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Physico-Chemical Limnology and Periphyton in a Warm-Water Stream  
Receiving Wastewater Treatment Plant Effluent

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## ABSTRACT

PHYSICO-CHEMICAL LIMNOLOGY AND PERIPHYTON IN A WARM-WATER STREAM  
RECEIVING WASTEWATER TREATMENT PLANT EFFLUENT

Physical, chemical, and biological parameters were monitored at five stations in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois, from 12 September 1969 through 7 September 1970 to characterize these streams as a periphyton habitat. Periphyton accrual and periphytic bacterial uptake kinetics studies continued until 10 December 1970 to determine the effect of the effluent from the Sullivan wastewater treatment plant on assimilation of dissolved organic matter by the periphyton community.

The results of the coordinated physical, chemical, and biological study of 34 parameters measured biweekly revealed that there was no gross evidence of any differences between the creek and river sampling sites as determined by these measurements. Wastewater treatment was of such high quality that the effluent was generally undetectable 2 km downstream from the outfall. Only nitrate-nitrogen, ammonia-nitrogen, and phosphorus (all forms) concentrations were directly attributable to the effluent in Asa Creek, the receiving stream.

Of eight parameters measured during the periphyton accrual study, four were found to be significantly different among stations. These included standing crop (biomass), organic content, and productivities (biomass and caloric value). The range of production efficiency of the periphyton community was 0.004 to 0.165% among the five stations. Intrastation correlations among these parameters revealed that the discharge of effluent into Asa Creek coupled with Asa Creek's low stream order resulted in not only a wide flux of physico-chemical conditions, but had a varied effect on the periphyton. Greater stability in the Kaskaskia River, a higher order stream, was reflected in more predictable levels of physico-chemical parameters and in more stable periphyton communities which developed there.

Planktonic and periphytic bacterial chemo-organotrophy, with acetate as the substrate, were measured and evaluated through enzyme kinetics analysis procedures. The maximum bacterial uptake velocity, the maximum natural substrate concentrations, and the substrate regeneration time of acetate, are presented.

Bacterial uptake kinetics experiments demonstrated that the periphyton, at stations influenced by the wastewater treatment plant effluent, assimilated two to three times more dissolved organic matter than at stations not influenced by the effluent. The influence of the treatment plant was not as apparent for assimilation by planktonic bacteria. This reinforced the premise that the attached community was the most sensitive to subtle changes in the aquatic environment.

Brigham, Allison Roeske

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Dr. Warren U. Brigham, Illinois Natural History Survey, assisted in the field and laboratory portions of this study and continued field investigations while I participated in a two-month course in tropical limnology. During preparation of the manuscript, he prepared the final figures and critically reviewed the manuscript.

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## INTRODUCTION

Streams are both biological and chemical individuals (Minckley 1963). In the years since Kolkwitz and Marrson (1908) presupposed that organisms were dependent, in relatively narrow limits, upon the chemical composition of the water for their distribution and ultimate development, the individuality of streams has been overlooked. Existing studies of wastewater treatment plant effluent assimilation by receiving streams generally have relied heavily upon the physical and chemical analyses of the effluents or the streams below the source of the outfall (Klein 1957, Mackenthun and Ingram 1967, Ball et al. 1963, Brehmer et al. 1968, Venkateswarlu 1969, MacCrimmon and Kelso 1970). Depression of dissolved oxygen, high concentrations of soluble phosphorus, free carbon dioxide, biochemical oxygen demand, ammonia, coliform bacteria, and suspended solids have been routinely applied to demonstrate the debilitating effect of wastewater discharges to streams. Chemical analyses, however, can only give a general impression of stream conditions. They do not determine the effects of pollution on biological life (Brehmer et al. 1963). The search for organisms or a community of organisms which would be useful as indicators of water quality continues. Even if such studies were not hampered by the overriding lack of information regarding the environmental requirements of various species and their resistance to various chemical substances (Williams 1964), the continued application of the "indicator organism" concept would be a gross oversimplification. In fact, the constant association with a high correlation between individual species and specific ranges of chemical or physical conditions has been difficult, if not impossible, to demonstrate (Blum 1972).

Many tributary streams in the midwest receive effluents from the wastewater treatment plants of small communities. The Asa Creek-Kaskaskia River system, Moultrie County, Illinois, was considered to be representative of this



generally occurring situation. A coordinated physical, chemical, and biological study emphasizing nutrient assimilation was undertaken in September, 1969. Cur-  
sory inspection indicated that there was no gross evidence of any differences  
between the creek and river sampling sites as determined by physical and chemi-  
cal measurements. Since the periphyton (Aufwuchs) community was considered by  
this author to constitute an intermediate level of utilization between subtle  
differences in water chemistry and the macrobiota, research efforts were con-  
centrated on the role of the periphyton community in the assimilation of the  
Sullivan wastewater treatment plant effluent.

The population dynamics of the algal members of the periphyton community  
have been used by various authors to characterize pollution (Kolkwitz and  
Marrson 1908; Butcher 1949, 1955; Fjerdinstad 1950; Neel 1953; Patrick 1953,  
1957). Butcher (1946, 1947), Cooke (1956), and Blum (1957) concluded that ex-  
posure times of less than two weeks precluded the development of a permanent or  
"climax" algal community. Minckley (1963) maintained, however, that there would  
continue to be a "perpetuated disclimax," *i. e.*, a set of communities adapted  
so that they could quickly recover from a chronic or severe depletion as soon  
as favorable conditions recurred. He believed that the term "climax" implied  
a successional sequence of events that might not occur in streams.

There was also considerable disagreement as to the overall effect of  
organic enrichment. Butcher (1947) observed that algae were reduced or elimi-  
nated for eight miles (12.9 km) below the source of pollution while Peters et  
al. (1968) rejected the hypothesis that the community structure might be grossly  
altered by the addition of nutrients from a wastewater treatment plant. They  
failed to observe any increase in production below the plant outfall as compared  
to other stations in the river. Further, Jolly and Chapman (1966) discovered  
that organisms in the "polluted" zone also occurred in the "clean" zones, but

## INTRODUCTION

Streams are both biological and chemical individuals (Minckley 1963). In the years since Kolkwitz and Marrson (1908) presupposed that organisms were dependent, in relatively narrow limits, upon the chemical composition of the water for their distribution and ultimate development, the individuality of streams has been overlooked. Existing studies of wastewater treatment plant effluent assimilation by receiving streams generally have relied heavily upon the physical and chemical analyses of the effluents or the streams below the source of the outfall (Klein 1957, Mackenthun and Ingram 1967, Ball et al. 1963, Brehmer et al. 1968, Venkateswarlu 1969, MacCrimmon and Kelso 1970). Depression of dissolved oxygen, high concentrations of soluble phosphorus, free carbon dioxide, biochemical oxygen demand, ammonia, coliform bacteria, and suspended solids have been routinely applied to demonstrate the debilitating effect of wastewater discharges to streams. Chemical analyses, however, can only give a general impression of stream conditions. They do not determine the effects of pollution on biological life (Brehmer et al. 1968). The search for organisms or a community of organisms which would be useful as indicators of water quality continues. Even if such studies were not hampered by the overriding lack of information regarding the environmental requirements of various species and their resistance to various chemical substances (Williams 1964), the continued application of the "indicator organism" concept would be a gross oversimplification. In fact, the constant association with a high correlation between individual species and specific ranges of chemical or physical conditions has been difficult, if not impossible, to demonstrate (Blum 1972).

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that certain clean-water forms were eliminated in the more disturbed zones. In other words indicator organisms were conspicuous by their absence. Patrick et al. (1954) and Patrick and Hohn (1956), working with the structure of diatom communities, concluded that the total percent of the population composed of dominant species was a valuable criterion for judging the effect of pollution.

The above-mentioned papers, however, are of a more descriptive nature. Little has been published concerning the assimilative capacity of the periphyton community in recovery from organic enrichment. Grzenda et al. (1968) determined the rates at which the periphyton community in the Red Cedar River, a warm-water stream in Michigan, fixed phosphorus and nitrogen. Ehrlich and Slack (1969) found that periphytic algae assimilated nitrate so rapidly in a laboratory system that its concentration was undetectable after three weeks. Toetz (1971) observed that a Ceratophyllum-periphyton community assimilated ammonium during both day and night while nitrate assimilation was strongly dependent upon light.

Few papers in the periphyton literature discussed the role of non-algal members of the community in the assimilation of organic matter. Allen (1971) described the chemo-organotrophy and nutritional interactions of epiphytic algae and bacteria on macrophytes in the littoral area of a lake in Michigan. The techniques he used were developed originally to describe the uptake kinetics of organic solutes by bacteria and algae in the plankton (Hobbie and Wright 1965, 1968; Wright and Hobbie 1965; Allen 1969; Hobbie and Crawford 1969a, 1969b). His was the only published modification of this technique for periphytic bacteria and algae. To date, these methods have not been applied to stream periphyton communities.

## DESCRIPTION OF THE STUDY AREA

Moultrie County has a continental climate typical of central Illinois. The annual mean temperature is 12.1 C. Normal precipitation averages 101 cm, 48% of which normally falls during the growing season May through September (Moats et al. 1969). Atypical climatological conditions prevailed during the field portions of the study. Comparing mean monthly temperatures during the study year with 1931-1963 mean monthly temperatures for Moultrie County (at Windsor) revealed that the temperature was significantly lower than that previously recorded. Total precipitation during the study exceeded the 37-year means by 14.5 cm. Above average rainfall during September, October, and April (two to three times the 37-year means) resulted in prolonged flooding, while the months following high precipitation had below average rainfall. Climatological data for the study period September, 1969 through October, 1970 are summarized in Table 1.

The Kaskaskia River has the second largest drainage area in Illinois, 14,120 km<sup>2</sup> (Doyle 1971). The source of the Kaskaskia River is in west-central Champaign County, Illinois, approximately 8 km northwest of the city of Champaign. It flows southwest through central and southern Illinois to empty into the Mississippi River near Chester, Randolph County, Illinois. The Kaskaskia River has two mainstream impoundments along its 563 km course, Carlyle Lake and Lake Shelbyville. The study area was located in the upper portion of the Kaskaskia River in Moultrie County, Illinois, approximately 448 km from the mouth of the river. Field work was completed before impoundment of Lake Shelbyville inundated portions of the study area.

Asa Creek originates 5 km north of Sullivan, Moultrie County, Illinois. It flows 14 km south by east to empty into the Kaskaskia River 4 km south south east of Sullivan. The drainage area of the Asa Creek basin is 74 km<sup>2</sup>. The

Table 1. Climatological data for the period September, 1969 through October, 1970. Mean temperature and precipitation data for the period 1931-1968 adapted from Moats *et al.* (1969). Minimum, maximum, and mean temperature, total precipitation, and sky data adapted from Illinois Natural History Survey Laboratory weather summaries, Sullivan, Moultrie County, Illinois. Discharge data adapted from the open files of the United States Geological Survey, Champaign, Champaign County, Illinois.

MONTH	TEMPERATURE (C)				PRECIPITATION (cm)		STREAM DISCHARGE (m <sup>3</sup> sec <sup>-1</sup> ) Mean	SKY (days)		
	Max.	Min.	Mean	1931-1968 Mean	Total	1931-1968 Mean		Clear	Partly Cloudy	Cloudy
September, 1969	32.8	5.6	18.3	20.0	17.9	8.1	0.05	8	12	10
October	28.9	-3.9	12.8	13.9	22.3	7.1	1.08	10	5	16
November	18.3	-9.4	3.9	6.1	4.4	7.8	0.18	7	5	18
December, 1969	10.6	-18.3	-2.2	0.0	2.4	6.7	0.07	3	8	20
January, 1970	16.1	-25.0	-5.6	-1.1	2.3	6.4	0.06	10	11	10
February	11.1	-20.0	-3.9	0.0	4.8	6.0	0.16	9	8	11
March	17.8	-11.7	1.7	5.0	6.3	8.6	0.19	7	12	12
April	27.8	-7.8	11.1	14.4	22.1	10.0	0.63	8	16	6
May	28.9	2.2	18.3	20.0	5.0	11.2	0.22	9	12	8
June	32.8	8.3	19.4	22.8	10.9	11.9	0.57	6	9	12
July	34.4	7.8	22.2	25.0	6.8	8.6	0.03	7	15	9
August	32.8	10.6	22.2	23.9	5.1	8.6	0.001	7	14	8
September	34.4	3.9	20.0	20.0	14.4	8.1	0.002	8	11	11
October, 1970	25.6	-2.8	11.7	13.9	6.0	7.1	0.0003	10	9	12

Sullivan wastewater treatment plant is a tertiary treatment facility with chlorination of the effluent. Effluent discharge averages 0.554 mgd ( $2,075 \text{ m}^3 \text{ day}^{-1}$ ) and enters Asa Creek 4.9 km from the mouth of the creek. Additional urban drainage enters Asa Creek via two storm drains, one located approximately 200 m upstream and the other, immediately downstream from the treatment plant outfall.

Five collecting stations were established in the Asa Creek-Kaskaskia River system. All stations were located within 10 km of Sullivan, Moultrie County, Illinois. Stations 1, 2, and 3 were located on Asa Creek; stations 4 and 5 on the Kaskaskia River. Station 4 was upstream from the confluence of Asa Creek with the Kaskaskia River. The map of the study area (Fig. 1) and the location of stations were made by reference to the United States Geological Survey topographic map of the Sullivan (Illinois) quadrangle (15 min. ser., 1935 ed.). The stations were located as follows:

Station 1; T14N, R5E, SE $\frac{1}{4}$ , NE $\frac{1}{4}$ , NE $\frac{1}{4}$ , Sec. 35

Station 2; T13N, R5E, SW $\frac{1}{4}$ , SW $\frac{1}{4}$ , SE $\frac{1}{4}$ , Sec. 12

Station 3; T13N, R5E, NE $\frac{1}{4}$ , SW $\frac{1}{4}$ , SW $\frac{1}{4}$ , Sec. 18

Station 4; T13N, R6E, NE $\frac{1}{4}$ , NW $\frac{1}{4}$ , SE $\frac{1}{4}$ , Sec. 17

Station 5; T13N, R5E, NE $\frac{1}{4}$ , NE $\frac{1}{4}$ , SE $\frac{1}{4}$ , Sec. 24

Station 1 (Fig. 2, upper left) was established 3.7 km upstream from the outfall of the Sullivan wastewater treatment plant. Stream width was approximately 2 m, water depth varied from 0.3 to 3.4 m, averaging 0.5 m, and the substrate was composed of soft ooze. An extensive bed of Sagittaria latifolia Willd. completely covered the stream bottom during warm months. The stream banks sloped steeply upward approximately 3 m and were bordered by cultivated fields. Numerous field tiles were evident protruding from the banks. A United States Geological Survey stream gage was located 10 m downstream from the station. Discharge data from this gage are presented in Table 1.

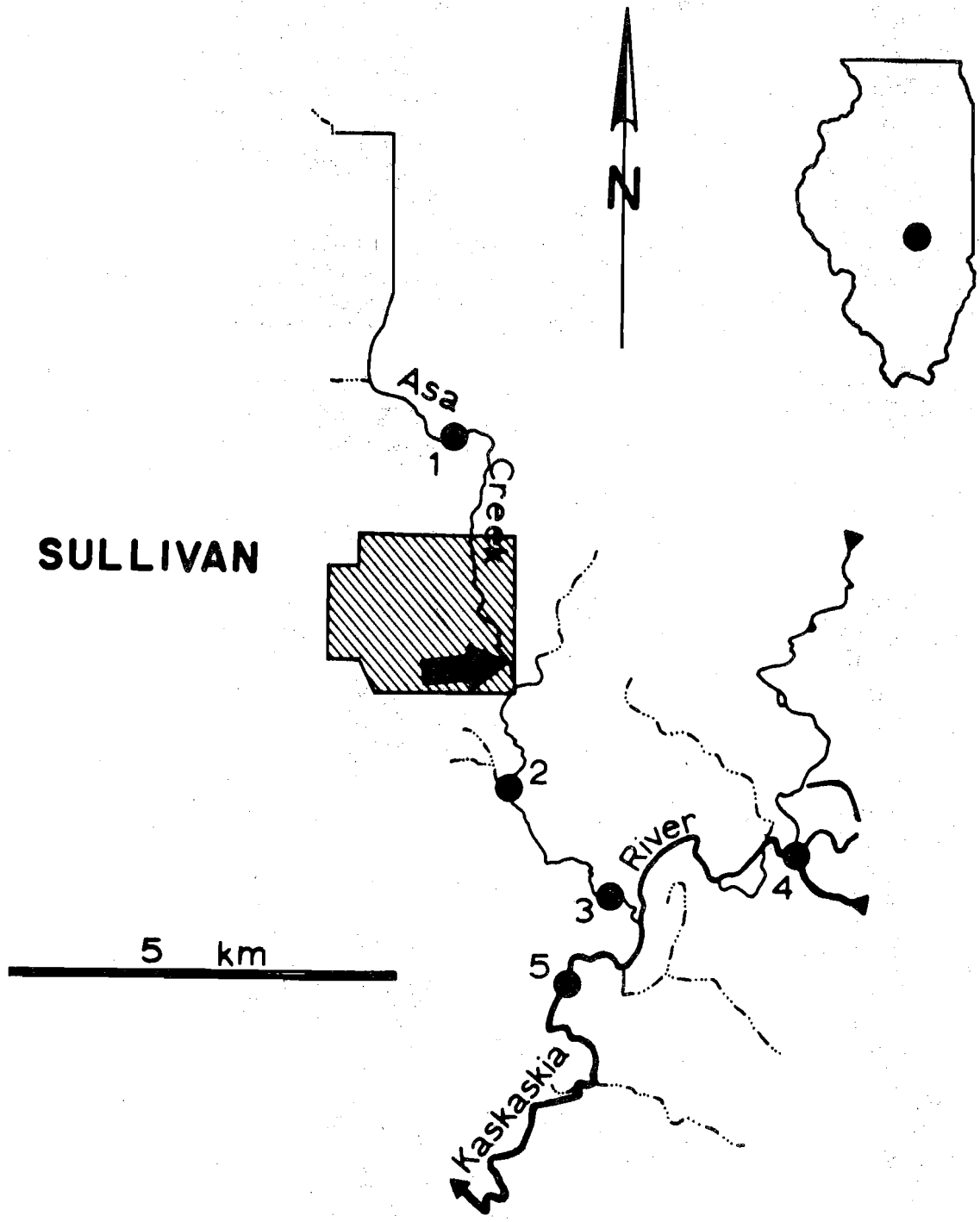


Figure 1. Map of the Asa Creek-Kaskaskia River system, Moultrie County, Illinois, showing the five collecting stations and the Sullivan wastewater treatment plant outfall (arrow).

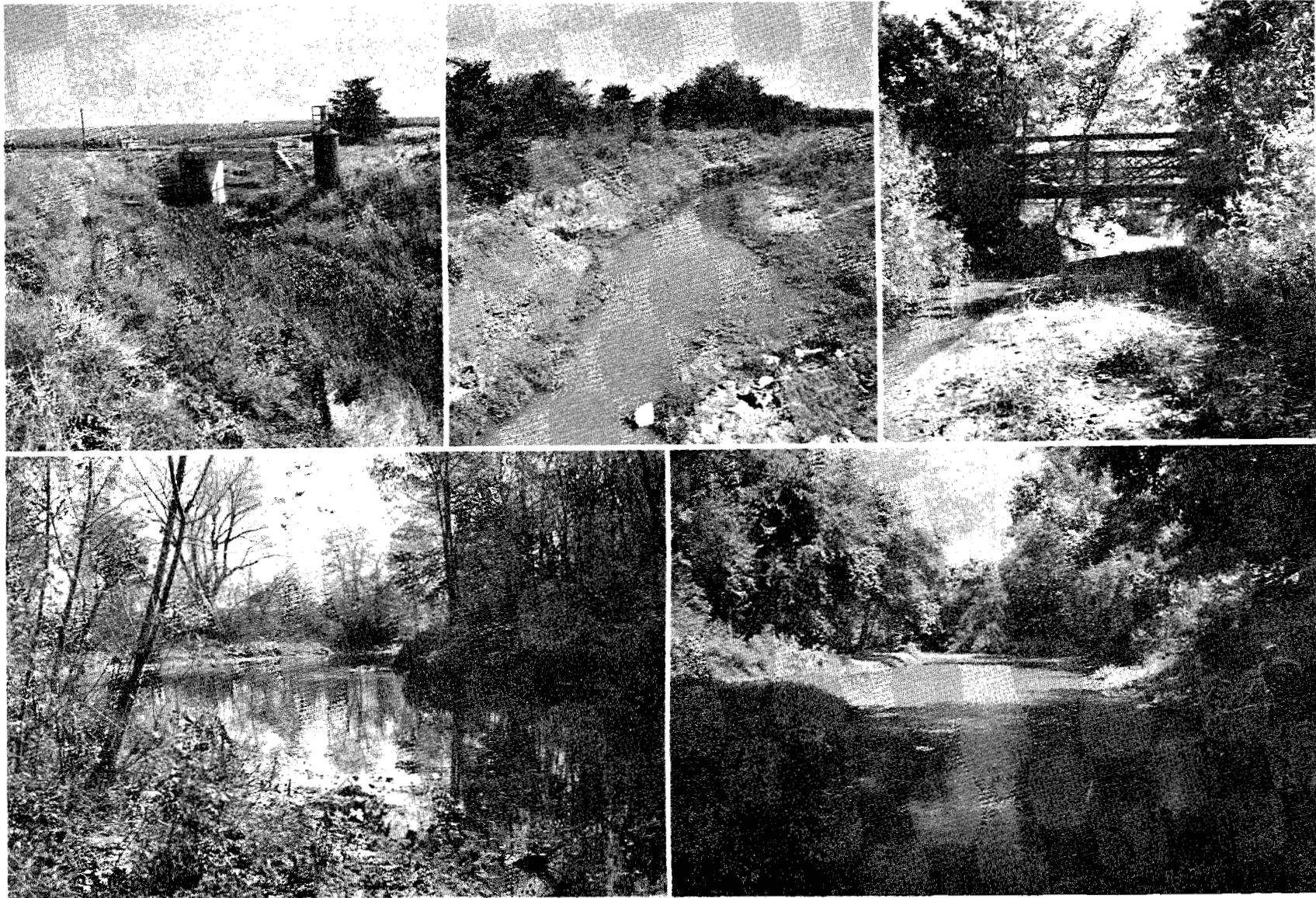


Figure 2. Collecting stations 1 through 5 (upper left to upper right, lower left to lower right, respectively) in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.



Station 2 (Fig. 2, upper center) was 2.1 km downstream from the outfall of the Sullivan wastewater treatment plant. Stream width ranged from 1 to 4 m, averaging 2 m, while water depth varied from 0.2 to 1 m, averaging 0.6 m. The substrate was composed of sand and gravel overlaid with soft ooze, especially along the stream margins. The east bank sloped upward gradually 2 m and an open pasture extended to the top of the bank. The west bank sloped steeply upward 3 to 4 m and culminated in a small stand of trees.

Station 3 (Fig. 2, upper right) was located 4.2 km downstream from the outfall of the Sullivan wastewater treatment plant. Stream width varied from 1 to 4 m, averaging 1 m, while water depth ranged from 0.1 to 3.5 m, averaging 0.3 m. The substrate was composed entirely of sand. The stream banks sloped steeply upward 3 m. Both banks were semi-wooded and lightly shaded the stream.

Station 4 (Fig. 2, lower left) was established 3.4 km upstream from the mouth of Asa Creek and immediately upstream from the mouth of Jonathan Creek. The river was approximately 25 m wide and 0.9 m deep with bottom materials of sand and gravel. The banks sloped gradually upward 1 m into narrow floodplain forests which shaded most of the river.

Station 5 (Fig. 2, lower right) was 1.9 km downstream from the mouth of Asa Creek. The width of the river averaged 20 m and water depth ranged from 0.1 to 5 m, averaging 0.3 m. The substrate was composed of sand, gravel, and rubble. A bed of Dianthera americana L. occurred immediately upstream from the station. The east bank sloped steeply upward 5 m while the west bank sloped gradually 2 m. Station 5 was located immediately upstream from the beginning of the complete clearing prior to the impounding of Lake Shelbyville. Consequently, the river at station 5 was more open to sunlight than at station 4.

## METHODS

The study was conducted from 12 September 1969 through 10 December 1970. Each of the five stations was sampled biweekly from the inception of the study through 7 September 1970. A periphyton accrual study was conducted from 9 July 1970 through 5 October 1970 at each of the five stations. Bacterial uptake kinetics experiments at stations 1, 2, 4, and 5 were conducted on 7, 8, 9, and 10 December 1970, respectively.

## Biweekly Measurements

The Illinois Natural History Survey maintains a weather station at its laboratory 8 km south south east of Sullivan, Moultrie County, Illinois. Mean daily air temperature, sky conditions, and precipitation data were obtained from monthly weather summaries. Weekly graphs of incident solar radiation recorded with a pyrhelimeter (Belfort Instrument Co., Baltimore, Maryland) were used to calculate the total amount of energy received during the periphyton accrual study.

Dissolved oxygen concentration measurements were taken in the field with a YSI model 54 dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio). This meter was calibrated in the field following the procedure outlined by the manufacturer. Oxygen saturations were calculated using Welch's (1948) table of saturation concentrations of dissolved oxygen at various water temperatures. Temperature-compensating thermocouple circuitry in the dissolved oxygen meter allowed water temperatures to be taken in conjunction with dissolved oxygen measurements.

Hydrogen ion concentrations, as  $pH$ , were measured electrometrically immediately upon returning to the laboratory with either a Sargent model PB portable  $pH$  meter (E. H. Sargent, Chicago, Illinois) or a Beckman Century SS laboratory meter (Beckman Instruments, Cedar Grove, New Jersey). Samples were maintained at, or slightly below, stream temperature until the  $pH$  measurement could be taken.

Specific conductance was determined with a model RA-2A conductivity meter (Beckman Instruments, Cedar Grove, New Jersey). Concentrations of total dissolved ionizable solids were calculated by interpolation from specific conductance values and water temperatures using a matrix supplied by the manufacturer.

A Hach model 2100 turbidimeter (Hach Chemical Corporation, Ames, Iowa) was used for turbidity measurements and sulfate determinations.

Total alkalinity, biochemical oxygen demand (BOD), calcium, free carbon dioxide, chemical oxygen demand (COD), chloride, total and fecal coliforms, iron, nitrogen (all forms), phosphorus (all forms), potassium, residue (all forms), silica, sodium, and sulfate analyses were performed following procedures outlined in Standard Methods for the Examination of Water and Wastewater (Amer. Publ. Health Assoc. et al. 1971).

A model DU spectrophotometer (Beckman Instruments, Cedar Grove, New Jersey), and Spectronic models 20 and 70 colorimeters (Bausch and Lomb, Rochester, New York) were used in colorimetric determinations.

In this study the material which passed through a Gelman type A 47 mm glass fiber filter (semi-colloidal range, Gelman Instruments, Ann Arbor, Michigan) was considered to be "dissolved." That which was retained on the filter (plankton and other particulate matter) was considered "particulate." Reed and Reed (1970) reported that glass fiber filters removed two times the seston by dry weight from water than centrifugation did. Surface water samples were filtered using the glass fiber filters in a Millipore filter apparatus. Generally two 1-liter portions from each sample were filtered. The glass fiber filters and two 100-ml portions of the filtrate were subjected to the potassium dichromate wet oxidation procedure described by Maciolek (1962). His conversion factor of 3.4 was used to convert oxygen consumed values to organic content (g-cal). Using benzoic acid Doyle (1971) determined an oxidation efficiency of 102% for this technique.

Three additional portions of water were filtered using the apparatus described above. Each of the glass fiber filters was homogenized in 90% acetone using an electric drill fitted with a teflon-coated tissue grinder. Concentrations of phytopigments were determined by the method of Parsons and Strickland as described by Strickland and Parsons (1968). An IBM 360 computer was used to perform the calculations. Mean values were reported.

Multiple correlation with missing data and one-way analysis of variance programs of the University of Illinois Statistically Oriented Users Programming and Consulting (SOUPAC) were used to analyze the 34 biweekly and the eight periphyton accrual parameters. Although the analysis of variance indicated whether or not there were any significant differences between stations for the biweekly and accrual parameters, it was necessary to use the Duncan Multiple-Range Test (Steel and Torrie 1960) to determine which stations were significantly different. In presenting the results of the Duncan Multiple-Range Test, any two means underscored by the same line are not significantly different at the 0.05 level.

#### Periphyton Accrual Study

Periphyton collections at the stations in the Asa Creek-Kaskaskia River system were analyzed to provide information regarding 1) standing crop (phytopigment concentrations, weight, and caloric value per unit area), 2) the net rate of production (weight and gram-calories per unit area per day), and 3) organic content (gram-calories per unit weight) of the accrued materials. Production efficiencies were calculated for algal components of the periphyton.

Standard glass microscope slides were used as the substrate for the development of the periphyton community. Considerable controversy surrounds the adequacy of glass slides as a suitable substrate for culturing periphyton, *i. e.*, are the communities which develop on artificial substrates substantially different

from those on natural substrates. While Young (1945) observed a distinct community on each type of artificial substrate he used, other investigators (Coe 1932, Coe and Allen 1937, Scheer 1945, Patrick et al. 1954, Peters 1959, Peters et al. 1968) demonstrated that there was little discernible difference in the communities which developed on glass slides versus natural substrates.

An important disadvantage to glass slides was that they favored certain diatoms (Godward 1937) and excluded or discouraged other taxa, chiefly blue-green algae (Sladeckova 1962). While limitations exist, the glass slide remains the most widely adopted artificial substrate because of uniformity, convenience, suitability to laboratory study, and above all, its usefulness in demonstrating major seasonal changes in production.

Sladeckova (1962) and Hohn (1966) reviewed the methods for collecting periphyton. In the present study, the glass slides were suspended vertically above the stream bottom in the apparatus described by Doyle (1971). Although Newcombe (1949, 1950) and Castenholz (1960) favored horizontal placement of glass slides because it yielded greater weights of material, King and Ball (1966) demonstrated that the greater weights were attributable to silt deposition. However, they observed no significant differences at the 0.05 level between horizontal and vertical placement. Oriented so that water flowed across the faces of the slides, the periphyton samplers were positioned at each station on 9 July 1970 and eight slides were removed from each station after 0.5, 1.5, 2, 3, 4, 5, 6, 7, 10, 13, 16, 20, 25, 35, 40, 50, 60, 70, and 81 day's exposure. The capacity of the slide-holding apparatus made it necessary to gather 6-day accrual data and then restart the study with sufficient slides to carry through to day 81, this time omitting the first six days of collecting. Thus a 7-day hiatus existed between the day 6 and day 7 collections. The last collections were made on 5 October 1970. Two slides were preserved in 4% formalin for reference; three for phyt pigment

determinations; three for biomass and organic content determinations.

For phytopigment analyses, the entire slide was extracted in darkness for 24 hours in 90% acetone and the concentrations of phytopigments were determined as described above. The remaining three slides were oven-dried at 98-103 C, cooled to ambient temperature in a desiccator, weighed to the nearest 0.1 mg, and subjected to the wet oxidation procedure described above. After oxidation, the slides were oven-dried and reweighed. The weight of periphyton was considered to be the difference of these weights.

#### Bacterial Uptake Kinetics

Samples for the estimation of the utilization of acetate by the periphyton were collected on 144 mm<sup>2</sup> glass coverslips exposed at stations 1, 2, 4, and 5 for two weeks prior to the sampling date. The coverslips were placed in pairs, back to back, vertically in small plexiglass holders which were then wired to a steel rod driven into the stream bottom. A surface water sample was taken for the simultaneous measurement of chemo-organotrophy of the plankton and also as a source of water to be filtered for the periphyton coverslips. Samples were returned to the laboratory at near-ambient stream temperatures within one half hour after collection.

The procedures for and the theory of the measurements of the bacterial utilization of acetate are outlined in detail by Hobbie and Wright (1965, 1968), Wright and Hobbie (1965), Allen (1969), and Hobbie and Crawford (1969a, 1969b) and are only briefly considered here. For plankton uptake experiments at each station, 5-ml portions of unfiltered stream water were pipetted into each of 20, 25-ml flasks. For periphyton uptake experiments, a coverslip was placed in each of 20, 25-ml flasks containing 5-ml of filtered stream water from that station. All samples, except blanks, were run in triplicate to account for any variability among periphyton coverslips or portions of plankton. An adsorption blank for

each concentration was treated identically as the samples except that blanks were immediately fixed with 4% formalin after the addition of the isotope. Increasing amounts of uniformly labeled acetate- $^{14}\text{C}$  (5, 10, 20, 50, and 100  $\mu\text{liter}$ ), equivalent to 41 to 820  $\mu\text{g liter}^{-1}$  at a specific activity of 0.934  $\mu\text{Ci ml}^{-1}$ , were added with micropipettes to the sample flasks. Blanks and samples were incubated in the dark on a rotary shaker for two hours at near in situ temperatures. After incubation the samples were immediately filtered onto Millipore HA membrane filters (0.45  $\mu$ ) under low vacuum (maximum 0.34 atm). For periphyton samples the coverslip was also retrieved. After storage in a desiccator, the filters or the filters plus coverslips were placed in vials containing 15 ml toluene solution (4 g PPO and 0.1 g POPOP  $\text{liter}^{-1}$  toluene) and counted for 100 min in a Packard Tri-Carb Liquid Scintillation Spectrophotometer (Packard Instruments, Downers Grove, Illinois). Samples were corrected for quenching and adsorption.

The maximum bacterial uptake velocity for acetate,  $V_{\text{max}}$ , maximum natural substrate concentrations of acetate,  $K_t + S_n$ , and the substrate regeneration time,  $T_t$ , for periphytic and planktonic bacteria were calculated using the equations contained in Allen (1969).

THE ASA CREEK-KASKASKIA RIVER SYSTEM  
AS A PERIPHYTON HABITAT

The installation of a sewage pre-treatment facility at Lucy Ellen Candies, Inc., Sullivan, Illinois, during autumn, 1969, reduced the BOD of its gelatinized starch and sugar waste 97% before it entered the city's sewers. Prior to this, the Sullivan wastewater treatment plant had been unable to comply with Illinois State Sanitary Water Board standards. Operation of the pre-treatment facility improved general conditions in Asa Creek, the receiving stream. Improvement in effluent quality, however, revealed that two storm drains discharged considerable amounts of toxic materials into Asa Creek. From 15 July 1970 to 20 May 1972, three major fish kills were directly attributable to these materials. Total phosphorus concentrations of  $972 \text{ mg liter}^{-1}$  as P;  $1.2 \text{ mg liter}^{-1}$  chromium,  $1.2 \text{ mg liter}^{-1}$  lead, and pH 12; and  $260 \text{ mg liter}^{-1}$  ammonia nitrogen were all found to be probable causes. These sporadic major spills and probable frequent low-level discharges of the same pollutants were possible causes of some of the otherwise unexplainable environmental perturbations noted in the field portions of this study.

Interstation Differences

The results of the biweekly measurements of 34 physical, chemical, and biological parameters are summarized in Table 2. The number of determinations, mean, standard deviation, and range of concentration for each parameter are given. These data are presented to provide baseline information for the collecting sites in the Asa Creek-Kaskaskia River system. To delineate further the interstation differences so that the influence of the wastewater treatment plant and major composition variations between creek and river habitats could be defined, a one-way analysis of variance was performed on the data. The Duncan Multiple-Range Test at the 0.05 level (Steel and Torrie 1960) was applied to yearly means of



Table 2. The number of determinations, mean, standard deviation (in parentheses), and range of concentration for 34 parameters measured biweekly from 12 September 1969 through 7 September 1970 at five collecting stations in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois (as mg liter<sup>-1</sup> except where other units are indicated).

PARAMETER	STATION 1	STATION 2	STATION 3	STATION 4	STATION 5
Air Temperature (C)	22 14.0(10.7) -5.0-27.8	22 14.0(10.7) -5.0-27.8	22 14.0(10.7) -5.0-27.8	22 14.0(10.7) -5.0-27.8	22 14.0(10.7) -5.0-27.8
Water Temperature (C)	20 13.3(8.8) -0.1-28.8	20 16.2(9.6) 2.0-33.1	19 15.0(10.5) -0.2-34.9	20 13.8(9.4) -0.4-27.2	21 15.3(10.2) -0.2-29.6
Turbidity (JTU)	23 25(22) 5-93	23 15(10) 4-38	23 14(11) 6-56	22 26(29) 3-135	23 27(21) 3-81
Dissolved Oxygen	20 9.5(4.1) 2.0-16.0	20 11.9(2.6) 8.2-17.0	20 11.5(3.0) 8.0-16.9	19 9.1(2.2) 5.6-14.7	21 9.7(2.1) 6.6-14.6
Dissolved Oxygen (% saturation)	20 86.9(34.8) 23.0-137.0	20 119.0(31.5) 81.0-225.0	19 108.9(29.3) 63.0-193.0	19 85.1(14.3) 68.0-123.0	21 93.5(15.7) 68.0-124.0
Free Carbon Dioxide	19 13.5(15.2) 3.1-70.	19 6.3(5.4) 0.0-21.5	19 5.0(4.9) 0.0-17.0	19 8.5(7.6) 0.0-33.	19 5.4(6.3) 0.0-28.
Total Alkalinity (as CaCO <sub>3</sub> )	23 257(33) 168-328	23 224(40) 140-279	23 219(42) 122-270	22 214(45) 112-291	22 218(42) 117-283
Hydrogen Ion (pH)	22 7.7(0.4) 6.8-8.3	22 8.0(0.4) 7.2-9.0	22 8.1(0.4) 7.2-8.9	21 7.8(0.4) 7.1-8.4	21 7.9(0.4) 7.2-8.4
Tot. Dis. Ioniz. Solids (as NaCl)	23 448(60) 319-513	23 442(86) 242-541	23 422(96) 213-515	22 510(172) 246-908	23 431(144) 246-844
GDD	23 19.32(20.34) 0.75-83.46	23 24.81(18.54) 1.49-72.17	23 20.95(16.86) 0.00-59.67	22 17.47(16.37) 0.00-61.31	23 19.16(14.05) 1.20-55.24

(continued on the next page)

Table 2. (continued)

PARAMETER		STATION 1		STATION 2		STATION 3		STATION 4		STATION 5
Chloride	22	16.0(3.5) 11.3-22.8	23	28.7(17.9) 13.1-82.7	23	27.6(15.3) 13.1-64.1	22	32.4(15.6) 11.4-65.5	23	31.3(14.3) 6.8-63.0
Sulfate	23	57(22) 10-105	23	68(18) 28-105	23	67(13) 39-91	22	132(102) 16-485	23	126(96) 27-435
Nitrate-Nitrogen	15	5.9 0.6-10.4	15	7.2 2.0-12.2	15	5.8 2.5-9.8	13	5.6 0.3-11.0	14	5.6 0.5-12.95
Nitrite-Nitrogen	22	0.046(0.096) 0.000-0.417	22	1.04(3.10) 0.000-12.68	22	1.18(3.03) 0.001-11.38	22	0.078(0.222) 0.001-0.985	21	0.726(3.22) 0.000-14.77
Ammonia-Nitrogen	22	0.074(0.156) 0.000-0.545	23	0.455(0.760) 0.002-2.66	22	0.196(0.427) 0.001-1.86	22	0.016(0.035) 0.001-0.150	23	0.035(0.057) 0.000-0.210
Organic-Nitrogen (as NH <sub>3</sub> -N)	22	0.252(0.397) 0.001-1.52	22	0.368(0.545) 0.001-1.63	22	0.393(0.670) 0.001-1.23	21	0.199(0.292) 0.001-0.887	23	0.246(0.349) 0.001-1.13
Total Phosphorus (as P)	23	0.197(0.244) 0.001-1.07	23	6.74(7.64) 0.068-19.38	23	3.25(3.87) 0.111-15.01	22	0.453(0.706) 0.011-2.77	23	0.735(1.23) 0.032-4.73
Sol. Orthophosphate (as P)	23	0.054(0.118) 0.001-0.554	23	4.10(5.57) 0.034-14.34	23	2.45(3.67) 0.004-13.34	22	0.137(0.246) 0.004-0.868	22	0.226(0.425) 0.031-2.01
Calcium	23	81.2(36.3) 36.1-161.1	23	87.2(35.0) 1.96-169.1	23	77.5(24.6) 24.0-106.6	22	103.2(59.9) 32.1-255.7	23	94.1(48.7) 28.1-228.1
Sodium	23	7.1(6.0) 1.2-23.5	23	19.2(18.5) 1.0-61.3	23	16.2(15.2) 2.8-54.2	22	22.1(22.6) 4.1-94.6	23	18.2(15.7) 3.3-70.8

(continued on the next page)

Table 2. (continued)

PARAMETER		STATION 1		STATION 2		STATION 3		STATION 4		STATION 5
Potassium	23	3.5(9.6) 0.0-46.5	23	10.0(18.6) 0.2-80.8	23	8.6(15.6) 0.1-65.2	22	6.9(16.8) 0.1-71.0	23	5.1(11.8) 0.0-51.8
Iron	23	1.61(1.70) 0.28-8.40	22	1.05(0.76) 0.44-2.92	23	0.96(0.81) 0.18-4.02	22	1.75(1.57) 0.16-6.20	23	1.58(1.18) 0.48-4.28
Silica	23	9.49(5.96) 0.76-18.77	22	10.23(7.70) 0.00-31.53	23	9.11(7.82) 0.72-35.91	22	8.37(5.53) 0.60-19.10	23	7.78(5.67) 0.60-19.1
Total Residue	8	550(205) 384-892	8	528(103) 448-748	6	672(235) 424-1,048	8	575(141) 440-780	8	546(120) 456-800
Filtrable Residue	8	414(136) 272-720	8	450(138) 252-720	6	489(105) 288-664	8	506(146) 300-740	8	474(143) 284-748
Non-filtrable Residue	8	136(148) 37-493	8	78(63) 20-204	6	184(192) 32-520	8	69(53) 24-160	8	74(49) 35-184
BOD	11	3.95(2.65) 0.00-7.70	11	5.54(2.69) 2.30-8.80	11	5.30(2.39) 2.10-9.00+	10	2.86(1.26) 0.55-4.80	11	2.89(1.43) 0.55-5.70
Total Coliforms (number per 100 ml)	14	7,286(7,353) 0-21,600	14	12,210(9,148) 1,400-29,208	14	12,948(10,918) 0-36,000	15	4,404(7,517) 0-25,600	15	7,370(11,890) 0-46,920
Fecal Coliforms (number per 100 ml)	12	26,175 (64,582) 0-226,000	12	79,656 (133,088) 1,367-411,000	12	24,618 (46,631) 333-167,700	13	54,859 (168,094) 0-612,000	12	47,572 (1,458,848) 0-510,000
Dis. Org. Matter (g-cal liter <sup>-1</sup> )	23	364(595) 26-2,622	21	370(482) 37-1,637	20	384(583) 33-2,216	22	308(528) 49-2,338	22	322(356) 28-1,044

(continued on the next page)

Table 2. (continued)

PARAMETER	STATION 1		STATION 2		STATION 3		STATION 4		STATION 5	
Part. Org. Matter (g-cal liter <sup>-1</sup> )	23	35.6(36.9) 7.6-146.5	20	32.7(20.9) 11.0-92.5	21	29.5(22.4) 9.0-70.3	21	36.4(49.0) 0.8-231.6	22	27.8(20.9) 1.4-600.
Chlorophyll a (µg liter <sup>-1</sup> )	22	28(47) 1-174	22	13(15) 1-66	22	19(24) 1-108	21	20(26) 1-111	22	17(18) 1-55
Chlorophyll b (µg liter <sup>-1</sup> )	22	10(18) 0-59	22	5(6) 1-23	22	7(7) 1-27	21	10(22) 1-105	22	5(4) 1-15
Chlorophyll c (µg liter <sup>-1</sup> )	22	22(46) 0-204	22	13(28) 0-113	22	52(161) 1-756	21	21(61) 0-283	22	21(62) 0-296

those parameters found to have significant interstation differences by the one-way analysis of variance procedure. The results of these analyses are presented in Table 3.

Of 34 parameters measured biweekly, only 13 were found to be significantly different among stations. Four of these, nitrate-nitrogen, ammonia-nitrogen, total phosphorus, and soluble orthophosphate, were significantly higher below the wastewater treatment plant outfall. Station 1 above the outfall had lower chloride and sodium levels, but a higher total alkalinity than the downstream stations. Sulfate concentrations were higher at the river sites. Relationships among stations were not as apparent for the remaining five parameters showing significant differences. The following discussion will be limited to consideration of those parameters found to be significantly different among stations.

#### Chloride

Mean chloride concentrations increased from 16.0 to 28.7 mg liter<sup>-1</sup> below the outfall of the Sullivan wastewater treatment plant. These high concentrations persisted in the Kaskaskia River (Tables 2 and 3). While the concentration decreased slightly downstream at station 3 in Asa Creek, the mean chloride levels were consistently higher at the Kaskaskia River stations, upstream and downstream from the mouth of Asa Creek. A slight decrease in chloride concentration occurred downstream at station 5. Doyle (1971) concluded that high chloride levels in the Kaskaskia River occurred presumably due to brine introductions from oil fields in the watershed. Wastewater contamination has been recognized as contributing to increased chloride concentrations in receiving streams (Kofoid 1903, Reinhard 1931, Roy 1955, Blum 1957, Klein 1957, Venkateswarlu 1969). MacCrimmon and Kelso (1970) and Brigham (1972) noted that high concentrations from wastewater contamination persisted for considerable distances downstream.



Table 3. (continued)

Total Phosphorus (as P)	6.74	3.25	<u>0.735</u>	0.197	<u>0.453</u>
Sol. Orthophos. (as P)	<u>4.10</u>	<u>2.45</u>	<u>0.226</u>	<u>0.054</u>	<u>0.137</u>
Nitrate-Nitrogen	7.2	<u>5.8</u>	<u>5.6</u>	<u>5.9</u>	<u>5.6</u>
			STATION		
	1	4	5	2	3
Free Carbon Dioxide	<u>13.5</u>	<u>8.5</u>	6.4	6.3	5.0
			STATION		
	3	2	5	4	1
Hydrogen Ion (pH)	<u>8.1</u>	<u>8.0</u>	<u>7.9</u>	<u>7.8</u>	<u>7.7</u>
			STATION		
	2	3	1	4	5
BOD	<u>5.54</u>	<u>5.30</u>	<u>3.95</u>	2.89	<u>2.80</u>

### Sodium

Mean concentrations of sodium increased from 7.1 to 19.2 mg liter<sup>-1</sup> below the outfall of the wastewater treatment plant. The pattern of changes in sodium levels was similar to that observed for chloride at the five collecting stations (Tables 2 and 3). Comparing sodium and chloride concentrations in the Asa Creek-Kaskaskia River system, however, it was apparent that sodium chloride was not the only salt contributing to the chloride concentrations, although it constituted by far the greatest percentage. Calcium, magnesium, and potassium salts, to name a few, undoubtedly contributed. The presence of oil fields in the watershed also contributed to higher sodium levels in the Kaskaskia River (Doyle 1971).

### Sulfate

Mean sulfate concentrations increased below the wastewater treatment plant outfall from 57 to 68 mg liter<sup>-1</sup>, but the increase was insignificant when compared to the sulfate levels existing in the Kaskaskia River (Tables 2 and 3). Although levels of sulfate were observed to decrease with increasing discharge at all stations, the loading of sulfate was believed to increase in the Kaskaskia River. Concurrent increases observed in calcium concentrations and total dissolved ionizable solids probably reflected the leaching from gypsum deposits at a large chemical plant approximately 60 km upstream. MacCrimmon and Kelso (1970) also recognized the effect of gypsum deposits on sulfate concentrations. They observed mean sulfate concentrations of 246 mg liter<sup>-1</sup> in the Grand River, Ontario, downstream from such deposits.



Total Alkalinity, Free Carbon Dioxide, and Hydrogen Ion Concentration

Concentrations of total alkalinity were within the range reported for warm-water streams in the midwest (Ball et al. 1968, Brehmer et al. 1968, Brigham 1972). Mean concentrations of bicarbonate were significantly higher at station 1 above the wastewater treatment plant outfall than at the other four stations (Tables 2 and 3). This was attributed to limited epiphytic algal production caused by shading of the stream by Sagittaria.

Mean concentrations of free carbon dioxide were highest at station 1 (Tables 2 and 3). Free carbon dioxide is generally highest in autumn due to bacterial decomposition of fallen leaves (Neel 1951, Roeske 1969, Hynes 1970, Brigham 1972). At station 1 bacterial decomposition of organic matter produced in the Sagittaria bed, the complete shading of the stream by its emergent leaves, coupled with minimal flow (Table 1) resulted in high levels of free carbon dioxide. Station 4 in the Kaskaskia River was deep, turbid, and bordered by flood-plain forest. Consequently free carbon dioxide concentrations were greater here than at the remaining stations, which were more shallow, clear, and open.

The relative consistency of mean pH values, especially in the presence of high concentrations of free carbon dioxide, attested to the buffering capacity of the water (Tables 2 and 3). The typical inverse relationship of pH and free carbon dioxide was observed (Table 3). Station 1, with the highest mean concentration of free carbon dioxide, had the lowest mean pH, and so forth.

#### Dissolved Oxygen

Mean dissolved oxygen concentrations ( $\text{mg liter}^{-1}$ ) and percent saturation were consistently high at all stations. Stations 2 and 3 downstream from the wastewater treatment plant outfall in Asa Creek had higher mean dissolved oxygen concentrations, 11.9 and 11.5  $\text{mg liter}^{-1}$ , respectively, than did station 1, upstream from the outfall and stations 4 and 5 in the Kaskaskia River, 9.5, 9.1,

and 9.7 mg liter<sup>-1</sup>, respectively. Station 5, however, was not significantly different from station 3 (Table 3).

Ball et al. (1968) observed that with reduced summer flow rates in the Red Cedar River, Michigan, dissolved oxygen concentrations fell from supersaturation to approximately 3.5 mg liter<sup>-1</sup> by midnight. Brigham (1972) found that dissolved oxygen concentrations plunged below 2.0 mg liter<sup>-1</sup> by midnight a few kilometers below the Urbana-Champaign wastewater treatment plant. Although diurnal pulses in dissolved oxygen were expected, especially at stations 2 and 3 in Asa Creek, 24-hour measurements were not taken. The low BOD values and the fact that concentrations of dissolved and particulate organic matter were not significantly different among stations led to the conclusion that nocturnal minima in dissolved oxygen concentrations caused by biotic respiration would not be severely limiting, even during peak production periods and low flow.

That sewage outfalls generally reduce the dissolved oxygen concentration in the receiving stream is a recognized phenomenon (Butcher 1940, 1949; Blum 1956; Venkateswarlu 1969; Brigham 1972). Low dissolved oxygen has been associated with high concentrations of unoxidized organic matter (Blum 1957). The situation in the Asa Creek-Kaskaskia River system was atypical in that the wastewater discharged was of sufficient quality to preclude the development of the characteristic ill effects in the receiving stream. Brehmer et al. (1968) observed that although the BOD increased in the Red Cedar River, Michigan, below Williamston, it did not seriously reduce the dissolved oxygen content of the water. They concluded that turbulent flow, dilution, reaeration, and the photosynthetic activity of the flora combined to compensate for the oxygen demand of the organic material in the process of stabilization.

#### Nitrate-Nitrogen and Ammonia-Nitrogen

Mean concentrations of both nitrate-nitrogen and ammonia-nitrogen were

significantly higher at station 2 below the outfall of the wastewater treatment plant than at the other four stations. Concentrations of these parameters were not significantly different at the other stations (Tables 2 and 3).

The Kaskaskia River has been termed a high nitrate watershed (Harmeson et al. unpublished). Since the output from wastewater treatment plants is reasonably constant, increased spring discharge would dilute nitrate concentrations. Harmeson et al. (unpublished) observed that this was not the situation in the Kaskaskia River. They determined that 88% of the nitrates were derived from soils and inorganic fertilizers, 7% from animal wastes, 4% from the atmosphere, and only 1% from wastewater treatment plant outfalls. Many authors have similarly recognized the importance of run-off from agricultural land during periods of increased discharge in the spring as a source of nitrates in streams (Blum 1956, Roeske 1969, Venkateswarlu 1969, Brigham 1972). Even so, the increase in nitrate-nitrogen concentration below the wastewater treatment plant outfall at station 2 was temporary. By the time the flow reached station 3, 4.2 km below the outfall, the effect of the wastewater treatment plant was not discernible. Cushing (1964) concluded that similar decreases in concentration resulted from coincident nutrient assimilation by the increasingly large autotrophic populations, including periphyton and macrophytes.

Although the mean concentration of ammonia-nitrogen was highest, 0.455 mg liter<sup>-1</sup>, at station 2, 2.1 km below the outfall of the wastewater treatment plant, the decreasing concentrations downstream at stations 3 and 5 reflected a progressive recovery of the stream from the organic loading at Sullivan (Tables 2 and 3). Roeske (1969) and Brigham (1972) observed similar situations in their studies on the Salt Fork system in Illinois. Oxidation generally proceeds from organic-nitrogen through ammonia and nitrite to nitrate (DeMarco et al. 1967). Since concentrations of organic-nitrogen were not significantly different at the

five collecting stations (Table 3), one must conclude that much of the organic-nitrogen-to-ammonia step in the oxidation of nitrogen took place within the Sullivan wastewater treatment plant. Further oxidation was rapid in the creek. Nitrite-nitrogen concentrations were not significantly different at the five collecting stations and ammonia-nitrogen and nitrate-nitrogen concentrations at station 3, 4.2 km below the outfall, were not significantly different from station 1 and the Kaskaskia River stations.

### Phosphorus

Although rivers have not exhibited marked depletions in phosphorus concentrations, fluctuations are characteristic (Blum 1956, MacCrimmon and Kelso 1970). Phosphorus is not a conservative element. Phosphorus dynamics in warm-water streams are complicated by pollution, variations in seasonal flows, adsorption, deposition, and assimilation by a complex biota (Ball *et al.* 1968, Keup 1968).

Mean concentrations of total phosphorus and soluble orthophosphate were significantly higher at stations 2 and 3, below the outfall of the wastewater treatment plant than at the other three stations (Tables 2 and 3). The wastewater treatment plant contributed mean concentrations of 6.54 and 4.05 mg liter<sup>-1</sup> as P, total phosphorus and soluble orthophosphate, respectively, to Asa Creek. Sewage, sludges, and effluents have been demonstrated to contain appreciable amounts of soluble and organic phosphates (Engelbrecht and Morgan 1959, 1961; Mackenthun *et al.* 1960; Srinath and Pillai 1966; Keup 1968; Roeske 1969; MacCrimmon and Kelso 1970; Brigham 1972).

The principal source of phosphorus in natural waters is run-off from the land surface. In heavily fertilized agricultural lands, great amounts of phosphorus in the simple orthophosphate form or adsorbed on clay particles, enter surface waters as run-off during heavy rainfall (Engelbrecht and Morgan 1961). Phosphorus is also scoured from benthic deposits and resuspended during periods

of high discharge (Keup 1968). Ball et al. (1968), however, observed that while phosphorus concentrations varied with discharge in the Red Cedar River, Michigan, the daily phosphorus transport remained essentially constant. Thus phosphorus loading was independent of run-off patterns. While agricultural drainage was considered to be the principal source of total and soluble phosphorus at stations 1 and 4, phosphorus concentrations at stations 2 and 3 reflected the presence of the effluent from the wastewater treatment plant. The increase in mean phosphorus concentrations between stations 4 and 5 demonstrated the persistence of this effect (Table 3).

#### Biochemical Oxygen Demand

The mean BOD was higher in Asa Creek, both upstream and downstream from the wastewater treatment plant outfall, than in the Kaskaskia River (Tables 2 and 3). In unpolluted streams there may be significant BOD attributable to allochthonous materials such as dead leaves (Hynes 1970). This was considered to be the principal source of BOD in the Kaskaskia River. The mean BOD of 3.95 mg liter<sup>-1</sup> at station 1 was the result of the conditions created by the Sagittaria bed described above. In addition, run-off from the surrounding agricultural land at station 1 contributed to the oxygen demand of the stream. The wastewater treatment plant effluent resulted in an approximate increase of 1.6 mg liter<sup>-1</sup> in the BOD of Asa Creek. Brehmer et al. (1968) observed a definite increase in BOD ranging from 4.6 to 8.8 mg liter<sup>-1</sup> in the Red Cedar River, Michigan, below the city of Williamston. Brigham (1972) reported a value comparable to that observed in this study. He determined that the Urbana-Champaign wastewater treatment plant contributed approximately 4 mg liter<sup>-1</sup> BOD to the Saline Ditch-Salt Fork system.

### Intrastation Correlations

A multiple correlation with missing data program was used to determine what, if any, intrastation correlations existed among the 34 parameters measured biweekly at five stations in the Asa Creek-Kaskaskia River system. It was hoped that this analysis would point out possible subtle interrelationships existing among parameters at a given station. Four hundred fifty-seven significant correlations (0.05 level or greater) were observed. A total of 228 correlations was found to be significant at the 0.05 level. One hundred twenty-one and 108 correlations were found to be significant at the 0.01 and 0.001 levels, respectively. These correlations are presented in Table 4.

The following discussion has been limited to several of those parameters which correlated significantly (0.05 level or greater) at all stations, at either the creek or river stations only (to characterize those habitats), and at stations 2 or 2 and 3 only, and at all stations except station 2 (to distinguish the effects of the wastewater treatment plant effluent).

#### All Stations

Water temperature followed air temperature, but was less extreme and less rapid. Increased soluble orthophosphate concentrations correlated directly with increases in total phosphorus. Free carbon dioxide and pH were inversely related at all stations. These are accepted phenomena in the aquatic environment. Increased air and water temperatures were accompanied by increased organic-nitrogen concentrations at all stations. The enhanced growth of periphyton and macrophytes in warm weather has been demonstrated to have a marked effect downstream because of the increased organic matter produced by plant photosynthesis and decomposition (Blum 1956). Iron and turbidity were also directly related at all stations. Minckley (1963) observed that the iron in Doe Run, Kentucky, originated through leaching of ferrous compounds in the soil and from iron-rich.

Table 4. The 34 by 34 multiple correlation with missing data matrix\*, by station, summarizing biweekly measurements of physical, chemical, and biological parameters at stations 1 through 5 in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois, from 12 September 1969 through 7 September 1970.

PARAMETER	AIR TEMP.	WATER TEMP.	TURBIDITY	DIS. OXY.	DIS. OXY. (% sat.)
Air Temperature	-----				
Water Temperature	CCCCC	-----			
Turbidity	A---B	A---A	-----		
Dissolved Oxygen	b--cc	b--cc	c--a	-----	
Dissolved Oxygen (% sat.)		-AA-A	c----	CBBA-	-----
Free Carbon Dioxide		----a	A-B--	a----	a---b
Total Alkalinity	-bb--	-bb--	-b-bc		-a---
pH	a----		b-aaa	BB---	BB---
Tot. Dis. Ioniz. Solids					
COD		-A---		----A	--AAA
Chloride		-A---			-A---
Sulfate		-B--A			ABB--
Nitrate-Nitrogen		-a-aa	c----	A----	----a
Nitrite-Nitrogen	--a--	--a--	C----	a-AB-	a----
Ammonia-Nitrogen	-A--A	AA--B	A----	a---a	
Organic-Nitrogen	AAAAB	BBAAB	-B-AA		-A--A
Total Phosphorus	A---b	BA--a		b---A	
Soluble Orthophosphate	---a-				
Calcium	-a----	-A---	--a--	b----	b----
Sodium					-A---
Potassium			C----		
Iron	---A-	----A	CACCC	b----	a----
Silica					----a
Total Residue	--ABA	A-AC-	A----	a----	a----
Filtrable Residue	-ABCB	---BA	---A-	---aa	
Non-Filtrable Residue			B----	---A	
BOD			A-a--	b----	a----
Total Coliforms	A----	---A-	A----	-a----	
Fecal Coliforms			A----	a----	-A----
Dis. Organic Matter			A----		
Part. Organic Matter			C--CC	a----	a----
Chlorophyll <u>a</u>	----A	----B	C----	a----	--B--
Chlorophyll <u>b</u>			A----		
Chlorophyll <u>c</u>					

\*A, b, and c represent 0.05, 0.01, and 0.001 levels of significance, respectively; positive or negative correlations are distinguished by upper or lower case letters, respectively.

(continued on the next page)

Table 4. (continued)

FREE CARBON DIOXIDE	TOT. ALK.	pH	TOT. DIS. IONIZ. SOLIDS	COD	CHLORIDE	SULFATE	NITRATE-NITROGEN
-----	-----						
cccc	-----B	-----	-----				
	B----		-----				
	BaB--		A--A-	-----			
--a--	-cb--	---AA		-A---	-----		
--a--		A-C--	---CC	a--AA		-----	
	--A--				--aa-		-----
-A--C				---A-	a----		b---A
A----							
						-A---	
A----	B----	b----		C----	---A	a----	
	A----	a----		C----		c----	
			-ACCC	-B----		---CC	
	Bcc--		-A-CC	BCABA	-CC--	b--CC	----b
	Aaa--			A----	-CB--	b----	a-aaa
-BC--	---ac	-abb-		A--A-		a-bA-	b---a
					---A-		---ba-
	B----			---A		b----	--a--
	B----			A----			
B----		b----					--a--
						-A---	
		---A-					c-ca-
A----	---ba	b----				a----	a----
C----	--a--	a----	---AB	--A--	--BA-	---AC	a--aa
	--a--				--C--	---A	a--aa
-A----					--A--		--a--

(continued on the next page)



Table 4. (continued)

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NITRITE- NITROGEN	AMMONIA- NITROGEN	ORGANIC- NITROGEN	TOT. PHOS.	SOL. ORTHO.	CALCIUM	SODIUM	POTASSIUM
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-----	----- -----C-----	-----	----- CCCCB	-----	-----	-----	-----
B-----			A-----	B-----	AA-CC	-----	-----
C-----				-----A		CBB--	-----
CA-----			-----A--	-----A--	-----A-	B--A-	C-----
A-----	--A--	--B--	-----B-	-----A--	-B---	B-----	
A-----	B-A--	--B--	-----A-	-----A--	-A---		
	A-A--		C---B	C---B	AA---	B--A-	C-----
		--A--				-B---	A---B
	B-CC-	-A-A-					-----C
C-----						B-----	CA-C-
B-----						A-----	B-----
B-----	A-----	--B--			---AC	--BBC	A--C-
A-----					---B	--C-B	B-AC-
-B-----						--A--	--CC-

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(continued on the next page)

Table 4. (continued)

IRON	SILICA	TOT. RES.	FILT. RES.	NON- FILT. RES.	BOD	TOTAL COLIFORMS	FECAL COLIFORMS
-----	-----	-----	-----	-----	-----	-----	-----
A--A-	-----	-----	-----	-----	-----	-----	-----
---A-	-----	-B-CC	-----	-----	-----	-----	-----
CA---	b--b-	A-A--	-a---	-----	-----	-----	-----
Aaa--	A-----	-----	-----	-----	-----	-----	-----
			--A--		A-----	-----	-----
	A-----						-----
C-----		A-A-C	-A--B	A-B--			
C--BA		-A-----	-A-----	B-----		C-----	
B-----				C-----	A-----	B-----	
A---A				Cb-----	-----A	A-----	
-C-----				-A-----			

(continued on the next page)

Table 4. (continued)

DIS. ORGANIC MATTER	PART. ORGANIC MATTER	CHLOROPHYLL <u>a</u>	CHLOROPHYLL <u>b</u>	CHLOROPHYLL <u>c</u>
----- BB--- -B-C- CB-C- ---C-	----- C-B-- B-B-A	----- C-CCB ---C-	----- B-BC-	-----

soil particles as turbidity. His total iron concentrations were greatest during periods of high discharge and turbidity, reflecting the presence of iron in the suspended sediments. Last, sodium and COD were directly related at all stations. The COD determination measured the oxygen equivalent of the portion of the organic matter in the water that was oxidized by potassium dichromate (Amer. Publ. Health Assoc. et al. 1971). Sources contributing to sodium concentrations, discussed above, would also add COD to the streams.

#### Creek or River Stations

At all stations in Asa Creek, sulfate and dissolved oxygen as percent saturation were directly related. While sulfate is typically abundant in hard waters, its natural concentrations can be augmented by wastewater treatment plant effluents (Hynes 1960). Sulfate concentrations were also inversely related to stream discharge (discussed above). Assuming relatively consistent loading from the wastewater treatment plant, low-flow conditions in warm weather (Table 1) at times of increased percent saturation from photosynthetic oxygen production accounted for this relationship.

A direct relation of sodium and potassium was noted. In lakes, the ratio of potassium to sodium has been demonstrated to decrease with increasing sodium concentrations (Hutchinson 1957). In Asa Creek, agricultural drainage and the wastewater treatment plant effluent offset this relationship.

The following parameters were directly related at station 1 above the outfall of the wastewater treatment plant, but inversely related below the outfall at stations 2 and 3: BOD with iron, and COD, sodium, and potassium with total alkalinity. Increased turbidity has been shown to produce increased iron concentrations (discussed above). Increased turbidity and the accompanying organic matter introduced into the stream during periods of high rainfall and run-off would increase BOD at station 1. A satisfactory explanation for the

direct relationship between COD, sodium, and potassium and total alkalinity at station 1 was not apparent, however. Total alkalinity was, however, significantly higher at station 1 than at the other four stations (Table 3). BOD and COD levels and sodium and potassium concentrations were higher below the wastewater treatment plant outfall (Table 2). Iron and bicarbonate concentrations were reduced by the water softening process in the Sullivan water treatment plant. Since domestic water use constituted the bulk of the effluent, base concentrations of iron and bicarbonate would be lower. Reduction of dilution water from upstream during low-flow periods would explain the inverse relationships observed.

At stations 4 and 5 in the Kaskaskia River, total dissolved ionizable solids concentrations were directly related to sulfate concentrations. Likewise, concentrations of calcium were significantly correlated with sulfate concentrations. The leaching from the extensive gypsum deposits 60 km upstream was strongly suspected as the source of calcium sulfate to the river. Concentrations of planktonic chlorophyll a were directly related to calcium, sulfate, and total dissolved ionizable solids concentrations. MacCrimmon and Kelso (1970) noted that the substantial sulfate concentrations (24 to 599 mg liter<sup>-1</sup>) observed in the Grand River, Ontario, could not be considered limiting to algal growth. Blum (1956) remarked that waters rich in calcium generally had a rich flora, although calcium was not the element which solely determined the presence of the principal species.

Low-flow conditions in the Kaskaskia River, resulting from below-normal precipitation (Table 1) during summer and autumn, 1970, coincided with the time of peak production. This produced an apparently spurious direct correlation observed between chloride and pH.

Run-off from the land during periods of high discharge was responsible

for the inverse relationships observed in the Kaskaskia River for filtrable residue with dissolved oxygen and for iron and particulate organic matter with total alkalinity. Blum (1957) associated low dissolved oxygen concentrations with high levels of unoxidized organic matter. The inverse relationship between alkalinity and stream discharge has been well established (Minckley 1963, Ball et al. 1968, MacCrimmon and Kelso 1970, Brigham 1972).

#### Effects of the Wastewater Treatment Plant

At station 1 and at the Kaskaskia River stations, the accepted inverse relationship between dissolved oxygen concentrations and air and water temperatures, pH and turbidity, and the direct relationship between turbidity and particulate organic matter were observed. At the nutrient-rich stations below the outfall of the wastewater treatment plant these relationships were obscured by increased algal production.

Planktonic chlorophylls a and b were directly related at stations 1, 4, and 5. Planktonic chlorophyll c correlated directly with free carbon dioxide, nitrite-nitrogen, iron, and non-filtrable residue at station 2. A satisfactory explanation for these correlations was not apparent.

At station 2 dissolved oxygen as percent saturation was directly related to chloride, sodium, and fecal coliform bacteria, but inversely related to total alkalinity. These relationships were the result of the low-flow conditions discussed above and the enhanced algal production (saturation of dissolved oxygen reaching 225%) below the outfall (Table 2). No further significance was attributed to them. The inverse relationship between total alkalinity and air and water temperature, however, indicated that there was increased algal production at station 2. Over and above the reduction in total alkalinity attributed to low-flow conditions in Asa Creek, further reduction occurred due to algal uptake of bicarbonate and free carbon dioxide as substrates for photosynthesis.

A number of other significant correlations existed only at station 2 (Table 4). These involved parameters generally associated with a wastewater treatment plant outfall: BOD, calcium, chloride, COD, coliform bacteria, filtrable residue, organic-nitrogen, sodium, and sulfate. The concentrations of these parameters in the effluent was presumed to be relatively constant, hence constant loading into Asa Creek. As discharge in the receiving stream increased and decreased according to local precipitation, the concentrations and values of these parameters below the point of complete effluent mixing would decrease and increase, respectively, in accordance with the amount of dilution water available. It was therefore not unusual that these parameters would exhibit a high degree of correlation since the behavior of each was a function of a common parameter, discharge (Table 4).

THE DYNAMICS OF THE ASA CREEK-KASKASKIA RIVER SYSTEM  
PERIPHYTON COMMUNITY

Since the periphyton community was considered by this author to constitute an intermediate level of utilization between subtle differences in water chemistry and the macrobiota, research efforts were concentrated on the role of the periphyton community in the assimilation of the Sullivan wastewater treatment plant effluent. While algae constituted a major portion of the periphyton, bacteria, protozoans, and other decomposers and microconsumers were responsible for considerable transformation and utilization of nutrients and dissolved organic matter.

Interstation Differences

Periphyton collections at stations in the Asa Creek-Kaskaskia River system were analyzed to determine standing crop (phytopigment concentrations, weight, and caloric value per unit area), the net rate of production (weight and gram-calories per unit area per day), and organic content (gram-calories per unit dry weight) of the accrued materials. Identical statistical analyses were performed as already described for the physical, chemical, and biological parameters measured biweekly.

Of eight parameters measured during the accrual study, four were found to be significantly different among stations (Table 5). The following discussion was limited to consideration of those parameters.

Standing Crop

For dry weight data, the mean standing crop of periphyton was significantly higher at station 4,  $569.6 \text{ mg dm}^{-2}$ , in the Kaskaskia River than at the four remaining stations (Table 5). The influences of the Dianthera bed and fluctuating low water levels at station 5 were believed to be the cause of the lower mean standing crop determined here than at station 4. The lowest mean standing



Table 5. Relationships among mean values of accrued standing crop and organic content, and productivity in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois, from 9 July 1970 through 5 October 1970. Any two means underscored by the same line are not significantly different by the Duncan Multiple-Range Test (0.05 level).

PARAMETER	STATION				
	2	3	1	5	4
Standing Crop ( $\mu\text{g}$ chlorophyll <u>a</u> $\text{dm}^{-2}$ )	Not Significantly Different				
Standing Crop ( $\mu\text{g}$ chlorophyll <u>b</u> $\text{dm}^{-2}$ )	Not Significantly Different				
Standing Crop ( $\mu\text{g}$ chlorophyll <u>c</u> $\text{dm}^{-2}$ )	Not Significantly Different				
Standing Crop ( $\text{g-cal}$ $\text{dm}^{-2}$ )	Not Significantly Different				
Standing Crop ( $\text{mg}$ $\text{dm}^{-2}$ )	<u>232.3</u>	<u>199.9</u>	<u>100.2</u>	<u>220.3</u>	569.6
Organic Content ( $\text{g-cal}$ $\text{mg}^{-1}$ )	<u>1.07</u>	<u>1.05</u>	<u>1.01</u>	<u>0.86</u>	0.60
Productivity ( $\text{g-cal}$ $\text{dm}^{-2}$ $\text{day}^{-1}$ )	34.5	<u>12.6</u>	<u>12.9</u>	<u>7.6</u>	<u>11.0</u>
PARAMETER	STATION				
	2	3	4	1	5
Productivity ( $\text{mg}$ $\text{dm}^{-2}$ $\text{day}^{-1}$ )	<u>32.4</u>	<u>20.8</u>	<u>20.2</u>	9.3	3.4

crop,  $100.2 \text{ mg dm}^{-2}$  was at station 1. Shading by the emergent leaves of the Sagittaria at station 1 (discussed above) was presumed to be the determining factor producing this low mean value.

Doyle (1971) observed a maximum standing crop of periphyton in the Kaskaskis River of  $1010 \text{ mg dm}^{-2}$  for a 73-day exposure period at the same time of year. The maximum standing crop at station 4 after a 70-day exposure was  $1633 \text{ mg dm}^{-2}$ , two to five times that observed at the other four stations (Fig. 3). Beyond the influence of the Urbana-Champaign wastewater treatment plant, Austin and Sollo (1969) observed that maximum community development had not been reached after an exposure of 35 days. Kevern et al. (1966) represented standing crops by a sigmoid curve which reached an upper asymptote after 60 days. While stations 4 and 5 followed this developmental pattern, the other three stations did not.

The upper asymptote, representing stabilization of the periphyton community, occurred when net production and accumulation were balanced by grazing and sloughing (Castenholz 1960, Kevern et al. 1966). Grazing by fish, amphibians, snails, and midge larvae have been cited as appreciably reducing the standing crop of periphyton (Young 1945, Dickman 1963, Austin and Sollo 1969, Doyle 1971); Brock (1967), however, emphasized that animal grazers were obligatory for nutrient cycling. He maintained that the organic carbon locked in the periphyton could be released only if animal grazers were available. Sloughing became an important factor in reducing the standing crop when the periphyton mat thickened to the point of causing decay of underlying layers, so that accumulations of trapped gases, coupled with water movements, allowed portions of the periphyton to float away (Butcher 1946, Kevern et al. 1966, Castenholz 1960).

Fluctuations and lower mean standing crops at stations 1, 2, 3, and 5 (Fig. 3) are attributed to sloughing, primarily, although some animal grazers were collected at station 2. Periphyton at stations 2 and 3 below the wastewater

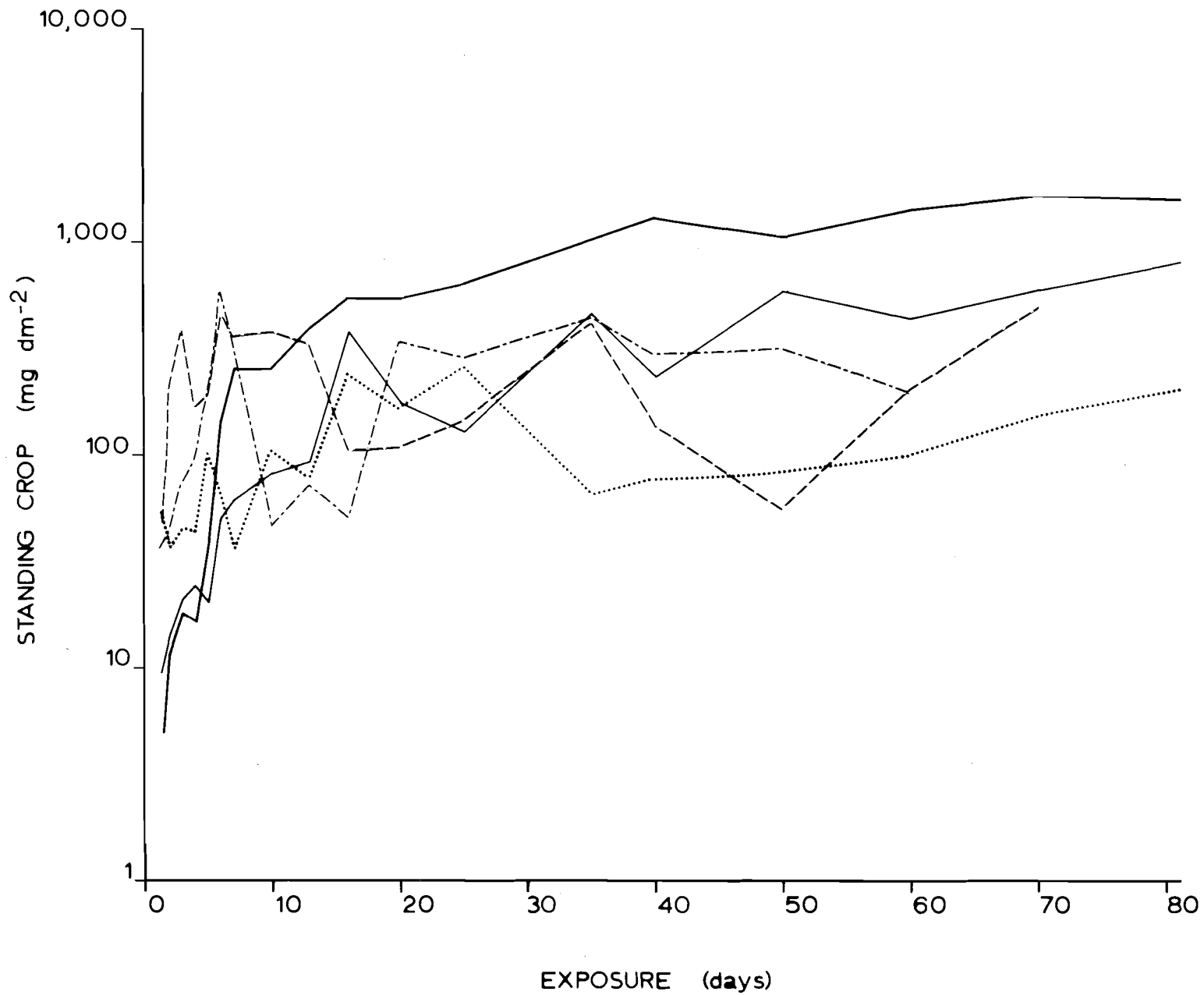


Figure 3. Standing crop of the periphyton community expressed as dry weight ( $\text{mg dm}^{-2}$ ) during the accrual period 9 July 1970 through 5 October 1970 at stations 1 through 5 (dotted, dashed, broken, wide and narrow solid lines, respectively) in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.

treatment plant outfall exhibited rapid substantial growth by day 6, 453 and 581 mg dm<sup>-2</sup>, respectively. Shortly thereafter, sloughing resulted in a severe decline in standing crop. The pattern of rapid growth followed by sloughing was repeated through the end of the study, although the maxima observed early in the study were reached and surpassed only once again (Fig. 3). Austin and Sollo (1969) also noted that maximum community development at stations in the Salt Fork system, influenced by the Urbana-Champaign wastewater treatment plant, was completed in two weeks or less.

#### Organic Content

The mean values of organic content of the periphyton ranged from 0.60 g-cal mg<sup>-1</sup> at station 4 to 1.07 g-cal mg<sup>-1</sup> at station 2 (Table 5). Austin and Sollo (1969) reported comparable, but slightly higher, means for periphyton, ranging from 0.90 to 1.20 g-cal mg<sup>-1</sup> in the Salt Fork system. Their highest levels were reported at stations influenced by the Urbana-Champaign wastewater treatment plant. Cummins and Wuycheck (1971) reported organic content values for periphyton ranging from 1.15 to 4.52 cal mg<sup>-1</sup>. Stations 1, 2, and 3 had significantly higher organic content values than station 4, in the Kaskaskia River upstream from the mouth of Asa Creek. Station 5, 1.9 km downstream from the confluence of Asa Creek and the Kaskaskia River, was intermediate in mean value of organic content (Table 5, Fig. 4). The enriching influence of the wastewater treatment plant was presumed to have had a positive effect on the organic content of the periphyton.

Brigham (1972) attributed an inverse relationship between the weight of attached materials per unit area of substrate and the caloric value of these attached materials to silt deposition on the substrates. This would cause weight increases without accompanying increases in caloric value. The glass slides were positioned vertically in the present study to minimize the adverse

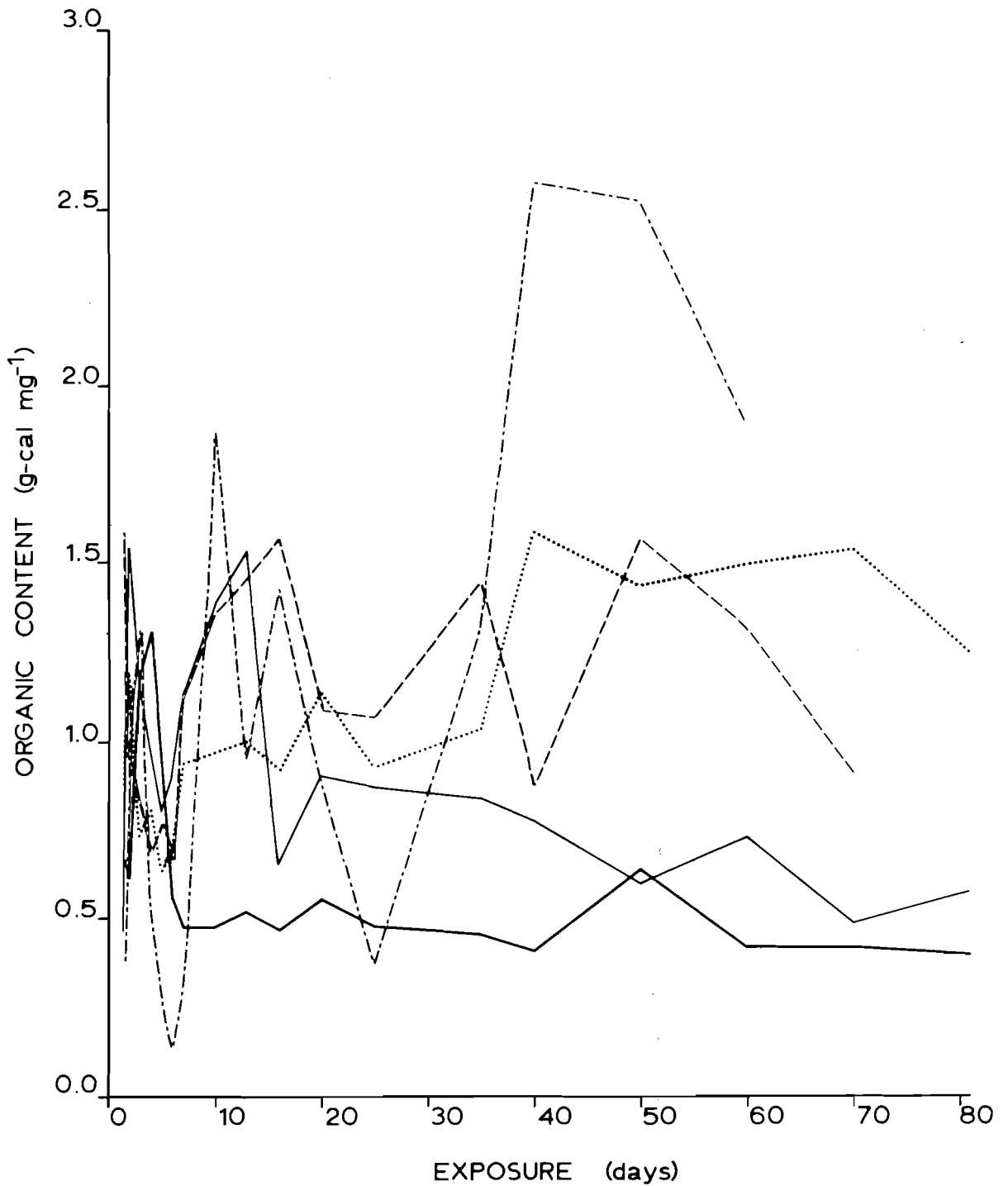


Figure 4. Organic content of the periphyton community (g-cal mg<sup>-1</sup> dry weight) during the accrual period 9 July 1970 through 5 October 1970 at stations 1 through 5 (dotted, dashed, broken, wide and narrow solid lines, respectively) in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.

effect of siltation. The results of the statistical analysis did not reveal any significant correlations, inverse or direct, between standing crop ( $\text{mg dm}^{-2}$ ) and organic content ( $\text{g-cal mg}^{-1}$ ) at any of the stations (Fig. 5).

During the first seven days of exposure, the organic content of the periphyton fluctuated widely. The maximum value for station 2,  $1.59 \text{ g-cal mg}^{-1}$ , was observed and values at the four other stations were among the highest observed during the accrual period (Fig. 4). Doyle (1971) attributed the very high organic contents observed on the first collection dates in the Kaskaskia River and an oxbow lake to the absence of silt. It would appear more reasonable to assume, however, that during the first days of exposure, heterotrophic organisms, principally bacteria and protozoans, would be among the first colonizers to exploit bare substrates. This would be especially true at station 2 below the wastewater treatment plant outfall. Variations in the rate at which these initial rapid colonizers were excluded and replaced by algae, would account for the wide fluctuations in the first week. Visual examination of the reference slides for the first week of exposure at all stations confirmed this observation. Butcher's (1947) observations that algae were reduced or eliminated below the source of pollution and that protozoans were abundant in the polluted zone in the River Trent lend credence to this hypothesis.

#### Productivity

The rates of production, calculated for each collection date, were expressed as biomass and caloric value per unit area per day ( $\text{mg dm}^{-2} \text{ day}^{-1}$  and  $\text{g-cal dm}^{-2} \text{ day}^{-1}$ , respectively). These data are presented in Figs. 6 and 7, respectively.

Mean productivity (biomass) was highest at station 2,  $32.4 \text{ mg dm}^{-2} \text{ day}^{-1}$ , below the wastewater treatment plant outfall, and decreased downstream to  $20.8 \text{ mg dm}^{-2} \text{ day}^{-1}$  at station 3. Lowest mean productivities were observed at stations 1 and 5,  $9.3$  and  $8.4 \text{ mg dm}^{-2} \text{ day}^{-1}$ , respectively. Productivity at

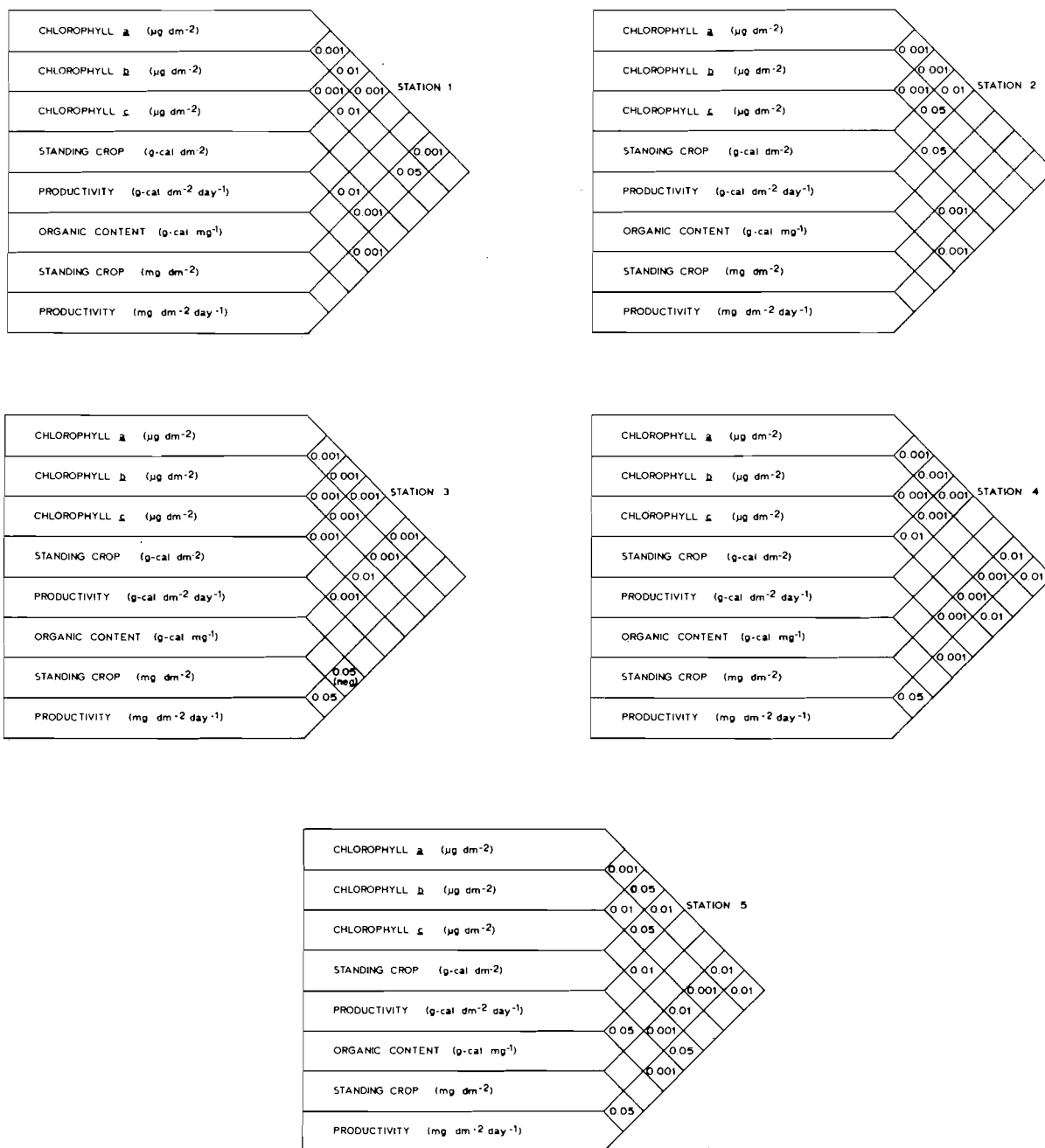


Figure 5. Results of multiple correlation with missing data analyses for periphyton during the accrual period 9 July 1970 through 5 October 1970 at five stations in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.

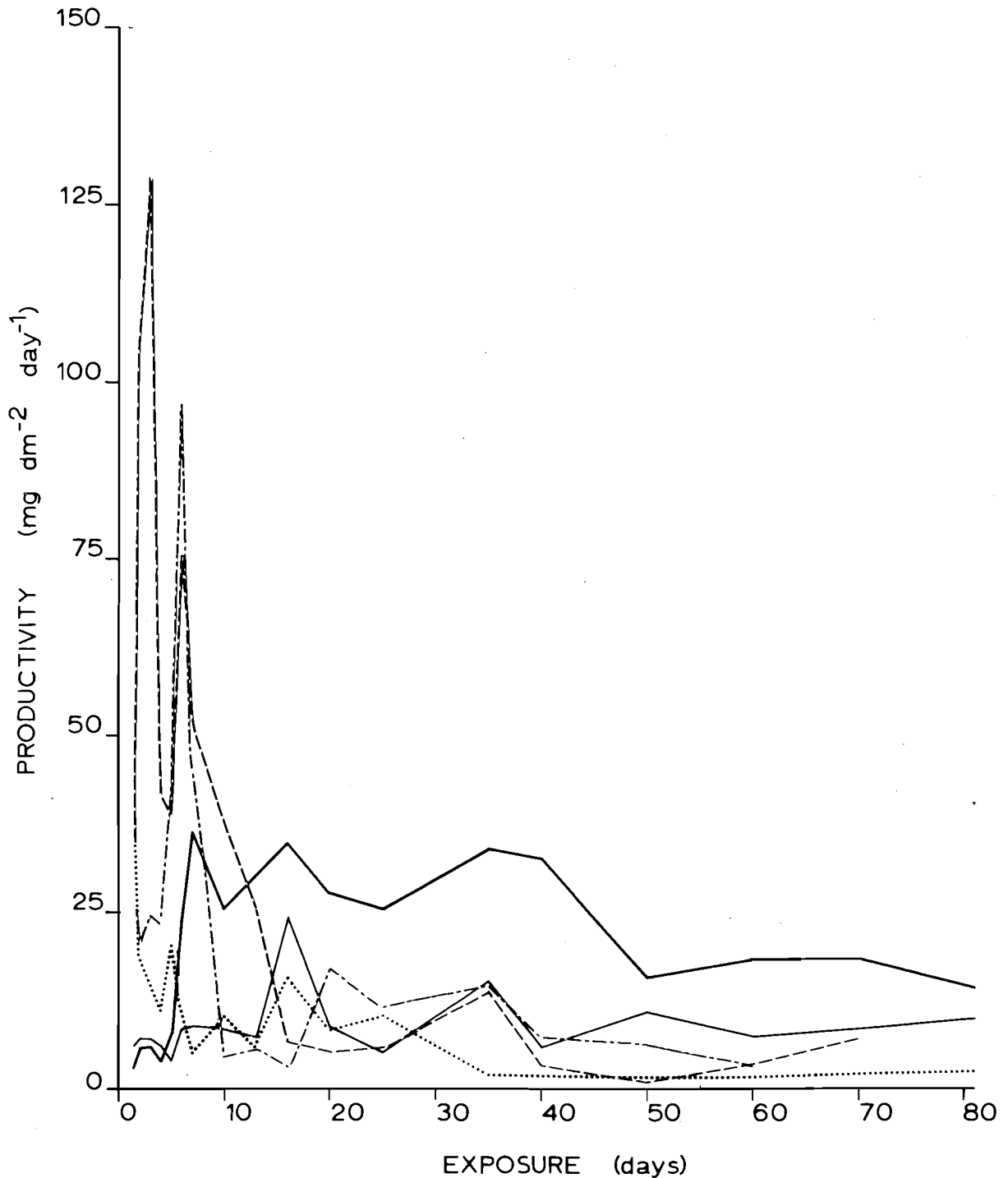


Figure 6. Productivity of the periphyton community ( $\text{mg dry weight dm}^{-2} \text{ day}^{-1}$ ) during the accrual period 9 July 1970 through 5 October 1970 at stations 1 through 5 (dotted, dashed, broken, wide and narrow solid lines, respectively) in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.



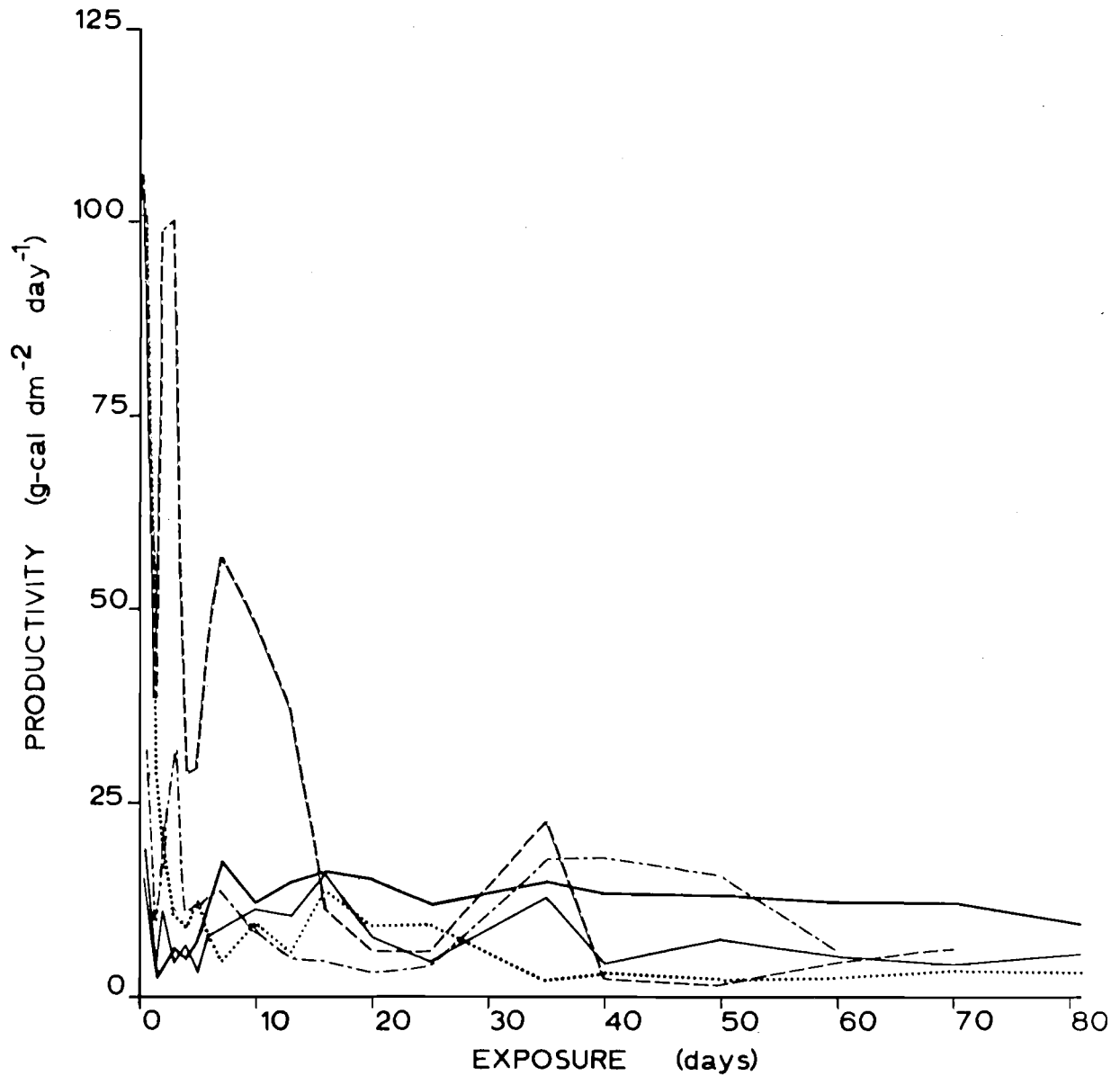


Figure 7. Productivity of the periphyton community ( $\text{g-cal dm}^{-2} \text{ day}^{-1}$ ) during the accrual period 9 July 1970 through 5 October 1970 at stations 1 through 5 (dotted, dashed, broken, wide and narrow solid lines, respectively) in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.

station 4,  $20.2 \text{ mg dm}^{-2} \text{ day}^{-1}$ , was intermediate between stations 2 and 3 and 1 and 5 (Table 5).

Doyle (1971) observed the maximum rates of production (biomass) in the associated floodplain pools of the Kaskaskia River within four days after exposure. In the Kaskaskia River productivity increased to day 12. After the initial high peak productivity, the rates declined and remained low through the end of his study period. In the present study, productivity at stations 1 through 5 peaked on days 1.5, 3, 6, 7, and 16, respectively (Fig. 6). At stations 1 and 5, the effects of fluctuating water levels and shading by emergent vegetation (discussed above) combined to reduce productivity. Doyle (1971) observed similar productivity inhibition for periphyton in floodplain pools with Lemna and Wolffia mats. After the peak productivity on day 7 at station 4, productivity declined slightly, but remained essentially constant. Generally, standing crop ( $\text{mg dm}^{-2}$ ) continued to increase gradually at station 4 through the end of the accrual period (Fig. 3).

Jolly and Chapman (1966) found that nutrient enrichment from a wastewater treatment plant produced luxuriant periphyton growth downstream. Peters et al. (1968), however, did not observe any increase in productivity below a wastewater treatment plant, compared with other stations. Productivity (biomass) at stations 2 and 3 exhibited extreme fluctuations and higher rates of production than at the other stations.

High nutrient levels at these stations (Table 2) enhanced periphyton growth. However, the repeated pattern of rapid growth followed by sloughing (discussed above) greatly reduced the productivity (biomass). Although production rates were determined for each collection date, the productivity values were a cumulative function, a given value being the record of what had transpired at that station to that date. Extreme highs and lows in standing crop

(Fig. 3) would adversely affect the rate calculated for a given date. In addition, although nutrient concentrations permitted almost unlimited periphyton growth below the wastewater treatment plant outfall, there was a thin line separating the ideal from the adverse. Discharge of toxic substances into Asa Creek (discussed above) was verified once at stations 2 and 3 during the accrual period and reduced the amount of periphyton which had accrued to that date (15 July 1970, accrual day 5) (Figs. 6 and 7).

Productivity (caloric value) was almost three times greater at station 2,  $34.5 \text{ g-cal dm}^{-2} \text{ day}^{-1}$ , than at the other four collecting stations, 7.6 to  $12.9 \text{ g-cal dm}^{-2} \text{ day}^{-1}$  (Table 5). Austin and Sollo (1969) reported comparable values. They observed the highest net production rates, approaching  $30 \text{ g-cal dm}^{-2} \text{ day}^{-1}$ , in the more polluted portions of the stream.

Understandably, good agreement was observed between the graphs of productivity as biomass and as caloric value. The combination of factors which resulted in the fluctuations observed in productivity (biomass) during the accrual period acted similarly to influence productivity (caloric value). One notable exception was observed for productivity (caloric value). Maximum values occurred at stations 1, 2, and 4, and values slightly below the maxima occurred at stations 3 and 5 on day 0.5, shown in Fig. 7. The graph of productivity (caloric value) in Austin and Sollo (1969) exhibited the identical phenomenon. Initial colonization of bare substrates by heterotrophic organisms (discussed above) was considered to be the cause of extremely high productivities (caloric value) observed at this time.

#### Production Efficiency

Production efficiencies for each collection date at each station were calculated after the data had already been statistically analyzed. For that reason, production efficiencies were not included in the one-way analysis of

variance and multiple correlation analyses. Production efficiencies (% g-cal cm<sup>-2</sup> periphyton of g-cal cm<sup>-2</sup> insolation) of the algal component of the periphyton at each collection date at the five sampling stations are presented in Fig. 8. The mean production efficiency was highest at station 2, 0.061%. Stations 1 and 5 had the lowest mean production efficiencies, 0.016% each; stations 3 and 4 intermediate, 0.025% each. Grzenda *et al.* (1968) reported periphyton production efficiencies between 0.003 and 0.245% for a warm-water stream in Michigan. The range observed in the Asa Creek-Kaskaskia River system was 0.004 to 0.165%.

Blum (1956) concluded that the best correlation between abundant nutrients and abundant phytoplankton occurred when the decrease in nutrients following the peak concentrations preceded the maximum development of phytoplankton by a few days or weeks. At station 2 nutrient levels were always higher than at the other four stations (Table 2) and production efficiency was highest during the first 13 days of colonization, then declined to within the ranges observed for the other stations (Fig. 8). Stockner (1968) observed a major peak of algal export from a thermal stream from April to August, and attributed it to the period of maximum production and growth. The repeated pattern of accumulation and sloughing was evident in production efficiencies at station 2. When sloughing reduced the amount of periphyton, the production efficiency declined. During regrowth of the periphyton, production efficiency continued to decrease. Only after a 10 to 15-day period of regrowth following sloughing, was production efficiency observed to rise significantly (Fig. 8, accrual days 16 through 35). The sloughing of significant amounts of periphyton composed primarily of algae would reduce the algal component severely. Initial recolonization by heterotrophic organisms would keep production efficiency low even while standing crop was increasing. When the algae again became sufficiently abundant,

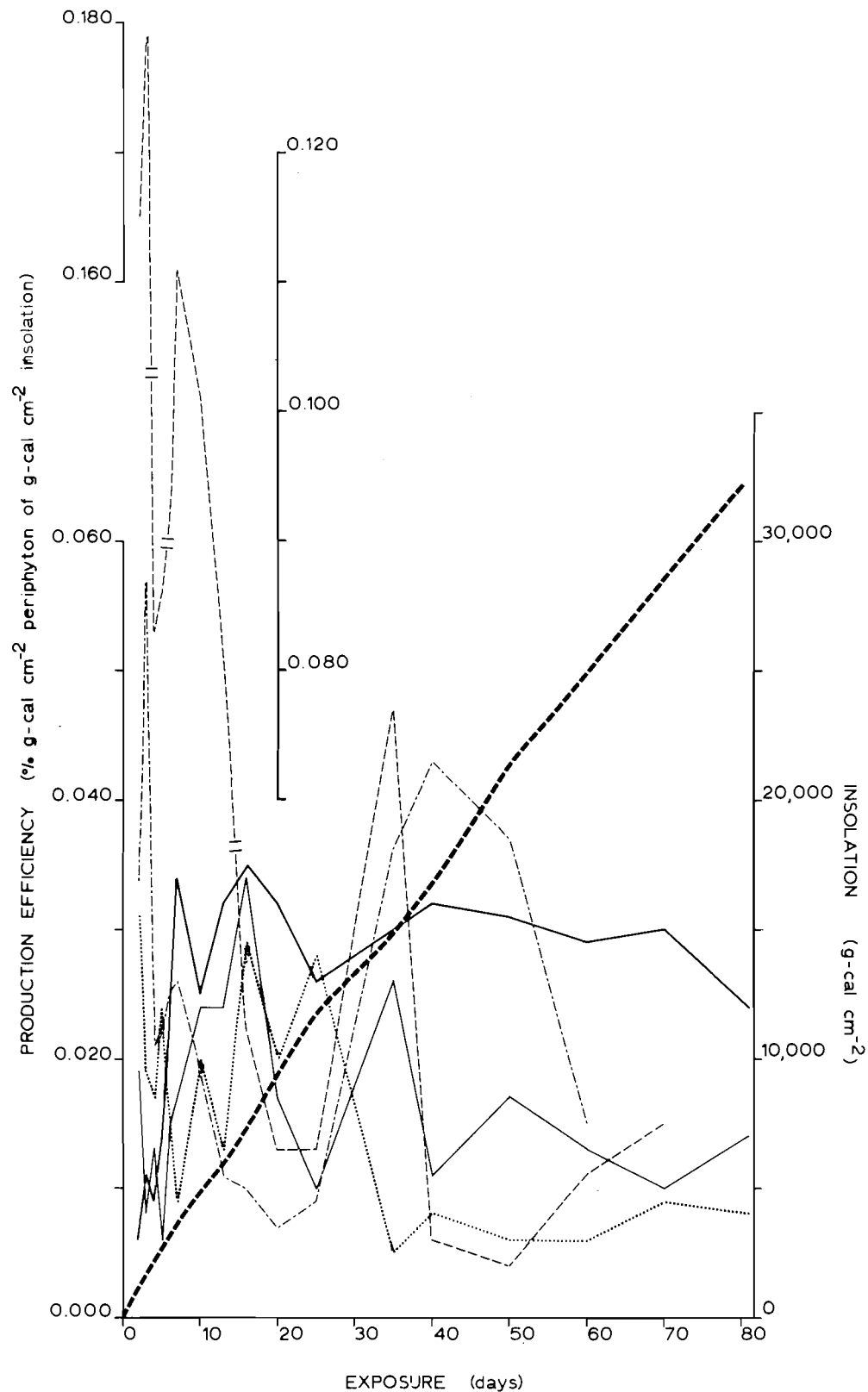


Figure 8. Production efficiency of the periphyton community during the accrual period 9 July 1970 through 5 October 1970 at stations 1 through 5 (dotted, dashed, broken, wide and narrow solid lines, respectively) in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois. Total solar insolation (heavy dashed line) from beginning of the accrual period, 9 July 1970, to each collection date.

production efficiency would begin to increase sharply. While not as pronounced, stations 1, 3, and 5 behaved similarly. Doyle (1971) observed that the production efficiency increased when sloughing occurred. He believed that the limited holding capacity of the substrate was responsible for the sloughing and that the efficiency of young algal cells produced during regrowth was greater.

Low light intensities, caused by shading by emergent vegetation in the present study, slower current velocities, and fluctuating low water levels have been described as reducing primary production (McIntire *et al.* 1964, Kevern and Ball 1965, McIntire 1966, Doyle 1971). These factors combined to keep production efficiency low at stations 1 and 5 by limiting algal growth.

At station 4 production efficiency reached three peaks (each peak followed by sloughing), but remained reasonably constant through the end of the accrual period. Doyle (1971) observed three peaks during his accrual period at the surface-OFT position in the Kaskaskia River which coincided with the three-phase development of the periphyton: first a Cocconeis-diatom layer, then an amorphous layer of silt and palmelloid algae, and finally the development of Cladophora. The periphyton communities he observed in other positions continued to increase in standing crop with time, but without major changes in structure.

#### Intrastation Correlations

Results of the multiple correlation with missing data analyses for the eight parameters measured for periphyton during the accrual period 9 July 1970 through 5 October 1970 are presented in Fig. 5. Results were grouped for discussion to characterize common factors among stations, differences between creek and river habitats, and the influence of the wastewater treatment plant.

#### All Stations

No significant differences in mean standing crops of phytopigments were

observed among the five stations (Table 5). At all stations, the standing crops of chlorophyll a ( $\mu\text{g dm}^{-2}$ ) were directly related to the standing crops of chlorophylls b and c ( $\mu\text{g dm}^{-2}$ ) and standing crops of chlorophyll b were directly related to standing crops of chlorophyll c (Fig. 5). All algae possess chlorophyll a. Only the Chlorophyceae and Euglenophyceae have chlorophyll b and only Bacillariophyceae, Phaeophyceae, and Dinophyceae have chlorophyll c (Smith 1950, Prescott 1956). The standing crops of chlorophyll a were therefore expected to be greater than the standing crops observed for chlorophylls b and c (Fig. 9). Although graphs of standing crops of these phytopigments generally had similar shapes, standing crops of chlorophyll b were not consistently greater than standing crops of chlorophyll c. During the accrual study, standing crops of chlorophyll c were often greater than those of chlorophyll b at stations 1, 4, and 5 (Fig. 9), principally reflecting large numbers of diatoms at these times.

McIntire (1968) found that the organic content of periphyton was related to species composition. He observed that dominance by blue-green algae gave the highest organic contents, diatoms the lowest, and mixtures of periphyton intermediate organic contents. Standing crops ( $\text{g-cal dm}^{-2}$ ) were intermediate to low at stations 1, 4, and 5 at the times when standing crops of chlorophyll c exceeded standing crops of chlorophyll b (Figs. 9 and 10).

The effect of sloughing following periods of maximum production was apparent at station 2. A severe decline in standing crop as organic content, from 481.4 to 162.0  $\text{g-cal dm}^{-2}$ , occurred between days 13 and 16. A loss of 500  $\text{g-cal dm}^{-2}$  occurred from days 35 to 40 (Fig. 10).

Standing crops of chlorophylls a and b were directly related at all stations to standing crops (organic content) (Fig. 5). McIntire's (1968) observation that species composition largely determined the organic content of the periphyton applied here. As accrual proceeded, the population dynamics of algae,

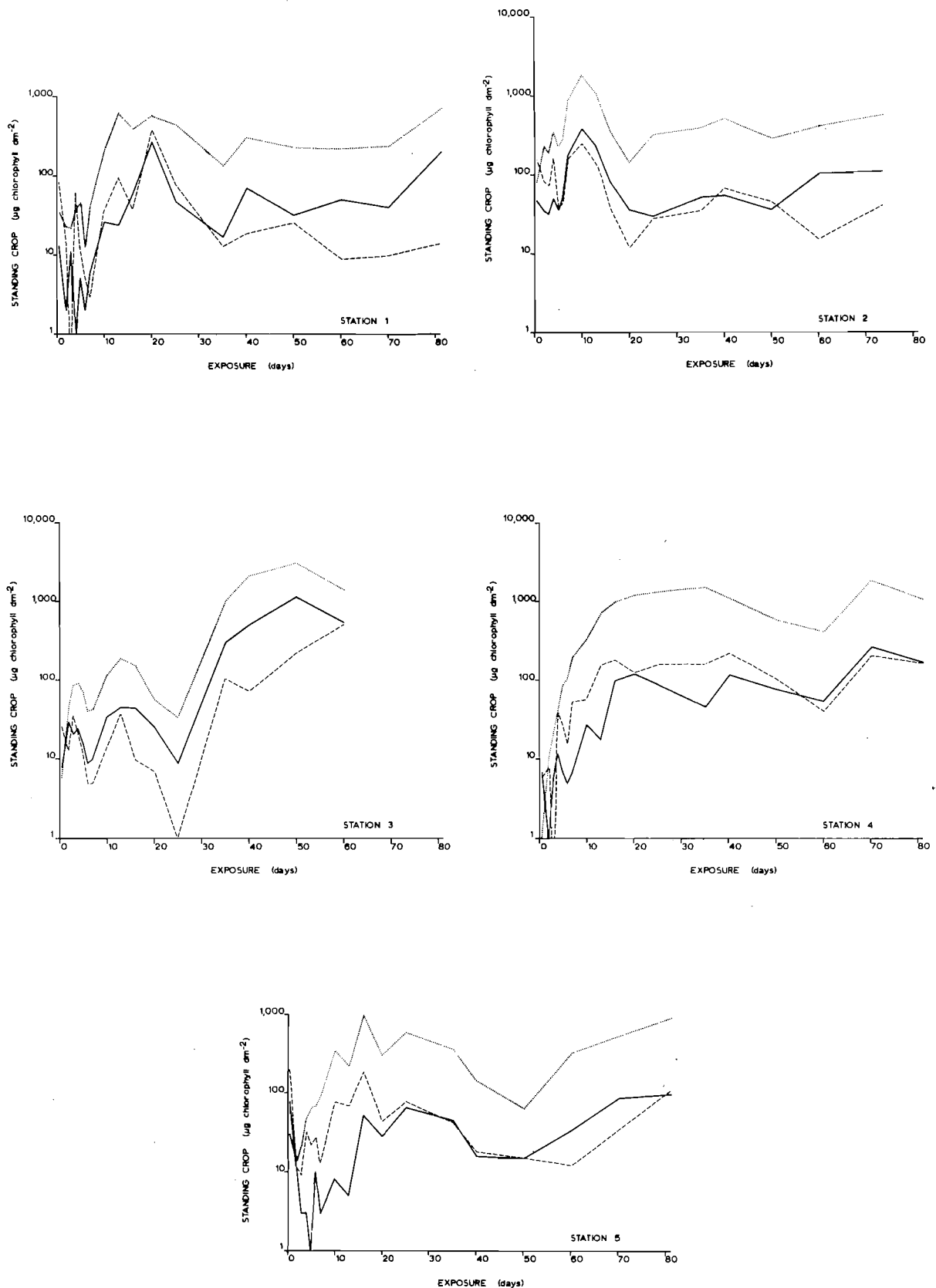


Figure 9. Standing crop of the periphyton community expressed as chlorophylls *a*, *b*, and *c* ( $\mu\text{g dm}^{-2}$ ), dotted, solid, and dashed lines, respectively, during the accrual period 9 July 1970 through 5 October 1970 at five stations in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.



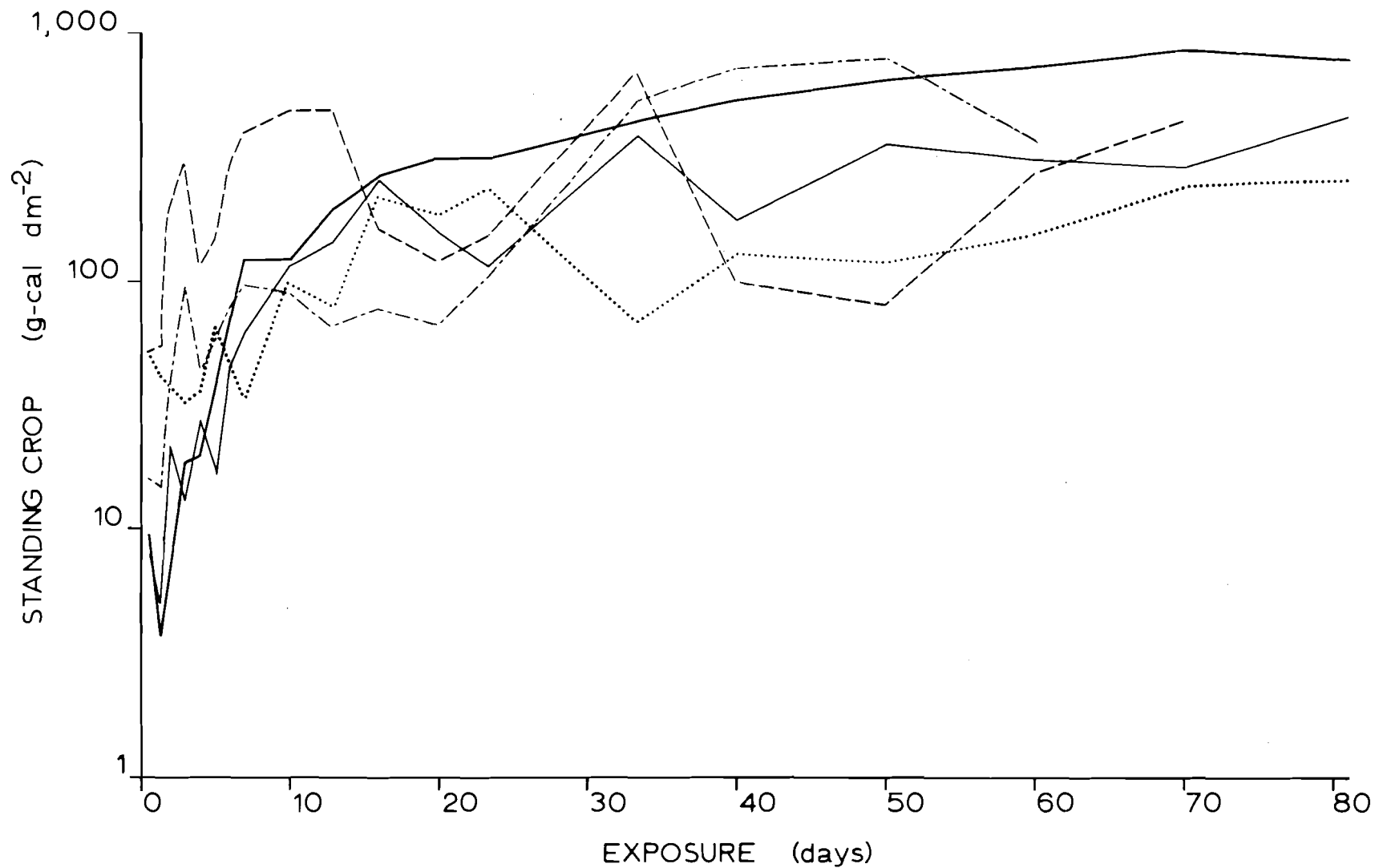


Figure 10. Standing crop of the periphyton community expressed as organic matter ( $\text{g-cal dm}^{-2}$ ) during the accrual period 9 July 1970 through 5 October 1970 at stations 1 through 5 (dotted, dashed, broken, wide and narrow solid lines, respectively) in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.

especially those rich in chlorophylls a and b, were largely responsible for determining the caloric content of the community. Phytopigment analyses, however, did not give any estimate of the heterotrophic components of the periphyton. In the Salt Fork system, which received the Urbana-Champaign wastewater treatment plant effluent, Austin and Sollo (1969) recorded a range of 8 to 500 g-cal dm<sup>-2</sup> for periphyton. Their highest values were below the plant outfall in an area composed primarily of periphytic bacteria and protozoans.

#### Creek or River Stations

There were no correlations observed to be peculiar to only Asa Creek stations.

At the Kaskaskia River stations only, the standing crops of chlorophyll a were directly related to productivity (biomass) (Fig. 5). The rate of photosynthesis of periphyton has been demonstrated to be a function of the chlorophyll a content (Waters 1961). In fact, reliable estimates of population densities have been obtained from phytopigment absorbance values, corrected for deviations from absorbancy-concentration relationships (Grzenda and Brehmer 1960).

Harrel and Dorris (1968) remarked that although many investigators have used the concept of self-purification with stream succession to evaluate pollution, they have not been successful in distinguishing the effects of pollution from the effects of natural succession in streams. In this instance, the lack of agreement between standing crops of chlorophyll a and productivity (biomass) at the creek stations was attributed to both pollution effects and natural succession. The effects of the wastewater treatment plant on standing crops and productivity of periphyton have been discussed in part, above.

Harrel and Dorris (1968) and Whiteside and McNatt (1972) observed that physico-chemical fluctuations decreased as stream order increased, indicating

greater stability in higher order streams. Asa Creek was determined to be an order 2 stream while the Kaskaskia River was an order 4 stream in the study area (using United States Geological Survey 1:62,500 topographic maps). The discharge of effluent from the wastewater treatment plant coupled with low stream order of Asa Creek resulted in wide flux of physico-chemical conditions which had a similarly varied effect on the periphyton. The greater stability in the higher stream order Kaskaskia River was reflected in the more predictable levels of physico-chemical parameters and in the more stable periphyton communities which developed there. This was particularly apparent in the regression lines between standing crops of chlorophyll a and biomass (Table 6, Fig. 11). The regression lines have a wide variation in slope. Stations 1 and 2 in Asa Creek had similar slopes, but were lower than stations 4, 5, and Doyle's (1971) line for the Kaskaskia River, all of which had similar slopes (Fig. 11). These differences have been attributed to variations in silt content and the disproportionate differences in sizes of the heterotrophic components of the periphyton (Doyle 1971).

The correlation coefficients (Table 6) calculated for stations 2 and 3 reflected the disrupting effect of the wastewater treatment plant on the periphyton community. While the correlation coefficient at station 1 was significant at the 0.001 level, the regression line was still the lowest among stations. The shading of the stream by emergent vegetation, fluctuating water levels, and low flow seriously limited primary production of the periphyton.

Standing crops of chlorophyll c correlated with standing crops (biomass) at the Kaskaskia River stations (Fig. 5). Dominant diatom communities have been recognized in river periphyton (Butcher 1932, 1940, 1946; Starrett and Patrick 1952; Cushing 1967; Peters et al. 1968). In the Kaskaskia River study area, Doyle (1971) described a community dominated by the diatom Cocconeis

Table 6. Correlation coefficients, confidence levels, and regression equations between chlorophyll  $a$  ( $\mu\text{g dm}^{-2}$ ) and dry weight ( $\text{mg dm}^{-2}$ ) for stations in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.

STATION	CORRELATION COEFFICIENT	CONFIDENCE LEVEL	REGRESSION EQUATION
1	0.72	0.001	$Y = 0.107x + 76.89$
2	0.44	Not Sig.	$Y = 0.150x + 172.2$
3	0.28	Not Sig.	$Y = 0.657x + 184.4$
4	0.76	0.001	$Y = 0.521x + 258.5$
5	0.65	0.01	$Y = 0.534x + 80.93$
Doyle (1971)*	0.80	0.05	$Y = 0.80x + 108.5$

\*Kaskaskia River Bottom Vertical Out

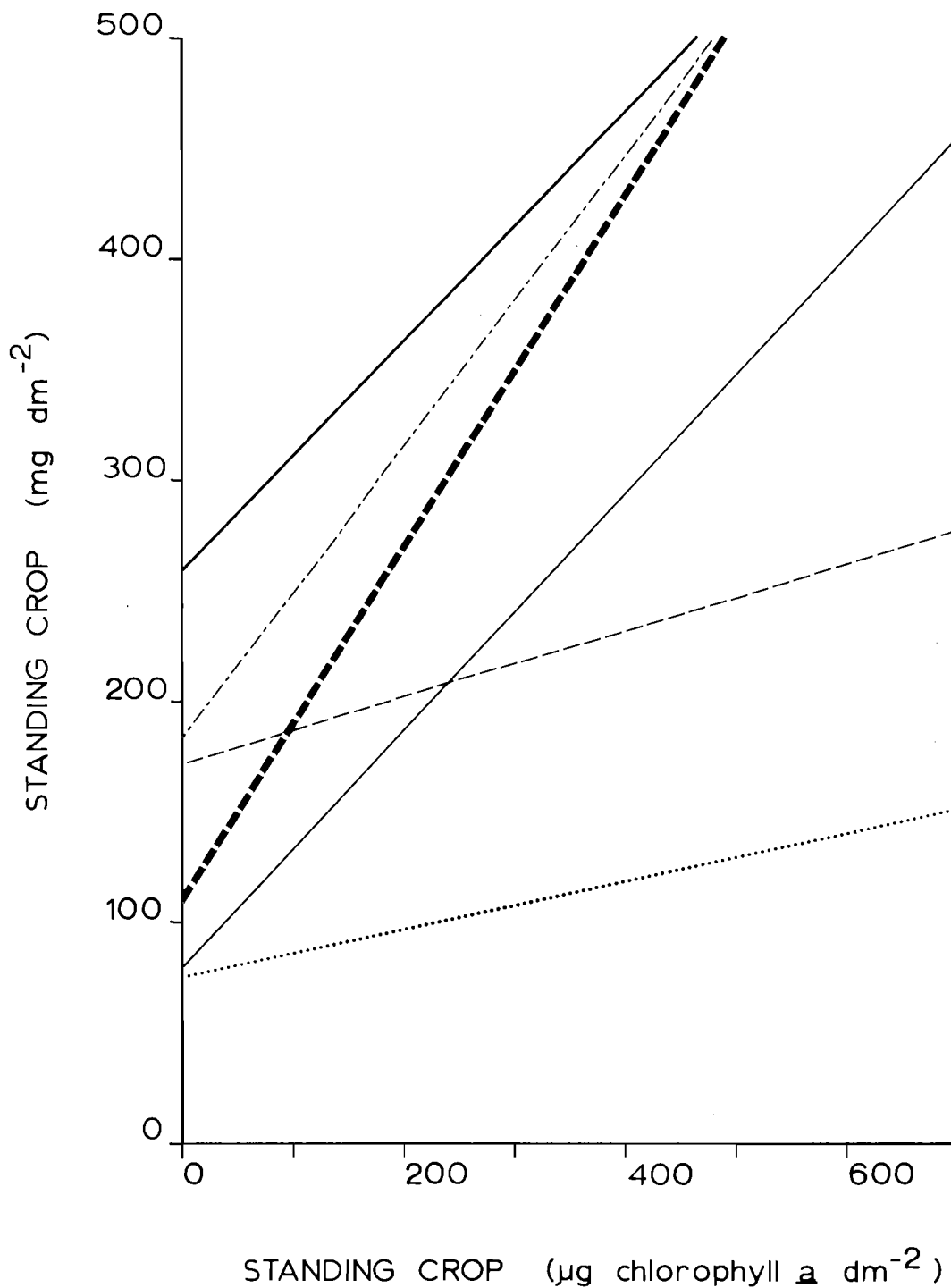


Figure 11. Regression lines between chlorophyll a ( $\mu\text{g dm}^{-2}$ ) and dry weight ( $\text{mg dm}^{-2}$ ) during the accrual period 9 July 1970 through 5 October 1970 at stations 1 through 5 (dotted, dashed, broken, wide and narrow solid lines, respectively) in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois, compared with Doyle's (1971) regression line for the Kaskaskia River (heavy dashed line).

placentula, with the filamentous green algae Cladophora glomerata and Stigeoclonium as other important members.

#### Effects of the Wastewater Treatment Plant

At station 1, upstream from the influence of the wastewater treatment plant, and stations 4 and 5, in the Kaskaskia River, standing crops of chlorophylls a and b were directly related to standing crop (biomass) (Fig. 5). Relationships between standing crops of chlorophyll a and standing crop (biomass), discussed above, have an extensive literature. The important members of the periphyton community which were responsible for the significant correlations between standing crops of chlorophyll a and standing crop (biomass) would also contain chlorophyll b. It was therefore not unusual that standing crops of this phytopigment would behave similarly to standing crops observed for chlorophyll a.

#### Station 3

A number of significant correlations were peculiar either to station 3 only or to all stations except station 3. This station in Asa Creek was 4.2 km downstream from the wastewater treatment plant outfall. The substrate was composed entirely of shifting sand. During the accrual period the water level was low (0.13 m or less) and the ripples of the sand were solid green with benthic algae. Between accrual days 20 and 25, the entire sampler was buried beneath the sand and standing crops of phytopigments decreased (Fig. 9).

At only station 3 standing crops of all phytopigments were directly related to organic content ( $\text{g-cal mg}^{-1}$ ). The only inverse relationship observed during the accrual study was at station 3 between organic content ( $\text{g-cal mg}^{-1}$ ) and productivity (biomass). At all stations except station 3, significant correlations between productivities as biomass and caloric value, and between

standing crops as biomass and caloric value were observed (Fig. 5).

Duffer and Dorris (1966) observed the highest chlorophyll a concentrations associated with a large boulder substrate and the lowest concentrations of chlorophyll a associated with shifting sand. However, shifting sand was a more suitable substrate for periphyton than the highly organic soft silt substrates at stations 1 and 2. In the present study, the maximum standing crops of chlorophylls a and b, and near-maximum standing crops of chlorophyll c were observed at station 3 (Fig. 9). Butcher (1947) found that while algae were reduced or eliminated below a source of pollution in the River Trent, their numbers peaked eight miles downstream. The predominance of benthic algae in the periphyton and the presence of those organically-rich algae described by McIntire (1963) (blue-greens and greens mixed with diatoms) accounted for the strong correlations observed for standing crops of chlorophylls a, b, and c with organic content, 0.001, 0.001, and 0.01 levels, respectively.

Since the periphyton sampler was often partially or totally buried by the sand, abrasion, in addition to sloughing, reduced the standing crop (biomass) at station 3 (Fig. 3). While this reduced productivity (biomass), rapid initial recolonization by heterotrophic organisms (abundant downstream from the wastewater treatment plant outfall) enhanced the organic content.

Productivities as biomass and caloric value, and standing crops of organic content and biomass correlated significantly (0.001 level) at stations 1, 2, 4, and 5 (Fig. 5). The lack of any significant correlations for these parameters at station 3 was considered to be a function of what physically occurred to the sampler (burial) rather than to any ecological conditions.

## PERIPHYTON NUTRIENT ASSIMILATION

Studies of assimilation by the periphyton community have been principally concerned with nutrient uptake and the fixation of inorganic nitrogen and phosphorus. Ewing and Dorris (1970) observed that the size and diversity of algal populations was not strongly correlated with nutrient concentrations. Chu (1943), however, found that nitrogen and phosphorus had a limiting effect on phytoplankton if concentrations were 0.1 or less and 0.009 or less mg liter<sup>-1</sup>, respectively. He further determined that the optimum growth for all algae was obtained with phosphorus concentrations from 0.09 to 1.8 mg liter<sup>-1</sup> and nitrate-nitrogen concentrations from 0.9 to 3.5 mg liter<sup>-1</sup>. Increased concentrations of total nitrogen and phosphorus in periphyton were concluded to be a "luxury" consumption, observed in enriched nitrogen-phosphorus environments (Stockner and Armstrong 1971). In northwestern Ontario they found the percent nitrogen to range from 2.29 to 3.14 and the percent phosphorus to range from 0.04 to 0.15 in periphyton. Kevern and Ball (1965) observed comparable mean organic phosphorus contents of 0.21% and mean nitrogen contents of 3.29% in periphyton in an artificial stream.

The stream biota was determined to be the primary mechanism for the assimilation of phosphorus by Keup (1968). Confer (1972) also observed that the periphyton community rapidly removed a large percentage of the phosphorus concentration. Using radioactive phosphorus Ball and Hooper (1963) concluded that an atom of <sup>32</sup>P traveled 450 to 11,235 yds (412 to 10,270 m) downstream before being assimilated.

The rate of phosphorus fixation by the periphyton was closely related to the growth rate of the community. Grzenda et al. (1968) observed that the two rates resembled an exponential relationship where the rate of phosphorus fixation equalled the growth rate of the periphyton colony raised to some



power. They determined that the minimum fixation rate of periphyton was  $0.7 \mu\text{g P dm}^{-2} \text{ day}^{-1}$  during January and that the maximum fixation rate was  $383 \mu\text{g P dm}^{-2} \text{ day}^{-1}$  during June. When the saturation concentration was reached within a periphyton colony on an artificial substrate, the amount of phosphorus fixed by the old standing crop was assumed to be negative compared to the quantity of phosphorus fixed by "new" growth.

Nitrogen concentrations stimulated algal growth and determined largely the amount of chlorophyll formed (Mackenthun and Ingram 1967, Cullimore and McCann 1972). Nitrogen concentrations beyond the optimum range inhibited the formation of chlorophyll in green algae (Mackenthun and Ingram 1967). Ehrlich and Slack (1969) observed that in a laboratory stream productivity (biomass) was greater for periphyton supplied with nutrients in an inorganic rather than an organic form. Standing crops of chlorophyll a increased rapidly, reached maximum levels in three weeks, then declined. They further noted that ammonia derived from organic compounds was partly assimilated by algae and partly nitrified by bacteria. The nitrate from the bacteria was then assimilated by the algae.

#### Bacterial Uptake Kinetics

Thomas and O'Connell (1966) concluded that their measurements of primary production by stream periphyton were not entirely representative of the benthic algae because bacteria were simultaneously oxidizing carbonaceous and nitrogenous materials. This consideration of the effects of heterotrophic organisms in periphyton studies is becoming more common. Hobbie and Crawford (1969a) determined that the heterotrophic bacteria in aquatic ecosystems were extremely important as transformers of soluble carbon into particulate forms available to higher organisms. They claimed that in reservoirs, the biomass production of heterotrophic bacteria might be as important as algal primary production.

Allen (1969) described further the intricate relationships between dissolved organic matter in the aquatic environment and the natural populations of bacteria and algae which were capable of producing, transforming, and utilizing it. He considered the annual cycle of production and utilization of dissolved organic matter.

Since the literature on bacterial uptake kinetics was not extensive, acetate was selected as the substrate because of its precedence in the literature and its ready availability to the author. The literature was almost exclusively restricted to planktonic uptake kinetics in lentic habitats. Allen's (1971) paper was the first, and only to date, to include periphyton. His study was conducted in a small Michigan lake. For these reasons, bacterial uptake kinetics were applied to stream periphyton, and included planktonic uptake for comparative purposes, so that the role of heterotrophic organisms in the assimilation of the wastewater treatment plant effluent could be determined.

In uptake-response kinetics of  $^{14}\text{C}$ -labeled organic compounds (Allen 1969, 1971), bacterial uptake, represented by first-order kinetics, is an active transport phenomenon, and occurs at low substrate concentrations ( $0.5 \text{ mg liter}^{-1}$  or less). Algal uptake (not measured directly in this study) is represented by zero-order diffusion and occurs at high substrate levels (greater than  $0.5 \text{ mg liter}^{-1}$ ).

Fig. 12 graphically illustrates the results of the planktonic and periphytic bacterial chemo-organotrophy with acetate as the substrate, measured at concentrations of  $41$  to  $820 \text{ } \mu\text{g liter}^{-1}$ , and evaluated through the enzyme kinetic analysis described by Allen (1969). Data for the bacterial uptake for plankton at station 2 were erratic and could not be calculated. For this reason station 2 was not included for plankton. Although substrate concentrations used in this study are higher than those reported in the literature, it was necessary because

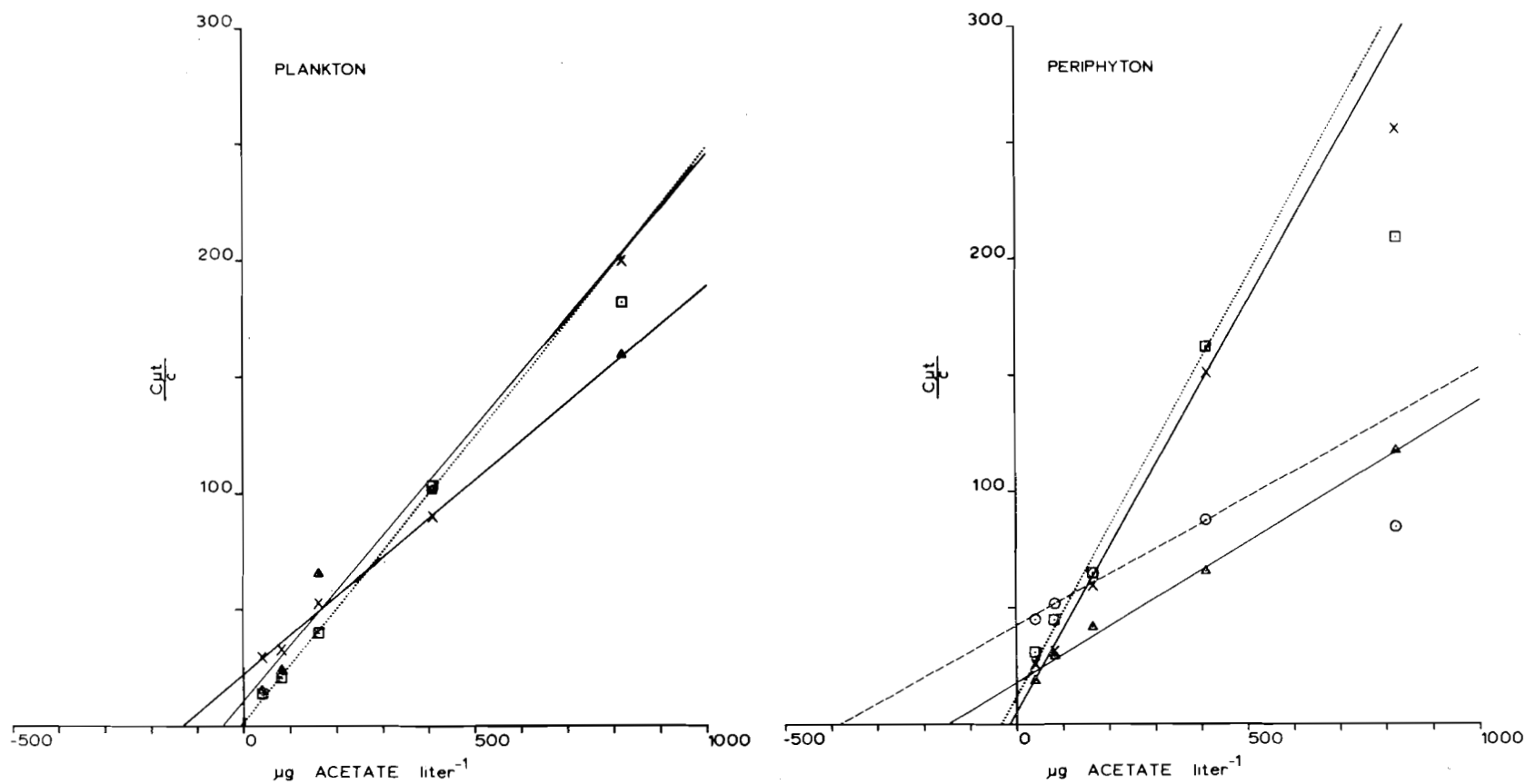


Figure 12. Planktonic and periphytic bacterial uptake of acetate at stations 1, 2, 4, and 5 (dotted, dashed, wide and narrow solid lines, respectively) on 7, 8, 9, and 10 December 1970, respectively, in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois. Regression equations presented in Table 7.

the natural substrate concentrations in the Asa Creek-Kaskaskia River system were believed to be higher than those previously reported.

The maximum bacterial uptake velocity of acetate ( $V_{max}$ ), the maximum natural substrate concentrations of acetate ( $K_t + S_n$ ), and the substrate regeneration time or turnover time of acetate ( $T_t$ ) calculated from the graphs in Fig. 12 are presented in Table 7. For plankton, the maximum bacterial uptake was similar, but lower at stations 1 and 5 than the maximum bacterial uptake velocity at station 4. Natural substrate concentrations of acetate and turnover times exhibited wide variations. Periphyton at stations 1 and 4 had similar, but lower maximum bacterial uptake velocities compared to stations 2 and 5. Again, natural substrate concentrations of acetate and turnover times varied widely.

The values obtained for the Asa Creek-Kaskaskia River system were compared with those reported in the literature (Table 7). Comparable seasonal ranges were used.

Since temperature regulates metabolic rates and growth, the low water temperatures would be expected to influence strongly the number of bacteria present. Consequently, one would anticipate winter determinations of the maximum bacterial uptake velocity to be significantly lower than at any other time of year. Natural substrate concentrations of acetate would be greater with low uptake and utilization, and turnover times would be lower.

This was most apparent in the winter uptake kinetics for Lake Erken reported by Wright and Hobbie (1966) (Table 7). Maximum bacterial uptake velocities and regeneration times were extremely low. Allen (1969) attributed low regeneration times to the presence of very small bacterial populations. Wright and Hobbie (1966) concurred that bacteria were largely absent and theorized that the uptake detected was due mainly to small phytoplankters with uptake mechanisms

Table 7. Maximum bacterial uptake velocity of acetate ( $V_{\max}$ ), maximum natural substrate concentrations of acetate ( $K_t + S_n$ ), and substrate regeneration time of acetate ( $T_t$ ) for periphytic and planktonic bacteria. Equations for regression lines are shown for stations in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois.

LOCATION	REGRESSION EQUATION	$V_{\max}$ ( $\mu\text{g liter}^{-1} \text{ hr}^{-1} \text{ dm}^{-2}$ )	$V_{\max}$ ( $\mu\text{g liter}^{-1} \text{ hr}^{-1}$ )	$K_t + S_n$ ( $\mu\text{g liter}^{-1}$ )	$T_t$ (hours)
<u>Periphyton</u>					
Station 1	$Y = 0.36x + 13.1$	194.4	-	36.3	13.1
Station 2	$Y = 0.11x + 42.8$	631.9	-	389.1	42.8
Station 4	$Y = 0.35x + 5.9$	201.4	-	16.9	5.9
Station 5	$Y = 0.12x + 18.3$	576.4	-	152.7	18.3
<u>Plankton</u>					
Station 1	$Y = 0.25x + 1.8$	-	4.1	73.5	1.8
Station 4	$Y = 0.17x + 22.6$	-	6.0	136.0	22.6
Station 5	$Y = 0.23x + 11.0$	-	4.3	47.1	11.0
Lake Löttsjön, Sweden (winter) (Allen 1969)		-	1 to 5	5 to 30	1 to 10
Lake Erken, Sweden (winter) (Wright and Hobbie 1966)		-	0.041 to 0.054	6 to 15	200 to 270
Lawrence Lake, Michigan (winter) (Allen 1971)					
<u>Scirpus</u> site		less 250	-	-	-
<u>Najas-Chara</u> site		500 to 1000	-	-	-

similar to those possessed by bacteria. Furthermore,  $T_t$  could be more related to changes in the physiological composition of the bacterial community than to total numbers (Allen 1969).

Maximum uptake velocities for planktonic bacteria in the Asa Creek-Kaskaskia River system were comparable to those reported by Allen (1969) for Lake Löttsjön (Table 7). He concluded that his values were higher than those observed by Wright and Hobbie (1966) because Lake Löttsjön was more eutrophic than Lake Erken. The higher natural substrate concentrations of acetate observed in the Asa Creek-Kaskaskia River system were credited to the agricultural and municipal drainage in the watershed.

Maximum bacterial uptake velocities for periphyton calculated for the Asa Creek-Kaskaskia River system were comparable to the winter values reported by Allen (1971) for epiphytic bacteria in Lawrence Lake, Michigan. The maximum observed bacterial uptake velocity for periphyton,  $631.9 \mu\text{g liter}^{-1} \text{hr}^{-1} \text{dm}^{-2}$ , was at station 2, 2.1 km downstream from the wastewater treatment plant outfall. The lowest maximum bacterial uptake velocities for periphyton were observed at stations 1 and 4,  $194.4$  and  $201.4 \mu\text{g liter}^{-1} \text{hr}^{-1} \text{dm}^{-2}$ , respectively. Both stations were upstream from the influence of the wastewater treatment plant. At station 5, in the Kaskaskia River, 1.9 km downstream from the mouth of Asa Creek, the maximum bacterial uptake velocity for periphyton was  $576.4 \mu\text{g liter}^{-1} \text{hr}^{-1} \text{dm}^{-2}$ , less than found for station 2, but still nearly three times that observed at stations 1 and 4 (Table 7).

Effluents from wastewater treatment plants have been shown to exert a damping effect on the annual fluctuation of water temperature below plant outfalls (Hynes 1960, Roeske 1969, Brigham 1972). Brigham (1972) observed that this damping effect could maintain biological activity at higher levels in winter than would otherwise be possible. For the periphyton at stations 1 and

4, the values of  $V_{\max}$  observed may be the natural winter levels for streams not enriched by wastewater treatment plant effluents. In winter the treatment plant effluent affected both the temperature and nutrient content of the stream further downstream (as witnessed by the significantly higher maximum bacterial uptake velocities for periphyton at stations 2 and 5). These or other relationships between stations 2 and 5 were not detected in the periphyton accrual study, perhaps because the study was completed before temperatures began to decline and the algae population was reduced.

Using glucose and acetate to compare the activities of epiphytic, benthic, and water-isolated bacteria, Strzekczyk and Mielczarek (1971) concluded that epiphytic bacteria exhibited the greatest metabolic activity, benthic bacteria the least activity, and the water-isolates an intermediate activity. Bacterial uptake kinetics for acetate in the Asa Creek-Kaskaskia River system (Table 7) supported their observations. The maximum uptake velocities for planktonic bacteria were generally lower than those observed for the periphytic bacteria at all stations. Likewise, the influence of the wastewater treatment plant was not as apparent for planktonic as for periphytic bacteria. This reinforced the premise that the attached community was the most sensitive to subtle changes in the aquatic environment.

## IMPORTANCE TO WATER QUALITY RESEARCH

Many small streams in the midwest receive effluents from the wastewater treatment plants of small communities. The Asa Creek-Kaskaskia River system, Moultrie County, Illinois, which receives effluent from the city of Sullivan, is considered to be representative of this generally occurring situation. Typically these communities are expanding, have inadequate wastewater treatment, and discharge into streams having insufficient dilution water. To the contrary, Sullivan has recently put a tertiary treatment facility with chlorination into operation. As advanced wastewater treatment becomes more common, instances similar to those encountered in Asa Creek will become more frequent.

The results of the coordinated physical, chemical, and biological study revealed that there was no gross evidence of any differences between the creek and river sampling sites as determined by these measurements. Wastewater treatment was of such high quality that the effluent was generally undetectable 2 km downstream from the outfall. Only nitrate-nitrogen, ammonia-nitrogen, and phosphorus (all forms) concentrations were directly attributable to the effluent in Asa Creek, the receiving stream.

Since the periphyton (Aufwuchs) community was considered to constitute intermediate nutrient utilization between subtle differences in water chemistry and the macrobiota, research efforts were concentrated on defining the role of the periphyton community in the assimilation of the Sullivan wastewater treatment plant effluent.

Bacterial uptake kinetics experiments demonstrated that the periphyton, at stations influenced by the wastewater treatment plant effluent, assimilated two to three times more dissolved organic matter than at stations not influenced by the effluent. The influence of the treatment plant was not as apparent for assimilation by planktonic bacteria. This reinforced the premise that the attached community was the most sensitive to subtle changes in the aquatic environment.



The periphyton is an attached community which develops best on hard substrates ( e.g. rocks and branches). In this study the periphyton community, by its role in the assimilation of dissolved organic matter, was shown to behave as an "in-stream" trickling filter. Disturbances such as stream straightening, channelization, and dredging seriously upset this natural process. These actions remove most desirable periphyton substrates from the stream. The silt-sand substrate which results from dredging, in addition to being unsuitable for periphyton, offers a much-reduced surface area for attachment than the gravel-rock-branches substrate of undredged streams.

Stream straightening and channelization exert a two-fold effect by producing a shorter stream channel. In addition to reducing the surface area available to the periphyton, they shorten contact time between the water in the stream and the periphyton community. All these actions seriously reduce the assimilative capacity of the stream to dissolved organic matter.

Results of this study further reinforced the importance of the periphyton community as a "biological monitor" of stream conditions. In water quality surveillance, use of periphyton community dynamics may not provide rapid estimates of catastrophic occurrences. For chronic effects, however, as demonstrated in the Asa Creek-Kaskaskia River system, the periphyton community was a sensitive indicator of subtle differences in water quality.

## SUMMARY

1. Physical, chemical, and biological parameters were monitored at five stations in the Asa Creek-Kaskaskia River system, Moultrie County, Illinois, from 12 September 1969 through 7 September 1970 to characterize these streams as a periphyton habitat. Periphyton accrual and periphytic bacterial uptake kinetics studies continued until 10 December 1970.
2. Stations 1, 2, and 3, in Asa Creek, were located 3.7 km upstream and 2.1 and 4.2 km downstream from the outfall of the Sullivan wastewater treatment plant, respectively. Stations 4 and 5, in the Kaskaskia River, were located 3.4 km upstream and 1.9 km downstream, respectively, from the mouth of Asa Creek.
3. Only 13 of 34 parameters measured biweekly were found to be significantly different among stations. Four of these, nitrate-nitrogen, ammonia-nitrogen, total phosphorus, and soluble orthophosphate, were significantly higher below the wastewater treatment plant outfall. Station 1 above the outfall had lower chloride and sodium levels, but a higher total alkalinity than the downstream stations. Sulfate concentrations were higher at the river sites. Relationships for the remaining five parameters showing significant differences among stations were discussed. Results of analyses of 21 other parameters are summarized.
4. Significant correlations (0.05 level or greater) among the 34 parameters measured biweekly which were common to all stations, which characterized the creek or river sites, and which distinguished the effect of the wastewater treatment plant are discussed.

At the Kaskaskia River stations, concentrations of planktonic chlorophyll a were directly related to calcium, sulfate, and total dissolved ionizable solids concentrations. Leaching from extensive gypsum deposits 60 km upstream was strongly suspected as the source of calcium sulfate to the river.

In Asa Creek below the wastewater treatment plant outfall, significant

correlations among BOD, calcium, chloride, COD, coliform bacteria, filtrable residue, organic-nitrogen, sodium, and sulfate were observed. These parameters exhibited a high degree of correlation since the behavior of each was a function of a common parameter, discharge.

5. Of eight parameters measured during the periphyton accrual study, four were found to be significantly different among stations. Standing crop (biomass) was higher in the Kaskaskia River at station 4, but organic content was lowest at that station. Productivity (caloric value) was greatest at station 2. Lowest productivities (biomass) were at stations 1 and 5.

6. The range of production efficiency of the periphyton community was 0.004 to 0.165% among the five stations. Highest mean production efficiencies were reported from station 2 below the outfall. Lowest mean production efficiencies were observed at stations 1 and 5.

7. Results of correlations among the eight parameters measured in the periphyton study were grouped for discussion to characterize common factors among stations, differences between creek and river habitats, and the influence of the wastewater treatment plant.

The discharge of effluent into Asa Creek coupled with its low stream order resulted in wide flux of physico-chemical conditions which had a similarly varied effect on the periphyton. Greater stability in the higher stream order Kaskaskia River was reflected in more predictable levels of physico-chemical parameters and in more stable periphyton communities which developed there.

8. Planktonic and periphytic bacterial chemo-organotrophy, with acetate as the substrate, were measured and evaluated through enzyme kinetics analysis procedures. The maximum bacterial uptake velocity, the maximum natural substrate

concentrations, and the substrate regeneration time of acetate are presented.

The maximum uptake velocities for planktonic bacteria were generally lower than those observed for the periphytic bacteria at all stations. Likewise, the influence of the wastewater treatment plant was not as apparent for planktonic as for periphytic bacteria.

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