cc: Fish Division
Educ.-Game
J. W. Leonard
Inst. for Fish. Research
F. F. Hooper
R. C. Ball
C. T. Yoder
D. S. Shetter
W. C. Latta

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A second report on translocation of radioactive phosphorus (P^{32}) in a Michigan trout stream

by

R. C. Ball, Department of Fisheries and Wildlife
Michigan State University

and

F. F. Hooper, Institute for Fisheries Research,
Michigan Department of Conservation

Introduction. Our activities during 1959 consisted of: (1) A recheck of the many data obtained from the radiophosphorus study in 1958. Repetition of the work was necessary, since the 1958 experiment was undertaken after a minimum period of planning. By repeating essentially the same experiment we obtained many data in replicate for two calendar years. This increased our confidence in certain results and allowed somewhat broader interpretations. During 1959 in many instances we were able to collect a larger series of invertebrate and fish samples. From these samples we are studying the variability in the amount of activity among animals of the same kind living at a given stream station at a particular time. (2) A number of new observations that were not a part of the work in 1958. These included (a) an intensive study of the particulate phosphorus in the stream water; (b) a more complete analysis of the methods of translocation of regenerated phosphorus through the food chain (for this purpose an exclosure was established in the stream enabling study of the spread of activity into an area affected very little by the isotope dosage); and (c) a more intensive study of the movement of radiophosphorus in and out of the invertebrates of various food niches.

Study Area and Techniques. The study area in 1959 was the same as in 1958. We felt there were many advantages in using the same area since many of our data would be in replicate. Factors such as stream morphometry, flow rate, amount of shade, etc., were nearly identical for the two experiments. In 1959 we added the radiophosphorus on July 8, nearly a month earlier than in 1958, to enable us to follow activity in the stream for a longer period. (Since the

corrected activity of many animals occupying higher levels in the food chain was still on the increase when observations were terminated in September of 1958, it was felt that an earlier treatment was highly desirable.)

The radiophosphorus was again released in 1959 in Section 21 of T. 31 N., R. 3 W., Cheboygan County, Michigan. (Figure 1) Sampling and routine monitoring of activity were again carried on downstream to the U.S. 27 bridge crossing this stream (Station 16, T. 33 N., R. 3 W., Section 14). Sampling stations in 1959 were identical to 1958 except that a new station, Station 12 E, was added adjacent to Station 12. Station 14 E was within an exclosure consisting of approximately one-third the width of a 150-foot long section of the stream channel. Here the stream was divided by a diversion of polyethylene film. (Figure 2) Water normally passing through the southern one-third of the stream bed was diverted to the northern two-thirds of the bed. The diversion was installed 24 hours before the isotope addition and was removed two hours after the P^{32} dosage had passed beyond this point in the stream (when water activity had fallen to background level).

As in 1958 a shipment of 23.1 millicuries of P^{32} was diluted with 55 gallons of stream water and the mixture was fed into the stream at a constant rate for 30 minutes. This gave a theoretical maximum concentration of 1.22×10^{-5} microcuries per milliliter which is the same theoretical dosage as in 1958.

Water Activity. Water activity curves (Figure 3) were similar to those of 1958. More precision was achieved in monitoring stream activity while the isotope was moving through the area by taking samples at five minute intervals. The maximum water activity reached a somewhat greater peak in 1959 than in 1958 (12 counts per milliliter in 1959 compared to 10.3 in 1958). Higher values were obtained because we were able to sample more nearly at the time of maximum activity and also because periphyton uptake was less at upstream stations in 1959. Maximum activity was higher during 1959 at Stations 5 and 11, but appeared to be approximately the same as in 1958 at Station 8. Because of

Figure 1. Map of section of West Branch of Sturgeon River used in experiment.

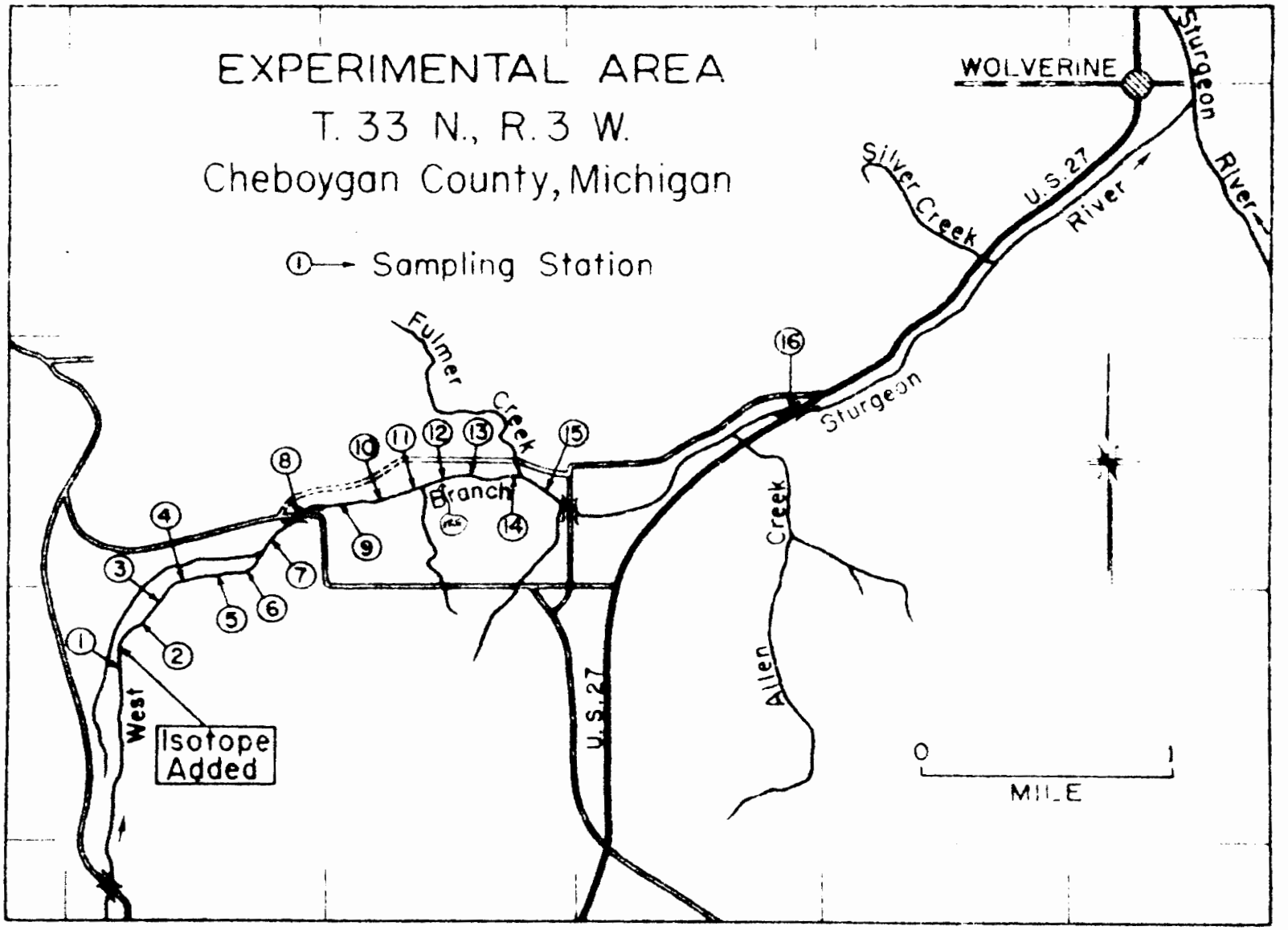
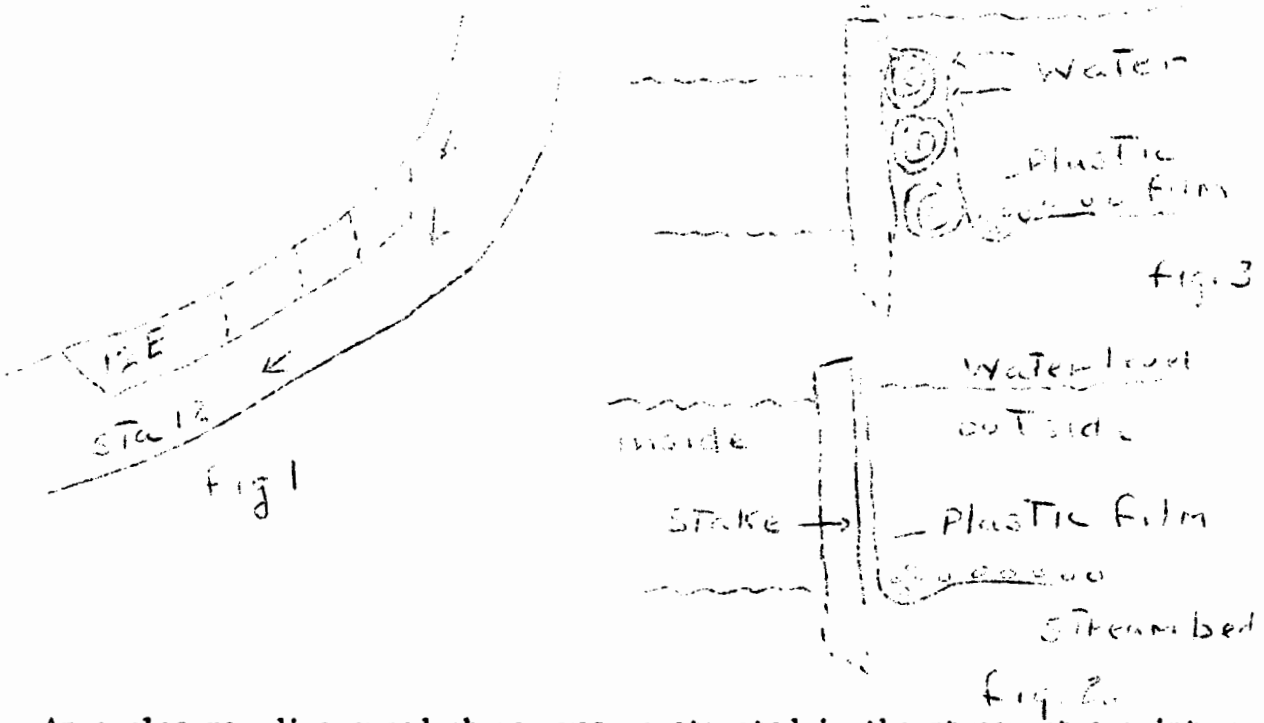

EXPERIMENTAL AREA
T. 33 N., R. 3 W.
Cheboygan County, Michigan
① → Sampling Station
WOLVERINE
Sturgeon River
Silver Creek
U.S. 27
Sturgeon River
Fulmer Creek
Branch
Allen Creek
U.S. 27
West
Isotope Added
0
1
MILE

Figure 2. Exclosure at Station 12. Area inside designated as 12 E. Area outside designated as 12.

Figure 2.

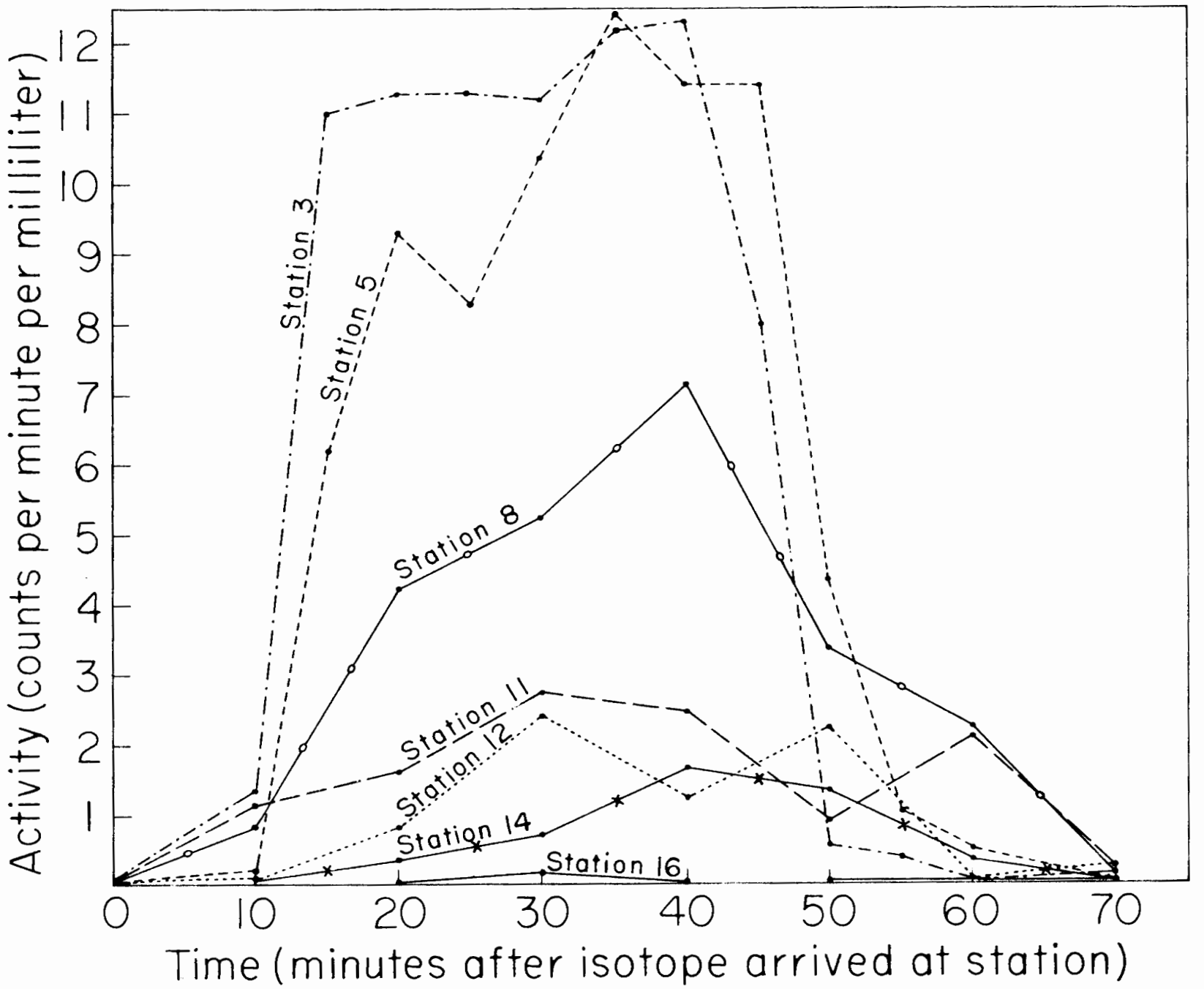


An exclosure, diagrammed above, was constructed in the stream at a point approximately a mile and a half below the point of entrance of the isotope. The purpose of this was to prevent the isotope-bearing water from going through an area of the stream and being taken up by the stream organisms. By this means we could estimate the amount of radioactive material moving out of the stream complex above and being taken up at this lower level in the stream under unmodified conditions.

The barrier was constructed of poles driven into the stream and covered with 6 mil polyethylene film, (Fig. 2 above). The enclosed area was divided into five sections by cross barriers as shown in Fig. 3, above. By the use of dyes it was determined that the mixing of surface waters of the stream with that of the inside was quite low. As can be seen in Fig. 11, some activity was found inside and it is presumed that this could have come into the area through the gravel substrate of the stream.

The exclosure was maintained intact during the period the isotope was flowing down the stream and for two hours after the last identifiable activity had passed. The upstream, downstream, and intermediate barriers were then removed and the water of the stream allowed to flow through the area. The film dividing the two parts of the stream (lengthwise) was kept in place throughout the remainder of the study. This was done to eliminate cross-stream migration of aquatic organisms. This did eliminate the possibility of the organisms collected within the exclosure coming from the immediately adjacent half of the stream, but it is possible that some of the invertebrates were washed into the area from upstream.

Figure 3. Total water activity at various collecting stations during passage of isotope. All counts corrected for background and decay.


Activity (counts per minute per milliliter)
1
2
3
4
5
6
7
8
9
10
11
12
Station 3
Station 5
Station 8
Station 11
Station 12
Station 14
Station 16
0
10
20
30
40
50
60
70
Time (minutes after isotope arrived at station)

more frequent sampling the activity curves for various stations were smoother and much more regular than those obtained in 1958.

By collecting a series of samples across the stream during passage of the isotope dose, it was learned that there was considerable horizontal variation in activity. Table 1 shows the activity in a transect at Station 8 after the peak in water activity had passed this station. In this instance activity was highest along the stream margin and lowest in midstream. This condition arises after the dosage in the center current has started to decrease and high activity water from the backwaters and eddies begins to feed back into the stream channel.

Table 1.--Water activity (counts per minute per milliliter) at different points in the stream channel during passage of isotope dose

Left Bank	Left Center	Right Center	Right Bank
3.0	2.1	2.3	3.3

In both 1958 and 1959 there was much greater rate of uptake of activity in the upstream stations than in the lower stations. Curves showing the total activity passing various stations fell logarithmically between Stations 1 and 5 during 1958; downstream the much reduced rate of decrease gave a decided break in the activity curve. In 1959 the break in the curve appears to have been farther downstream than in 1958 (between Stations 8 and 11); in addition a somewhat smaller decrease in activity occurred between Stations 3 and 5. These differences also seem to be related to the greater phosphorus pool within the crop of periphyton upstream from Station 8 in 1958 and to a slightly greater pool at Station 8 and between Stations 8 and 11 in 1959. The extent of periphyton growth and presumably the size of the phosphorus pool in the upper stream sections in 1959 differed from that observed in 1958. This resulted from differences in the amount of fertilizer added before the isotope release and from a change in the place and manner in which the fertilizer was added.

In the first year of the program, 1958, soluble fertilizer was fed into the stream at the rate of 170 micrograms per liter in both the upper and middle zones of the experimental area for several days prior to the addition of the isotope. This was done to increase the production of periphyton and thus assure the presence of it in large enough amounts to take up the isotope within the experimental area. From the first year's data it was apparent that there was no need for concern that any appreciable amount of radioactive material would leave the area and as a result only a token amount of fertilizer was put in the stream during 1959, prior to the addition of isotope. The small amounts were added at the site of entrance of the isotope and at Station 8 two days prior to the treatment.

As a result of this change in procedure it was presumed that there was a smaller pool of natural phosphorus in 1959 than in 1958. The uptake pattern of the isotope supported this conclusion.

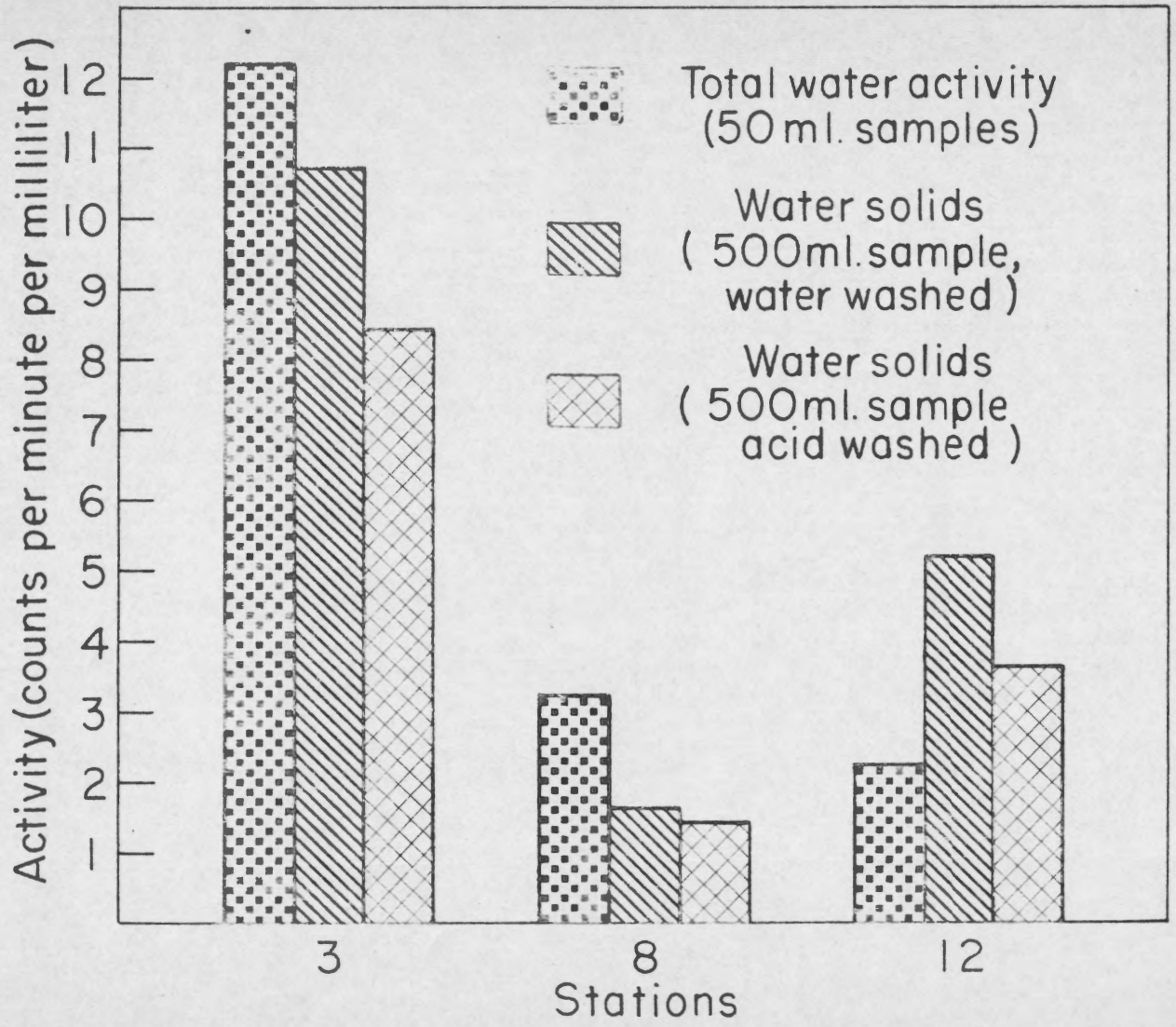
In 1959 statistically significant counts of activity were not obtained from water samples collected at Station 16 (U.S. 27 bridge). Thus activity was rather completely removed in passing through the experimental area. Since a significant count was obtained at Station 16 in 1958, removal of the isotope was perhaps more complete in 1959.

Activity of water solids

In 1959, 500 ml. water samples were collected from the stream while the isotope dose was moving downstream. Samples were filtered through a millipore filter. Solids collected on the filter were washed with one-tenth normal hydrochloric acid and were then dried, mounted on a planchette and counted.

A large percentage of the water activity was in the form of solids. (Figure 4) At Station 3 approximately two-thirds of the water activity was in the form of solids and data collected at Station 8 after the peak water activity had passed the station indicates that the solids made up 50 percent of the total activity. Data from Station 12 appear anomalous since total water activity was lower than the activity of solids, but this difference is probably

Figure 4. Total water activity, and the activity of water-washed and acid-washed solids filtered from stream water by millipore filter. Counts corrected for background and decay.


Activity (counts per minute per milliliter)
1
2
3
4
5
6
7
8
9
10
11
12
Total water activity
(50 ml. samples)
Water solids
(500ml. sample,
water washed)
Water solids
(500ml. sample
acid washed)
3
8
12
Stations

due to a small difference in time of collection of these 2 samples.

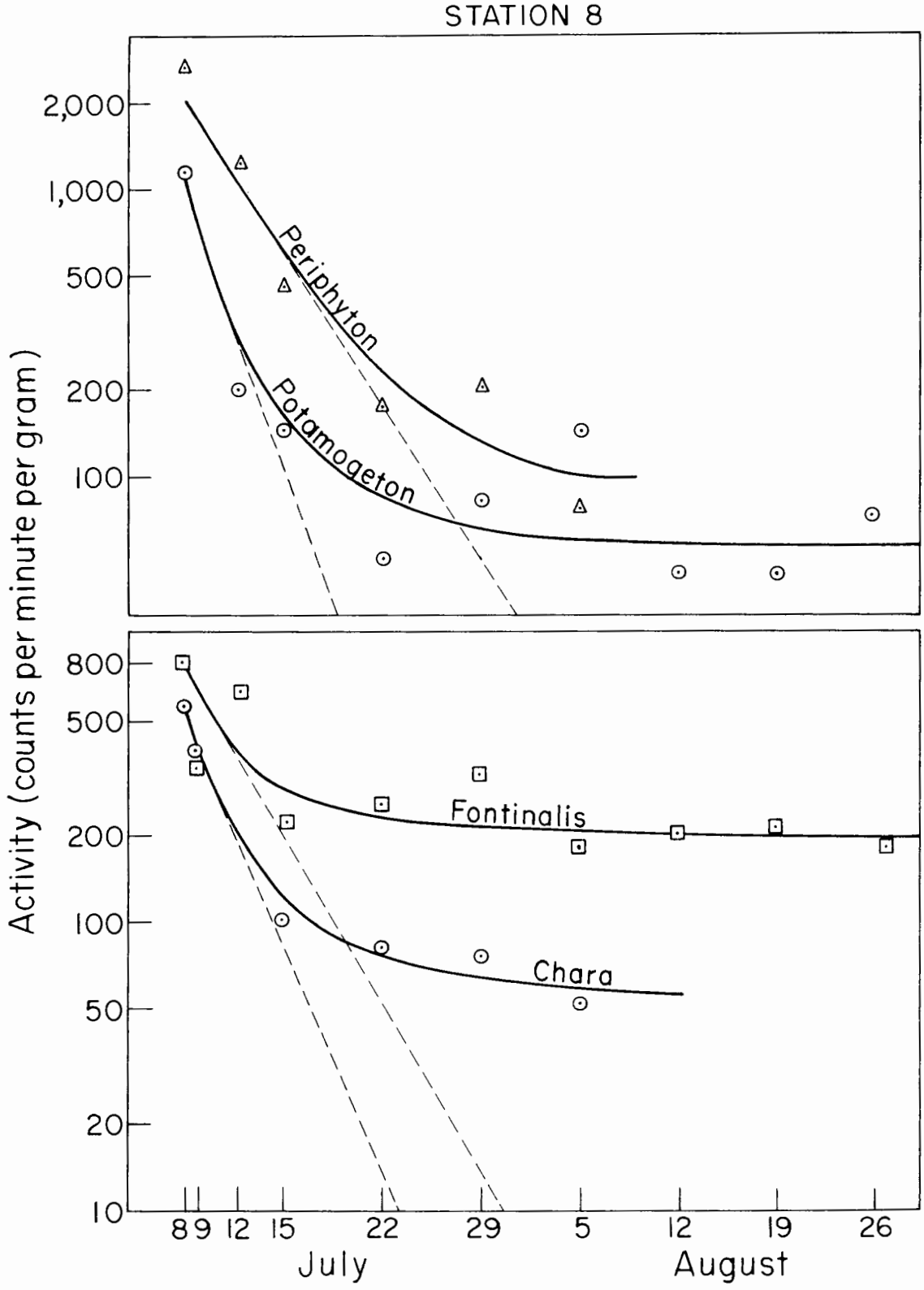
Autoradiographs made from millipore filter papers showed activity to be concentrated in very small particles that were uniformly distributed over the paper. The distribution of active particles strongly suggest that the activity was concentrated largely in water bacteria or small diatom cells. Only a small fraction of the activity on the paper was removed by washing the solids in dilute acid, so it is to be assumed that very little activity was absorbed on solid particles.

Periphyton. As in 1958, artificial substrates of plastic were placed in the stream and allowed to grow a covering of periphyton. After an incubation period in the stream, substrates were removed, periphyton was scraped off, dried, weighed,and its activity determined. Methods used were the same as were employed in 1958 except that greater precision was achieved by using a sensitive balance in weighing periphyton. At Station 8 a sufficient number of substrates were introduced into the stream before isotope treatment to permit us to remove for analysis one or more substrates each week for a period of approximately one month. This gave a continuous record of periphyton activity after the isotope dosage. (Figure 5) Comparable data were not obtained in 1958.

Figure 5 shows that activity decreased at a logarithmic rate during the first week but at a much reduced rate thereafter, indicating that the periphyton, like other aquatic plants, reaches a plateau of activity. Such a plateau presumably is caused by intake from the water of regenerated (re-cycled) phosphorus that re-enters the stream for a considerable period of time after dosage. In Figure 5 the logarithmic rate of decrease during the first week has been extrapolated to the X axis and a smooth curve has been fitted to the activity values by inspection. If the plateau in the activity curve is due to regenerated phosphorus taken in by plants, then an estimate of the amount of activity in the periphyton which comes from regenerated material can be obtained by subtracting the activity under the projected logarithmic curve from the actual activity curve. If this is done, it appears that the amount of activity within

Activity of plants collected at Station 8.

Figure 5. The dotted lines represent the extrapolation of the logarithmic rate of decrease of activity during early stages of experiment.


STATION 8
Activity (counts per minute per gram)
2,000
1,000
500
200
100
Periphyton
Potamogeton
800
500
200
100
50
20
10
Fontinalis
Chara
8 9 12 15 22 29 5 12 19 26
July
August

the periphyton cells coming from water uptake of regenerated material was approximately constant during the last three weeks in which measurements were made. A constant activity from regenerated phosphorus suggests that the cells have equilibrated to a nearly constant amount of phosphorus in the water environment. This equilibration level of phosphorus appears to be characteristic of all plant growth in the stream and will be discussed further in the section dealing with other vegetation. It should be pointed out, however, that activity loss curves during the logarithmic phase of decrease are also influenced by loss because of biological dilution, e.g., growth of cells, and secondly, by loss due to sloughing of old cells. Once the substrates have equilibrated (i.e., have lost the "excess" activity from the original dosage) further decline in activity represents only a fall in the regenerated phosphorus of the stream water.

Supporting evidence for a relatively constant equilibrium level of regenerated phosphorus comes from measurements made of the activity of substrates removed from the stream temporarily during treatment but replaced immediately after the isotope dosage had passed the station. Such substrates tend to pick up and hold a relatively constant amount of activity. (Figure 6) Their activity level, however, is much below the level of activity of control substrates which remained in the stream during treatment. Further analysis of data collected in 1959 should show whether or not the equilibrium level of substrates placed in the stream after dosage tend to show upstream-downstream differences.

Analysis of the activity changes in the periphyton at Station 3, 8, 12, and 14 suggests that the most upstream station, Station 3, picked up a relatively small amount of activity which then decreased rapidly to an equilibrium level, (Figure 7), whereas equilibration was much slower at Station 8. In both 1958 and 1959 the most downstream station studied (Station 14) tended to lose activity for the first 24 hours and thereafter showed an activity increase. The initial decrease may represent a loss of some of the excess activity above the stream equilibrium level while the later increase may be due to slow rise

Figure 6. Comparison of activity of periphyton substrates at stations 3 and 8. Counts corrected for background and decay.

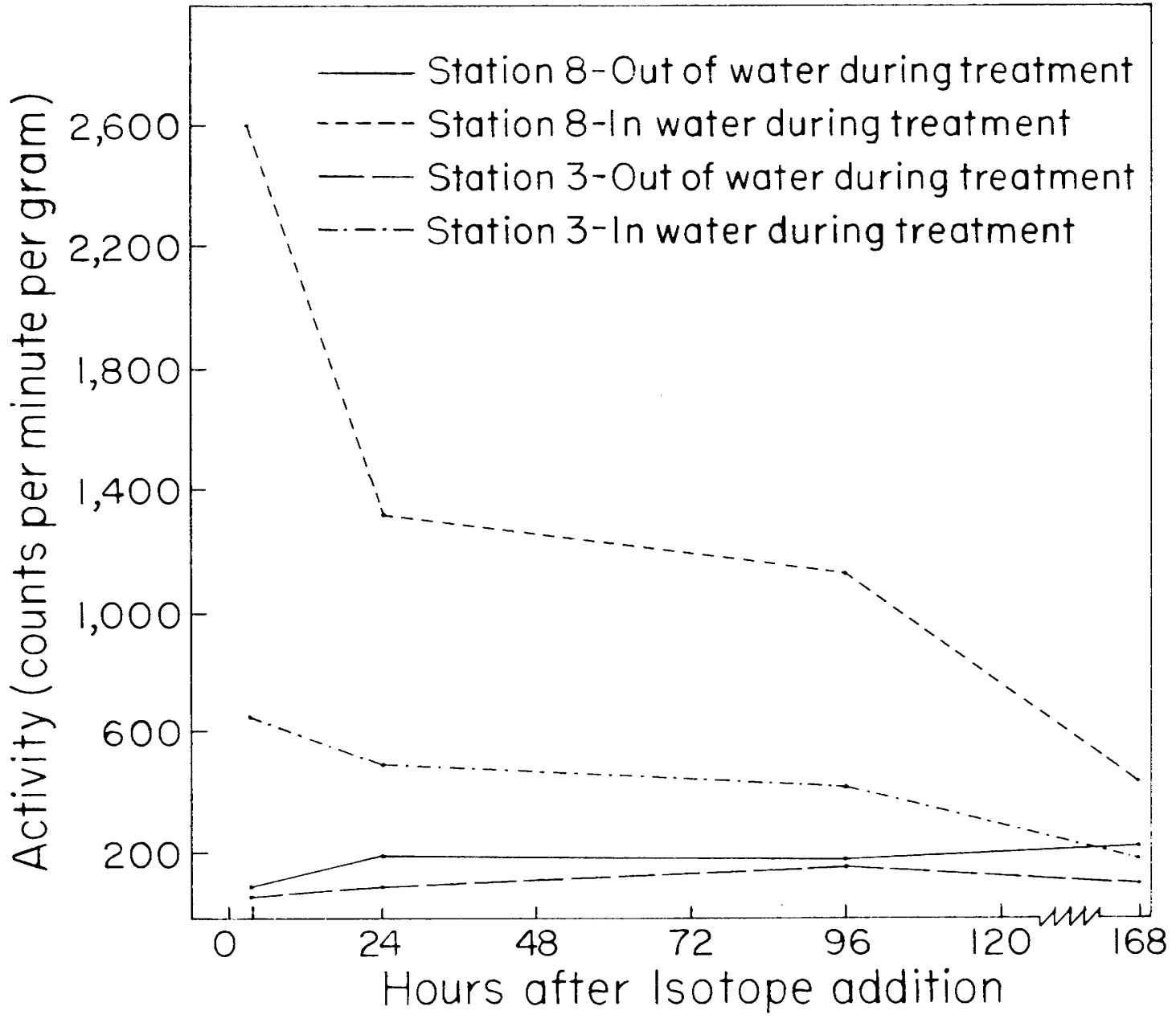
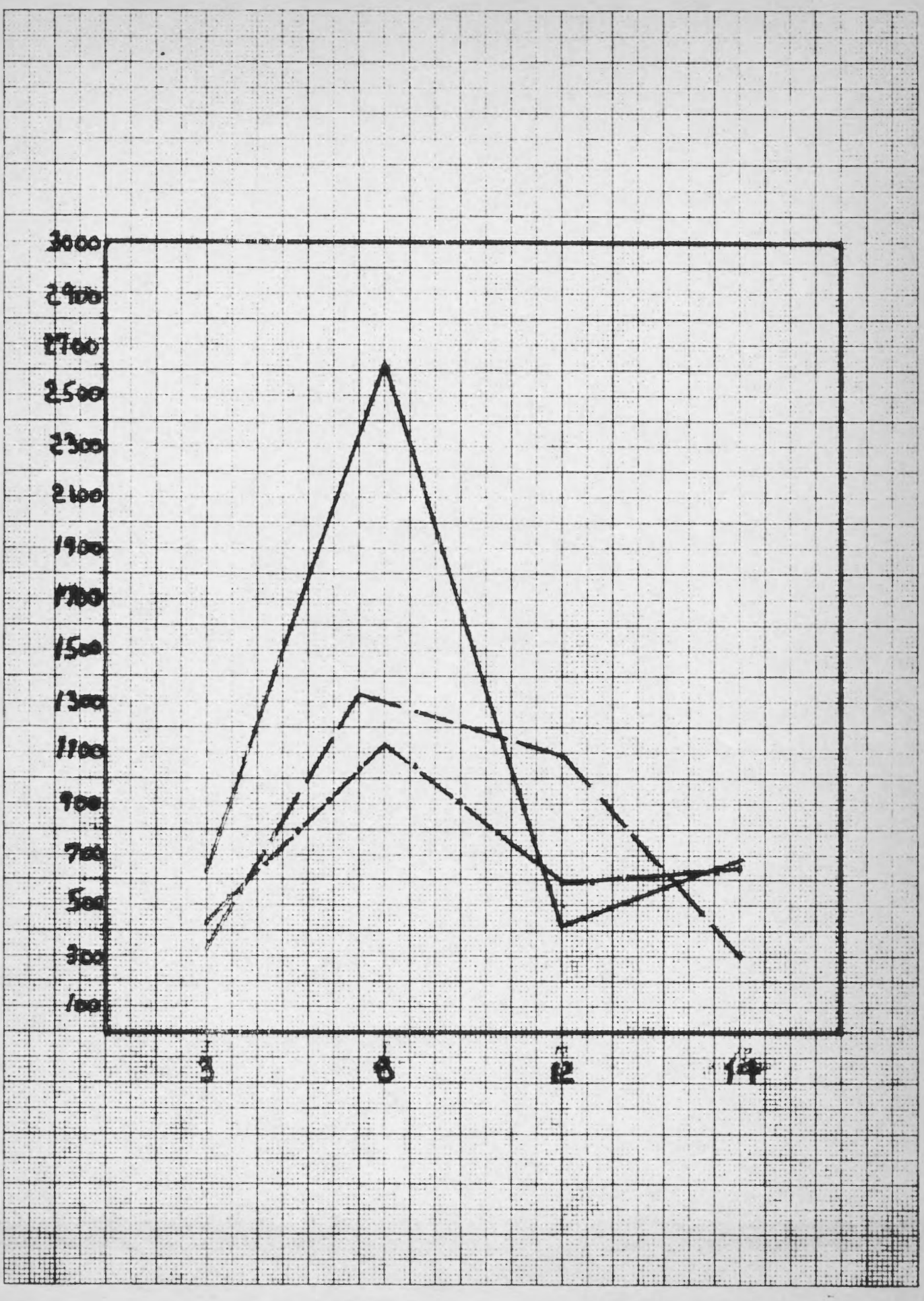

Station 8-Out of water during treatment
Station 8-In water during treatment
Station 3-Out of water during treatment
Station 3-In water during treatment
Activity (counts per minute per gram)
2,600
2,200
1,800
1,400
1,000
600
200
0
24
48
72
96
120
168
Hours after Isotope addition

Figure 7. Activity of periphyton at stations 3, 8, 12, and 14 on three dates. Counts corrected for background and decay.



in the regenerated phosphorus present at this point in the stream.

Uptake of radiophosphorus by periphyton was considerably lower in 1959 than in 1958 at Stations 3, 5, and 14. In 1958 at Station 3 the activity of substrates collected on the day of the isotope dosage was 7,972 counts per minute per gram as compared to 650 at this station on July 8, 1959. At Station 14 the count was 1,900 counts per minute per gram in 1958, as compared to 700 in 1959. Only at Station 8 was the initial uptake in the range of 1958 value. Here it was 2,600 counts per minute per gram in 1959 (somewhat higher than the 2,200 counts recorded in 1958).

The much greater uptake in 1958 particularly at the upstream stations almost certainly was due to difference in the fertilization treatment administered before the isotope was released. The fertilizer increased the standing crop of periphyton and the pool of phosphorus in the upstream areas. The larger pool of phosphorus made possible the more complete removal of the radioactive phosphorus atoms.

Uptake by other aquatic plants. Curves showing the decrease in activity with time (Figure 8) for Chara, Potamogeton and Fontinalis were strikingly similar to the activity curves for periphyton. All of these aquatic plants showed an initial logarithmic decrease in activity followed in from four to ten days by an equilibration phase in which there was little or no further fall in activity. The aquatic moss, Fontinalis, seems to have reached the equilibration phase somewhat sooner than the other species and to have equilibrated at a higher level of activity than either Chara or Potamogeton. Periphyton had the greatest initial uptake of activity and was last in reaching a equilibration stage. Potamogeton had an initial uptake somewhat higher than Fontinalis and Chara but an equilibration level below Fontinalis and within about the same range or lower than Chara. Curves for Fontinalis and Potamogeton suggest that the equilibration level was maintained throughout the month of August or for approximately 50 days after isotope addition.

If the source of activity in the environment at a given station is assumed to be the same for all of these types of plants then it would appear that the activity level at equilibration is proportional to the capacity of the plant to concentrate phosphorus from the water. The moss Fontinalis thus appears to have the ability to maintain a higher concentration of phosphorus than other species. A somewhat higher initial uptake of the isotope dosage by Potamogeton and periphyton suggested that the passage of marked atoms into the plant cell was more rapid for these forms than for Chara and Fontinalis. This in turn may have been related to a relatively high concentration of phosphorus in the cells of Potamogeton and periphyton.

The uptake level of isotopes by plants other than periphyton was considerably higher in 1959 than in 1958. At Station 8 the activity after treatment on July 8, 1959 was 1,200 counts per minute per gram for Potamogeton compared to 400 counts per minute in 1958. Comparable counts for Fontinalis were 800 in 1959 and 400 in 1958. The activity of Chara was only slightly higher in 1959 (550 counts per minute per gram) than in 1958 (500 counts per minute per gram). The higher activity in 1959 most likely was due to the decreased periphyton uptake. It would seem that the pre-fertilization in 1958 built a large phosphorus pool in the periphyton as compared to the size of the phosphorus pool in Potamogeton, Chara and Fontinalis. In competition for phosphorus it appears that periphyton is better adapted to utilize sudden and temporary increases in fertility than other types of trout stream vegetation. Fontinalis and Potamogeton are not able to increase their phosphorus pool during such temporary enrichments. These plants probably obtain a larger percentage of the naturally occurring phosphorus during periods when the periphyton crop is low.

Activity of bottom invertebrates. Somewhat better methods used in collecting and preparing samples in 1959 reduced much of the variability encountered in the 1958 bottom fauna data. The high variability in the activity of snails (Physa) persisted. This perhaps is due to an omniverous food habit. A somewhat more consistent pattern of activity uptake by other species began to

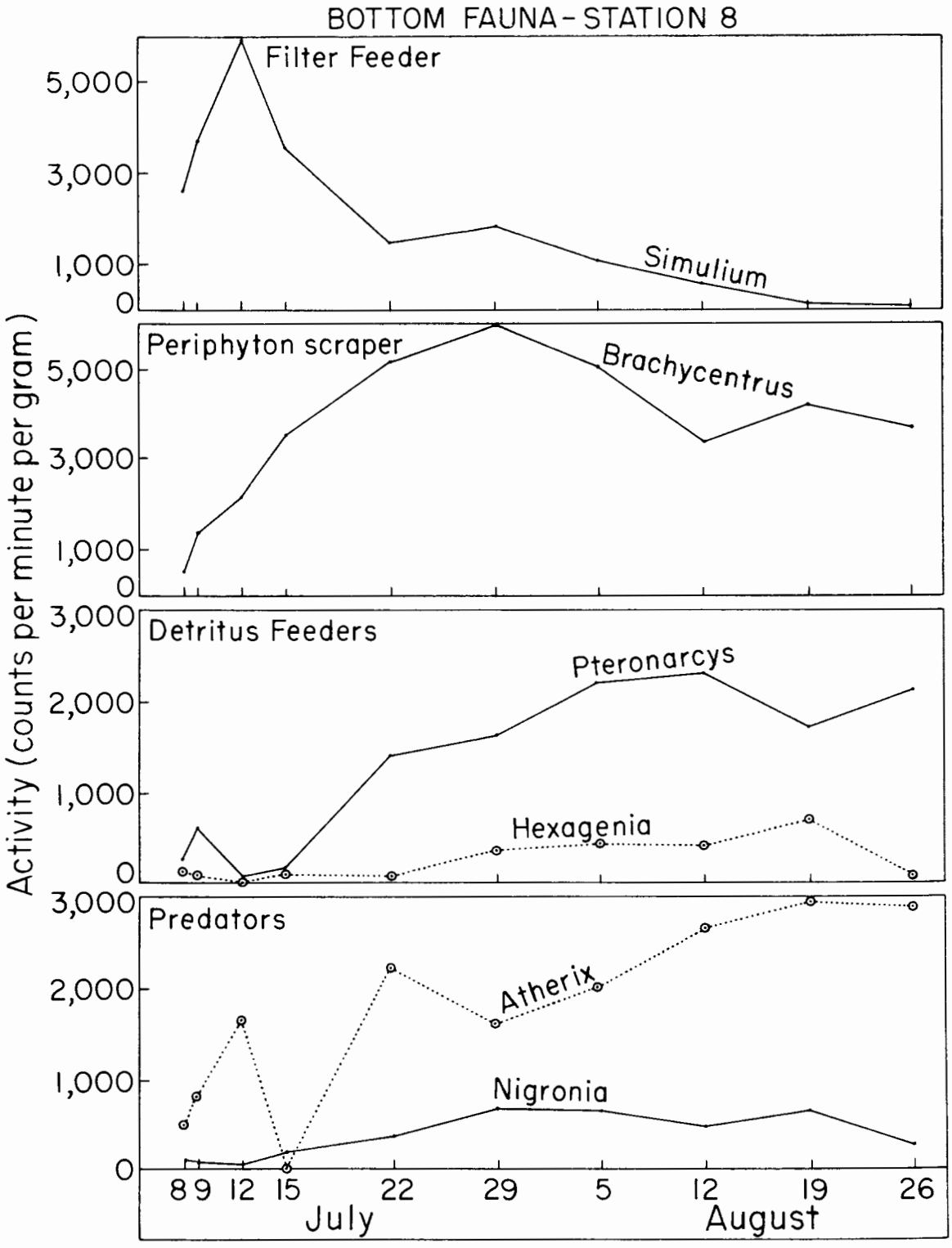
emerge which appears to be related to food habits.

Invertebrates studied. The black fly (Simulium) again showed an extremely high uptake on the day of isotope addition. Its activity decreased at a logarithmic rate thereafter, indicating there was very little uptake from regenerated materials and its source of activity had disappeared. (Figure 9) It now seems that the initial high activity of black flies was due to their filtering activity whereby they removed a considerable proportion of the water activity present in the form of solids. The caddis fly (Brachycentrus) which is a true plant feeder and subsists by removal of periphyton from rocks, had a maximum activity approximately 20 days after dosage--a similar activity pattern was shown by a heptageniid mayfly collected at Station 3. In the case of two predators, the snipe fly (Atherix) and larvae of the fish fly (Nigronia) activity continued to increase during most of the period of study. Similarly the activity of larvae of Hexagenia increased during most of the summer suggesting that this species, which is an ooze browser, must continue to pick up the radioactive detritus which settles into backwaters for a considerable period. Larvae of the stone fly (Pteronarcys) feeds upon debris that collects in backwaters and eddies. Its activity also continued to increase most of the summer.

The maximum activity of the invertebrates other than the black flies collected at Station 8 in 1959 was very close to or slightly higher than the maximum activity found in 1958. Since the periphyton activity in 1959 at this station was only slightly higher than in 1958, good agreement would be expected between the two years in the activity of primary and secondary consumers. The maximum activity of black flies at Station 8 was less than one-half that recorded in 1958. Since these insects are filter feeders and dependent on the drift from an upstream area, a lower activity in 1959 would have been predicted for black flies on the basis of a decrease in periphyton activity in 1959.

Fish. High variability persisted in the activity of fish in 1959 despite efforts to standardize collecting and sampling procedures. All the fish were from a restricted area of a given station and were of approximately the same

Figure 9. Activity of bottom-dwelling invertebrates representing four food niches. Counts corrected for background and decay.


BOTTOM FAUNA - STATION 8
Filter Feeder
Simulium
Periphyton scraper
Brachycentrus
Detritus Feeders
Pteronarcys
Hexagenia
Predators
Atherix
Nigronia
Activity (counts per minute per gram)
5,000
3,000
1,000
0
8 9 12 15 22 29 5 12 19 26
July
August

size (4 to 6 inches). Figure 10 shows that two specimens collected at the same time at the same station vary markedly in activity. In the case of brown trout any pattern of uptake or activity loss during the summer was obscured by this variation between individuals. In the case of muddlers there were some indications of a slow rise in activity, however, here also there is considerable variability. Such differences between individuals can only be due to variation in the activity of food consumed. Since at any one time there was considerable difference in the activity of the animals of various food niches, these differences in fish activity probably reflect nothing more than differences in the past feeding experience of individual fish. For example, a brown trout which had fed heavily upon periphyton-feeding caddis flies prior to August 5 might well have had a count in excess of 12,000 counts per minute per gram, whereas the activity of a fish which had fed upon detritus feeders such as Hexagenia might have been 2,000 counts per minute per gram or less.

The activity of the brook lamprey was somewhat less variable, particularly at Station 8. Activity never reached the high level encountered in trout and muddlers. It increased at a more or less steady rate after treatment. The rather slow activity increase and the low level of activity at its maximum is more suggestive of the uptake found in detritus feeders such as Hexagenia than of a periphyton feeder or a filter feeder. The activity of brook lampreys seemed to be somewhat higher in the downstream station (Station 12) than upstream (Station 8). This perhaps is due to a greater amount of radioactive detritus reaching the downstream station.

Exclosure Experiment. Difference in activity of water inside and outside the plastic exclosure during passage of the isotope dosage is shown in Figure 11. Clearly the exclosure only delayed the time of entrance and greatly reduced the amount of activity entering the test area. It is apparent that slow seepage of activity into the exclosure continued for a considerable period although such activity was of a low level. The activity curves of invertebrates collected in and outside the area are of interest and throw some light upon the

Figure 10. Activity of fish and lampreys following treatment of the stream with P^{32}. Counts corrected for background and decay.

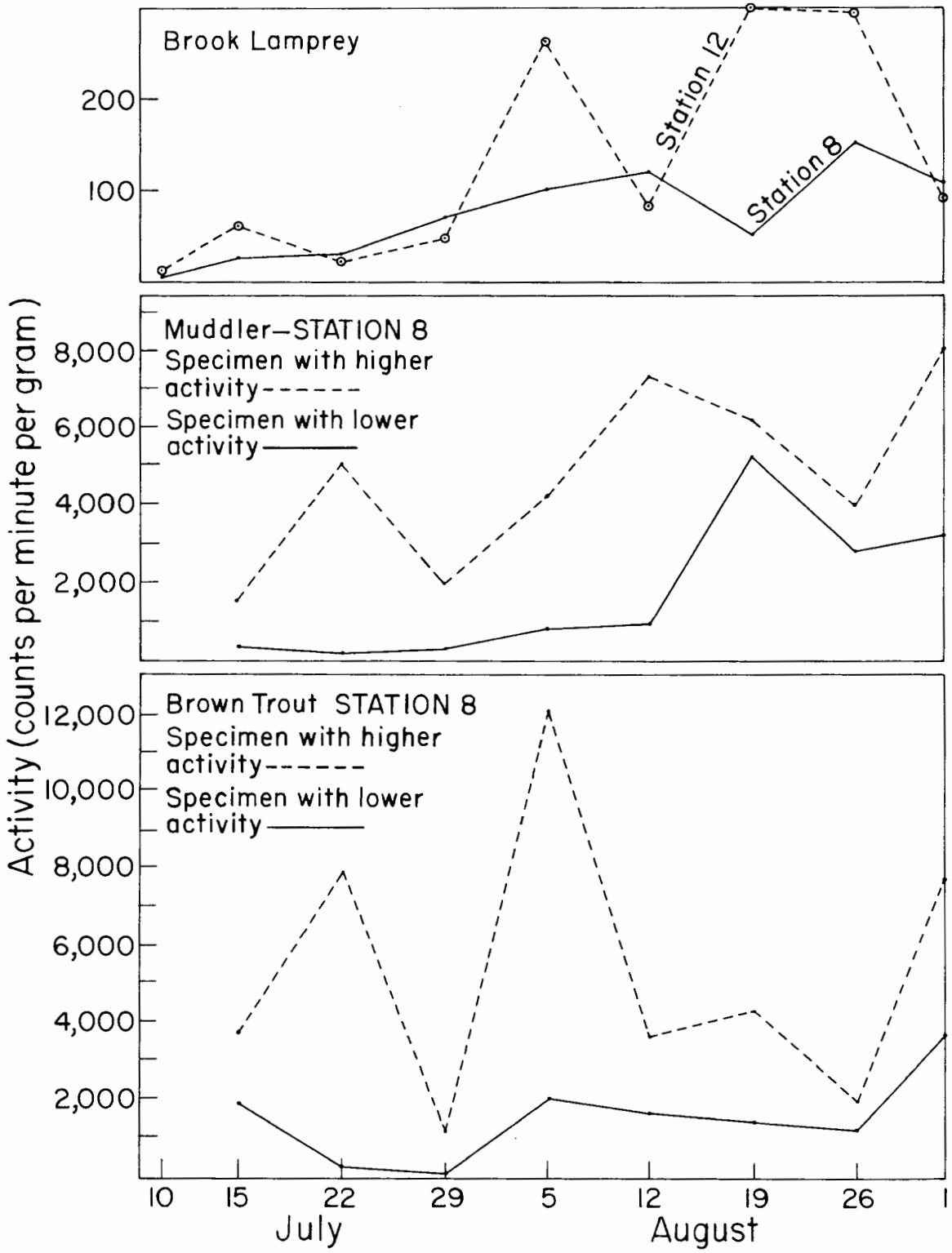
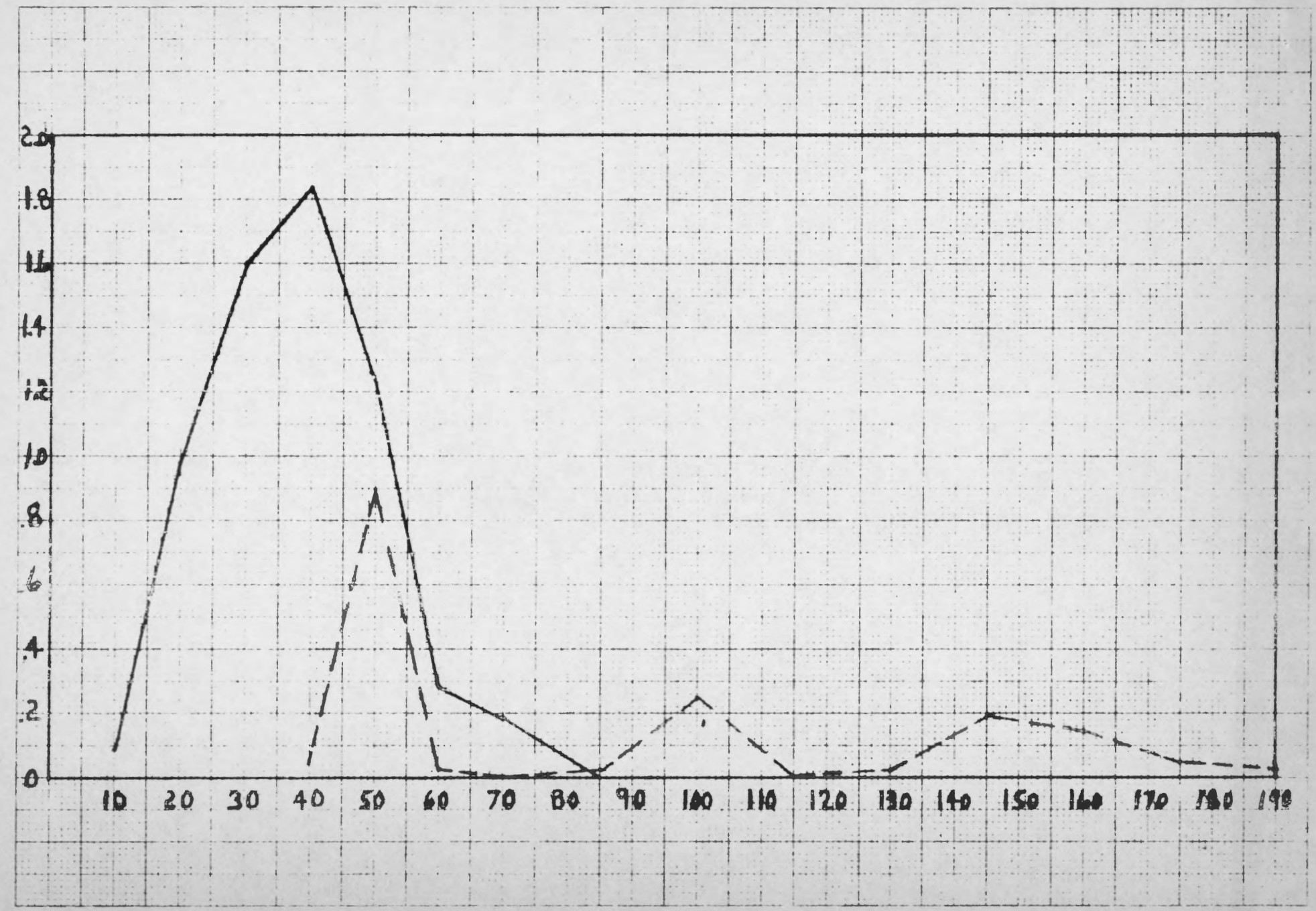
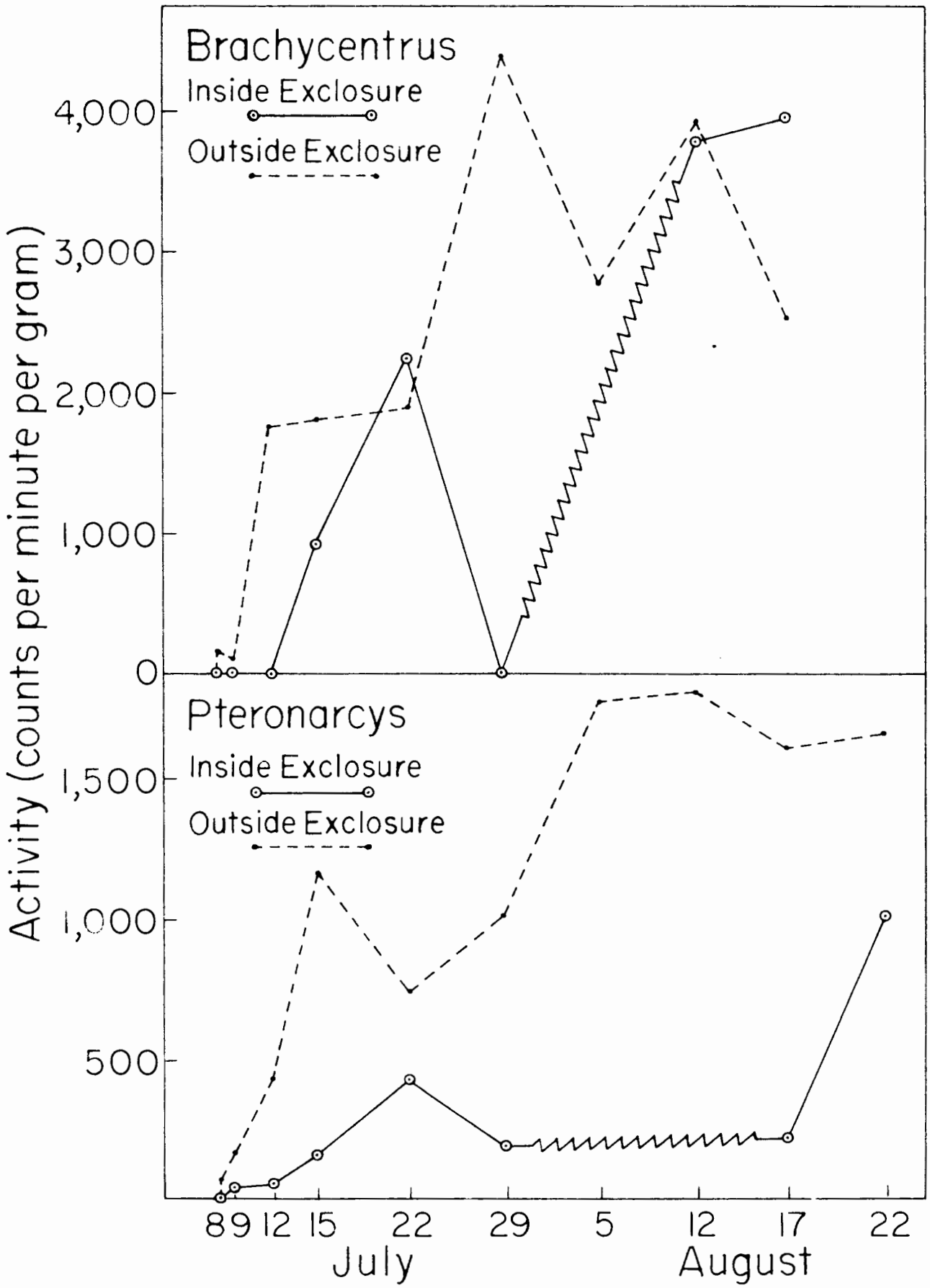

Brook Lamprey
Station 12
Station 8
200
100
Muddler—STATION 8
Specimen with higher activity------
Specimen with lower activity——
8,000
6,000
4,000
2,000
Brown Trout STATION 8
Specimen with higher activity------
Specimen with lower activity——
12,000
10,000
8,000
6,000
4,000
2,000
Activity (counts per minute per gram)
10
15
22
29
5
12
19
26
1
July
August

Figure 11. Water activity inside and outside of exclosure during passage of isotope down stream. Counts corrected for background and decay.


2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190

means by which the isotope moves from point to point within the stream. The rate of activity uptake was much greater for the first four days after treatment, outside than inside the plastic exclosure, for both Brachycentrus and Pteronarcys. (Figure 12) It was only after July 12 that significant activity was encountered in these invertebrates within the exclosure. Thereafter their activity was quite erratic. The failure of activity to increase during the first four days suggest that there was little or no high-activity food available within the exclosure for these insects. The rise in activity after July 12 may have been due either to (1) migration of individuals into the area from the surrounding stream or (2) the movement or growth of radioactive food material within the area subsequent to isotope treatment. Individuals within the exclosure became increasingly hard to collect. Data were missing for some of the collecting dates after July 29 and the data in general were too meager to permit a decision as to which of the two possibilities is correct. Further analysis of the exclosure data may provide an explanation.

Figure 12. Activity of the caddis fly, Brachycentrus and the stone fly, Pteronarcys inside and outside of exclosure. Counts corrected for background and decay.


Brachycentrus
Inside Exclosure
Outside Exclosure
4,000
3,000
2,000
1,000
0
Pteronarcys
Inside Exclosure
Outside Exclosure
1,500
1,000
500
Activity (counts per minute per gram)
8 9 12 15 22 29 5 12 17 22
July
August

Proposed Technical Program

Renewal Request AT (11-1) 655

"Study of Productivity in a Stream Ecosystem"

Our basic program aims are the same as outlined in the original proposal. Certain of these have been met in the two years work, others still remain to be worked out, and additional problems have been presented as a result of the research.

The program carried out during 1959 made several contributions to our knowledge of the rate and method of movement of nutrient phosphorus, as indicated by the movement of P^{32}, through the several trophic levels of a stream ecosystem and estimates of the rate of biological uptake and regeneration will be possible from the data.

The rate at which the P^{32} is incorporated into the several aquatic biological phases has been explored and estimates made, but the exact status or nature of the medium of transfer is not well understood. From autoradiographs of membrane filters it would appear that the particulate matter in which the P^{32} is incorporated is made up of organisms of bacteria-size or the smallest of the nanno-plankton.

To determine the exact method of movement of the nutrient through the stream system we are planning to incorporate the entire mass of P^{32} into a bacteria culture and release this into the stream in a media-free state. In the work of the past two years we have put the isotope into the stream in the inorganic form and it has been taken up immediately by the periphyton diatoms and moved down the stream system by re-cycling through the trophic structure.

By putting the P^{32} into the stream in organic form we believe we will be able to more thoroughly explore the methods and mechanism of biological uptake, assimilation, release, and transport of an isotope within the stream ecosystem.

The program offers many possibilities, and some important uncertainties, in the pre-treatment mass culturing of bacteria. In view of the possible, but not anticipated, failure to establish a completely satisfactory pre-treatment program we are planning an alternative program that can be activated on very short notice and which will give equally needed information on the basic concepts of this program. It is as follows:

From work done recently on marl lakes (Hooper) and in our laboratory (Ball) in an artificial stream it has been determined that certain of the versenes will (1) release to the water phosphorus bound in bottom deposits of a lake and (2) almost quantitatively release bound (adsorbed?) phosphorus into the circulating water system of the artificial stream. Thus we believe further careful study of the effects of the chelating compounds on the release of bound ions and their possibility of keeping such ions in the moving phase of a stream system will contribute important knowledge concerning the movement, distribution, and perhaps dispersal of an isotope.

We are planning to continue to explore the interrelationships of light intensity to quantitative uptake of nutrients. At the beginning of the 1959 program we put considerable faith and effort into the development of the anthracene-dianthracene methods of light evaluation. This effort was largely wasted and yielded little acceptable data. We expect to modify our methods of estimating the standing crop of primary producers, primary and secondary consumers, and our estimates of production within these trophic levels to give us a more sophisticated estimate of production within the stream system.