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NITRIFICATION IN FOUR ACIDIC STREAMS IN SOUTHERN NEW JERSEY

U.S. GEOLOGICAL SURVEY

Water-Resources Investigations 77-121

Prepared in cooperation with the State of New Jersey, Department of Environmental Protection

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16. Abstracts Chemical and microbiological data from four characteristically acidic streams in southern New Jersey were used to evaluate seven factors related to the proclivity of each stream to nitrify under the influence of secondary sewage effluent. pH, water temperature, and dissolved oxygen were used as indicators of stream conditions while neutralization of alkalinity, nitrogen species trends, BOD/NOD incubations, and nitrifying bacteria densities served as actual nitrification indicators. Hay Stack Brook showed strong evidence for nitrification on the basis of all four nitrification indicators while Landing Creek was nitrifying little if at all. Hammonton Creek is apparently nitrifying, but because of the uncertainty in the downstream trends of the nitrogen species, and a lower level of alkalinity neutralization, it is nitrifying less than Hay Stack Brook. Squankum Branch showed some evidence for nitrification on the basis of the BOD/NOD incubations. The acidic character of these streams does not appear to be an exclusive factor in determining whether the stream will undergo nitrification.

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The following factors may be used to convert the English units published herein to the International System of Units (SI).

Multiply English Units	By	To Obtain SI Units
	Length	
inches (in)	2.54	centimeters (cm)
feet (ft)	30.48	centimeters (cm)
miles (mi)	1.609	kilometers (km)
	Area	
square miles (mi^2)	2.590	square kilometers (km^2)
	Flow	
cubic feet per second (ft^3/s)	.02832	cubic meters per second (m^3/s)
million gallons per day (mgd)	.04381	cubic meters per second (m^3/s)

NITRIFICATION IN FOUR ACIDIC STREAMS IN SOUTHERN NEW JERSEY

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ABSTRACT

Four characteristically acidic streams in southern New Jersey were investigated to determine the effect of secondary sewage effluent on nitrification in the receiving waters. Chemical and microbiological data were obtained at four sites on each stream. From these data seven factors were evaluated to determine the proclivity of each stream to nitrify. pH, water temperature, and dissolved oxygen were used to describe the general condition of the streams, while neutralization of alkalinity, nitrogen species concentration trends, biological and nitrogenous oxygen demand incubations, and nitrifying bacteria densities were used to determine the actual presence of nitrification in each stream. Each stream had a unique distribution of conditions, making it possible to qualitatively rank the streams according to their proclivity to nitrify. Hay Stack Brook showed strong evidence for nitrification on the basis of all four nitrification indicators, whereas Landing Creek showed little, if any, evidence of nitrification. Hammonton Creek is apparently nitrifying, but because of the uncertainty in the downstream trends of the nitrogen species and a lower level of alkalinity neutralization, it is nitrifying less than Hay Stack Brook. Squankum Branch also showed some evidence for nitrification, mostly on the basis of the biological and nitrogenous oxygen demand incubations. Thus, although these streams are acidic in character, acidity does not appear to be an exclusive factor in determining whether a stream will undergo nitrification.

INTRODUCTION

The introduction of concentrated liquid wastes into natural waters can have a significant deoxygenating effect on a receiving stream. Secondary waste-water treatment plants have been designed to decrease the amount of oxidizable carbonaceous material discharged into receiving streams so as to minimize their effect on the dissolved-oxygen concentration of a stream. A properly designed and operated waste-water treatment plant can remove most of the oxidizable carbonaceous material, but a significant amount of biological oxygen demand (BOD) can still remain in the effluent. This second component of the waste load consists of oxidizable nitrogenous material made up of reduced forms of nitrogen, which can be oxidized to nitrite and(or) nitrate via nitrification. The biological oxidation of ammonia to nitrite and nitrate is termed nitrification.

The rate and degree of nitrification in a receiving stream are dependent upon several environmental factors including pH, temperature, water chemistry, and the amount of particulate matter. The purpose of this study was to determine the effect of secondary effluents on nitrification in acid streams in southern New Jersey.

Acknowledgments

The authors wish to express their appreciation to Dr. S. D. Faust of Rutgers University, who directed the master's research (Ram, 1975) upon which this report is based. Special thanks go to the supervisors of the four waste-water treatment plants who were most helpful during the sample collection phase of the project.

BACKGROUND

The principal organisms responsible for the oxidation of reduced nitrogen compounds are the autotrophic nitrifying bacteria (Verstraete and Alexander, 1973). The autotrophic nitrifying bacteria belong to the family Nitrobacteraceae. Of the seven genera classified by Bergey (Bergey, 1974), only Nitrosomonas and Nitrobacter are reported frequently enough to be considered in this report (Tuffey, 1973; Bergey, 1974). Nitrosomonas is responsible for the oxidation of ammonia to nitrite while Nitrobacter oxidizes nitrite to nitrate.

Nitrification consumes oxygen. Stoichiometrically three atoms of oxygen are required for each atom of nitrogen oxidized to nitrite or 3.43 parts of oxygen by weight to 1 part nitrogen (equation 1). Similarly, 1.14 parts of oxygen are required for every part of nitrite oxidized to nitrate (equation 2). The actual amount of oxygen consumed in nitrification, however, varies from the stoichiometric value because oxygen is evolved in protoplasm synthesis. Measurements by Wezernak and Gannon (1967) showed the values to be 3.22 mg/L and 1.11 mg/L, respectively.

$$2NH_4^+ + 3O_2 \xrightarrow{\textit{Nitrosomonas}} 2NO_2^- + 2H_2O + 4H^+ + 66{,}500 \text{ calories} \quad (1)$$

(Hofman and Lees, 1951)

$$2NO_2^- + O_2 \xrightarrow{\textit{Nitrobacter}} 2NO_3^- + 17{,}000 \text{ calories} \quad (2)$$

(Lees and Simpson, 1956)

Nitrifying bacteria are for the most part autotrophic (Verstraete and Alexander, 1973), oxidizing inorganic nitrogen compounds for energy and using carbonates, bicarbonates, or carbon dioxide for biosynthesis. The literature indicates that phosphate, magnesium, calcium, iron, and copper are also required for bacterial growth (Van Droogenbroech and Laudelout, 1967; Aleem and Alexander, 1969; Loveless and Painter, 1967; Lees, 1947, 1951). The rate of growth of bacteria can either be stimulated or inhibited by a variety of inorganic and organic compounds (Lees and Simpson, 1956; Delwiche and Finstein, 1965; Downing, Painter, and Knowles, 1964; Lees, 1947, 1951; McBeath, 1962; Downing, Tomlinson, and Truesdale, 1964). Some organic compounds that have a stimulating effect on nitrification and growth, cannot, however, substitute for carbon dioxide as sources of carbon for biosynthesis.

Nitrifying bacteria are noted for their long generation time which is the interval in which one cell develops and completely divides into two cells. The generation time of microorganisms is dependent upon the conditions of growth and the nature of the specific organisms. The generation times for Nitrosomonas have been reported to range from 30 hours to 94 hours (Alexander, 1961; Buswell and others, 1953; McBeath, 1962; Loveless and Painter, 1967), while Nitrobacter generation times range from less than 1 to 15 hours (Tuffey, 1973; Boon and Laudelout, 1962; Buswell, and others, 1950).

Previous studies have shown that nitrification can occur from 5° to 40°C and that the optimum temperature range is from 25° to 30°C (Bergey, 1974). In general, the rate of nitrification increases with increasing temperature up to a limiting temperature. Because nitrification proceeds faster at higher temperatures, this study was conducted during the summer months. The temperatures recorded in this study ranged from 15° to 26.5°C. Nitrification, can occur during colder months but to a much lesser extent.

Nitrifying bacteria are obligate aerobes. In general, the rate of nitrification increases with increasing concentration of dissolved oxygen up to a critical level of 0.5 mg/L above which a further increase in concentration has little effect (Downing, Painter, and Knowles, 1964). Although nitrification is retarded below the 0.5 mg/L dissolved-oxygen level, lack of oxygen for long periods of time does not appear to be lethal (Downing, Painter, and Knowles, 1964).

As with dissolved oxygen and temperature, the pH of a potentially nitrifying medium falls into a certain range for optimum results. Although there is considerable controversy about the exact optimal values, a generally accepted pH range for nitrifying bacteria is from 6.0 to 8.5 and an optimum value from 7.5 to 8.0 (Bergey, 1974). Since the rate of nitrification falls off rapidly with decreasing pH, nitrification does not occur to any significant extent in acidic water.

Nitrification in Streams and Below Waste-Water Outfalls

Nitrogen may enter streams naturally or as the result of man's activities. The entry of nitrogen into streams resulting from man's activities may be subdivided into intentional and unintentional sources. Of the latter, agricultural runoff comprises a large part and may contain fertilizers as well as nitrogen compounds inherent in the soil itself. Leaching of nitrogen compounds into ground water which reappears as surface water also contributes to nitrogen in streams. Fluctuations in the nitrate content of water supplies may in part be atrributable to soil leaching associated with rainfall (Feth, 1966). The major intentional source of nitrogen in streams is the direct addition of primary and secondary effluents from sewage treatment plants. Rainwater and Thatcher (1960) state that unpolluted water seldom contains greater than 10 mg/L as nitrogen of nitrogen compounds. George and Hastings (1951) cite 5 to 10 mg/L as common upper limits.

The primary sources of nitrogen in sewage are the end products of nitrogen metabolism in man. Industrial wastes may be a minor source of nitrogen. Nitrogen compounds in sewage include amino acids, proteins, and their degradation products, and proteoses, peptones, and peptides. The largest single source of nitrogen in sewage is urea. Hanson and Flynn (1964) reported that urea comprised up to 88 percent of the organic constituents of normal urine. Urea is hydrolyzed to ammonia by various microorganisms. Hanson and Flynn (1964) determined that free ammonia comprised the greatest part of the nitrogen balance of the sewage studied, and urea was usually the second most abundant constituent. Of the total nitrogen in domestic sewage, ammonia and urea comprised approximately 85 percent of the total (Hanson and Flynn, 1964).

Wezernak and Gannon (1967) reported that secondary treatment plants typically produce effluents containing 10 to 20 mg/L NH_4^+-N. Tuffey (1973) reported a typical range of 10 to 30 mg/L.

Nitrification may occur to some extent in sludge flocs in activated sludge plants and in the film of percolating filters (Montgomery and Borne, 1966). Although nitrifiers are present in activated sludge digesters, nitrification does not occur to any significant extent because of insufficient detention time in the digester. The presence of nitrifying bacteria in effluents from trickling filters is substantiated by the use of a trickling filter as a seed for nitrification experiments. This, however, merely indicates the possibility of the presence of nitrifiers in effluents from secondary waste-treatment plants. It does not guarantee their role in the occurrence of nitrification in the stream below the sewage outfall.

The introduction of ammonia from waste-treatment effluents represents a potential energy supply for nitrifying bacteria. If oxygen is available and other environmental conditions are favorable, a vigorous nitrifying flora will develop which will oxidize the ammonium ion to nitrite and nitrate. These nitrifying bacteria may be either suspended or attached to solid substrates. Previous investigators indicate that the latter is of greater significance (Tuffey, 1973; and Tuffey, and others, 1974). Tuffey (1973) cites two New Jersey streams, Mine Brook and Beaver Brook, as examples of streams with luxurient surface growths of nitrifiers. Nitrification in these streams was rapid and complete. Tuffey concluded that it was occurring as a result of surface acitvity.

Nitrification can also result from nitrifiers suspended in the water phase provided that the nitrifying bacteria remain suspended and that they have sufficient time for growth. Both conditions are satisfied in a tidal estuary where tidal activity keeps the nitrifiers in suspension and provides the long detention time required for growth (Tuffey, 1973). Tuffey reasons that headwaters and small tributaries nitrify because of surface activity while estuaries nitrify in the water phase by suspended bacteria. It was concluded that transitional zones exist between these extremes which are not clearly delineated.

Tuffey, Hunter, and Matulewich (1974) put forth the idea of nitrifying zones. They suggested that nitrification, occurring at a level significant enough so that it must be included in a dissolved-oxygen or water quality model, does not occur along the entire length of a polluted river, but occurs in identifiable zones. The key element in the existence of a nitrifying zone is, again, a residence time sufficiently long to develop a substantial population.

The rate of nitrification may vary from stream to stream and also from section to section in the same stream depending upon the combined influence of all the environmental factors (Ruchhoft and others, 1948). The concentration of nitrifying organisms is a function of the immediate history of the water in the reach of the stream considered. Stratton and McCarty (1967) suggest that if active nitrification is in progress or has recently been completed in the stream or estuary under study, a relatively large number of nitrifying organisms will be present in the water. If, however, little nitrification has occurred prior to the study, very few nitrifying organisms will be present in the water. It was added that when the initial concentration of ammonia oxidizing organisms is small there may be a considerable time lag between the introduction of ammonia and the reduction of a significant quantity of nitrite. Once in progress, however, nitrification will proceed at a steady rate provided that the dissolved-oxygen concentration remains above the critical value (Jenkins, 1969).

The oxidation of inorganic nitrogen may represent a major deoxygenating component in waters which receive significant nitrogenous loads. The amount of oxygen utilized in the oxidation of nitrogenous material alone (nitrogenous oxygen demand, NOD), is important in small streams that receive relatively large volumes of secondary effluents and during the low flow, warm weather periods of the year (Wezernak, and Gannon, 1968). The occurrence of nitrification, then, is of great significance to the dissolved-oxygen balance of streams.

The products of nitrification are nitric acid and water. The production of nitric acid can therefore increase the acidity of an unbuffered nitrifying environment. Under acid conditions, nitrification proceeds slowly. Therefore, serious acidification of environmental waters attributable to nitrification does not occur because nitrification is self-limiting.

APPROACH

The literature previously cited indicates that nitrification should not occur to a significant extent below pH values of about 6.0. Thus, little, if any, biologically mediated oxidation of ammonia to nitrate in acid streams of southern New Jersey would be expected. As secondary waste-water effluents can raise the pH value of a receiving stream, the authors conjectured that the introduction of an alkaline waste-water effluent could raise the pH value in a receiving stream sufficiently to permit nitrification. In addition, a secondary waste-water effluent would favor the occurrence of nitrification in the receiving stream by supplying nitrogenous substrate for the nitrifying bacteria and nitrifying bacteria directly into the receiving stream.

In order to determine the effect of secondary effluents on nitrification in acid streams, four streams in southern New Jersey were selected for investigation. Physical, biological and chemical parameters were measured to assess the occurrence of nitrification and to determine its contribution as an oxygen sink in each of the streams studied. Chemical constituents were measured to follow the chemical transformations occurring in the streams and biological determinations were conducted to evaluate the presence of nitrifying organisms in the streams and the waste water effluents.

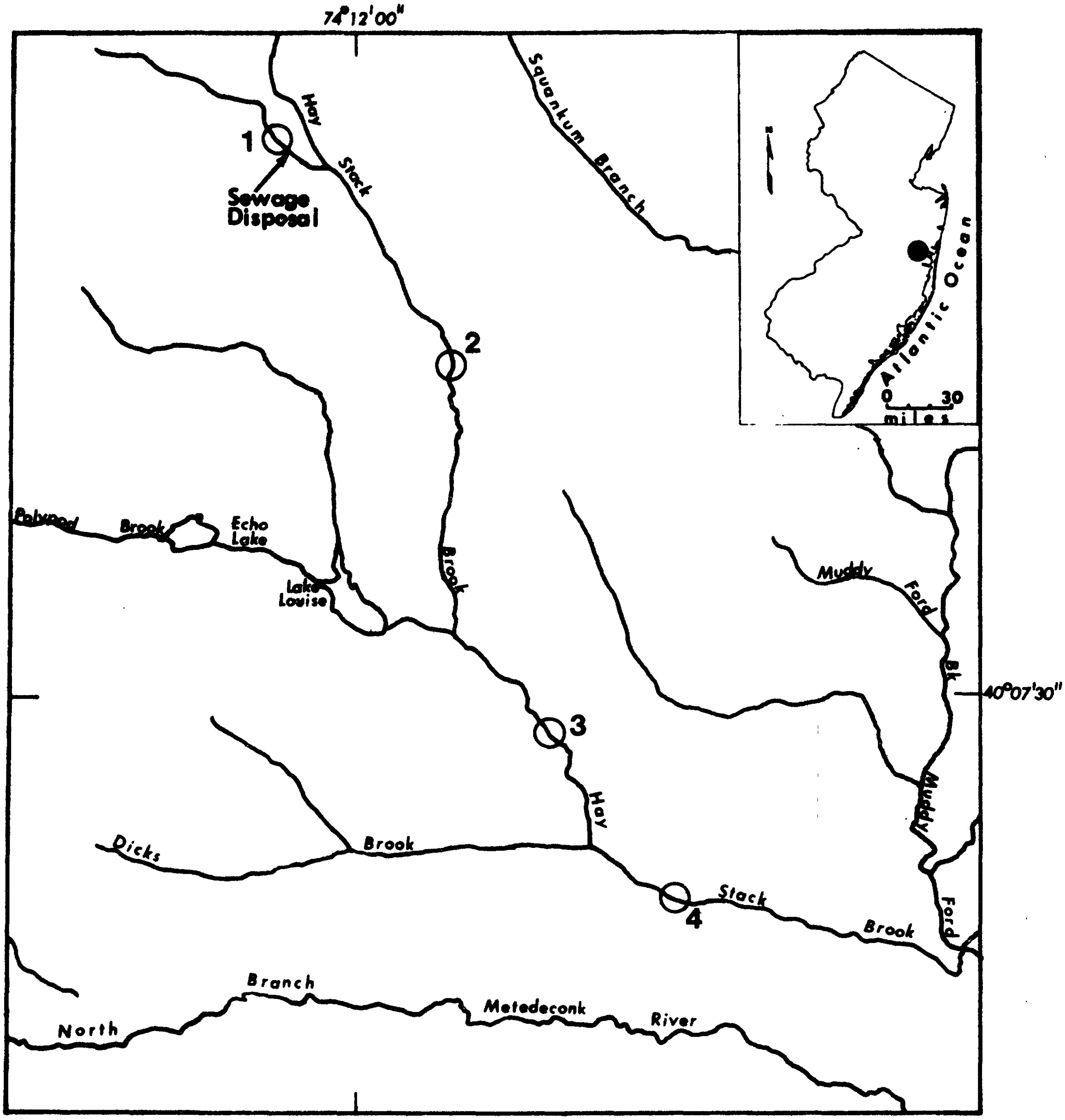
Sampling Sites

The four streams selected for the New Jersey field studies are: Hammonton Creek near the town of Hammonton, the Squankum Branch of the Great Egg Harbor River near Williamstown, Landing Creek near Egg Harbor City, and Hay Stack Brook near Lakewood (figs. 1, 2, 3, 4). These streams were chosen because the secondary effluents from waste treatment plants form a large segment of the total streamflow and significantly affect the oxygen resources of each stream. Dilution then, would not obscure the possibility of observing nitrification below the sewage outfalls. Table 1 lists the names of each of the four waste water treatment plants located on the streams studied. Figure 5 shows the relative distances between sampling sites and the sewage outfalls.

Sampling stations were established both above and below the waste-water discharge point to each stream. Samples were collected at each site on two separate occasions approximately 1 month apart.

RESULTS

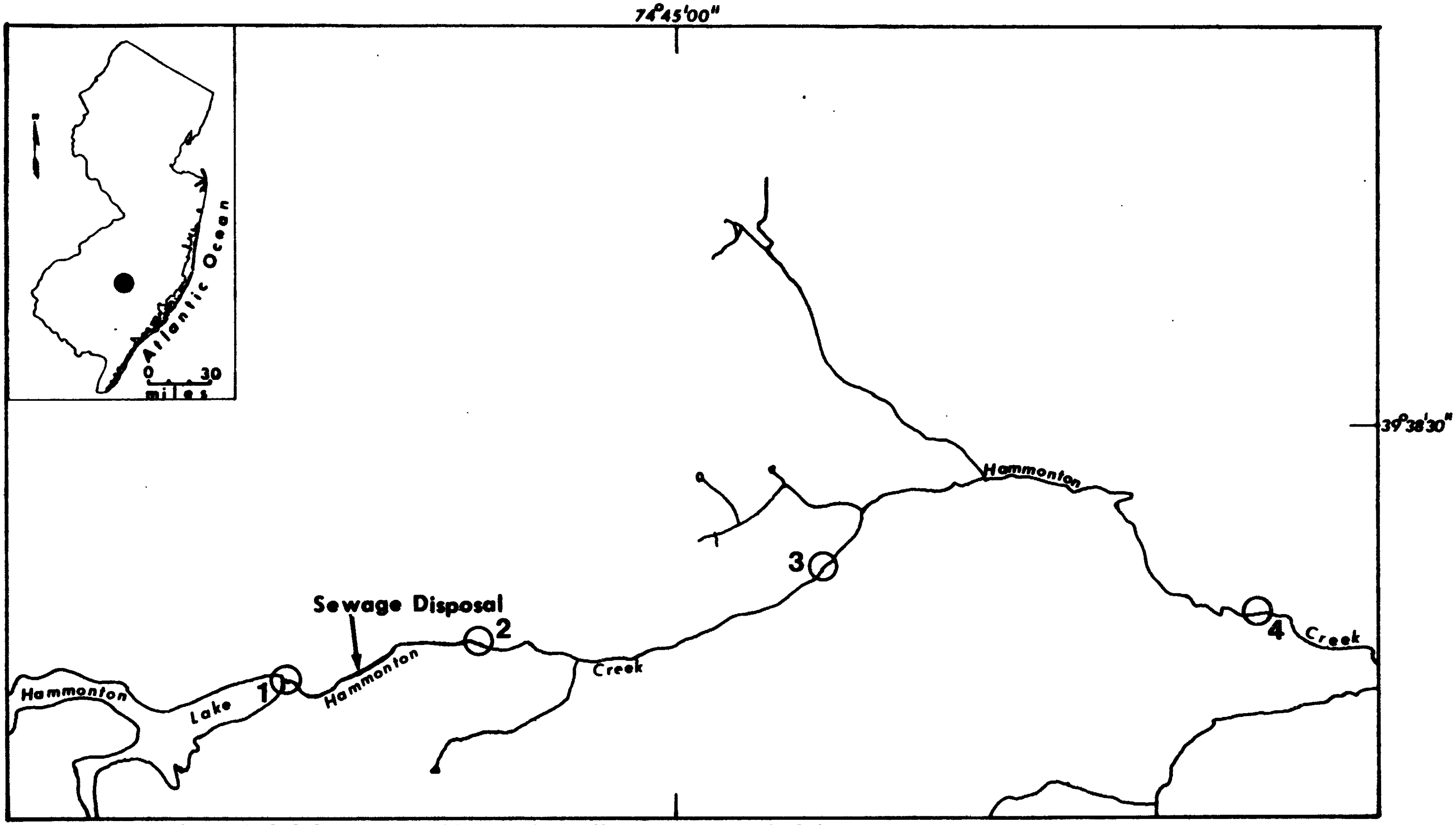
The chemical analyses data are summarized in tables 2, 3, 4, and 5. The data clearly show the significant impact of the sewage-plant effluents on the receiving streams. A dissolved oxygen sag was observed in all the streams studied resulting from the oxidation of carbonaceous and nitrogenous waste materials from the sewage effluents. At no time did the dissolved-oxygen level fall below the 0.5 mg/L level, thus indicating that oxygen was not limiting to nitrification in the water phase. The total organic carbon (TOC) and carbonaceous biological oxygen demands increased below most of the sewage effluents reflecting the input of carbonaceous waste materials. Increases in chloride, turbidity, and dissolved-solids



Base adapted from U.S.G.S. topographic quads Lakewood and Farmingdale.

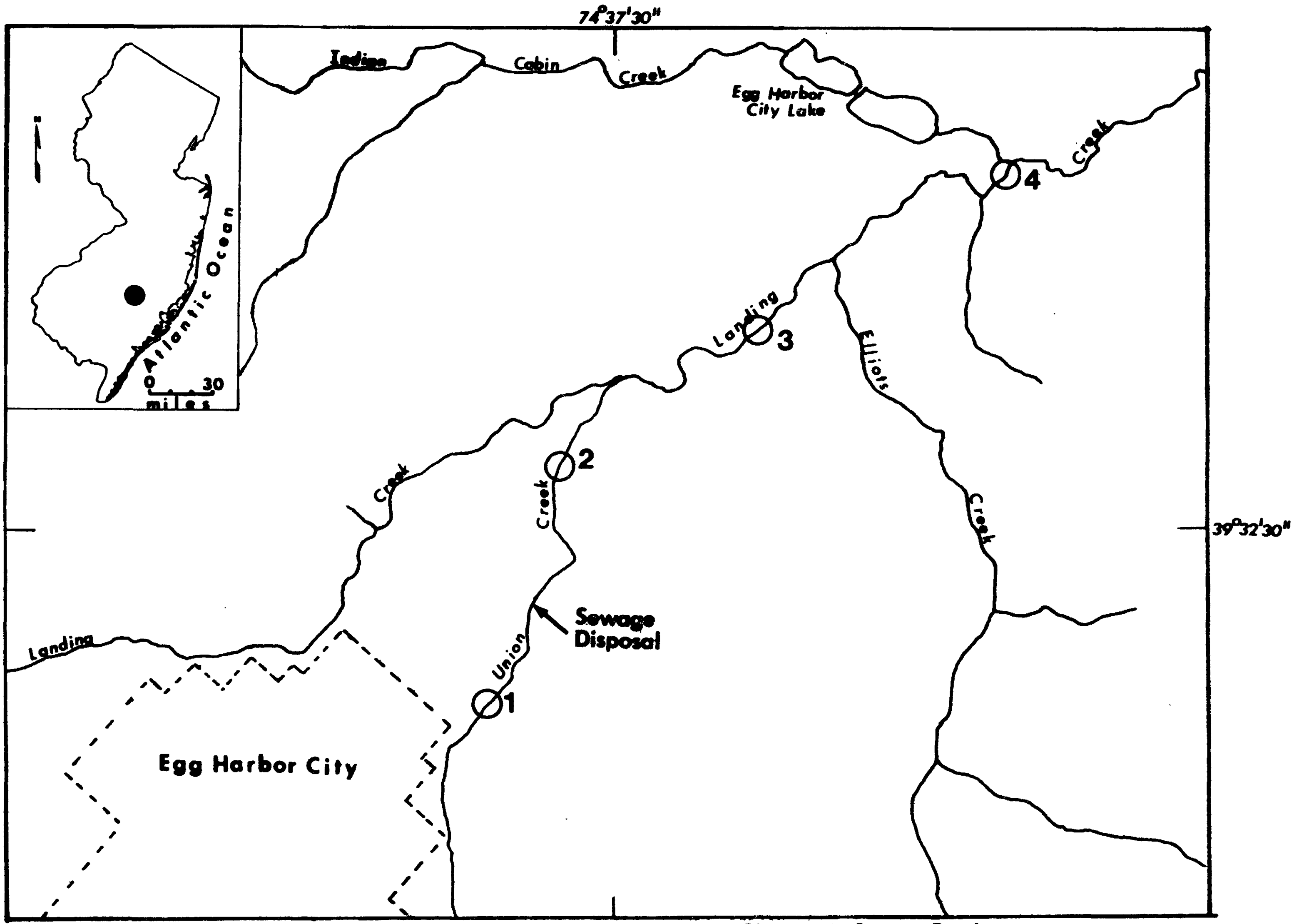
0 1/2 1 Mile

Figure 1.--Sampling sites on Hay Stack Brook.



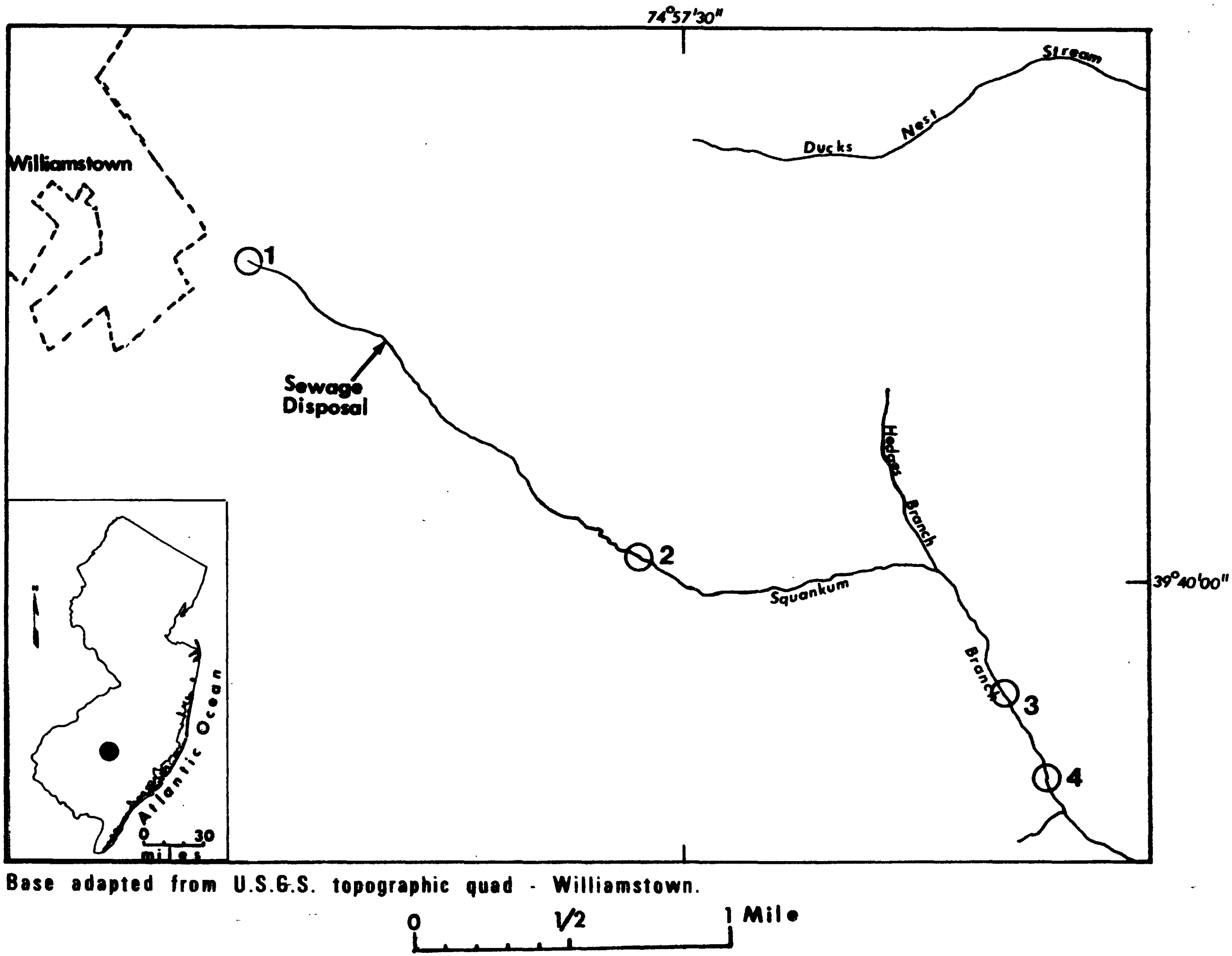
Base adapted from U.S.G.S. topographic quads - Hammonton and Atison.

Figure 2.--Sampling sites on Hammonton Creek



Base adapted from U.S.G.S. topographic quads - Egg Harbor City and Green Bank.

Figure 3.--Sampling sites on Landing Creek.



Base adapted from U.S.G.S. topographic quad - Williamstown.

Figure 4.--Sampling sites on Squankum Branch.

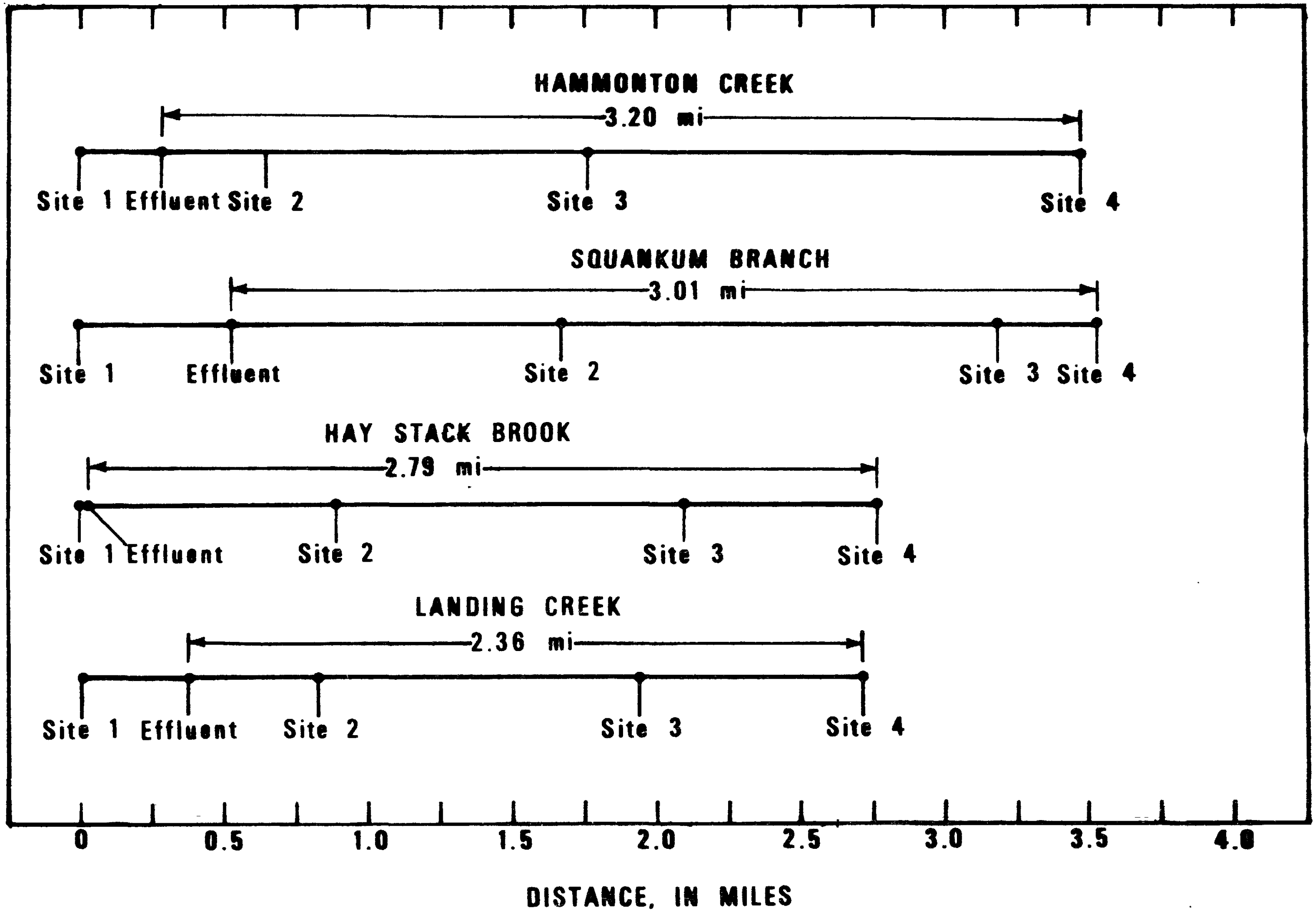


Figure 5.--Distances between sampling sites.

concentration in the streams below the sewage effluents were also observed.

Flow data indicated that significant volumes of effluent entered the streams in comparison with their total flows. The waste water effluent comprised about 10 percent of the total flow at site 4 and comprised between 20 and 60 percent of the flow at site 2.

Table 1.--Waste-treatment plants

Name of plant	Point of discharge	Secondary treatment
Hammonton Municipal Waste Treatment Plant.	Hammonton Creek	Two slow trickling filters. One high rate trickling filter.
Monroe Municipal Utilities Authority.	Squankum Branch of the Great Egg Harbor	One high rate trickling filter.
Maximum Sewer Company.	Hay Stack Brook	One high rate trickling filter.
Egg Harbor City Municipal Plant.	Landing Creek	One standard rate trickling filter.

pH

The effect of the introduction of sewage effluent on the pH value of the receiving streams studied can be placed into three categories:

Table 2.--Chemical analyses for Hay Stack Brook.

Site	Date	Time	Dis-charge (ft^3/s)	Temp (°c)	Spec. cond. (umhos)	pH units	Dis-solved oxygen (mg/L)	Total nitrite (N) (mg/L)	Total nitrate (N) (mg/L)	Total kjeld. (N) (mg/L)	Total ammo-nia (N) (mg/L)	Total organic nitro-gen (N) (mg/L)	Total nitro-gen (N) (mg/L)	Total phos-phorus (P) (mg/L)	Total ortho phos-phorus (P) (mg/L)	Total organic carbon (mg/L)	Dis-solved organic carbon (mg/L)
Hay Stack	7/30/74	0730	0.19	17.0	92	5.6	7.8	0.02	2.0	0.43	0.27	0.16	2.4	0.01	0.0	7.8	7.8
Brook 1	9/23/74	1000	0.33	17.0	94	6.4	8.3	0.00	2.1	0.58	0.45	0.13	2.7	0.24	0.01	4.5	4.4
Effluent	7/30/74		0.54			7.0											
	9/23/74		0.27			7.1											
	9/28/74	1150						0.06	0.05	24	21	3	24	9.4	6.5	25	
Hay Stack	7/30/74	0900	1.49	20.0	331	7.1	2.3										
Brook 2		0930	1.49	20.0	287	7.1	2.3	0.04	0.26	>9.5	9.5	>0.0	>9.8	3.2	2.9	20	10
	9/23/74	0720	2.20		224	6.8		0.04	0.32	>7.7	7.7	>0.0	>8.0	2.6	1.8	13	12
		0800	2.20	15.5	205	6.8	3.5										
		0805	2.20	15.5	202	6.8	3.5	0.04	0.35	>7.8	7.8	>0.0	>8.2	2.0	1.9	15	12
Hay Stack	7/30/74	1030	4.35	22.0	166	6.5	3.0	0.19	1.6	>2.9	2.9	>0.0	>4.5	1.3	1.1	25	9.9
Brook 3	9/23/74	1700	5.9		120			0.18	1.6	>1.9	1.9	>0.0	>3.5	0.82	0.65		12
		1808	5.9	17.0	119	6.4	4.1	0.17	1.6	>1.8	1.8	>0.0	>3.4	0.81	0.62	13	10
Hay Stack	7/30/74	1115	4.45	21.3	140	6.5	4.0	0.05	2.2	>1.1	1.1	>0.0	>3.3	0.87	0.75	28	
Brook 4	9/23/74	2025	6.0		117			0.01	3.2	>1.0	1.0	>0.0	>4.2	0.66	0.58	12	11
		2241	6.0	15.8	112	6.4	5.3	0.10	2.0	>1.0	1.0	>0.0	>3.0	0.67	0.53	9.7	8.9

Site	Date	Alka-lin-ity (CO3) (mg/L)	Bicar-bonate (HCO3) (mg/L)	Total cal-cium (mg/L)	Total magne-sium (mg/L)	Total sodium (mg/L)	Total potas-sium (mg/L)	Dis-solved chloride (mg/L)	Dis-solved sulfate (mg/L)	Total fluor-ide (mg/L)	Dis-solved silica (mg/L)	Dis-solved solids (mg/L)	Total iron (ug/L)	Dis-solved iron (ug/L)	Total man-ganese (ug/L)	Dis-solved man-ganese (ug/L)
Hay Stack	7/30/74	4.0	5	7.0	2.0	4.1	2.5	8.2	12	0.2	6.3	62	2800		260	
Brook 1	9/23/74	7.0	9	6.9	5.2	4.0	2.6	8.5	13	0.0	6.8	84	6200		180	
Effluent	9/28/74		241				12					350				
Hay Stack	7/30/74	32	39					24	24		14	163		170		70
Brook 2		75	91	13	2.4	26	5.2	25	23	0.5	14	164	1100		120	
	9/23/74	52	64	10	5.0	18	4.2	16	16	0.1	12	151	1500		120	
								16	15		12	150		690		60
		51	62	9.5	4.4	17	4.0	16	15	0.1	12	151	2000		120	
Hay Stack	7/30/74	12	15	7.6	3.2	14	4.1	15	16	0.2	8.8	110	1700		90	
Brook 3	9/23/74	15	18	6.2	3.6	10	2.9	22	15	0.2	7.4	99	800		60	
		13	16	5.9	3.6	9.4	2.9	12	11	0.2	7.0	94	800		70	
Hay Stack	7/30/74	10	12	6.7	2.0	12	3.5	15	15	0.3	8.7	97	9500		90	
Brook 4	9/23/74	8	10	5.5	2.0	10	3.0	11	12	0.2	7.3	81	950		70	
		10	12	8.0	5.6	9.0	2.7	10	11	0.1	7.4	87	900		60	

Table 3.--Chemical analyses for Hammonton Creek.

Site	Date	Time	Dis-charge (ft³/s)	Temp (°c)	Spec. cond. (umhos)	pH units	Dis-solved oxygen (mg/L)	Total nitrite (N) (mg/L)	Total nitrate (N) (mg/L)	Total kjeld. (N) (mg/L)	Total ammo-nia (N) (mg/L)	Total organic nitro-gen (N) (mg/L)	Total nitro-gen (N) (mg/L)	Total phos-phorus (P) (mg/L)	Total ortho phos-phorus (P) (mg/L)	Total organic carbon (mg/L)	Dis-solved organic carbon (mg/L)
Hammonton	7/17/74	0815	1.09	26.5	65	6.9		0.01	0.08	0.46	0.41	0.05	0.55	0.02	0.01	9.2	4.2
Creek 1	8/28/74	0700	1.39	26.0	65	7.1	7.5	0.00	0.02	0.44	0.44	0.00	0.26	0.02	0.01	27	9.4
Effluent	7/17/74		0.96			6.6											
	8/28/74		0.80			6.2											
Hammonton	7/17/74	1000	4.06	20.0	159	6.1	5.8	0.07	3.1	3.5	3.2	0.3	6.7	2.3	1.8	15	13
Creek 2		1015	4.06	20.0	154	6.1	5.8										
	8/28/74	0830	4.54	20.9	127	6.2	5.3										
		0900	4.54	20.9	123	6.2	5.3	0.09	2.7	1.9	1.7	0.2	4.7	1.5	1.4	16	16
Hammonton	7/17/74	1130	5.75	20.0	140	6.2	4.2	0.23	2.2	>4.1	4.1	>0.0	>6.3	2.2	1.9	12	10
Creek 3	8/28/74	0930	7.8	20.0	152	6.4	1.2	0.04	1.9	>3.2	3.2	>0.0	>5.1	2.0	1.8	15	12
Hammonton	7/17/74	1330	6.67	20.0	145	6.1	4.4	0.10	2.0	>4.1	4.1	>0.0	>6.1	2.6	2.6	15	15
Creek 4	8/28/74	1030	10.8	21.0	137	6.4	2.8	0.08	2.1	3.4	3.0	0.4	5.6	1.6	1.4	11	11

Site	Date	Alka-lin-ity (CO3) (mg/L)	Bicar-bonate (HCO3) (mg/L)	Total cal-cium (mg/L)	Total magne-sium (mg/L)	Total sodium (mg/L)	Total potas-sium (mg/L)	Dis-solved chloride (mg/L)	Dis-solved sulfate (mg/L)	Total fluor-ide (mg/L)	Dis-solved silica (mg/L)	Dis-solved solids (mg/L)	Total iron (ug/L)	Dis-solved iron (ug/L)	Total man-ganese (ug/L)	Dis-solved man-ganese (ug/L)
Hammonton	7/17/74	16	19	8.6	1.8	5.3	1.1	7.3	4.9	0.1	3.1	30	1400		30	
Creek 1	8/28/74	14	17	3.5	2.0	5.4	1.0	7.3	2.5	0.1	4.1	51	2300		190	
Effluent																
Hammonton	7/17/74	14	17	7.5	2.1	17	3.7	25	14	0.3	8.3	106	1100		60	
Creek 2		14	17					13	7.8		6.3	78		720		30
	8/28/74	15	18					14	7.5		7.1	83		640		80
		3	4	4.0	2.0	12	2.5	14	7.8	0.2	7.2	96	1300		80	
Hammonton	7/17/74	21	26	7.8	1.8	15	3.3	18	12	0.2	7.0	79	580		60	
Creek 3	8/28/74	7	9	4.2	1.9	16	2.8	17	8.7	0.2	7.7	89	800		220	
Hammonton	7/17/74	19	23	10	1.7	14	3.5	18	14	0.4	6.4	87	1500		20	
Creek 4	8/28/74	12	15	6.0	1.6	13	2.8	15	10	0.1	7.0	81	830		480	

Table 4.--Chemical analyses for Squankum Branch.

Site	Date	Time	Dis-charge (ft^3/s)	Temp (°c)	Spec. cond. (umhos)	pH units	Dis-solved oxygen (mg/L)	Total nitrite (N) (mg/L)	Total nitrate (N) (mg/L)	Total kjeld. (N) (mg/L)	Total ammo-nia (N) (mg/L)	Total organic nitro-gen (N) (mg/L)	Total nitro-gen (N) (mg/L)	Total phos-phorus (P) (mg/L)	Total ortho phos-phorus (P) (mg/L)	Total organic carbon (mg/L)	Dis-solved organic carbon (mg/L)
Squankum Branch 1	8/15/74	0730	0.13	19.0	128	6.5	5.0	0.05	1.1	0.72	0.44	0.28	1.8	0.22	0.18		11
	9/10/74	0700	0.10	22.0	306	6.3	4.7	0.04	1.8	0.38	0.19	0.19	2.2	0.04	0.01		9.2
Effluent	8/05/74		1.08	23.0		6.8											
	9/10/74		1.00			6.9											
Squankum Branch 2	8/05/74	0915	2.66	17.0	148	5.9	5.0	0.12	2.0	3.8	3.8	0.0	5.9	1.2	1.2		14
		0930	2.66	17.0	145	5.9	5.0										
	9/10/74	0930	2.54	19.0	304	6.7	2.0	0.00	0.02	>12	12	>0.0	>12	3.4	2.9	9.6	5.0
		0935	2.54	19.0	280	6.7	2.0										
Squankum Branch 3	8/05/74	1100		18.0	116	6.1	1.5	0.08	0.21	4.0	3.7	0.3	4.3	1.2	0.93	20	18
	9/10/74	1000		21.0	247	6.7	1.1	0.01	0.03	>11	11	>0.0	>11	3.6	3.2	18	16
Squankum Branch 4	8/05/74	1200		17.0	118	6.3		0.05	0.09	4.5	4.3	0.2	4.6	1.2	1.1	11	
	9/10/74	1100			210	6.7	2.4	0.12	0.13	>8.9	8.9	>0.0	>9.2	3.0	2.6	21	13

Site	Date	Alka-lin-ity (CO3) (mg/L)	Bicar-bonate (HCO3) (mg/L)	Total cal-cium (mg/L)	Total magne-sium (mg/L)	Total sodium (mg/L)	Total potas-sium (mg/L)	Dis-solved chloride (mg/L)	Dis-solved sulfate (mg/L)	Total fluor-ide (mg/L)	Dis-solved silica (mg/L)	Dis-solved solids (mg/L)	Total iron (ug/L)	Dis-solved iron (ug/L)	Total man-ganese (ug/L)	Dis-solved man-ganese (ug/L)
Squankum Branch 1	8/05/74	25	31	11	3.3	5.5	3.8	6.3	21	0.3	3.2	106	320		70	
	9/10/74	50	61	26	8.0	14	5.8	15	51	0.2	5.4	188	1000		110	
Effluent																
Squankum Branch 2	8/05/74	28	34	7.0	3.1	13	3.5	14	14	0.2	5.9	116	240		60	
	--	27	33					14	15		5.8	103		170		310
	9/10/74	79	96	16	4.8	25	8.0	23	13	0.3	8.1	153	300		30	
		85	104					23	15		8.3	156		270		60
Squankum Branch 3	8/05/74	17	21	5.3	2.5	11	3.2	10	24	0.4	4.7	106	900		90	
	9/10/74	66	80	13	4.0	25	4.8	19	17	0.2	8.9	130	490		30	
Squankum Branch 4	8/05/74	23	28	4.3	2.2	12	3.3	10	20	0.3	4.7	100	740		90	
	9/10/74	59	72	7.0	4.0	23	5.0	17	15	0.3	8.5	127	2500		80	

Table 5.--Chemical analyses for Union Creek - Landing Creek.

Site	Date	Time	Dis-charge (ft^3/s)	Temp (°c)	Spec. cond. (umhos)	pH units	Dis-solved oxygen (mg/L)	Total nitrite (N) (mg/L)	Total nitrate (N) (mg/L)	Total kjeld. (N) (mg/L)	Total ammo-nia (N) (mg/L)	Total organic nitro-gen (N) (mg/L)	Total nitro-gen (N) (mg/L)	Total phos-phorus (P) (mg/L)	Total ortho phos-phorus (P) (mg/L)	Total organic carbon (mg/L)	Dis-solved organic carbon (mg/L)
Landing	7/22/74	0745	0.57	15.0	110	5.5	8.2	0.01	1.1	0.17	0.15	0.02	1.3	0.01	0.01		11
Creek 1	9/06/74	0730	1.01	16.5	86	6.0	0.9	0.00	0.9	0.18	0.02	0.16	1.1	0.01	0.01	8.1	3.7
Effluent	7/22/74		0.38														
	9/06/74		1.65	23.0		7.0											
Landing	7/22/74	1000	1.29	17.0	141	6.7	4.7								1.7		
Creek 2		1030	1.29	17.0	147	6.7	4.7	0.11	1.4	1.5	1.4	0.10	2.9	2.2		4.7	
	9/06/74	0930	2.67	18.0		6.5	5.6								0.9		
		1015	2.67	18.0	140	6.5	5.6	0.04	0.75	0.93	0.7	0.23	1.7	1.1		11	
Landing	7/22/74	1130	5.81	16.0	103	6.7	6.6	0.06	0.93	>0.99	0.99	>0.00	>1.9	0.96	0.87	11	4.1
Creek 3	9/06/74	1115	6.26	17.0	73	5.5	6.4	0.00	0.04	0.09	0.07	0.02	0.1	0.31	0.05	11	10
Landing	7/22/74	1400	6.89	17.0	85	6.4	6.6	0.02	0.82	>0.98	0.98	>0.00	>1.8	0.71	0.63	8.0	7.5
Creek 4	9/06/74	1215	16.2	17.0	69	5.5	6.4	0.01	0.45	0.42	0.41	0.01	0.88	0.31	0.23	14	9.4

Site	Date	Alka-lin-ity (CO3) (mg/L)	Bicar-bonate (HCO3) (mg/L)	Total cal-cium (mg/L)	Total magne-sium (mg/L)	Total sodium (mg/L)	Total potas-sium (mg/L)	Dis-solved chloride (mg/L)	Dis-solved sulfate (mg/L)	Total fluor-ide (mg/L)	Dis-solved silica (mg/L)	Dis-solved solids (mg/L)	Total iron (ug/L)	Dis-solved iron (ug/L)	Total man-ganese (ug/L)	Dis-solved man-ganese (ug/L)
Landing	7/22/74	1.0	1.0	5.0	1.8	4.0	0.7	7.4	9.7	0.1	9.3	60	390		50	
Creek 1	9/06/74	5.0	6.0	7.0	1.9	4.5	1.3	8.0	15	0.3	7.5	73	320		20	
Effluent																
Landing	7/22/74	13	16					16	15		19	103		370		40
Creek 2		14	17	9.0	1.9	15	2.8	16	15	0.6	20	111	570		40	
	9/06/74	14	17					11	13		6.8	108		450		40
		19	23	8.7	2.5	13	2.0	10	13	0.7	11.0	81	590		20	
Landing	7/22/74	7	8.0	8.0	1.7	10	1.7	12	12	0.3	17.0	81	1200	30		
Creek 3	9/06/74	4	5.0	3.4	1.3	6.0	1.4	9.1	11	0.3	11.0	73	1200	20		
Landing	7/22/74	3	4.0	0.5	1.4	8.0	1.2	11	12	0.3	15.0	76	1200	10		
Creek 4	9/06/74	2	3.0	5.2	1.2	5.5	1.0	8.3	8.9	0.3	5.4	48	1100	20		

1. The pH value of the effluent was greater than that of the stream above the outfall (site 1) and subsquently the pH value in the stream below the sewage outfall was higher.
2. The pH value of the effluent was less than the upstream value and consequently the pH value of the stream below the sewage outfall was lower.
3. The pH value of the effluent was greater than that of the upstream site, but the pH value of the stream below the outfall showed a decrease.

The pH of the mixed water in Hay Stack Brook, Landing Creek and the second sampling of Squankum Branch was greater than the pH of the upstream water. The pH of the mixed water in Hammonton Creek and the first sampling of Squankum Branch was lower than the pH of the upstream water. Therefore, five of the eight samplings indicated that the introduction of effluent into the receiving waters produced an initial overall increase in the pH value of the stream. As the stream passed downstream sites 3 and 4, the pH values were returning toward their original values at site 1.

Although none of the pH values in these streams was in the optimum range for nitrification, most values did lie above 6.0 indicating that nitrification could occur. The most favorable pH conditions for nitrification occurred for both samplings of Hay Stack Brook, the first sampling of Landing Creek, and the second sampling of Squankum Branch where the mean pH values were all greater than 6.5. The mean pH values observed in the other samplings of the remaining three streams ranged from 5.8 to 6.3.

Temperature

The potential effect of temperature on nitrifying bacterial density can be illustrated by the data from Hay Stack Brook. The mean water temperature at the time of the first sampling was 20.1°C while the mean value for the second sampling was 16.3°C. The number of nitrifying bacteria found in the water phase during the first sampling was considerably more than found during the second sampling, although the temperature was not necessarily the only factor contributing to this observation.

Alkalinity

At pH values below 8.0, alkalinity can be defined as follows:

$$\text{Total alkalinity} = [HCO_3^-] + 2[CO_3^{-2}] + [OH^-] - [H_3O^+]. \quad (3)$$

Both samplings of Hay Stack Brook and Landing Creek showed an average sixfold increase in alkalinity at site 2. This added alkalinity, which could be expected to serve as a possible buffering agent, apparently had little sustained effect on the pH of the streams as evidenced by the trend toward progressively lower pH values downstream.

The amount of alkalinity consumed in neutralizing nitrous acid produced from nitrification is an indication of the extent to which nitrification is occurring in a stream. Symons, Weibel, and Robeck (1967) observed that the oxidation of 1 mg/L of NH_4^+ - N resulted in a decrease in alkalinity equal to 6.5 mg/L as $CaCO_3$. The amount of ammonia oxidized within each stream section can be determined on the basis of an increase in oxidation products (Wezernak and Gannon, 1968). If one assumes that the alkalinity of additional water being added as ground or surface runoff is equal to the alkalinity in the stream above the treatment plant then, provided no nitrification takes place, the expected amount of nitrite and nitrate any point downstream would be

$$[NO_2^- + NO_3^-]_{expected} = \frac{[NO_2^- + NO_3^-]_i Q_i + [NO_2^- + NO_3^-]_i Q_a}{Q_d}, \quad (4)$$

where Q = flow (cubic feet per second),
i = initial or upstream values,
a = additional ground water and(or) surface water values, and
d = downstream values.

Any nitrite and nitrate measured in excess of this amount could then be attributed to increases in oxidation products by nitrification. Therefore the amount of ammonia oxidized is equal to

$$[NH_4^+]_{\text{oxidized via nitrification}} = [NO_2^- + NO_3^-]_{\text{measured}} - [NO_2^- + NO_3^-]_{\text{expected}}. \quad (5)$$

If this quantity is positive, then nitrification is indicated. The amount of alkalinity in milligrams per liter neutralized as the result of nitrification is then calculated from

$$[\text{Alkalinity neutralization}]_{\text{attributable to nitrification}} = 6.5 \times [NH_4^+ - N]_{\text{oxidized}} \quad . \qquad (6)$$

The results of these calculations are summarized in table 6. Calculations for Squankum Branch are not included because discharge data are not available for the two most downstream sites. Only Hay Stack Brook and Hammonton Creek show signs of nitrification on the basis of alkalinity neutralization, and the rate of nitrification appears to be increasing as the stream passes site 4. The fraction of alkalinity neutralized as the result of ammonia oxidation, however, is quite small. The large decrease in alkalinity between sites 2 and 3 in Hay Stack Brook is mostly the result of dilution.

In Hammonton Creek there is little change in the alkalinity because the stream above the treatment plant and the effluent have about the same level of alkalinity. The alkalinity change in Landing Creek is totally the result of dilution because no nitrification is indicated, and the alkalinity of the effluent is considerably higher than that of the stream above the treatment plant. Although no calculations could be made for Squankum Branch, the alkalinity data indicate that little, if any, nitrification is occurring because the nitrite and nitrate concentrations, both above the treatment plant and in the effluent, are much higher than at any of the downstream sites.

Nitrogen Species

Since the process of nitrification involves a breakdown of ammonia followed by an increase in nitrite and nitrate, a stream undergoing perceivable nitrification should reflect these transformations downstream of a pollution input. These transformations, however, do not always proceed in an orderly fashion in nature. Various occurrences may confuse and complicate the interpretation of these nitrogen transformations. The concentration of total nitrogen species in a particular stream may decrease below the pollution input resulting from dilution as the discharge of the stream increases. Ammonia may increase from the decomposition of organic nitrogen.

Table 6.--Neutralization of alkalinity attributable to nitrification.
[I, first sampling; II, second sampling]

	SITE 1			SITE 2			SITE 3					SITE 4				
Stream	Dis-charge[1] (ft³/s)	Alka-linity[1] (CaCO3) (mg/L)	NO2 + NO3[1] (mg/L)	Dis-charge[1] (ft³/s)	Alka-linity[1] (CaCO3) (mg/L)	NO2 + NO3[1] (mg/L)	Dis-charge[1] (ft³/s)	Alka-linity[1] (CaCO3) (mg/L)	NO2 + NO3[1] (mg/L)	NH4[2] oxi-dized site 2 to site 3 (mg/L)	Alka-linity[3] neu-tralized site 2 to site 3 (mg/L)	Dis-charge[1] (ft³/s)	Alka-linity[1] (CaCO3) (mg/L)	NO2 + NO3[1] (mg/L)	NH4[2] oxi-dized site 2 to site 4 (mg/L)	Alka-linity[3] neu-tralized site 2 to site 4 (mg/L)
Haystack I	0.19	4	2.02	1.49	75	0.30	4.35	12	1.79	+0.36	2.3	4.45	10	2.25	+0.81	5.3
Haystack II	0.33	7	2.10	2.20	51	0.39	5.9	13	1.77	+0.32	2.1	6.0	10	2.10	+0.64	4.2
Hammonton I	1.09	16	0.09	4.06	14	3.17	5.75	21	2.43	+0.17	1.1	6.67	19	2.10	+0.14	0.9
Hammonton II	1.39	14	0.02	4.54	3	2.79	7.8	7	1.94	+0.31	2.0	10.8	12	2.13	+1.00	6.5
Landing I	0.57	1	1.11	1.29	14	1.51	5.81	7	0.99	-0.21	0	6.89	4	0.84	-0.34	0
Landing II	1.01	5	0.90	2.67	19	0.79	6.26	3	0.04	-0.81	0	16.2	2	0.46	-0.42	0
Squankum I	0.13	25	1.15	2.66	28	2.12		17	0.29				23	0.14		
Squankum II	0.10	50	1.84	2.54	79	0.02		66	0.04				59	0.25		

[1] - Measured stream values

[2] - $[NH_4^{+1}]_{oxidized} = [NO_2^{-1} - N + NO_3^{-1} - N]_{measured} - [NO_2^{-1} - N + NO_3^{-1} - N]_{expected}$

[3] - Alkalinity neutralized = $6.5 \times [NH_4^{+1}]_{oxidized}$

Assimilation of ammonia by plants and algae, ammonia exchange between the mud and water phases, microanaerobes mediating the reduction of NO_3^- - N to nitrogen gas, or unknown pollutional inputs in ground water may also affect the nitrogen balance of the stream.

Despite the complications discussed above, nitrification patterns were observed in several of the streams (figs. 6 to 13). Hay Stack Brook encountered all of the expected nitrogen transformations below the waste water input. The increase in the oxidation products in Hammonton and Landing Creeks indicate that nitrification was occurring in these streams although the increases in both the ammonia concentrations and the total nitrogen concentrations in Hammonton Creek and Landing Creek are unexplained. The increase in oxidation products in Hammonton Creek supported the 20 day incubation findings that nitrification was occurring in this stream. Although nitrogen transformations were observed in Landing Creek, NOD values were comparatively small indicating that nitrification was not occurring to a significant extent in this stream. The determination of the occurrence of nitrification in Squankum Branch could not be based upon the changes of nitrogen species because of insufficient data.

Organic Nitrogen

Organic nitrogen is an additional nitrogen constituent in water and is composed of, among other things, amino acids, polypeptides, and proteins. Since organic nitrogen can be decomposed to ammonia, the presence of any significant amount must be considered in evaluating nitrogen transformation attributable to nitrification. For all the sites sampled in this study, organic nitrogen was an insignificant component of the total nitrogen content of the stream.

Organic Carbon

In general, both the total- and dissolved-organic content of the stream at site 2 reflect the high carbon input of the treatment plant. No discernible patterns are evident except that as far downstream as site 4 some values are still greater than values observed above the sewage treatment plants.

Biological Oxygen Demand

The results of the microbiological analyses are given in tables 7 and 8. In all streams, the nitrogenous oxygen demand experimentally determined over 20 days (NOD_e) showed a substantial increase at site 2 over site 1 as a result of the input from the treatment plant. The largest increase was in Hay

Stack Brook while the smallest increase was in Landing Creek. The NOD_e tended to decrease in the downstream direction, however, notable exceptions were site 4 in both Hammonton Creek and Squankum Branch.

If the experimentally determined NOD is divided by the total BOD (carbonaceous + nitrogenous) determined over the same 20 day time period, the fraction of oxygen consumed from nitrification is obtained (Ram, 1975).

$$\phi = NOD_e/BOD_t \tag{7}$$

where

NOD_e = nitrogenous oxygen demand determined experimentally from 20-day incubations, and

BOD_t = total 20-day biological oxygen demand (carbonaceous and nitrogenous).

At all locations with the exception of site 3 for the second sampling of Landing Creek, the percentage of oxygen utilized by nitrification was smallest at site 1. Because of the small amount of NOD observed at all sites in this sampling, the experimental values may represent a higher degree of error.

The NOD represented over 50 percent of the total oxygen consumed in 33 percent of the samples and therefore confirms that NOD can be a significant deoxygenating component in a nitrifying stream. Since 3.22 mg/L oxygen are required to oxidize ammonia to nitrite and 1.11 mg/L oxygen are required to oxidize nitrite to nitrate, calculated NOD can be determined if the concentrations of ammonia and nitrite are known.

$$NOD_{calculated} = 4.33 \times [NH_4^+] + 1.11 \times [NO_2^-] \tag{8}$$

If NOD_{calc} is assumed to be the ultimate NOD of the water sample then the quantity, NOD_e/NOD_{calc} represents the fraction of the maximum potential NOD oxidized at each location. Values greater than 1.0 are attributable to experimental error in the

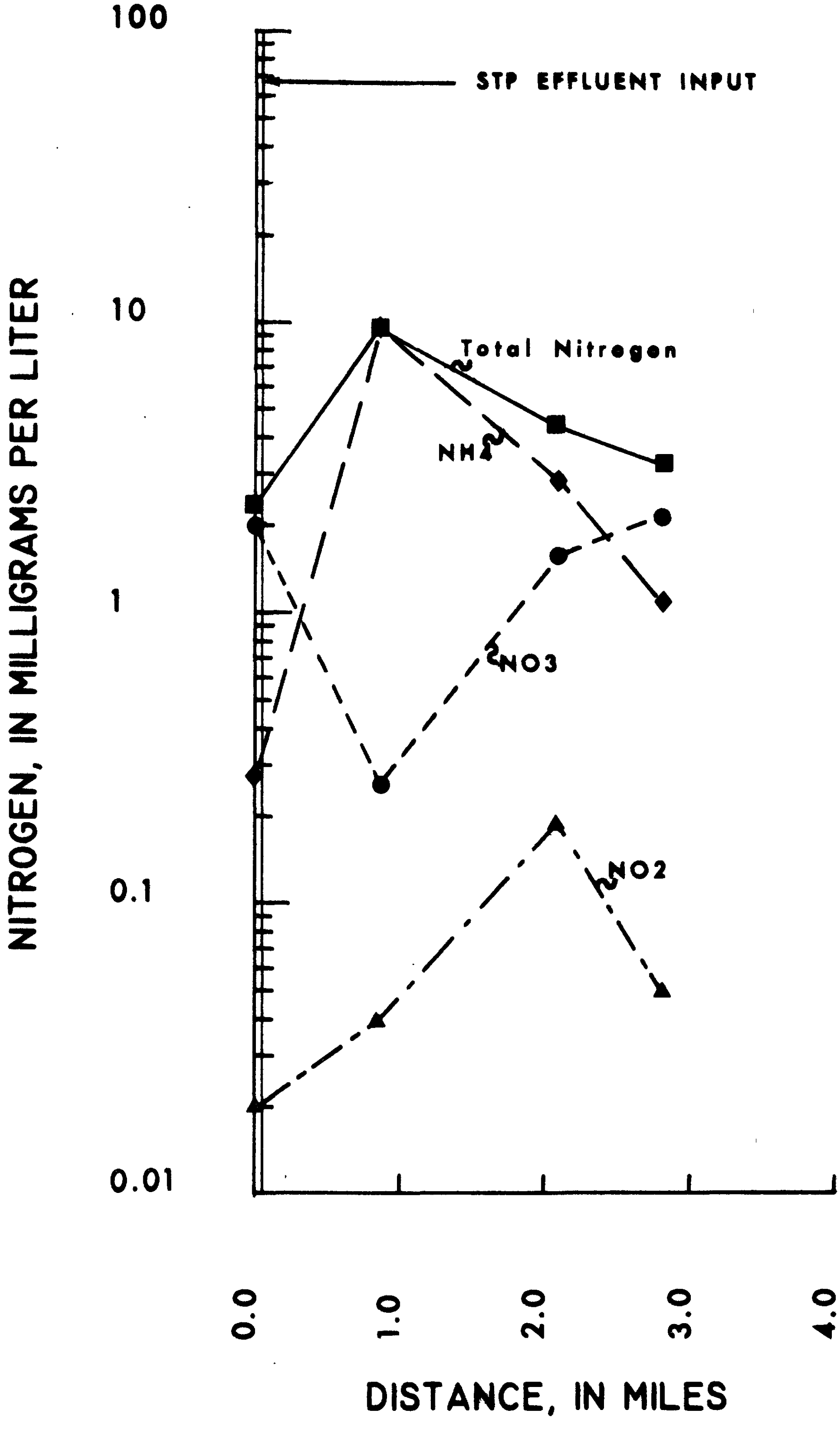


Figure 6.--Nitrogen transformations: Hay Stack Brook I.

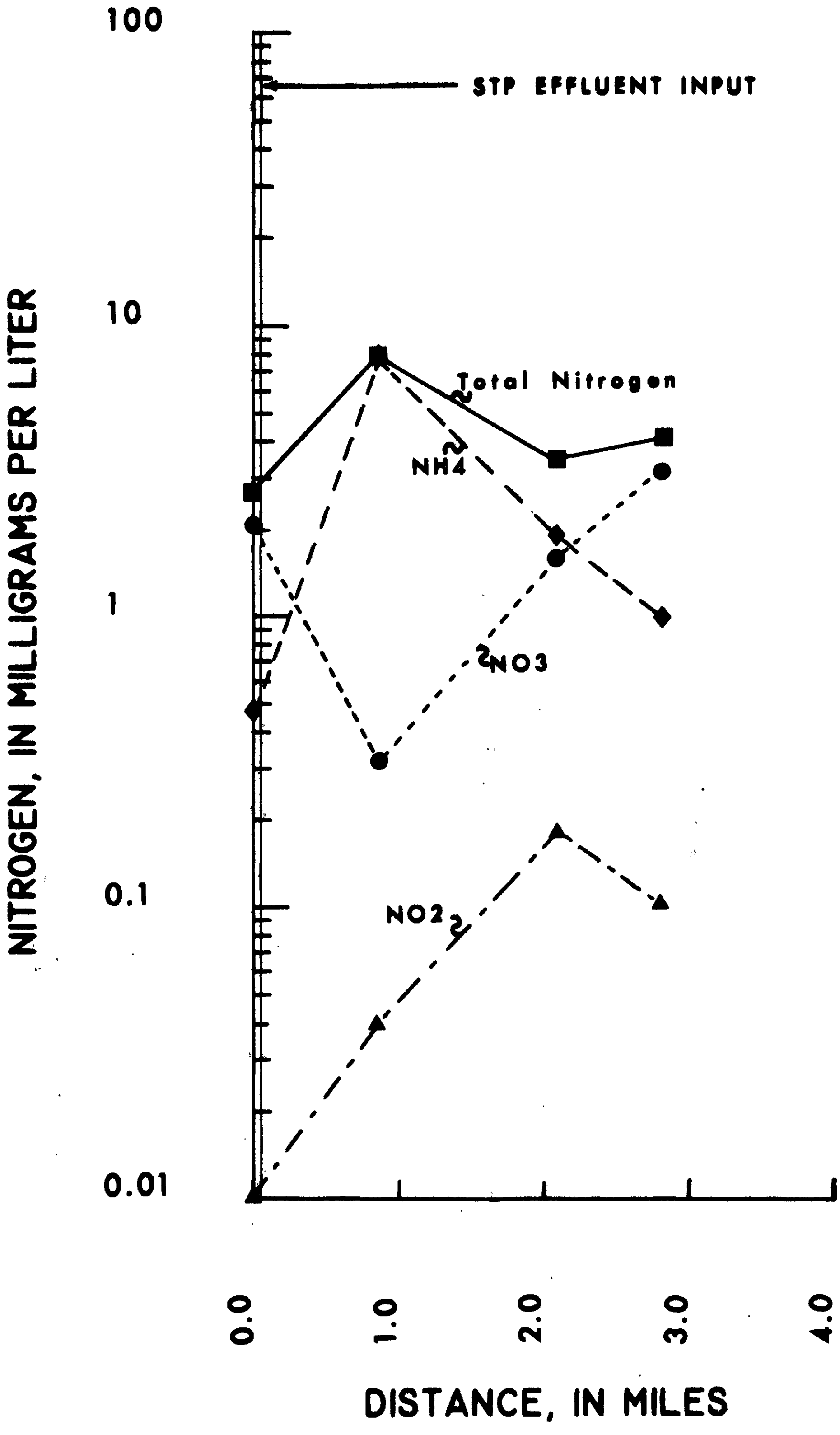


Figure 7.--Nitrogen transformations: Hay Stack Brook II.

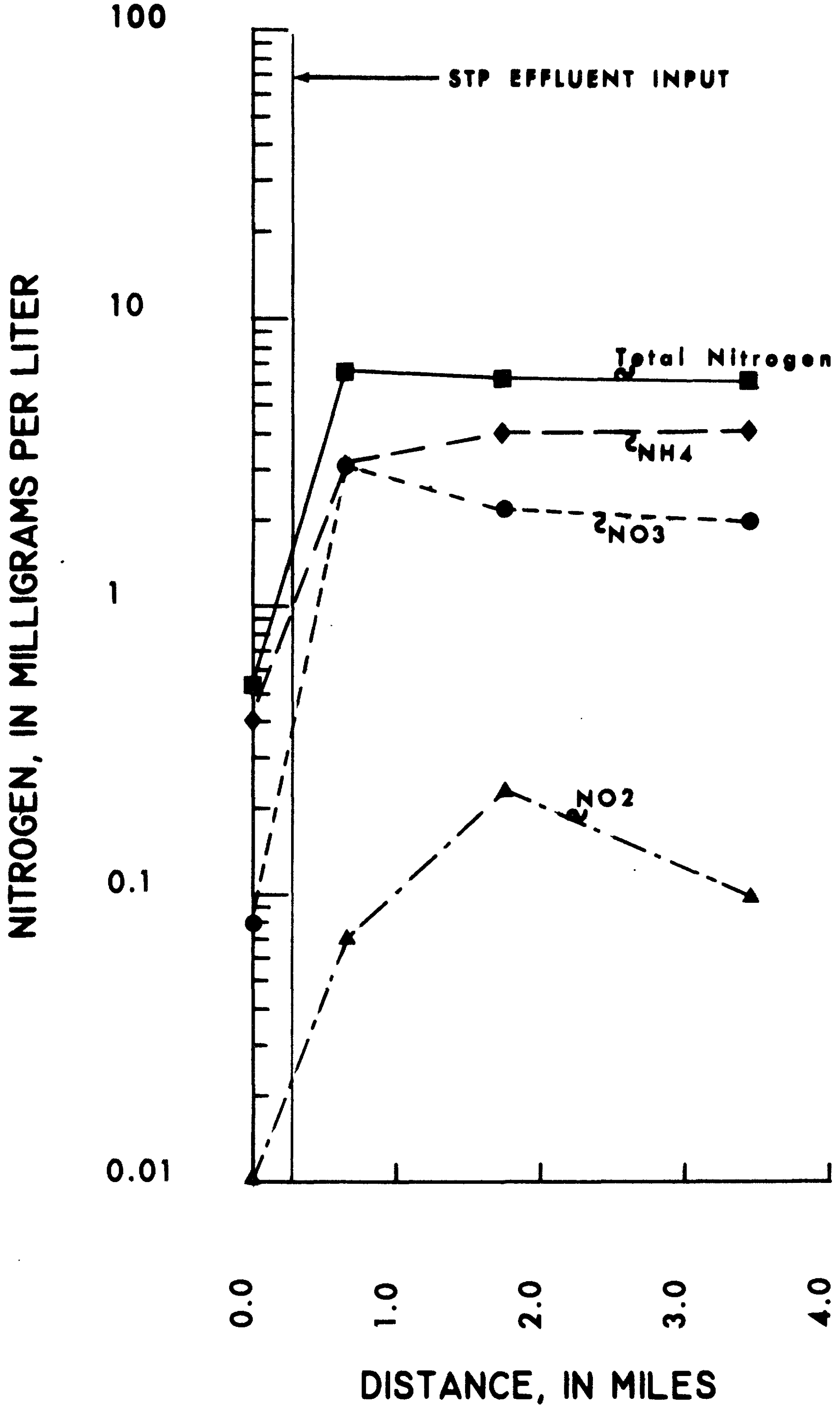


Figure 8.--Nitrogen transformations: Hammonton Creek I.

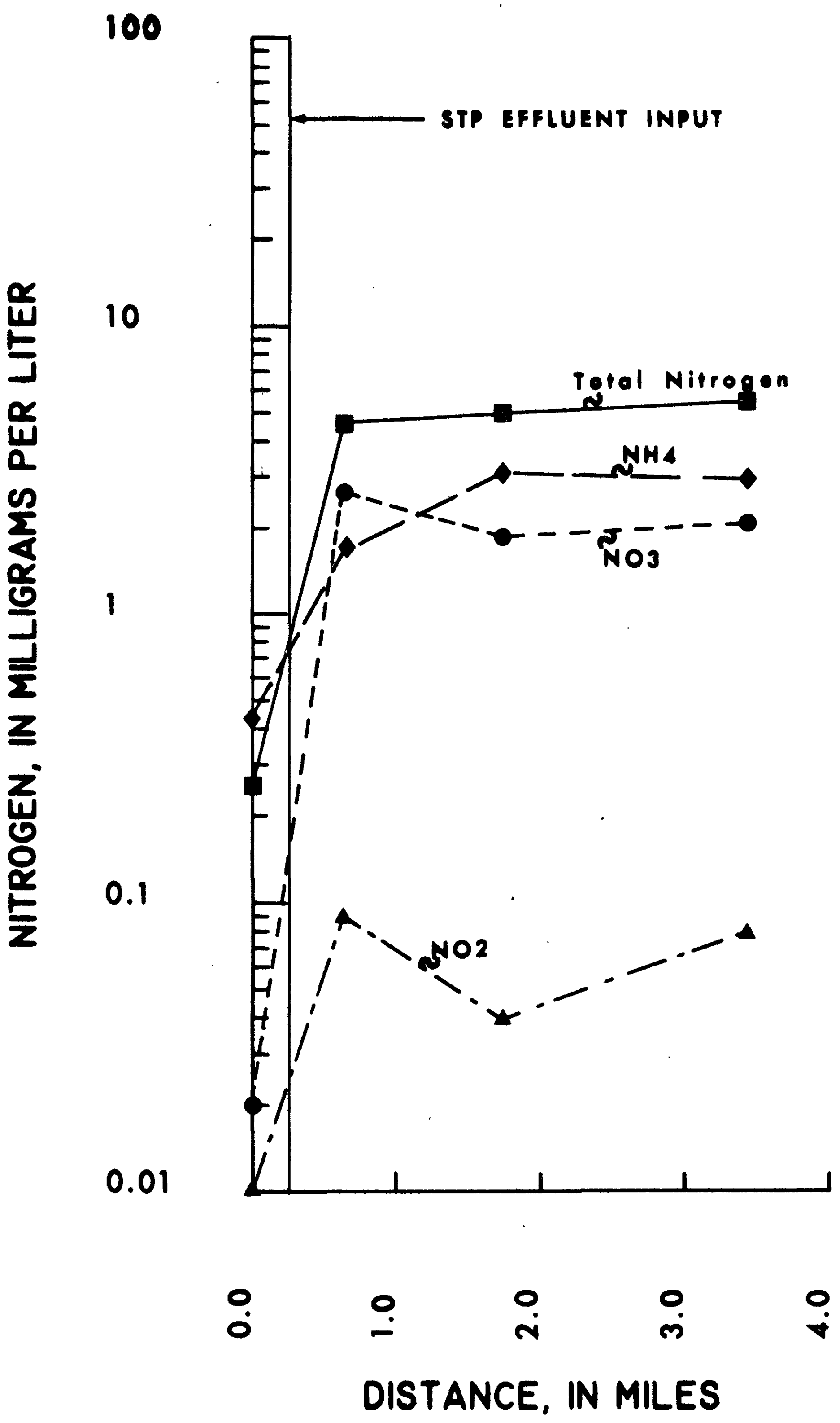


Figure 9.--Nitrogen transformations: Hammonton Creek II.

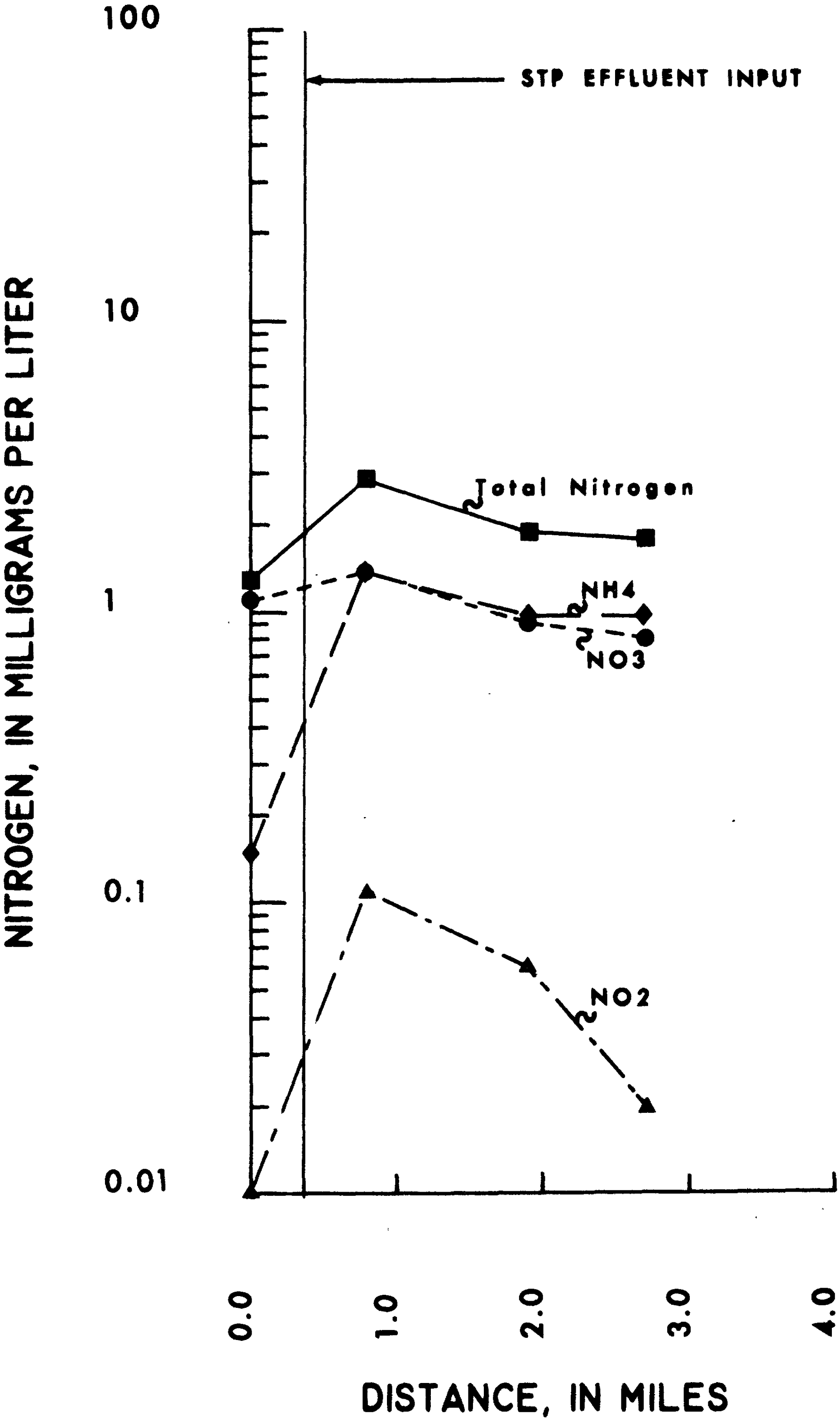


Figure 10.--Nitrogen transformations: Landing Creek I.

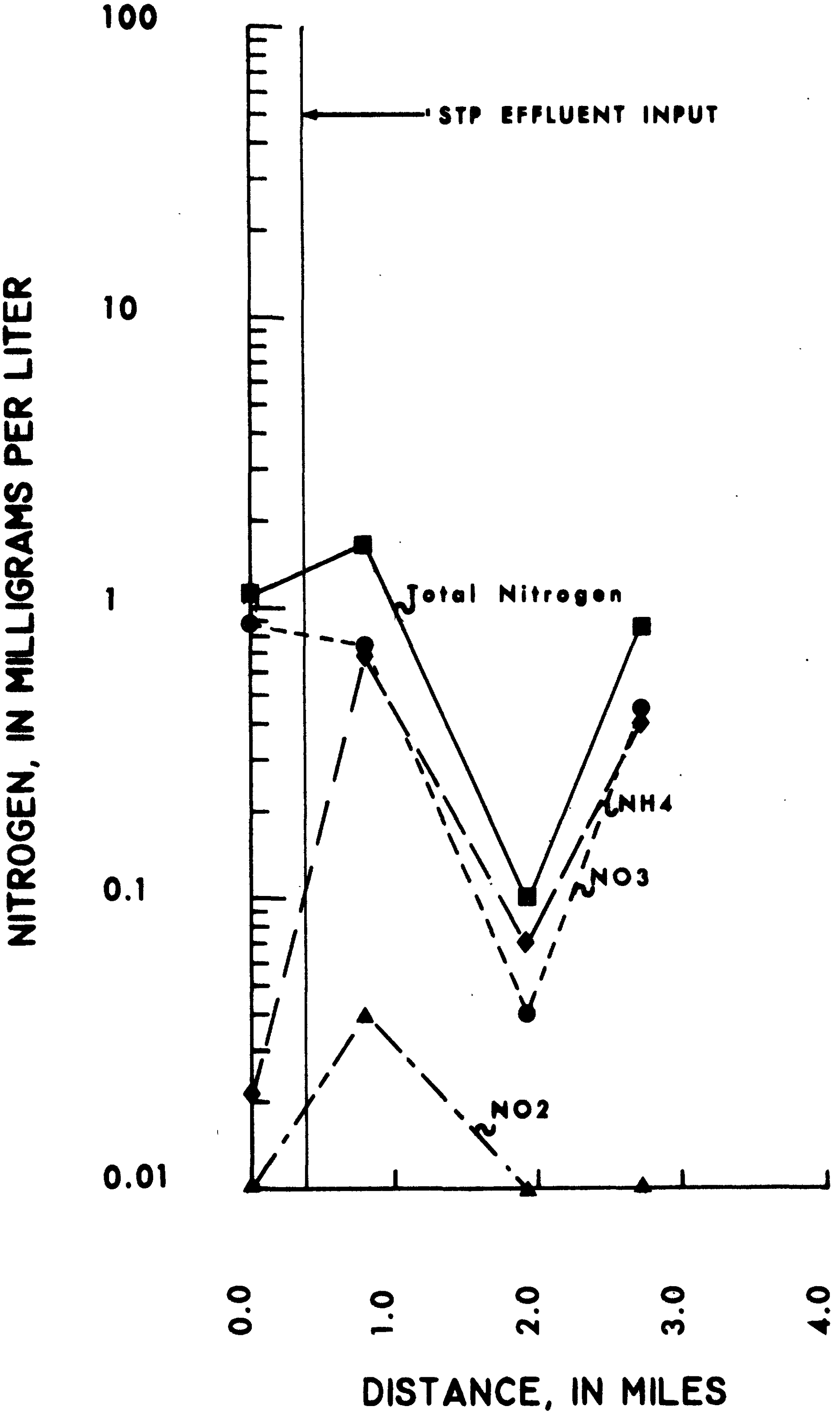


Figure 11. Nitrogen transformations: Landing Creek II.

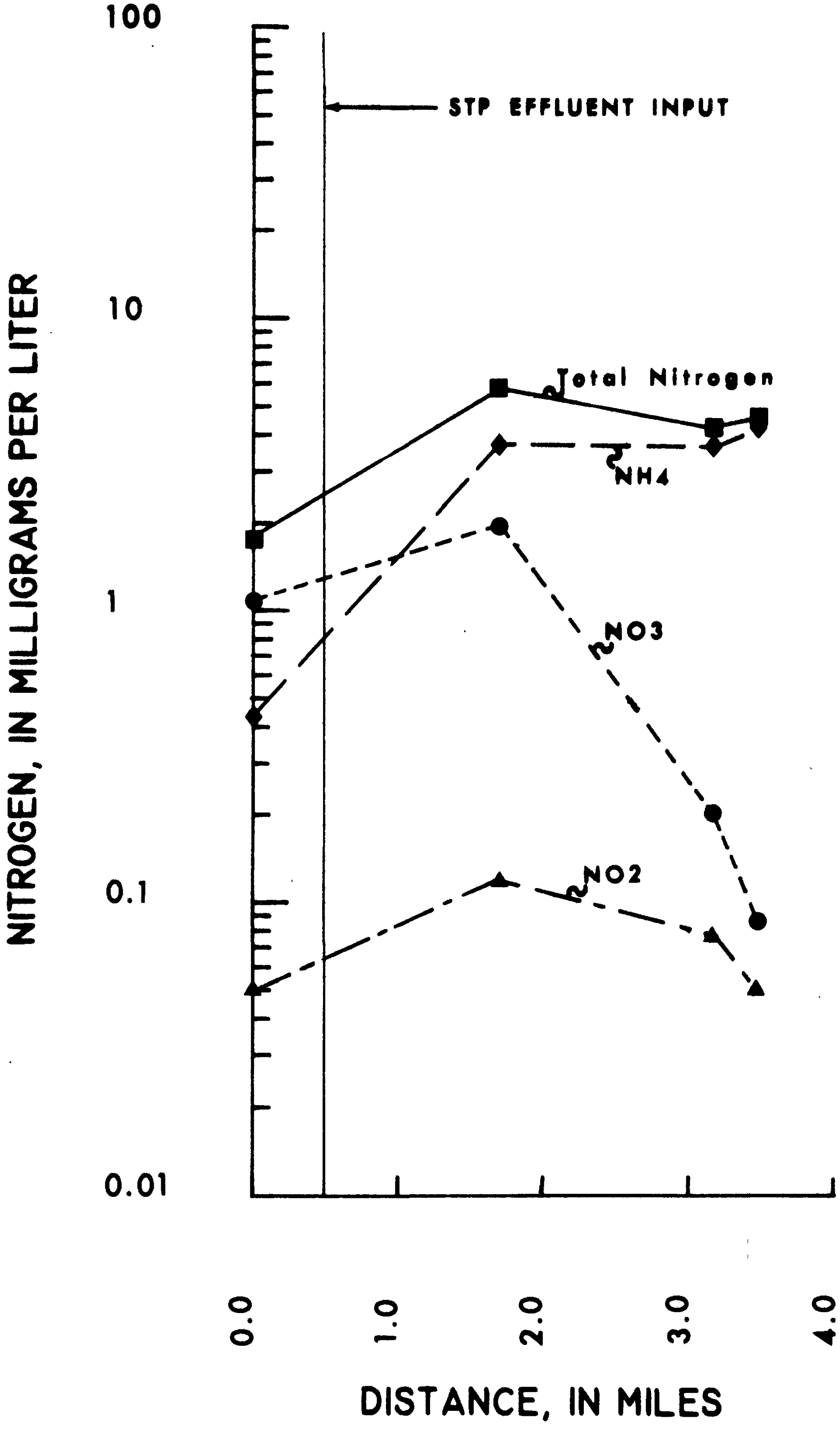


Figure 12.--Nitrogen transformations: Squankum Branch I.

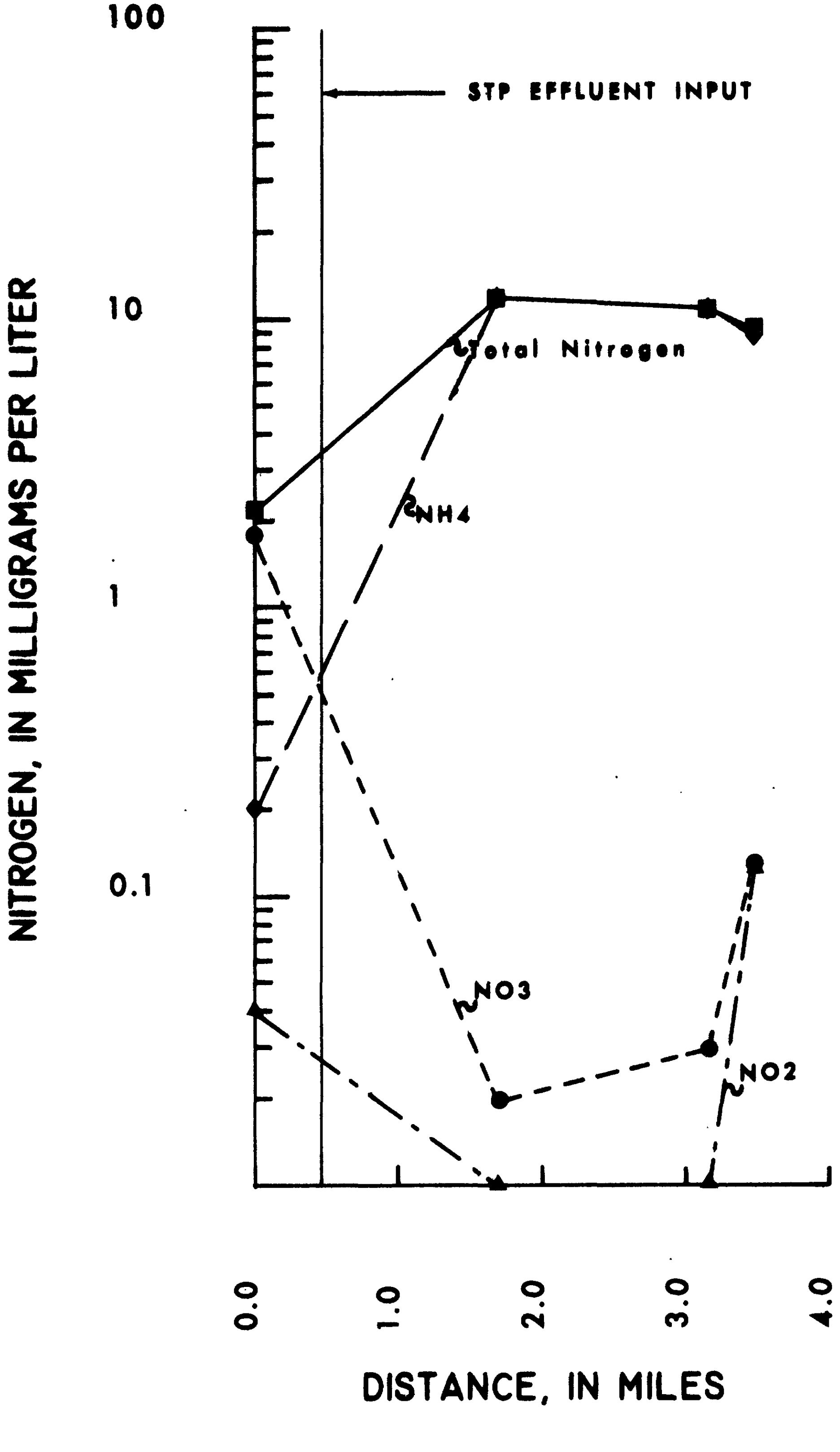


Figure 13.--Nitrogen transformations: Squankum Branch II.

Table 7.--Biological analyses for Hay Stack Brook and Hammonton Creek.

	Date	BOD_5	BOD_t	BOD_{carb}	NOD_e	NOD_e / BOD_t	NOD_{calc}	NOD_e / NOD_{calc}	Nitrosomonas colonies/mL	Nitrosomonas in mud colonies/g	Nitrobacter colonies/mL	Nitrobacter in mud colonies/g
Hay Stack Brook 1	7/30/74	1.0	1.8	1.6	0.2	0.11	1.2	0.17	5.5		0.0	
	9/23/74	30.0	33.0	33.0	0.0	0.0	2.0	0.00	0.8	5.0×10^2	0.3	3.44×10^2
Effluent	7/30/74											
	9/23/74								1.34		0.0	
Hay Stack Brook 2	7/30/74	3.2	48.5	12.2	36.3	0.75	41.2	0.88	5100.		8.4	
	9/23/74	20.0	57.3	28.0	29.3	0.51	33.8	0.87	1590.	1.6×10^7	0.6	1.53×10^4
Hay Stack Brook 3	7/30/74	4.4	26.4	10.8	15.6	0.59	12.8	1.22	2980.		6.2	
	9/23/74	17.5	56.1	23.2	32.9	0.59	8.0	4.11	1910.	1.9×10^5	4.5	1.55×10^3
Hay Stack Brook 4	7/30/74	2.6	11.7	6.8	4.9	0.42	4.8	1.02	1910.		2.3	
	9/23/74	22.5	31.2	28.8	2.4	0.08	4.4	0.56	1860.		0.3	
Hammonton Creek 1	7/17/74	1.1	4.5	4.4	0.1	0.02	1.8	0.06	0.0		0.0	
	8/28/74	3.1	9.0	7.2	1.8	0.2	1.9	0.95	2.3		1.1	
Effluent	8/28/74								0.0			
Hammonton Creek 2	7/17/74	3.3	28.3	15.6	12.7	0.45	13.9	0.91	208.		0.0	
	8/28/74	4.5	15.3	9.4	5.9	0.39	7.5	0.79	2260.		0.8	
Hammonton Creek 3	7/17/74	3.7	22.6	16.6	6.0	0.27	18.0	0.33	582.		2.3	
	8/28/74	5.6	22.6	13.8	8.8	0.39	13.9	0.63	1890.		2.3	
Hammonton Creek 4	7/17/74	2.2	28.3	6.1	22.2	0.78	17.9	1.24	70.2		2.3	
	8/28/74	3.7	19.1	7.0	12.1	0.63	13.1	0.92	6220.		4.5	

BOD_t = Total 20 day biological oxygen demand

BOD_{carb} = $BOD_t - NOD_e$

NOD_e = 20 day nitrogenous oxygen demand

NOD_{calc} = $4.33 \times [NH_4^+] + 1.11 \times [NO_2^-]$

Table 8.--Biological analyses for Union Creek - Landing Creek and Squankum Branch.

	Date	BOD_5	BOD_t	BOD_{carb}	NOD_e	NOD_e / BOD_t	NOD_{calc}	NOD_e / NOD_{calc}	Nitrosomonas colonies/mL	Nitrobacter colonies/mL
Landing Creek 1	7/22/74	0.6	1.7	1.6	0.1	0.06	0.7	0.14	0.9	0.1
	9/6/74	1.2	2.0	2.2	0.0	0	0.1	0.0	2.3	0
Effluent										
Landing Creek 2	7/22/74	3.2	13.0	8.1	4.9	0.37	6.2	0.79	1030.	0
	9/6/74	3.2	9.6	7.2	2.4	0.25	3.1	0.77	1150.	0
Landing Creek 3	7/22/74	1.7	6.8	4.1	2.7	0.40	4.4	0.61	191.	0
	9/6/74	1.2	2.8	2.8	0	0	0.3	0.00	159.	0
Landing Creek 4	7/22/74	1.4	5.9	3.4	2.5	0.42	4.3	0.58	301.	0
	9/6/74	1.2	2.7	2.5	0.2	0.07	1.8	0.11	103.	0.3
Squankum Branch 1	8/5/74	4.8	11.7	9.3	2.4	0.21	2.0	1.20	298.	191.
	9/10/74	2.4	6.1	5.7	0.4	0.07	0.9	0.44	16.	2.3
Effluent										
Squankum Branch 2	8/5/74	10.4	41.9	21.3	20.6	0.49	16.6	1.24	77.	3.8
	9/10/74	47.0	88.7	67.8	20.9	0.24	52.0	0.40	8.4	0.1
Squankum Branch 3	8/5/74	8.1	29.5	16.7	12.8	0.43	16.0	0.80	138.	3.8
	9/10/74	11.0	34.1	22.5	11.6	0.34	47.6	0.24	5.8	0.6
Squankum Branch 4	8/5/74	8.0	36.4	15.6	20.8	0.57	18.7	1.11	138.	23.
	9/10/74	8.6	46.8	17.3	29.5	0.63	38.7	0.76	385.	4.5

BOD_t = Total 20 day biological oxygen demand

$BOD_{carb} = BOD_t - BOD_e$

NOD_e = 20 day nitrogenous oxygen demand

$NOD_{calc} = 4.33 \times [NH_4^+] + 1.11 \times [NO_2^-]$

laboratory determination of ammonia. The fraction of NOD oxidized provides a means of evaluating the extent of nitrification. Squankum Branch and Hay Stack Brook experienced the largest nitrogenous oxygen demands in the laboratory incubations, although the fractions of NOD oxidized in these streams were variable. More than 70 percent of the mean NOD was oxidized in Hay Stack Brook, the first sampling of Squankum Branch, and the second sampling of Hammonton Creek suggesting that these streams were nitrifying.

We found that 53 and 22 percent of the potential NOD was oxidized in the first and second samplings, respectively, of Landing Creek; 46 percent in the second sampling of Squankum Branch; and 58 percent in the first sampling of Hammonton Creek, thus indicating that these streams were nitrifying at a slower rate than Hay Stack Brook.

Hammonton Creek contained a smaller concentration of nitrogenous material than did Hay Stack Brook and Squankum Branch. Landing Creek contained an even smaller amount. The experimental nitrogenous oxygen demands were consequently smaller in Hammonton Creek, and even less so in Landing Creek. In terms of BOD and NOD, therefore, nitrification occurred during the second sampling of Hammonton Creek, the first sampling of Squankum Branch, and both samplings of Haystack Brook.

Laboratory BOD incubations indicated that Landing Creek was nitrifying, but only to a very small extent, probably because the water phase contained only a small amount of oxidizable substrate and because the environmental conditions were not favorable in Landing Creek during the second sampling. The mean pH values for the first and second samplings of Landing Creek were 6.6 and 5.8, respectfully, indicating that nitrification was not favored during the second sampling of Landing Creek.

The cumulative oxygen consumption in inhibited and uninhibited BOD bottles is shown in figures 14 to 20. The inhibited bottles contained 0.5 mg/L of allylthiourea (ATU) to inhibit oxygen consumption attributable to nitrification. The difference between the inhibited and uninhibited curves, then, represents the nitrogenous oxygen demand. The time of initiation of nitrification may be estimated from these graphs. The estimated time of initiation of nitrification ranged from less than 2 days to more than 10 days. In 34 percent of the samples, initiation of nitrification was not discernable at all.

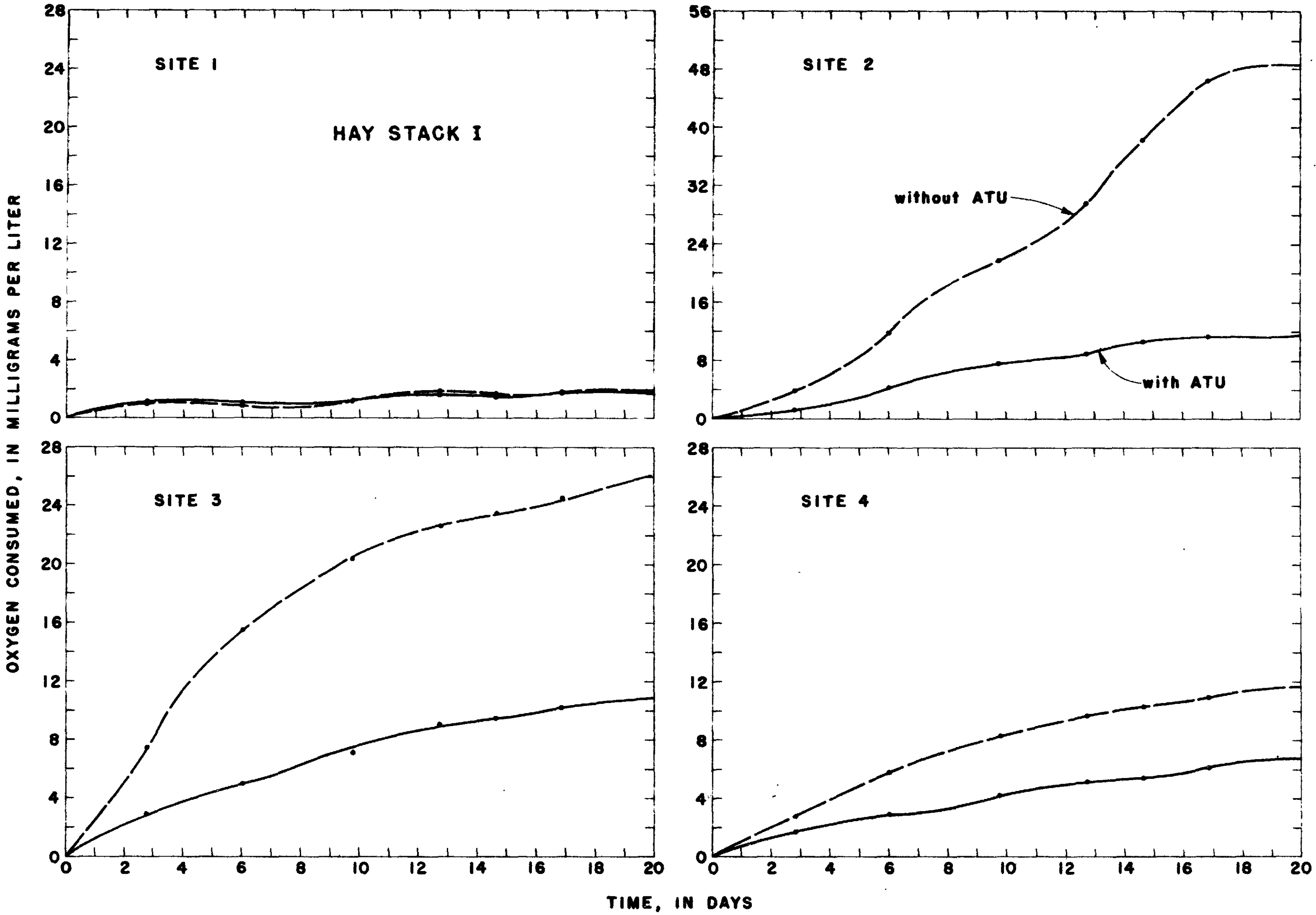


Figure 14.--Oxygen consumption curves for ATU inhibited and uninhibited water samples: Hay Stack Brook, first sampling.

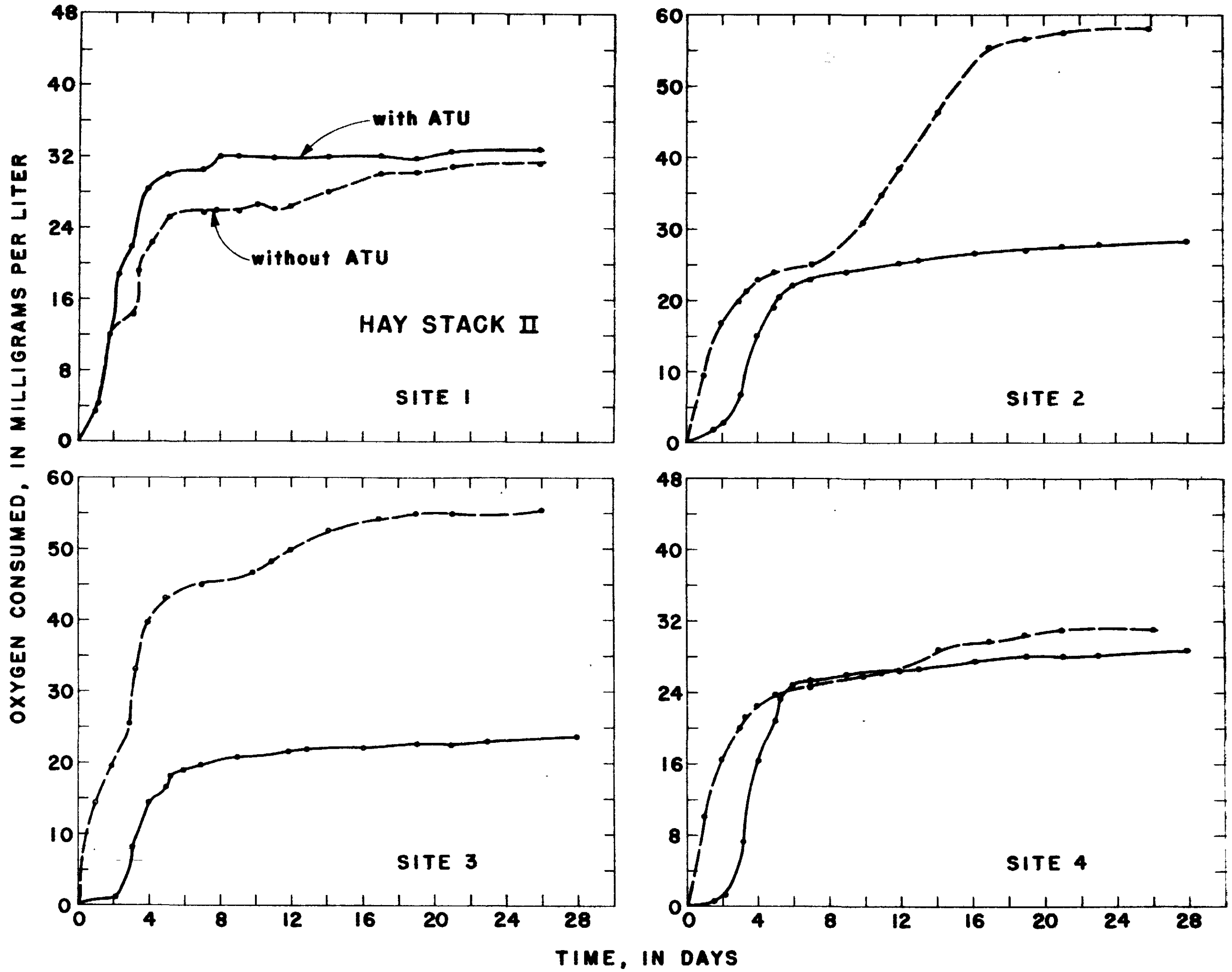


Figure 15.--Oxygen consumption curves for ATU inhibited and uninhibited water samples: Hay Stack Brook, second sampling .

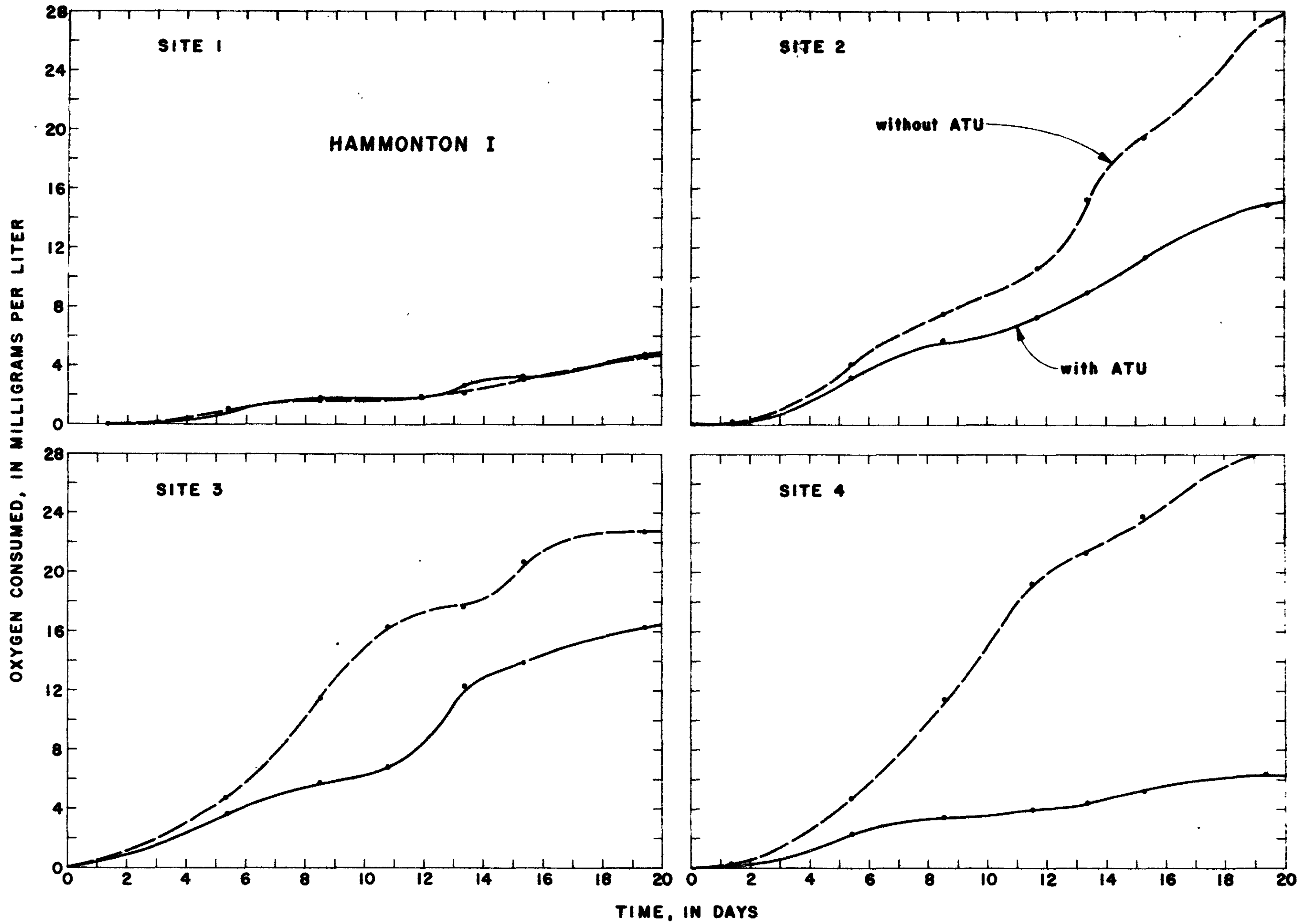


Figure 16.--Oxygen consumption curves for ATU inhibited and uninhibited water samples: Hammonton Creek, first sampling.

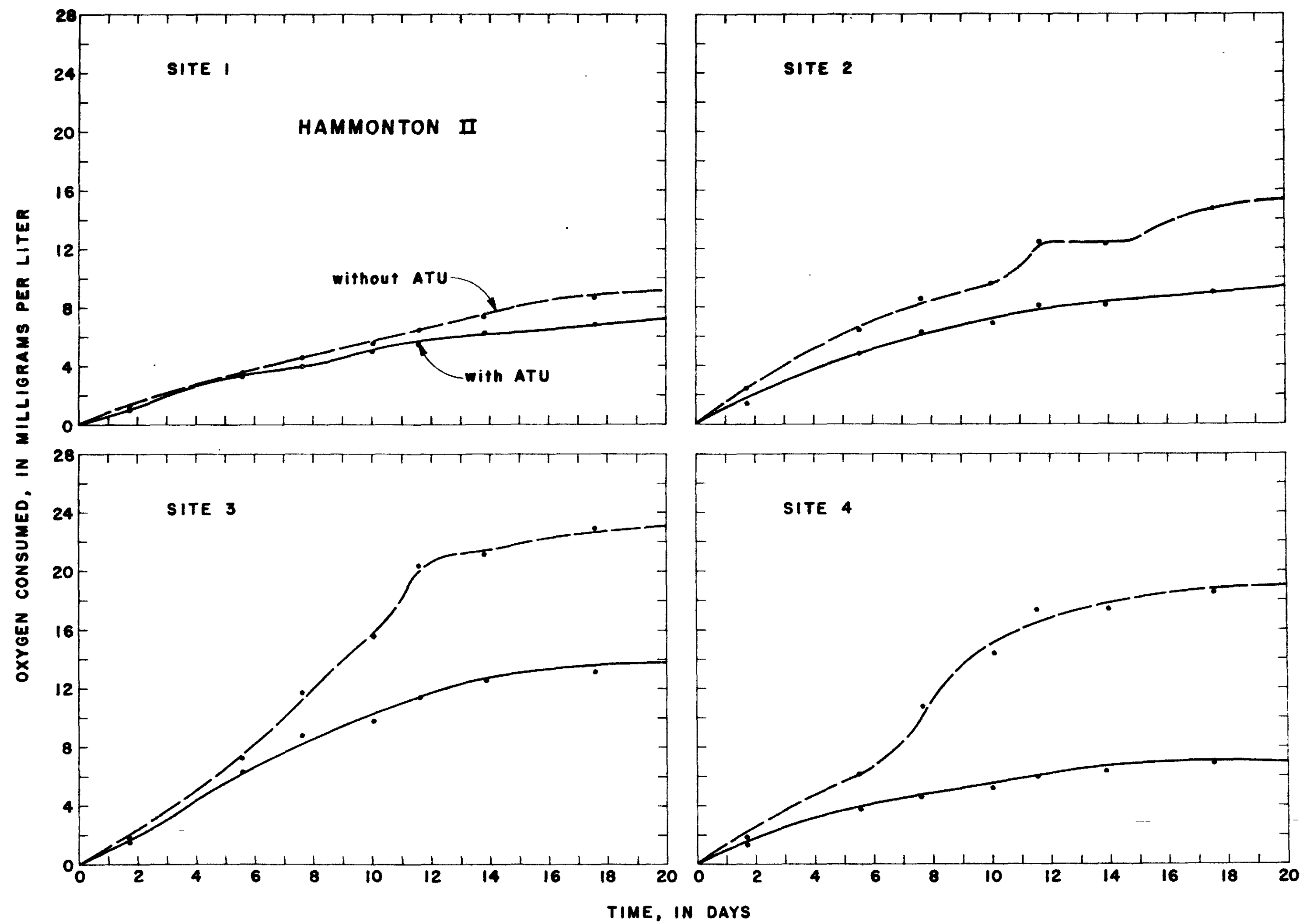


Figure 17.--Oxygen consumption curves for ATU inhibited and uninhibited water samples: Hammonton Creek, second sampling.

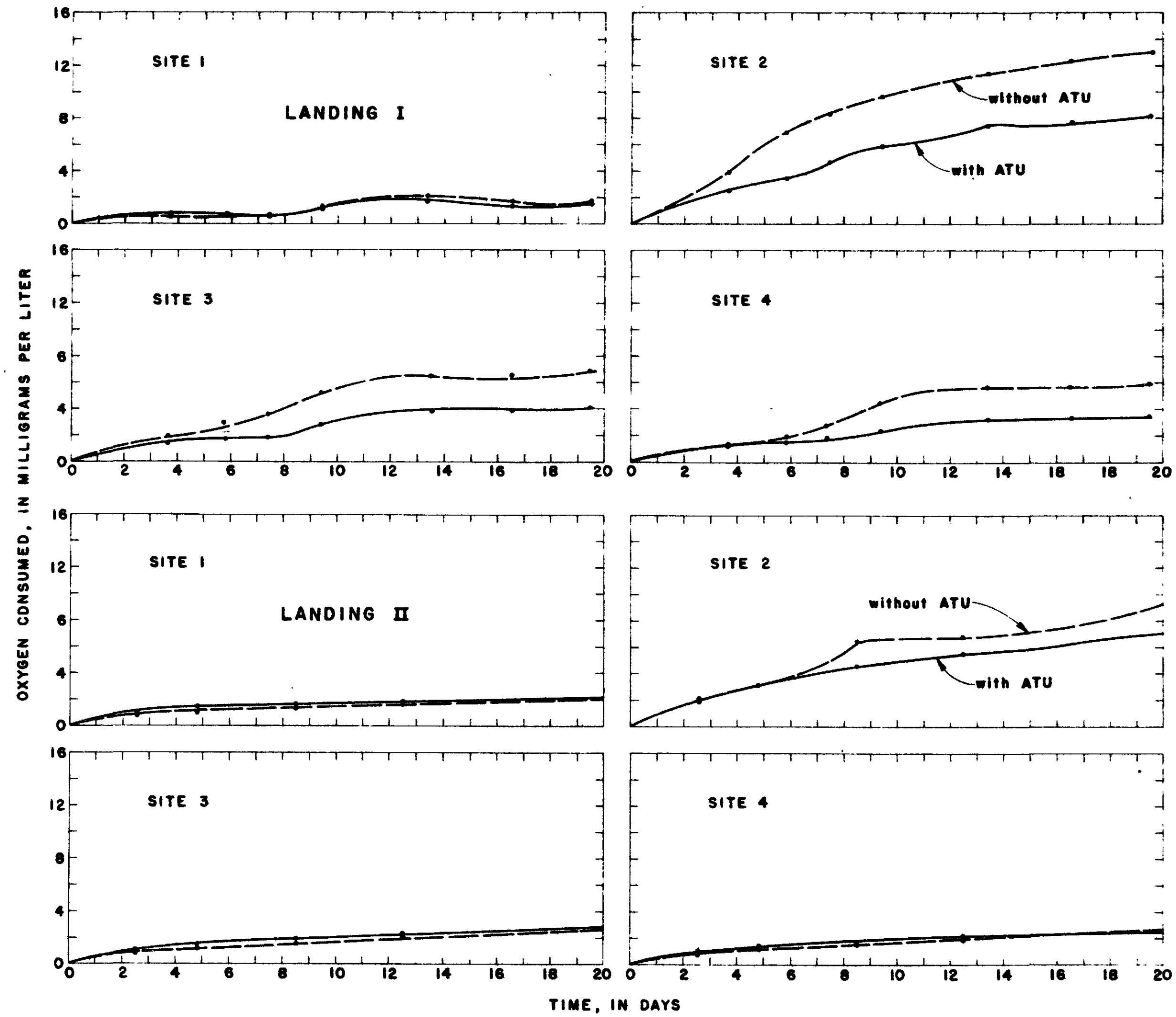


Figure 18.--Oxygen consumption curves for ATU inhibited and uninhibited water samples: Landing Creek, first sampling.

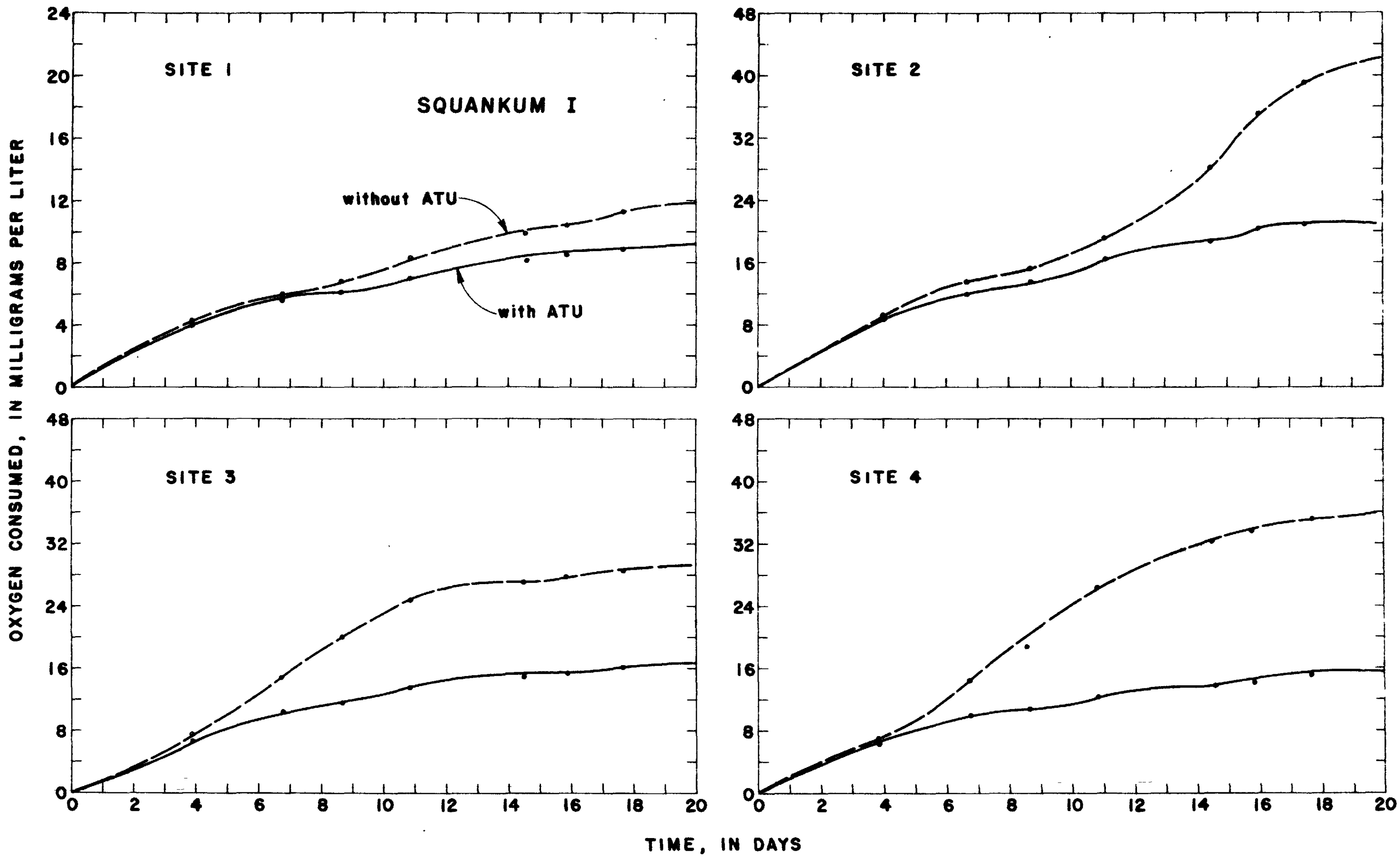


Figure 19.--Oxygen consumption curves for ATU inhibited and uninhibited water samples: Squankum Branch, first sampling.

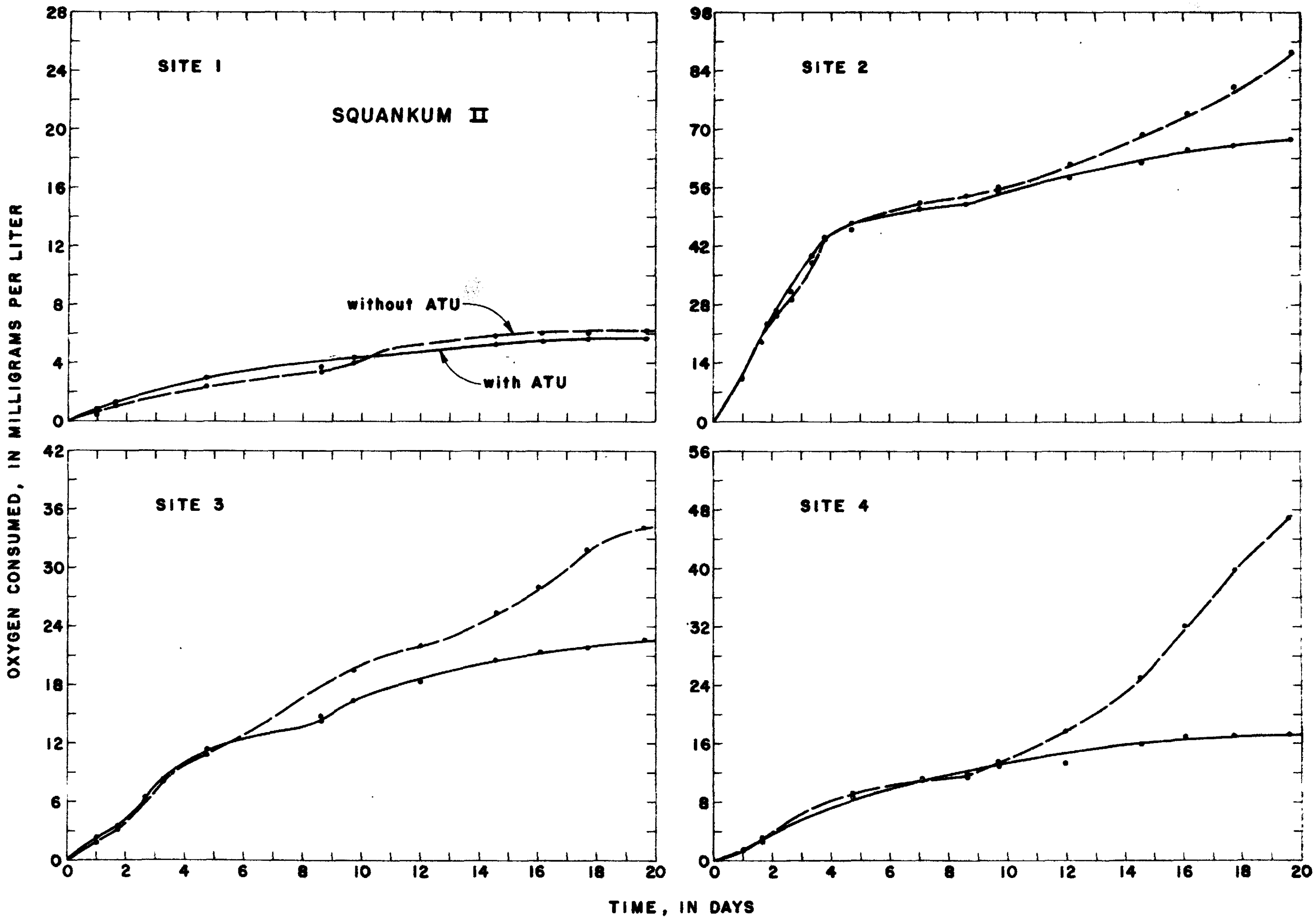


Figure 20.--Oxygen consumption curves for ATU inhibited and uninhibited water samples: Squankum Branch, second sampling.

Bacteriological Enumeration

The presence of a large nitrifying flora in a stream supports the potential for NH_4^+ - N to be nitrified. Studies have indicated that nitrification occurs in streams as a result of activity at the mud-water interface rather than from the activity of nitrifying bacteria in the water phase and that the bacteria found in the water phase results from bottom scour (Tuffey, 1973; Finstein and Matulewich, 1974; Tuffey, Hunter, and Matulewich, 1974).

The number of bacteria present in the water phase of each of the rivers studied is shown in tables 7 and 8. The numbers represent MPN (most probable number) values determined from 35 day incubations at 28°C. In all streams except Squankum Brook, the population of nitrifying bacteria increased markedly below the sewage treatment plant. No obvious trend in the change in the populations of nitrifying bacteria with distance are apparent. In almost all cases, the population of Nitrosomonas substantially exceeded Nitrobacter. The mean ratio of Nitrosomonas: Nitrobacter for all sites was 728 and ranged from 1.6 to 7,153.

Hay Stack Brook and Hammonton Creek displayed the highest numbers of nitrifying bacteria. The mean numbers of nitrifying bacteria in these streams were 1.8 X 10^3 and 2.6 X 10^3 total nitrifying organisms/mL for Hammonton and Hay Stack Brook, respectively. The large numbers of bacteria in Hay Stack Brook support the results of the laboratory BOD incubations and observed nitrogen transformations that perceivable nitrification was occurring in this stream. Despite the large number of Nitrosomonas bacteria observed in the water phase of Hammonton Creek, however, BOD incubations indicated that nitrification in this stream occurred to a lesser extent than in Hay Stack Brook and Squankum Branch.

Despite the small numbers of nitrifying organisms in Squankum Branch, BOD incubations indicated that perceivable nitrification was occurring in that stream. This supported the hypotheses that nitrification in streams did not occur by way of the bacteria suspended in the water phase, but rather it occurred by way of activity at the mud and plant-water interface. The small numbers of nitrifying bacteria in Landing Creek supported the BOD results that perceivable nitrification was not occurring in that stream.

The bacterial population data indicated that some streams having relatively large numbers of nitrifying bacteria in the water phase were found to be nitrifying extensively while others also having large numbers of nitrifying bacteria were not. On the other hand, streams having relatively few numbers of nitrifying bacteria were found to be perceivably nitrifying. The large numbers of nitrifying bacteria observed in streams not experiencing extensive nitrification might be explained by the presence of large numbers of persistent inactive forms. The small numbers of nitrifying bacteria observed in the streams undergoing extensive nitrification could be related to the scouring of surface bacteria into the water phase. Therefore, the presence or absence of nitrifying bacteria in the water phase is not an indication of the occurrence or nonoccurrence of nitrification.

The growth of Nitrosomonas and Nitrobacter over a 56-day incubation period at 28°C was determined for water-phase samples taken during the second sampling of Hay Stack Brook and are shown in figures 21 and 22. The populations of Nitrosomonas at sites 1-3 reached their maximum numbers by the 24th day confirming earlier work by Matulewich, Strom, and Finstein (1974). An anomalous growth pattern was observed for site 4 which had a secondary growth increase in Nitrosomonas organisms between the 30th and 42nd day of incubation. The populations of Nitrobacter at sites 1, 2, and 4 all showed a steady increase in numbers beyond the 35th day of incubation and site 3 showed a marked growth beyond the 24th day of incubation. These results corroborate the findings of Matulewich, Strom, and Finstein (1974) that the growth of Nitrobacter can continue beyond the 35th day incubation at 28°C.

The presence of nitrifiers in the mud-water interface was also determined for Hay Stack Brook (table 6). At site 2 there was an increase in the number of nitrifying bacteria by a factor of 10 in the muds below the treatment plant relative to site 1. In all places, the population of Nitrosomonas in the mud exceeded the population of Nitrobacter. The mud phase populations of both species exceeded the water phase populations from a factor of 10 at site 3 to a factor of 10 at site 2. Fifty-six-day incubation results for Hay Stack Brook showed that the maximum population of Nitrosomonas was obtained after 28 days while continued growth was observed for Nitrobacter beyond the 35th day of incubation.

Both the increased populations of nitrifying bacteria and the greater nitrogenous oxygen demands below the waste water outfalls of the streams suggested that these effluents exerted some effect on nitrification. The direct input of nitrogenous wastes attributable to the waste water effluent lead to increased nitrogenous oxygen demands in the streams resulting from nitrification. The increased nitrifying population below the waste water effluent was not a result of the direct input of nitrifying bacteria by the waste water effluent since only 1.4

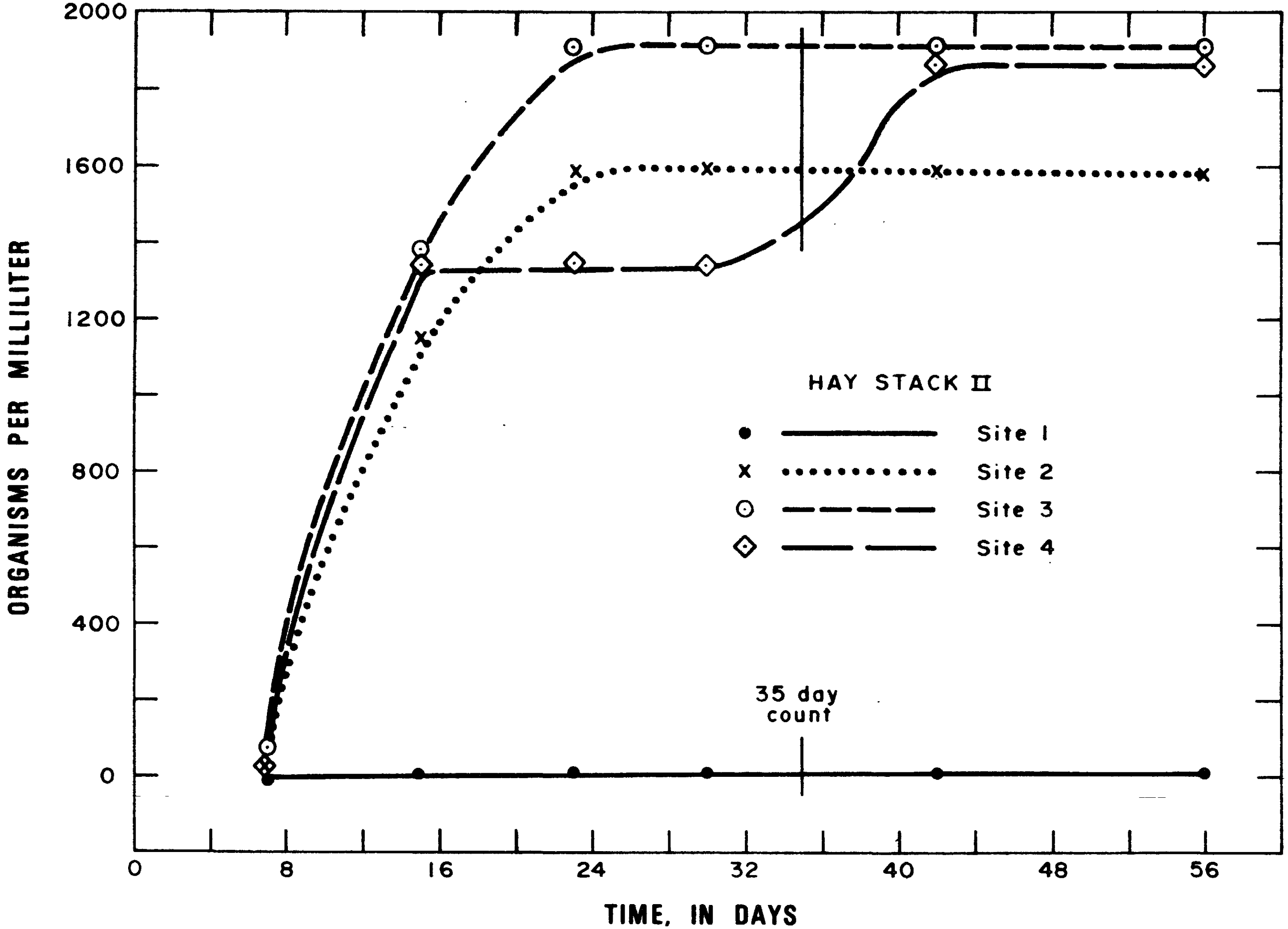


Figure 21.--Nitrosomonas water incubation: Hay Stack Brook II.

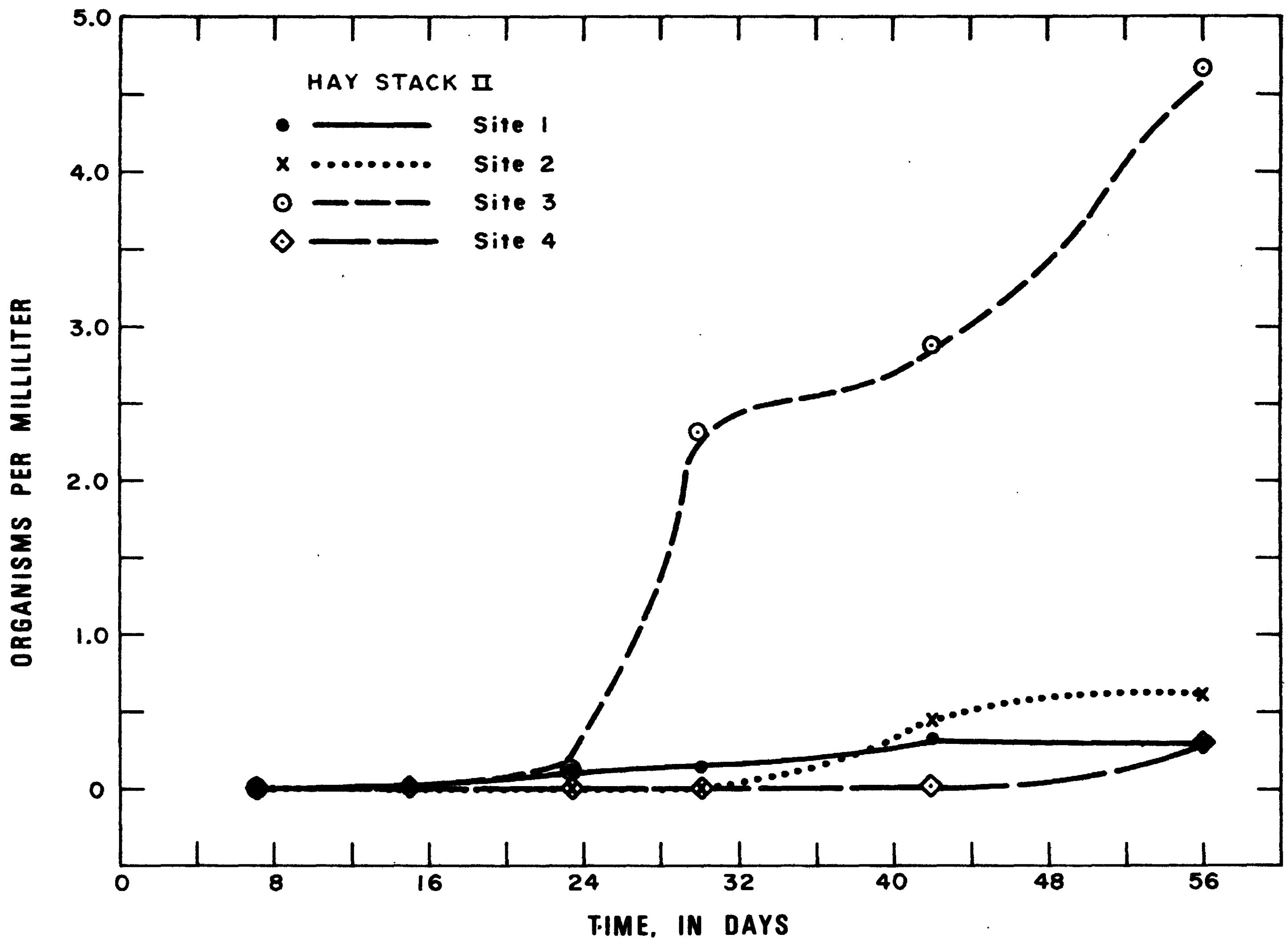


Figure 22.--Nitrobacter water incubation: Hay Stack Brook II.

Nitrosomonas organisms/mL were found in the effluent from Hay Stack and no other nitrifying organisms were found in any of the other effluents. Therefore, the large numbers of nitrifying organisms below the sewage outfalls probably resulted from their proliferation because of the increased nitrogen substrate rather than from a direct input of nitrifying bacteria.

SUMMARY

The characteristic acidity of southern New Jersey streams does not appear to be an exclusive factor in determining whether a stream will nitrify or to what extent. The occurrence and extent of nitrification in a stream is a function of several physical, chemical, and biological properties. As the number of factors increases, the number of possible interactions also increases, the results of which can either enhance or inhibit nitrification. In this report we have evaluated seven such factors which appear to be adequate in establishing the presence or absence of nitrification in a stream.

The temperatures of streams in southern New Jersey will generally afford favorable conditions for nitrification during the warm months of the year and possibly unfavorable conditions during the winter months.

None of the streams studied in this report had dissolved oxygen concentrations below the critical 0.5 mg/L level, thus oxygen is not a limiting factor at any time during the year. Because of the large numbers of nitrifying bacteria found in the surface sediments it was assumed that oxygen was not limiting there as well.

Most streams in southern New Jersey are characteristically acidic and since the pH range for optimal nitrification is between 7 and 9 (Bergey, 1974) it is not surprising that unfavorable conditions prevail in the streams studied. The addition of the sewage effluent to the stream has only a short term effect on the pH value. If a slight increase occurs, it tends to drop back towards the original value just a few miles downstream from the treatment plant.

On the basis of these three properties, (temperature, dissolved-oxygen concentration, and pH) conditions in all four streams studied are usually such that nitrification could probably occur but under slightly less than favorable conditions.

Although the assumptions regarding background concentrations in the calculation of the amount of alkalinity being neutralized during the nitrification process is somewhat

Table 9.--Summary of stream conditions and properties affecting the occurrence of nitrification.

	Conditions				Nitrification Indicators			
	Temperature[1]	Dissolved oxygen	pH[2]	Neutralization of alkalinity	Nitrogen species	20 Day BOD incubations	Nitrifying[3] bacteria	Stream nitrifying
Hay Stack I	F	O	F	+	+++	+++	+++	YES
Hay Stack II	F	O	F	+	+++	+++	+++	YES
Hammonton I	F	O	U	+	?	+	+++	YES
Hammonton II	F	O	U	+	?	+++	+++	YES
Landing I	F	O	F	-	?	+	+	NO
Landing II	F	O	U	-	?	-	+	NO
Squankum I	F	O	U	-	?	+++	+	YES
Squankum II	F	O	F	-	?	+	+	YES

1 - Favorable temperature conditions probably degrade to unfavorable conditions during winter months.
2 - pH values above 6.5 were arbitrarily considered as favorable conditions while values below 6.5 were considered
- unfavorable conditions.
3 - Nitrifying populations greater than 10^3 organism/mL were arbitrarily considered as favorable indicators for nitrification.

O - Optimum conditions for nitrification
F - Favorable conditions for nitrification
U - Unfavorable conditions for nitrification

+++ - Indicates probable occurrence of nitrification
\+ - Indicates possible occurrence of nitrification
\- - Indicates nitrification unlikely
? - Could not be used in assessing nitrification

tenuous, the indicated neutralization occurring in Hay Stack Brook and Hammonton Creek is nonetheless complementary of other positive indicators for the occurrence of nitrification. Landing Creek and Squankum Branch showed no evidence of alkalinity neutralization.

The downstream trends of the concentrations of the various nitrogen species suggest optimum conditions for nitrification in Hay Stack Brook, but uncertain conditions for Hammonton Creek, Landing Creek, and Squankum Branch. Because nitrification in nature may not be an orderly process, the absence of the expected trends may or may not preclude nitrification.

Table 9 is a summary of the seven factors evaluated for the four streams in this study. Each stream had a unique distribution of conditions with minor differences between sampling, making it possible to qualitatively rate the streams in order of their potential for nitrification. Although it is difficult to rank the relative importance of these indicators, it can be seen that Hay Stack Brook appears to be nitrifying the most extensively while Landing Creek gave little, if any, evidence for nitrification. Both Hammonton Creek and Squankum Branch are nitrifying, however, the relative extent of nitrification was hard to assess.

Thus the characteristic acidity of southern New Jersey streams does not appear to an exclusive factor in determining whether a stream will nitrify or to what extent. Each stream will assume its own regime for nitrification based on the interactions of several parameters.

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