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DETERMINATION OF A NITROGEN-PHOSPHORUS BUDGET FOR BAYOU TEXAR, PENSACOLA, FLORIDA

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ABSTRACT

DETERMINATION OF A NITROGEN-PHOSPHORUS BUDGET FOR BAYOU TEXAR, PENSACOLA, FLORIDA

The extent of nitrogen and phosphorus inputs and their effects on algal productivity in Bayou Texar, Pensacola, Escambia County, Florida, has been under investigation since June, 1971. To date, results indicate Carpenter's Creek to be the major source of nutrient input in this estuarine bayou. In general, nitrogen and phosphorus concentrations are highest at the north end of the Bayou and decrease southward--a pattern which is directly related to carbon fixation rates, but inversely related to the extent of nitrogen fixation by the nitrogen fixing organisms in the Bayou. Preliminary results also suggest presence of phosphorus in the sediment muds and replenishment of water column phosphorus by phosphorus from the sediment. Currently, all phases of the project are being continued in order to determine seasonal trends as well as other sources of nutrient input. Concerted efforts are being directed toward the kinetics of phosphorus exchange between the sediment and the water column.

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Introduction

In recent years, extensive estuarine waters of northwest Florida have been showing alarming increases in levels of degradation. Causes are similar to those already described in the case of other estuaries (Ketchum, 1969; Carpenter, et al, 1969), and include industrial and domestic wastes, as well as those resulting from bottom dredging.

Of particular interest in these waters are the bayou estuaries which penetrate and interlace a number of urban areas, and are particularly the subjects of industrial and domestic waste inputs. Such inputs soon cause cultural eutrophication and general degradation of these important waterways.

Bayou Texar, Pensacola, Escambia County, Florida, is such a bayou, extending northward from Pensacola Bay (Figure 1). This body of water is rapidly becoming eutrophic due to siltation and nutrient enrichment from a number of sources. The principal inflow source is Carpenter's Creek which enters the northern limits of the bayou. This stream, in turn, receives a large volume of natural run-off, as well as effluents, from a number of sewerage treatment lift stations. In addition to Carpenter's Creek, run-off from fertilized lawns of private dwellings, located in the Bayou Texar watershed, are suspected sources of nutrients into this body of water. Such enrichments are rapidly converting this suburban bayou into a shallow, eutrophic system unfit for recreational and aesthetic purposes. This trend is evidenced by recent restrictions placed on recreational activities in this body of water by the Florida Department of Health and Rehabilitation Services, as well as by the fact that numerous fish kills have been reported there during the past three years.

This project which was started in June, 1971 was partially funded by the Office of Water Resources Research under Annual Allotment Agreement Number 14-31-0001-3509 for the summer months of 1971. Concerted efforts have been directed toward frequent sampling for nutrient level determinations, as well as toward primary productivity studies. In addition, exploratory efforts have been directed toward the determination of the extent of nitrogen fixation in the water column and bayou muds, as well as the extent of nutrient accumulation in the sediments. These results have justified our decision to intensify our efforts in these areas in the future. Culture work, in progress since summer, has been modified and will be continued through the duration of the

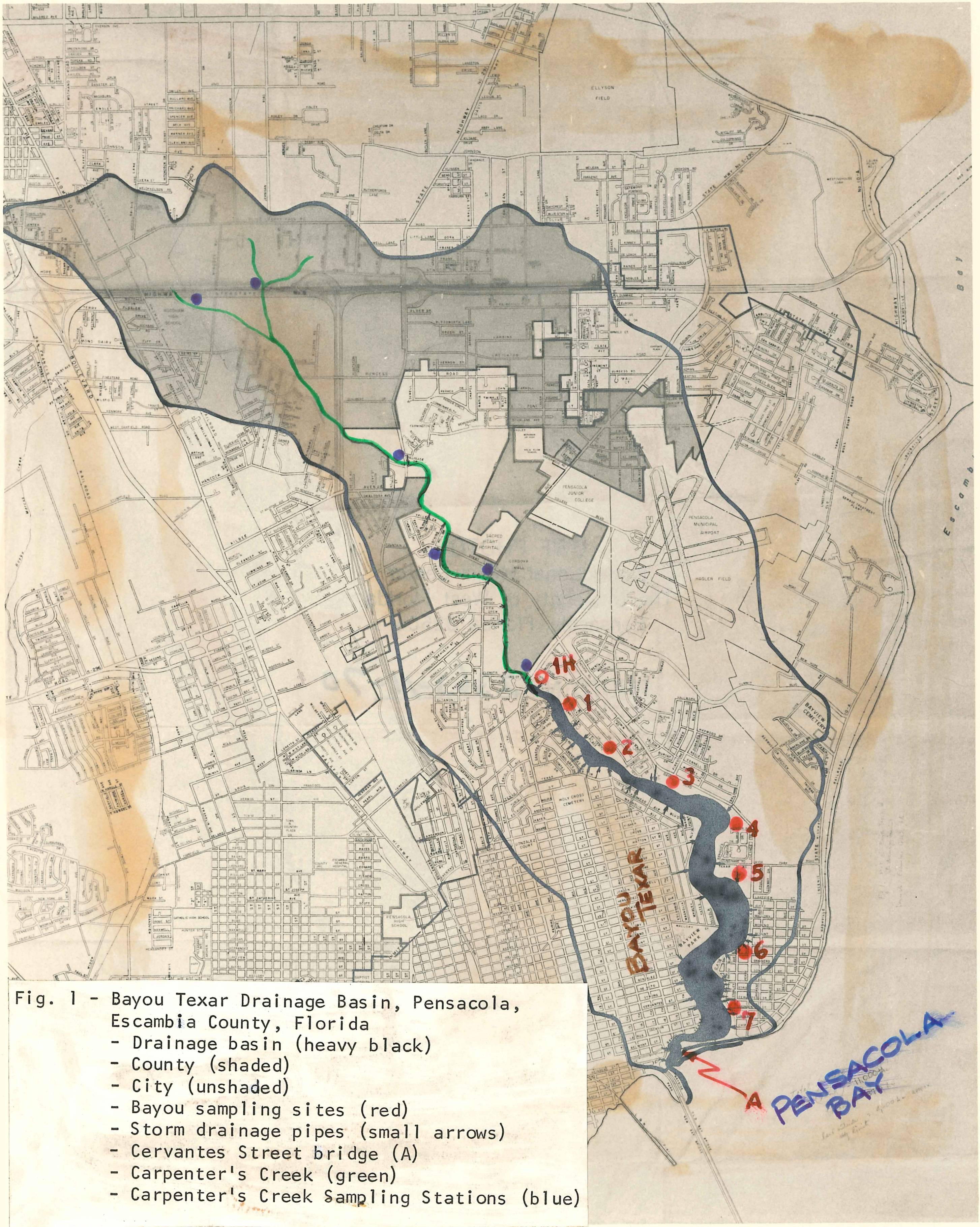

Fig. 1 - Bayou Texar Drainage Basin, Pensacola, Escambia County, Florida
- Drainage basin (heavy black)
- County (shaded)
- City (unshaded)
- Bayou sampling sites (red)
- Storm drainage pipes (small arrows)
- Cervantes Street bridge (A)
- Carpenter's Creek (green)
- Carpenter's Creek Sampling Stations (blue)

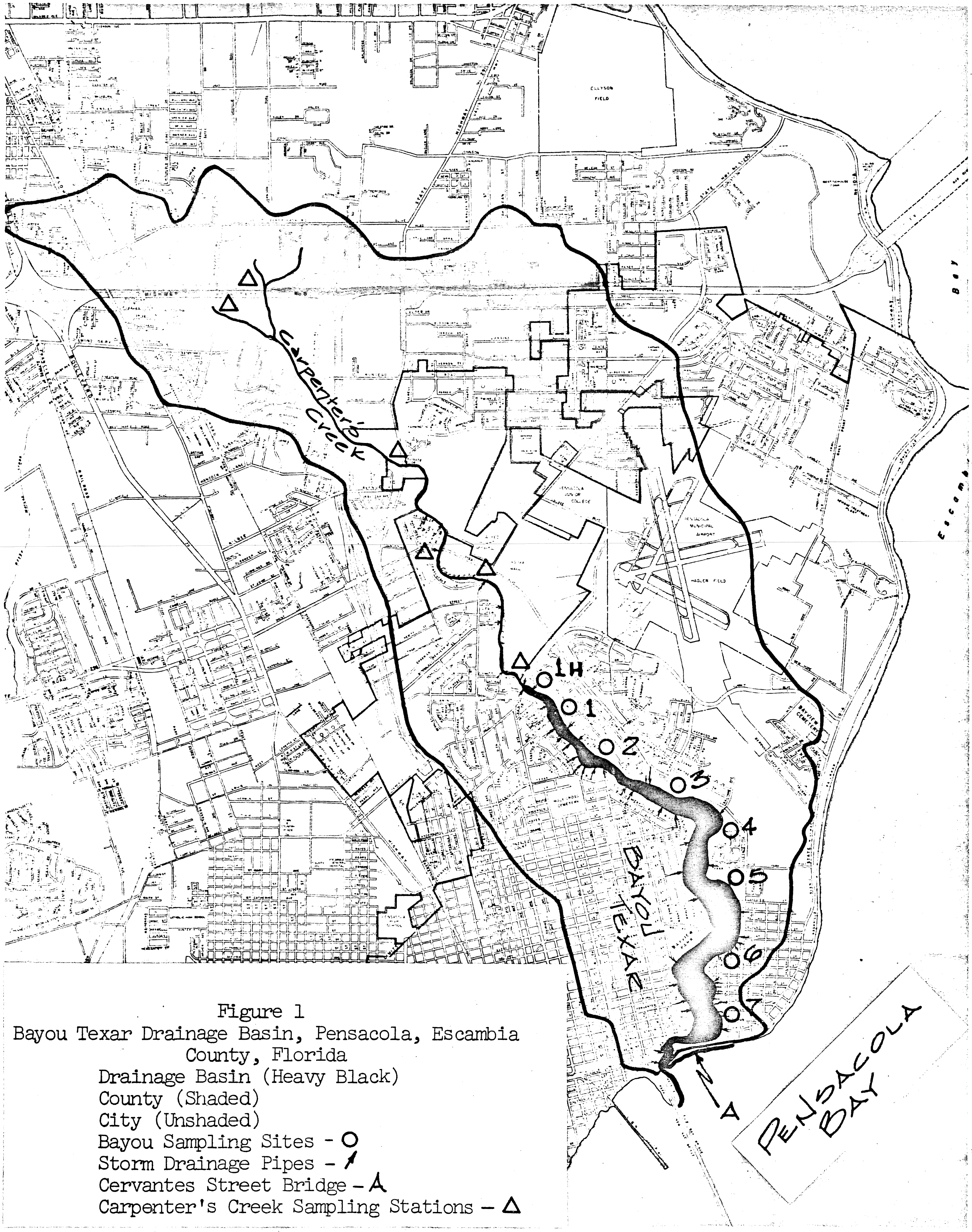

Figure 1
Bayou Texar Drainage Basin, Pensacola, Escambia County, Florida
Drainage Basin (Heavy Black)
County (Shaded)
City (Unshaded)
Bayou Sampling Sites - O
Storm Drainage Pipes - ↗
Cervantes Street Bridge - A
Carpenter's Creek Sampling Stations - △

project.[1] This should give valuable information concerning the effects of Carpenter's Creek water on the primary productivity of Bayou Texar.

Information presented here is actually a report on one season of a planned twelve-season study. We expect to put much effort into determining spatial and temporal factors involved, and the effects of such factors on nutrient inputs and on primary productivity of Bayou Texar. It is expected that information gathered during this study will aid in the recommendation of specific alleviation methods for Bayou Texar and other nearby bayou-estuaries, such as Bayou Grande and Bayou Chico, afflicted with many of the same problems.

Methods

All data collection has been against a background of physical-chemical parameters measured at each collection site. These include light, temperature, salinity, conductivity, alkalinity, pH and dissolved oxygen. Light measurements were made using a TSK light meter, and a Beckman salinometer was employed in the measurement of temperatures, salinities, and conductivities. Alkalinity determinations were made using standard techniques, and pH and dissolved oxygen determinations made using an Orion portable pH meter, and a Delta scientific field oxygen meter, respectively.

Water samples for nutrient analysis were collected weekly from seven sites (Figure 1). Collections were made in acid-rinsed polyethylene bottles and kept on ice until returned to the laboratory for analysis. These samples were filtered (HA Millipore filters, 0.47μ pore size) and analyzed for ammonia, employing the method described by Solórzano (1969); for nitrite using the technique suggested by Strickland and Parsons (1968); and for nitrate, the method of Kahn and Brezenski (1967). For phosphate determinations the methods described by Strickland and Parsons (1968) were principally employed. Orthophosphate levels were determined by reaction with an acidified molybdate solution to form a phosphomolybdate heteropoly acid, the concentration of whose reduced form (phosphomolybdenum blue)was then determined spectrophotometrically. It is anticipated that starting in the Spring of 1972 assays will also include those for organic nitrogen and phosphorus. With the former, measurements will employ the micro-Kjeldahl method of Miller and Miller (1948), and the analysis of the ammonia released by the technique to which reference has been made above. In

[1]This research is being continued under the sponsorship of the University of West Florida. A Matching Grant Proposal for a second phase was submitted for FY 1973 (B-016-FLA).

the case of organic phosphorus, the acidified samples are oxidized and the resulting orthophosphate analyzed as already described.

In conjunction with the Carpenter's Creek inflow point, as well as sediments further down the bayou, preliminary investigations now under way are designed to measure the extent of phosphorus accumulation in the sediments. For this reason, bottom samples are taken at stations 1, 4, and 6 (Figure 1) and the muds analyzed for phosphorus, using techniques essentially the same as those already described for water samples. However, some modifications to adapt these procedures to sediment analysis have been described by Jackson (1958), and Harwood, et al (1969).

In addition to the assay techniques already described for various nitrogen species, some preliminary experiments employing the acetylene reduction method were employed to determine the extent of nitrogen fixation by bacterial and algal populations in the Bayou Texar ecosystem. Some of the results of this work, as conducted by Mr. Michael Eaton of The University of West Florida, are presented here. This method promises to serve as a diagnostic tool in the estimation of nitrogen levels in an aquatic system, as well as a technique for the quantification of nitrogen inputs into such system through fixation.

The technique employed is essentially that described by Stewart, Fitzgerald, and Burris (1968), as modified by Edmisten and Eaton. Samples were collected in 125 ml Erlenmeyer flasks sealed with serum stoppers. Each of these was innoculated with 20 ml of acetylene and analyzed by gas phase chromatography using a Beckman GC-5 Gas Chromatograph. A standard ethylene production curve was established utilizing an incubation period of twenty-four hours. This time duration was used to yield optimum production.

Sediment samples were flushed with helium to maintain an anaerobic atmosphere and then incubated in total darkness in order to eliminate the possibility of fixation by photic organisms. Water samples were processed in unconcentrated form, and incubated under aerobic conditions in the presence of sunlight. Finally, because of their abundance on the shores of the Bayou, samples of *Myrica cerifera L.* (Wax Myrtle) and attached algae were also tested by the acetylene reduction technique. Data thus produced were evaluated for ethylene peak height per gram (or per milliliter of sample) per microliter of gas phase of sample introduced into the gas chromatograph.

This exploratory work extended over six sampling periods, of which three involved water and three involved sediment samples. The stations at which samples were collected were the same as those at which collections were made for nutrient analysis (Stations 1-7, Figure 1). One additional station, 1H, was located just north of Station 1 (Figure 1).

Following incubation of samples, 5 ml aliquots of trichloral acetic acid (TCA) or mercurous chloride were added to each flask to prevent further nitrogen fixation. It was discovered, however, that the addition of TCA to sediment samples resulted in its reaction with carbonaceous materials, such as oyster shells, and the release of excessive amounts of CO_2. Therefore, in the case of sediment samples, only mercurous chloride was used to terminate the fixation activity. Additional difficulties encountered in the use of the acetylene reduction technique included the presence of background ethylene found as an impurity in the acetylene used for innoculation. Therefore, ethylene control peak heights were used to correct all ethylene peak heights.

In conjunction with the evaluation of nutrient levels in the bayou, experiments have been under way to measure the effects of the Carpenter's Creek water on the rates of carbon fixation by the phytoplankton in the Bayou. The approach taken is threefold: 1) measurements of temporal and spatial variations in surface primary productivity; 2) measurement of the specific effects of Carpenter's Creek water on the Bayou primary productivity through field and laboratory culture experiments; and 3) measurements, through culture experiments, of the effects of augmentation of nitrogen and phosphorus levels on the carbon fixation rates in the Bayou. Once again, all work described is conducted against a background of physical-chemical data as previously stated. To these have been added the collection of water samples to determine algal cell numbers to correlate with carbon fixation data.

It must be stated that certain design modifications have been implemented in this work since the original proposal was submitted last spring. These modifications were made following examination of data collected throughout the summer.

1. <u>Temporal and spatial variations in surface productivity</u>.

Water samples are collected just below the surface using a nonmetalic sampler. Duplicate light and single dark bottles containing water samples from stations 1, 2, 4 and 6 (in contrast to the original design using all seven stations) are innoculated with ^{14}C sodium bicarbonate and incubated at station 4 from 10 A.M. to 2 P.M. (Figure 1). In addition to these, diel studies are conducted on a quarterly basis at stations 2, 4, and 6 in order to provide additional information on the carbon cycle and on diel variations in primary productivity. Information collected during the dark part of the diel gives some estimate of community respiration not made available through tracer studies.

2. Effects of Carpenter's Creek water on carbon fixation in the Bayou.

Bayou water is innoculated with 5, 25 and 50 ml aliquots of filtered Carpenter's Creek water collected near the mouth of the creek (Figure 1). Same aliquots using filtered spring water are used as controls. This is designed to compensate for cell-free volume, and changes in salinity and alkalinity. Once again, the procedure used is that involving duplicate light and single dark bottles as previously described. This procedure is now conducted monthly with collections at stations 4 and 6 and incubations at station 4. The initial design involved sample collection and incubation at all seven stations once each week.

3. Effects of Carpenter's Creek nutrients on carbon fixation in the Bayou.

The approach employed here is essentially the same as that outlined in part 2 above, and is aimed at determining the effects of Carpenter's Creek nitrogen and phosphorus species on primary productivity of Bayou Texar. Creek water is analyzed for inorganic nitrogen and phosphorus compounds, and solutions containing the same concentrations of these nutrients as the Creek are prepared using spring water. These are used to innoculate bottles of Bayou water collected from station 6 in the same experimental and control dilutions described in part 2 above. These cultures are then incubated in the laboratory. This experiment, which is supplementary to those originally proposed, is being conducted twice each month.

Results and Discussion

1. Nutrients

Results of assays for nitrate and ammonia levels in the surface and bottom waters during the summer months appear in Figures 2 and 3, Table 1; for phosphates in Figure 4 and Table 1. Reference to these figures suggests a decrease in

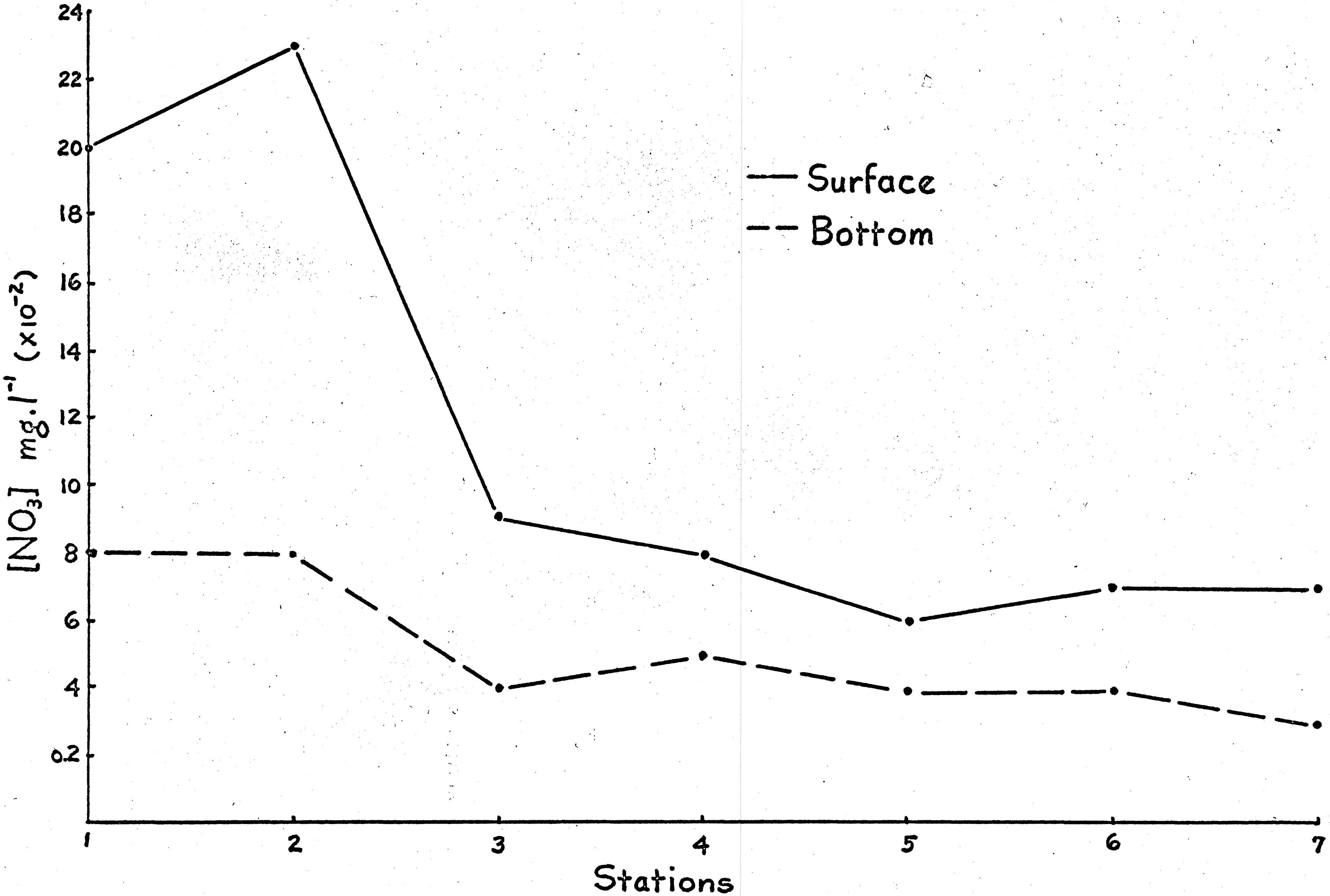

Figure 2 - Surface and bottom nitrate concentrations. Each point represents a mean of 17 determinations between May 20, 1971 and September 29, 1971.

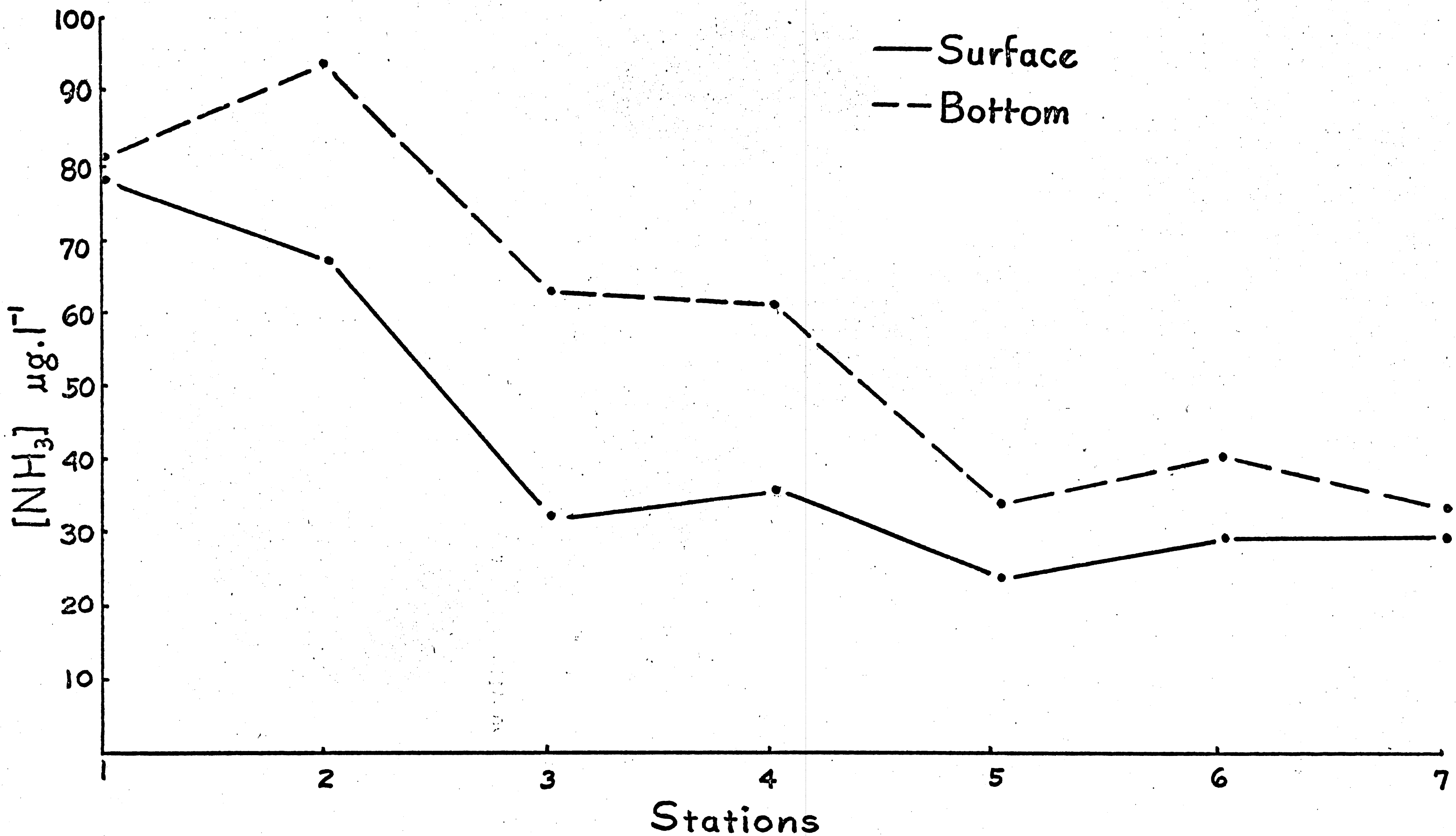

Figure 3 - Surface and bottom ammonia concentrations. Each point represents a mean of 21 determinations between May 20, 1971 and September 29, 1971.

Table 1 - Mean values for NO_3-N, NH_3-N, and PO_4-P for surface (S) and bottom (B) Bayou Texar water samples from May 29, 1971 to September 29, 1971.

Stations		NO_3-N in mg. l^{-1}*	NH_3-N in μg. l^{-1}*	PO_4-P in μg. l^{-1}**
1	S	0.20	78	14
	B	0.08	81	9
2	S	0.23	67	11
	B	0.08	94	9
3	S	0.09	32	13
	B	0.04	63	6
4	S	0.08	36	9
	B	0.05	61	6
5	S	0.06	24	9
	B	0.04	34	8
6	S	0.07	29	9
	B	0.04	40	8
7	S	0.07	29	8
	B	0.03	33	9

* Values represent means of 21 determinations.
** Values represent means of 17 determinations.

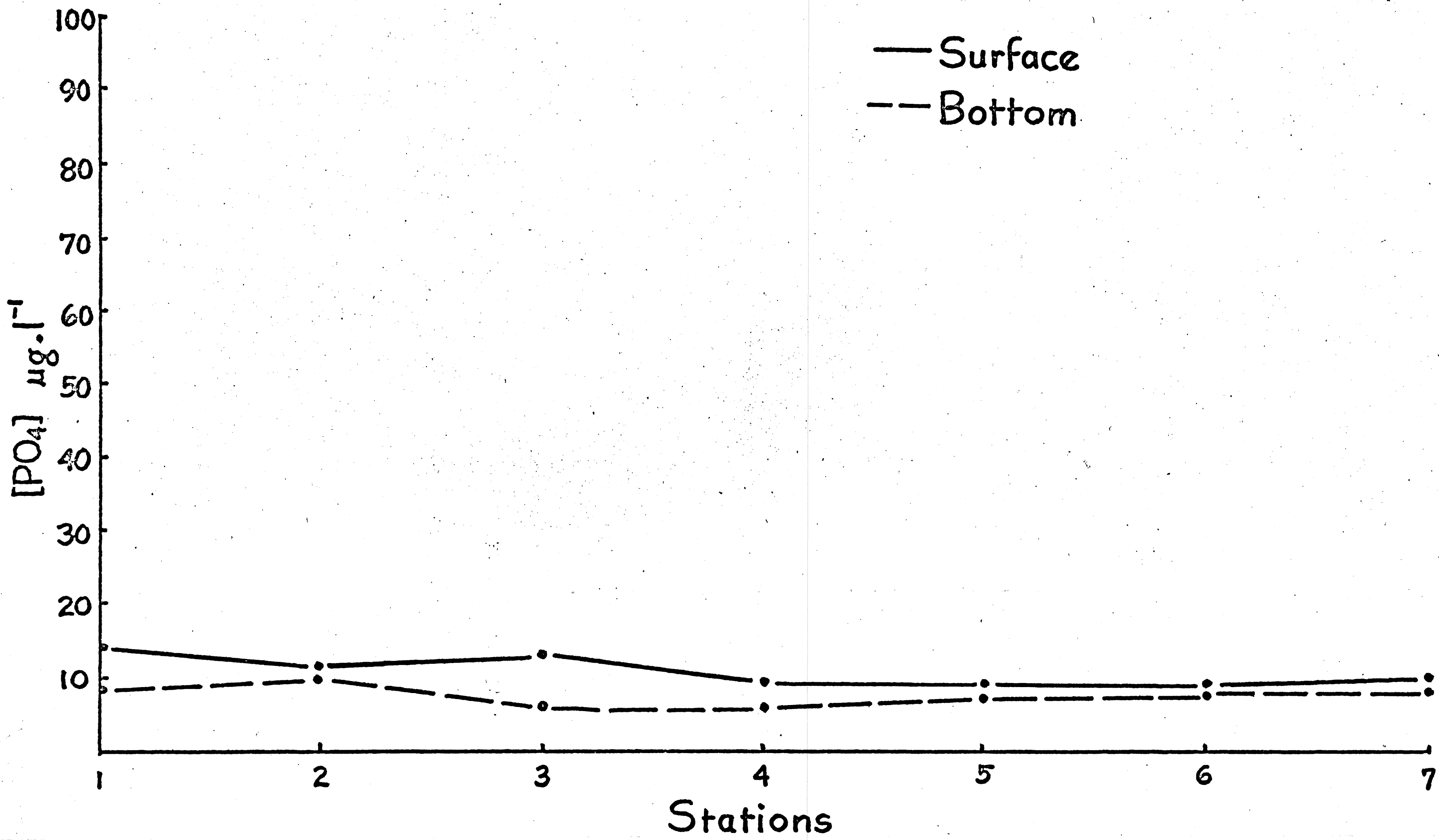

Figure 4 - Surface and bottom orthophosphate concentrations. Each point represents a mean of 17 determinations between May 20, 1971 and September 29, 1971.

nutrient levels from station 1 to station 7 (Figure 1), although the steep rate of decrease declines beyond station 2. Both nitrate and ammonia levels start at relatively high levels at station 1 reflecting the extent of nutrient input from Carpenter's Creek. Surface and bottom ammonia concentrations follow much the same pattern, although concentrations in the bottom waters were consistently higher for all stations, except at station 1 at which point there seemed to be little difference between surface and bottom ammonia concentrations. This pattern is most likely due to the extent of organic matter accumulation and decomposition in the sediment. This is supported by dissolved oxygen data showing a significant decrease from surface to bottom (Figure 5). At station 1, sedimentation takes the form of sand and silt, and the effects of Carpenter's Creek flow prevents accumulation of lighter particles including organic materials. Examination of sediments at this site indicates a layer of sand and silt, ranging from 25-30 cm thick, covering a layer of partially decomposed organic materials mixed with silt and loam. Presumably, this is the result of the straightening of the Carpenter's Creek channel for mosquito control purposes, as well as the result of increased construction activities in the Bayou Texar drainage basin during recent years.

It is interesting to note that this pattern of nitrate and ammonia distribution in the bottom waters shows an inverse relationship with the extent of nitrogen fixation in the Bayou muds. Results of preliminary studies indicate that the extent of nitrogen fixation was consistently lower at the upper Bayou stations (stations1H, 1, 2) than at stations 3-6 (Figure 6). Brezonik, et al (1971) have shown that an inverse relationship exists between available nitrogenous nutrients and nitrogen fixation by aquatic plants. Therefore, substrates with high concentration of organic substances, and accompanying high ammonia levels resulting from decomposition, do not show high levels of nitrogen fixation. However, it should be noted that, although as mentioned above, and sand-silt substrate at station 1 would be expected to show relatively high nitrogen fixation activity, the high nitrogenous input from Carpenter's Creek acts as an inhibiting factor in this respect.

Assays for nitrogen fixation in the water column have not been successful so far, although water samples taken from regions of dense phytoplankton concentration during blooms showed evidence of nitrogen fixation. It is suspected that more difficulty will be encountered in estimating fixation in the water column than in the sediment. This is partly due to the inhibitory effects of oxygen on the nitrogenase system of nitrogen fixers (Drozd and Postgate, 1969; Stewart, 1970), as well as the difficulties encountered in the concentration of water samples without the destruction of plant cells involved in the fixation process.

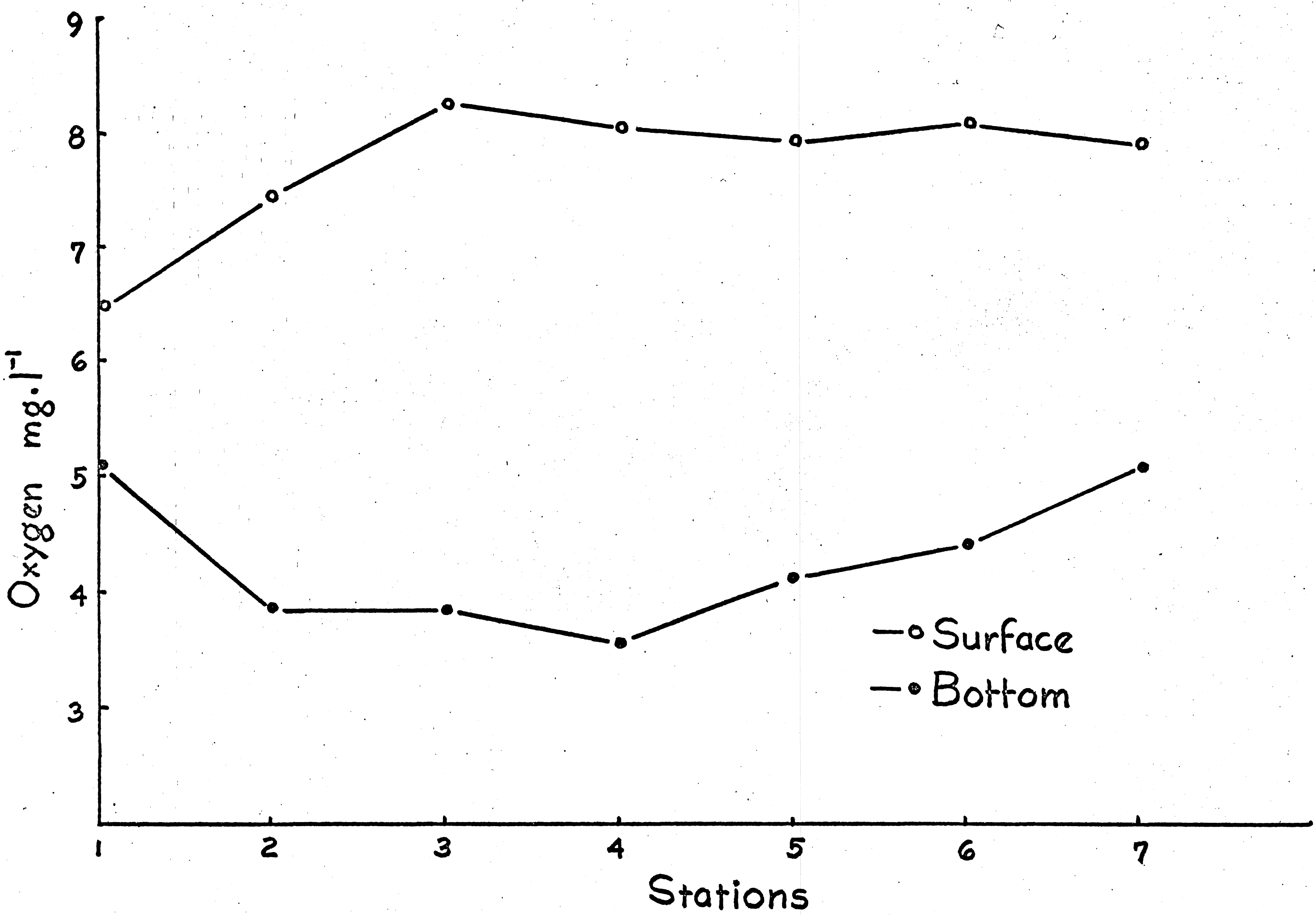

Figure 5 - Mean surface and bottom oxygen values from May through October, 1971.

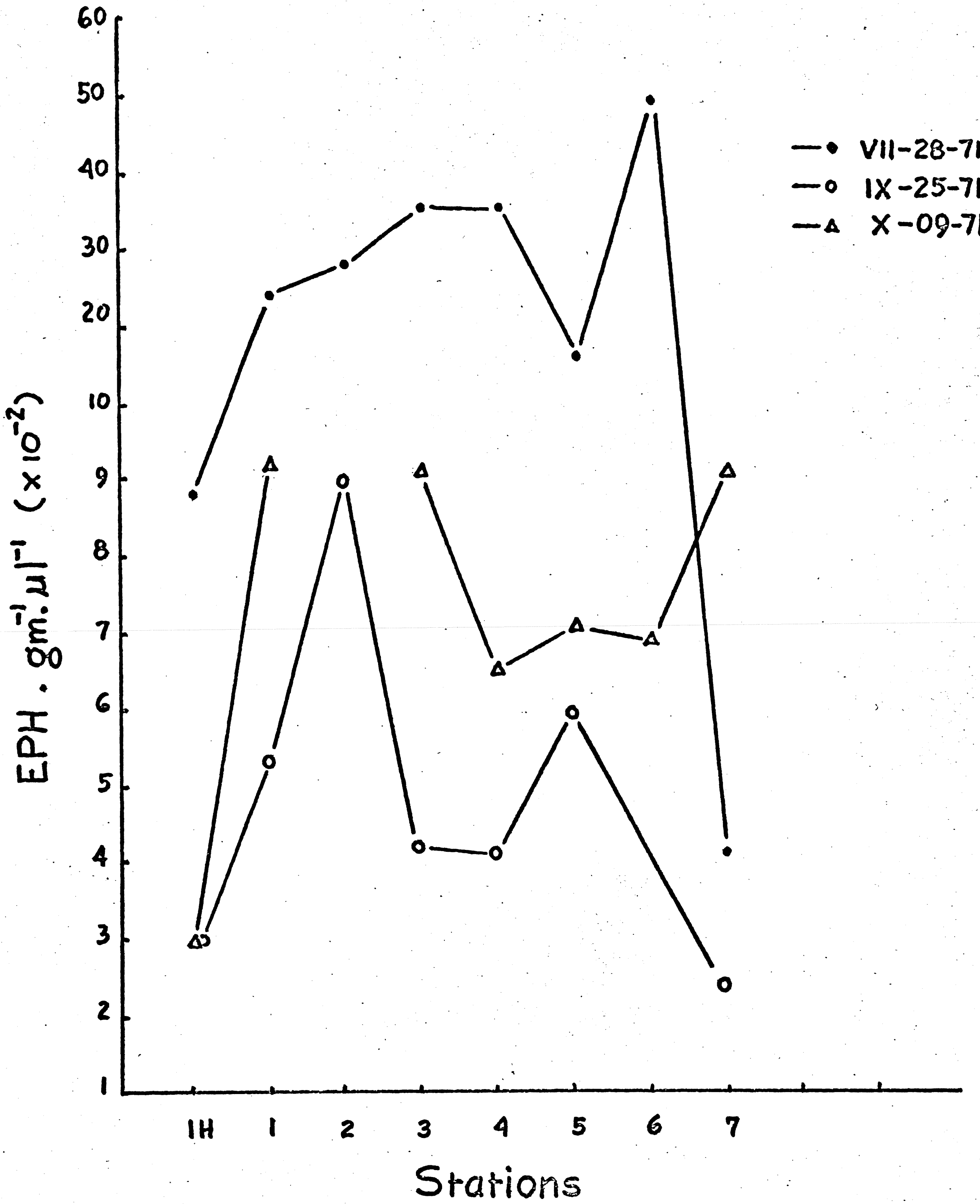

Figure 6 - Mean ethylene peak height (EPH) per gram of sample per microliter of gas phase of sample introduced into the gas chromatograph for three sampling dates. Sediment samples were taken at a depth of 5-15 cm.

So far, results of these preliminary experiments seem to justify further and more concerted efforts in this direction. It is hoped that, if additional funds are made available, factors such as seasonal variations in the extent of nitrogen fixation by plant cells will be investigated. Results of experiments to date suggest that such variations do indeed exist and that warmer summer temperatures apparently cause an increase in fixation rates. Furthermore, results of the same experiments, although as yet not quantified in terms of grams of nitrogen per unit volume per unit time, do suggest that nitrogen fixation by attached algae, sediment bacteria and algae, and shoreline vascular plants, is a significant source of natural nitrogen input into Bayou Texar. Therefore, more frequent sampling and quantification of results would also be very desirable.

Examination of the data on phosphorus concentrations in the Bayou indicate that the basic patterns present are essentially the same as those shown for nitrates and ammonia, although the magnitude of variations in phosphorus concentrations between upper and lower stations may not be significant (Figure 4). During the latter part of the summer; once data from a number of sampling periods were examined, it was noted that the extent of dissolved inorganic phosphorus in the Bayou Texar water column seemed to remain fairly constant in spite of the occurrence of numerous and extensive algal blooms. Such blooms were, in turn, evidenced by variations in the pattern of surface oxygen concentration, variations in algal cell numbers and distribution, as well as through primary productivity studies. Therefore, experiments were initiated to delineate the dynamics of phosphorus exchange between the sediment and the water column. Such an exchange is reported by Pomeroy, et al (1965) in estuarine systems; and the results of preliminary experiments in Bayou Texar strongly suggest that such an exchange does indeed occur at times of water column phosphorus level depletion by the phytoplankton. At present concerted efforts are being directed in this area in order to qualify and quantify the extent of this exchange.

One more observation should be made from nutrient data collected thus far. Reference to Figures 2, 3 and 4, and Table 1 indicate a sharp drop and a leveling off of nutrient concentrations beyond station 2. It should be pointed out that nutrient readings are taken at surface and bottom depths only. Therefore, this sharp drop, and subsequent stabilization in nutrient levels, is most likely due to more extensive mixing and turnover of the water column beyond station 2. This hypothesis is supported by conductivity data collected at the same time as the water samples. Bayou Texar is a relatively shallow bayou and there is much boating and other recreational activity in the lower parts of the system during the summer. Furthermore, wind, current, and tidal factors tend to have greater turnover effects in the lower (southern most) regions of the Bayou.

2. Primary Productivity

The most interesting and significant conclusion apparent from the Bayou Texar primary productivity data for the summer months was the erratic nature of the rate of carbon fixation from one sampling period to the next (Figures 7, 8). There are indications, however, that seasonal patterns do exist and merit further investigation. For example mean monthly values for carbon fixation at station 4 show a definite rise from June through September. At station 6, this trend, although not as marked, is still apparent (Figure 9).

So far, all indications point to an unusually high rate of primary productivity in Bayou Texar. Figures for the summer months indicate levels as high as 300 mg $C \cdot m^{-3} \cdot hr^{-1}$ at station 2 during August (Figure 10), and increasing to 400 mg $C \cdot m^{-3} \cdot hr^{-1}$ for the same site during September (Figure 11). Examination of spatial trends point to high levels of fixation at all stations sampled. However, sites of greatest activity are at stations 2 and 4. Beyond this point, mean fixation rates decrease and level off (Figures 10, 11). These trends are in agreement with nutrient profiles already presented (Figures 2, 3, and 4, Table 1).

Carpenter's Creek flow patterns and nutrient inputs have their most significant effects at the upper Bayou stations. Our nutrient, salinity and conductivity data present evidence of distinct stratification in reference to conductivity and nutrients at these upper stations; and more uniformity and mixing in the lower stations. Therefore, station 1, which is nearest to the Carpenter's Creek inflow, is influenced by the dynamics of this inflow, and therefore shows less than optimum conditions for maximum primary productivity. At the lower Bayou stations the increased levels of mixing caused by increased effects of winds and tides also dilute the surface and bottom nutrient levels. This causes lower and less erratic rates of carbon fixation by the Bayou phytoplankton community. Stations 2 and 4 present conditions which are relatively free from some of the limitations indicated above, and, therefore, present the most favorable conditions for high rates of carbon fixation (Figures 10, 11).

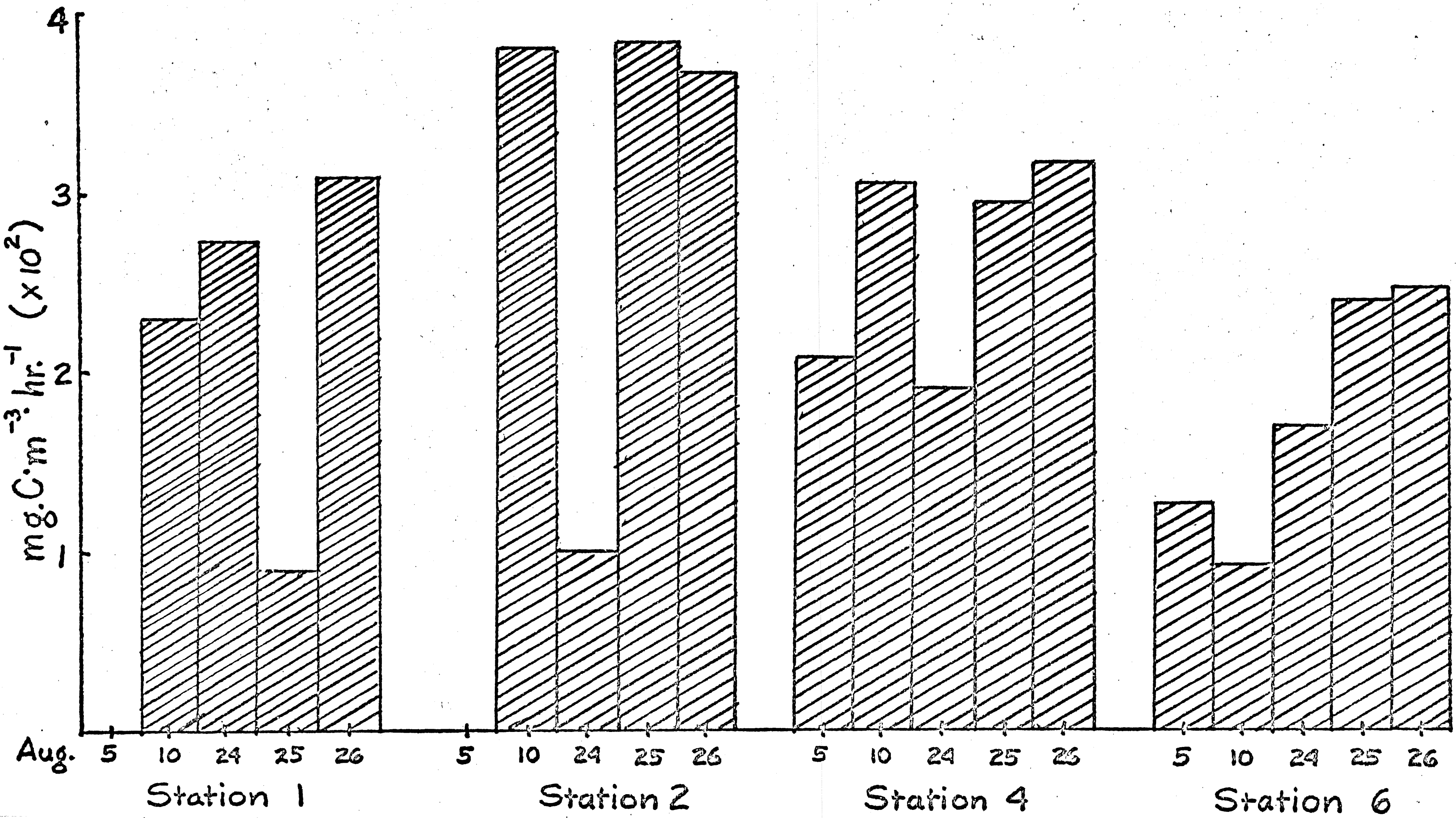

Figure 7 - Daily variations in carbon fixation rates for 4 stations during 4-5 sampling dates in the month of August, 1971.

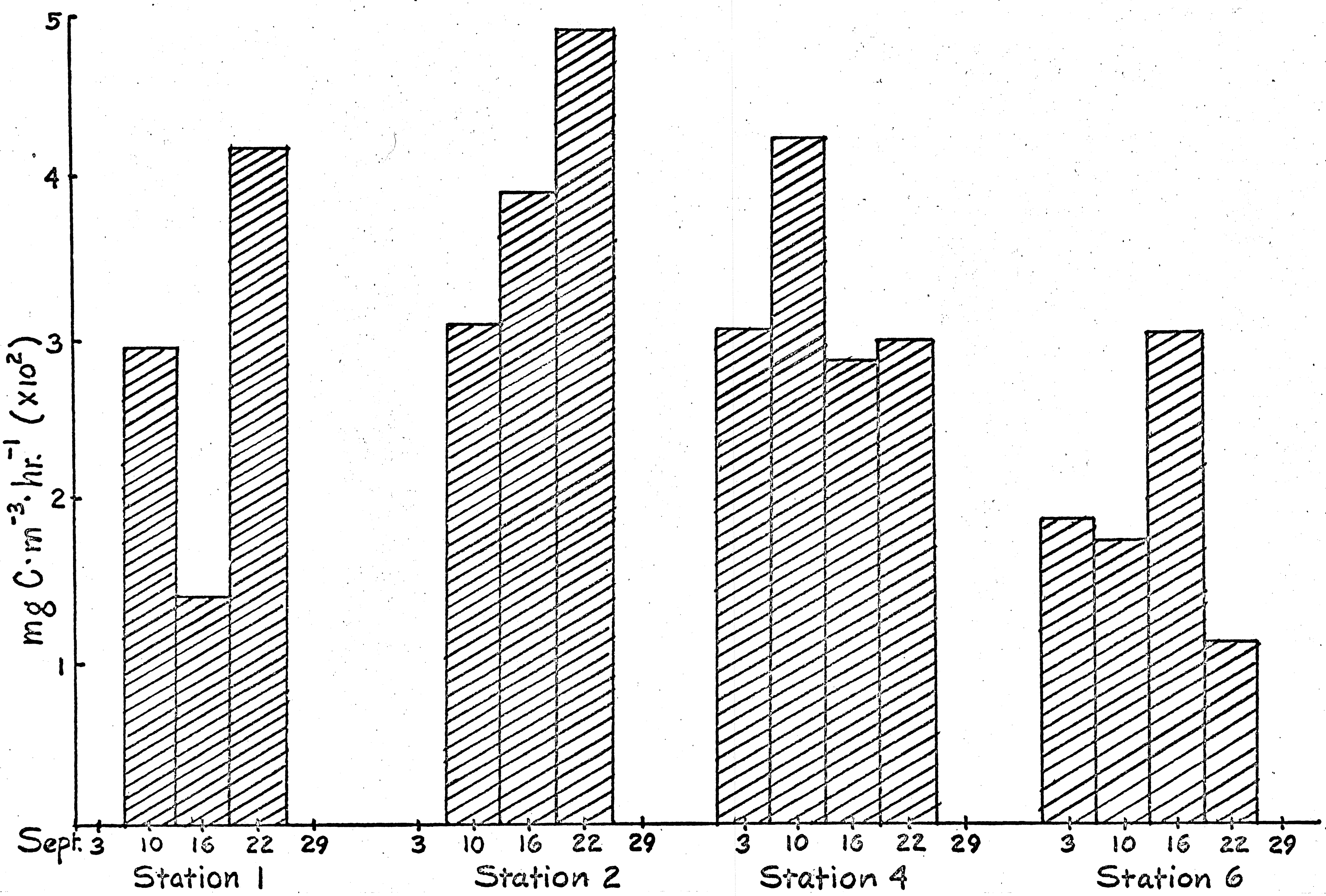

Figure 8 - Daily variations in carbon fixation rates for 4 stations during 3-4 sampling dates in the month of September, 1971.

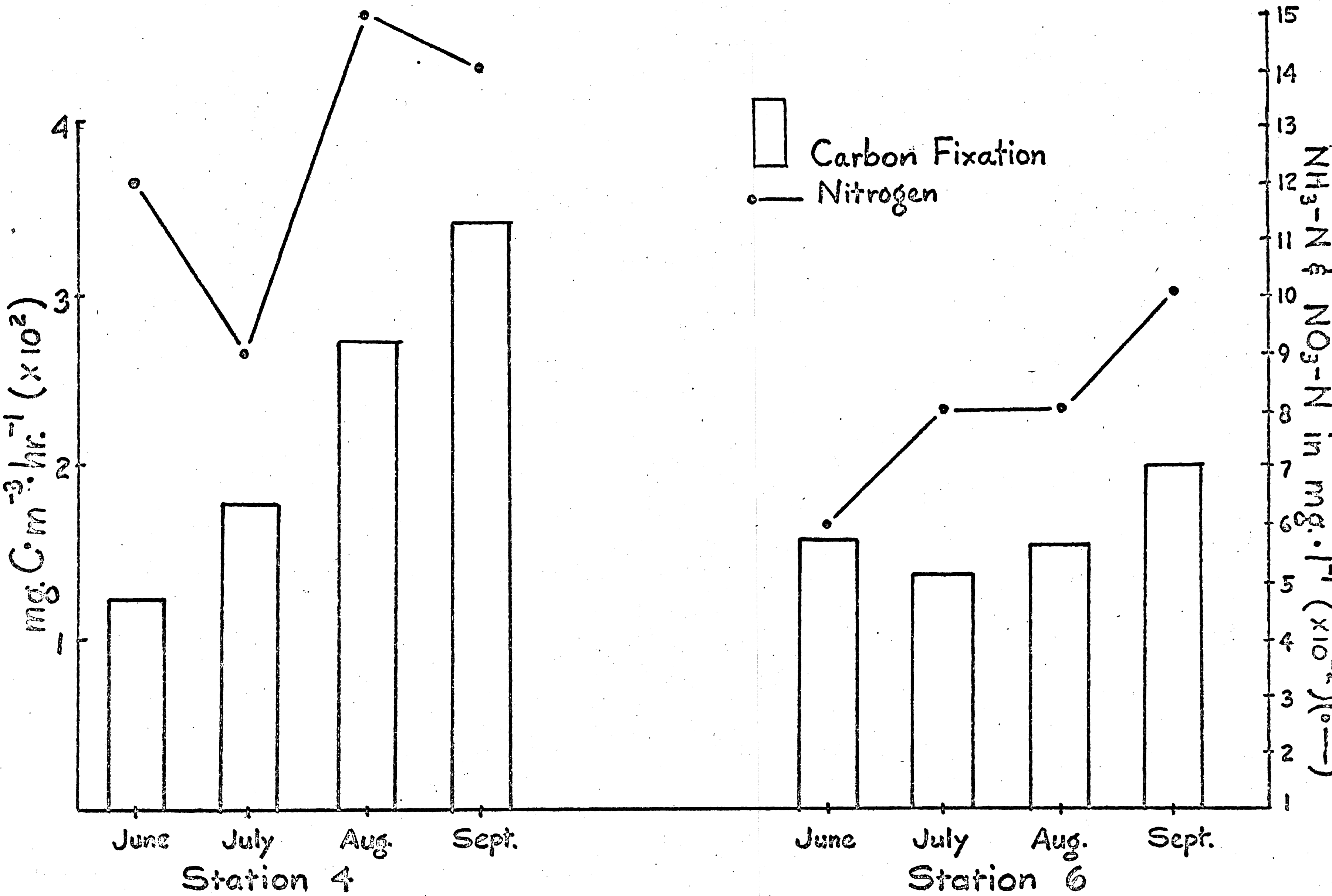

Figure 9 - Mean carbon fixation values and nitrogen as NH_3 and NO_3 for stations 4 and 6 from June through September, 1971.

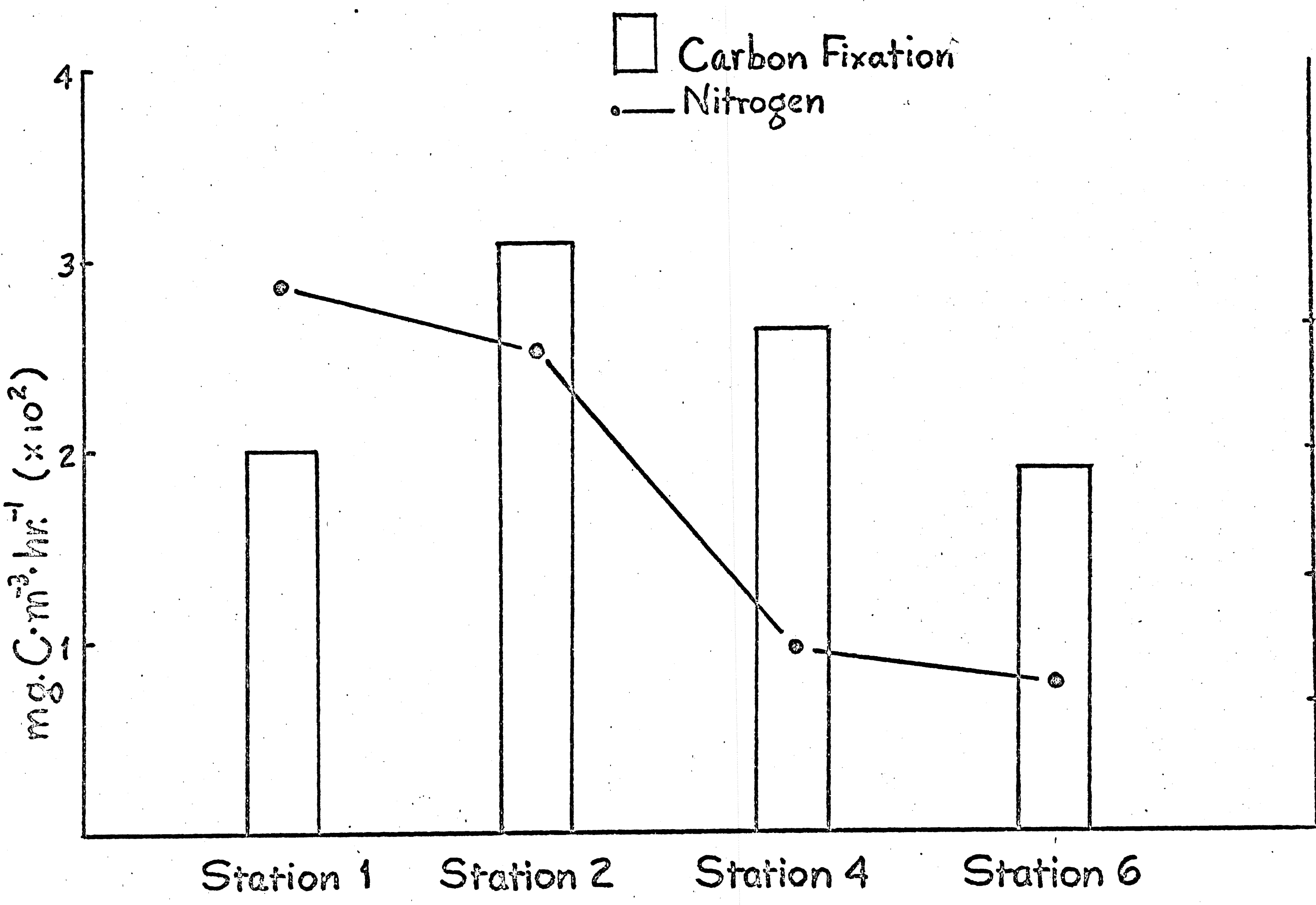

Figure 10 - Mean carbon fixation values and nitrogen as NH_3 and NO_3 for stations 1, 2, 4 and 6 for August, 1971.

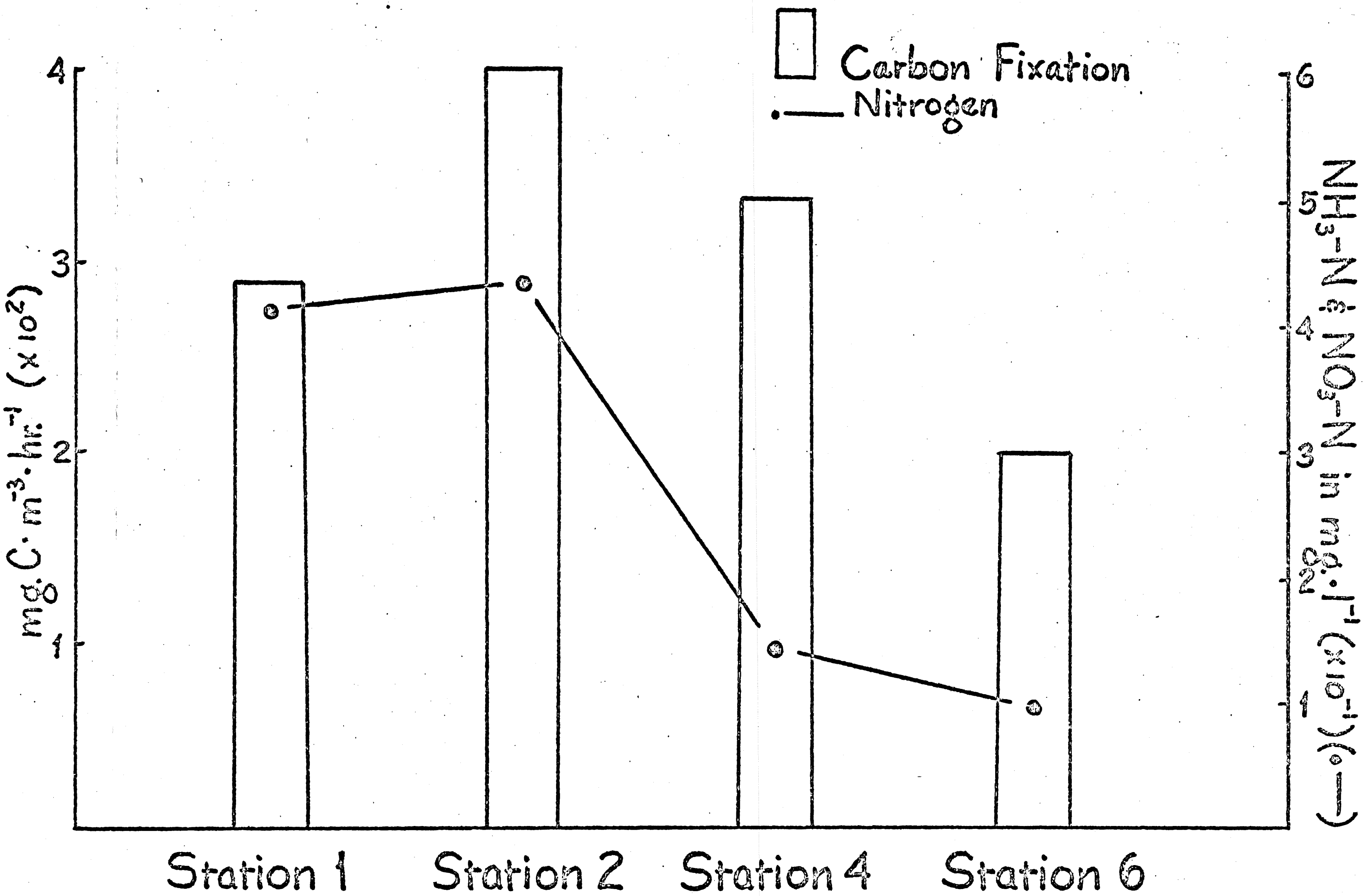

Figure 11 - Mean carbon fixation values and nitrogen as NH_3 and NO_3 for stations 1, 2, 4, and 6 for September, 1971.

Culture experiments involving innoculations of water from stations 4 and 6 with 5, 25 and 50 ml aliquots of Carpenter's Creek water, have shown some interesting results. From June through August, such innoculations stimulated primary productivity at station 6. At this site throughout September, however, the same procedure had inhibitory results. At station 4, such innoculations produced erratic results throughout the summer months (Table 2).

Stimulatory effects of Carpenter's Creek water at station 6 are most likely the result of nutrification of waters which have a comparatively low nutrient content (Figures 2, 3, 4, Table 1). The erratic results obtained at station 4 seem difficult to explain. These inhibitory effects were first thought to be due to the presence of heavy metals or pesticides in the Carpenter's Creek water. Therefore, a sampling program was initiated to determine the extent of such substances, if any, in Carpenter's Creek. Samples taken from six stations (Figure 1) from mid-July through October showed only trace amounts of these substances (Tables 3, 4). Therefore, the inhibitory effects observed at times at both stations 4 and 6 are now being investigated as possibly being caused by high nitrogen and phosphorus content of the Creek water. Culture experiments are now being conducted <u>in situ</u>, as well as in the laboratory, using 5, 25 and 50 ml aliquots of nutrient solutions made to the same nitrogen and phosphorus concentrations as found regularly in waters from Carpenter's Creek, to investigate the possibility that such nitrogen and phosphorus concentrations may have inhibitory effects on the primary productivity of the Bayou phytoplankton.

At the present, all experiments for which results have been presented here are being continued. In addition, the extent of nutrient inputs from rain water, land runoff, and sediments are also being quantified. It is expected that continued efforts in these directions will enable observation of additional spatial and temporal patterns, if any, in the Bayou Texar nutrient and carbon fixation profiles. This will make possible recommendation of alleviation techniques for the nutrification problems of Bayou Texar and similar estuarine bayous.

Table 2 - Results of culture experiments involving innoculations of Bayou Texar water with water from Carpenter's Creek. Cultures were incubated *in situ* at Station 4 between 10:30 A.M. and 2:30 P.M.

Culture Dates	Station 4 Aliquots of Carpenter's Creek Water			Station 6 Aliquots of Carpenter's Creek Water		
	5 ml	25 ml	50 ml	5 ml	25 ml	50 ml
6-11-71	--	--	--	--	--	--
6-18-71	--	--	--	+	+	--
6-24-71	+	--	--	+	+	+
6-30-71	--	--	+	+	+	+
7-07-71	+	+	--	+	+	+
7-13-71	--	+	+	0	+	+
7-29-71	+	+	--	+	+	+
8-05-71	--	--	--	0	+	+
9-03-71	--	--	--	--	+	+
9-10-71	--	--	--	--	--	--
9-16-71	--	--	--	--	--	--
Total +	3	3	2	5	8	7
Total --	8	8	9	4	3	4
Total Observations	11	11	11	11	11	11

+ = Significant increase in carbon fixation over control.

-- = Significant decrease in carbon fixation over control.

0 = No significant difference in carbon fixation over control.

Table 3 - Carpenter's Creek yield of DDT and Dieldrin in water samples collected between August, 1971 and October, 1971.

Sampling Dates	DDT (PPB)	Dieldrin (PPB)
8-11-71	N.D.	N.D.
8-18-71	N.D.	T
8-25-71	0.0019	0.0059
9-01-71	0.0042	0.0100
9-15-71	N.D.	0.0020
9-24-71	N.D.	T
9-29-71	N.D.	0.004
10-07-71	N.D.	<0.009
10-14-71	N.D.	0.006
10-21-71	N.D.	0.002

N.D. = not detectable

T = trace

Table 4 - Carpenter's Creek yield of heavy metals in water samples collected between July, 1971 and October, 1971. Results are in parts per million (ppm).

Sampling Dates	Cu	Fe	Pb	Zn	Hg
7-13-71	0.05	0.20	0.5	0.22	0.01
8-11-71	0.05	0.80	0.5	0.21	0.04
8-18-71	0.05	0.10	0.5	0.21	0.15
8-25-71	0.05	4.29	0.5	0.24	0.09
9-01-71	0.05	1.00	0.5	0.20	0.07
9-15-71	0.05	0.40	0.5	0.24	0.04
9-22-71	0.05	0.68	0.5	0.22	0.04
9-29-71	0.05	0.66	0.5	0.19	0.18
10-09-71	0.02	0.42	0.5	0.05	0.06
10-14-71	0.02	0.42	0.5	0.05	0.10
10-24-71	0.02	0.46	0.5	0.06	0.04

Acknowledgements

We are grateful to Mr. Michael Eaton of the University of West Florida for his involvement in our project and providing us with the data from his nitrogen fixation studies. We also wish to express our appreciation to Dr. Thomas S. Hopkins of the University of West Florida for providing equipment and supplies. Grateful appreciation is also extended to the Bayou Texar Association for equipment and evaluated services.

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