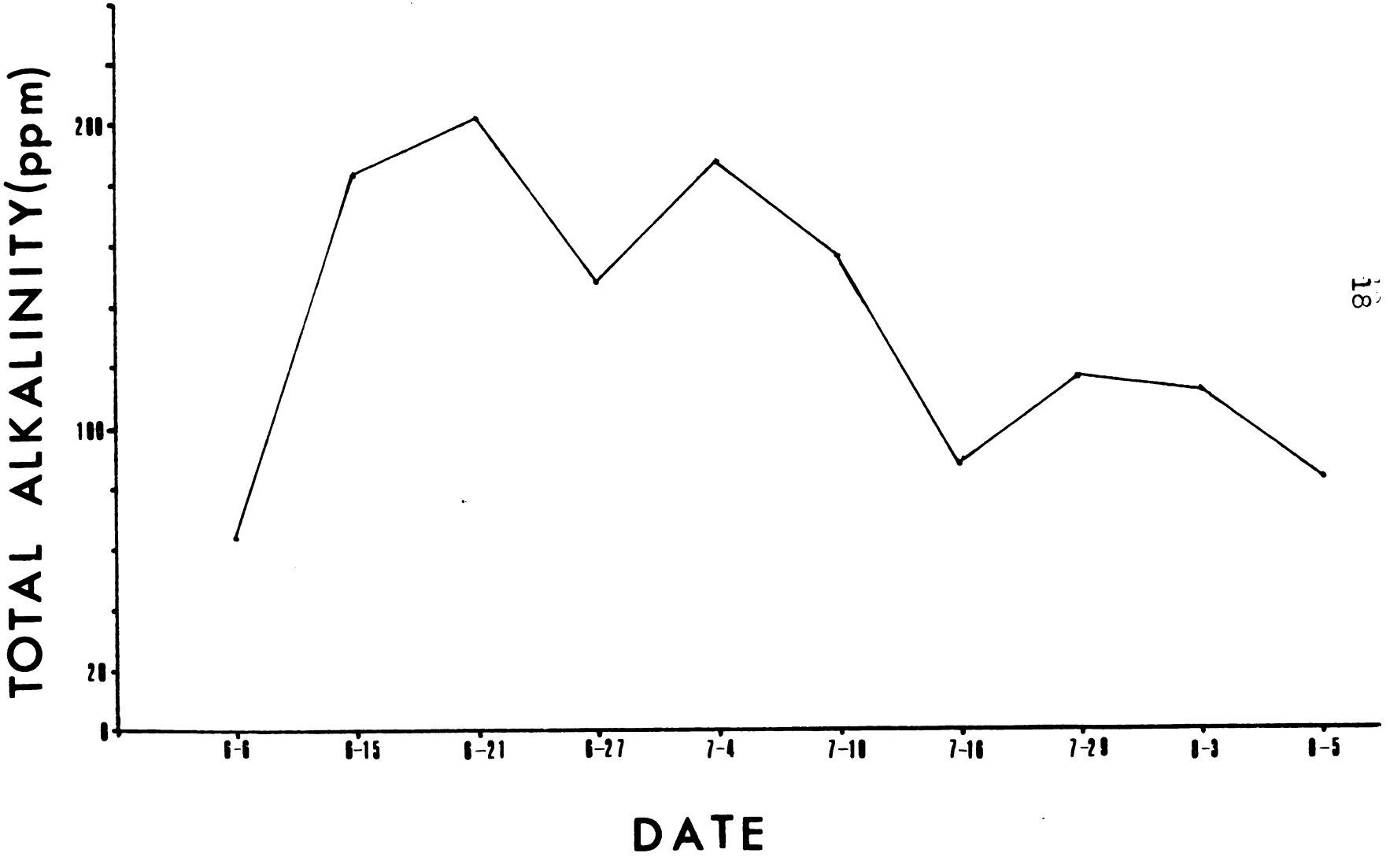
Table 2. Nutrient medium.

NUTRIENT	STOCK SOLUTION	STOCK ADDED TO MEDIA
$MgSO_4 \cdot 7H_2O$*	25mg/l	25ml/l
$NaSiO_3$*	20mg/l	20ml/l
$Na(HCO_3)_2$*	160mg/l	10ml/l
K_2HPO_4	5mg/l	5ml/l
Ferric citrate*	1mg/l	100ml/l
+ Citric acid	1mg/l	
$MnSO_4$**	0.03mg/l	0.3ml/l
$ZnSO_4$	50mg/l	1ml/l
$CuSO_4 \cdot 5H_2O$**	5mg/l	1ml/l
H_3BO_3	0.6mg/l	1ml/l
$MnCl_2 \cdot 4H_2O$**	0.4mg/l	1ml/l
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$**	10mg/l	1ml/l
EDTA	1.5g/l	2ml/l
Vitamin B^{12}	100mg/l	0.33ml/l
Biotin	1mg/l	0.33ml/l

*Prescott(1968)

**Bonner(1952)

Figure 5. Total alkalinity of the stream water for the two months preceding experimentation.


TOTAL ALKALINITY(ppm)
200
100
20
0
6-6
6-15
6-21
6-27
7-4
7-10
7-16
7-28
8-3
8-5
DATE

pH. pH was determined, using a Leeds and Northrup pH meter. The pH varied between 7.9 and 8.5: Most of the variability was due to determinations being made at different time of day. The pH varied with the amount of CO_2 dissolved in the water.

CO_2. The carbon dioxide concentrations in the water were determined from the pH and total alkalinity, using Moore's nomograph. The amount of free CO_2 held by the water decreased during the two months before experimentation (Figure 6). This was probably due to the greater standing crop of the growing community. The carbon dioxide concentration showed a marked diurnal cycle (Figure 7). The concentration of free CO_2 decreased at the onset of the photoperiod as the algae started photosynthesizing, until there was no free CO_2 in the water. After the photoperiod, the CO_2 concentration increased to a miximum of 3.3 ppm.

Phosphorus. Total and dissolved orthophosphate was determined colorimetrically by the stannous chloride method (APHA, 1971). Total orthophosphate was determined directly, while dissolved orthophosphate was determined after filtering water samples through a 0.45 u membrane filter. All phosphorus was reported as P. The phosphorus concentration decreased from 0.20 ppm initially to 0.01 at the time of experimentation (Figure 8). The fluctuations and large increase in phosphorus between 4 June and 10 June may have been caused by sloughing off of some algae or mixing of the algal residue in the bottom of the reservoirs with

Figure 6. Free carbon dioxide concentrations for the two months preceding experimentation.

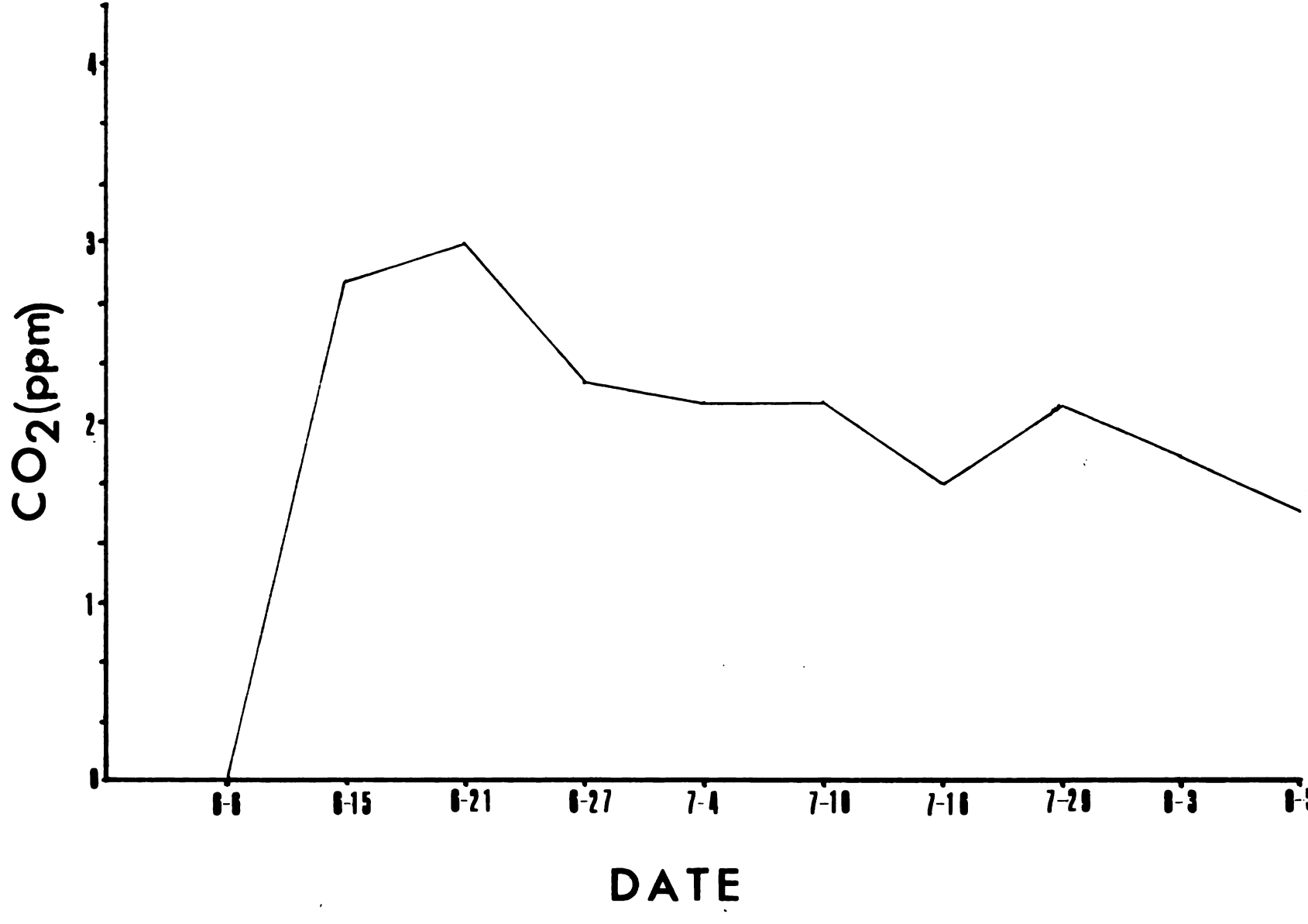
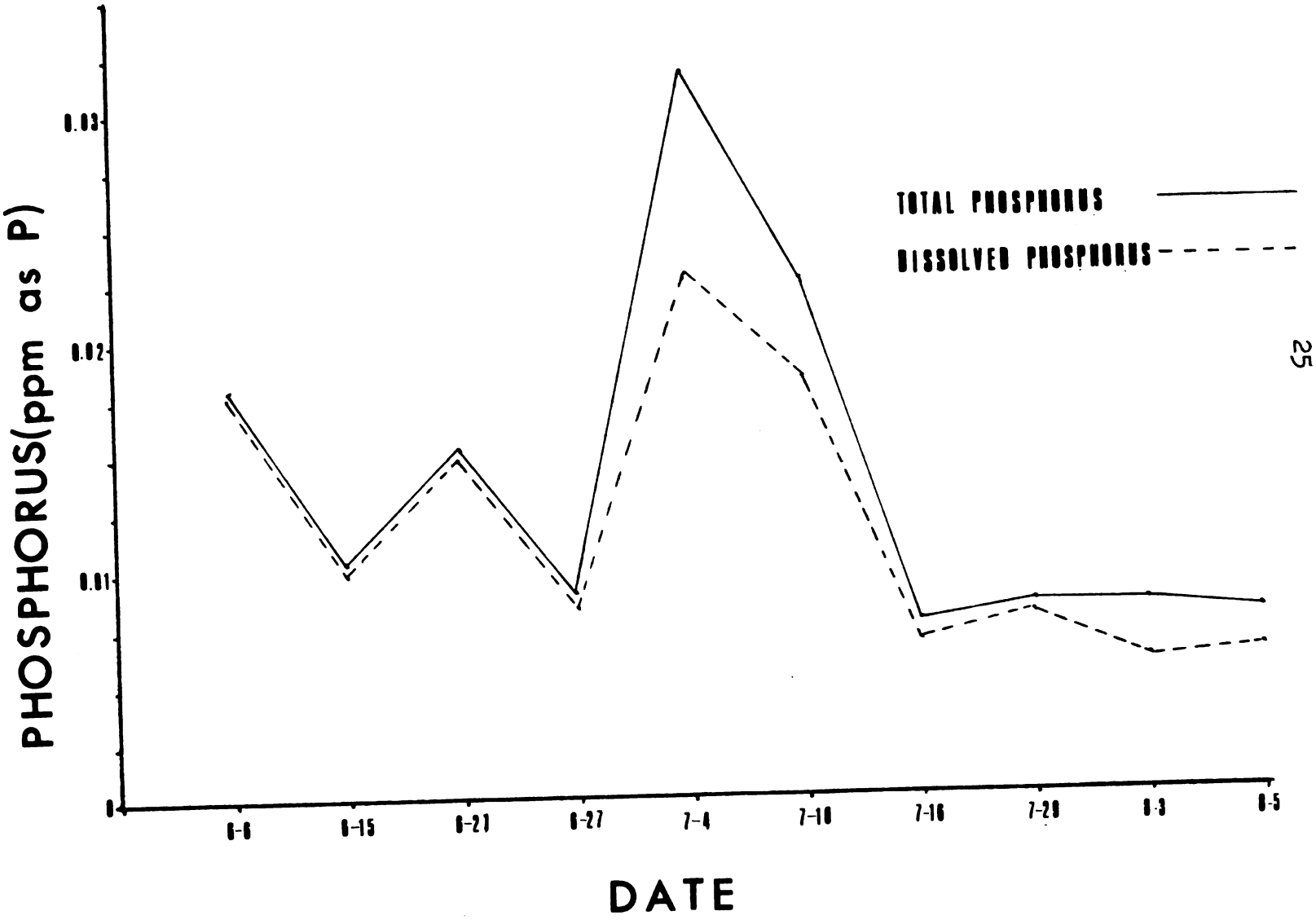

CO_2(ppm)
4
3
2
1
0
6-8
6-15
6-21
6-27
7-4
7-10
7-16
7-28
8-3
8-5
DATE

Figure 7. Diurnal carbon dioxide cycle for water in the reservoir just under the return pipe from the stream, on 21 June 1971.

Figure 8. Total and dissolved orthophosphate in the stream water for the two months preceding experimentation.


PHOSPHORUS(ppm as P)
0.03
0.02
0.01
0
TOTAL PHOSPHORUS
DISSOLVED PHOSPHORUS
6-6
6-15
6-21
6-27
7-4
7-10
7-16
7-28
8-3
8-5
DATE

the water. The phosphorus level was constant the last three weeks before the experiment. The phosphorus level in the stream was about the same as the level in the Red Cedar River, a local stream. This level was not as low as what might be expected in a severely phosphorus limited trout stream environment, but was low enough to allow for rapid sorption but also good colonization.

Stream colonization. The term periphyton, often used for the algae that cover rocks and other submerged substrata, is ambiguous, so, to avoid confusion, the encrusting algal forms growing on stream substrata will be referred to as epilithic algae.

Stream colonization was initiated by seeding the stream with rocks covered with epilithic algae. Rocks taken from the Red Cedar River; T.4N., R.2W., NW¼, SW¼ Section 13 Lansing Township, Ingham County, Michigan during September 1970 were placed in the stream reservoir. Within two months there was a flocculent growth of green and bluegreen algae, where the current did not sweep them away. Three months after seeding the algal community had developed into a very diverse community of epilithic and non-epilithic algae (Table 3). The diversity of the algal community decreased (Table 3) so that by August 1971 there was a homogeneous epilithic community of only a few species (Table 3 and Figure 9). The stream bottom and cylinders were covered with a dense film of Cocconeis placentula, which gave the stream bottom a distinct brown cast. Embedded in the diatom matrix were the other two

Table 3. Algal genera present in the artificial stream:

Dec. 15, 1970	Apr. 21, 1971	August 3, 1971
Nostoc	Chlorella	Chlorella*
Scenedesmus	Gleotrichia	Scenedesmus*
Ulothrix	Anabaena	Anabaena
Cosmarium	Nostoc	Chlorococcum
Chlorococcum	Ankistrocesmus	Cladophora
Mugeotea	Tabelaria	Nostoc
Gleocapsa	Mugeotea	Rhizoclonium
Meridion	Meridion	Cocconeis placentula*+
Chlamydomonas	Nitzscia	
Pandorina	Diatoma	
Microcystis	Scenedesmus	
Euglena	Cladophora	
Pinnularia		
Navicula		
Desmidium		
Anabaena		
Pemium		
Roya		
Chlorella		
Cladophora		
Rhizoclonium		
Spyrogyra		
Ceratium		
Tribonema		
Netrium		
Gonatozygon		
Nitzschia		
Gyrosigma		
Fragilaria		
Stephanodiscus		

*Dominant species

+Patrick (1966)

Figure 9. A photograph of the epilithic algal community and associated Tendipedidae larvae.

dominant genera, Chlorella and Scendedesmus. There were a few long strands of Cladophora but most of these were removed so that they would not interfere with the determination of bottom surface area.

Besides the algal population, the stream supported a population of protozoans and other small invertebrates (Table 4). The most numerous animals were the midges of the family Tendipedidae, which formed tubes out of the Chlorella and Scenedesmus and grazed on the green algae (Figure 9). There were clear areas around the midge tubes where the algae had been grazed off, down to the diatom base.

Table 4. Animal groups represented in the artificial stream at the time of the experiment.

Tardigrada
Cladocera
Rotatoria
Nematoda
Protozoa
Rhabdocoela
Planaria
Ostracoda
Gastrotrichia
Chironominae

DETERMINATION OF BOTTOM SURFACE AREA

Tracer. Phosphorus is generally found in small quantities as soluble orthophosphate in most natural, unpolluted aquatic systems (Round, 1970). A low concentration of phosphorus can limit growth in most species of algae (Round, 1970). Because phosphorus is so often limiting, many species of algae have evolved ways of absorbing excess phosphorus when it is available and storing it within the cell until it is needed (Goldberg et al., 1951). It has also been found that phosphorus limited algae can absorb as much as 25 times as much phosphorus as algae grown in an environment with sufficient phosphorus (Fitzgerald, 1966).

It is this ability of algae to remove phosphorus from water that makes phosphorus an ideal tracer to measure water-algae interactions in a lotic system, where there is a short contact time between algae and water.

It was thought that the colonized surface area of a small rocky stream could be calculated by knowing the amount of phosphorus sorbed per unit area and the total quantity of phosphorus sorbed.

Phosphorus has been used as a tracer in phosphorus limited streams before, but it was found that it was difficult to detect the stable phosphorus, even when large amounts were used (Ball and Hooper, 1961).

Stable phosphorus in algae can be detected by hot water extraction in conjunction with the stannous chloride colorimetric method of detection. But, because of the large amounts

of algae required for analysis, it is difficult to use extraction methods for epilithic algae (Fitzgerald, 1966).

Phosphorus has a radioactive isotope that has excellent tracer qualities. Phosphorus-32 has a single, high-energy B^- particle (1.7 Mev) and a half-life of 14.3 days, which is long enough to work with but not long enough to make disposal a problem (Comar, 1956). By using ^{32}P one can obtain a measurement of the amount of phosphorus taken up by algae and the amount remaining in the water.

Phosphorus-32 was added from a liter, polyethylene bottle equipped with a bottom mounted disseminator and shut-off clamp (Figure 10a). The dissemenator was a 17 cm piece of ¼ inch PCV pipe that had been perforated with small holes to allow an even addition of phosphorus tracer across the entire stream width, in one minute.

Three and one half uCi ($7.72X10^7$ cpm) were mixed in 1 liter of 0.001 N HCl to avoid adsorption to the sides of the delivery bottle. One half gram of fluorescein dye was added so that the radioactive phosphorus could be followed visually.

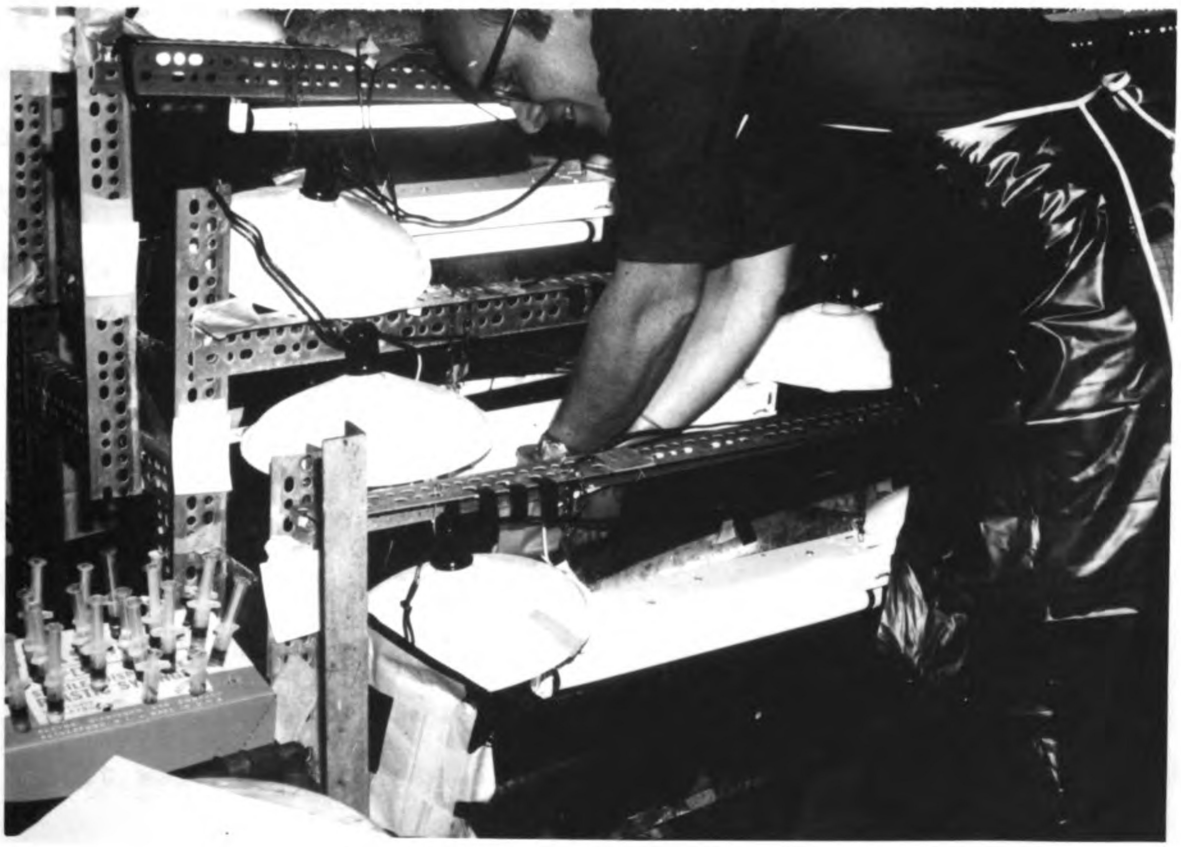
Water sampling. Stream water was sampled just before the baffle in each trough. Using disposable plastic, 5 ml-syringes several personnel removed 4 ml water samples according to a predetermined time schedule (Figure 10b). Each syringe contained one drop of concentrated HCl to prevent adsorption of ^{32}P to the sides of the syringe.

The initial water sample was taken as the dye front passed each sampling station and the time recorded. The

Figure 10. (a) Photograph of tracer delivery bottle. (b) Photograph of a person taking a water sample.

a

b



time was used for the samplers to keep track of time between samples. Samples were taken according to the following schedule: Samples were taken at 30 second intervals for eight minutes or a total of 17 samples; Then three samples were taken at one minute intervals, three at five-minute intervals and a final sample was taken 36 minutes after the dye front passed each station, making a total of 24 samples.

Water samples were mixed in the syringes and a subsample of three ml placed in planchets with retaining circles and dried at 43.5 C. After drying, ten drops of water and five drops of normal hexane were added to spread the sample evenly across the bottom of the planchet. After drying for two hours at 43.5 C, the samples were ready for counting.

Algal sampling. Algal samples were taken by colonized three by one inch glass slides that had been placed in the stream six months before the experiment was run (Figure 2b). Nine slides were arranged evenly in each trough giving a total of 54 slides in the stream. The slides were arranged so that activity, dry weight, and organic matter could be analyzed with a randomized block analysis of variance.

As soon as the water sampling was completed and all of the phosphorus-32 tracer had been collected, the glass slides were removed from the stream. The algae was scraped and washed from each slide into a tared, 2-inch aluminum planchet with a 3 cm diameter wax retaining circle in the center to insure constant counting geometry.

It was found in preliminary experiments, that if a three

ml sample was added to the planchet, without a retaining circle, the sample would dry in concentric rings very near the lip of the planchet, causing a loss in activity and a large amount of variability from sample to sample. Algae was placed in the planchet with enough water to spread it evenly in the retaining circle. After drying at 43.5 C for one hour, ten drops of water and five drops of normal hexane were added to spread the algae evenly on the bottom of the planchet for counting (Friedlander et al, 1956). Samples were dried in an oven at 43.5 C for two hours before counting.

Detection of activity in samples. The activity of water and algal samples was detected, using an end-window, gas flow Geiger-counter, equipped with an anticoincidence circuit to reduce background. The background, determined by counting a series of stream water samples before the tracer was added, was found to be 1.5 cpm. This level was not significant and caused no problems in counting.

Samples were counted for a total of 10,000 counts or ten minutes. Most algal samples were active enough to give 10,000 counts in ten minutes. Some water samples were not this active, but most of these samples were near background levels and did not enter into any calculations. Counting 10,000 total counts gives 2% error with α=0.05 (Chase, 1970)

Throughout the experiment relative activities were used instead of absolute activities because error is added with each correction to obtain absolute activity. Background, counter efficiency, sample geometry and backscattering were

all held constant.

Counter dead time caused loss of ionizing events in proportion to the activity of each sample. The higher the activity of the sample, the greater the coincidence loss. Using a split standard source, the counter dead time was calculated to be:

$$T=1.5928X10^{-6}\ cpm^{-1}$$

The true count rate was calculated, using the following equation.

$$R=\frac{r}{1-rT}$$

where R=true count rate
r=observed count rate
T=counter dead time

Self absorption varied from sample to sample, depending on the sample thickness as mg/cm^2. There was no correction needed for the water samples, but the algal samples varied enough that a selfabsorption correction factor was necessary. Construction of a selfabsorption curve for algae was almost impossible so the selfabsorption correction factor was calculated from a selfabsorption curve for NaCl (Friedlander, 1956). This is not a perfect correction for the density of algal cells, but is a good approximation. The correction factor was only two or three percent of the total activity.

Since ^{32}P has a half-life of 14.3 days, the greatest correction factor was for decay. Time of counting was recorded for each sample and all samples were corrected to the time the tracer was added to the stream, using the following equation:

$$A = A_o e^{-\lambda t}$$

where A=corrected activity
A_o=observed activity
λ=decay constant for ^{32}P ($0.0000336\ min^{-1}$)
t=time of decay

Weighing. After counting, the algal samples were prepared for weighing by drying in an oven at 45 C and a dessicator alternately until there was no weight change used to calculate the weight of the organic matter.

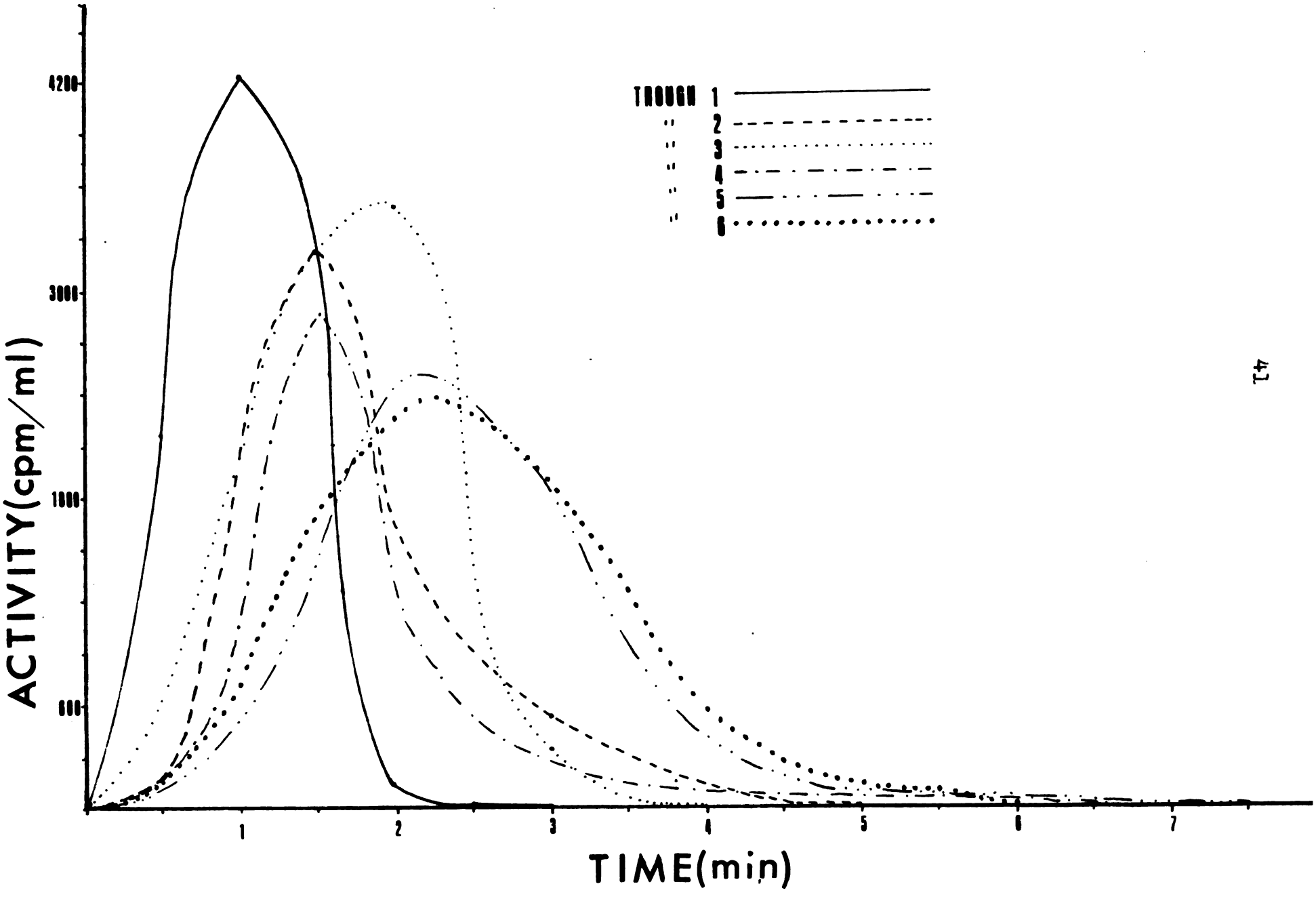
Calculation of activity in tracer pulse. Calculation of the amount of phosphorus tracer absorbed by the algae was calculated by subtracting the total activity leaving the stream from the total activity added.

Calculation of the total activity in the water leaving the stream was complicated by the fact that while passing through the stream, the activity pulse became elongated and diluted so that the remaining activity was contained in a larger volume of water.

The total activity of the tracer pulse was calculated by plotting the specific activity (cpm/ml) as a function of time (min) on large pieces of graph paper. The area under the curve, measured with a polar planimeter, was expressed as cpm/ml-min (Figure 12). By knowing the stream rate of flow (20.0 liter/min) the total activity in the tracer pulse was calculated. The activity of the tracer pulse was also calculated at the end of each trough.

Calculation of bottom surface area. With a few as-

Figure 11. Reduced versions of the large graphs used to calculate the activity in the tracer pulse at the end of each trough.


TROUGH 1
" 2
" 3
" 4
" 5
" 6
4200
3000
1000
000
ACTIVITY(cpm/ml)
1
2
3
4
5
6
7
TIME(min)

sumptions the bottom surface area can be calculated from the amount of activity removed per unit area of colonized surface area and the total amount of activity removed from the water by the algae. The total activity removed divided by the activity removed per unit of area will give the bottom surface area.

It must be assumed that the bottom area to be determined is completely covered with algae and there is little superflous filamentous algae, which will bias the estimate of bottom area. It must also be assumed that the algae in the study area all remove phosphorus at the same rate. It is also assumed that the tracer is instantly and completely mixed with the stream water, assuring constant contact with the epilithic algal mat. The change in phosphorus tracer concentration has been shown to cause a change in uptake by the algae.

The time of contact between tracer and algae and concentration of tracer also determines how much phosphorus is sorbed. The greater the contact time and the concentration of phosphorus, the greater the amount the algae are able to remove. Since the area is calculated by the amount of phosphorus removed by the algal community, there is an implied decrease in water phosphorus concentration.

Since this cannot be assumed to remain constant it must be controlled out of the experiment. To avoid inconsistancies from changing tracer concentrations the stream study area was divided into subsections, much like is done in the

calculus, where smaller and smaller sections of the whole are studied and assumed to form a continuum. Because of sampling limitations, the smallest subsection possible was one trough.

The rate of uptake by algae is influenced by many factors. In a natural system these factors could not be controlled, but in an artificial stream they can be controlled out as variable parameters. Different types of algae take up phosphorus at different rates (Goldberg et al., 1951). If the algal community is homogeneous, the sorption will be quite similar throughout the system.

Nutrient uptake by algae is also influenced by water temperature and current (Davis and Foster, 1958). Light also affects the ability of algae to absorb phosphorus. Some species of Chlorella have been found to absorb phosphorus five times as fast in the dark as in the light (Fitzgerald, 1966). Light, temperature, current, and community homogeneity were held fairly constant throughout the entire stream for any point in time.

RESULTS

So much phosphorus was added in the present study, that there was no measurable change in the tracer concentration from the initial spike to the pulse leaving the stream (Figure 13). The apparent decrease in the pulse activity during the first trough was due to a sampling error.

The variability in sampling made it appear that there was more activity at the end of all the troughs, but the first. The phenomena of over-calculating the activity of the pulse is unexplained, but may be due to an uneven flow of tracer. If the ^{32}P was not evenly distributed in the water and samples were taken from the more concentrated area an over estimation of the total activity would occur. This is possible because water samples were taken just under the surface to avoid taking algae in the samples. The tracer-dye solution had a tendency to float along the surface in the first trough, but seemed to be mixed after falling into the second trough. If the tracer had not been thoroughly mixed the above mentioned sampling bias could have occurred.

Activity sorbed by algae. The activity removed by the algae on each sampling slide was quite variable (Figure 14). There was a slight increase in the activity removed per slide the farther the slide was from the source of the stream (Figure 14). The least squares regression was not very significant ($0.10<F<0.25$).

The average amount of activity removed by the algae on the slides in each trough seemed to increase from one

Figure 12. Activity in tracer pulse at the end of each trough.

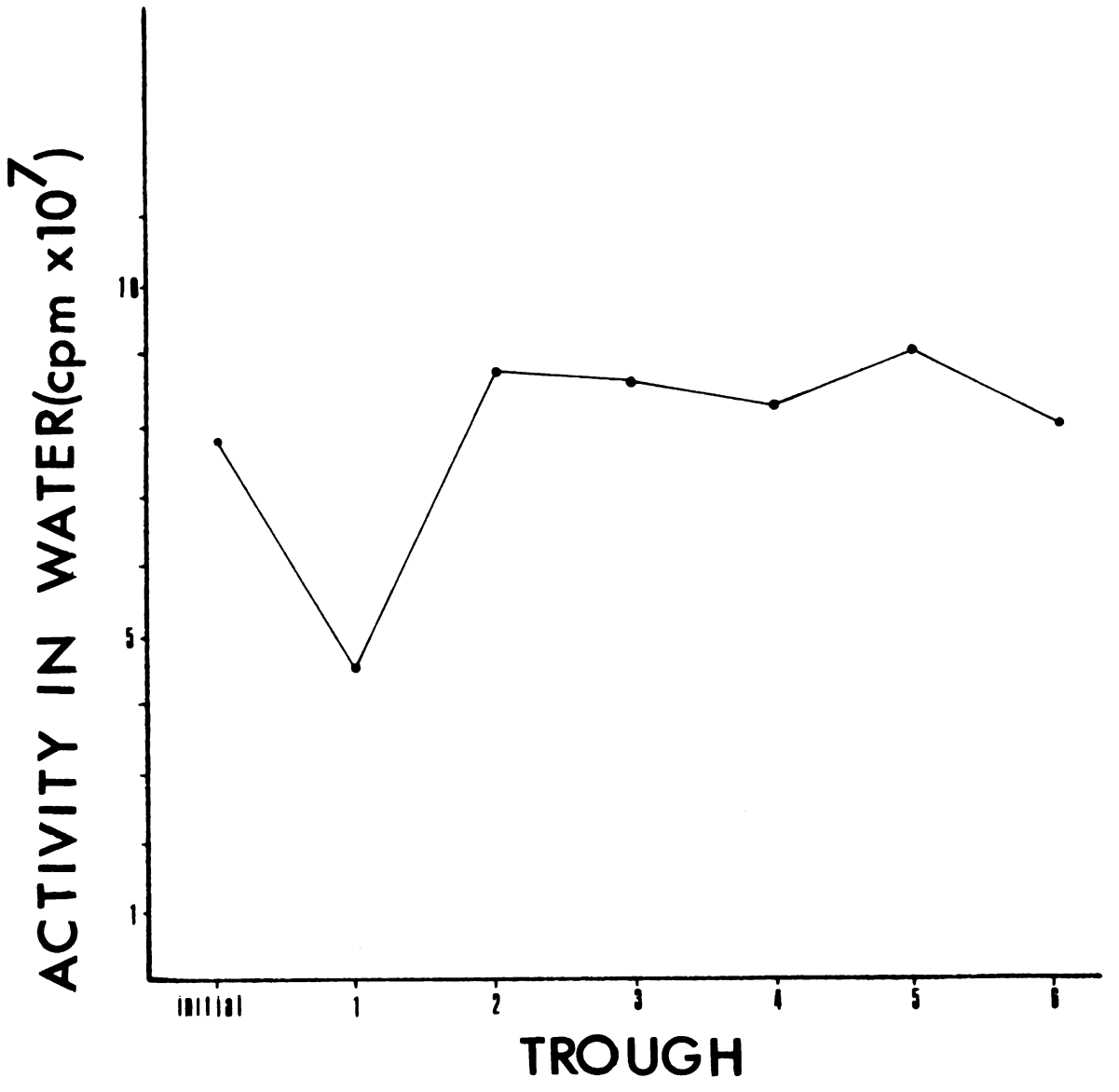
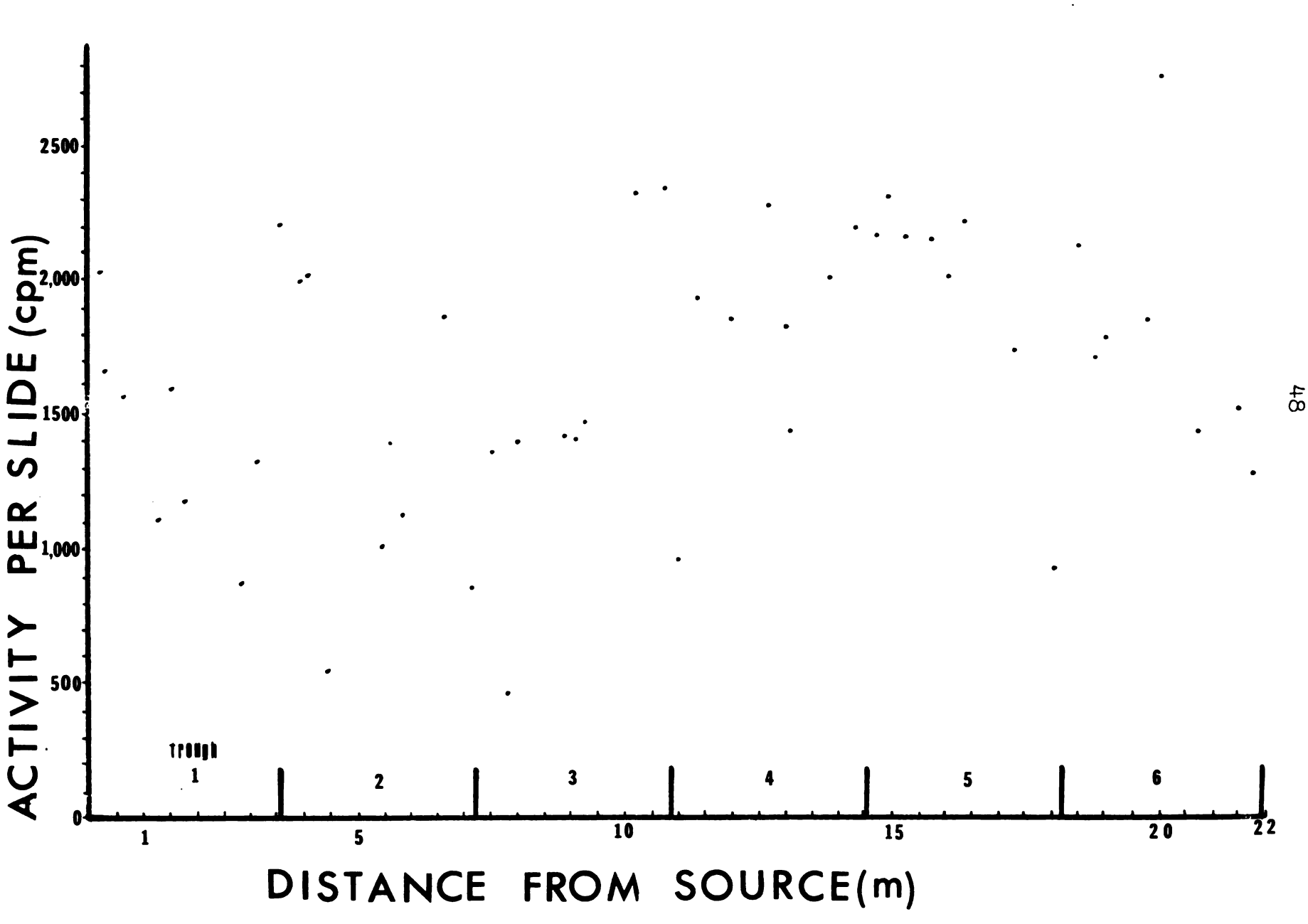

ACTIVITY IN WATER(cpm x10^7)
10
5
1
initial
1
2
3
4
5
6
TROUGH

Figure 13. The activity per slide as a function of distance from the stream source with a linear regression least squares line (Y=1544.89+14.44X). The regression line was not considered to be significant ($0.10<P<0.25$).


ACTIVITY PER SLIDE (cpm)
2500
2,000
1500
1,000
500
0
trough
1
2
3
4
5
6
1
5
10
15
20
22
DISTANCE FROM SOURCE(m)

trough to the next (Table 5) but the variance within troughs was so great that no difference could be demonstrated. The variance showed a decreasing trend from one trough to the next (Table 5). The increasing removal of activity and the decrease in variance may be due to the fact that the activity was better mixed in the water or there was an increased contact time so the tracer pulse became elongated. The calculated coefficient of varience for each trough (Table 5) seems to indicate that there was a true decrease in the variance and not just a change due to the difference in activity sorbed.

A LSR test at the 0.05 level failed to show any difference in the mean activity removed in each trough (Table 5). It can be concluded that there was no significant difference in the amount of activity absorbed over the distance of the stream. This may have been due, in part, to the fact that there was a small decrease in activity in the tracer pulse.

Dry weight. The dry weight of algae on the sampling slides varied considerably from slide to slide (Figure 15). Attempts to fit linear polynomial regression curves to the data failed. There were no curves with a significant regression.

Dry weight was analyzed with a randomized block analysis of variance, blocked over troughs. No significant difference could be shown between the mean dry weights of different troughs. There was a trend for the variance to decrease

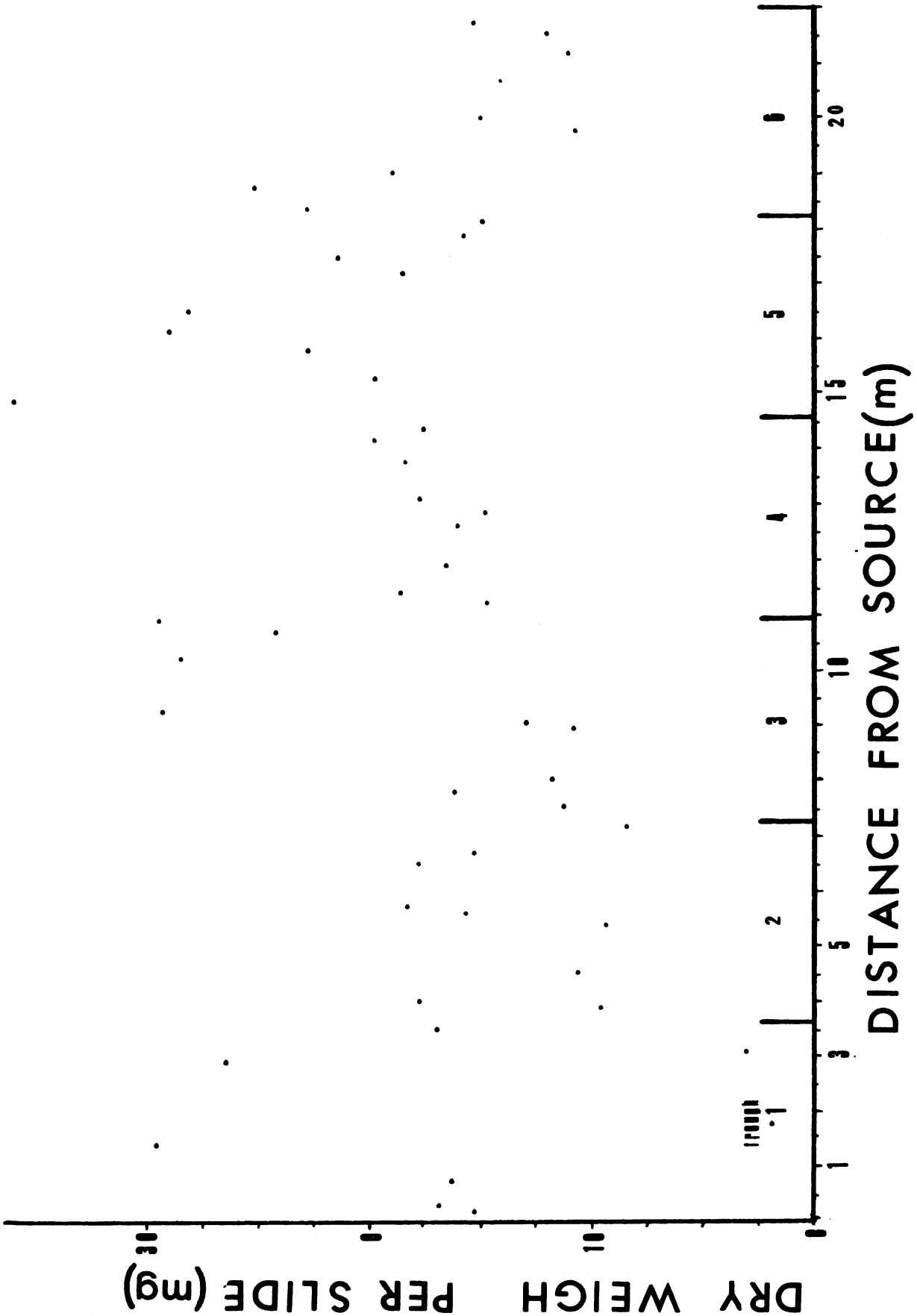
Table 7. Mean activity per slide in each trough with the estimated standard error and coefficient of variance for each trough. The mean activities are compared by L.S.R.

TROUGH	ACTIVITY	S	$\frac{S}{\bar{X}}(100)$
1	1368.12cpm	255.35cpm	18.40%
2	1403.38 "	183.02 "	13.04"
3	1552.70 "	129.68 "	8.35"
4	1942.88 "	87.20 "	4.48"
5	1874.51	141.76 "	7.56"
6	1775.51 "	159.62 "	8.79"

L.S.R. .05

TROUGH	1	2	3	4	5	6
$\bar{X}$	1368.12	1403.38	1552.70	1775.50	1874.50	1942.88

Figure 14. Dry weight as a function of distance from source.


DRY WEIGH PER SLIDE (mg)
DISTANCE FROM SOURCE(m)

in the troughs farthest from the stream source, but calculation of the coefficient of variation showed that the decrease was mainly due to the decrease in average dry weight per trough (Table 6). The average dry weight per trough seemed to increase with distance from the source (Figure 15) but this could not be shown statistically because of the variance within troughs.

The mean dry weight per slide increased from trough two until trough six where the mean dry weight decreased (Table 6, Figure 16). No significant difference could be shown between troughs using a LSR test.

Organic matter. The organic matter was highly correlated with dry weight (Table 7) as would be expected. The organic matter was studied in the experiment because it was thought that an increase in organic matter not in proportion to dry weight would indicate an algal colony of green and blue-green algae instead of diatoms. It was thought that this may have an influence on ^{32}P uptake.

The organic matter was distributed almost directly proportional to the dry weight, which indicated that the algal population was homogeneous throughout the entire stream. The average organic matter per trough followed the same pattern as that of dry weight with a decrease in the second trough and a great increase in trough 5 (Figures 17 and 18). The variance did not show any trends and there was considerable variation within the troughs (Table 8).

Table 6. Mean dry weight of algae per sampling slide with standard error and coefficient of variation.

TROUGH	DRY WEIGHT	S	$\frac{S(100)}{\bar{X}}$
1	16.80mg	3.11mg	18.51%
2	13.69 "	1.20 "	8.76"
3	18.30 "	2.75 "	15.03"
4	17.14 "	3.28 "	19.31"
5	23.04 "	2.21 "	9.59"
6	16.11 "	1.49 "	9.24"

Figure 15. Mean dry weight of algae per sampling slide in each trough.

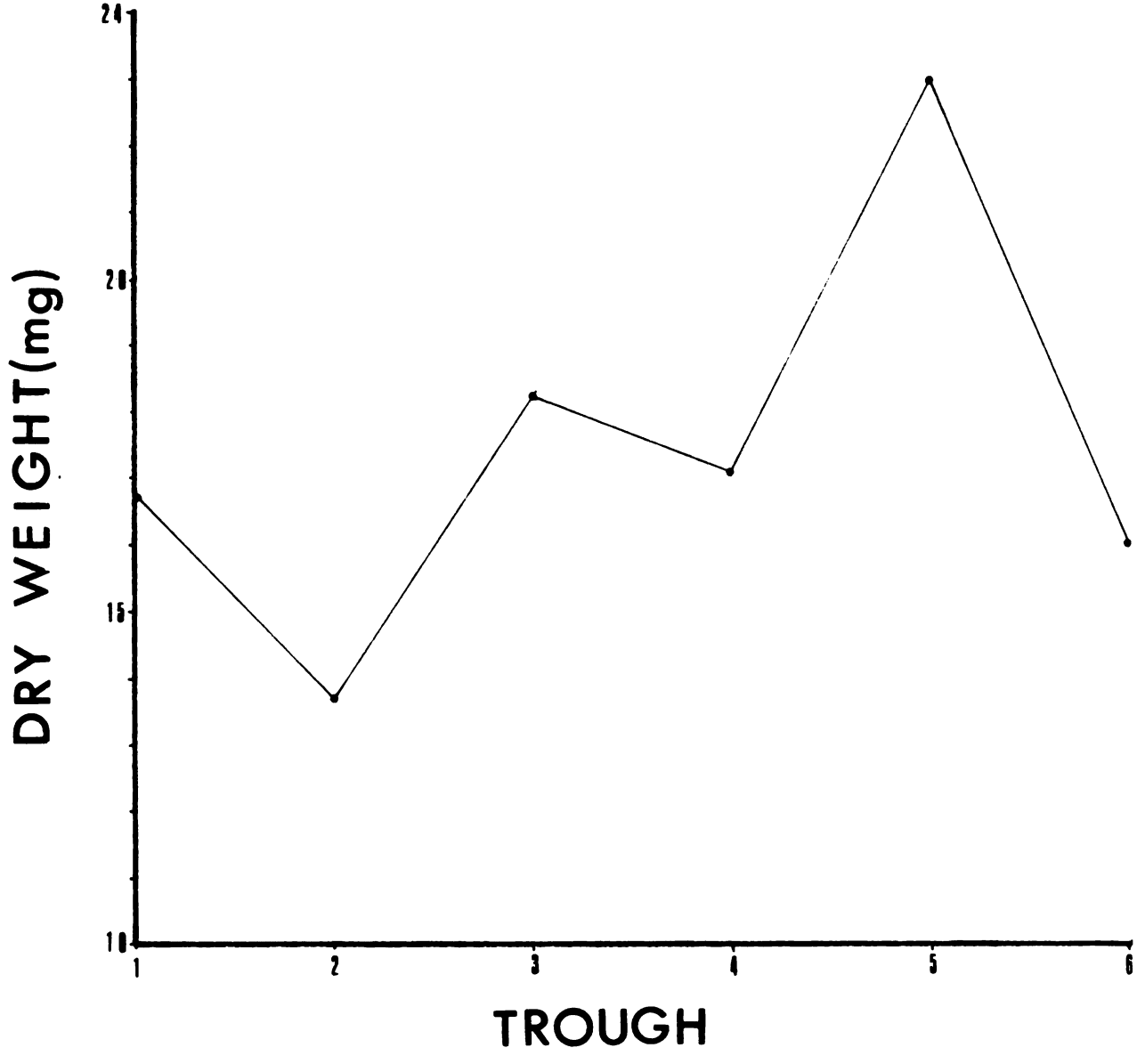

DRY WEIGHT(mg)
24
20
15
10
1
2
3
4
5
6
TROUGH

Table 7. Correlation matrix of intercorrelations between the dry weight, organic matter, distance from source and activity of each slide.

	1	2	3	4
1	1.000	0.905*	0.150	0.316
2	0.905*	1.000	0.087	0.371
3	0.150	0.087	1.000	0.284
4	0.316	0.371	0.284	1.000

1=Dry weight
2=Organic matter
3=Distance
4=Activity

*Significant correlation

Figure 16. Organic matter as a function of distance from the source of the stream.

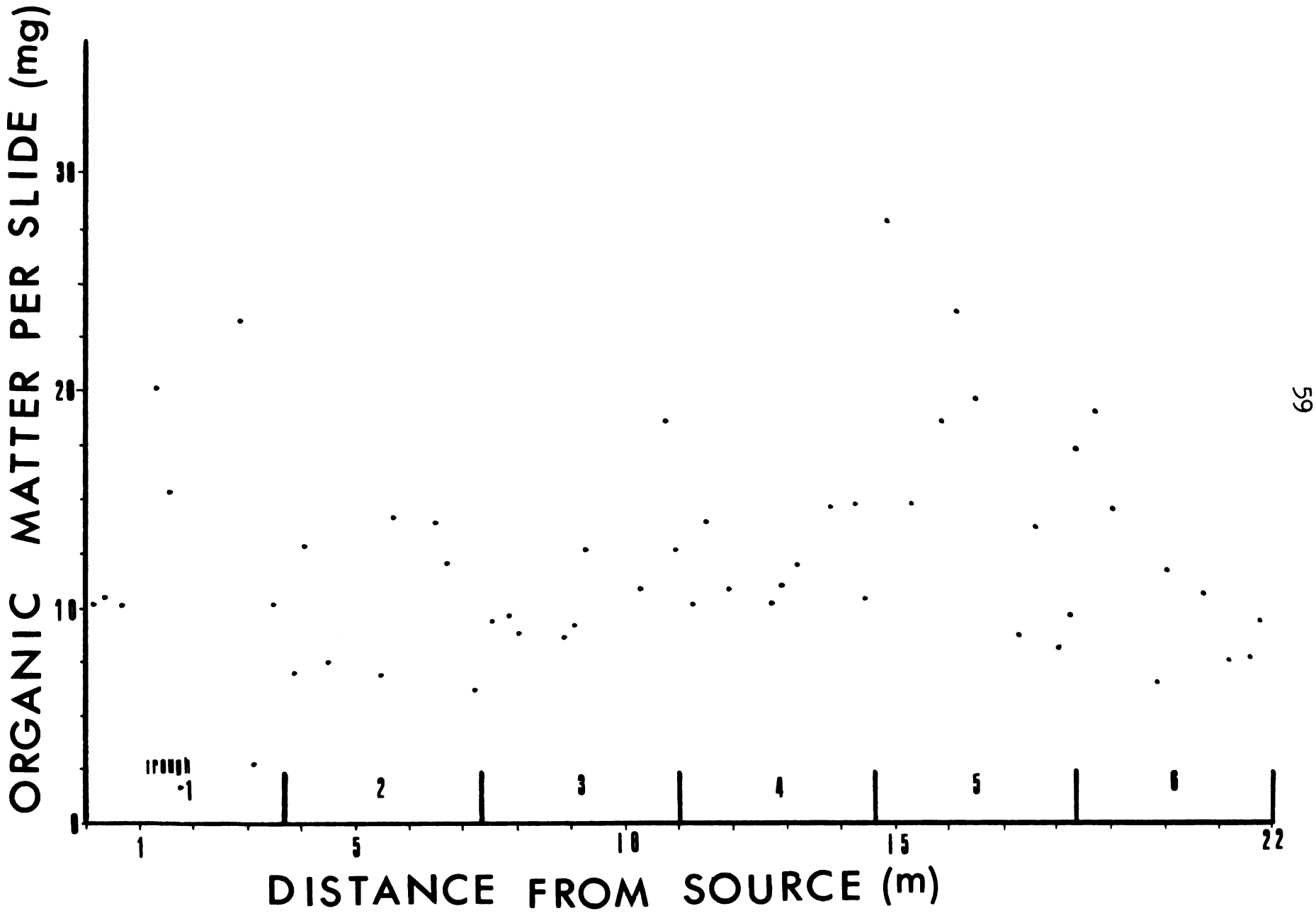

ORGANIC MATTER PER SLIDE (mg)
30
20
10
0
trough
-1
2
3
4
5
6
1
5
10
15
22
DISTANCE FROM SOURCE (m)

Figure 17. Mean weight of organic matter per slide in each trough.

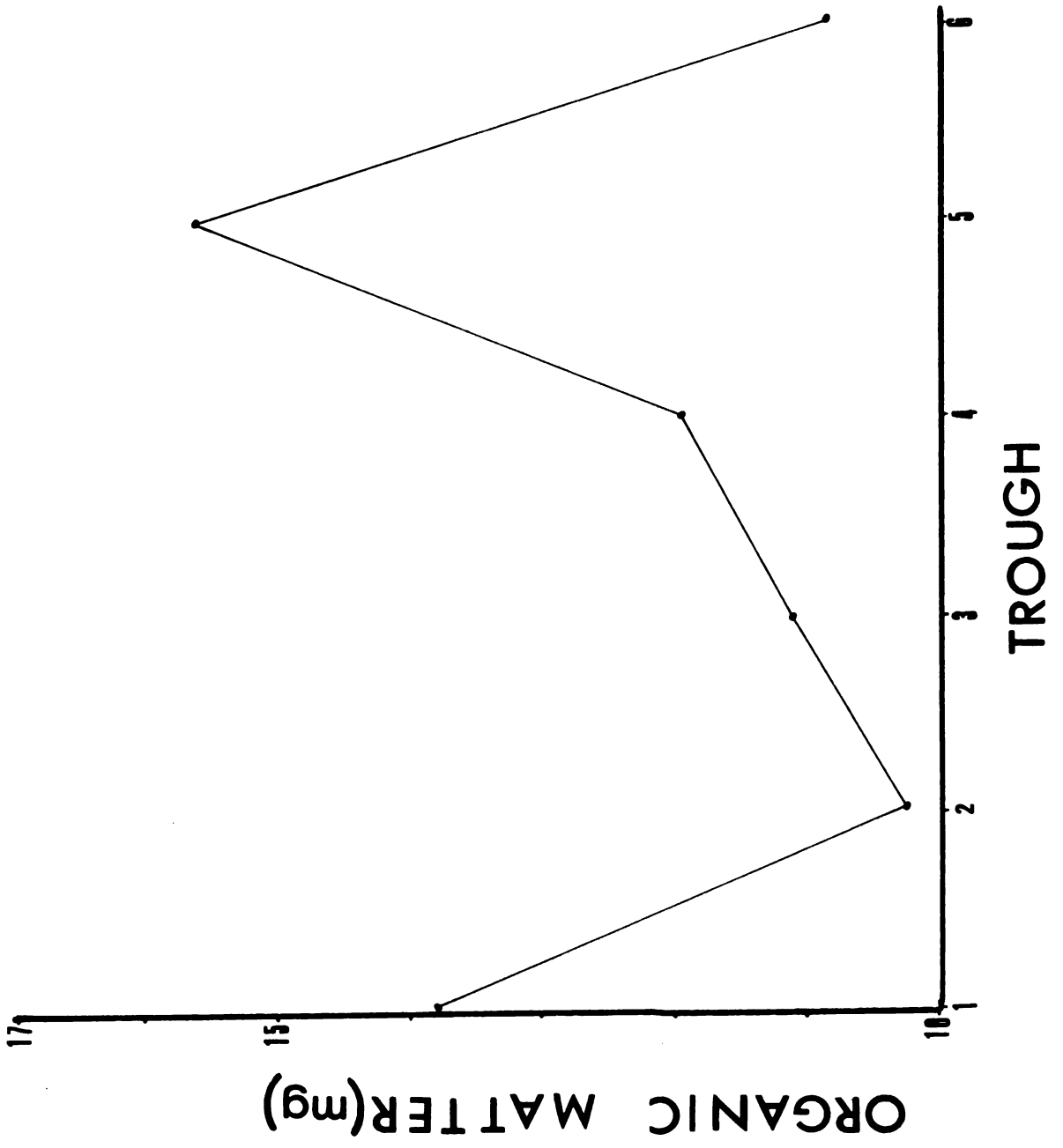

ORGANIC MATTER(mg)
17
15
10
1
2
3
4
5
6
TROUGH

Table 7. Mean weight of organic matter on each sampling slide with standard error and coefficient of variation.

TROUGH	ORGANIC MATTER	S	$\frac{S(100)}{\bar{X}}$
1	13.92mg	1.20mg	8.62%
2	10.23 "	4.24 "	41.45"
3	11.12 "	1.00 "	9.01"
4	11.92 "	0.58 "	4.86"
5	15.64 "	2.26 "	14.45"
6	16.66 "	2.65 "	22.73"

Relationship of activity to dry weight and organic matter. Studies by Rose and Cushing (1970) showed that nutrient sorption by epilithic algae was a surface phenomenon. Rose and Cushing were using relatively thick algal mats of about 500-600 u. Even though the algal mats used in the present study were much thinner, it was assumed that the sorption of phosphorus would be a surface phenomenon. Activity seemed to be correlated with dry weight and organic matter (Figures 19 and 20). However, a multiple regression correlation showed that activity was not significantly correlated with dry weight or organic matter (Table 7). The inability to show significant correlation was probably due to the great variability in the samples. It is probably true that the thickness of the algal mat would not affect the nutrient uptake if the layer of epilithic algae is thicker than some critical value. This is because if the algae on the substratum were very thin, giving rise to uncovered places, sorption would be decreased.

Figure 18. Activity as a function of dry weight.

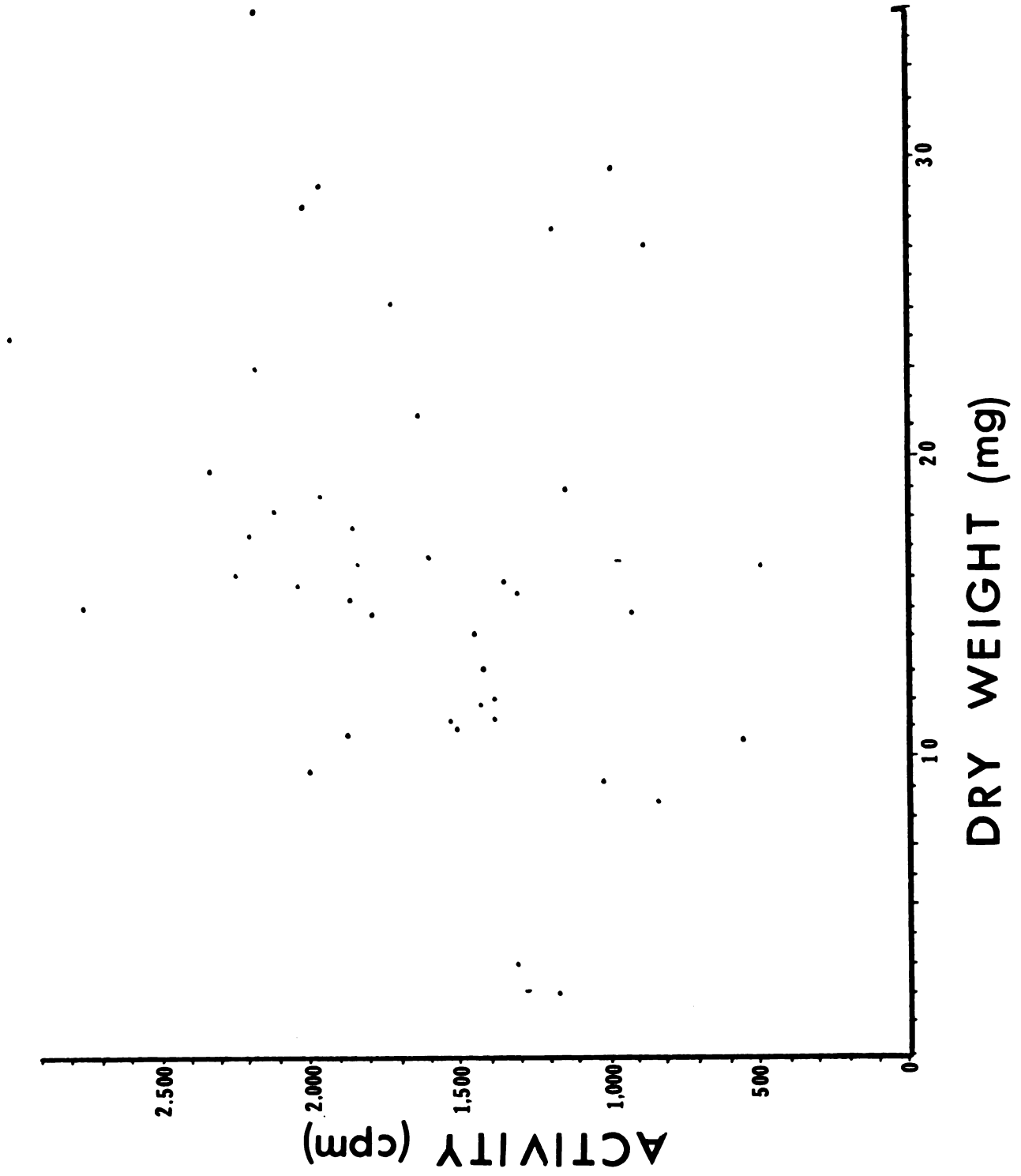
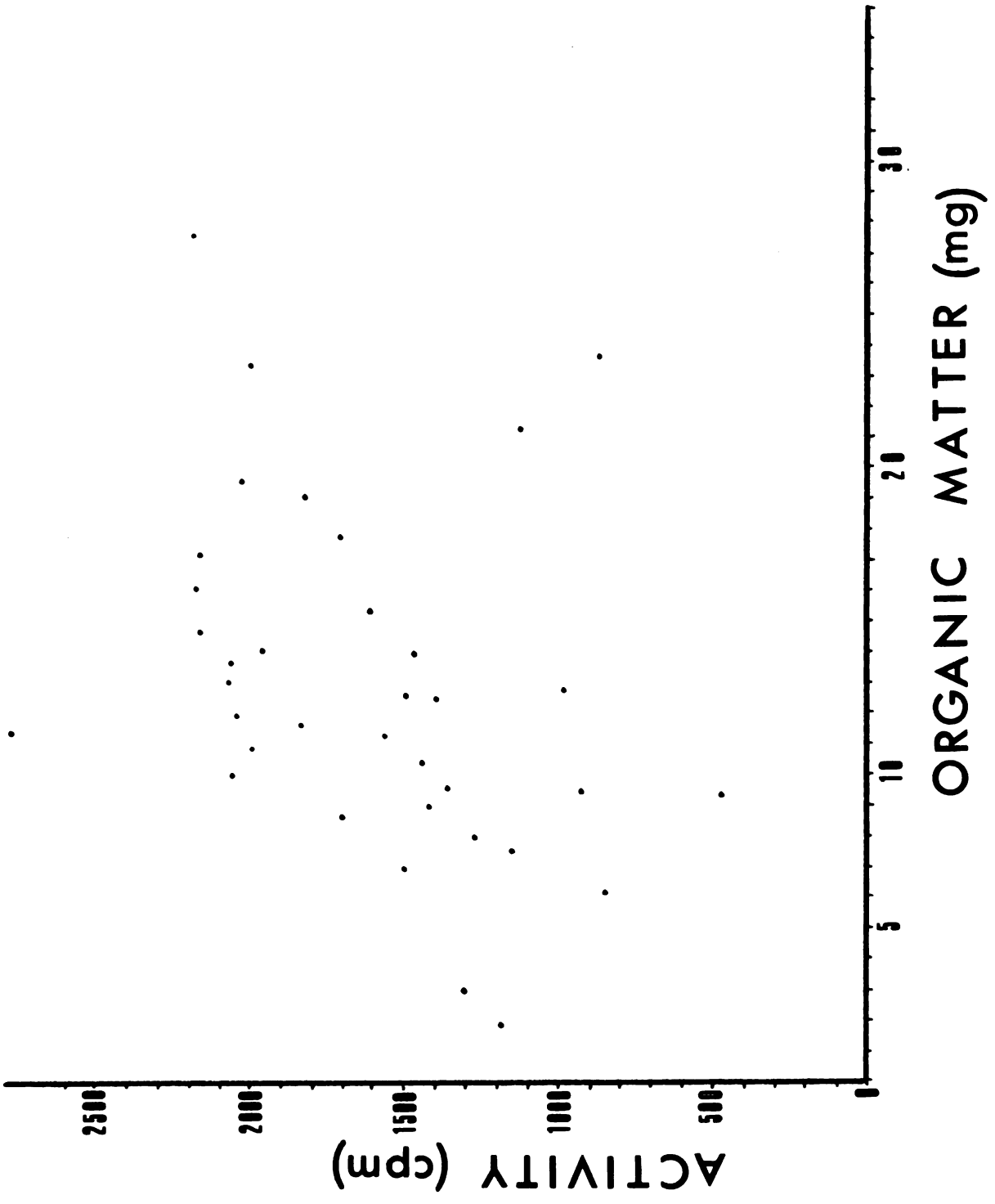

ACTIVITY (cpm)
2,500
2,000
1,500
1,000
500
0
10
20
30
DRY WEIGHT (mg)

Figure 19. Activity as a function of organic matter.


ACTIVITY (cpm)
2500
2000
1500
1000
500
0
5
10
20
30
ORGANIC MATTER (mg)

Back calculation of total phosphorus-32 tracer removed. By knowing the mean activity removed per unit of area and the total stream area, the activity of phosphorus-32 tracer removed in the stream system was calculated.

The glass sampling substrata and attached algae removed an average of 87.38 $cpmXcm^{-2}$. Using this mean and assuming the sorption onto the rest of the bottom was the same, the calculated activity of tracer removed was $6.09X10^{6}$ cpm. This was only 12.5% of the activity added in tracer pulse. This low coefficient of sorption coupled with the variability of the water samples, made it impossible to calculate the colonized bottom surface area by tracer sorption.

SUMMARY

The artificial stream functioned well as an experimental unit. Algae colonized well and a homogeneous eplithic algal community went through a series of successional stages and stabilized as a diatom dominated community that completely encrusted the entire stream bottom.

Since no experiments of this type had been attempted before, it was difficult to determine the amount of P-32 tracer to be added. The estimate of the needed amount, from pilot studies, was high. This made it impossible to determine the bottom surface area by phosphorus sorption because only a small amount of the tracer was removed by the stream system.

Phosphorus sorption was found to be strictly a surface phenomenon, not influenced by the thickness of the algal mat.

The use of phosphorus-32 as a tracer in artificial streams is promising, not only for determining bottom area but for determining the effects of different parameters on phosphorus uptake by algae.

Any future experiments with ^{32}P as a tracer must find a way to reduce the variance.

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